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Progress in epithelial ovarian carcinoma.
Has the outcome been improved?

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Introduction
Ovarian cancer is a complex neoplasm composed of different histological grades and types, and is the leading cause of death from gynecologic malignancies in Western countries. Epithelial ovarian cancer (EOC) is the most common histological type of ovarian malignancy. In some areas of this malignancy considerable progress has been made during the past 30 years, in others there is stagnation. Has the outcome been improved?

Progress
Although the etiology of the disease is still mostly obscure, several involved risk factors are known. Certain reproductive factors, such as age at menopause and infertility, and lifestyle factors as cigarette smoking, obesity, diet and hormone replacement therapy may contribute to a greater risk of ovarian cancer, whereas pregnancy, pelvic surgery and oral contraceptive use, reduce the risk. Advances in cancer genetics has allowed the identification of mutations within the BRCA1 and BRCA2 genes. Women who have these mutations have a significantly increased lifetime risk (range 15-60%) of ovarian malignancies. Subsequent clinical genetic testing for mutations in the genes associated with hereditary breast-ovarian cancer and Lynch/hereditary nonpolyposis colorectal cancer syndrome were made available. About 5-10% of ovarian cancers are due to a hereditary risk. Risk-reducing surgery consisting of bilateral salpingo-oophorectomy is offered to women who carry the BRCA mutations. Chemoprevention (mainly oral contraceptive use) and/or intensified surveillance are alternative approaches.

Some understanding has been gained of the proteins and pathways involved in the early stages of malignant transformation and metastasis of ovarian carcinoma [1]. Multiple recent publications deal with clinical proteomics [2] and its possible relevance for risk assessment, early detection and management of ovarian cancer, though the new discoveries are not yet clinically applicable.

Transvaginal sonography and the serum CA125 biomarker are currently helpful in distinguishing benign from malignant adnexal masses. CA125 is extremely valuable for monitoring treatment response and diagnosis of recurrent disease. In addition, modern imaging modalities, such as PET/CT, greatly assist in the preoperative evaluation and follow-up of ovarian cancer patients.

In 1988 the FIGO surgical staging system was introduced. Among its advantages was the more accurate identification of malignancies truly confined to the ovaries. Several prognostic factors of ovarian carcinoma, in addition to stage, have been identified and include age, performance status, presence of large volume of ascites, histological tumor type, grade and microvessel density analysis.

In the surgical management of ovarian cancer the concept of cytoreduction (debunking) has been introduced and accepted based on numerous retrospective studies that indicate that patients with optimal cytoreduction have a better prognosis. Throughout the years the definition of optimal cytoreduction has been modified and today consists of residual tumor nodules of up to 1 cm and preferably of no macroscopic residual disease. Several studies indicate that women operated on by gynecologic oncologists have a more favorable outcome.

Based on phase III trials the standard adjuvant chemotherapy has been established as well [3]. According to the guidelines of the 1994 NIH Consensus Conference [4] management of advanced ovarian carcinoma should include surgical staging, optimal cytoreduction followed by chemotherapy with platinum combined with taxane. It has been recognized that surgically determined early ovarian cancer patients can be treated conservatively. In young women, the uterus and in some instances the uninvolved ovary may be retained thus preserving fertility. Such patients do not require postoperative chemotherapy. Using currently available assisted reproductive technology pregnancies and term deliveries have been reported in these cases.
Following initial surgery, the great majority of patients with epithelial ovarian carcinoma will receive standard combined chemotherapy i.e., platinum (carboplatin or cisplatin) and a taxane (paclitaxel or docetaxel). Patients with recurrence are usually treated with several additional chemotherapy regimens during the course of the disease. They include newly developed chemotherapy agents such as etoposide, liposomal doxorubicin, gemcitabine, and topotecan.

Several treatment modifications are currently practiced. Neoadjuvant chemotherapy prior to cytoreductive surgery is used in selective cases of advanced ovarian cancer. Phase III trials have confirmed a significant advantage in progression-free and overall survival for initial adjuvant intraperitoneal chemotherapy in optimally cytoreduced advanced ovarian cancer. This treatment combined with postoperative IV chemotherapy was endorsed in January 2006 by the National Cancer Institute in the USA as the preferred treatment method for advanced ovarian cancer. However, it is noteworthy that this treatment regimen is more toxic and is associated with reduced short-term quality of life.

Novel treatments are being explored including immunotherapy, gene therapy, anti-angiogenic therapy and treatment by signal transduction inhibitors. Extreme drug resistance-directed chemotherapy may improve outcome in recurrent ovarian carcinoma patients [5]. Attempted approaches to achieve longer clinical complete remissions include consolidation and maintenance therapy. Microarray technologies may in the future provide valuable expression data for classifying ovarian cancer and insight into molecular changes in ovarian cancer that could be exploited in new treatment strategies.

**Stagnation**

Only 25% of ovarian cancers are detected in Stage I. However, when diagnosed in this stage, up to 90% of patients can be cured with conventional therapy. Therefore early diagnosis could markedly improve the overall survival of ovarian cancer patients.

Although transvaginal ultrasound and the marker CA125 are very useful in the diagnosis of ovarian malignancy, each of these modalities lacks the sensitivity and specificity to serve alone or in combination as a screening test. Currently no screening test for ovarian carcinoma with a high sensitivity and high enough specificity to avoid the harmful effects of false-positive results is available [6].

The quest for optimal cytoreduction in ovarian carcinoma is well ingrained in the gynecologic oncology community although no prospective randomized trials have ever been performed to show whether the benefit of this procedure is due to the aggressive surgery or to inherent biological properties of the tumor that allow cytoreduction. The concept of cytoreduction is not unchallenged. The results of some investigations do not support this surgical approach [7]. Therefore the justification for radical operations that often consist of procedures that involve other organ systems and that may be followed by a non-negligible complication rate is still debated. The Scottish Randomised Trial in Ovarian Cancer surgical study examined the impact on progression-free survival (PFS) of cytoreductive surgery and international variations in surgical practice in 889 patients [8]. One of the main conclusions of this study was that the increased PFS associated with optimal surgery is limited to patients with less advanced disease, supporting case selection rather than aggressive cytoreduction in all patients irrespective of disease extent. As so eloquently worded by two most prominent gynecologic oncologists [9]: “Many feel that the pendulum is now swinging toward … either neoadjuvant chemotherapy or less than ultraradical debulking among women with advanced ovarian cancer”.

Survival is not compromised with neoadjuvant chemotherapy but there is as yet no good evidence that for women with advanced epithelial ovarian cancer, it is superior to conventional cytoreductive surgery and platinum-based chemotherapy. Yet this approach does distinguish between responders and nonresponders to standard platin/taxane chemotherapy. Whether cytoreductive surgery is of value in nonresponders or whether they should be offered alternative chemotherapeutic agents or experimental treatment modalities remains to be proven.

Currently, objective responses are observed in approximately 60-80% of patients after initial surgery and standard adjuvant chemotherapy, but ultimately more than 80% of them recur. Each one of the subsequent lines of chemotherapy regimens has a different toxicity profile, and requires intensive monitoring and frequent hospitalizations for management of side-effects. The response rates to additional chemotherapy regimens are similarly low and can induce tumor remission in 20% to 30% of patients but after relapse the median survival time is only about two years. Patients with recurrence are not curable, complete responses are very rarely reported and long lasting responses are very seldom observed. Therefore the goal of salvage chemotherapy is palliation. How many additional cycles of chemotherapy should be used in patients with platinum-refractory or platinum-resistant recurrence has not been prospectively studied and their benefits over palliation have not been proven [10]. Some authors are of the opinion that continuous provision of futile cure-oriented therapy at the end of life is rarely justified [11] and that it involves significant cost increase with no appreciable improvement in survival [12].

The relatively few studies that assessed effect of ovarian cancer and its treatment on the patients and their caregivers [13] indicate that significant alterations occur in the quality of life of patients during treatment and follow-up. Long-term survivors of ovarian cancer frequently experience chronic fear of disease recurrence, significant sexual dysfunction, and identity disturbances [14]. Today many aspects of patient care such as emotional support, helping with daily activities, administration of medications and of special nutrition, rely on family, community, and social service
resources. These stressful demands and responsibilities have major emotional and physical impact on caregivers as well. Quality of life endpoints should be included in clinical trials of cancer therapies to supplement standard endpoints such as tumor response and overall survival.

How much have the aggressive surgical approach and modern initial and salvage chemotherapy regimens contributed to an improvement in outcome?

The US Survival, Epidemiology and End Results (SEER) cancer database is the most comprehensive source of information on cancer incidence and survival in the USA. It covers about 26% of the United States population, uses several quality control measures, ensures accuracy, completeness of reporting and is considered the standard for quality in cancer registries around the world.

Barnholtz et al. [15] used the SEER database to examine overall survival in 32,845 EOC patients from 1973 to 1997, with follow-up through the end of 1999.

Only a 4% increase in 5-year relative survival from 39% in 1980-1989 to 43% in 1990-1997 (p ≤ 0.05) was observed. Women who were older than 60 years had a significantly worse prognosis than those who were younger than 60 years at the time of diagnosis. Chan JK et al. [16] also estimated the change in the 5-year disease-specific survival rates of 26,753 women with non-clear cell EOC during the 14-year period 1988-2001 (across three intervals, 1988-1992, 1993-1997, and 1998-2001) registered in the SEER database. An overall increase in overall survival from 42.5% to 45.8% and in patients with advanced stage (III-IV) disease from 25.4% to 29.4% (p < .001) was observed. No improvements were observed for clear cell carcinoma. Although the increases in both studies are statistically significant they are small, consist of only about 4-5%, and may not be clinically notable. Chan et al. [17] also analyzed the outcome of 6,152 early stage (I-II) EOC patients during 1988-2001 obtained from the SEER database. A non significant (p = 0.076) increase in the 3-year disease-specific survivals across the above-mentioned three intervals (from 86.1 to 87.2 to 88.8%) was observed.

of those early-stage patients who underwent staging procedures with lymphadenectomy, there was also no improvement in survival over the three study period intervals (from 93.2 to 93.5 to 93.1%; p = 0.978). This is in line with a recent prospective randomized study that found that in women with advanced ovarian carcinoma who were optimally debulked systematic pelvic and aortic lymphadenectomy improves PFS but not overall survival [18].

The SEER cancer database demonstrates a marginal improvement in the death rate of women with ovarian cancer from 10 per 100,000 of the population to just over 9 per 100,000 [19] over the 30 years preceding 2003.

Similar outcome results were seen in smaller European registries. Data of 4,564 ovarian cancer patients (including non EOCs) documented from 1978 to 2000 in the Munich Cancer Registry [20] showed an improvement in survival in Stage I and II. However only a 6% improvement was seen in overall relative 5-year survival of ovarian cancer. It increased from 42.9 during 1978-1988 to 49.0% after 1998. As in other studies relative survival decreased with increasing age at time of diagnosis. Gondos A et al. [21] examined age-specific trends in 5-year relative survival of 2,260 ovarian cancer patients from 1979 to 2003, using data from the population-based Cancer Registry of Saarland, Germany. They found an improvement of 14%, mainly due to increased survival in the younger age groups (< 54 years). Analysis according to stage and histological type, important confounders that might influence outcome, is not reported in this study. Thus the improvement in the younger age group is possibly due to the modern effective treatment of non-EOC tumors prevalent in this age group.

According to the latest ovarian cancer statistics from the statistics team of GLOBOCAN 2002, a hardly noticeable decrease in mortality throughout the period from 1975 to 2005 is seen in the United Kingdom (Figure 1).
The FIGO Annual Report on the results of treatment in gynecological cancer includes survival results of EOC from individual institutions in five continents. The overall absolute survival of patients during the period 1990-1992 was 41.6% and during the period 1996-1998 it increased by about 5% and was 46.4% [22].

In Israel during the period 1998-2004 about 300 new ovarian malignancies were diagnosed yearly and about 230 deaths from this malignancy occurred each year [23].

It thus seems that the advances in ovarian cancer have not translated into a considerable increased survival and most patients with epithelial ovarian cancer still die of their disease.

These gloomy results of the treatment outcome of ovarian cancer are not presented in order to dishearten gynecologic oncologists who daily relentlessly deal with ovarian cancer, but are intended to encourage accelerated research leading to the detection of 1) Highly specific effective methods for early detection of ovarian cancer; 2) A better understanding of the natural history of ovarian cancer, the biological and immunological processes leading to its oncogenesis and to the ability to harness them for prevention and treatment of this malignancy and 3) Identification of novel effective drugs and methods that will allow more effective individualization of treatment according to biological properties of the tumor.

References


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Preservation of fertility in reproductive-age women with the diagnosis of cancer

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Summary

Over the past few decades the number of young, reproductive age cancer survivors has increased as a result of improved and less destructive cancer treatments. Certain types of cancers are predominantly diagnosed among reproductive age women and a small proportion of cancers originating in the reproductive tract are also detected in this age group. Treatment in the past used to be definitive and in most cases led to sterility. In recent years, improved medical treatments and more conservative surgical approaches have been introduced increasing the number of young survivors of cancer treatment. These less invasive treatments seem to be associated with similar survival rates and fertility can be preserved in most cases. This has led to studies evaluating the reproductive options of these women. Conservative surgical techniques, the use of chemotherapeutic agents with a reduced gonadotoxical side-effect profile, and the application of more focused radiation therapy are associated with maintenance of fertility. In addition, assisted reproductive technology (ART) has undergone tremendous improvements and now offers several alternatives to those who wish to maintain fertility before or even after cancer therapy. This review summarizes the fertility sparing medical and surgical as well as ART options that reproductive age women desiring to maintain fertility may utilize if they face cancer therapy.

Key words: Cancer; Fertility, surgery; Chemotherapy; Radiation; Assisted reproductive technology; Cryopreservation.

Introduction

Cancer is generally associated with older age. This diagnosis is usually not expected among reproductive age women. Certain cancer types however typically occur among children or young adults and a small proportion of cancers that are otherwise more prevalent among older women are diagnosed in the reproductive age group [1]. In the vast majority of cases, cancer treatment used to result in sterility [2]. Surgery was definitive and chemotherapeutic agents were used to destroy the ovarian follicles [2].

In recent years, more conservative therapies have been introduced and with the development of new chemotherapeutic agents the salvage of ovarian function has become possible. This not only leads to improved survival rates, but to a need to address the fertility concerns of these patients as well. For example, almost two-thirds of Hodgkin's lymphomas and over 75% of acute lymphocytic leukemias are diagnosed in patients under the age of 44 [1]. Survival rates for Hodgkin’s lymphoma improved from 75-88% over the past three decades [1]. Leukemia survival rates improved from 37% to 51% in the same period [1]. This means that there is a large group of reproductive age women whose reproductive options have to be discussed prior to or after chemo- or radiation therapy.

Cancers of the female genital tract may also affect young women. Forty-two percent of cervical cancers, 9% of uterine cancers and 13% of ovarian cancers are diagnosed among women under the age of 45 [1]. Survival rates are excellent for cervical cancer (83% when diagnosed under the age of 45) and endometrial cancer (91% if diagnosed under the age of 45). Overall survival rates have increased for ovarian cancer as well (from 36% to 45% between 1975-2003), and 5-year survival rates are even better (73%) when the diagnosis is made under the age of 45 [1]. A conservative surgical approach and careful selection of chemo- or radiation therapy therefore is important for these women if fertility needs to be maintained.

This review will discuss the surgical, medical and assisted reproductive technology (ART) options of those women who are diagnosed with cancer during the reproductive years. Some of our own cases will be used to illustrate the importance of a conservative approach when it does not compromise the long-term health benefits of the patient.

Surgical options

Cervical cancer

Case 1

NA, a 34-year-old nulliparous woman, was evaluated at our clinic for primary infertility of male origin. She had previously undergone two unsuccessful IVF cycles. Her cervical cytology in 1999 showed atypical cells and a cervical biopsy detected cervical cancer. Her full staging evaluation revealed Stage IB epithelial cervical cancer. Since she had no children at the time of the diagnosis she...
opted to undergo fertility sparing radical trachelectomy. The lymph nodes were negative for cancer and the margin of the surgical specimen was also free of disease therefore no adjuvant therapy was administered. Her postoperative follow-up showed no evidence of recurrence. She maintained regular cycles after the procedure and two years after the operation underwent two further IVF cycles. During the second attempt five oocytes were retrieved and three embryos were transferred. This treatment was successful and a singleton gestation was achieved. After an uneventful pregnancy she delivered a healthy girl at term.

Malignant transformation in the cervical epithelium is a slow process and it usually takes several years for invasive cancer to develop. Human papilloma virus infection plays an important role in this process. Well-described precancerous lesions (cervical intraepithelial neoplasia I-III) precede the development of cancer. Effective screening techniques are available that accurately identify precancerous lesions as well as cancer [3].

Cervical cancer is staged clinically. During the exam, the size of the primary lesion and its spread to adjacent organs (parametrium, uterus, bladder, rectum) are evaluated. Early stage cervical cancer (Stage I and II) has traditionally been managed surgically. Radical hysterectomy with sampling and if needed removal of the pelvic and paraaortic lymph nodes has been the treatment of choice. Pre- and postoperative radiation, and more recently chemotherapy further improve outcome [4]. While radical surgery is associated with good survival rates, these women are rendered infertile, as the uterus is removed.

In developed countries, the introduction of cervical cytology was associated with early detection of cancer and reduced morbidity and mortality due to treatment and the disease itself [3]. Detection of early-stage cancer, when the disease is localized to the cervix (Stage I-II), allows a more conservative surgical approach without compromising survival. In these cases the removal of the cervix, upper vagina and parametrium with adequate lymph node sampling has been associated with similar survival rates to radical hysterectomy [5]. Radical trachelectomy can be carried out through the vagina or by the abdominal route [6, 7].

Vaginal radical trachelectomy requires additional surgical training. It is associated with a higher rate of intraoperative complications (injury to the bladder, ureter, bowel), and less parametrial resection. Recurrence in the parametrium has been described following this procedure [8]. In order to improve outcome and reduce complications, radical trachelectomy via the abdominal route was introduced. The procedure is very similar to radical hysterectomy, but with this procedure the uterus is left in place. Its blood supply is maintained via the vessels that run in the infundibulopelvic ligament.

This conservative approach preserves fertility, as the uterus remains functional. Spontaneous and ART pregnancies have been reported following both the vaginal and abdominal procedure [9, 10].

Some of the patients undergoing radical trachelectomy will require in vitro fertilization (IVF). IVF following the removal of the parametrium and cervix can be particularly challenging. The ovaries lose their support and their position might change. This could interfere with the transvaginal oocyte collection. It is also questionable how well the ovarian vessels can perfuse the uterus, whether the endometrium is able to undergo the necessary changes to allow successful implantation. Earlier studies reported average birthweight among infants born following radical trachelectomy, suggesting that adequate blood supply is maintained [11]. In some cases stenosis or complete obstruction of the remaining “cervical” canal may develop. Such stenosis may cause scarring which might interfere with the embryo transfer. The passage through the canal needs to be evaluated prior to initiating stimulation.

The shortening of the cervix may put the ongoing pregnancy at risk. Preterm delivery and preterm premature rupture of membranes have both been reported to occur with higher frequency. This is most likely due to the lack of protection offered by the cervix and mucous plug [11]. Several groups routinely place a cerclage to add support to the remnants of the cervix [11]. The use of cerclage is controversial even in cases where it is placed to prevent preterm delivery [12]. The potential benefits of cerclage placement under these circumstances require further evaluation.

In order to reduce the load on the lower uterine segment and cervix following radical trachelectomy, a singleton pregnancy is the desired outcome and therefore the number of embryos transferred needs to be limited, preferably to one.

These pregnancies should be followed by a high-risk obstetrician, and delivery by cesarean section should especially be considered in those cases where a cerclage has been placed.

**Ovarian tumors**

**Case 2**

AK, a 32-year-old nulliparous woman, at the age of 22 underwent a left salpingo-oophorectomy for an ovarian cyst that was thought to be a mature teratoma prior to the surgery. The final pathologic exam showed papillary cystocarcinoma of the ovary. Following this report she underwent a formal staging procedure with lymph node biopsies, peritoneal washings and omentectomy, and also received six cycles of chemotherapy (cyclophosphamide, cisplatinum, epirubicin). Tumor markers were followed and pelvic ultrasounds (US) were performed at regular intervals. Six years after the initial diagnosis she underwent a right cystectomy for an endometrioma. Eight years after the diagnosis and treatment of ovarian cancer she was having regular cycles, and since her right tube was blocked she underwent IVF treatment. The treatment was successful but she miscarried at eight weeks. Subsequently she underwent two further IVF attempts that were unsuccessful. She always responded well to stimu-
lation and produced 8-11 eggs. During the treatments, follow-up of the tumor markers was continued. She is now scheduled to undergo treatment for the fourth time.

There are various types of ovarian lesions. The appropriate treatment primarily depends on the histology. Benign lesions are generally treated by cystectomy or if it is not possible by oophorectomy. Malignant ovarian tumors have generally been treated by bilateral salpingo-oophorectomy and hysterectomy, and a complete staging procedure with pelvic washings, lymph node biopsies and omentectomy.

In well-selected cases, ovarian cancers however could be managed more conservatively. Ovarian germ cell tumors (2% of ovarian cancers) typically affect young women. For a long time surgery followed by radiation therapy was the standard care. Radiation however destroys ovarian follicles and induces premature ovarian failure. Since most of these patients have not had a chance to start a family, therapy that preserves ovarian activity would be preferable. Therefore chemotherapy has been evaluated instead of radiation for the treatment of germ cell tumors.

Several large studies have reported similarly excellent survival rates with chemo- and radiation therapy. Brewer et al., followed 26 women who were treated by surgery and bleomycin, cisplatin, and etoposide chemotherapy for ovarian dysgerminoma (16 patients underwent conservative surgery [unilateral salpingo-oophorectomy] and ten had hysterectomy and bilateral salpingo-oophorectomy). After a median follow-up of 89 months 96% of these women remained disease free. Among those who had conservative surgery, 14/16 maintained regular menstrual cycles post chemotherapy. Five pregnancies were reported in this group without any birth defects [13]. Gershenson et al. followed 132 survivors of ovarian germ cell cancer; 71 of these women had fertility sparing surgery, 87.3% of them reported regular menstrual cycles post treatment (surgery plus chemotherapy), and 24 of the survivors had 37 children during the follow-up period [14]. Gershenson in an earlier report evaluated reproductive outcome among 40 germ cell cancer survivors following fertility sparing surgery and chemotherapy (most commonly vincristine, dactinomycin, cyclophosphamide). Twenty-seven of 40 women maintained regular menstrual cycles, 13 had menstrual difficulties of which three were considered severe; 11/16 patients who wished to have children were successful and delivered 22 children altogether [15]. These reports provide good evidence for the use of conservative surgery in combination with chemotherapy for those young women who are diagnosed with early or even advanced stage ovarian germ cell tumor and desire to maintain fertility.

The answer is not this straight forward when epithelial ovarian cancer is diagnosed in reproductive age women. This type of cancer has traditionally been treated by hysterectomy, bilateral salpingo-oophorectomy and formal staging with the aim of maximal tumor debulking as most of them are diagnosed in an advanced stage. Some of these cancers are however diagnosed when they are still localized to the ovary. Unilateral salpingo-oophorectomy with staging followed by chemotherapy could be a conservative and fertility sparing option if it did not affect survival.

Schilder et al., studied 52 reproductive-age women (mean age 26 years) who were diagnosed with Stage IA-IC epithelial ovarian cancer and were managed by unilateral salpingo-oophorectomy with or without adjuvant chemotherapy. Cisplatin/taxol or carboplatin/taxol were given to 19/52 of these women. Five patients were diagnosed with recurrence and had to be treated again. Fifty of 52 women were alive and well after a median of 68 months follow-up. 5-year survival was estimated to be 98%. Seventeen of 24 women attempting pregnancy conceived and have delivered 26 children [16]. It appears that in well-selected cases unilateral salpingo-oophorectomy with appropriate staging could be offered to those young women who desire future fertility. It is important that these women be followed by tumor markers and regular pelvic US for evidence of recurrence. They also need to be made aware of the excess risk that they take even if it appears to be small.

Borderline ovarian tumors (15% of ovarian tumors) are histologically a special group of ovarian tumors. In these cases the epithelium undergoes atypical proliferation but the stroma is not infiltrated. If fertility is desired, conservative surgery with or without adjuvant therapy can be offered. Wong et al., reviewed the outcome of 247 cases of ovarian borderline tumors; 33% of the women were managed by unilateral salpingo-oophorectomy, 15% by simple cystectomy, and the rest by bilateral salpingo-oophorectomy and hysterectomy. After a mean follow-up of 59 months six recurrences were found and the overall survival was 98% [17]. In another large series of borderline ovarian tumors, the potential benefit of adjuvant therapy was evaluated. Of the 370 cases, 77 women were treated with fertility sparing surgery. This study found no evidence for adjuvant chemotherapy to improve long-term survival [18]. Crispens et al., in their series of low malignant potential ovarian tumors showed that survival depended on optimal cytoreduction and also reported no benefit of non-surgical treatment alternatives [19]. These reports suggest that borderline ovarian tumors can be successfully managed by surgery alone. Conservative surgery is an alternative in those cases where fertility maintenance is desired. The ideal surgical approach has not been determined yet. Tinelli et al. evaluated recurrence and pregnancy rates in 43 women treated by conservative laparoscopic surgery for borderline ovarian tumors. The recurrence rate was 7%. Half of the patients successfully achieved pregnancy and over half of these pregnancies were spontaneous conceptions [20]. Maneo et al. compared long-term outcome in 62 women with borderline ovarian tumors following conservative surgical treatment either by laparoscopy (N = 30) or laparotomy (N = 32). Recurrences were detected in 11/30 and 7/32 cases. In the laparoscopic surgery group relapses were found more often when the initial cyst was greater than 5 cm [21]. Conservative laparoscopic management
of borderline ovarian tumors should be reserved for experienced surgeons who are able to perform appropriate staging during laparoscopic exploration. In addition, cases where the cyst is large (> 5 cm) seem to have a better prognosis when managed by laparotomy.

These reports suggest that in well-selected cases when future fertility is desired ovarian tumors (even epithelial cancer) can be managed by conservative, fertility-sparing surgery in combination with chemotherapy (when needed). In most cases spontaneous pregnancy may follow. Ovulation induction has not been shown to increase recurrence rates in these selected cases [22]. Appropriate patient counseling is very important and close follow-up with tumor markers and US is also a crucial part of the care of these women.

Ovarian lateral position prior to radiation

Radiation therapy alone or in combination with surgery might be required to treat certain cancers (e.g., cervical cancer, Hodgkin’s lymphoma). Radiation is known to have a dose-dependent toxic effect on ovarian follicles. Radiation in doses over 800 cGy will always result in ovarian failure, but lower doses could also be associated with sterilization [23]. The full effect of lower doses depends on the patient’s age and the cumulative dose delivered directly to the ovaries. When pelvic or lower abdominal radiation is planned the position of the ovaries out of the radiation field may preserve ovarian function. By displacing the ovaries out of the field of radiation the dose delivered to them can be significantly reduced. Transposition can be performed at the time of laparotomy or laparoscopy and typically the ovaries are removed laterally out of the radiation field. For best results the ovaries should be placed at least 3 cm out of the field [24]. If the transposition is closer to the field or if the ovaries migrate back to their original position ovarian failure may develop. Ovarian function could also be affected by vascular compromise as a result of the procedure [25]. Those ovaries that maintain their cyclic function at the completion of therapy will produce a sufficient amount of estradiol and progesterone needed for an endometrial cycle. The unusual position of the ovaries however may lead to infertility. Further surgeries might be needed if ART is required to achieve a pregnancy.

Medical options

Progestin therapy of endometrial cancer

Case 3

HMB, a nulliparous woman, was seen in our clinic following years of infertility evaluation and treatment. She had been diagnosed with polycystic ovary syndrome (PCOS) based on irregular cycles, hirsutism and elevated testosterone levels. Her body mass index (BMI) was 33.8 kg/m². Initially, she had been treated with clomiphene citrate and intrauterine inseminations. She had six treatment cycles that were unsuccessful. Following the inseminations she was scheduled to undergo IVF when an endometrial polyp was detected on US. Hysteroscopic polypectomy was performed. Histologic evaluation showed a well-differentiated carcinoma and areas of atypical hyperplasia. At this point she was counseled to have definitive therapy by removal of the uterus. Since she wished to have children she chose conservative management with high-dose gestagen (medroxyprogesterone acetate (MPA), 100 mg daily). Over the six-month gestagen therapy she underwent repeat biopsies, which showed that the disease had regressed. On completion of her treatment she underwent IVF in our center. Clomiphene citrate in combination with gonadotropins was used. Four eggs were retrieved and two embryos were transferred. Her treatment was successful and after an uneventful pregnancy she gave birth to a healthy baby boy. A year after delivery she returned to discuss a possible second treatment. She was advised again about the potential risks that she would take by delaying definitive therapy but she wanted to undergo a second IVF treatment. Her endometrial biopsy at this point showed complex hyperplasia without atypia. She was given 100 mg of MPA for three months and the histology regressed to simple hyperplasia. She underwent IVF using the same stimulation protocol. This time two eggs were retrieved and one embryo was transferred. Her treatment was successful again and she gave birth to another healthy male at term. She did not desire any more children at this point and is discussing definitive treatment options with her oncologist.

About 10% of endometrial cancers are diagnosed in premenopausal women [1]. Most of these cases are diagnosed among women with PCOS. Anovulation and extended periods of unopposed estrogen exposure are common with this syndrome and are linked to endometrial cancer. Most of these cancers are endometrioid type and are well differentiated. Endometrial cancer is generally treated surgically with the removal of the uterus which would render women desiring to maintain fertility infertile. As these tumors are mostly hormone-dependent and express receptors for both estrogen and progesterone, medical therapy using high-dose progestins could be offered if fertility maintenance is desired. Randall et al. managed 29 women with well-differentiated endometrial carcinoma or atypical endometrial hyperplasia using high-dose progestins (megestrol acetate or medroxyprogesterone acetate [26]). The disease has not progressed in any of the cases. Regression was seen in all cases except for four where the disease persisted. Twenty-five of these women were managed for infertility later on and five of them conceived successfully [26]. Gotlieb et al. followed 13 women with a diagnosis of endometrial cancer who chose medical therapy. Treatment with high-dose megestrol acetate or MPA was administered for a minimum of three months. Five women developed local recurrence over a mean follow-up of 82 months. Six out of 13 women successfully conceived either on their own or using ART [27]. Further case reports/case series lend support for the use of high-dose progestin for the conservative treatment of well-differentiated endometrial cancer
when fertility is desired [28, 29]. Fukuda et al. have shown that endometrial cancers that are well differentiated are more likely to express steroid receptors and positive immunohistochemistry for the progesterone receptor was associated with less invasion, longer disease-free and overall survival [30]. It is also important to mention that not all cases end positively. Vinker et al. reported a case where a well-differentiated endometrial carcinoma was initially managed medically. Due to the lack of response definitive surgical treatment was performed and even adjuvant radiation had to be used for advanced stage disease [31]. At this point several issues need to be answered. Are all patients with localized well-differentiated endometrial cancer candidates for medical therapy? Which preparation should be used, at what dose, and for how long? Based on published reports it appears that a minimum of three months is required to have a positive effect. If the disease persists continuation with a higher dose could be attempted but definitive therapy should seriously be considered. It is also not clear how these patients should be evaluated (“staged”) pretherapy. Should US or MRI findings influence the decision? On one issue most experts seem to agree. Once childbearing has been completed definitive therapy by removal of the uterus should follow.

Prevention of chemotherapy related toxic effects

Chemotherapy works by destroying rapidly dividing cancer cells, but it also destroys other sensitive cells as well. Chemotherapy often negatively influences gonadal function. Chemotherapy induces loss of follicles and leads to ovarian fibrosis. The full effect depends on the patient’s age, gonadal function prior to such therapy and most importantly on the agent itself and duration of treatment. Certain agents are well known for toxic ovarian effects (e.g., cyclophosphamide, busulfan, phenylalanine mustard) while others have no significant ovarian impact (e.g., methotrexate, 5-fluorouracil) [32]. Depending on the exact drug regimen fertility may be compromised to different degrees. Byrne et al. assessed the effect of childhood chemotherapy on fertility among survivors and compared it to controls. Cancer survivors were less likely to achieve pregnancy (RR: 0.85 [95% CI: 0.78-0.92]). In this group of patients alkylating agent use alone had no significant effect but when combined with radiation reduced fertility was observed [33]. Byrne et al. in a different paper assessed the risk of premature ovarian failure among adolescent cancer treatment survivors. When treatment was administered between ages 13-19 the risk of premature menopause was increased fourfold when compared to age-matched controls. In this group of patients the risk of ovarian failure was increased 9-fold compared to controls. Cancer survivors were less likely to achieve pregnancy (RR: 0.85 [95% CI: 0.78-0.92]). In this group of patients alkylating agent use alone had no significant effect but when combined with radiation reduced fertility was observed [33]. Byrne et al. in a different paper assessed the risk of premature ovarian failure among adolescent cancer treatment survivors. 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Since the use of chemotherapy cannot be avoided in all cases efforts have been made to reduce the toxic reproductive effects. It is presumed that dividing cells are more sensitive to chemotherapy and therefore several drugs that “suspend” ovarian activity have been evaluated in combination with chemotherapy.

Whitehead et al., followed nine women on oral contraceptives (OC) undergoing chemotherapy. Seven of the nine women reported some degree of menstrual irregularity following chemotherapy, therefore OCs alone did not seem to provide the needed protection [35].

Blumenfeld et al. evaluated the effect of depot gonadotropin releasing hormone agonist (GnRHa) in combination with chemotherapy for Hodgkin’s lymphoma (n = 65). Post therapy ovarian function was compared with a control group of women undergoing the same chemotherapy for the same disease. GnRHa was not administered in the control group. Ninety-seven percent of women in the GnRHa group and 63% of the control women resumed regular menses upon discontinuation of chemotherapy [36]. Potolog-Nahari et al. evaluated the combination of GnRHa and GnRH antagonist in nine women undergoing chemotherapy. In 89% of them gonadotropin levels returned to baseline and they reported regular menstruation [37]. Cyclophosphamide is often used in women with lupus. Somers et al. found that in women receiving monthly cyclophosphamide for severe lupus GnRHa administration was associated with reduced risk of ovarian failure when compared to age-matched controls undergoing the same therapy without GnRHa (5% vs 30%) [38]. Castelo-Branco et al. reported significantly fewer cases of premature ovarian failure in patients undergoing chemotherapy for Hodgkin’s disease when they also received GnRHa in combination with tibolone (10% vs 23%) [39]. These reports are very encouraging and suggest that by rendering the ovary inactive follicles may be saved and ovarian function could be maintained when chemotherapy with known ovarian toxicity has to be used.

To be complete we also mention that animal experiments show promising results with apoptosis inhibitors (e.g., shingosine-1 phosphate) but it is beyond the scope of this paper to enter the details of these experiments [40].

Assisted reproductive technology (ART): options prior to chemotherapy

Embryo cryopreservation

Case 4

JJ, a 30-year old nulliparous woman, was referred to us by her oncologist. Prior to seeing us she had had a tumor removed from her right inguinal region. Histopathologic exam showed a synovial sarcoma. She was scheduled to undergo chemo- and radiation therapy. She came to us for a consultation to review her options to maintain fertility in light of the therapy she was scheduled to undergo. After discussing her options (egg, ovarian tissue or embryo freezing), we decided to proceed with IVF and elective embryo cryopreservation. The oncologist was not aware of any adverse effect of stimulation on the course of her disease. Ten eggs were retrieved after stimulation.
using an antagonist protocol. Seven eggs were successfully fertilized using ICSI, and all seven pronuclear stage embryos were frozen. She has completed her treatment and is currently well two years after the initial diagnosis. If she remains disease free she plans to undergo frozen embryo transfer in the future.

Embryo cryopreservation is a well established technique in IVF centers. In humans the first pregnancy was reported in 1983 and the first delivery in 1985 [41, 42]. Embryo cryopreservation is primarily used to store the surplus embryos created during the fresh cycle but in some cases all embryos are frozen electively. Freezing can be performed at different developmental stages (two pronucleus, 2-8 cells, blastocyst). Freezing techniques have undergone a lot of changes and today most IVF centers use slow freezing protocols. Permeable cryoprotectants like 1,2-propanediol (PROH), dimethyl-sulfoxide (DMSO) or glycerin are combined with non-permeable agents such as sucrose in most protocols. First, the cells are dehydrated and after equilibration, the embryos are loaded into plastic straws or cryovials. Computer-assisted freezers are used to achieve adequate cooling. Vitrification (ultra-rapid freezing) is an alternative to slow cooling. Cooling rates and the cryoprotectants used differ between vitrification and slow freezing. During vitrification, a glass-like solidification of the freezing solution is achieved by using a high concentration of the cryoprotectants. Vitrification was first used to cryopreserve murine embryos by Rall and colleagues [43]. Today it is used for both embryo cryopreservation and oocyte freezing [44, 45].

Beyond the different technical approaches, the developmental stage of frozen embryos also has great impact on survival [46]. The highest survival rate was observed for zygotes (86.5%), followed by day 2 (61.7%) and day 3 (43.1%) embryos. In women under the age of 36, cryopreservation at the 4-cell stage was associated with the best outcome (implantation rate of 30.9%) following the transfer of a single frozen-thawed embryo [47].

The main benefit of embryo cryopreservation is its well established nature. Thousands of children born following the transfer of frozen-thawed embryos prove its efficacy and safety. High survival rates of the cryopreserved embryos usually allow several transfer attempts and therefore offer the possibility of having more children as a result of a single stimulation. In those cases where stimulation is not allowed embryo cryopreservation cannot be offered unless immature oocytes are retrieved and matured in the laboratory for IVF. Embryo freezing is also not the ideal option for those who are not in a stable relationship at the time of treatment unless they agree to fertilization with donor sperm.

**Oocyte freezing**

Oocyte freezing would be a good option for those who are single at the time of cancer therapy and do not want to use donor sperm to fertilize their eggs. Stimulation is not always important prior to obtaining eggs for freezing, therefore patients with hormone-sensitive tumors could utilize this method as well. Oocyte cryopreservation is a relatively new technique, and its results have not been as consistent as results with embryo freezing [48, 49]. It however has additional benefits when compared to embryo cryopreservation [50]. First, women who lose ovarian function due to surgery, chemotherapy, or radiotherapy could maintain their fertility via oocyte cryopreservation. Second, ethical, religious and legal issues surrounding embryo cryopreservation can be avoided if oocytes are frozen.

Unfortunately, mature human oocytes have lower survival rates than do embryos when similar freezing protocols are used [51]. The cytoplasmic membrane of the oocytes has fewer submembranous actin microfilaments and therefore is more fragile during cryopreservation and therefore more difficult. Freezing and thawing may result in the disturbance of the meiotic spindle, which could lead to chromosomal dispersion, failure of normal fertilization, and failure to develop [53]. The meiotic spindle is crucial for the events following fertilization, including completion of meiosis, second polar body formation, migration of the pronuclei, and formation of the first mitotic spindle [54]. Another problem with frozen-thawed oocytes is the hardening of the zona pellucida, caused by premature release of cortical granules. It can prohibit the entry of the spermatozoon [55]. To prevent this, intracytoplasmic sperm injection (ICSI) has been used to assist fertilization two to three hours after thawing [56].

Oocyte freezing is routinely performed two to three hours postretrieval [57]. For slow freezing, Porcu *et al.* used 1.5 mol/l 1,2-propanediol (PROH) with a sucrose concentration of 0.2 mol/l and obtained a 59% post-thaw survival rate [56]. Fabbri *et al.* showed that increasing the sucrose concentration to 0.3 mol/l and exposing oocytes for 15 min to cryoprotectants yielded higher oocyte survival rates (82%) [58]. Borini and colleagues achieved a 17.2% pregnancy rate per embryo transfer (ET) with embryos obtained from frozen oocytes [59]. If vitrification is used, ethylene glycol is the primary choice of cryoprotectant [60].

Oocyte survival rates depend on the type and concentration of cryoprotectant used, on the freezing protocol (slow vs vitrification) and on the stage of development. Cryopreservation of immature (GV-stage) oocytes is also on the horizon [61]. Freezing of immature oocytes (primordial follicle level) could lead to even better results since the metabolic rate in these eggs is lower, there is no zona pellucida, and the meiotic spindle has not formed yet [62].

**Ovarian tissue freezing**

Cryopreservation of ovarian tissue has several advantages over cryopreservation of oocytes or embryos. The premenopausal human ovarian cortex contains large numbers of primordial follicles and by cryopreserving
ovarian tissue its endocrine function can also be preserved. Furthermore, ovarian tissue can be collected relatively easily by laparoscopy or laparotomy at any time during the menstrual cycle. Since stimulation is not required prior to surgery it can also be performed in those cancer cases where stimulation would otherwise not be allowed. And finally, primordial follicles appear to be less sensitive to cryopreservation when compared to mature oocytes. This is most likely associated with the low metabolic rate of tissues. It also has to be pointed out that in the case of cancers that may also involve the ovary reintro-duction of cancer is a potential risk after transplanta-
tion.

Ovarian tissue needs to be prepared for the freezing process. First the ovarian medullary tissue is removed, and then the remaining tissue is cut into strips (1–5 mm ×1 mm×1 mm) using optical tweezers. Usually, ovarian tissue is cryopreserved by slow freezing, which leads to excellent results [63-65]. During the slow protocol DMSO or DMSO/PROH is used as cryoprotectant with or without adding sucrose as a non-permeable cryopro-
tectant. Similarly to embryo or oocyte cryopreservation, vitrification with a higher concentration of cryoprotec-
tants has been evaluated with ovarian tissue as well. It is however still difficult to adopt a vitrification protocol to ovarian tissue because of the different physical structures and cell types in human ovarian tissue [66]. Ovarian frag-
ments contain various types of cells, have high cell density, and also have an intact vascular system. Improvements in the freezing technology have led to improved survival rates of follicles and recent studies report morphologically intact follicles in excess of 80% [62, 66-68]. Success rates depend on the cryoprotectant, its concentration, the freezing protocol (slow vs vitrification) and the transplantation or in vitro maturation tech-
nique. Smaller or larger pieces can be frozen. Cryodam-
age is less likely with small cortical fragments but since they lack adequate blood supply ischemia is a major problem following transplantation. Blood supply can be maintained when larger pieces are frozen, but follicle loss due to insufficient freezing becomes an issue in this case [69].

After thawing the tissue can either be transferred to its original site (orthotopic transplantation) or to a different anatomic location (heterotopic transplantation). Successful ortho- and heterotopic transplantsations have both been reported. Oktay et al. described two cases where ovarian tissue was transplanted to the forehead following pelvic radiation or chemotherapy. Follicular activity with cyclic changes in estradiol and progesterone hormones was doc-
umented in these cases [70]. In 2004 the same group pub-
lished results with ovarian tissue transplantation into the subcutaneous layer of the lower abdomen. Three months after the surgery, follicular and hormonal activity could be demonstrated. In repeat cycles of stimulations al-
together 20 eggs were retrieved. Immature eggs had to be matured in vitro, but fertilization and embryo develop-
ment were achieved [65]. Donnez and colleagues reported a successful spontaneous pregnancy following the transplantation of ovarian tissue to its original anatomic location [63].

Work is still in progress to refine techniques of in vitro maturation of frozen-thawed immature oocytes, and the frozen-thawed ovarian cortical tissue slices. Mikkelsen and colleagues achieved a 24% clinical pregnancy rate per oocyte collection with in vitro matured fresh GV oocytes [71]. Children born after IVM appear to be healthy. These data, taken together, suggest that in the future, immature oocyte retrieval combined with freezing and IVM could replace conventional IVF in selected patients and could play a particularly important role among cancer patients.

Prechemotherapy stimulation

Steroid hormones negatively affect the course of certain types of cancer, while such an association is not evident with others. Estradiol levels significantly increase during stimulation, therefore in the case of hormone sen-
sitive cancers (e.g., breast cancer) ovarian stimulation prior to cancer treatment is not recommended. If a cancer with steroid hormone sensitivity is detected the patient may still undergo natural cycle IVF when stimulation is not used, but even if everything goes well a single embryo can be stored frozen. Without stimulation immu-

ture eggs can be retrieved and cryopreserved for later use. The freezing-thawing and the need for in vitro maturation limits its clinical use.

In cases of estrogen-sensitive tumors ovarian stimula-
tion with the combination of an aromatase inhibitor was evaluated in a prospective cohort study by Azim et al. Patients with invasive breast cancer desiring to preserve fertility by embryo cryopreservation prior to initiating chemotherapy were enrolled. They were assigned to either letrozole plus follicle stimulating hormone (FSH) or anastrazole plus FSH. Peak estradiol level was signif-
icantly lower in the letrozole (5 mg) group (427.78 ± 278.24 pg/ml vs 1325.89 ± 833.17 pg/ml) [72]. Oktay et al. prospectively evaluated stimulation outcome with tamoxifen, tamoxifen plus FSH or letrozole plus FSH among women with invasive breast cancer prior to start-
ing chemotherapy. Significantly more oocytes were retrieved and more embryos were frozen in the groups where FSH was administered as well. Peak estradiol levels were lower in the tamoxifen only and letrozole plus FSH groups when compared to the tamoxifen plus FSH group. Recurrence rates were similar in the three stimulation groups when compared to a control group of women not undergoing stimulation prior to chemother-
apy [73]. In a different study, Oktay et al. compared stimulation outcome with letrozole plus FSH in women with breast cancer to an age-matched control group of women free of breast disease undergoing IVF using stan-
dard stimulation protocols. Despite a significantly lower peak, estradiol level stimulation outcome and the number of available embryos were comparable [74].
Based on still small numbers of cases it appears that aromatase inhibitors in combination with FSH can be used in women desiring fertility preservation who are scheduled to start chemotherapy of a hormone sensitive tumor.

Ovarian stimulation has no known negative effect on the course of other types of cancers. If a short delay with chemo- or radiation therapy does not compromise cancer treatment ovarian stimulation, retrieval of mature eggs and the cryopreservation of oocytes, fertilized oocytes or embryos can be offered. Currently, embryo cryopreservation is considered to be the most effective among these options [69].

Options after chemotherapy: Post chemotherapy stimulation

Case 5

DBM, a 32-year old nulliparous woman, was diagnosed with gastric cancer at the age of 24. She had had part of her stomach resected and received chemotherapy for six months. Post-therapy she had transient amenorrhea, but has reported regular 28-day cycles over the past three years. She came to us for infertility evaluation. During the elevated normal semen parameters were found and an HSG revealed blocked tubes. Her early cycle FSH was 12.6 IU/l. She underwent ovarian stimulation with daily 300 IU gonadotropins for IVF. This cycle was cancelled due to the presence of a dominant follicle. In a repeat cycle, stimulation with higher dose gonadotropins was initiated. After 18 days of stimulation (total of 5,275 IU gonadotropins) two eggs were retrieved and fertilized successfully. She had two embryos transferred but the treatment was not successful. The use of donor oocytes as an alternative to further stimulations was discussed with her due to the poor response during stimulation. The patient agreed to the use of donor eggs. She had two blastocysts transferred in an artificially supported cycle. The treatment resulted in a singleton intrauterine pregnancy that was unfortunately miscarried at seven weeks. She is now scheduled to undergo a frozen embryo transfer cycle.

Regular ovarian activity may be preserved after chemo- or radiation therapy. Prior to any ART treatment ovarian reserve is assessed by various studies to help in choosing the optimal stimulation. Various hormone measurements (early cycle FSH, inhibin B, anti-Mullerian hormone), dynamic tests, and ultrasound findings (ovarian volume and antral follicle count) can be used as ovarian reserve tests. Cutoff levels were established in the general infertile population but we might not be able to apply these cutoff values in cancer survivors. Ovarian reserve tests do need to be evaluated among cancer survivors too [75]. Similarly to stimulation options prior to cancer treatment, a decision about stimulation needs to be made based on the hormone sensitivity of the primary tumor.

Donor oocyte use/adoption

When cancer treatment results in ovarian failure and none of the pretreatment options to maintain fertility could be or were utilized the patient is left with two options. If the uterus is preserved she can undergo IVF treatment with donor oocytes. In most countries donor cycles are regulated. The donor must be a healthy, fertile young woman (< 35 years) who is willing to undergo treatment and offers her oocytes for use by someone else. Simultaneously with the donor’s cycle the recipient’s endometrium also has to be prepared for implantation. Oral, transdermal or injectable estradiol and vaginal or muscular progesterone preparations are used for this purpose. If the uterus is intact excellent pregnancy rates can be achieved. Radiation therapy however may compromise uterine function. Radiation may damage the uterine vessels and therefore could compromise endometrial development. Pelvic radiation has been associated with increased miscarriage, preterm delivery rates, and low birthweight. This effect is also dose dependent [76].

As a last resort adoption is also an option for cancer survivors if they have no other solutions to start a family.

Summary

While cancer is rare among young women, when the diagnosis is made it can be devastating for several reasons. Even with improved treatment options the outcome is not always positive. Some of these young reproductive-age women have not had a chance to start a family prior to the diagnosis and treatment in a lot of the cases will induce sterility. Over the past decades emphasis was placed on developing surgical and medical methods that could maintain fertility in these women. Rapidly improving ART also provides hope for them. Therefore, it is very important for those involved in taking care of young cancer patients to be familiar with the available options. This often requires a multi-disciplinary approach by internists, surgeons, oncologists and infertility experts. Future medical and ART improvements could provide even safer and more effective fertility solutions for the survivors of cancer therapy during the reproductive years.

References

Preservation of fertility in reproductive-age women with the diagnosis of cancer


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Accuracy of frozen section diagnosis at surgery in pre-malignant and malignant lesions of the endometrium

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West Kent Cancer Centre, Maidstone Hospital, Maidstone Kent (U.K.)

Summary

Objective: The purpose of this study was to correlate the histological diagnosis made during intraoperative frozen section examination of hysterectomies with atypical hyperplasia or carcinoma, with the definitive paraffin section histology. Study Design: Frozen section pathology results of patients with a preoperative biopsy showing atypical hyperplasia or endometrial carcinoma (87 patients) were compared retrospectively with paraffin section pathology findings. Those patients with curettage specimens showing atypical hyperplasia or curettings suspicious of endometrioid carcinoma had intraoperative frozen section to determine whether an invasive lesion was present and whether they required pelvic lymphadenectomy. The purpose of frozen section assessment in those patients who had a preoperative curette specimen showing endometrial carcinoma was to identify poor prognostic pathological factors related to histological subtype, grade, depth of myometrial invasion and cervical involvement. Results: The correlation between frozen sections and paraffin histology in patients with endometrial carcinoma was 98.6% (69/70) for histological sub-type and 84.3% (59/70) for grade of differentiation. Depth of myometrial invasion was accurately diagnosed in 94.3% (66/70) while cervical involvement was accurately assessed in 86.7% (52/60). Of the 37 patients with atypical hyperplasia or suspicious curettings on preoperative curettage who had intraoperative frozen section, 23 patients had invasive malignancy, which was confirmed in subsequent paraffin sections. Of the remaining 14 patients with a non-malignant frozen section diagnosis, 11 were confirmed with paraffin sections while three had a small well differentiated invasive lesion, two were FIGO Stage 1a and one had microscopic invasion into the myometrium. Conclusion: Intraoperative frozen section is a useful procedure to identify poor prognostic pathological factors as well as to diagnose endometrial cancer in patients undergoing hysterectomy for a preoperative biopsy diagnosis of atypical hyperplasia.

Key words: Frozen section; Endometrial carcinoma; Endometrial hyperplasia.

Introduction

Endometrial cancer is the most common malignancy of the female genital tract in the United States with over 40,000 new cases and 7,000 deaths in 2004 [1]. In the United Kingdom and Ireland uterine cancer accounted for around one in 30 cancer cases and one in 50 cancer deaths in the 1990s, and is the fifth most common cancer in women, with over 90% occurring in women over the age of 50 years [2]. The main risk factors for endometrial carcinoma are associated with prolonged or increased exposure of the uterus to oestrogen and include early age at menarche, low parity, late age at menopause, anovulatory cycles, and obesity as well as unopposed administered oestrogens [2]. There is also a 2-3 fold increase risk of endometrial cancer in women treated with tamoxifen for breast cancer [3, 4].

Endometrial hyperplasia is characterised by an increased endometrial thickness with glandular crowding. In the presence of cytologic atypia the potential for malignancy may be as high as 30% [5-8]. The work of Kurman et al. suggested a 1.6% risk of progression to cancer in the absence of cytological atypia compared to a risk of 23% in the presence of atypia [5], and a similar progression rate of 25% was found by Ferenczy and Gelfand [9]. A common diagnostic problem arises when atypical hyperplasia is diagnosed in the preoperative biopsy histology as it may be difficult to distinguish between atypical hyperplasia and a well differentiated adenocarcinoma, especially in small biopsy specimens. Several morphological features have been described which, when seen in a biopsy, are predictive of myometrial invasion in the uterus [10]. However underdiagnosis of adenocarcinoma and limitations of sampling probably account for the fact that adenocarcinoma is found in 17-43% of hysterectomy specimens performed for a preoperative diagnosis of atypical hyperplasia [8, 11].

The cornerstone of treatment for endometrial cancer is total abdominal hysterectomy and bilateral salpingo-oophorectomy. Certain pathological characteristics are associated with a higher risk of nodal involvement and when present are normally regarded as an indication for surgical staging by pelvic lymphadenectomy. These factors include depth of myometrial invasion > 50%, tumour grades 2 or 3, high risk histological subtypes and cervical or adnexal involvement. These patients may also receive post-op adjuvant radiotherapy.

The aim of this study was to establish the accuracy of intraoperative frozen section, when compared to definitive paraffin histology, in identifying those endometrial...
carcinoma patients with poor prognostic factors who require pelvic lymphadenectomy. Secondly, we assessed the accuracy of intraoperative frozen sections in those patients undergoing hysterectomy for atypical hyperplasia or suspicious curettings but no definite preoperative diagnosis of carcinoma, in identifying carcinoma and poor prognostic factors requiring lymphadenectomy.

Materials and Methods

One hundred and sixty-two patients with a preoperative diagnosis of endometrial carcinoma or of atypical hyperplasia/curettings 'suspicious of endometrioid carcinoma' were treated at the Kent Cancer Centre in Maidstone, Kent, UK from December 2002 to December 2005. Patient ages ranged from 37 to 88 years with a median age of 66 years. Of these patients 87 had frozen sections performed at the time of surgery and were included for analysis. Of the 162 patients there were 46 patients with a preoperative diagnosis of atypical hyperplasia/suspicious curettings but no definite diagnosis of carcinoma. Of these 46 patients 37 had intraoperative frozen section assessment. Intraoperative examination of hysterectomies and attached adnexal structures was performed to identify patients at high risk of regional lymph node metastasis. Specifically, gross dissection and subsequent frozen section microscopy were performed to determine depth of myometrial invasion, cervical involvement and adnexal involvement by tumour. In addition, tumour subtype and grade were assessed and compared with the preoperative curettag diagnosis. Upon receipt of the fresh specimen in the laboratory the external surfaces of the uterus and adnexal structures were briefly examined and the adnexa were then sliced with inspection of the cut surfaces. Any adnexal nodules suspicious of metastasis that were visible with the naked eye were submitted as frozen sections. The uterus was then bisected in the coronal plane followed by sequential transverse slicing at 3 mm intervals through each half of the uterus. During this dissection the depth of tumour invasion into the myometrium and any involvement of the cervix were assessed with the naked eye. Tissue slices incorporating the maximum depth of myometrial invasion were submitted as frozen sections (either as one or two sections depending on the thickness of the uterine wall). One section of the cervix was normally also examined, though this was omitted when the tumour was clearly invading into the outer half of the myometrium. Tissue slices were placed on metal chucks in cryomatrix embedding medium and frozen with Envirotech 1,1,1,2-tetrafluoroethane freezer spray. Sections with a thickness of 4 μ were cut in a Leica CM 1900 semi-automated cryostat and stained with haematoxylin and eosin before presentation to the reporting pathologist. Between one to five frozen sections were examined per case and the whole process from receipt to telephoned result was 10 to 15 minutes in duration. After formalin fixation further sections were taken for routine processing and paraffin embedding (depending on the case, typically four more sections of cervix, four to five of tumour, one of background endometrium and four of the adnexal structures). The paraffin section report and frozen section result were reviewed by an experienced gynaecological pathologist.

Results

Eighty-seven frozen sections cases were performed during the study period and all cases subsequently had definitive paraffin histology. Four cases had benign histology on both frozen section and paraffin assessment. One of these cases was referred with a small focus of well differentiated endometrioid adenocarcinoma and three with atypical hyperplasia on polypoid curettings. Neither intraoperative frozen section assessment nor subsequent paraffin histology showed any residual pathology.

Thirteen cases had a frozen section diagnosis of atypical hyperplasia. Ten of these were confirmed on paraffin while three cases also showed an underlying coexisting well differentiated endometrioid adenocarcinoma. Two of these carcinomas were confined to the endometrium and the other showed a small 5 mm focus of infiltration into the inner half of the myometrium. Although the invasion was not detected at frozen section, neither of these cases were undertreated as lymphadenectomy was not indicated for such small tumours.

Histological subtyping

Seventy cases had a frozen section diagnosis of endometrial carcinoma and of these 65 were diagnosed as endometrioid, three were serous papillary, one was clear cell and one was mixed Mullerian tumour. Correlation of frozen and paraffin assessment of histological subtype was almost perfect for these cases although one case diagnosed as a poorly differentiated endometrioid adenocarcinoma on frozen section was subsequently shown to be poorly differentiated mixed serous/endometrioid on paraffin histology. Management was not affected by the minor discrepancy in this case as both diagnoses were of poorly differentiated tumours for which lymphadenectomy was indicated. Overall correlation for histological subtyping was 98.6%.

Histological grade

The frozen section assessment of grade for the 65 cases of endometrioid adenocarcinoma was 36 as grade 1, 26 as grade 2, and three as grade 3. There was a 100% correlation for assessment of tumour grade between frozen section and paraffin sections for grade three cases (and the five non-endometrioid cases were all considered grade 3 by definition). Thirty-one out of 36 well differentiated and 20 out of 26 moderately differentiated endometrioid tumours also correlated for grade. Five cases diagnosed as grade 1 were subsequently upgraded to grade 2: in two of these there was no myometrial invasion so treatment was not affected by the undergrading but the other three cases were undertreated. Three cases of frozen section grade 2 were downgraded to grade 1 on paraffin but all three underwent lymphadenectomy: for involvement of the outer half of the myometrium in two cases and endocervical glands in the other. Three cases of frozen section grade 2 were upgraded to grade 3 after paraffin histology, but all three had lymphadenectomy due to other poor pathological features of deep myometrial, cervical and adnexal involvement. The correlation
for grading in our series was 84.3%, and only three out of 70 carcinomas were undertreated because of undergrading (Table 1).

Table 1. — Correlation for grade between frozen section and paraffin histology.

<table>
<thead>
<tr>
<th>Frozen section</th>
<th>Paraffin histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1</td>
</tr>
<tr>
<td>Grade 1 (n = 36)</td>
<td>31</td>
</tr>
<tr>
<td>Grade 2 (n = 26)</td>
<td>3</td>
</tr>
<tr>
<td>Grade 3 (n = 3)</td>
<td>0</td>
</tr>
<tr>
<td>Other histological types (n = 5)</td>
<td>0</td>
</tr>
</tbody>
</table>

* clinically important errors.

Depth of myometrial invasion

The accuracy of assessment of the depth of myometrial invasion by tumour was also investigated. Of 70 cases of carcinoma identified intraoperatively, nine were reported as limited to the endometrium on frozen section. In the subsequent paraffin sections three of these cases showed a small focus of invasion into the inner half of the myometrium. One of these cases was a well-differentiated tumour and so management was not affected but the other two cases were microinvasive grade 2 tumours which would undergo lymphadenectomy at our institution. Forty cases were reported as invading the inner half of the myometrium at frozen section assessment and 39 of these correlated with the paraffin histology. One case was a well-differentiated tumour which infiltrated into the outer half of the myometrium and was undertreated. Nineteen cases were reported as infiltrating the outer half of the myometrium, and these were all confirmed on paraffin sections. There was one case of serosal breach and one case with ovarian deposits, which were diagnosed on both frozen and paraffin sections. Correlation for the assessment of depth of myometrial invasion was 94.3% (66 out of 70 cases). Misinterpretation of the depth of invasion at frozen section led to inappropriate management in three cases (Table 2).

Table 2. — Correlation for myometrial invasion between frozen section and paraffin histology.

<table>
<thead>
<tr>
<th>Frozen section</th>
<th>Paraffin histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No myometrial invasion</td>
</tr>
<tr>
<td>Paraffin histology</td>
<td>6</td>
</tr>
</tbody>
</table>

Cervical involvement

A comparison was made of the frozen section and paraffin section assessment of cervical involvement by tumour. A total of 60 cases (out of 70 cases of carcinoma) underwent cervical assessment by intraoperative examination of a frozen section. Ten cases did not have frozen section assessment of the cervix as they already had adverse prognostic factors prompting lymphadenectomy. Eight of 51 cases, which were reported as negative for cervical involvement on frozen section, were subsequently found to have endocervical involvement in paraffin sections (four showed gland and superficial stromal involvement and four showed only gland involvement). In four of these cases the degree of involvement in the paraffin sections was a microscopic deposit up to only 1 mm across and all of these were discontinuous with the uterine lesion. A fifth case was a 3 mm focus of endocervical gland involvement continuous with the lower edge of the uterine tumour. Frozen sections positively identified four cases with endocervical gland involvement and five with endocervical stromal invasion and this was confirmed in paraffin sections. Overall accuracy of assessment of cervical involvement by tumour at frozen section was 86.7% (Table 3). Misinterpretation of cervical involvement at frozen section assessment led to inappropriate management in four cases while the other four cases had lymphadenectomy performed on other histological grounds.

Table 3. — Correlation between frozen section and paraffin histology for cervical involvement.

<table>
<thead>
<tr>
<th>Frozen section</th>
<th>Paraffin histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No cervical involvement</td>
</tr>
<tr>
<td>No cervical involvement (n = 51)</td>
<td>43</td>
</tr>
<tr>
<td>Endocervical glands (n = 4)</td>
<td>0</td>
</tr>
<tr>
<td>Stromal involvement (n = 5)</td>
<td>0</td>
</tr>
</tbody>
</table>

* clinically significant.

The overall correlations between frozen section and paraffin section assessment of the various factors described for the series of 70 cases of carcinoma identified at frozen section are detailed in Table 4.

Table 4. — Overall correlation between frozen section and paraffin histology for cases of endometrial cancer.

| Histological subtypes | 69 out of 70 cases | 98.6% |
| Depth of myometrial invasion | 66 out of 70 cases | 94.3% |
| Cervical involvement | 52 out of 60 cases | 86.7% |

A subgroup of 46 patients were operated on with a pre-operative biopsy histology of either atypical hyperplasia (33 cases) or suspicious curettings, but not diagnostic of carcinoma (13 cases). Thirty-seven of these cases had intraoperative frozen sections and all had definitive paraffin histology.

Of these 37 cases, three cases showed no residual pathology on both frozen section and paraffin examination. Eleven were reported as showing atypical hyperplasia on frozen section and eight of these diagnoses were unchanged in the paraffin sections. In the three discrepant cases a small focus of co-existent well differentiated endometrioid adenocarcinoma was detected in the paraffin sections (Table 5). Two tumours were limited to the
endometrium while the other showed a microscopic focus of invasion of 5 mm depth into the inner half of the myometrium. In all three cases surgical staging with lymphadenectomy was not indicated.

Table 5. — Correlation between frozen sections and paraffin histology in cases with a preoperative diagnosis of atypical hyperplasia/suspicious curettings.

<table>
<thead>
<tr>
<th>(n = 37)</th>
<th>Atypical hyperplasia</th>
<th>Invasive tumour</th>
<th>No residual abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen sections</td>
<td>11</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>Paraffin histology</td>
<td>8</td>
<td>26</td>
<td>3</td>
</tr>
</tbody>
</table>

At frozen section an endometrial carcinoma was detected in 23 of the 37 cases with a preoperative diagnosis of atypical hyperplasia/suspicious curettings and all of these were confirmed in subsequent paraffin sections. Table 6 details the pathological characteristics of the 26 invasive tumours ultimately detected in those patients with no definite preoperative diagnosis of malignancy who underwent frozen section as well as three cases which were not identified at frozen section as detailed above. Fourteen cases had adverse factors requiring lymphadenectomy and eight of these were picked up during frozen section assessment. Of the six cases that were undertreated, three cases had a microscopic deposit on endocervical glands while one case was a moderately differentiated carcinoma thought to be limited to the endometrium but was found to have a small focus invading the inner half of the myometrium. Two well-differentiated adenocarcinomas diagnosed as Stage 1b on frozen section were subsequently found to be moderately differentiated in one case and with cervical stromal deposits in the other.

Table 6. — Pathological characteristics of the 26 invasive tumours ultimately detected on paraffin sections in the subgroup of 37 patients with a preoperative diagnosis of atypical hyperplasia/suspicious curettings who underwent frozen section.

<table>
<thead>
<tr>
<th>Grading</th>
<th>Paraffin section findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>18</td>
</tr>
<tr>
<td>Grade 2</td>
<td>6</td>
</tr>
<tr>
<td>Grade 3</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Depth of myometrial invasion (DMI)</th>
<th>Paraffin section findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>2</td>
</tr>
<tr>
<td>Inner 1/2</td>
<td>20</td>
</tr>
<tr>
<td>Outer 1/2</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cervical involvement</th>
<th>Paraffin section findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>18</td>
</tr>
<tr>
<td>Endocervical glands</td>
<td>5</td>
</tr>
<tr>
<td>Stroma</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

Pelvic lymphadenectomy plays an important role in surgical staging of endometrial carcinoma. The therapeutic role of lymphadenectomy is still debated. A retrospective study by Kilgore et al. of 649 surgically managed patients with endometrial adenocarcinoma showed a significantly better overall survival for patients managed with multiple-site pelvic node sampling relative to patients without node sampling (p = 0.0002) [12]. There was also a better survival for both low-risk and high-risk cancer groups (p = 0.026 and 0.0006, respectively). It is anticipated that some questions relating to the benefit of lymphadenectomy in endometrial cancer may be answered following publication of results from the ASTEC multicentre randomised controlled trial investigating the impact of lymphadenectomy and adjuvant external beam radiotherapy in the treatment of endometrial cancer [13].

In our centre FIGO Stage 1a and 1b well differentiated carcinomas and FIGO Stage 1a moderately differentiated carcinomas are treated and staged by total abdominal hysterectomy, bilateral salpingo-oophorectomy and peritoneal washing cytology. Complete pelvic lymphadenectomy is performed for cases of poorly differentiated endometrioid tumours and high-risk histological subtypes (serous, clear cell, squamous, mixed Mullerian tumours) irrespective of stage; for Stage 1c or above irrespective of the grade and for moderately differentiated endometrioid tumours Stage 1b or above [14]. Postoperative adjuvant external beam radiotherapy is normally given to node positive cases while node negative Stage 2 cases are treated with adjuvant vault brachytherapy alone. The latter is in accordance with a number of reports suggesting that if pelvic lymph nodes are negative for tumour it is reasonable to omit external beam therapy and administer brachytherapy alone to prevent vault recurrence, saving treatment time, cost and reducing potential side-effects from combined treatment [15-20].

Intraoperative frozen section assessment is performed in many centres to identify patients with poor prognostic factors who require lymphadenectomy and to identify low-risk patients who may be spared extensive surgery and possible complications and morbidity. Our results have shown a good correlation between frozen sections and paraffin histology in the assessment of factors, which influence the decision to perform lymphadenectomy. Frozen section assessment in our series was 98.6% accurate for histological subtype, 84.3% accurate for grade, 94.3% for depth of myometrial invasion and 86.7% for assessment of cervical involvement. This is comparable with the published literature reporting accuracies of assessment of 60-98% for grade, 80-96.6% for depth of myometrial invasion and 60-94% for cervical involvement [21-31].

There was a frozen section accuracy rate of 84.3% in assessing tumour grade in our series. Reports in the literature have frequently shown a poor correlation between the grade of carcinoma in preoperative curettings or pipele biopsy and the final grade in the resected uterus, and the poorest correlation is for biopsy grade 1 tumours which may subsequently be upgraded in 20-40% of cases [32,33]. Several of the cases in our series tended to show a somewhat better differentiated component of tumour lining the cavity with relatively more solid areas in the myoinvasive component. Of the five cases, which were upgraded from grade 1 to grade 2 in our series, the dis-
crepancy in grading led to undertreatment in three cases. Three cases were downgraded from grade 2 to 1 and three were upgraded from grade 2 to 3 but in all of these cases there were other poor prognostic factors prompting lymphadenectomy.

The overall accuracy of frozen sections in assessing the depth of myometrial invasion was 94.3% in our series. Discrepancy in the assessment of depth led to undertreatment in only three cases according to our protocol (two small grade 2 tumour infiltrating the inner half of the myometrium and a grade 1 tumour with outer half myometrial invasion). There was 95.5% accuracy in detecting those tumours infiltrating into the outer half of the myometrium in our series. This compares very favourably with the reported accuracy of preoperative MRI in detecting invasion into the outer half of the myometrium (i.e. Stage 1e) with reported results of 81-95% for overall accuracy, 80-82% for sensitivity and 91-100% for specificity [34-37]. We would also make the point that intraoperative frozen section is quicker and much less costly than preoperative MRI.

In our series frozen sections were 86.7% accurate in assessing cervical involvement by tumour when compared to the paraffin section histology. Unsurprisingly there was a lack of sensitivity for detection of microscopic disease in the cervix found only after thorough paraffin section sampling. Indeed, four of the eight foci of cervical involvement not detected at frozen section measured no more than 1 mm across and a fifth measured only 3 mm. Interestingly, most of these cases were microscopic deposits discontinuous from the uterine tumour suggesting a metastatic pathogenesis by detachment from the main tumour, transit through the endocervical canal and implantation into the endocervical mucosa. One could argue that the biological significance of such microscopic disease in the cervix is questionable but follow-up of a large number of patients showing only microscopic cervical disease would be required for clarification. The overall correlation of 86.7% is comparable with other similar studies reporting a range of 60-94% [22, 27, 31]. Studies of preoperative MRI have suggested a radiological accuracy of assessment of cervical involvement of 80-95% with a sensitivity of 33-80% and a specificity of 96-100% [34-37]. The sensitivity and specificity in our series were 52.9% and 100%, respectively, but it is of note that in our study frozen section assessment of the cervix was not performed in ten cases because other obvious poor prognostic indicators were present and so these cases were excluded from analysis of accuracy, sensitivity and specificity.

Of the subgroup of patients (n = 46) referred with a preoperative biopsy histology of atypical hyperplasia or suspicious curettings but no definite diagnosis of cancer, 37 had intraoperative frozen section assessment which detected 23 cases of cancer which were all confirmed on subsequent paraffin histology (Table 5). Eleven were reported as atypical hyperplasia on frozen section but three of these cases were subsequently shown on paraffin histology to coexist with well-differentiated endometroid adenocarcinomas of Stage 1a (two cases) and 1b. This discrepancy did not lead to undertreatment of the three cases, which were still low-risk. Of the initial 46 cases in this sub-group, paraffin sections ultimately showed 32 cases of carcinoma and lymphadenectomy was indicated in 18 of them. These cases would not normally have been referred to and treated in a Cancer Centre.

In conclusion this series supports other published data reporting that intraoperative frozen section assessment of hysterectomy specimens is an accurate method for the detection of risk factors considered as indications for full surgical staging of endometrial cancer. We also believe that frozen section assessment of hysterectomies removed for a preoperative diagnosis of atypical hyperplasia or suspicion of malignancy is justified to identify the significant fraction requiring lymphadenectomy and spare low-risk patients the morbidity of lymphadenectomy.

References


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p16 expression in Paget’s disease of the breast

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Breast Unit, Erasme Hospital, Free University of Brussels (ULB) (Belgium)

Summary

Background: Paget’s disease of the nipple is generally associated with an underlying invasive cancer or an underlying ductal carcinoma in situ. Epidermotropic theory maintains that Paget’s cells are derived from an underlying mammary in situ adenocarcinoma. Because p16 protein plays a major role in cell-cycle control and in tumoral cell mobility, we analyzed p16 expression in Paget’s disease of the nipple and in associated underlying ductal carcinoma in situ. Methods: The expression of p16 protein was analyzed by immunohistochemistry in eight cases of Paget’s disease of the nipple with associated underlying ductal carcinoma in situ. The Student’s t-test (2-tailed) was used to establish the equality of means. Results: The expression of p16 protein was observed in 87.5% (7/8 cases) both in the nipple disease and in the associated underlying ductal carcinoma in situ. The difference between the two populations was not statistically significant. In normal breast tissue, no expression of the protein was observed. Conclusion: The positive p16 expression in Paget’s disease of the nipple and the underlined ductal carcinoma in situ and its role in cell motility lead us to propose a role of p16 in the spread of this disease.

Key words: p16; Paget’s disease; Breast; Carcinoma; Intraductal carcinoma.

Introduction

Paget first described Paget’s disease of the nipple in 1874 after he noticed that changes in the skin adjacent to the nipple preceded the development of an underlying breast cancer [1]. In 1881, Thin observed that the nipple lesion contained malignant cells that were related to an underlying cancer [2] and suggested the process of intraductal extension of cancer through the major lactiferous sinuses that we know today as “pagetoid spread”. Paget’s disease may occur in the nipple in conjunction with an invasive cancer mass, with underlying ductal carcinoma in situ (DCIS), or alone without any underlying invasive breast carcinoma or DCIS. The associated underlying cancer may be located centrally in the breast adjacent to the nipple or peripherally in the breast. The most widely accepted hypothesis regarding the origin of Paget’s cells is the epidermotropic theory, which maintains that Paget’s cells are derived from an underlying mammary in situ adenocarcinoma [3, 4].

The p16 protein plays a major role in the cell-cycle control by way of cyclin D-dependant kinase4 (cdk4). Despite conflicting results, it has been suggested that p16 protein expression is associated with accelerated tumor growth and poor clinical outcome in breast carcinoma [5]. In addition, in uterine cervical cancer, colorectal cancer, and basal cell carcinoma, it has been demonstrated that elevated p16 expression accompanies the tumor invasion front and could play a role in tumor cell hypermobility [6-9]. As Paget’s cells are considered to be intraepidermally hypermobile and migratory tumoral cells originating from underlying in situ intraductal carcinoma, the aim of the present study was to investigate p16 expression in Paget’s disease of the nipple and in the associated underlying DCIS to determine if p16 could play a role in this disease by modulation of cell invasion. These data have not yet been published.

Materials and Methods

Patients

Tumor sample were obtained from eight patients with primary Paget’s disease of the breast who had undergone mastectomy or central lumpectomy with sentinel lymph node dissection at the Erasme Hospital (Brussels, Belgium). All patients gave their consent for this study and the experimental research was performed with the approval of the local ethics committee.

Histologic evaluation

To establish immunohistochemistry, 4-μm sections were cut sequentially from the archival specimens and mounted onto superfrost-treated slides (Menzel-Glaser, Braunschweig, Germany). The slides were dried overnight at 37°C before deparaffinization in xylene and and rehydrated with graded ethanols. For the p16 antibody, the antigen retrieval method was used with an incubation period of 60 min in a hot water bath at 95-99°C with citrate buffer (pH 6.0). The slides were then cooled in the buffer for 20 min at room temperature; 0.3% H2O2 was added to the slides, which were incubated at room temperature for 30 min. After rinsing with tris-buffered saline (TBS), pH 7.6, normal horse serum was added to each slide for 20 min. The mouse monoclonal antibodies diluted in TBS (monoclonal p16 antibody, clone 16P04, dilution 1/50; Neomarkers, Fremont, CA, USA) were added to each slide and incubated for 60 min at room temperature. After rinsing, twice for 5 min in TBS, the slides were incubated for 30 min with diluted biotinylated secondary antibody. The sections were again washed for 5 min in TBS buffer and incubated with an avidin-biotin-peroxidase complex for 30 min (Vectastain Elite ABC kit; Vector,
of Paget’s disease of the nipple and associated DCIS (Figure 1). One of the three underlying invasive carcinomas was positive (Table 1).

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Paget’s nipple</th>
<th>Associated DCIS</th>
<th>Associated invasive carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 positivity</td>
<td>7/8</td>
<td>7/8</td>
<td>1/3</td>
</tr>
<tr>
<td>H score</td>
<td>165 ± 126</td>
<td>110 ± 97</td>
<td>33 ± 57</td>
</tr>
</tbody>
</table>

p value for H score Paget nipple/DCIS: p = 0.34.
p value for H score Paget nipple/invasive carcinoma: p = 0.04.

No difference was found to be significant (t-test for equality of means, p > 0.02) between Paget’s disease of the nipple and the associated DCIS or invasive carcinoma.

Discussion

Both decreased and increased p16 expression have been described in primary human breast cancer, but these differences in expression have not been well correlated with clinical outcome [5]. In other human cancers, the loss of p16 expression, regardless of the mechanism, appears to confer a grave prognosis presumably because of more rapid cell growth and an increased mutation rate in p16 null cells [11].

Our study is the first to describe p16 overexpression in Paget’s breast disease and in the underlying DCIS. High levels of p16 expression have been associated with loss of retinoblastomo-protein (pRB) expression in both primary cancers and cell lines of various kinds, presumably due to loss of a feedback loop and to pRB expression being transcriptionally repressed by ectopic p16 expression [12]. This loss of feedback would be associated with a higher cell proliferation rates and could explain why p16 without gene mutation is associated with larger primary tumors with a poor prognosis in breast cancers [5].

Because p16 is up-regulated at the invasive front of the majority of basal cell carcinomas with an infiltrative pattern and accompanied by cessation of proliferation [8], a similar role could be associated with the spread of the underlying DCIS to the nipple.

We may also consider the possibility that mutations in p16 might result in an effective protein that was overexpressed in a compensatory manner. In vitro, adenovirus-mediated p16/CDKN2 gene transfer suppresses glioma invasion and growth [13, 14]. Therefore, the loss of p16-controlled cell proliferation and invasion in the underlying DCIS could suggest the process of intraductal extension of cancer through the major lactiferous sinuses that is known today as “pagetoid spread” [15].

Research into the proliferation index regarding p16 expression in DCIS and in Paget’s lesions as well as into gene mutations in p16 may contribute to an understanding of this protein in Paget’s breast disease.

In summary, the similar p16 overexpression in the two cell populations confirms that they share the same
immunohistochemical profile [16]. The leading role of p16 in cell invasion and motility could explain the pagetoid spread of this disease but this will have to be verified in future studies.

Acknowledgements

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References


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Immunohistochemical expression of MMP-2, MMP-9 and COX-2 in Stage IA malignant polyps of the endometrium

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Introduction

Endometrial cancer is the most common gynecologic cancer in developed countries [1]. It usually affects postmenopausal women and manifests as uterine bleeding early in the course of disease. Therefore, it is usually identified in FIGO Stage I [2]. Tumor type, histologic grade, myometrial invasion, lymphovascular space involvement and lymph node metastasis are important prognostic factors [3, 4]. The extracellular matrix and basal membrane are the main barriers to tumor progression.

Matrix metalloproteinases (MMP) are a group of zinc-dependent endopeptidases which play a pivotal role in the degradation of extracellular matrix [4]. The extracellular matrix is composed of type I, type II and type III collagen. The basement membrane is especially composed of type IV collagen. These are degraded particularly by gelatinases (MMP-2 and MMP-9) in tumor invasion [4, 5]. The frequency of MMP-2 and MMP-9 expression is reported to be increased with advancing histologic grade and with increasing depth of myometrial invasion [6-8]. Endometrial cancer invading the basal membrane and myometrium by gelatinases penetrates to lymphovascular spaces and disseminates. The role of gelatinases in this sequence has been well-established in recent reports [6-8].

Cyclooxygenases (COX) synthesize prostaglandins from arachidonic acid. COX-1 is constitutively expressed in most of the tissues [9]. However, COX-2 transcription can be induced by cytokines, growth factors, phorbol esters and mitogens. COX-2 is found to be upregulated in malignant cells [10, 11]. COX-2 is related to angiogenesis, tumor growth and progression [12]. COX-2 is reported to be overexpressed in endometrium cancer [13] and it is proposed that COX-2 inhibitors may be of benefit in the treatment of endometrial cancer [14].

Malignant endometrial polyps are rarely encountered [15]. Seventy-eight percent of endometrial polyps are benign, and 13% are with endometrial hyperplasia without atypia. Polyps with endometrial hyperplasia with atypia and carcinomatous polyps comprise 1% and 2% of endometrial polyps, respectively [16]. The low prevalence of malignant endometrial polyps undervalues its entity and makes it difficult to study. It is not known whether malignant endometrial polyps are different from malignancies not arising in a polyp [17]. Malignant polyps may have access to the lymphovascular system even though they are low-stage [17]. Characteristics of malignancies arising in polyps may be different from malignancies not associated with polyps. In the present study, immunohistochemical expressions of gelatinases and COX-2 were studied in benign endometrial polyps, early-stage malignant endometrial polyps and early-stage endometrial cancer. This may give clues regarding interaction of malignant endometrial polyp tumors (MEP) and endometrial cancer not associated with polyps (ECNAP) with extracellular matrix.

Material and Methods

Specimens of eight MEP, eight ECNAP and 16 benign endometrial polyps were included in the study. All the malignant specimens were grade 1, endometrioid type endometrium cancer in FIGO Stage IA. We defined a malignant polyp according to Coeman et al.‘s strict criteria [18]. Coeman et al. reported that the pedicle and surrounding endometrium must be benign and the carcinoma must be confined to the polyp surface to recognize malignancy originating in a polyp [18].

Summary

Objective: To study whether endometrioid type malignant endometrial polyps (MEP) are different from endometrium cancer not associated with polyps (ECNAP) in means of immunohistochemical expressions of MMP-2, MMP-9 and COX-2.

Methods: Archived tissue samples of eight MEP, eight ECNAP and 16 benign endometrial polyps were selected and immunohistochemically analyzed for MMP-2, MMP-9 and COX-2 expression. Results: MMP-2 and MMP-9 were overexpressed in ECNAP compared to MEP and benign endometrial polyps (p < 0.05). MMP-2 and MMP-9 expressions were not different in the malignant part of MEP, benign part of MEP and benign endometrial polyps. COX-2 expression was found to be higher in benign lesions, although this was not statistically significant. Conclusion: Similar immunohistochemical expression of MMP-2, MMP-9 and COX-2 within a polyp and with benign polyps may indicate an immunohistochemically indolent characteristic of MEP.

Key words: Endometrial polyp; Endometrium cancer; Cyclooxygenase 2; Matrix metalloproteinase 2.
Immunohistochemical analysis for MMP-2, MMP-9 and COX-2 were performed on formalin-fixed, paraffin-embedded archival tissue using the streptavidin-biotin-peroxidase technique. For all cases, a 4 μm histological section was deparaffinized in xylene and dehydrated in descending dilution of ethanol. For antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked by 20 min of incubation with 0.3% hydrogen peroxide. Slides were tested with MMP-2 antibody (1:100, rabbit polyclonal, LabVision, USA), MMP-9 antibody (1:100, rabbit polyclonal, Lab Vision, Fremont, CA, USA) and COX-2 antibody (1:100 Epitep specific rabbit antibody, Lab Vision, Fremont, CA, USA). Sections were counterstained with Mayer’s hematoxylin, and mounted with mounting medium. The positive control for MMP-2 and MMP-9 was placental tissue. Tissue of colon cancer from CA, USA), and after incubation the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer’s hematoxylin, and mounted with mounting medium. The positive control for MMP-2 and MMP-9 was placental tissue. Tissue of colon cancer from mice served as the positive control in the COX-2 immunostaining.

Two independent observers blinded for clinical data analyzed the staining for MMP-2, MMP-9 and COX-2. Scoring was done on a point scale, the IRS [19]. Staining intensity (weak, 1 point; moderate, 2 points; strong, 3 points) and percentage groups of positive tumor cells (< 10%, 1 point; 11%-50%, 2 points; 51%-80%, 3 points; > 80%, 4 points) were multiplied to achieve a score between 1 and 12. IRS for MMP-2, MMP-9 and COX-2 were calculated in benign polyps, ECNAP and MEP. Two IRS for each case were calculated for each MEP. Results were analyzed by the One-way ANOVA test and chi-square test. The level of statistical significance was chosen to be p < 0.05. Statistical analysis was performed using the SPSS 13.0 software program (SPSS, Chicago, IL, USA).

Results

Cox-2 expression

None of the examined specimens had a maximum IRS of 12. Mean of COX-2 IRS was 3.2 ± 2.0 points, 2.8 ± 1.9 points, 3.5 ± 2.5 points and 4.3 ± 1.4 points in ECNAP, malignant part of MEp, benign part of MEp and benign endometrial polyp, respectively (p < 0.05) (Figure 1). Percentage of stained cells was similar in all groups. Intensity of staining in benign lesions was higher than malignant lesions (p < 0.05); COX-2 was stained strongly in benign endometrial polyps compared to ECNAP and the malignant part of MEp (p < 0.05), while intensity of COX-2 staining in benign endometrial polyps and the benign part of MEp was similar. There was no difference in intensity of COX-2 staining within the benign and malignant parts of the each MEP (Table 1).

MMP-2 expression

None of the examined specimens had a maximum IRS of 12. Mean of MMP-2 IRS was 3.7 ± 0.7 points, 2.8 ± 1.8 points, 2.1 ± 1.8 points, 1.5 ± 1.3 points in ECNAP, malignant part of MEp, benign part of MEp and benign endometrial polyp, respectively (p < 0.05). IRS of MMP-2 in endometrium cancer is higher than benign polyps (p < 0.05) and malignant polyps (p > 0.05) (Figure 2). Percentage of stained cells and intensity of stained cells were significantly different in ECNAP and the malignant section of MEP than benign lesions (Table 1).

MMP-9 Expression

None of the examined specimens had a maximum IRS of 12. Mean of MMP-9 IRS was 4.2 ± 1.2 points, 2.7 ± 1.9 points, 2.2 ± 2.1 points, 1.9 ± 1.5 points in ECNAP, the malignant part of MEp, the benign part of MEp and benign endometrial polyps, respectively (p < 0.05). IRS was found to be higher than benign endometrial polyps (p < 0.05) and MEP (p > 0.05) (Figure 3). Percentage of stained cells and intensity of stained cells were significantly different in ECNAP and malignant sections of MEP than benign lesions (Table 1).

Discussion

In the present study, immunohistochemical expression of important markers which have prognostic value and are involved in the pathogenesis of endometrial cancer were compared in ECNAP, MEP and benign endometrial polyps.
COX-2 expression is reported to be increased in endometrial carcinoma and it is proposed that COX-2 inhibits apoptosis, enhances metastases and angiogenesis [12, 13, 20-22]. However, it is not known whether COX-2 expression is a late or an early step in the development of endometrial carcinogenesis [13]. COX-2 expression was found to be similar in benign polyps, MEP, and Stage IA endometrial carcinoma in our study. Orejuela et al. compared expression of COX-2 in biopsy samples of endometrial cancer, endometrial hyperplasia, and normal endometria and found no statistically significant increase in COX-2 expression in the endometrial cancer cases or endometrial hyperplasia samples [23]. They have also reported that COX-2 expression was noticeably greater in the superficial layer of normal epithelium. Interestingly, immunohistochemical expression of COX-2 was remarkable in benign endometrial polyps in our study. In contrast to these findings, Nasir et al. reported that COX-2
expression increases as the severity of the disorder changes from endometrial hyperplasia to invasive endometrial cancer [24]. Further, studies are needed to evaluate the expression of COX-2 and to clarify its role in the process of endometrial hyperplasia and early-stage endometrium cancer.

Upregulation of MMP-2 and MMP-9 in endometrial cancer have been associated with increased myometrial invasion, higher grade, metastasis and poor prognosis in previous reports [7, 8, 25]. Serous carcinomas usually arise in polyps and have a worse prognosis than other types of carcinomas, but it is not known whether similar types of carcinomas that begin in a polyp are different from carcinomas not associated with polyps [17, 26]. IRS
of MMP-2 and MMP-9 increased from benign endometrial polyps to endometrial cancer. Immunohistochemical expression of MMP-2 and MMP-9 in MEP were moderate between benign polyps and endometrial cancer in the present study, suggesting that early endometrioid endometrial cancer developing in a polyp may be more indolent. Immunohistochemical expression of MMP-2 and MMP-9 was not statistically different in benign and malignant sections within a polyp, indicating that invasiveness potential is not increased in malignant parts of polyps in early-stage endometrium cancer. However, gelatinases which play a role in tumor progression are overexpressed in ECNAP.

In conclusion, we found that MMP-2 and MMP-9 are
overexpressed in early-stage endometrial cancer. However, immunohistochemical expression of COX was not different. COX-2 may be involved in the pathogenesis of endometrium cancer in later stages of tumoral development. Similar immunohistochemical expression of MMP-2, MMP-9 and COX-2 within a polyp and with benign polyps may indicate an immunohistochemically indolent characteristic of MEP.

References


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Expression of the epidermal growth factor system in endometrial cancer

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Introduction

Endometrial cancer is the most common malignancy of the female genital tract. Overall, about 2% to 3% of women develop endometrial cancer during their lifetime [1]. Endometrial cancer is a malignancy that occurs primarily in postmenopausal women. It most often occurs in the sixth and seventh decades of life, at an average age of 60 years. Several risk factors have been identified (nulliparity, late menopause, obesity, unopposed estrogen therapy, atypical endometrial hyperplasia, diabetes mellitus and tamoxifen therapy). Most of these risk factors are related to prolonged, unopposed estrogen stimulation of the endometrium [2].

The overall survival rate for endometrial cancer is 84%; this reflects its early clinical declaration [1].

A wide variety of prognostic factors (age, stage, tumor grade, histologic type and depth of myometrial invasion) have been described and evaluated in detail.

During the last decade efforts have focused on attempting to identify cytokinetic or molecular events that correlate with the malignant potential of endometrial cancers. Several laboratories have evaluated the expression of oncogenes and tumor suppressor genes.

The epidermal growth factor (EGF) system is a type I growth factor family consisting of four receptors: epidermal growth factor receptor (EGFR) (also called c-erbB-1, HER-1), c-erbB-2 (also called HER-2), c-erbB-3 (also called HER-3), c-erbB-4 (also called HER-4). The receptors are transmembrane glycoproteins with an extracellular ligand-binding domain, a transmembrane region and an intracellular domain. The intracellular domains of c-erbB-1, c-erbB-2 and c-erbB-4 display tyrosine kinase activity. Activation of the receptors induces dimerization. C-erbB-1 and c-erbB-4 form either homo- or heterodimers, whereas c-erbB-2 functions as a cofactor for the other receptors, and c-erbB-3 needs heterodimerization because of its lack of tyrosine kinase activity. There are at least 11 known ligands for the EGF system [3].

The EGF system is ubiquitous in human organs and plays fundamental roles in diverse processes such as embryogenesis, development, proliferation, differentiation, cell motility and survival [4, 5].

The EGF system signaling network induces a wide variety of biologic responses and is involved in many physiologic and pathologic conditions. The biologic outcome of signaling through the EGF system depends on the cellular and environmental context. Dysregulation of the EGF signaling network is implicated in multiple human pathologies, of which the role of EGF in cancer is the best characterized, particularly for c-erbB-1 and c-erbB-2 [5].

Only a limited number of studies concerning the EGF system and the endometrium have been published. In these studies one or few members of the EGF system have been investigated [6-9]. Most of these studies are based on immunohistochemistry of single biopsies from women undergoing hysterectomy for benign indications.

The aim of our study was to describe the expression of c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 in endometrial cancer tissue and its correlation with clinicopathologic features and prognosis of the patients.

Summary

The aim of our study was to describe the expression of c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 in endometrial cancer tissue and its correlation with clinicopathologic features and prognosis of the patients. One hundred and six cases of endometrial cancer were identified from the archives of the Department of Obstetrics and Gynecology of the University of Patras. Tissue specimens from endometrial lesions were immunostained for c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4. Statistical analyses were performed using the chi square test, Kaplan-Meier method and Cox analysis. We found a significant association between c-erbB-1 expression and patient survival. A reverse correlation was found between tumor grade and c-erbB-1 expression. Tumor grade was not significantly correlated with the expression of the remaining three receptors. Stage of the tumor showed no relationship with the expression of these receptors. The ability to predict increased risks of advanced disease, recurrence, and death from abnormal molecular markers detected in curettage or endometrial biopsy specimens will facilitate pretreatment referral of these patients to gynecologic oncologists for definitive surgical treatment.

Key words: Endometrial cancer; EGF system; c-erbB receptors; Prognosis.
Expression of the epidermal growth factor system in endometrial cancer

Materials and Methods

Case selection

Between May 1991 and December 2004, about 106 women with histologically confirmed endometrial cancer were referred to the Department of Gynecologic Oncology of the University of Patras Medical School.

Among them, 12 patients denied surgical intervention and they underwent radiotherapy.

Ninety-four patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. Lymph node sampling and cytologic tests of the peritoneal fluid were performed in all patients. All staging procedures were performed by a gynecologic oncologist.

Histopathology and immunohistochemistry

Three pathologists reviewed all hematoxylin-eosin stained sections. Staging was determined using the surgical staging system for endometrial cancer established by the International Federation of Obstetrics and Gynecology (FIGO). Tumor histologic classification was performed using the criteria of the World Health Organization (WHO).

Formalin-fixed paraffin-embedded tissue sections representative of the tumor in each case, were immunostained using the biotin-streptavidin peroxidase method. Sections were quenched with H2O2 (0.6%) in 100% methanol for 20 min to inhibit endogenous peroxidase activity. Microwave pretreatment was used to unmask epitopes. Non specific binding was blocked by incubating the sections in TBS solution containing 3% BSA. The following antibodies against c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 were used: a) anti-EGFR mouse monoclonal antibody (Santa Cruz Biotechnology Inc, UK) in a dilution 1:20, b) anti-HER-2 mouse monoclonal antibody (Biogenex), in a dilution 1:100, c) anti-HER-3 mouse polyclonal antibody (Santa Cruz Biotechnology Inc, UK) in a dilution 1:100 and d) anti-HER-4 mouse polyclonal antibody (Santa Cruz Biotechnology Inc, UK) in a dilution 1:200.

Labeling was detected using the streptavidine-biotin complex method, while 3',3' diaminovenzidine (DAB) was used as a chromogen. Sections were counterstained with hematoxylin. Negative staining controls were included in which no primary antibody had been added.

Evaluation section-immunopositivity was done by three pathologists through a Zeiss light microscope. Immunostaining of 5% of the tumor cells was considered as an optimized cut-off for tumor positivity (we chose this evaluation system because there is no acceptable scoring system for endometrial cancer as there is for breast cancer). The staining for c-erbB-1, c-erbB-3 and c-erbB-4 was mainly cytoplasmic, though nuclear staining was also detected in some of the cases (Figures 1A, 2A, and 2B). The staining for c-erbB-2 was membrane, although cytoplasmic staining was also detected in some cases, only the membrane immunostaining was considered as positive (Figure 1B).

Statistical analysis

Statistical analyses were performed using the SPSS-13 for Windows. The association between c-erbB-1, c-erbB-2, c-erbB-3 and c-erbB-4 expression, as well as between molecule expression and other clinicopathological markers was analyzed using the chi square test. Life tables were calculated according to the Kaplan-Meier method. The survival time was calculated from the date of the initial diagnosis. Multivariate survival analyses were performed with the Cox proportional hazards model, entering the following covariates: a) c-erbB-1 expression (negative vs positive), b) c-erbB-2 expression (negative vs positive), c) c-erbB-3 expression (negative vs positive), d) c-erbB-4 expression (negative vs positive), e) tumor stage (I, II, III and IV), and f) tumor grade (I, II and III). In Cox regression analysis a p of 0.05 was adopted as the limit for inclusion of a covariate. All statistical tests were two-sided. For each method every p < 0.05 was considered as statistically significant.
Results

Clinical findings

Median age at diagnosis of endometrial cancer was 64.2 years (range 39-89 years). Median weight was 74.5 kg (range 65-89 kg). Time of follow-up ranged from 11 to 168 months (mean 76 months). None of the patients had received hormonal therapy.

Histopathologic findings

Our study included 106 cases of endometrial adenocarcinomas who had been diagnosed by endometrial curetage. Among the 106 cases, we had 31 grade 1 (29.2%), 51 grade 2 (48.1%), and 24 grade 3 carcinomas (22.6%).

In the majority of cases (94 from 106) the diagnosis was followed by total abdominal hysterectomy with bilateral salpingo-oophorectomy. Among the 94 patients, we had 64 (60.4%) in Stage I, 15 (14.2%) in Stage II, ten (9.4%) in Stage III and five (4.7%) in Stage IV, according to the FIGO classification.

The histopathologic findings of 106 endometrial cancers are summarized in Table 1.

Immunohistochemical findings

For c-erbB-1, 66 cases (62.3%) were positive and 40 (37.7%) were negative. For c-erbB-2, 69 cases (65.1%) were positive and 37 (34.9%) were negative. For c-erbB-3, 72 cases (67.9%) were positive and 34 (32.1%) were negative. For c-erbB-4, 78 cases (73.6%) were positive and 28 (26.4%) were negative.

Table 1. — Histopathologic findings.

<table>
<thead>
<tr>
<th></th>
<th>Case numbers</th>
<th>Percentage (%)</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60 years</td>
<td>38</td>
<td>35.8</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>68</td>
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<tr>
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<tr>
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<td><strong>Ovarian metastasis</strong></td>
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</table>
Statistical analysis

A significant association was found between c-erbB-1 and c-erbB2 expression (Pearson correlation 0.369, p < 0.001), as well as between c-erbB-1 expression and the expression of c-erbB-3 and c-erbB-4 proteins (Pearson correlation 0.341 and 0.505, respectively and p < 0.001 in both cases). A significant correlation also emerged between the expression of c-erbB-2 and c-erbB-3 receptors (Pearson correlation 0.557, p < 0.001). Moreover, the expression of c-erbB-2 and c-erbB-4 was strongly correlated with one another (Pearson correlation 0.235, p = 0.015). Finally, c-erbB-3 and c-erbB-4 positivity also had a strong association with each other (Pearson correlation 0.368, p < 0.001). Finally, the correlation between the expression of c-erbB-2 and c-erbB-4 approached borderline (p = 0.055).

There were no significant differences in EGF system expression between endometrioid and non-endometrioid histological subtypes. Possibly, this was due to the small number of cases of non-endometrioid subtypes in our study.

A reverse correlation was found between tumor grade and c-erbB-1 expression (Pearson correlation = -0.2, p = 0.040). Tumor grade was not significantly correlated with the expression of the rest three receptors (p = 0.317, p = 0.171, p = 0.206 for c-erbB-2, c-erbB-3 and c-erbB-4 respectively). Stage of the tumor showed no relationship with the expression of the c-erbB receptors.

Kaplan-Meier plots revealed a significant association between c-erbB-1 expression and patient survival (p = 0.011). For the other three receptors, Kaplan-Meier plots showed a tendency toward decreasing survival with protein expression, though not statistically significant.

To evaluate the potent correlation between the molecule expression and patient outcome, a Cox regression analysis was also performed. According to this, c-erbB-1 is a significant independent prognostic factor for patient death (ExpB = 4.93, p = 0.043), which means that patients whose carcinomas express c-erbB-1 are 4.93 times more likely to die of their disease, compared with those whose tumors do not express c-erbB-1. C-erbB-1 was also significantly correlated with patient death (Pearson correlation 0.302, p = 0.002). In logistic regression analysis, c-erbB-1 was found to be a predictor factor for patient death (p = 0.016 ExpB = 0.140, 95% CI for ExpB: 0.028-0.693). C-erbB-2 was also strongly correlated with patients death in bivariate analysis (Pearson correlation 0.209, p = 0.036), but no significant association was found in multivariate Cox regression analysis.

No association emerged between c-erbB-3 and c-erbB-4 expression and patient death in either bivariate (Pearson correlation 0.131, p = 0.195) or multivariate Cox regression analysis (p = 0.622 and p = 0.161, respectively).

Discussion

The EGF system signaling network induces a wide variety of biologic responses and is involved in many physiologic and pathologic conditions. The biologic outcome of signaling through the EGF system depends on the cellular and environmental context. Dysregulation of the EGF signaling network is implicated in multiple human pathologies, of which the role of EGF in cancer is the best characterized, particularly for c-erbB-1 and c-erbB-2 [5].

The four receptors have different levels during the menstrual cycle. C-erbB-1 shows the highest value in the early proliferative phase, c-erbB-2 and c-erbB-4 in the early secretory phase and c-erbB-3 in the late secretory phase [9]. The results that emerged from our study indicate that the four receptors are also overexpressed in endometrial adenocarcinomas of postmenopausal women.

C-erbB-1 is localized to the basal part of the surface epithelial cells [6, 9], only in stromal cells, or both to epithelial and stromal cells [10]. It is overexpressed in multiple human malignancies, including cancers of the breast, head, neck, lung and gliomas [11]. In our study c-erbB-1 was the only molecule whose expression was proven to have a prognostic factor for patient survival even in multivariate (Cox) analysis. Moreover its expression was correlated with tumor grade, a finding that agrees with other published studies.

C-erbB-2 is localized basolaterally, and solely to the glands and epithelium [9]. With no direct ligand identified to date, c-erbB-2 functions as a preferred partner for heterodimerization with other members of the EGF system, and thus plays an important role in coordinating the EGF system signaling network that is responsible for regulating cell growth and differentiation [12]. Overexpression of c-erbB-2 has been found to play a role in cellular transformation, tumorigenesis, and metastasis [13]. It is overexpressed in many types of cancer and has been shown to be an indicator of more aggressive disease and poorer prognosis in patients with breast cancer [14], while it is also overexpressed in some stomach and ovarian carcinomas [15, 16]. Opinions in the literature regarding the prognostic value of c-erbB-2 overexpression in endometrial cancer are conflicting, and the marker has been correlated with unfavorable prognosis in some studies [17], but not in others [18]. Possible explanations of the lack of concordance in the prognostic value of c-erbB-2 expression among the studies include differences in populations studied, techniques used, antibodies used, or interpretation of results. The conflicting results reported in the literature about its possible prognostic role, the lack of independent prediction of patient outcome, the subjectivity in its measurement, and the concerns expressed regarding its reproducibility would minimize the potential role of c-erbB-2 as a marker in the preoperative evaluation of patients with endometrial cancer. According to our findings, c-erbB-2 seemed to have a strong relationship with patient death, though this relationship was not conserved in multivariate analysis. However its role in the appearance of a more aggressive phenotype of the tumor cells should always be kept in mind.
C-erbB-3 and c-erbB-4 are receptors activated by the neuregulin family of ligands, which comprises a number of different isoforms of NEU differentiation factor/Hereregulin [19].

C-erbB-3 is localized in the epithelium [9]. It is expressed in some breast and gastric carcinomas [20, 21], while little is known regarding its precise intracellular function. In our study the expression of c-erbB-3 receptor had no association either with tumor grade and stage, or with the outcome of the disease.

C-erbB-4 is the most recently identified member of the family, and is localized to epithelial and stromal cells [7-9, 22]. It is expressed in much adult and fetal tissue - lining epithelia of skin, gastrointestinal, urinary reproductive and respiratory tracts, skeletal muscle, circulatory, endocrine and nervous systems, as well as in the majority of ovarian cancers [22]. In our study the expression of c-erbB-4 receptor had no association either with tumor grade and stage, or with the outcome of the disease.

The most interesting observation of our study was the strong association of all four receptors with each other, which implies their potent common or consecutive activation and overexpression during the malignant process, maybe with a different role in each step of cell transformation.

The ability to predict increased risks of advanced disease, recurrence, and death from abnormal molecular markers detected in curettage or endometrial biopsy specimens will facilitate pretreatment referral of these patients with endometrial cancer to gynecologic oncologists for definitive surgical treatment. Prospective studies with an appropriate panel of antibodies could lead to better definition of risk groups.

Conclusion

The c-erbB-1 gene has the ability to predict patient survival in endometrial cancer cases. The ability to predict increased risks of advanced disease, recurrence, and death from abnormal molecular markers detected in curettage or endometrial biopsy specimens will facilitate pretreatment referral of these patients to gynecologic oncologists for definitive surgical treatment.

References


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Vulval cancer: what is an adequate surgical margin?

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Summary

Objective: To determine the accuracy of naked eye assessment of surgical margins after formalin fixation in vulval cancer in comparison with microscopic assessment. Design: Retrospective review. Setting: The Gynaecological Cancer Centre, St Bartholomew’s Hospital, London, UK. Population: Patients with primary vulval cancer who underwent surgery from 1997 to 2006. Methods: Histopathology reports were reviewed and data on surgical margins were analysed. After formalin fixation, pathologists analysed surgical margins and measured them with a ruler. This measurement was compared with microscopic measurement. Other clinicopathologic variables were also recorded and compared. Main outcome measure: Comparison between macroscopic and microscopic measurement, and the relation to clinicopathological variables. Results: Naked eye assessment of surgical margins was within 2 mm of correlated microscopic measurement in 29 patients (Group 1). In ten patients the macroscopic measurement of clear margins was less than the microscopic (Group 2). In the remaining 11 cases (22%) naked eye observation overestimated the normal skin margins (Group 3). Seven patients from this group eventually fell into the unfavourable prognostic category of surgical margins < 8 mm. The presence of LVSI was significantly more frequent in Group 3 than in the other two groups (p = 0.01). The difference between other variables of the study groups was statistically non-significant. Conclusion: Our study demonstrates that naked eye assessment of surgical margins after formalin fixation is inaccurate and that surgical margins are often inadequate. We conclude that tumours with LVSI should be considered for a wider surgical excision.

Key words: Vulval cancer; Surgical margin; LVSI.

Introduction

Vulval cancer constitutes only 4% of female genital tract cancers but represents major clinical dilemmas. Although the majority of patients are over 70, often with multiple medical comorbidities, 20% of patients are under the age of 50. In the United Kingdom, the incidence of vulval cancer has shown an increasing trend since the mid-1990s and has now reached a similar level to that of 1975 with approximately 1,000 diagnoses each year and 380 deaths from disease [1]. In the United States the incidence of vulval carcinoma has increased by 20% since 1973 and the incidence of in situ vulval cancer by a striking 411% [2].

Radical excision of the primary lesion with unilateral or bilateral inguinofemoral lymph node dissection continues to be the standard of care for patients with vulval cancer. The traditional butterfly en-bloc resection of the vulva and groins described by Antoine Basset in 1912 and promoted by Stanley Way in the 1940s, was associated with high cure rates but significant morbidity [3]. A reduction in radicality was achieved during the last century to preserve sexual function and to reduce postoperative morbidity including wound dehiscence, infection, and disfiguration. Radical wide local excision with separate groin incision(s) eventually became the standard of surgical care by reducing morbidity without compromising survival [4-7]. Further reduction in morbidity in the groins has more recently been proposed using sentinel node techniques or ultrasound-guided fine needle aspiration cytology [8, 9].

Reducing the extent of surgical resection is not without risk. In spite of clear surgical margins, recurrence of vulval cancer is frequent (12-37%) with more than half of cases having a local component [10]. Surgical margin status, depth of invasion, size of tumour, pattern of infiltration (pushing vs spray pattern), and lymphovascular space invasion (LVSI) have been established for some time as the most important prognostic factors for local recurrence [11]. Tumour-free surgical margins < 8 mm measured in a formalin-fixed pathology specimen are clearly associated with significant risk for local recurrence, while margins ≥ 8 mm are not [12, 13]. On the basis of these findings, many clinical guidelines and textbooks recommend that a surgical margin of 1-1.5 cm be removed with the tumour at the time of surgery [8, 14, 15]. However, others have criticised this practice as there is a high rate (50%) of pathological margins < 8 mm in spite of removing 1 cm of an apparent clear surgical margin intraoperatively [8].

Naked eye estimation of surgical margins is the most important step in planning resection of vulval cancer. However, to date there are no data on the correlation of macroscopic assessment and the microscopic measurement of surgical margins. The aim of this study was to determine the accuracy of naked eye assessment of surgical margins after formalin fixation in comparison with microscopic measurement.
Patients and Methods

A retrospective analysis of hospital notes of patients who underwent surgical treatment for primary vulval cancer at St Bartholomew’s Hospital during the period 1997-2006 was performed. The departmental policy of removing an apparently tumour-free surgical margin at least 1 cm during the operative procedure was applied to all patients. Histopathology reports were reviewed and data on surgical margins analysed. Patients with incomplete data on surgical margins were excluded from the study.

After formalin fixation, pathologists analysed the lateral surgical margins and measured them with a ruler in the course of routine macroscopic examination of the specimen. This macroscopic assessment of the lateral surgical margins was compared with microscopic measurement which was taken by an ocular micrometer. Data on deep surgical margins were not included in this study. Other clinicopathologic variables, including age, stage, grade, tumour diameter, presence of LSVI, site of tumour, and type of operation were also recorded. For statistical analysis, Fisher’s exact test, the chi-square test, and unpaired t-test were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego CA, USA).

Results

Data of 50 consecutive patients with primary vulval cancer were available for analysis. The mean age of patients was 70 years (24-89 years). Thirty-one patients were staged as FIGO Stage II, 12 as Stage III and seven as Stage IV A. Thirty-eight patients (76%) underwent some form of radical vulvectomy (total or hemi-vulvectomy, anterior or posterior horseshoe vulvectomy) and the rest of the patients (24%) received wide local excision (WLE). Forty-five patients underwent bilateral groin node dissection, three received unilateral lymphadenectomy, and two patients did not undergo this procedure due to the palliative nature of their surgical procedure. All cancers were squamous in type: 34% were grade 1, 40% grade 2, and 26% grade 3.

Naked eye assessment of surgical margins after formalin fixation was within 2 mm of microscopic measurement in 29 of the 50 patients (58% - Group 1). In ten patients (20%) the pathologist estimated the margins closer than under the microscope (Group 2). In the remaining 11 cases (22%), naked eye observation underestimated the normal skin margin, (Group 3). The difference between the naked eye and microscopic measurement ranged from 2.5-15 mm (40 to 70%). Seven of these 11 patients from Group 2 (14% of all 50 cases) had an inadequate microscopic margin less than 8 mm.

The presence of LVSI was significantly more common in Group 3 than in the other two groups (Group 3 - 5/11 pts vs Groups 1&2 – 2/30 pts; p = 0.01) (Table 1). The difference between other variables of the study groups was statistically non-significant: tumours in Group 3 (closer microscopic margins) were more likely to be

<table>
<thead>
<tr>
<th>Adequate assessment (Group 1+2)</th>
<th>Inadequate assessment (Group 3)</th>
<th>p value</th>
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<tr>
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<tr>
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</tbody>
</table>

Table 1. — Clinicopathologic characteristics of the study groups.

LVSI = lymphovascular space invasion; LS = lichen sclerosus; VIN = vulval intraepithelial neoplasia.

Table 2. — Difference between the means of depth of invasion in the study groups (unpaired t-test, p = 0.6518).

Table 3. — Difference between the means of tumour diameter in the study groups (unpaired t-test, p = 0.3762).
central than those in Groups 1 or 2 (7/11 vs 14/28 and 3/8); grade 1 and 3 tumours were more common in tumours in Group 3 than in Groups 1 or 2. In terms of skin abnormalities around the cancer, the presence of lichen sclerosus was more common in Group 3 (5/10) than in Groups 1 and 2 (7/22 and 2/8). In contrast, vulval intraepithelial neoplasia (VIN) was least frequent in Group 3 (4/10) than Groups 1 and 2 (14/22 and 5/8). The difference between the means of depth of invasion and tumour diameter in the study groups was not statistically significant (Tables 2 and 3).

Discussion

Local recurrence is by far the most common site of treatment failure in vulval cancer, with 53-86% of all recurrences developing in the vulva [10]. In spite of clear surgical margins approximately 20% of patients develop local recurrence after primary surgical treatment [10, 11]. There are three different patterns of local recurrence described: primary tumour site recurrence (within 2 cm of the previous incision), distant local recurrence (more than 2 cm from the scar), and skin bridge recurrence [11]. Rouzier et al. found that a close or positive margin was significantly more common in patients with primary tumour site recurrence than in more distant local recurrences. Using microscopic measurement of surgical margins in formalin-fixed specimens, Heaps et al. reported that 21 of the 44 patients with microscopic margins < 8 mm developed local recurrence (47.7%), as opposed to none of the 91 patients with margins ≥ 8 mm [12]. More recently their results have been supported by other publications [8, 13]. De Hullu et al. however found that in spite of their policy to remove a clear surgical margin of at least 1 cm, the pathological evaluation of the specimens revealed that only 50% of patients had clear margins > 8 mm, resulting in significant undertreatment [8]. To date there is a dearth of evidence in the literature on the exact accuracy of naked eye assessment of surgical margins. Interestingly, we expected that depth of tumour invasion and diameter of tumour would correlate with the inaccuracy of naked eye assessment, but the correlation was not statistically significant.

Marking the surgical margins on the vulva prior to surgical excision is an important but not always straightforward step of the operation. Tumour size, the patient’s age, comorbidity and patient’s wishes together with proximity to the urethra, rectum, and clitoris may compromise clear surgical margins. On the other hand, an adequate tumour-free surgical margin must be removed to reduce the risk of local recurrence. Difficulties in correctly planning surgical margins prior to surgical excision may include positional stretching of vulval skin in the lithotomy position and tissue retraction of surgical specimens after surgical excision. Misinterpretation of the surrounding epithelial changes, e.g., inflammation, lichen sclerosus, and VIN3 by the surgeon can also result in undertreatment or overtreatment. Shrinkage of surgical specimens during formalin fixation has been well documented and up to 50% shrinkage has been recorded [16]. Microscopic subcuticular invasive tumour foci or tumour emboli in lymphovascular spaces under the apparent normal margin may also alter the efficacy of naked eye assessment [16].

In our study we analysed the accuracy of naked eye assessment of surgical margins after formalin fixation, therefore positional retraction of skin and shrinkage of specimens in formalin did not alter our results. We found that in 58% of the cases there was a strong correlation between macroscopic and microscopic measurement. In 20% of the cases the naked eye method measured surgical margins even smaller than microscopic measurement. However, in 22% of cases surgical margins were inadequately assessed and were overestimated by the naked eye. In seven of these 11 patients, the closest microscopic margin was measured < 8 mm. Although these seven patients (14% of all patients) were thought to have an adequate surgical margin at the time of surgery, they eventually fell into an unfavourable prognostic group with a high risk of local recurrence. We found that the presence of LVSI in inadequately assessed tumours was significantly more frequent than in tumours with adequate margin assessment.

Our study demonstrates that macroscopic assessment of surgical margins is inaccurate and surgical margins are often inadequate when aiming for a 1 cm apparently tumour-free surgical margin. We think that as general principle, the 1 cm clear margin is inadequate when planning a vulval excision. As part of the preoperative assessment mapping biopsies around the tumour in the apparent normal surgical margin may play a role in individual surgical planning. Our results demonstrate that tumours with LVSI should be considered for a wider surgical excision than 1 cm. Prospective studies on preoperative assessment of surgical margins of vulval cancer are required to determine an adequate surgical margin.

References


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Bcl-2 oncogene expression in estrogen receptor-positive and negative breast carcinoma

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Summary

Purpose: The aim of this study was to evaluate Bcl-2 oncogene expression in estrogen receptor (ER)-positive and negative breast carcinomas. Methods: A study involving 72 cases of infiltrating ductal carcinoma of the breast in postmenopausal women divided into two groups: Group A (ER positive, n = 37) and Group B (ER negative, n = 35). Immunohistochemical analysis of bcl-2 expression was carried out semiquantitatively based on the percentage of stained tumoral cells and the intensity of staining. The chi-square test was used in the statistical analysis of the data and significance was established at p < 0.05. Results: Bcl-2 oncogene expression was statistically greater in tumors of Group A (59.5%) compared to those of Group B (8.6%), (p < 0.001). Conclusion: Bcl-2 had a significantly greater expression in the ER-positive breast tumors compared to ER-negative tumors.

Key words: Breast; Cancer; Apoptosis; Estrogen receptor; Bcl-2.

Introduction

Breast carcinoma is a heterogeneous disease with a wide variety of histopathological characteristics, presenting unpredictable clinical behavior and prognosis [1]. A considerable number of patients with early breast cancer treated with local therapy alone suffer recurrences; however, there are, unfortunately, still no predictive and/or prognostic factors that are able to unequivocally identify which patients will respond to adjuvant therapy and which will have an unfavorable prognosis [2].

Estrogen receptors are expressed in around 60-65% of breast cancer cases, and in these cases, a relatively better prognosis can be expected compared with tumors that do not express them [3, 4]. This association of estrogen (ER)-positive tumors with a better prognosis may involve other molecular markers related to the proliferation and apoptosis of tumor cells [5-7]. A significant association has been shown between Ki-67 expression and negativity for estrogen receptors [8]. On the other hand, a positive expression of the bcl-2 protein has been associated with ER-positive breast cancer cells and a more favorable prognosis [9].

The bcl-2 oncogene, although suppressing apoptosis, paradoxically appears to be associated with a better prognosis, and its expression may be a marker of the response to endocrine therapy [10]. This association between bcl-2 expression and breast cancer prognosis has not yet been clarified. A study in women with ER-positive breast cancer treated with tamoxifen reported an increase in bcl-2 expression and a reduction in cell proliferation [9]. However, there is a scarcity of studies correlating bcl-2 with hormone receptor-positive and negative breast cancer tumors, which led to the conception of the present study.

Material and Methods

This study includes tumor samples from 72 patients who had been postmenopausal for at least two years and who were receiving care at the Mastology Department of the Federal University of Piauí. The patients were submitted to surgical treatment between 1999 and 2004 for ER-positive or ER-negative infiltrating ductal carcinoma. None of the patients had undergone any prior treatment. The study was approved by the Institutional Review Board of the Federal University of Piauí. Following hematoxylin-eosin staining and confirmation of the diagnosis of invasive ductal carcinoma, paraffin blocks containing the samples underwent histochemical analysis to evaluate estrogen and progesterone receptor status. Tumors with nuclear staining measured semiquantitatively as high (≥10% immunoreactive cells) were considered positive [8]. The cases were then divided into two groups: Group A: ER-positive, n = 37; and Group B: ER-negative, n = 35. Patients ranged in age from 52 to 82 years (mean 69.73) in Group A and from 51 to 86 years (mean 63.49) in Group B. The size of the tumors in the two groups ranged from 2 to 5 cm, Stages I and II. Immunohistochemistry (Dako ABC) was carried out to identify bcl-2 oncogene expression (mouse anti-human bcl-2 oncoprotein clone 124, Dako, code number M0887, 1:50). Bcl-2 expression was evaluated under light microscopy by two independent observers, who were blinded with respect to group identification. These observers performed semiquantitative counts of the cells with positively stained cytoplasm (magnification 400x) using a Nikon optical microscope, model Eclipse E200. Bcl-2 immunoreaction was evaluated according to the criteria established by Van Slooten et al. [11], taking the following parameters into consideration: intensity of cell coloration (I) and fraction of stained neoplastic cells (F). Intensity of cell staining was classified as: 0 (negative), 1 (weakly stained), 2 (moderately

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stained) and 3 (strongly stained). The fraction of stained cells was classified as: I (0-25%), II (25-75%) or III (75-100%). The final score was the result of the combination of the two parameters (I and F) and ranged from 0 to 6. Cases with a final score ≥ 3 were classified as positive for bcl-2 (11). In all cases, brownish staining in the cytoplasm was adopted as the standard for positivity. Evaluation began at the location that had the greatest quantity of stained cells, after which the other microscopic fields were selected at random, and staining intensity and the percentage of stained tumor cells were assessed, resulting in a final score [11]. In all cases, lymphoid cells accompanying the tumors served as internal positive controls of the immunohistochemical reaction. Statistical analysis was carried out on the bcl-2 data using the chi-square test. Significance was established at p < 0.05.

**Results**

Under light microscopy, the ER-positive tumors had an immunohistochemical reaction for the bcl-2 oncogene, as expressed by numerous cells containing cytoplasm with intense brown staining. In comparison, the hormone receptor-negative tumors showed no such reaction (Figures 1 and 2). Analysis of bcl-2 oncogene expression was positive in 22 (59.5%) ER-positive tumors (Group A) compared with only three (8.6%) tumors in Group B (Table 1). This difference was statistically significant (p < 0.001).

**Discussion**

The classic morphological prognostic factors of breast cancer, although subject to diverse influences, still play a relevant role in predicting the biological behavior of some of these cancers [5]. Nevertheless, there is a continual search for molecular markers capable of predicting therapeutic response and prognosis in breast cancer [2].

ER and PR, expressed in around 60-70% of cases of breast cancer, have been used with the principal objective of predicting response to adjuvant therapy [12, 13]; however, it is known that ER-positive tumors are more likely to be well-differentiated and to present a better prognosis than ER-negative tumors [4]. This difference between ER-positive and ER-negative tumors may be associated with the expression of molecular markers such as those related to apoptosis of breast cancer cells [9].

In the present study, there was a highly significant expression of the bcl-2 oncogene in ER-positive breast tumors compared with hormone receptor-negative tumors. Likewise, some studies have shown that there is a strong positive correlation between ER and bcl-2 immunoreactivity [5, 10, 12], raising the hypothesis that bcl-2 is an ER-regulated gene [6]. On the other hand, an inverse correlation has been reported between bcl-2 protein expression and cell proliferative activity [9].

Some authors have failed to confirm any relationship between bcl-2 oncogene and cell proliferation and prognosis in breast cancer [14]. A tentative explanation for this lack of correlation may be in the difficulty to quantify bcl-2 expression, which would result in low accuracy and reproducibility of this marker [2]. The hypothesis that loss of antigenicity may result from material having been kept in storage [15] was not observed in the blocks used in this study. Thus, the present study showed that ER-positive breast cancer cells significantly express bcl-2 compared to negative tumors.

<table>
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<tr>
<th>Group</th>
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<th>Positive n (%)</th>
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<td>A</td>
<td>15 (40.5)</td>
<td>22 (59.5)</td>
<td>37 (100.0)</td>
</tr>
<tr>
<td>B</td>
<td>32 (91.4)</td>
<td>3 (8.6)</td>
<td>35 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>47 (65.3)</td>
<td>25 (34.7)</td>
<td>72 (100.0)</td>
</tr>
</tbody>
</table>

The percentage of cells with positive bcl-2 expression in Group A was significantly greater when compared to those of Group B (p < 0.001).
Bozzietti et al. [16] reported a correlation in bcl-2 expression, evaluated by immunocytochemistry on fine-needle aspirates from primary breast carcinoma, with favorable prognostic features such as ER and PR expression, p53 negativity, a low Ki-67 index, and high tumor differentiation [16]. Bcl-2 oncogene expression occurs in 40-80% of breast carcinomas [17] and its strong correlation with estrogen receptor status suggests a potential role for bcl-2 expression as a modulator of the response to adjuvant therapy in breast cancer [18, 19]. ER-positive tumors treated with tamoxifen have shown an increase in bcl-2 expression and a reduction in cell proliferation, which in this case may explain the association between bcl-2 and a favorable prognosis in breast cancer [9].

Studies involving the effects of selective estrogen receptor modulators (SERMs), both in normal breast tissue and in neoplastic tissue, may help clarify the correlation between cell proliferation, apoptosis and prognosis in breast cancer [20]. Bcl-2, despite being an anti-apoptotic protein, is associated with a favorable prognosis [9], which in this case may explain the association between bcl-2 and a favorable prognosis in breast cancer [9].

Further studies are required to better clarify the association between bcl-2 and other markers and prognosis in breast cancer.

References


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CT colonography to detect rectosigmoid involvement in patients with primary ovarian cancer

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Summary

Introduction: We retrospectively evaluated the performance of preoperative computed tomographic (CT) colonography to detect tumor involvement of the rectosigmoid wall and predict the need for rectosigmoid resection in patients with primary ovarian cancer. Methods: Thirty-three patients with primary ovarian cancer who underwent preoperative CT colonographic examination were evaluated. The images of the examination were analyzed and compared with the subsequent surgical findings. Results: All abnormal findings (malignant infiltration of the rectosigmoid mucosa and extrinsic compression) revealed by conventional colonoscopy were correctly observed as extrinsic compression using CT colonography. The sensitivity, specificity, positive predictive value and negative predictive value of CT colonography for the prediction of rectosigmoid resection were 100%, 64.7%, 72.7%, and 100%, respectively. Though conventional colonoscopic examinations could not be completed in five patients because of the presence of extrinsic stenosis and occlusion at the sigmoid colon, CT colonography enabled the entire large bowel to be examined in these patients. Conclusions: This preliminary study showed that the CT colonographic examination is feasible and safe. CT colonography seems to have several advantages over conventional colonoscopy for the detection of rectosigmoid involvement in patients with advanced ovarian cancer. For confirmation of the efficacy of CT colonography, further large prospective studies are needed.

Key words: Ovarian cancer; Computed tomographic colonography; Rectosigmoid resection.

Introduction

Advanced ovarian cancer spreads intraperitoneally in areas where ascites stagnate, like in the paracolic gutters, the dome of the diaphragm, along the greater omentum, and in the pelvis [1]. Therefore, ovarian and disseminated tumors frequently present with gastrointestinal involvement. The effectiveness of optimal cytoreductive surgery for the management of advanced ovarian cancer has been accepted, and an apparent survival benefit for patients whose largest residual tumor is no greater than 1 cm has been demonstrated [2]. Bowel resection is frequently required to achieve optimal cytoreduction [3, 4]. Some investigators have studied the roles of preoperative sigmoidoscopy or colonoscopy in patients with ovarian cancer [5-7]. However, endoscopic techniques are associated with some major problems, including considerable patient discomfort and pain. In addition, failure to visualize the entire colorectal surface possibly occurs in not just a few patients with advanced ovarian cancer because of the presence of extrinsic stenosis and occlusion of the large bowel arising from ovarian and disseminated tumors.

Computed tomographic (CT) colonography is a new tool that combines abdominal helical CT scanning and virtual reality computer technology; the reconstructed images provide a simulation of the luminal surface of the large bowel as viewed during a colonoscopy [8]. Visualization of the entire large bowel, even in the presence of stenosing lesions, and the ability to assess extracolonic abdominal and pelvic organs are the most relevant advantages of this procedure [9-11].

In this retrospective study, we evaluated the performance of preoperative CT colonography for detecting rectosigmoid involvement and predicting the need for rectosigmoid resection in patients with primary ovarian cancer.

Materials and Methods

From May 2005 to June 2007, 35 patients with a high suspicion of primary ovarian cancer had preoperative CT colonographic examinations. CT colonography was indicated in patients with a fixed pelvic mass and at least two additional features from suspected ovarian tumor on ultrasound and magnetic resonance imaging, elevated CA125 value (> 500 U/ml) or presence of ascites. This was a modification of criteria for inclusion previously described by Gornall et al. [5]. The exclusion criteria included the presence of metastatic ovarian cancers that had already been diagnosed before admission to our department, impending colorectal obstruction, and the performance of a barium examination in the preceding 14 days. All patients underwent debulking surgeries at the gynecologic department of our institution. As the final histopathological examination confirmed primary colon cancer metastatic to the ovary in one patient, and borderline tumor of the ovary in another patient; these two patients were excluded from the analysis. Thus, a total of 33 patients were evaluated in this study.

Almost all patients underwent the CT colonography following the conventional colonoscopy two days before surgery. Before the colonoscopic examination, each patient was given a standard preparation consisting of polyethylene glycol 4000 electrolyte solution, diluted in two liters of water. The colonoscopies were performed by endoscopists on staff at our institution. A standard videendoscope (CF-H260A1, Olympus, Tokyo, Japan) was used in all cases. Informed consent was obtained from each patient.

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As soon as the conventional colonoscopy was finished, the patient was transferred to the CT suite. After the intravenous administration of butylscopolamine (20 mg), a rectal catheter was inserted for colonic insufflation with room air. CT colonography was performed using a 16-detector row CT scanner (Somatom Sensation 16; Siemens, Erlangen, Germany) and the following parameters: 0.75-mm slice collimation, 120 kVp, 160 mAs for the supine position and 35 mAs for the prone position, and a table feed/rotation of 15 mm. In each patient, the entire large bowel (as displayed on a topogram) was scanned in the cephalocaudal direction. Contrast material was intravenously administered, unless contraindicated. Scanning was started 40 seconds after the start of an injection of iodinated contrast material (Iohexol, Omnipaque 300; Daiichiseiyaku, Tokyo, Japan) using a power injector (Dual shot type-D; Nemoto Kyorindo, Tokyo, Japan) through a 20-gauge cannula at a rate of 4.0 ml/sec. Images were obtained with the patient in supine and prone positions. The acquired images were processed using a commercially available workstation (Virtual Place Advance 300, version 3.0041; Aze, Tokyo, Japan). The staff radiologists created three-dimensional (3D) reconstructions and performed the interpretations of the 3D images.

Findings of CT colonography were reported as “normal findings or only minor abnormal findings”, “extrinsic compression associated with smooth surface” or “extrinsic compression associated with irregular surface”. We considered the rectosigmoid wall to be involved when abnormal findings (“extrinsic compression associated with smooth surface” and “extrinsic compression associated with irregular surface”) were observed.

All the surgeries were performed at the gynecologic department of our institution. Twenty-one patients underwent primary debulking surgery. Twelve patients with Stage IV disease (with pleural effusion, lung metastasis and liver metastasis) or with a poor performance status were treated using neoadjuvant chemotherapy, followed by interval debulking surgery. Surgical specimens were fixed in 10% buffered formaldehyde solution and routinely processed. In cases that rectosigmoid resections were successfully performed in all the patients. All abnormal findings in the rectosigmoid arising from ovarian tumor and disseminated tumor spread, which were revealed by conventional colonoscopy (malignant infiltration of the rectosigmoid mucosa and extrinsic compression), were correctly detected as extrinsic compression using CT colonography. A typical example of a CT colonography image in a patient with advanced ovarian cancer is demonstrated in Figure 1.

Comparison of CT colonographic findings in the patients performed with or without rectosigmoid resection is shown in Table 2. CT colonography visualized extrinsic compression associated with an irregular surface in two patients. This CT colonographic visualization corresponded to a tumor infiltration of the rectosigmoid mucosa, as confirmed histopathologically in the surgical specimens (Figure 2). Though conventional colonoscopy was not completed in five cases (15.2%) because of the presence of stenosis and occlusion at the sigmoid colon, CT colonography was successfully used to examine the entire large bowel in these cases. Consequently, CT colonography revealed that the stenosis and occlusion at the sigmoid colon was caused by ovarian and disseminated tumor involvement of the rectosigmoid wall in all five patients.

Among the 33 patients in this study, 26 patients had residual tumor with a maximal diameter of < 1 cm, of which 20 patients had no visible tumor. Rectosigmoid resections were performed in 17 patients. CT colonography showed abnormal findings in all the 12 patients who underwent rectosigmoid resection.

Evaluated on the basis of preoperative CT colonographic examinations, rectosigmoid resections were per-
Figure 1. — Left ovarian cancer and disseminated tumor involvement of the cul-de-sac in a 44-year-old female. (A) Schematic representation of the air-filled colon generated from a computed tomographic (CT) scan, showing extrinsic compression in the lower rectum (arrows). (B) Axial two-dimensional CT image showing a left ovarian tumor (To) and a disseminated tumor in the cul-de-sac (Td). U, uterus. (C) Coronal two-dimensional CT image showing an endoluminal projection in the rectum arising from disseminated tumor involvement of the cul-de-sac. The dotted line represents the centerline and is automatically generated to assist virtual navigation. (D) Conventional colonoscopy image showing extrinsic compression in the rectum. (E) Three-dimensional virtual colonoscopy image showing extrinsic compression with a smooth mucosal surface in the rectum. (F) Macroscopic dorsal view of an en bloc low anterior resection of the uterus, adnexa, pelvic tumor, and rectosigmoid. Rectal extrinsic compression arising from the disseminated tumor in the cul-de-sac can be seen (arrows). Histopathological sections of the specimen showed tumor infiltration into the proper muscularis of the rectal wall. The primary left ovarian tumor (To) was histopathologically confirmed to be a mucinous adenocarcinoma.
formed in 16 out of 22 patients with abnormal findings. The sensitivity and specificity of CT colonography for the prediction of rectosigmoid resection were 100% and 64.7%, respectively. The positive predictive value (PPV) and negative predictive value (NPV) were 72.7% and 100%, respectively.

Discussion
The effectiveness of optimal cytoreductive surgery for the management of advanced epithelial ovarian cancer has been commonly accepted, and multiple studies have reported that bowel resection is frequently required to achieve optimal cytoreduction [3, 4]. Some investigators have reported that the rates of bowel resection during the treatment of advanced ovarian cancer have increased to over 40% [2, 4]. Colorectal resection is the most commonly performed procedure for ensuring the complete removal of all visible tumors, a factor associated with the improved survival of patients with advanced ovarian cancer. Eisenkop et al. described the use of a modified pelvic exenteration with a low rectal anastomosis in 85 (52.1%) of 163 patients with Stage III or IV ovarian cancer [2]. The complete removal of all visible tumors was possible in 139 (85.3%) of the 163 patients. Hertel et al. evaluated 100 patients with Stage III ovarian cancer who underwent rectosigmoid resection for the removal of macroscopic suspected tumor involvement of the cul-de-
Tumor involvement of the colorectal wall was confirmed histopathologically in 73% of the patients. They concluded that pelvic en bloc surgery with rectosigmoid resection was necessary if complete debulking was the aim of the operations for advanced ovarian cancer. In the present study, tumor involvement of the colorectal wall was histopathologically confirmed in 12 (75%) of 16 patients who underwent rectosigmoid resection. Since the present study included only patients with a high suspicion of advanced ovarian cancer, the performance rate of rectosigmoid resection was relatively high compared with previous studies using endoscopic examinations [5-7].

With the development of new methods for image processing of the volumetric datasets obtained during spiral CT, interest in the potential of CT for the detection of colorectal cancer has become widespread [8]. Currently, CT colonography is seen as a promising imaging modality for the evaluation of the large bowel, in which spiral CT data sets can be used to generate two-dimensional images as well as virtual 3D, endoscopic-like views of the large bowel [8-11]. This preliminary study showed that CT colonography following conventional colonography is feasible and safe, and 3D reconstructions were successfully performed in all 33 patients. CT colonography showed extrinsic compression associated with an irregular surface in two patients. A correspondence between this CT colonographic finding and tumor involvement of the rectosigmoid mucosa was histopathologically confirmed in the surgical specimens.

Both prospective and retrospective studies have investigated the use of preoperative bowel examinations, including colonoscopy and barium enema, in patients with suspected ovarian cancer [5-7, 12]. Most of these studies concluded that assessing which patients have tumor involvement of the colorectal wall and predicting which patients will require colorectal surgery are difficult, but in reality preoperative bowel examinations have been performed in patients with ovarian cancer at a number of institutions. We also performed routine preoperative colonoscopy in all the patients with supposed advanced ovarian malignancy. Petru et al. reported that endoscopy was capable of showing malignant involvement of the colorectal mucosa in only 4.3% of the patients with ovarian malignancies in a series of 254 patients with a suspected adnexal mass; thus, they reported that the sensitivity of preoperative endoscopy for predicting bowel surgery was low (6% for colonoscopy and 38% for sigmoidoscopy) [6]. As they considered only the cases with malignant involvement of the colorectal mucosa to have tumor involvement of the colorectal wall, the sensitivity was too low to predict the need for colorectal surgery. However, 28 (35.9%) of the 78 patients in whom a preoperative endoscopy showed abnormal findings (17 with malignant involvement of the colorectal mucosa and 61 with colorectal extrinsic compression) underwent bowel surgery. On the other hand, 27 (15.3%) of the 176 patients in whom a preoperative endoscopy revealed normal findings or only minor abnormalities, underwent bowel surgery. In another study of 30 patients with suspected ovarian malignancies, preoperative sigmoidoscopy had a PPV of 100% and a NPV of 84% [5]. In this study, we considered the rectosigmoid wall to be involved when abnormal findings (“extrinsic compression associated with smooth surface” and “extrinsic compression associated with irregular surface”) were observed. Consequently, the sensitivity, specificity, PPV, and NPV of CT colonography for predicting rectosigmoid resection were 100%, 64.7%, 72.7%, and 100%, respectively.

CT colonography has several advantages over conventional colonoscopy. Firstly, CT colonography promises to have high patient acceptability (because of its non-invasiveness, quick examination time, and lack of intravenous sedation). Secondly, CT colonography enables the entire large bowel to be examined, even when a conventional colonoscopy cannot be completed. A complete colonoscopy provides the most thorough evaluation of the large bowel, with the added benefit of allowing biopsies of regions suspected of tumor involvement [9]. However, visualization of the entire large bowel using conventional colonoscopy can be interrupted by several factors, including the existence of occlusive lesions, the endoscopist’s lack of skill, and the patient’s intolerance of the procedure. Conventional colonoscopy reportedly fails to visualize the cecum in 4.3% of patients with primary ovarian cancer [7]. As the present series included only the patients with advanced ovarian cancer, the rate of incompleteness of conventional colonoscopy was relatively high (15.2%). However, CT colonography successfully examined the entire large bowel in these patients, and revealed that the stenosis and occlusion at the sigmoid colon was due to ovarian and disseminated tumor involvement of the colonic wall. It is reported that CT colonography is more effective than a barium enema for completing colon examinations in patients with incomplete colonoscopies because excessive air in the colon can make the performance of a barium enema difficult and untenable [9]. In this study, almost all patients underwent colonoscopic and CT colonographic examinations two days before surgery so that they would not need to receive bowel preparations on two separate occasions. We prefer using CT colonography to a barium enema when colonoscopy fails. Another benefit of CT colonography is the ability to provide extracolonic abdominal and pelvic findings [10]. Serracino-Inglott et al. reported that CT colonography detected extracolonic malignancies in 8% of 103 patients presenting with symptoms suggestive of colorectal pathology, including one patient with a primary ovarian tumor with uterine and colonic invasion [11]. We consider that this ability could be used to advantage in the preoperative evaluation of cases in which ovarian and disseminated tumors are suspicious for rectosigmoid involvement. The CT colonography images visualizing the tumors, large bowel, and afferent and efferent vessels could help in the planning for an en bloc resection.

All 3D images of CT colonography could be reviewed on a workstation at any time, assisting in the interpreta-
tion of the images and the planning of an appropriate surgical approach. The performance of preoperative modalities to detect rectosigmoid involvement may assist in surgical planning for the management of advanced ovarian cancer, enabling surgeries to be refined and performed more safely and improving the percentage of optimal cytoreductions that can be performed. Since CT colonographic technology is evolving, further improvements in the display of imaging data and methods that enable more efficient interpretations can be expected [11]. This preliminary study showed that the CT colonographic examination is feasible and safe, and that CT colonography seems to have several advantages over conventional colonoscopy for the detection of rectosigmoid involvement in patients with advanced ovarian cancer. However, the number of cases in the present study was too small to draw any final conclusions regarding the real value of this modality. Further large prospective studies are needed.

References


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Gonadotropins and female sex steroid hormones in cyst fluid and serum from patients with ovarian tumors

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Summary

The objective of the present study was to determine the concentrations of LH, FSH, 17β-estradiol and progesterone in ovarian cyst fluid and serum from patients with benign and malignant ovarian tumors and to assess the correlation of the gonadotropin and female sex steroid hormone concentrations with menopausal and tumor status. Ovarian cyst fluid and blood samples were prospectively collected from 103 patients with ovarian tumors. Seventy-four of the patients had benign ovarian tumors while 29 patients had malignant ovarian tumors. Malignant ovarian tumors showed significantly higher LH and FSH cyst fluid concentrations compared to concentrations from patients with benign tumors. Within the malignant subset, LH and FSH concentrations correlated with increasing FIGO stage and grade. Furthermore, LH and FSH cyst fluid concentrations showed strong correlations (r > 0.62) with serum concentrations in case of malignant tumors, especially in postmenopausal women, but not in case of benign tumors. The highest gonadotropin concentrations were observed in cyst fluid from malignant ovarian tumors. The most probable explanation for this is an increased vascular permeability within the cysts. Supportive evidence for such an increased vascular permeability is our previous finding of significantly higher VEGF concentrations in cyst fluid from malignant ovarian tumors. The possibility of ectopic production of LH and FSH by malignant ovarian tissue cannot completely be ruled out.

Key words: Gonadotropins; Female sex steroids; Ovarian tumor; Cyst fluid; Menopausal status.

Introduction

The majority of epithelial ovarian tumors, benign as well as malignant, present as cystic structures, with or without solid components [1]. Ovarian cysts contain variable amounts of fluid, most probably secreted by the ovarian surface epithelium (OSE) cells [2]. The macroscopic characteristics of cyst fluid reflect the differentiation of the OSE into one of the coelomic epithelium derivatives (serous, mucinous, endometrioid) [2-4]. There are only few studies on the determination of various analytes present in ovarian cyst fluid. A number of these studies involve measurement of gonadotropins or female sex steroid hormones [5-11]. Most of these studies focused on the differentiation between functional and neoplastic cysts. The studies that aimed to discriminate between benign and malignant tumors report conflicting results [7, 9-11].

The present study was designed to determine gonadotropin (LH, FSH) and female sex steroid hormone (17β-estradiol, progesterone) concentrations in cyst fluid and serum from patients with benign or malignant ovarian tumors, and to assess the correlation of these hormone levels with tumor and menopausal status and their relationship with known prognostic factors.

Materials and Methods

Cyst fluid collection

One hundred and three patients with an ovarian tumor planned for surgical removal were informed about the background and objectives of the present study. After having given their informed consent these patients were included in the cyst fluid collection procedure in accordance with the guidelines of the ethical and institutional board of the Radboud University Nijmegen Medical Centre. Immediately after surgical removal, the tumor was transported to the pathology laboratory where aseptic fine needle aspiration was performed to collect cyst fluid. Next, the cooled fluid sample was transported to the Department of Chemical Endocrinology, and after centrifugation at 3000 x g for 10 min, the supernatant was collected and immediately stored at −35°C. From 61 of these patients blood was collected up to four weeks before surgery, centrifuged at 2000 x g for 15 min and the serum was stored at −35°C until assayed. A gynecologic pathologist performed the histopathologic diagnoses according to WHO criteria.

Immunoassays

Determination of LH and FSH concentrations in cyst fluid and serum was performed with the random access analyzer AxSym (Abbott Laboratories, Chicago, IL, USA), a fully automated analyzer system based on microparticle enzyme immunoassay (MEIA) technology. Cyst fluid and serum 17β-estradiol and progesterone concentrations were measured with in-house radioimmunoassay (RIA) procedures [12]. Prior to RIA, the samples were extracted twice with diethylether and the dried extracts subjected to Sephadex LH-20 chromatography for isolation of the 17β-estradiol and progesterone containing fractions. The minimum detectable concentrations of 17β-estradiol and progesterone were 75 pmol/l and 1.3 nmol/l, respectively. The precision in terms of within- and between-assay...
coefficients of variation for means of duplicate determinations for 17β-estradiol was 4.3% and 7.9%, and 4.1% and 9.1% for progesterone. Reference values for gonadotropins and sex steroids in blood in our laboratory were for postmenopausal patients: 12-75 IU/l for LH; 37-140 IU/l for FSH; < 200 pmol/l for 17β-estradiol, and < 1.3 nmol/l for progesterone.

Statistics

Results are given individually as concentration and as median concentration and range (minimum-maximum). Assay results below the detection limits of the immunoassays for the different components in the clinical specimens were considered half of the detection limit of the assay. Significance of differences in concentrations of the various analytes between the histopathologic subgroups was tested using the Mann-Whitney-U test for independent samples (level of significance p < 0.05). Correlations between cyst fluid and serum concentrations were calculated using the Spearman rank correlation coefficient. The receiver operating characteristic (ROC) curve analysis was performed on LH and FSH cyst fluid concentrations determined in cyst fluid from benign and malignant ovarian tumors for calculating areas under the curve (AUC) and statistical significance for comparisons of various subsets according to histopathology or menopausal status.

Results

Table 1 presents the histopathologic results of 103 ovarian tumors from which 29 malignant and 74 benign ovarian cysts were aspirated for the collection of fluid. Three malignant cysts with metastatic localization in the ovary of a primary tumor elsewhere (two breast, one colon) were excluded from cyst fluid analysis. In up to 30 cases only a subset of the hormonal parameters could be analyzed due to the viscosity of the cyst fluids or too small sample volumes.

Table 1. — Histopathological diagnosis of ovarian cysts and aspirated cyst fluids (n = 103).

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Malignant</th>
<th>Benign</th>
<th>AUC 95% CI</th>
<th>Significance p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>serous cystadenocarcinoma</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>mucinous cystadenocarcinoma</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>endometrioid carcinoma</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>undifferentiated cystadenocarcinoma</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>metastatic*</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>mucinous cystadenoma</td>
<td>21</td>
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<td>dermoid cyst</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>functional cyst</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cystic endometriosis</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total benign</td>
<td>74</td>
<td></td>
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</tr>
</tbody>
</table>

N = number of tumors in each group; * = excluded from cyst fluid analysis.

Cyst fluid gonadotropin and steroid concentrations according to menopausal status and histopathologic subtype

Figures 1A-D show the LH, FSH, 17β-estradiol, and progesterone concentrations as measured in cyst fluids from 45 premenopausal and 25 postmenopausal women. The 45 premenopausal women could further be subdivided into 37 women with benign tumors (4 histopathology subtypes) and eight women with malignant tumors (3 histopathology subtypes). The 25 postmenopausal women had benign tumors in 13 cases (2 histopathology subtypes) while the 12 other women had malignant ovarian cysts from four different histopathology subtypes.

The LH cyst fluid concentrations (Figure 1A) from all the benign tumors were invariably lower than 2.6 IU/l (except 3 cases) whereas more than half the number of all malignant cysts (13 out of 20) were higher than that concentration. After excluding the invariably low malignant mucinous cyst LH results (n = 6, significantly different from the other pre- and postmenopausal serous cyst LH and FSH results, p < 0.05), 13 out of the 14 malignant cysts were higher than 2.6 IU of LH per liter. Comparable results were found for FSH, with only three out of the 50 benign ovarian cyst fluids and half the number of all the malignant cysts higher than 16 IU/l (Figure 1B).

Table 2. — ROC curve analysis for LH and FSH cyst fluid concentrations determined in cyst fluid from benign and malignant ovarian tumors; Areas under the curve (AUC) and statistical significance for comparisons of various subsets according to histopathology or menopausal status.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Benign No.</th>
<th>Malignant No.</th>
<th>AUC 95% CI</th>
<th>Significance p</th>
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</tr>
<tr>
<td>LH</td>
<td>50</td>
<td>20</td>
<td>0.849</td>
<td>0.733-0.964</td>
</tr>
<tr>
<td>FSH</td>
<td>50</td>
<td>20</td>
<td>0.884</td>
<td>0.805-0.963</td>
</tr>
<tr>
<td>Prenopausal women</td>
<td></td>
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</tr>
<tr>
<td>LH</td>
<td>37</td>
<td>8</td>
<td>0.747</td>
<td>0.527-0.967</td>
</tr>
<tr>
<td>FSH</td>
<td>37</td>
<td>8</td>
<td>0.883</td>
<td>0.768-0.998</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>13</td>
<td>12</td>
<td>0.891</td>
<td>0.750-1.032</td>
</tr>
<tr>
<td>FSH</td>
<td>13</td>
<td>12</td>
<td>0.865</td>
<td>0.724-1.007</td>
</tr>
<tr>
<td>All benign versus malignant serous and others (mucinous excluded) *</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LH</td>
<td>50</td>
<td>14</td>
<td>0.994</td>
<td>0.982-1.006</td>
</tr>
<tr>
<td>FSH</td>
<td>50</td>
<td>14</td>
<td>0.951</td>
<td>0.902-0.999</td>
</tr>
<tr>
<td>All benign versus malignant mucinous-only *</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>LH</td>
<td>50</td>
<td>6</td>
<td>0.508</td>
<td>0.309-0.708</td>
</tr>
<tr>
<td>FSH</td>
<td>50</td>
<td>6</td>
<td>0.728</td>
<td>0.580-0.877</td>
</tr>
</tbody>
</table>

* Because of the observed significant differences in cyst fluid gonadotropin concentrations of mucinous and the other histopathology types in malignant ovarian tumors, gonadotropin results were separately analyzed for subsets 'mucinous excluded' and 'mucinous-only'. No such concentration differences are present between histopathologic subsets of benign tumors.
those of the malignant mucinous cyst results gave non-significant AUCs of 0.51 and 0.73.

**Correlation between cyst fluid and serum gonadotropin concentrations**

Separately for both premenopausal and postmenopausal women the LH and FSH cyst fluid concentrations of the benign and malignant histopathologic subgroups were plotted against the corresponding LH and FSH serum concentrations (Figure 2). In both pre- and postmenopausal women with benign ovarian tumors, weak positive and negative correlations ranging between –0.128 and 0.284 were found for cyst fluid LH and FSH and their respective serum concentrations. With exception of the weak negative correlation between FSH in cyst fluid and serum from premenopausal women (r = -0.014), rather strong positive correlations (range 0.629-0.710) were found for cyst fluid LH and FSH and their respective serum concentrations in pre- and postmenopausal patients with malignant ovarian tumors. No such correlations could be observed in the case of 17β-estradiol or progesterone (data not shown).

**Clinicopathologic characteristics**

Table 3 presents gonadotropin and female sex steroid hormone cyst fluid concentrations related to tumor FIGO stage, histopathologic subgroup, and tumor differentiation grade. The median LH and FSH cyst fluid concentrations were significantly higher in FIGO Stages II, III, IV, malignant serous and histologic grade 2, 3 as compared to Stage I, malignant mucinous and grade 1, respectively. Although the median 17β-estradiol concentration was higher, and that of progesterone lower in Stage II, III, IV, malignant serous and grade 2, 3 as compared to those of stage 1, malignant mucinous and grade 1, respectively, these differences were not statistically significant.

**Discussion**

The present study intended to determine gonadotropins (LH, FSH) and female sex steroid hormones (17β-estradiol, progesterone) in cyst fluid from the different histopathology subgroups of ovarian tumors and to correlate these results with the clinical value. We observed significantly higher median LH and FSH cyst fluid concentrations in malignant compared to benign tumors. Reimer et al. also found higher FSH levels in malignant cysts [10]. However, in their study LH levels were found to be equal for benign and malignant cases. The inclusion in their study of four borderline tumors in the malignant group (n = 7) is a possible explanation for this difference.
Krämer et al. describe higher cyst fluid LH and FSH concentrations in the serous malignant than in the serous benign subtype [9]. In that study mucinous cyst fluids were excluded due to viscosity of the samples. Although we experienced similar problems with mucinous fluids, we were able to determine gonadotropin and steroid levels in both benign as well as malignant mucinous tumors. In benign tumors no differences were found for gonadotropin levels between both subtypes. However, significantly higher LH and FSH concentrations were found in cyst fluids from malignant serous compared to malignant mucinous tumors. To our opinion it seems unlikely that assay problems form the basis of this difference, as the difference was not noticed between the benign mucinous and serous cysts. The over-representation of higher tumor stages (in which higher gonadotropin concentrations were found) in the serous subgroup (2 FIGO Stage I and 8 FIGO Stage II, III, IV serous, compared to 7 FIGO Stage I and 1 FIGO Stage II, III, IV mucinous) probably is a better explanation for this finding. Higher LH and FSH cyst fluid concentrations in malignant tumors might result from local production of LH and FSH by the tumor tissue, although there are no studies describing LH or FSH production by tumor tissue epithelium. Differences in gonadotropin cyst fluid concentrations between malignant and benign tumors may also be the result of differences in vascular permeability. Due to increased angiogenesis with more permeable vessels in case of malignancy, higher serum LH and FSH concentrations (especially in postmenopausal women).

Figure 2. — LH and FSH cyst fluid concentrations in benign and malignant histopathologic subgroups of premenopausal and postmenopausal women as a function of the corresponding serum concentrations.

Table 3. — Gonadotropin and steroid concentrations in cyst fluid related to histopathologic characteristics in malignant ovarian tumors.

<table>
<thead>
<tr>
<th>FIGO</th>
<th>LH</th>
<th>FSH</th>
<th>17p-estradiol</th>
<th>progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>IU/l</td>
<td>IU/l</td>
<td>pmol/l</td>
<td>nmol/l</td>
</tr>
<tr>
<td>median</td>
<td>2.2</td>
<td>9.7</td>
<td>315</td>
<td>29</td>
</tr>
<tr>
<td>range</td>
<td>0.2-10</td>
<td>2.1-34</td>
<td>50-120000</td>
<td>0.6-84</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Stage II-III-IV</td>
<td>median</td>
<td>8.3</td>
<td>28A</td>
<td>560</td>
</tr>
<tr>
<td>range</td>
<td>0.1-18</td>
<td>6.7-68</td>
<td>130-210000</td>
<td>1.9-150</td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Histology serous</td>
<td>median</td>
<td>8.3C</td>
<td>39D</td>
<td>430</td>
</tr>
<tr>
<td>range</td>
<td>6.1-18</td>
<td>9.2-68</td>
<td>130-210000</td>
<td>1.9-150</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Mucinous</td>
<td>median</td>
<td>0.64</td>
<td>5.45</td>
<td>150</td>
</tr>
<tr>
<td>range</td>
<td>0.15-1.3</td>
<td>2.1-13.2</td>
<td>50-120000</td>
<td>3-44</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Grade 1</td>
<td>median</td>
<td>0.85</td>
<td>3.6</td>
<td>205</td>
</tr>
<tr>
<td>range</td>
<td>0.2-1.3</td>
<td>2.1-7.4</td>
<td>50-120000</td>
<td>4.2-84</td>
</tr>
<tr>
<td>N</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Grade 2-3</td>
<td>median</td>
<td>8.1F</td>
<td>23.5F</td>
<td>420</td>
</tr>
<tr>
<td>range</td>
<td>0.1-18</td>
<td>6.7-68</td>
<td>100-210000</td>
<td>0.6-150</td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

A, B = Significantly higher (p < 0.05) cyst fluid concentrations were found in FIGO Stage II, III, IV compared to FIGO Stage I for LH and FSH; C, D = significantly higher (p < 0.05) cyst fluid concentrations were found in malignant serous compared to malignant mucinous tumors for LH and FSH; E, F = Significantly higher (p < 0.05) cyst fluid concentrations were found in histologic grade 2-3 compared to grade 1 tumors for LH and FSH.
may passively diffuse from the serum into the cyst fluid compartment. This hypothesis is in line with the strong positive correlation between malignant and benign ovarian cysts. The indication of differences in vascular permeability between malignant and benign ovarian cysts is supported by significantly higher Vascular Endothelial Growth Factor (VEGF) levels we earlier found in cyst fluid from malignant tumors compared with benign tumors [13]. VEGF acts not only as an activator of angiogenesis, but also leads to increased vascular permeability.

Our study did not observe significant differences in 17β-estradiol and progesterone cyst fluid concentrations between the histopathologic subgroups. These results are in line with those from Gaetje and Popp who determined 17β-estradiol and progesterone in cyst fluid from ovarian tumors [7]. However, it contradicts the results of the study by Reimer et al. who reported significantly lower 17β-estradiol levels in cyst fluid from malignant tumors compared to those found in non-malignant cysts [10]. Ivarsson et al. describe production of 17β-estradiol and progesterone by normal ovarian surface epithelium cells in vitro from pre- as well as postmenopausal patients [14]. It is reasonable to assume that the finding of endogenous production of steroids by ovarian surface epithelium is a normal feature of epithelial cells that is still present during malignant transformation, rather than a tumor specific property [14]. Results from the present study indicate only limited potential prognostic value (in terms of correlation with FIGO stage or histologic grade) for cyst fluid gonadotropins or steroids in malignant tumors. Although significantly higher median cyst fluid LH and FSH concentrations were found in higher tumor stages and high-grade tumors, the results were too scattered to draw conclusions for individual patients.

Progesterone levels tended to be higher in fluid from prognostically more favorable tumors (lower stage and better histologic grade). Other studies describe a more favorable prognosis for tumors positive for the progesterone receptor compared to those that did not express this receptor [15, 16]. However, the relationship between tumors that are positive for the progesterone receptor and their cyst fluid progesterone levels remains unclear as well as their impact on prognosis, and might be the subject of future studies. In conclusion, higher gonadotropin concentrations were found in cyst fluid from malignant tumors compared to those from benign tumors. The correlation of cyst fluid and serum gonadotropins in malignant tumors might be explained by increased vascular permeability. Future studies have to determine whether the differences in ovarian cyst fluid gonadotropin concentrations might be of clinical interest. The therapeutic use of LHRH agonists in the treatment of ovarian cancer has been suggested [17, 18]. Further exploration of the efficacy of such a treatment has to be performed to find out whether cyst fluid gonadotropin levels might be of additional value in determining which proportion of the patients may benefit from such a treatment.
Platin sensitivity and long-term survival in Stage III epithelial ovarian cancer patients

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Department of Obstetrics and Gynecology, Gynecologic Oncology Unit, E. Wolfson Medical Center, Holon, Israel, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv (Israel)

Summary

Purpose: The aim of the present study was to assess the effect of platin sensitivity on long-term survival of Stage III epithelial ovarian cancer (EOC) patients. Methods: The records of all histologically confirmed Stage III EOC and PPC patients diagnosed during 1995-2006 were reviewed. A comparison of selected characteristics was made between long-term (> 5 years) and short-term (< 3 years) survivors. Results: Among 58 Stage III patients, 20 had long-term and 18 short-term survival. The rate of platin sensitive patients in long-term survivors was significantly higher than in short-term survivors (95.0% vs 27.8%, p < 0.001). The sensitivity and specificity of platin sensitivity for long-term survival was 95% and 72.2%, respectively, and the positive and negative predictive value was 79.2% and 92.8%, respectively. No statistically significant difference between the groups was found with regard to other selected characteristics. Conclusion: The rate of platin sensitive patients was significantly higher among long-term survivors than among short-term survivors but the specificity and positive predictive value of platin sensitivity for long-term survival prediction were relatively low precluding its practical clinical use.

Key words: Epithelial ovarian cancer; Platin sensitivity; Primary peritoneal carcinoma; Long-term survival; Short-term survival.

Introduction

Epithelial ovarian cancer (EOC) is a major cause of morbidity and mortality among gynecological malignancies [1] and is responsible for more than 100,000 annual deaths around the world [2]. Stage, tumor grade and post-operative residual disease are the most important prognostic factors [3, 4]. About 75% of EOC patients have advanced disease (FIGO Stage III or IV) at diagnosis. Treatment consists of cytoreductive surgery and adjuvant platin-based chemotherapy. Though the majority of patients achieve complete clinical remission, most of them recur. The median survival time for patients after recurrence is approximately two years [5]. Primary peritoneal carcinoma (PPC) behaves and is treated in a similar manner [6]. The overall 5-year survival rate for women with advanced EOC and PPC disease is only 13%-29% [7-9]. Nevertheless, with modern treatment, long-term survival can be achieved in some of them [10].

The aim of the present study was to assess the effect of platin sensitivity on long-term survival of Stage III EOC and PPC patients.

Material and Methods

The records of all histologically confirmed EOC and PPC patients diagnosed during the 11-year period from 1995 to 2006 were located. Records of Stage III patients were reviewed and clinicopathological data were retrieved after obtaining institutional review board approval. A comparison was made between patients who survived, with or without disease, more than five years (long-term survivors) and patients who died within three years after diagnosis (short-term survivors). Date of primary surgery was considered as date of diagnosis. For the purpose of analysis EOC and PPC patients were combined.

All patients had debulking surgery followed by combination chemotherapy with platin and paclitaxel. Patients were categorized as being platinum-sensitive if the platin-free interval prior to recurrence was more than six months.

Differences between the groups were calculated by chi square analysis or Fisher’s exact test when appropriate.

Results

During the study period 82 histologically proven EOC and eight PPC patients were diagnosed. Of these 90 patients 58 (64.4%) had Stage III disease. Among the Stage III patients 20 had long-term and 18 short-term survival. PPC was diagnosed in four long-term and four short-term survivors.

The mean age of long-term and short-term survivors was similar being 63.7 ± 7.2 (range 46-74) and 62.8 ± 13.4 (range 37-78), respectively. Platin-sensitivity and other selected characteristics of the two patient groups are presented in Table 1. No statistically significant difference between the groups was found with regard to age ≤ 50, parity, main complaint, duration of complaint, body mass index, preoperative CA 125 level, estimated amount of ascites at the original operation, histological type and diameter of residual disease.

All short-term survivors had sub-Stage IIIC disease at diagnosis compared to 80.0% of the long-term survivors (p = 0.06, Fisher’s exact test). The rate of platin-sensitive patients was 95.0% in long-term and only 27.8% in short-term survivors. This difference was highly significant (p < 0.001). The sensitivity and specificity of platin sensitivity for long-term survival was 95% and 72.2%, respectively, and the positive and negative predictive value was 79.2% and 92.8%, respectively.
Table 1. — Selected characteristics of long- and short-term survivors.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Long-term</th>
<th>Short-term</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>20</td>
<td>18</td>
<td>100.0</td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td>4</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>7</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>9</td>
<td>45.0</td>
</tr>
<tr>
<td>Main complaint</td>
<td>Abdominal pain ± distension</td>
<td>16</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>4</td>
<td>20.0</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>≤ 1</td>
<td>7</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>5</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>8</td>
<td>40.0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>18.5-24.9</td>
<td>8</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>25-30+</td>
<td>12</td>
<td>60.0</td>
</tr>
<tr>
<td>Preoperative CA 125 (IU/ml)</td>
<td>&lt; 500</td>
<td>6</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>500-1000</td>
<td>5</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>1000+</td>
<td>9</td>
<td>45.0</td>
</tr>
<tr>
<td>Sub-Stage III</td>
<td>A, B</td>
<td>4</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>16</td>
<td>80.0</td>
</tr>
<tr>
<td>Ascites (ml)</td>
<td>None</td>
<td>3</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>&lt; 1000</td>
<td>8</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>1000+</td>
<td>9</td>
<td>45.0</td>
</tr>
<tr>
<td>Grade</td>
<td>1-2</td>
<td>6</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14</td>
<td>70.0</td>
</tr>
<tr>
<td>Histological type</td>
<td>Serous</td>
<td>19</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>Non-serous</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Residual disease (cm)</td>
<td>None</td>
<td>5</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>≤ 2</td>
<td>7</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>&gt; 2</td>
<td>8</td>
<td>40.0</td>
</tr>
<tr>
<td>Platin sensitive</td>
<td>Yes</td>
<td>19</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1</td>
<td>5.0</td>
</tr>
</tbody>
</table>

NS = not significant.

Discussion

Our main finding was that in women with Stage III EOC and PPC there was a highly significant larger percentage of platin-sensitive patients among long-term (> 5 years) than among short-term (< 3 years) survivors (95.0% vs 27.8% respectively; p < 0.001).

It has been previously shown that the most important favorable prognostic factors in advanced EOC and PPC, granting an improved chance for long-term survival, include younger age, cell type other than mucinous and clear cell, lower stage, well differentiated tumor, absence of ascites and smaller residual tumor following primary cytoreductive surgery [11, 12].

While there was a lower rate of sub-Stage IIIC patients among the long-term survivors when compared to short-term survivors (p = 0.06), the other mentioned favorable factors were not found to be significant predictors of long-term survival in our study.

It has been shown that obesity [13] is a risk factor and that parity has a significant protective effect [14] in ovarian carcinoma, but we have not found them to be predictors of long-term survival. Preoperative CA 125 level has been shown in some investigations to be of prognostic significance [15, 16] but it also seems not to be a predictor of long-term survival.

Inconsistent results have been obtained in studies dealing with the effect of symptom duration on prognosis. In some studies longer duration of symptoms unfavorably affects prognosis [17, 18] while in others no effect on survival was observed [19-23]. Our finding that duration of symptoms is not a predictor of long-term survival concurs with the latter studies.

Some of our findings are in contrast to a similar study by Kaern et al. [24] who compared 28 short-term survivors to 23 long-term survivors. Their definition of long-term survival was identical to ours but they defined short-term survival as death from ovarian cancer within 18 months from diagnosis. They found that the absence of ascites, debulking surgery to < 1 cm residual disease, clear-cell histology and normal postoperative prechemotherapy CA 125 levels to be of prognostic importance. In addition they found that negative Ki-67 expression also predicted a more favorable prognosis. Goff et al. [25], who compared 22 patients with Stage III ovarian cancer followed for a median of 66 months with 30 with a median survival of 18 months, reported that long-term survivors were more likely to have had an optimal cytoreduction and lower levels of Ki-67 antigen expression and less likely to overexpress p53 than were short-term survivors. These biomarkers and BRCA mutation status were not assessed in our study.

The main weaknesses of our study are its retrospective nature, the unequal arms for Stage IIIC disease and the long study period which allows for heterogeneity of the chemotherapy employed as second line or after relapse. The lack of association between most of the selected clinopathological characteristics in our study and long-term survival may possibly be due to the small sample size. However in spite of the small sample size, platin sensitivity was a highly significant predictor of long-term survival. This factor has not been assessed in previous studies that compared long-term to short-term survivors. It is in line with that of Hunter et al. [26] who showed that cytoreductive surgery probably has only a small effect on the survival of women with advanced ovarian cancer but platinum-containing chemotherapy improved median survival time substantially.

While the rate of platin-sensitive patients was significantly higher among long-term survivors it should be noted that among short-term survivors about 25% of the patients were also platin sensitive. Thus the sensitivity and negative predictive value of platin sensitivity were high, however the specificity and positive predictive value were relatively low. Therefore the clinical useful-
ness of platin sensitivity as a predictor of long-term survival is limited. An accurate predictor of long-term survival could alleviate anxiety and be used to reassure EOC patients. Regrettfully, no single characteristic assessed by us has demonstrated sufficient accuracy for any definitive clinical prediction of long-term survival. Microarray gene profiling [27] may in the future be used for more accurate prediction of long-term survival.

References


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Evaluation of prognostic significance in extracapsular spread of pelvic lymph node metastasis in patients with cervical cancer

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Summary

Background: Extracapsular spread of lymph node (LN) metastasis has been shown as a negative prognostic factor in cancers of several organs. This study was performed to clarify the prognostic significance of extracapsular spread of pelvic lymph node metastases in patients who underwent radical hysterectomy and pelvic paraaortic lymphadenectomy for International Federation of Gynecology and Obstetrics (FIGO) Stage I-II cervical carcinoma. Methods: Ninety-five patients were treated with radical hysterectomy and pelvic paraaortic lymphadenectomy for Stage I-II cervical carcinoma. Twenty-one cases with positive nodes of the tumor and lymph nodes were reviewed. The description of the pattern of metastasis present in the node was focused on: maximal diameter of metastasis was compared with the maximal diameter of the node, the capsular integrity, and the type of immune response. The prognostic significance of extracapsular spread (ECS), maximal diameter of metastasis and the type of immune response of pelvic metastases was evaluated with respect to disease-free survival (DFS), overall survival, and the pattern of disease recurrence. Results: ECS was seen in 52.4% (11/21) of the cases. The 5-year DFS rate in patients with ECS was significantly lower compared to patients without ECS (63.4% vs 100%; p = 0.03). To assess the independent impact of ECS on overall survival, the multivariate Cox regression model was not significantly different. Conclusion: From data in our study and those obtained from the literature, the occurrence of ECS should be given in the pathology report. Including ECS in the classification of nodal involvement might be helpful in better prognostic discrimination of patients with metastatic lymph nodes.

Key words: Cervix carcinoma; Pelvic metastasis; Extracapsular spread.

Introduction

Patients with FIGO Stage I and IIa are preferentially treated with radical hysterectomy and pelvic lymph node dissection, achieving 5-year survival rates of approximately 88% [1, 2]. In spite of early disease state, a number of patients will recur, and most of these will eventually die of the disease. Therefore, determination of prognostic factors is required for discrimination between high and low-risk groups among this group. Individual parameters that predict poor prognosis have been the subject of many publications [1-4]. Studies indicate that the presence of lymph node metastases (LNM) is an independent prognostic factor for overall and disease-free survival (DFS), and local recurrence in carcinoma of the uterine cervix [5]. We showed that 22.1% of patients with Stage I-II cervical cancer had positive pelvic nodes.

Studies have shown that different types of lymph node metastases have different prognostic significance in the head and neck, breast, and vulvar carcinoma [6-8]. Despite this, there are only a limited number of studies in the literature addressing the prognostic significance of the pathologic pattern of pelvic lymph node metastases in early-stage cervical carcinoma [9-13].

The aim of this study was to correlate the pathologic patterns of pelvic lymph node metastases according to survival and recurrence rates in early-stage cervical carcinoma.

Material and Method

During the period from 1995 to 2000, 95 patients with early-stage cervical cancer were managed surgically at Ankara Oncology Research and Education Hospital.

We included patients with Stage I-II who were clinically staged as recommended by FIGO and excluded patients with paraaortic lymph node involvement. Histologic classification was performed according to the World Health Organization classification [14].

All patients underwent a staging laparotomy, radical hysterectomy, and bilateral pelvic and paraaortic lymphadenectomy. All surviving patients were followed-up at least 60 months with a median follow-up of 100 months (range, 5-120 months). Pathological and clinical data and follow-up information were retrieved from the medical records. The selection of postoperative adjuvant therapy was at the discretion of the attending gynecologic oncologist in charge of the patient. Generally, patients with LNM, parametrial invasion, positive or close surgical margins, lymphovascular space invasion (LVSI) with deep stromal invasion were considered for adjuvant radiotherapy (RT) or chemoradiotherapy (CT+ RT). A total of 76 patients received external beam radiation consisting of pelvic radiotherapy followed by vaginal brachytherapy or chemoradiotherapy. The protocols of postoperative chemotherapy included cisplatin-based regimens for four to six courses. Pelvic radiation was given at daily 2 Gy for a total dose of 50 Gy. Remote afterloading of intravaginal iridium-192 brachytherapy was placed one to two weeks after completion of external RT, delivering in 21 Gy/7 fractions within two weeks.

A chart review was performed to determine clinical outcomes including time to recurrence, salvage therapies, and survival.
All 21 patients with positive nodes had slides of the tumor and lymph nodes reviewed. A maximum of four positive pelvic lymph nodes was reviewed in every patient. When there were more than four, the node with the maximal diameter was always reviewed plus another three randomly chosen nodes. Owing to the retrospective nature of the study, only two of the three dimensions of the lymph nodes were available for measurements on the histologic section. The description of the pattern of metastasis present in the node was focused on: the maximal diameter of metastasis compared with maximal diameter of the node, capsular integrity, and type of immune response.

Extracapsular metastasis was defined as the extension of tumor through the capsule of the lymph node into the perinodal tissue. A patient had extracapsular metastasis if the above histologic pattern was present at least in one lymph node. The patterns of immune response were defined as follows: 1) lymphocyte predominance, when there was an increased number of small lymphocytes throughout the cortex, paracortex, and medullary regions; 2) germinal center predominance, when germinal centers containing large lymphoid cells and mitotic figures extended throughout the cortex and paracortex; 3) Unstimulated, when there were lymphoid follicles without germinal centers in the cortex and hypocellular paracortical areas [15, 16]. When there was a mixed immune pattern within an individual node, the lymph node was designated according to that pattern occupying the greatest area within it. When there were different lymph nodes with different types of immune pattern within the same patient, we classified the case according to the worst pattern reported in the literature for other tumors [16].

For the analysis of the diameter of lymph node metastasis, a cutoff of 2 mm was chosen on the basis of the existing literature on other tumors [8], which defined as micrometastases those measuring < 2 mm.

Statistics

Statistical analysis was performed using the “SPSS 10.05 for Windows” computer program. The follow-up interval was calculated in months and defined as the time between the date of surgery and date of the event (death, distant or local recurrence) or last follow-up. When DFS is used as an endpoint, an event ends in death, distant or local recurrence. The follow-up interval was calculated in months and defined as the time between the date of surgery and date of the event. DFS rates were calculated by the Kaplan-Meier method, and the log rank test was used for comparison. The stepwise Cox proportional hazard model was used to assess the independent prognostic and predictive factors affecting DFS; p values of less than 0.05 derived from two-tailed tests were considered significant.

Results

Median age was 48 (19-74) years and five-year survival was 87.1%. Characteristics of patients are shown in Table 1.

Eleven patients (52.4%) with pelvic lymph node involvement showed ECS of the metastatic deposits. Twelve patients (57.1%) with pelvic lymph node metastasis showed greater than 2 mm diameter of metastasis, and eight (38.1%) patients had lymphocyte predominance of immune pattern. Twelve patients (61.9%) had other immunologic patterns (Table 2).

The 5-year DFS rate in patients with ECS was 63.64% compared to 100% in patients without ECS (p = 0.0392). The 5-year DFS was not significantly different for immunologic pattern and maximal diameter of metastatic lymph nodes (Table 2).

To assess the independent impact of ECS on overall survival, multivariate analysis with Cox’s regression model was not significantly different (p = 0.985).

Discussion

The association between size or volume of lymph node metastases and prognosis has also been previously demonstrated in vulvar [6] and prostate cancer [17]. The presence of a tumor growing outside the capsule of the lymph node has been demonstrated to be of prognostic significance in squamous carcinoma of the head and neck [7], in the breast [8], and vulvar cancer [6].

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients</th>
<th>Pelvic lymph node positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>48 (19-74)</td>
<td>50 (33-64)</td>
</tr>
<tr>
<td>FIGO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>67 (70.5)</td>
<td>17 (80.9)</td>
</tr>
<tr>
<td>Stage II</td>
<td>28 (29.5)</td>
<td>4 (19.1)</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>79 (83.2)</td>
<td>17 (81)</td>
</tr>
<tr>
<td>Non-squamous</td>
<td>16 (16.8)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Tumor diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2</td>
<td>19 (20)</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>2-4</td>
<td>60 (63.2)</td>
<td>11 (51.4)</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>16 (16.8)</td>
<td>7 (33.3)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50 (52.6)</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>2-3</td>
<td>45 (47.4)</td>
<td>12 (57.1)</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>72 (75.8)</td>
<td>10 (47.6)</td>
</tr>
<tr>
<td>Yes</td>
<td>23 (24.2)</td>
<td>11 (52.4)</td>
</tr>
<tr>
<td>Parametrial involvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>81 (85.3)</td>
<td>18 (85.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>14 (14.7)</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Pelvic nodes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>74 (77.9)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21 (22.1)</td>
<td></td>
</tr>
<tr>
<td>Surgical margins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>89 (93.7)</td>
<td>17 (81)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (6.3)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Other - 1/3 stromal invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63 (66.3)</td>
<td>13 (61.9)</td>
</tr>
<tr>
<td>Yes</td>
<td>32 (33.7)</td>
<td>8 (38.1)</td>
</tr>
</tbody>
</table>

Table 2. — Univariate analysis of lymph node characteristics for 5-year disease-free survival (DFS).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
<th>5-year DFS</th>
<th>p-log rank</th>
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<tbody>
<tr>
<td>Extracapsular spread</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10 (47.6)</td>
<td>100</td>
<td>0.0392</td>
</tr>
<tr>
<td>Yes</td>
<td>11 (52.4)</td>
<td>63.64</td>
<td></td>
</tr>
<tr>
<td>Maximal diameter of lymph node</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2</td>
<td>9 (42.9)</td>
<td>100</td>
<td>0.0629</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>12 (57.1)</td>
<td>60.67</td>
<td></td>
</tr>
<tr>
<td>Immunologic pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphosit predominance</td>
<td>8 (38.1)</td>
<td>62.50</td>
<td>0.0880</td>
</tr>
<tr>
<td>Other</td>
<td>13 (61.9)</td>
<td>92.31</td>
<td></td>
</tr>
</tbody>
</table>
For nodal positive carcinoma of the uterine cervix, the frequency of ECS has been reported to be 41.8%, 47.3% and 30.9%, respectively [9, 10, 13]. In our study, the frequency of ECS was 52.4%. The differences in the frequency of ECS in these studies might be caused by the different numbers of cases included.

In our limited series, we showed reduced 5-year DFS in node-positive patients representing ECS (p = 0.03). To assess the independent impact of ECS on overall survival, Cox’s multivariate regression model was not significantly different. Five-year survival was not significantly different for immunologic pattern and maximal diameter of metastatic lymph nodes. However the relatively small number of patients seen in this study limits the power of our calculations. Thus, further studies dealing with the immunologic pattern and maximal diameter of metastatic lymph nodes might be of interest.

Samlal et al. [10] have reported a reduced 5-year disease-specific survival in their study of 134 node-positive patients (FIGO-Stage IB and IIA) representing ECS (56% vs 78%). However, they failed to demonstrate any significance. Tinga et al. [12] reported a reduced overall survival in patients who had positive nodes with ECS.

Two larger studies dealing with the parameters of ECS in cervical carcinoma are the report of Morice et al. [9] and Horn et al. [13] which demonstrated a significant prognostic impact of ECS. Morice et al. [9] examined 138 patients with pelvic and paraaortic lymph node involvement. These authors reported an overall 3-year survival rate of 75% for patients without and of 40% for those with ECS (p < 0.0001). Horn et al. [13] examined 256 surgically treated cervical carcinoma patients with Stage pT1b, pT2b. They showed that patients with pelvic lymph node involvement and the occurrence of ECS represented a significant reduction in 5-year recurrence free survival rate (59.7% vs 67.2%, p = 0.04) as well as a significantly lower 5-year overall survival rate (33.5% vs 60.5%, p < 0.001). In their study, ECS represented as independent prognostic factor in multivariate analysis.

From data in our study and those obtained from the literature, the occurrence of ECS should be given in the pathology report. The fact of ECS in the classification of nodal involvement might be helpful in better prognostic discrimination of patients with metastatic lymph nodes.

References

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Excessive pap smears due to opportunistic cervical cancer screening

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Summary

The study aimed to analyze the Pap smears carried out for cervical cancer screening according to Ministry of Health guidelines. All smear tests carried out within the public health system in Campinas in 2003 were analyzed. All tests that did not conform to the guidelines were considered excessive. The guidelines recommend screening once every three years for all women aged 25 to 59 after they have received two negative smears. This study showed that the majority of women initiated screening prior to 25 years of age and the periodicity was predominantly annual, followed by biannual tests. In conclusion, 63.4% of tests were excessive. The screening coverage was 14.76%, but if all the tests had been performed as recommended, the final coverage over three years could have reached 65.4%. Thus it is possible to increase the coverage with the available resources since the screening works like an organized program.

Key words: Cervical cancer screening; Vaginal smear; Public health administration; Women’s healthcare; Screening programs.

Introduction

Cervical cancer is the third most common type of cancer among Brazilian women, with only skin (non-melanoma) and breast cancer being more common [1]. This form of cancer is an important public health issue, since its incidence may be reduced through efficient screening programs [2-4].

Cervical intraepithelial neoplasia is a consequence of the action of the human papilloma virus (HPV), and its prevalence is closely associated with early sexual initiation and sexual activity with various partners [5,6]. Cervical epithelial lesions pass through various stages before becoming invasive carcinomas. Early detection may be carried out with the Papanicolaou test, thereby permitting therapeutic measures that may lead to a cure in as many as 100% of cases [7, 8].

Despite the existence of a cervical cancer-screening program, 19,260 new cases are estimated to have occurred in Brazil in 2006 [1]. Moreover, there is no evidence of any reduction in the mortality rate from this type of cancer [9]. The Brazilian program recommends screening women 25-59 years of age once every three years following two negative control tests [1]. Considering that indicators of incidence and mortality suggest that the preventive actions carried out are ineffective, it is possible that the recommended guidelines are not being adequately followed. This study analyzed the age at which women are being screened and the interval between tests. From this data, it is possible to estimate the number of screening tests that are being carried out outside the age group and periodicity established by the Brazilian screening program and, consequently, what constitutes excessive testing.

Materials and Methods

This cross-sectional study included women who were clients of the Brazilian public health system in Campinas, which is composed of 47 healthcare centers in five districts: north, east, south, northwest and southwest. Data from eight referral outpatient departments were also analyzed.

The study population comprised all the women who had been submitted to a Pap smear for cervical cancer screening within the Brazilian public health system in Campinas between January and December 2003. Samples were collected by a nurse or doctor, and were sent to the Cytopathology Laboratory of the Women’s Integrated Healthcare Center (CAISM) at the State University of Campinas (UNICAMP).

Exclusion criteria comprised:

Women undergoing follow-up at cervical pathology outpatient departments; those who had undergone Pap smear testing at the outpatient department of UNICAMP’s Teaching Hospital or at CAISM; hysterectomized women; those who had previously undergone radiotherapy; those who had been submitted to cauterization; women who had a previous abnormal Pap smear result; and women undergoing post-treatment follow-up. After applying the exclusion criteria, 54,338 Pap smear results were analyzed.

In 2003, regulations in force required that a sample from the cervical canal should be collected using an appropriate brush and a sample from the ectocervix should be obtained using an Ayre spatula. Cytological diagnosis was established according to the Bethesda System [10].

Pap smear results were obtained from the data system of CAISM’s Cytopathology Laboratory, which registers the information directly from the request form and the results of the Pap smear. This form was edited to transmit data using barcode scanners. This information system has features that automatically test data consistency. For each cell type, either squamous or glandular, only one diagnosis was accepted.

Calculation of the excessive cytology tests was made by using the study carried out by van Ballegooijen et al. [4] as a reference, and adjusting it for the data available in the database.

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of the Cytopathology Laboratory of UNICAMP. Based on the Ministry of Health guidelines, excessive testing was defined as:
- All tests carried out outside the target age group, i.e. performed in women ≤ 24 or ≥ 60 years of age.
- Whenever the interval between the previous screening control and the current one was less than three years; in these cases, information was recorded on the length of the interval: two years, one year or less than one year.

To calculate the total number of tests in excess, the following formula was constructed: \((2/3 \cdot (b - a) + \frac{1}{2} \cdot c + d + e)\) based on the following assumptions (Table 1). Since a woman’s second control should have been carried out one year after the first, to correct the calculation of excessiveness, exams carried out for the first time the previous year were subtracted from the total of all tests carried out at intervals of one year. Since the data regarding the number of tests carried out for the first time in 2002 were unavailable and the data system was unable to identify the tests that represented second screenings, it was assumed that the total number of first tests carried out in 2002 would be the same as the number performed in 2003. Therefore, the calculation of excessive testing was corrected by subtracting the number of tests carried out for the first time in 2003 (a) from the total number of tests performed after an interval of one year (b) in the 25-59 year age group.

Therefore, taking a three-year periodicity as a reference, in three years women should have undergone one screening. In the case of women who underwent screening at an interval of one year, we considered two-thirds of these tests to be excessive after subtracting the cases of second screening \((2/3 \cdot (b - a))\), since in three years three controls would have been carried out, but of these three, only one would have been necessary. In the case of women who underwent screening after an interval of two years \((c)\), we considered 50% of these tests to be excessive, since in three years two screening tests would have been performed, with only one being necessary \((\frac{1}{2} \cdot c)\). For the purposes of calculation, intervals of less than one year were considered as a year.

Tests carried out at intervals of three years or more were not considered excessive, nor were the tests in which no information was available with respect to periodicity. Tests in which quality was unsatisfactory \((d)\) were included in the sum of tests in excess, since they failed to contribute towards the prevention of cervical cancer and resulted in the test having to be repeated. Finally, the tests performed outside the target age-range were added to the total \((e)\).

**Results**

The total of studied tests was 54,338. Data showed that 24.6% of women were under 25 years of age, 68.8% were between 25 and 59 years of age, and 6.5% were 60 years of age or older. Of these women, 9.1% had undergone Pap smear testing for the first time. The majority of tests (44.5%) were carried out one year after the previous screening; 25.9% two years afterwards; 8.8% three years later; and 7.2% after four years or more. A further 1.3% of tests had no information with respect to the time since the previous screening.

Of the women who had undergone screening for the first time, 76.2% were under 25 years of age, 8.0% were aged 25-29, and 15.8% were 30 years of age or older. The quality of the smears was considered satisfactory in 49.8% of cases, and unsatisfactory in 2.1% of samples. The remaining 48.2% of cases were considered satisfactory but with limitations.

<table>
<thead>
<tr>
<th>Equation symbol</th>
<th>Age (years)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>25 - 59</td>
<td>First screening</td>
</tr>
<tr>
<td>b</td>
<td>25 - 59</td>
<td>Screening at an interval of ≤ 1 year since previous test</td>
</tr>
<tr>
<td>c</td>
<td>25 - 59</td>
<td>Screening at an interval of 2 years since previous test</td>
</tr>
<tr>
<td>d</td>
<td>25 - 59</td>
<td>Quality of tests unsatisfactory</td>
</tr>
<tr>
<td>e</td>
<td>&lt; 25 or &gt; 59</td>
<td>Quality of tests unsatisfactory</td>
</tr>
</tbody>
</table>

| Table 1.— Parameters for the calculation of excessive Pap smears based on the following formula: excessive tests = \((2/3 \cdot (b - a) + \frac{1}{2} \cdot c + d + e)\). |

| Total number of tests | 54,338 |
| Tests in excess | 34,465 |
| Percentage of the population aged 25-59 covered | 14.76% |
| Percentage of the population that would be covered annually if all tests had been performed in women within the age-group recommended by the Ministry of Health | 21.8% |

**Discussion**

Cervical cancer begins as a low-grade intraepithelial lesion that may evolve to a more serious lesion over an average period of 10-15 years. This means that the serious lesions are more prevalent in women who have been sexually active for more than ten years, i.e. older women [11]. In young women, low-grade lesions are more prevalent. Moreover, these have a high rate of spontaneous regression. The more severe intraepithelial lesions...
have a greater potential to progress to invasive carcinoma; however, they are rare in younger women [12, 13].

In the eighties, a study carried out by the International Agency for Research on Cancer (IARC) found that initiating screening at 20 years of age leads to a reduction of 1-2% in the accumulated rate of invasive carcinoma compared to screening from 25 years of age [14]. Therefore, beginning screening prior to age 25 means diagnosing predominantly less severe lesions, the majority of which regress spontaneously, consequently leading to a system that is poorly effective in reducing mortality rates from invasive cancer. Moreover, the rare cases of invasive carcinoma that occur in this age-group are normally very aggressive and very probably would occur irrespective of screening [15].

The same occurs in the case of screening at close intervals, since less severe lesions remain for less time. Therefore, repeating the test annually leads to the detection of predominantly low-grade lesions [13]. On the other hand, the more severe precursor lesions may take more than ten years to progress, therefore, women who undergo periodic screening will have various opportunities at which to detect these lesions. These were the conclusions reached in the IARC study, which showed that the reduction in the incidence of cervical cancer does not vary significantly when controls are annual or triennial, 93% and 91%, respectively; not justifying, therefore, the implementation of an annual screening program [14].

From the point of view of public health, the effectiveness of a cervical cancer-screening program is known to depend on the percentage of the population covered by the program. To achieve coverage of 80%, for example, 27% of women in the target age-group would have to be submitted to a Pap smear in one year, while in the following two years, the tests would be carried out in different women, attaining 80% of the total female population over a three-year period. However, this is not what is occurring. According to the results of this study, 73.6% of tests were carried out less than three years after a previous screening test, not including first screenings, i.e. there is a large contingency of women who undergo Pap smears every year.

In Campinas, the percentage of the population covered in 2003 was 10.19%, well below the expected rate for effective screening. Moreover, the age group with greatest coverage was between 20 and 34 years of age, an age at which less severe lesions are the most common.

Analysis of the age distribution of the women who carried out tests for the first time showed that the majority were 15 to 19 years of age, followed by the 20 to 24-year age-group, i.e. below the age recommended by the Ministry of Health [1].

Another important finding from this study refers to the quality of smears. Quality was found to be satisfactory but with some restrictions in 48% of all tests carried out, while a further 2% of smears were unsatisfactory. This results in an important waste factor, as well as additional discomfort to the women, since tests often have to be repeated. It is a fact that the low quality of smears is prejudicial to the performance of cervical cancer screening, since it leads to a greater rate of false-negative results and, consequently, a greater risk for the population [16].

A total of 54,338 tests were carried out of which 63.4% were considered in excess, i.e. a further 34,465 women from the target population could have been screened if the Ministry of Health guidelines had been followed. This would increase screening coverage and lead to a more effective control of this form of cancer with less need to increase healthcare resources.

The number of tests carried out in women 25-59 years of age, excluding those in which quality was unsatisfactory, was 36,755. The population in Campinas in this age group was 249,062; therefore, the percentage of the population covered by screening was 14.76% in 2003. If all the tests carried out in 2003 had been performed in the age bracket recommended by the Ministry of Health, the final coverage in that year would have been 21.8%. Thus, over three years, this coverage could have reached 65.4% if no woman had repeated the test in that period of time. This would be considered a high rate since it did not include tests carried out in the private sector.

Nevertheless, the situation of the city of Campinas may be considered privileged compared to the rest of the country, since specific data for Campinas show a reduction of 37.5% in the mortality rate for cervical cancer in the periods 1992-1993 and 1997-1998 [17].

The results of this study show that cervical cancer screening in Campinas does not adhere to the guidelines of the Ministry of Health. The majority of women began screening before reaching 25 years of age and most women carried out the test annually or biannually. With respect to the number of tests performed, annual screening coverage was low.

Therefore, to optimize cervical cancer screening, the best solution would not be to reduce the frequency of Pap smears, but to redistribute these tests to women who have not been reached by the program.

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References


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Prognostic factors and adjuvant therapy in uterine carcinosarcoma

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Summary

Purpose of investigation: The objective of this retrospective study was to investigate prognostic variables and impact of adjuvant therapy in uterine carcinosarcoma. Methods: The clinical information and pathological confirmation were reviewed for cases with uterine carcinosarcoma from 1984 to 2005. A total of 45 patients were eligible for analysis. Results: The median follow-up for survivors was 84 months. Five-year overall survival and progression-free survival (PFS) rates were 36.5% and 33.8%, respectively for Stage I-IV. Distant site metastasis with/without pelvic failure occurred in 83.3% of those with recurrence/progression. By multivariate analysis, older age (p = 0.001) and more than half of myometrial invasion (p = 0.002) were significant predictors of death, while only myometrial invasion (p = 0.022) was significantly associated with PFS. Stratified analyses demonstrated a monotonic trend of chemotherapy or chemoradiation to decrease death. Conclusions: Our results suggested that age and depth of myometrial invasion were significant prognostic factors, and chemotherapy or chemoradiation seemed to be beneficial for uterine carcinosarcoma.

Key words: Uterine carcinosarcoma; Chemotherapy, Chemoradiation, Malignant mixed müllerian tumor, Prognostic factor.

Introduction

Uterine carcinosarcoma (CS) is an aggressive neoplasm of the female genital tract, which comprises 4% of malignancies of the uterine corpus. According to a study of Surveillance, Epidemiology, and End Results from the United States, the incidence of CS (or malignant mixed müllerian tumor) was 1.71, 4.28 and 0.99 per 100,000 women/years in white, black or the other races, while a population-based study from Norway showed a trend of increasing incidence and mortality in CS over time [1, 2]. This malignancy has biphasic epithelial and mesenchymal components. The histogenesis remains unclear, but recent immunohistochemical and molecular genetic studies have attributed CS to a metaplastic carcinoma [3]. CS has been recognized as an aggressive subtype of endometrial cancer, and the carcinomatous component seems to be the driving force of malignant behavior [3, 4].

Clinical Stage I or II CS (grossly confined to the uterus) are often upstaged (30-61%) at the time of comprehensive surgical staging. The rates of pelvic or paraaortic lymph node metastasis ranges from 13.2% to 90% according to clinical stage. The prognosis has been poor even in early stage (44-74% of 5-year overall survival [OS] in International Federation of Gynecology and Obstetrics (FIGO) Stage I/II), and 5-year OS ranges from 6-38% in Stage I-IV [4-9]. The outcome is disappointing with currently available chemotherapeutic agents or radiotherapy (RT) [10]. Moreover, efforts to improve outcome of uterine CS are hindered by its rarity.

In this study, we aimed to investigate the prognostic factors of CS and evaluate the impact of adjuvant therapy on survival in CS from the 21-year experience of a tertiary referral medical center in Taiwan.

Materials and Methods

Patients

We retrospectively reviewed the hospital medical records and pathological slides, through a search of the disease code database (International Classification of Diseases of Oncology [ICDO]) and Systematized Nomenclature of Medicine (SNOMED) code in Chang Gung Memorial Hospital from June 1984 to January 2005. The diagnosis of CS was based on the World Health Organization’s classification, and all primary uterine CS contained malignant elements of both epithelial and stromal light microscopic appearance [11].

Surgical and postoperative treatment

Although this retrospective study spanned two decades, it has been our policy that all patients with histologically confirmed CS should undergo surgery unless an unresectable situation is clinically obvious. Limited distant nodal or upper abdominal metastasis did not preclude an initial surgical intervention. If medically feasible, surgical staging consisting of washing cytology, abdominal total hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic lymph node dissection with/without paraaortic lymph node (PALN) dissection, omentectomy, and appendectomy were performed. In the case of incomplete surgical staging, stage was assigned on a basis of available pathologic findings, and unevaluated areas were considered negative. For patients with deep myometrial invasion, cervical extension,
or retroperitoneal nodal metastasis usually received postoperative RT, and chemotherapy (CT) was given concurrently or sequentially preceding RT. Adjuvant CT alone might be recommended to Stage IA-B. CT regimens usually involved combinations of ifosfamide, cisplatin or doxorubicin. Hormone or molecular targeted therapy has been used in recent years for inoperable cases according to molecular diagnosis of targeted protein and gene expression studies.

**Statistical analysis**

Survival curves (OS and progression-free survival [PFS]) were generated using the Kaplan-Meier method. Cox’s proportional hazard model was used to implement multivariable analysis while variables were screened by univariate analysis in advance. Hazard ratio (HR) of factors associated with death or recurrence/progression was calculated by incidence density ratio with a 95% confidence interval (CI). The cut-off point of a continuous variable was determined by receiver operating curve (ROC) analysis to get optimal differentiation between groups. Dummy variables were designed for those independent categorical variables in the Cox regression analysis. A test of monotonic trend was performed to examine an increasing pattern of variables from the lowest to the highest relative risk. A p value of less than 0.05 was considered statistically significant [12, 13].

**Results**

**Patient characteristics and treatment**

A total of 45 patients were eligible for analysis. The median age of the study population was 58 years old (range 36-85), and the most common initial manifestation (86.8%) was abnormal vaginal bleeding or postmenopausal bleeding. Nineteen (43.2%) of the 44 study patients whose FIGO Stage could be assigned were in Stage I, two in Stage II (4.5%), 15 in Stage III (34.1%), and eight were in Stage IV (18.2%). A total of 45.5% (15/33) cases with lymph node metastasis, 26.7% (4/15) cases with PALN metastasis, 30% (12/40) with adnexal involvement, and 73.8% (31/42) with more than half of myometrial invasion were recorded (Table 1).

**Treatment**

Of the 45 study patients, 42 received primary surgery, and for the remaining three imaging studies confirmed the diagnosis from endometrial sampling (apparently with advanced disease - unresectable IVB). Adjuvant CT, RT or both was administered in 14 (31.8%), six (13.6%) and 12 (27.3%) of all patients, while the remaining 12 did not receive adjuvant therapy and one unknown adjuvant therapy (Table 2). Among the 21 Stage I/II patients, seven did not receive adjuvant therapy, ten received CT, one received RT, two received CT-RT, and one unknown adjuvant therapy. Of the 21 Stage III/IV patients receiving primary surgery, 18 received postoperative adjuvant therapy.

**Clinical outcome and pattern of failure**

The median follow-up for survivors was 84 months (range, 6-215). Median time to recurrence/progression was eight months (range, 0-68). The 5-year OS and PFS was 36.6% and 33.8% in the whole series, and 59.7% and 60.1% in Stage I/II, respectively. A single Stage IA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SD 58.6 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>Median, range 58, 36-85</td>
</tr>
<tr>
<td>Stage</td>
<td>I 19 (43.2%)</td>
</tr>
<tr>
<td></td>
<td>II 2 (4.5%)</td>
</tr>
<tr>
<td></td>
<td>III 15 (34.1%)</td>
</tr>
<tr>
<td></td>
<td>IV 8 (18.2%)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>No 18 (54.6%)</td>
</tr>
<tr>
<td>Pelvic lymph node metastasis</td>
<td>No 21 (63.6%)</td>
</tr>
<tr>
<td>Para-aortic lymph node metastasis</td>
<td>No 11 (73.3%)</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td>No 30 (71.4%)</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Homologous 32 (71.1%)</td>
</tr>
<tr>
<td>Lymphovascular permeation</td>
<td>No 21 (50%)</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td>Endometrium only 2 (4.8%)</td>
</tr>
<tr>
<td>Preoperative CA-125 (U/ml)</td>
<td>≤ 35 13 (52%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 35 12 (48%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total (n)</th>
<th>Stage I (n)</th>
<th>Stage II (n)</th>
<th>Stage III (n)</th>
<th>Stage IV (n)</th>
<th>Stage unknown (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hysterectomy</td>
<td>45</td>
<td>3 (0)</td>
<td>0 (0)</td>
<td>0 (2)</td>
<td>2 (1)</td>
<td></td>
</tr>
<tr>
<td>Lymphadenectomy</td>
<td>45</td>
<td>12 (5)</td>
<td>0 (2)</td>
<td>2 (4)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant Therapy</td>
<td>45</td>
<td>19 (12)</td>
<td>2 (1)</td>
<td>15 (8)</td>
<td>3 (1)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>14 (10)</td>
<td>1 (2)</td>
<td>0 (1)</td>
<td>4 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>6 (1)</td>
<td>0 (0)</td>
<td>4 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT-RT</td>
<td>12 (2)</td>
<td>0 (7)</td>
<td>3 (3)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Prognostic factors and adjuvant therapy in uterine carcinosarcoma

One patient receiving surgery without adjuvant therapy was recurrence-free at six months. One of the three (33.3%) Stage IB patients who received surgery only experienced recurrence, while two of the remaining seven (28.7%) with Stage IB relapsed despite postoperative CT. One each of Stage IC patients receiving surgery alone or adjuvant RT had a recurrence and died, as did two of the other three (66.7%) receiving CT. The ages of the three deceased Stage IB patients were 64, 58, and 76 years, respectively. Two Stage IC patients received CT-RT and remained alive and recurrence-free. Twenty-one of the 22 (95.5%) Stage IC-IV patients without adjuvant CT-RT failed. Of the known 24 sites of failure, 83.3% involved distant sites with/without local recurrence/progression (Table 3).

Among the four patients with PALN metastasis, two cases received adjuvant CT and extended field RT according to institutional guidelines. One of them has no evidence of disease 29 months later, while the other died from hepatic failure after the third CT. Only one patient with recurrence was salvaged successfully. The single survivor after CS relapse was a Stage IIIIC patient who encountered recurrence in a left supravacular node one month after adjuvant RT, and she remained with no evidence of disease for 83 months after salvage RT.

Univariate and multivariate analysis

Older age, lower parity, advanced stage, adnexal metastasis, heterologous element, lymphovascular permeation, deeper myometrial invasion and no adjuvant CT were significant adverse factors for OS by univariate analysis (Table 4). However, only older age (HR: 9.84 [95% CI, 485

---

**Table 3.** Recurrence/progression rates and pattern by stage and adjuvant treatment (n = 44) a.

<table>
<thead>
<tr>
<th>Stage I &amp; II</th>
<th>Total</th>
<th>Patients with recurrence/progression</th>
<th>Site of recurrence/progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of pts.</td>
<td>N (%)</td>
<td>Pelvic N (%)</td>
</tr>
<tr>
<td>Adjuvant Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>3 (42.9%)</td>
<td>1</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>10</td>
<td>5 (50%)</td>
<td>1</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>1</td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td>CT-RT</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21 (100%)</td>
<td>10 (47.6%)</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stage III &amp; IV</th>
<th>Total</th>
<th>Patients with recurrence/progression</th>
<th>Site of recurrence/progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of pts.</td>
<td>N (%)</td>
<td>Pelvic N (%)</td>
</tr>
<tr>
<td>Adjuvant Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>3</td>
<td>3 (100%)</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>5</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>CT-RT</td>
<td>10</td>
<td>7 (70%)</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>23 (100%)</td>
<td>20 (87%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

CT-RT, chemotherapy and radiotherapy. a One unstaged patient unlisted here had no adjuvant therapy and expired 4 months later.

**Table 4.** Univariate analysis of prognostic factors in uterine carcinosarcoma.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>5-year PFS p (log rank)</th>
<th>5-year OS p (log rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.192</td>
<td>49.9%</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 58</td>
<td>22</td>
<td>39.8%</td>
<td>0.341</td>
</tr>
<tr>
<td>≥ 58</td>
<td>23</td>
<td>28.1%</td>
<td>0.033</td>
</tr>
<tr>
<td>Parity (0-9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.001</td>
<td>34</td>
<td>66.9%</td>
<td>66.4%</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>21</td>
<td>13.3%</td>
<td>17.8%</td>
</tr>
<tr>
<td>III-IV</td>
<td>23</td>
<td>13.3%</td>
<td>17.8%</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>0.05</td>
<td>50.0%</td>
<td>0.098</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>52.6%</td>
<td>52.6%</td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>19.1%</td>
<td>25.7%</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td>0.003</td>
<td>51.4%</td>
<td>0.005</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>48.1%</td>
<td>30 mo</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>24 mo</td>
<td>30 mo</td>
</tr>
<tr>
<td>Differentiation Homologous</td>
<td>0.023</td>
<td>49.3%</td>
<td>0.009</td>
</tr>
<tr>
<td>Heterologous</td>
<td>13</td>
<td>7.7%</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular permeation</td>
<td>0.008</td>
<td>37.5%</td>
<td>0.037</td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>52.3%</td>
<td>37.5%</td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>17.9%</td>
<td>26.5%</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>13 mo</td>
<td>0.002</td>
</tr>
<tr>
<td>Inner half</td>
<td>9</td>
<td>77.9%</td>
<td>0.001</td>
</tr>
<tr>
<td>Outer half</td>
<td>31</td>
<td>17.4%</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Properative CA-125

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>5-year PFS p (log rank)</th>
<th>5-year OS p (log rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>0.017</td>
<td>&lt; 0.001</td>
<td>0.153</td>
</tr>
<tr>
<td>≤ 35</td>
<td>13</td>
<td>59.2%</td>
<td>0.042</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>12</td>
<td>32.6%</td>
<td>26.4%</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>0.001</td>
<td>21.4%</td>
<td>0.014</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>13.0%</td>
<td>21.4%</td>
</tr>
<tr>
<td>Yes</td>
<td>26</td>
<td>45.5%</td>
<td>44.4%</td>
</tr>
<tr>
<td>Adjuvant radiotherapy</td>
<td>0.821</td>
<td>37.9%</td>
<td>0.686</td>
</tr>
<tr>
<td>No</td>
<td>26</td>
<td>36.5%</td>
<td>37.9%</td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>26.7%</td>
<td>31.3%</td>
</tr>
<tr>
<td>Adjuvant CT-RT</td>
<td>0.235</td>
<td>33.9%</td>
<td>0.610</td>
</tr>
<tr>
<td>No</td>
<td>32</td>
<td>29.4%</td>
<td>33.9%</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>61.7%</td>
<td>38.9%</td>
</tr>
</tbody>
</table>

PFS: progression-free survival; OS: overall survival; CT-RT: chemotherapy and radiotherapy.
2.43-39.87), $p = 0.001$) and deeper than half of myometrial invasion (HR: $7.87 \ [1.39-44.59]$, $p = 0.002$) were significant predictors of death under the Cox proportional hazards model (Table 5). Advanced Stage (III-IV vs I-II, HR: $3.71 \ [95\% \ CI, 0.95-14.50]$, $p = 0.059$) revealed only marginal statistical significance for death.

Likewise, decreased parity, advanced stage, lymph node metastasis, adnexal involvement, heterologous element, lymphovascular permeation, and deeper myometrial invasion were adversely associated with PFS and adjuvant CT was favorably related to PFS in univariate analysis (Table 4). Among multivariate analyses, only deep myometrial invasion (outer half vs no/inner half, HR: $11.81 \ [1.42-97.92]$, $p = 0.022$) showed independent prognostic significance, while adjuvant CT was marginally associated with improved PFS ($p = 0.074$) (Table 5).

Preoperative increased CA-125 serum level was significantly associated with poor OS ($p < 0.001$) and PFS ($p = 0.017$) as a continuous variable although we could not define a cut-off point with the ROC curve with this limited sample size ($n = 25$). Patients with preoperative CA-125 $>35$ U/ml (normal upper limit in general) had a worse PFS as compared with those with $\leq 35$ U/ml by univariate analysis (Table 4). However, it was not selected by multivariate analysis.

Table 5. — Multivariate analysis of overall and progression-free survival evaluation of prognostic factors in uterine carcinosarcoma.

<table>
<thead>
<tr>
<th>Overall survival</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (58 vs &lt; 58)</td>
<td>9.84 (2.43-39.87)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stage (III-IV vs I-II)</td>
<td>3.71 (0.95-14.50)</td>
<td>0.059</td>
</tr>
<tr>
<td>Myometrial invasion (outer half vs no/inner half)</td>
<td>7.87 (1.39-44.59)</td>
<td>0.002</td>
</tr>
<tr>
<td>Progression-free survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myometrial invasion (outer half vs no/inner half)</td>
<td>11.81 (1.42-97.92)</td>
<td>0.022</td>
</tr>
<tr>
<td>Adjuvant chemotherapy (yes vs no)</td>
<td>0.261 (0.06-1.14)</td>
<td>0.074</td>
</tr>
</tbody>
</table>

Table 6. — Stratified analysis of adjuvant chemotherapy or chemoradiation in age groups on overall survival.

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Age (years)</th>
<th>CT</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 58 Yes</td>
<td>2</td>
<td>569</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>58 Yes</td>
<td>8</td>
<td>474</td>
<td>3.93 (0.81-19.16)</td>
<td>0.090</td>
</tr>
<tr>
<td>No 6</td>
<td>72</td>
<td>6.84 (1.24-37.86)</td>
<td>0.027</td>
<td></td>
</tr>
</tbody>
</table>

Model 2

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>CT-RT</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 58 Yes</td>
<td>6</td>
<td>293</td>
<td>1.08 (0.29-3.97)</td>
</tr>
<tr>
<td>58 Yes</td>
<td>5</td>
<td>768</td>
<td>1.00</td>
</tr>
<tr>
<td>No 1</td>
<td>1</td>
<td>111</td>
<td>1.00 (0.11-9.14)</td>
</tr>
<tr>
<td>No 16</td>
<td>768</td>
<td>4.23 (1.36-13.21)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**Stratified analysis**

We performed stratified analyses using the monotonic trend in OS from the different risk groups (age) selected by multivariate analysis under the presence or absence of adjuvant CT or CT-RT. Myometrial invasion was excluded for stratified analysis due to the strong correlation with adjuvant CT. According to the optimal stratification of age (< 58 vs $\geq 58$ years) in different models, a monotonic trend toward increased death in these patients with uterine CS not receiving adjuvant CT ($p = 0.005$) or CT-RT ($p = 0.006$) was noted (Table 6).

**Discussion**

Uterine CS is one of the most aggressive uterine tumors, with a high potential of hematogenous and lymphatic spread resulting in poor survival. Despite multimodality treatment, the 5-year survival in various reports ranges from 6-38% [4-9]. Therefore, it is very important to identify prognostic variables and appropriate adjuvant therapy for this highly lethal malignancy.

A large Gynecologic Oncology Group (GOG) prospective surgical-pathological study of 453 clinical Stage I-II uterine sarcomas found that histologic type (homologous vs heterologous), adnexal spread, lymph node metastasis, and grade of sarcomatous component were significant prognostic factors for CS ($n = 301$) based on multivariate analysis [5]. An Italian multicenter retrospective study ($n = 118$) identified surgical stage, depth of myometrial invasion, and lymphovascular space involvement as significant predictors of outcome [8].

Our data indicated that 45.5% of the patients who underwent LN dissection had pelvic node metastasis and 26.7% had PALN metastasis. In addition, 20 of 24 (83.3%) patients with recurrence/progression involved a distant failure. Because of the high probability of lymphatic and hematogenous metastasis in uterine CS, it is imperative that adjuvant systemic therapy be utilized after surgery. Older age and deeper myometrial invasion were independent predictors of death, and only myometrial invasion was significantly correlated with PFS by multivariate analysis. Our results are similar to previous series except for older age, which might reflect the worse tumor biology, poor performance status and treatment intolerance [5-8].

The role of adjuvant therapy in uterine CS has not been clearly established. Several retrospective reports demonstrated favorable local control with no influence on survival benefit from adjuvant RT. Gerszten et al. reported that both decreased local and distant failure rates were found in Stage I/II uterine CS receiving adjuvant RT compared to surgery alone [14]. In contrast, Callister et al. noted that adjuvant pelvic RT decreased pelvic recurrences, but no survival benefit in a large retrospective series of Stage I-III CS ($n = 300$) [15]. Manolitsas et al. reported a 5-year survival rate of 74% with postoperative CT-RT for 38 clinical Stage I-II CS [16]. Menczer et al. reported that 41 of 49 (83.7%) uterine CS with Stage I-
IV had postoperative adjuvant treatment including CT (n = 10), RT (n = 21) and sequential CT-RT (n = 10). Sequential CT-RT had a significant decrease in mortality when compared to CT alone (p = 0.049) but was not significant compared to whole pelvic RT alone (p = 0.4) after controlling for stage [17]. Sutton et al. reported a 5-year OS of 62% for Stage I-II CS with adjuvant CT alone using ifosfamide and cisplatin. Although the regimen seemed tolerable, pelvic relapse remained problematic [18].

In our study, adjuvant CT revealed a marginal progression-free survival benefit in multivariate analysis. Stratified analyses demonstrated a monotonic trend of CT or CT-RT in various risk groups to decrease death. Adjuvant CT could be useful for Stage IB in younger age (< 58 years), while CT or RT alone was obviously inadequate for Stage IC (3 of 4 died). Indeed, 21 of the 22 (95.5%) Stage IC-IV patients without adjuvant CT-RT failed. A recent GOG phase III study found that adjuvant CT (cisplatin, ifosfamide, and mesna [CIM]) reduced the recurrence rate and marginally significantly prolonged OS in optimally debulked uterine Stage I-IV CS patients as compared to whole abdominal irradiation (WAI). However, due to a high relapse rate (CIM 49%, WAI 55%) and poor OS, CT-RT is at least what can be done before the emergence of new effective systemic therapies [19]. With regard to the selection of CT regimens, three GOG randomized phase III trials with CT including uterine sarcoma or CS have been reported [20-22]. The earlier studies showed that combination arms (adriamycin with dimethyl triazenoimidazole carboxamide or cisplatin with ifosfamide) had better response rates than single agent adriamycin or cisplatin but no survival benefit [20, 21]. However, a recent phase III trial demonstrated that ifosfamide with paclitaxel compared with ifosfamide alone decreased 31% HR of death (13.5 vs 8.4 months, p = 0.03) and 29% HR of progression (5.8 vs 3.6 months, p = 0.03) in advanced, persistent or recurrent uterine CS [22]. Other agents such as topotecan appeared disappointing (response rate of 10%) [23]. Conventional cytotoxic and radiotherapeutic options have brought about limitation and challenges for clinical investigators toward uterine CS, thus exploring novel targeted therapies is necessary [24, 25].

The limitations of our study series are the retrospective nature, small sample size, long time span, and heterogeneous chemotherapeutic regimens. Nevertheless, the current study has added to the literature of results of consecutive uterine CS patients diagnosed and treated under the same surgical policy and principle of selecting adjuvant therapy in a tertiary referral medical center. Fighting a rare disease, every piece of deliberately collected information should be valued to support future prospective studies.

Conclusion

Age and depth of myometrial invasion were significant prognostic factors for CS, and adjuvant CT or CT-RT might be beneficial for outcome. This retrospective study suggests that all Stage IC-IV CS need adjuvant CT-RT, while optimal treatment for Stage IA-IB should be further elucidated. Prospective studies are warranted to validate the role of adjuvant therapy and to identify optimal CT regimens as well as novel targeted agents to overcome the high lethality of uterine CS.

References


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e-mail: sh46erry@ms6.hinet.net
Staining characterization by immunohistochemistry of tumor cancer antigen in patients with endometrial cancer

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¹Department of Gynecology, ²Department of Pathology, Ankara Oncology Education and Research Hospital, Ankara (Turkey)

Summary

Objective: The aim of the present study was to evaluate the correlation between the pattern of cancer antigen (CA-125) expression by immunohistochemistry and pathologic parameters in endometrial carcinoma. Methods: Seventy-two cases of primary uterine carcinomas, 66 endometrioid carcinoma and six non-endometrioid, were analyzed by immunohistochemistry for CA-125 expression. Myometrial invasion was evaluated by assessing the percentage of myometrial thickness involved at the site of deepest tumor extension. Presence or absence of vascular invasion, cervical stromal invasion, lymph node metastasis, and ovarian metastasis from endometrial cancer was assessed. Tumor size was measured by the maximum diameter. Peritoneal washings were examined for the presence or absence of cancer cells. The extent and location of immunohistochemical staining for CA-125 was assessed according to the immunoreactive score (IRS) that evaluated the proportion of cells expressing CA-125 and the intensity of staining. Percentage of the cancer area stained in high-power fields was examined. Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong); percentage of positive cells examined was scored as 0 (negative), 1 (< 10%), 2 (11-50%), 3 (51-80%), and 4 (> 80%). The two scores were multiplied and the IRS (values from 0-12) was determined: 0 as negative, values 1-3 as weak, values 4,6 as positive, and multiplication values 8, 9, 12 as strongly positive. Results: Of the 72 patients, 66 (91.7%) had endometrioid carcinoma and six (8.3%) had non-endometrioid carcinoma. Of the seventy-two patients, 38 (52.7%) had surgical Stage I disease, 12 (16.7%) had Stage II, 16 (22.2%) had Stage III disease, and six (8.4%) had Stage IV disease. Ten (14.7%) of the 68 patients who underwent lymphadenectomy had positive nodes. Nine (12.5%) of 72 patients had positive peritoneal cytologic findings. Forty-eight (66.7%) patients had deep myometrial invasion, 29 (40.3%) had lymphovascular invasion, 25 (34.7%) had cervical stromal involvement, and 12 (16.7%) had ovarian metastasis. Twenty-eight (38.9%) patients had grade 1, 25 (34.7%) had grade 2, and 19 (26.4%) had grade 3 disease. Fifty-nine (81.9%) patients had a tumor size greater than 2 cm. Negative staining was noted in ten (13.9%) tumors, weakly positive in 23 (31.9%), positive in 16 (22.3%) and strongly positive in 23 (31.9%). Grade 0 intensity was found in nine (12.5%) tumors, grade 1 in 16 (22.3%), grade 2 in 21 (29.16), and grade 3 in 26 (36.11). Negative percentage of positive cells examined was found in nine (12.5%) tumors, < 10% in 19 (26.38%), 11-50% in 18(25%), 51-80% in 13 (18.05%), > 80 in 13 (18.05%). We found that intensity, percentage of positive stained cells, and IRS correlated with deep myometrial invasion (p < 0.05). Conclusions: Intensity, percentage of positive stained cells for CA-125, and IRS can be used to determine the need for abdominal hysterectomy and lymphadenectomy for staging in endometrial cancer.

Key words: Endometrial carcinoma; CA-125; Immunohistochemistry.

Introduction

The tumor antigen CA-125 is expressed in the derivates of epithelium of müllerian origin (fallopian tube, cervix, and endometrium) and mesothelial cells lining (coelomic epithelium derivates) the peritoneum, pleura, and pericardium [1]. The frequency and tissue distribution of CA-125 expression in normal, hyperplastic, and neoplastic endometrium has been reported [2-4]. The endometrium has been shown to express high levels of CA-125 with an immunostaining [5] and high cytosolic tissue concentrations that are approximately 20-fold higher than normal ovarian tissue [6]. Weintraub et al. reported that CA-125 is an exocrine product of endometrial epithelial cells and is normally prevented from entry into the circulation. The plasma levels may be of endometrial origin only when the membrane barriers are damaged [7]. The use of CA-125 as a single diagnostic and prognostic tool for endometrial carcinoma has been restricted by the fact that it is produced by the peritoneum, normal cycle endometrium, and gestational endometrium. However, the pattern of CA-125 staining may be useful in distinguishing between low- and high-risk endometrial malignancies. Our study was designed to assess the intensity and distribution of staining correlation between pathologic parameters in endometrial cancer.

Materials and Methods

The study population consisted of 72 patients who were primarily treated by total abdominal hysterectomy and salpingo-oophorectomy, and bilateral pelvic and paraaortic lymphadenectomy for International Federation of Gynecology and Obstetrics (FIGO) Stage I-IV endometrial carcinoma between January 2002 and December 2005 at Ankara Oncology Education and Research Hospital.

Surgical procedures for endometrial cancers in our institution are defined as extended surgical staging consisting of washing cytology, total abdominal hysterectomy and bilateral salpingo-oophorectomy, with full pelvic and paraaortic lymphadenectomy. The tumors were surgically staged according to the FIGO staging system [8]. Endometrioid adenocarcinomas were graded according to FIGO classification; undifferentiated carcinoma, clear cell carcinoma, and papillary serous carcinoma were classified as grade 3 because the prognosis in these histo-
logic types is reported to be poor [9]. The histologic classification recommended by the World Health Organization Classification of Tumors was used [10]. Histologic types of endometrial cancer in the present study included endometrioid carcinoma, undifferentiated carcinoma, clear cell carcinoma, and papillary serous carcinoma; they were categorized as endometrioid and non-endometrioid (clear cell carcinoma, papillary serous carcinoma, undifferentiated carcinoma) groups. Formalin-fixed hematoxylin and eosin-stained 5-μm slides of tumor tissue from the same patients were performed again and reviewed by two senior pathologists to verify the diagnosis. Myometrial invasion was evaluated by assessing the percentage of myometrial thickness involved at the site of deepest tumor extension. Presence or absence of vascular invasion, cervical stromal invasion, lymph node metastasis, and ovarian metastasis from endometrial cancer was assessed. Tumor size was measured by its maximum diameter. Peritoneal washings were examined for the presence or absence of cancer cells.

**Immunohistochemistry of CA-125**

Briefly, 4-μm unstained sections from each of all 72 patients were prepared for immunohistochemical staining. After deparaffinization and rehydration, sections were placed in 3% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidase, and then autoclaved at 121°C in citrate buffer (10 mM, pH 6.0) for six minutes for antigen activation. After cooling at room temperature for 30 minutes the specimens were nonspecifically blocked by incubation with UltraV block for five minutes and endogenous avidin/biotin blocking kit for ten minutes each at room temperature. Sections were then incubated with anti CA-125 mouse monoclonal antibody (NeoMarkers, 1/20, Ab-1, Clone OV 185:1) for two hours at room temperature. Immunohistochemical staining was performed using a Standard avidin- biotin- peroxidase (Lab Vision); 3,3’-diaminobenzidine was used as the chromogen. All sections were counterstained with Mayer’s hematoxylin. Sections of ovarian serous carcinoma were used as positive controls.

The extent and location of immunohistochemical staining for CA-125 were assessed according to the IRS that evaluated the proportion of cells expressing CA-125 and intensity of staining [11]. The percentage of the cancer areas stained in high-power fields was examined. Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong); percentage of positive cells examined was scored as 0 (negative), 1 (< 10%), 2 (11-50%), 3 (51-80%), and 4 (> 80%). The two scores were multiplied and the IRS (value from 0-12) was determined: 0 as negative, 1-3 values as weak, 4,6 as positive, and values 8,9,12 as strongly positive.

**Statistical Analyses**

Statistical analyses were performed using the “SPSS 10.05 for Windows” computer program. All variables were analyzed statistically as categorical covariates. Several risk factors were evaluated in univariate analysis for any association with intensity, distribution, and IRS of CA-125 immunohistochemical staining; p values less than 0.05 derived from two-tailed tests were considered significant.

**Results**

Seventy-two women underwent surgery for endometrial adenocarcinoma during the 48-month period. Ages ranged from 35 to 87 years, with a mean age of 58.59 ± 9.87 years.

Four patients (5.6%) with grade 1 or grade 2 tumors underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy with collection of peritoneal fluid for cytologic testing. Sixty-eight (94.4%) patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy and bilateral pelvic together with periaortic lymphadenectomy, biopsies or debulking, or different combinations of these. All patients had peritoneal fluid collected for cytologic testing.

Table 1 shows the clinicopathologic profile of patients with endometrial carcinoma. Of the 72 patients, 66 (91.7%) had endometrioid carcinoma and six (8.3%) had non-endometrioid carcinoma. Of the seventy-two patients, 38 (52.7%) had surgical Stage I disease, 12 (16.7%) had Stage II, 16 (22.2%) had Stage III disease, six (8.4%) had Stage IV disease. Among patients with Stage I disease, one had Stage IA, 15 had Stage IB, and 22 had Stage IC disease. Ten (14.7%) of the 68 patients who underwent lymphadenectomy had positive nodes. Nine (12.5%) of the total 72 patients had positive peritoneal cytologic findings. Forty-eight (66.7%) patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of cases (%) n: 72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.59 ± 9.87 (35-87)</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>38 (52.7)</td>
</tr>
<tr>
<td>II</td>
<td>12 (16.7)</td>
</tr>
<tr>
<td>III</td>
<td>16 (22.2)</td>
</tr>
<tr>
<td>IV</td>
<td>6 (8.4)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>28 (38.9)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>25 (34.7)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>19 (26.4)</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>&lt; 1/2</td>
<td>23 (31.9)</td>
</tr>
<tr>
<td>≥ 1/2</td>
<td>48 (66.7)</td>
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<tr>
<td>Lymphovascular invasion</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>43 (59.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>29 (40.3)</td>
</tr>
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<td>Histologic subtype</td>
<td></td>
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<tr>
<td>Endometrioid</td>
<td>66 (91.7)</td>
</tr>
<tr>
<td>Non-endometrioid</td>
<td>6 (8.3)</td>
</tr>
<tr>
<td>Ovarian metastasis</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>60 (83.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>12 (16.7)</td>
</tr>
<tr>
<td>Lymph-node metastasis*</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>58 (85.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (14.7)</td>
</tr>
<tr>
<td>Washing cytology</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>63 (87.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (12.5)</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>47 (65.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>25 (34.7)</td>
</tr>
<tr>
<td>Tumor diameter (cm)</td>
<td></td>
</tr>
<tr>
<td>≤ 2</td>
<td>13 (18.1)</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>59 (81.9)</td>
</tr>
</tbody>
</table>

* No. of cases: 68.
had deep myometrial invasion, 29 (40.3%) had lymphovascular invasion, 25 (34.7%) had cervical stromal involvement, and 12 (16.7%) had ovarian metastasis. Twenty-eight (38.9%) patients had grade 1, 25 (34.7%) had grade 2, and 19 (26.4%) had grade 3 disease. Fifty-nine (81.9%) patients had a tumor with size greater than 2 cm.

Intensity varied from trace to very strong. Immunostaining was confined to the glandular epithelial cells and was present in the lumen, apical border (Figure 1). No staining of the basal border was observed in any case of endometrioid carcinoma.

Negative staining was noted in ten (13.9%) tumors, weakly positive in 23 (31.9%), positive in 16 (22.3%) and strongly positive in 23 (31.9%).

Grade 0 intensity was found in nine (12.5%) tumors, grade 1 in 16 (22.3%), grade 2 in 21 (29.16), and grade 3 in 26 (36.11).

A negative percentage of positive cells examined were found in nine (12.5%) tumors, < 10% in 19 (26.38%), 11-50% in 18 (25%), 51-80% in 13 (18.05%), and > 80 in 13 (18.05%).

We found that intensity, percentage of positive stained cells and IRS correlated with deep myometrial invasion (p < 0.05) (Table 2).

### Discussion

CA-125 has been localized by immunohistochemical techniques to the apical surface of secretory endometrial glands. Secretions in the endometrial gland lumina are intensely positive [12]. No staining of normal stromal or decidual cells has been demonstrated. CA-125 immunostaining in endometrial cancers has been reported in few studies [4].

The distribution and expression of CA-125 suggests that the antigen was a secretory product of normal endometrium [3]. CA-125 was expressed by the glandular tissue in the endometrial carcinomas and accumulated on the apical cell surface and in cytoplasm. Tumors with solid features had a lesser glandular component and had less CA-125 expression. Endometrioid grade 1 adenocarcinomas were more likely to express CA-125. Tumors of the clear cell and papillary serous histologic types contributed disproportionately to the positive staining seen in grade 2 and grade 3 tumors [3]. In our study, 25 of 28 (89.2%) endometrioid grade 1 tumors were CA-125 positive and the CA-125 immunostaining was intense in low-grade and early-stage endometrial carcinoma.

Berchuck et al. evaluated CA-125 expression in endometrial adenocarcinomas using a histologic score that evaluated the proportion of cells expressing CA-125 and the intensity of staining [13]. A high CA-125 score correlated with the presence of lymph-node metastases and increased metastatic potential. In our study, positive CA-125 staining (IRS > 0) significantly correlated with deep myometrial invasion (p < 0.05).

Nur et al. [14] showed that patients with endometrial carcinoma and solitary metastases to the ovaries with lymph-node involvement had lower IRS. Thus in cases of endometrial carcinoma as the tumor becomes aggressive, it loses its functional capabilities. The source of elevated serum CA-125 levels in such patients may be due to secretions by mesothelial cells rather than neoplastic endometrial cells.

### Table 2. — Relationship between intensity, distribution, and immunoreactivescore (IRS) of immunohistochemical CA-125 staining and pathologic variables of prognostic significance in patients with endometrial carcinoma.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Intensity of staining (≥ 1 grade)</th>
<th>Distribution of staining (1-100%)</th>
<th>IRS (≥ 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 50</td>
<td>10 (90.9)</td>
<td>10 (90.9)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>53 (86.9)</td>
<td>0.584</td>
<td>52 (85.2)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>46 (90.2)</td>
<td>46 (90.2)</td>
<td>45 (88.2)</td>
</tr>
<tr>
<td>III-IV</td>
<td>17 (81)</td>
<td>0.240</td>
<td>17 (81)</td>
</tr>
<tr>
<td>Tumor Size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2 cm</td>
<td>11 (84.6)</td>
<td>11 (84.6)</td>
<td>11 (84.6)</td>
</tr>
<tr>
<td>&gt; 2 cm</td>
<td>52 (88.1)</td>
<td>0.514</td>
<td>51 (86.4)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>57 (86.4)</td>
<td>57 (86.4)</td>
<td>56 (84.8)</td>
</tr>
<tr>
<td>Non-endometrioid</td>
<td>6 (100)</td>
<td>0.435</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25 (89.3)</td>
<td>25 (89.3)</td>
<td>25 (89.3)</td>
</tr>
<tr>
<td>2, 3</td>
<td>38 (86.4)</td>
<td>0.509</td>
<td>37 (84.1)</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1/2</td>
<td>18 (75)</td>
<td>18 (75)</td>
<td>17 (70.8)</td>
</tr>
<tr>
<td>≥ 1/2</td>
<td>45 (93.8)</td>
<td>0.032</td>
<td>45 (93.8)</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41 (87.2)</td>
<td>41 (87.2)</td>
<td>40 (85.1)</td>
</tr>
<tr>
<td>Yes</td>
<td>22 (88)</td>
<td>0.620</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Ovarian metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>53 (88.3)</td>
<td>53 (88.3)</td>
<td>52 (86.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (83.3)</td>
<td>0.466</td>
<td>10 (83.3)</td>
</tr>
<tr>
<td>Washing cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>55 (87.3)</td>
<td>55 (87.3)</td>
<td>54 (85.7)</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (88.9)</td>
<td>0.688</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td>LVI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>37 (86)</td>
<td>37 (86)</td>
<td>36 (83.7)</td>
</tr>
<tr>
<td>Yes</td>
<td>26 (89.7)</td>
<td>0.471</td>
<td>26 (89.7)</td>
</tr>
<tr>
<td>Lymph-node metastasis</td>
<td></td>
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<tr>
<td>No</td>
<td>51 (87.9)</td>
<td>51 (87.9)</td>
<td>50 (86.2)</td>
</tr>
<tr>
<td>Yes</td>
<td>8 (80)</td>
<td>0.395</td>
<td>8 (80)</td>
</tr>
</tbody>
</table>

Figure 1. — Positive staining for CA-125 is prominent at the apical cell surface (x 200).
The prognosis of endometrial carcinoma has major implications on patient management. In our study, intensity and distribution of staining correlated with deep myometrial invasion (p < 0.05). Intensity, percentage of positive stained cells, and IRS can be used to determine the need for abdominal hysterectomy and lymphadenectomy for staging in endometrial cancer. Further studies are required to correlate this hypothesis.

References


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Comorbidity and age affect treatment policy for cervical cancer: a population-based study in the south of the Netherlands, 1995-2004

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Summary

Objective. The aim of this study was to estimate the effects of age and comorbidity on the choice of treatment modalities and prognosis for patients with cervical cancer. Methods. All patients with cervical cancer newly diagnosed between 1995 and 2004 (n = 775) were selected from the population-based Eindhoven Cancer Registry. Time trends in treatment modalities and differences in treatment between older and younger patients, and those with and without comorbidity were evaluated. Results. Older patients with FIGO Stages IB–IIA, elderly and those with comorbidity underwent less surgery. In multivariate survival analysis, age had independent prognostic value. For patients with FIGO Stages IB2, IIB–IVA, age affected the choice of chemoradiation significantly. According to multivariate survival analysis, comorbidity and FIGO stage were independent prognostic factors. Conclusion. Older patients with cervical cancer and those with comorbidity were treated less aggressively. Because of the ever-increasing role of comorbidity in clinical decision-making for increasingly older patients in the near future, development of age-specific guidelines incorporating levels and management of specific comorbidity seems warranted.

Key words: Cervical cancer; Comorbidity; Radical hysterectomy; Radiotherapy; Chemoradiation.

Introduction

As in most northwestern European populations, the incidence of and mortality from cervical cancer have been decreasing in the Netherlands [1]. The main risk factor for cervical cancer, Human Papillomavirus (HPV), is found in almost all patients with cervical cancer, being strongly related to sexual behaviour, especially with multiple partners and early age at first intercourse [2]. Smoking markedly affects risk while a large number of live births and oral contraceptive use are also risk indicators [3, 4].

According to the national recommendations in 1990 for FIGO Stage IB and IIA cervical cancer, primary surgery and radiotherapy were equal therapeutic options, the choice depending mainly on patient characteristics such as age and comorbidity. Radiotherapy was the treatment of first choice for FIGO Stages IIB–IVA [5]. In 1999 the American National Cancer Institute (NCI) announced that adding chemotherapy to radiation therapy was highly recommended. This statement was based on five clinical trials which demonstrated the superiority of combined platinum-based chemoradiation over radiotherapy alone for patients with high risk and/or locally advanced cervical cancer [6-10]. A Dutch trial combining radiotherapy with hyperthermia also resulted in a significant improvement in the 3-year overall survival for patients with FIGO Stages IIB–IVA [11]. Therefore, from 2004 on the revised national guideline recommends primary chemoradiation or radiotherapy combined with hyperthermia for patients with FIGO Stage IB2, IIB and higher [12].

In general, treatment guidelines are based on the results of clinical trials from which patients with comorbidity and/or older age are often excluded. However, treatment of individual patients will be affected by age and comorbidity [13]. Therefore, we studied the influence of age and comorbidity on the treatment modalities chosen and the ultimate survival of unselected patients with cervical cancer.

Materials and Methods

Data collection

All patients with cervical cancer diagnosed between 1 January 1995 and 31 December 2004 (n = 775) were selected from the Eindhoven Cancer Registry, where data is recorded on all patients newly diagnosed with cancer in the southern part of the Netherlands, an area with 2.3 million inhabitants that is served by the Comprehensive Cancer Centre South (IKZ). It consists of ten community hospitals at 16 sites and two large radiotherapy institutes in Tilburg and Eindhoven.
Table 1.—Classification of comorbidity, according to an adapted list of Charlson et al. [14].

<table>
<thead>
<tr>
<th>Comorbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous malignancies (except basal cell skin carcinoma and cervix carcinoma in situ)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary diseases</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
</tr>
<tr>
<td>− Myocardial infarction</td>
</tr>
<tr>
<td>− Heart failure</td>
</tr>
<tr>
<td>− Angina pectoris</td>
</tr>
<tr>
<td>− Intermittent claudication</td>
</tr>
<tr>
<td>− Abdominal aneurysm</td>
</tr>
<tr>
<td>− Cardiomyopathy</td>
</tr>
<tr>
<td>− Valve prosthesis (aorta or mitralis)</td>
</tr>
<tr>
<td>Cerebrovascular diseases</td>
</tr>
<tr>
<td>− Cerebrovascular accident</td>
</tr>
<tr>
<td>− Hemiplegia</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Digestive tract diseases</td>
</tr>
<tr>
<td>− Ulcerative disease (only registered since 1997)</td>
</tr>
<tr>
<td>− Patients who underwent major surgery for ulcerative disease (Billroth I or II)</td>
</tr>
<tr>
<td>− Chronic inflammatory diseases (Crohn’s disease, ulcerative colitis except polyposis coli)</td>
</tr>
<tr>
<td>Liver disease (cirrhosis, hepatitis)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>− Urinary tract diseases</td>
</tr>
<tr>
<td>− Connective tissue diseases</td>
</tr>
<tr>
<td>− Dementia</td>
</tr>
<tr>
<td>− Chronic infections</td>
</tr>
</tbody>
</table>

After notification from the pathological laboratories, trained registration clerks collect information from the medical records on diagnosis, tumour stage and treatment. To explore the increasing complexity of oncological care in an older population, serious comorbidity with prognostic impact at the time of cancer diagnosis has been recorded for all patients since 1993, according to a slightly modified version of the Charlson index (Table 1) [14]. Information on comorbidity is obtained from reports on previous admissions, letters from and to other specialists, the medical history, and preoperative screening. In the absence of information on comorbidity in the patient files, the registrars have to code this as ‘unknown’. Patients for whom comorbidity was unknown were excluded from the survival analyses (n = 37 with FIGO Stage IB-IIA and n = 37 with FIGO Stage IB2, IIB-IVA).

Tumour stage was defined according to the FIGO staging system, based on preoperative clinical information. Only patients with FIGO Stage IB-IVA were included for further analysis of treatment and survival. Because of the different treatment recommendations, the patients were divided into two groups: FIGO Stages IB (excluding IB2)-IIA and FIGO Stages IB2, IIB-IVA. FIGO Stage IB2 was included in the Stage group IIB-IVA because treatment of FIGO IB2 is considered to be chemoradiation since the publication of the National Cancer Institute in 1999 [10]. Although FIGO Stage IB was divided into Stages IB1 and IB2 in 1997, this modification has been included in the cancer registry only since 1999 [15].

Treatment of patients with FIGO Stages IB-IIA was classified as surgery (± radiotherapy, ± chemotherapy), radiotherapy (± chemotherapy) and other/none (palliative, lymph node dissection only, chemotherapy only, metastasectomy and unknown therapy). Treatment for FIGO Stages IB2, IIB-IVA was classified as radiotherapy, chemoradiation (including radiotherapy combined with hyperthermia, n = 2), surgery (± radiotherapy, ± chemotherapy) and other/none (palliative, lymph node dissection only, chemotherapy only, metastasectomy and unknown therapy).

Socioeconomic status (SES) was considered to be a possible confounder. The SES of each patient was defined at the neighbourhood level (based on postal code of residence, 17 households on average) combining mean household income and mean value of the house, derived from individual fiscal data made available at an aggregated level. Postal codes were assigned to three SES categories: low (1st-3rd decile), intermediate (4th-7th decile) and high (8th-10th decile). Postal codes of institutions, such as nursing homes, were assigned to a separate category and excluded from the analyses of SES (n = 39).

Vital status was available up to January 1, 2006. In addition to passive follow-up via the hospitals, this information was also obtained through the National Genealogical Office and the Municipality Administration Database, where all deceased and emigrated persons in the Netherlands are registered via the civil municipal registries.

Statistical analysis

The prevalence of comorbidity was analysed according to age, dividing younger patients and the elderly (< 70 and ≥ 70 years). Time trends in treatment modalities and differences in treatment between patients with and without comorbidity were assessed by Chi-square analysis overall, and by age group. Crude 5-year survival rates were computed, survival time being the time from diagnosis to death or January 1, 2006. The log-rank test was performed to evaluate significant differences between survival curves in univariate analyses. A multivariate Cox regression model was constructed for evaluation of the independent prognostic effects of age and comorbidity on survival. The independent prognostic effects of age and comorbidity were first estimated using a model without treatment modality. Then treatment was included in the model in order to investigate whether the prognostic effects of age and comorbidity could be fully explained by the treatment modality chosen. The prognostic effect of the number of comorbid conditions was also evaluated. The prognostic impact of specific diseases and combinations of diseases could not be evaluated because the number of patients in each subgroup was too small. Hazard ratios (HR) and 90% confidence intervals (CI) were calculated. Due to the small number of patients in each subgroup, p-values of 0.10 were considered significant. The period of diagnosis, SES and FIGO stage were divided into categories and entered into the model as dummy variables using a stepwise approach. Variables were considered confounders and included in the model when the regression coefficient of the variable of interest (treatment) changed by more than 10%. Separate analyses were performed for survival of those with Stages IB-IIA and Stages IB2, IIB-IVA. Furthermore, relative survival was calculated to estimate differences between the two age groups as a measure of disease-specific survival using the Ederer II method in STATA version 9.2 [16]. Relative survival is the ratio between crude and expected survival and approaches disease-specific survival. Relative survival was used only to estimate differences between age groups since overcorrection would occur if patients without comorbidity were compared with the general population.
Results

General

All patients with cervical cancer diagnosed between 1 January 1995 and 31 December 2004 (n = 775) were included in this study. The median age of the patients in this study was 48 years (range 15-100), 81% being younger than 70 years at diagnosis. Most patients presented with FIGO Stage IB (excluding IB2)-IIA (37%, n = 288), followed by 28% of patients with FIGO IA (n = 200) and 26% of patients with FIGO Stages IB2, IIB-IVA (n = 214). Six percent of the patients presented with metastatic disease (n = 46). FIGO stage was unknown in 3% of cases (n = 27).

The proportion of patients with one or more comorbid conditions at the time of diagnosis was 18% for patients aged < 70 and 59% for patients aged ≥ 70 (p < 0.001). The most frequent comorbidity in both age categories was hypertension. Cardiovascular diseases and diabetes were also very common among those aged ≥ 70 (Table 2).

Table 2. — Number and type of comorbid conditions present in newly diagnosed patients with cervical cancer in south-eastern Netherlands, 1995-2004, according to age group.

<table>
<thead>
<tr>
<th>Number of comorbid conditions</th>
<th>&lt; 70 yrs a (%)*</th>
<th>≥ 70 yrs a (%)*</th>
<th>Total a (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>408 (65)</td>
<td>43 (29)</td>
<td>451 (58)</td>
</tr>
<tr>
<td>1</td>
<td>84 (13)</td>
<td>49 (33)</td>
<td>133 (17)</td>
</tr>
<tr>
<td>2 or more</td>
<td>27 (4)</td>
<td>40 (27)</td>
<td>67 (9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>106 (17)</td>
<td>18 (12)</td>
<td>124 (16)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of comorbid condition</th>
<th>&lt; 70 yrs a (%)*</th>
<th>≥ 70 yrs a (%)*</th>
<th>Total a (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous cancer</td>
<td>20 (3)</td>
<td>15 (10)</td>
<td>35 (5)</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>22 (4)</td>
<td>38 (25)</td>
<td>60 (8)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>36 (6)</td>
<td>42 (28)</td>
<td>78 (10)</td>
</tr>
<tr>
<td>COPD</td>
<td>23 (4)</td>
<td>9 (6)</td>
<td>32 (4)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>24 (4)</td>
<td>28 (19)</td>
<td>52 (7)</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>4 (1)</td>
<td>10 (7)</td>
<td>14 (2)</td>
</tr>
<tr>
<td>Dementia</td>
<td>0 (0)</td>
<td>3 (2)</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>Digestive tract</td>
<td>7 (1)</td>
<td>1 (1)</td>
<td>8 (1)</td>
</tr>
<tr>
<td>Other</td>
<td>14 (2)</td>
<td>6 (4)</td>
<td>20 (3)</td>
</tr>
</tbody>
</table>

* One patient may have more than one comorbid condition, so the total of all comorbid conditions can be more than 100% (i.e., more than the number of patients in the study).

Table 3. — Treatment of cervical cancer in south-eastern Netherlands according to FIGO stage, age and comorbidity, 1995-2004.

<table>
<thead>
<tr>
<th>Age</th>
<th>Comorbid conditions</th>
<th>FIGO IB-IIA</th>
<th>FIGO IB2, IIB-IVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surgery</td>
<td>RT*</td>
<td>Other/none</td>
</tr>
<tr>
<td>&lt; 70</td>
<td>145 (92)</td>
<td>8 (5)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>1+</td>
<td>33 (69)</td>
<td>13 (27)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>21 (62)</td>
<td>3 (9)</td>
<td>10 (29)</td>
</tr>
<tr>
<td>≥ 70</td>
<td>14 (41)</td>
<td>18 (53)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>unknown</td>
<td>1 (33)</td>
<td>0 (0)</td>
<td>2 (67)</td>
</tr>
</tbody>
</table>

*RT = radiotherapy, CHEMRT = chemoradiation (including 2 patients who received radiotherapy + hyperthermia).

Crude five-year survival rates were significantly worse for patients aged ≥ 70 (50% vs 80%, respectively), for patients with FIGO Stage IIA (65% versus 78% for FIGO IB and 79% for FIGO IB1, respectively), and for patients with comorbidity (83% without, 66% with one, and 48% with two or more comorbid conditions) (Table 4). Survival for patients with FIGO Stage IB-IIA receiving primary radiotherapy was 47% versus 81% for those who underwent primary surgery. No effect on survival was found for period of diagnosis or SES. According to multivariate survival analyses, age was the only independent prognostic indicator (Table 4). The risk of dying increased by 2% with every additional year in age. The hazard ratios for age and comorbidity did not change when primary treatment was introduced into the model.

FIGO IB2, IIB-IVA

Median age of patients with FIGO Stages IB2, IIB-IVA was 57 years (range 28-94 years). Patients aged ≥ 70 suffered comorbidity more frequently than patients aged < 70 (64% vs 21%, p < 0.001). Especially age had a significant influence on the choice of treatment: 28% of patients aged < 70 received chemoradiation, 30% of those without comorbidity and 38% of those with at least one comorbidity. Only 3% of patients aged ≥ 70 received chemoradiation, 5% of those without comorbidity and none of those with at least one comorbidity (p < 0.001) (Table 3). Differences in the use of chemoradiation according to the presence of comorbidity, within both age categories, were not significant. A small group of patients with FIGO Stages IB2, IIB-IVA without comorbidity underwent surgery more often than patients with one or more comorbid conditions (n = 18 vs n = 2, p < 0.001). The use of chemoradiation increased from 9% in the period 1995-1997 to 32% in the period 2001-2004 (p = 0.01), i.e., 41% of patients aged < 70 and 5% of patients aged ≥ 70 (p = 0.02) in the latter period. Analysing the time trend per year revealed that the use of chemoradiation had already started to increase from 1999, the year of the clinical alerts of the NCI (p = 0.01). The number of patients who received radiotherapy combined with hyperthermia was too small (n = 2) to reveal a time trend.

FIGO IB (excluding IB2)-IIA

Median age of patients with FIGO Stages IB-IIA was 47 years (range 24-88 years). Patients aged ≥ 70 exhibited comorbidity more frequently than patients aged < 70 (76% vs 23%, p < 0.001). Both age and presence of comorbidity had a significant influence on the choice of treatment modality. Eighty-three percent of patients aged < 70 underwent surgery as the primary treatment, i.e., 92% of those without comorbidity and 69% with at least one comorbidity condition (p < 0.001). In contrast, only 46% of patients aged ≥ 70 years underwent primary surgery: 73% of those without comorbidity and 41% with at least one comorbidity condition (p = 0.006) (Table 3).

Five-year relative survival for patients aged ≥ 70 was 61% versus 81% for patients aged < 70 years (p = 0.005).
Five-year relative survival for patients aged ≥ 70 was worse compared to patients aged < 70 (32% vs 51%, p = 0.05). According to univariate analysis, five-year crude survival was significantly worse for patients aged ≥ 70 (24%, compared to 48% for patients aged < 70), those with one comorbid condition (24%, compared to 42% without comorbidity), for patients with FIGO IIIA (33%), IIIB (23%) and IVA (16%) compared to patients with FIGO IB2 or IIB (54% and 55%, respectively) and for those receiving radiotherapy (38% compared to 49% for patients receiving chemoradiation and 57% for surgery) (Table 4). No effect was found for period of diagnosis and SES. According to multivariate survival analysis, comorbidity and FIGO were independent prognostic factors (Table 4). The risk of dying for patients with one comorbid condition was twice as high as that for patients without comorbidity. Furthermore, the risks of death of patients diagnosed with FIGO IIIA, IIIB, and IVA were 2.0, 3.5 and 7.7 times higher respectively, compared to patients diagnosed with FIGO IB. The hazard ratios for age and comorbidity did not change when treatment was introduced into the model.

Discussion

Substantial variations were found in the treatment of women with cervical cancer in this retrospective population-based study. In previous studies concerning patients with FIGO Stage IB (excluding IB2)-IIA cervical cancer, primary surgery and radiotherapy were shown to be equal therapeutic options, resulting in similar outcomes [17, 18]. However, the present study showed that for elderly patients, especially in the presence of comorbidity, radiotherapy remained the treatment of first choice. For patients with FIGO Stages IB2, IIB-IVA cervical cancer, age especially influenced the therapy of choice: radiotherapy or chemoradiation. Only 5% of patients aged 70 years or older received chemoradiation versus 41% of patients younger than 70 years in the period 2001-2004. As a matter of fact, chemoradiation was proposed as a superior alternative to radiotherapy alone in 1999 but was only incorporated in the guidelines in 2004. It is known that the elderly are less likely to be included in clinical trials and to receive aggressive therapy because of considerations concerning patient safety [19, 20]. In addition, older women are more likely than their younger counterparts to refuse aggressive treatment [21, 22]. We found that older patients and patients with comorbidity were indeed treated differently compared to younger
patients and patients without comorbidity with both lower and higher FIGO stages. Both the patient’s and the doctor’s preference might play a major role in the explanation of this phenomenon. We had no further information on this topic.

Relative survival (adjusting for survival in the general population of the same age) for patients with FIGO Stage IB (excluding IB2)-IIA older than 70 years was worse than that for their younger counterparts, which may be explained by the higher proportion of FIGO IIA tumours in older patients (p < 0.001). However, also according to a multivariate analysis age was the only independent prognostic indicator after adjustment for other prognostic factors as comorbidity and treatment, which has also been confirmed in another recent study [23]. In FIGO Stages IB2, IIB-IVA, prognosis was determined by the number of comorbid conditions and FIGO stage. Patients with one comorbid condition exhibited worse survival compared to patients without comorbidity. In contrast, the increased risk of death for the rather small group of patients with multiple comorbid conditions did not reach statistical significance. Furthermore, no change was seen in the hazard ratio for age when treatment was included in the model. Treatment was not an independent prognostic factor for either stage group, which could indicate that the right treatment modality was in general offered to the right patient. Nevertheless, worse survival was found for patients who received ‘other therapies’ or no therapy and most of these patients were elderly patients.

As cervical cancer is assigned a FIGO stage based on specified clinical tests, it is not uncommon for the physician to have other non-specified tests at their disposal (CT scan, MRI). Often it is known that the patient has metastatic disease but the FIGO stage can not officially be upstaged based on these findings. However, the treatment choice certainly is affected. This bias is present and may be a major confounder for any analysis of stage-adjusted outcome based on treatment modality.

Although this population-based study has the advantage of avoiding selection bias, detailed and uniform information on the performance status of the patient, adherence to protocol (dose reduction, treatment delay) for radio- and/or chemotherapy and treatment-related complications were not available. These and other factors which determine frailty, for example cognitive disorders, might also affect the choice of treatment and prognosis of the patients.

Although severity of comorbidity was not recorded, misclassification of comorbidity seems to be limited because the concomitant conditions are recorded routinely by trained registry personnel directly from the medical records of the patients, thereby using a variety of sources. A validation study of breast cancer patients showed some under-registration, mainly for less severe cardiovascular conditions [24]. Furthermore, not all cases of non insulin-dependent diabetes are subclinical, implying that the prognosis of patients without diabetes might therefore be underestimated. The true effects of comorbidity on treatment choice and survival may thus be stronger than described here.

In conclusion, in cervical cancer, treatment modalities chosen but also prognosis differed between younger and older patients and between patients with and without comorbidity. Attention should be directed toward treatment in relation to ageing and comorbidity. In an increasingly older population (on the basis of recent numbers of population growth it is estimated that the number of women over 65 years will increase by 23% [25]), comorbidity and other factors that determine frailty - such as performance status - will probably play an increasing role in clinical decision-making and outcome. Development of age-specific guidelines, which incorporate levels of comorbidity and for example performance score, may therefore be warranted. Furthermore, this may lead to an increased awareness of comorbidity among physicians.

References


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Prognostic markers of low-grade squamous intraepithelial lesions: the role of topoisomerase IIα and active caspase-3

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¹Department of Gynecology, and ²Department of Pathology, Federal University of São Paulo (UNIFESP-EPM), São Paulo (Brazil)

Summary

Purpose: To study the relationship between topoisomerase IIα, active caspase-3 expressions and HPV DNA in uterine cervices with low-grade squamous intraepithelial lesions (LSIL). Methods: Forty women with LSIL and 32 without cervical neoplasia diagnosed through cytologic and histopathologic examination were evaluated regarding topoisomerase IIα and active caspase-3 expressions and HPV DNA detection using PCR (GP5/GP6) in cervicovaginal smears. Results: The mean percentage of cells immunomarked by topoisomerase in the group with LSIL was 11.62% while in the control it was 4.13% (p < 0.0001). In the presence of HPV DNA, topoisomerase expression was higher in the group with productive viral infection than in nonneoplastic tissue (p = 0.004). Caspase-3 expression was observed in 17 patients with LSIL (42.5%) and in five without cervical neoplasia (15.63%). Conclusion: The use of topoisomerase IIα and active caspase-3 in cervical biopsies may help to define the prognosis of HPV cervical infection.

Key words: Topoisomerase II alpha; Caspase-3; HPV; Neoplasia.

Introduction

Human papillomavirus is highly prevalent in sexually active women. Persistence of the viral infection is associated with a higher risk for development of intrap epithelial lesions, mainly in the presence of high-risk viral types [1, 2]. The “ideal” screening test should initially target persistent high-risk HPV infections with superimposed cellular dysregulation, also detecting higher-grade cervical intraepithelial neoplasia (CIN) or invasive cancer, searching for the real potentially progressive oncogenic lesions [3]. Viral infection stimulates cell proliferation, while the host generates defense mechanisms against multiplication of viral particles through activation of apoptosis. DNA-topoisomerases I and II are nuclear enzymes present in all cells that act on condensation, chromosomal segregation and genic expression [4, 5]. Apoptosis involves caspases, pro-apoptotic proteases which destroy essential proteins or activate toxic proteins. Some are initiators and others, effectors, activated by other caspases [6-8]. Once activated, caspase-3 generates lysis of cell proteins, such as polymerases and epidermal growth factor receptor [9, 10]. Among the identified caspases in humans, caspase-3 is the one best related to apoptosis.

The present study was aimed to evaluate topoisomerase IIα and active caspase-3 immunohistochemical expressions in addition to HPV DNA presence in patients with low-grade squamous intraepithelial lesions (LSIL) and without cervical neoplasia, in the search for defining markers of productive viral infection and the potentiality for clinical progression.

Materials and Methods

Seventy-two patients examined in the Gynecology Department of the Federal University of São Paulo (UNIFESP-EPM) were selected: 40 with LSIL, diagnosed through cytologic, colposcopic and histopathologic examination, and 32 without HPV-induced cytohistopathologic lesions. Women with previous treatment of the lower genital tract, immunosuppressed, or pregnant were excluded. The project was approved by the Ethical Committee of the Institution.

Patients were submitted to collection of material for cervicovaginal smears and for detection of HPV DNA, colposcopic evaluation, and tissue sampling of anormal findings. Both groups included only women with concordant cytohistopathologic diagnoses. Biomolecular HPV detection was performed by PCR (polymerase chain reaction) with Gp5/Gp6 primer.

Immunohistochemical reactions for topoisomerase IIα and active caspase-3 determination were performed with the biotin-streptavidine-peroxidase method. After hydration and blockade, incubation with monoclonal human anti-topoisomerase II alpha antibody (DakoCytomation S/A Denmark, code M7186, 1:50 titer) and polyclonal rabbit anti-active caspase antibody (Chemicon International Inc., 1:25 titer) was carried out. The slides were incubated overnight at a temperature of 2° to 8°C and then washed in PBS and incubated using the Kit System-HPR (DakoCytomation K0690). After this step, revelation with the chromogen 3,3-diaminobenzidine (DAB) (Sigma Chemical Co., St. Louis, MO, USA) and staining with Harris hematoxylin (Merck, Darmstadt, Germany) followed.

The staining for topoisomerase IIα was considered positive in the cells whose nuclear staining was brownish, and negative in the absence of staining or in those weekly stained. Active caspase-3 expression was considered positive in the cells whose nuclear and cytoplasmic staining was brownish, and negative in its absence. Counting was manual by two independent observers, with a minimum of near 200 cells, as described by HAFIAN et al. [11].

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Comparison of the quantitative variable means between the case and control group was performed with the Student’s t-test for two independent samples. Fisher’s exact test was utilized for the analysis of homogeneity and of association in contingency tables. In both tests, the values \( p \leq 0.05 \) were regarded as statistically significant.

**Results**

Seventy-two patients, 40 with LSIL and 32 controls without neoplasia, were evaluated analyzing immunohistochemistry together with HPV testing. Analyzing topoisomerase II\(\alpha\), a mean of 11.62% immunomarked cells was observed in the case group and 4.13% in the control, with epithelial distribution of stained cells specially in the basal and parabasal layers (\( p < 0.0001 \)) (Table 1). Immunohistochemical evaluation of active caspase-3 revealed nuclear and cytoplasmic marking by brownish poorly delimited staining, more evident in the epithelial layers near the basal layer, of difficult quantification. Active caspase-3 expression was evident in 17 (42.5%) patients with LSIL and in five (15.63%) control patients (\( p = 0.020 \)) (Table 1). Topoisomerase II\(\alpha\) expression in patients with positive caspase-3 was 12.05%, while it was 6.71% in those negative for caspase-3 (\( p = 0.0024 \)).

Table 1. — Distribution of patients in the case and control groups according to topoisomerase II\(\alpha\) and active caspase-3 expression.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean %</th>
<th>SD</th>
<th>( p )</th>
<th>N</th>
<th>Active caspase-3+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSIL</td>
<td>11.62</td>
<td>7.31</td>
<td>&lt;0.0001</td>
<td>17</td>
<td>42.50</td>
<td>40</td>
</tr>
<tr>
<td>control</td>
<td>4.13</td>
<td>3.48</td>
<td></td>
<td>5</td>
<td>15.63</td>
<td>32</td>
</tr>
</tbody>
</table>

\( ^* = \) Fisher’s exact test; \( ^\dagger = \) Student’s t-test; SD = standard deviation; LSIL = low-grade squamous intraepithelial lesions; N = number of patients.

Table 2. — Distribution of HPV infected patients in the case and control groups according to topoisomerase II\(\alpha\) and active caspase-3 expression.

<table>
<thead>
<tr>
<th>Group</th>
<th>LSIL, HPV+</th>
<th>Control, HPV+</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topoisomerase II(\alpha)</td>
<td>11.94%</td>
<td>4.31%</td>
<td>0.004 (^*)</td>
</tr>
<tr>
<td>Active caspase-3</td>
<td>9 (34.6%)</td>
<td>3 (15.8%)</td>
<td>0.191 (^*)</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>19</td>
<td>45</td>
</tr>
</tbody>
</table>

\( ^* = \) Fisher’s exact test; \( ^\dagger = \) Student’s t-test; LSIL = low-grade squamous intraepithelial lesions.

**Discussion**

The use of molecular markers in cervical intraepithelial lesions would offer a better analysis of risk for productive HPV infection. In this study a significant difference between mean topoisomerase II\(\alpha\) and active caspase-3 positivity indices was observed for patients with and without cytohistologic LSIL, which may suggest an increase in apoptosis in answer to atypias. There seems to be an association between increased proliferative activity and degree of apoptosis.

Several studies are concordant with our results regarding presence of HPV DNA [12-18]. The high prevalence of HPV DNA in our control group may be explained by the fact of dealing with a specialized outpatient clinic. Possible explanations for the high proportion of LSIL cases on cytohistopathologic examination with a negative result for HPV DNA by PCR in this study are: 1) viral clearance in the interval between the reference cytohistopathologic examination and sample collection for PCR; 2) false-positive diagnosis of LSIL, not very probably due to confirmation of the cases by an experienced pathologist; 3) LSIL cases without HPV, constituting a real biologic entity, as suggested by the study of Burger et al. [4] small amount of cells collected for PCR, because it was performed after collection of material for cytopathologic examination, with frequent bleeding and local inflammatory processes [19, 20].

In the presence of HPV DNA, the expression of topoisomerase II\(\alpha\) in the group with productive viral infection was higher than in nonneoplastic tissue. Samples with a higher mean topoisomerase II\(\alpha\) index expressed more active caspase-3, and were more evident in the case group. The use of topoisomerase II\(\alpha\) may help define which patients with viral infection will produce cervical lesions. There is a need for prospective studies with numerous LSIL to associate this finding with real progressive lesions and even to establish new guidelines for the management of these patients.

**References**


Prognostic markers of low-grade squamous intraepithelial lesions: the role of topoisomerase IIα and active caspase-3


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Extraovarian peritoneal serous papillary carcinoma mimicking colonic obstruction


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Summary

Involvement of the colon by extraovarian peritoneal serous papillary carcinoma (EPSPC) is considered as rare. During a 10-year period the records of five female patients with a mean age of 73.4 years who were admitted for colonic obstruction due to EPSPC were reviewed. Preoperative and postoperative data were studied. All patients presented with symptoms of colonic obstruction and high concentrations of CA-125. Involvement of the sigmoid colon was demonstrated preoperatively both in CT and colonoscopy. Operative findings of multiple peritoneal implantations involving the surface of the ovaries in two cases, the greater omentum in three cases and invasion of the sigmoid colon in all cases prompted us to perform sigmoidectomy and omentectomy in all cases with bilateral salpingo-oophorectomy in four of them. All patients received adjuvant paclitaxel plus platinum-based combination chemotherapy.

Key words: Extraovarian peritoneal serous papillary carcinoma; Staging; Surgery; Chemotherapy.

Introduction

Extraovarian peritoneal serous papillary carcinoma (EPSPC) is a rare neoplasm mainly characterized by diffuse peritoneal spread while sparing or minimally involving the ovaries. Initially described by Swerdlow in 1959 [1] as a “mesothelioma” resembling ovarian cancer and further categorized by Feuer et al in 1989 [2] in “normal-sized ovarian carcinoma syndrome”, the EPSPC is currently defined by criteria developed by the Gynecologic Oncology Group (GOG) [3]: a) both ovaries must be either normal in size or enlarged by a benign process (4 cm in largest diameter), b) involvement of extraovarian sites must be greater than that on the surface of either ovary, c) microscopically, the ovarian component must be nonexistent or confined to the ovarian surface with no evidence of cortical invasion or involving the ovarian surface and underlying cortical stroma but less than 5 x 5 mm or less than 5 x 5 mm within the ovarian substance associated with or without surface disease and d) the histologic and cytologic characteristics of the tumor must be predominantly of the serous type, similar or identical to ovarian serous papillary adenocarcinoma of any grade.

While distinct from malignant mesotheliomas these tumors are preoperatively diagnosed as ovarian malignancy with exploratory laparotomy revealing diffuse abdominal carcinomatosis without any obvious ovarian primary neoplasm. EPSPC are histologically indistinguishable from serous papillary ovarian carcinoma although the ovaries are minimally, if at all, involved on gross examination.

In the present study we retrospectively reviewed five cases presenting as colonic obstruction and eventually diagnosed as EPSPC.

Materials and Methods

The medical records of female patients presenting with colonic obstruction due to EPSPC were retrospectively reviewed during the past decade (1996-2006). Clinical presentation, preoperative imaging workup with abdominopelvic computed tomography (CT) and flexible colonoscopy, as well as operative findings and surgical procedures performed were analyzed. Routine histologic sections (hematoxylin and eosin, H&E) and immunohistochemistry for cytokeratin, epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA) and other markers (B72.3, WT1, Leu-M1, calretinin) were reviewed. Disease staging was determined according to the classification of the International Federation of Gynecology and Obstetrics (FIGO). Adjuvant combination chemotherapy was initiated in all patients one month after surgery in the form of paclitaxel (175 mg/m2) plus cisplatin (75 mg/m2) every three weeks for six cycles.

Results

Five female patients with a mean age of 73.4 years presented with clinical manifestations of colonic obstruction and were admitted to our department for further evaluation and treatment. Clinical presentation, past surgical history and findings of preoperative imaging workup are presented in Table 1, while operative findings and the surgical procedure carried out are presented in Table 2.

Three patients (cases # 1, 3, 5) had a hysterectomy performed in the past for benign lesions. All female patients presented with abdominal discomfort, usually in the hypogastrium, constipation and abdominal distension. The concentrations of CA-125 were elevated in four patients. Abdominopelvic CT in all cases revealed a thickening of the sigmoid colon wall with concomitant diffuse nodularity of the parietal peritoneum and ascites in two patients. Double-contrast enema in two cases
**Table 1. — Clinical characteristics and preoperative diagnostic workup of female patients with EPSPC.**

<table>
<thead>
<tr>
<th>n</th>
<th>Age (y)</th>
<th>Prior procedures</th>
<th>Presenting symptoms</th>
<th>Clinical Findings</th>
<th>Biological Markers (U/ml)</th>
<th>Imaging findings</th>
<th>Colonscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>79</td>
<td>Hysterectomy (uterine prolapse)</td>
<td>Abdominal distension</td>
<td>Abdominal distension</td>
<td>CT: Thickening of the sigmoid colon.</td>
<td><strong>CT:</strong> Thickening of the sigmoid colon.</td>
<td>Extramural compression of the sigmoid colon.</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td>-</td>
<td>Change in bowel habits</td>
<td>Ascites</td>
<td>CA125: 400</td>
<td>CT: Nodular infiltration of the omentum and pelvic peritoneum, thickening of the sigmoid colon, Ascites.</td>
<td>Stenosis of the sigmoid colon.</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>Hysterectomy (bleeding uterine fibroids)</td>
<td>Lower abdominal discomfort</td>
<td>Tenderness of the left lower quadrant of the abdomen</td>
<td>CA125: 500</td>
<td>CT: Smudgy, large mass involving the sigmoid colon.</td>
<td>Stenosis of the sigmoid colon.</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>-</td>
<td>Loss of appetite - nausea</td>
<td>Ascites</td>
<td>CA 125: 600</td>
<td>DCE: Irregularity of the sigmoid colon.</td>
<td>Extramural compression of the sigmoid.</td>
</tr>
<tr>
<td>5</td>
<td>62</td>
<td>Abdominal hysterectomy (uterine fibroids)</td>
<td>Weight loss</td>
<td>Tenderness of the lower abdomen</td>
<td>CA 19-9: 80</td>
<td>CT: Mural thickening of the sigmoid colon, Fine nodular infiltration of the greater omentum and mesentry.</td>
<td>Stenosis of the sigmoid colon.</td>
</tr>
</tbody>
</table>

* CT: computed tomography, DCE: double-contrast enema.

**Table 2. — Operative findings and surgical procedure performed in patients with EPSPC.**

<table>
<thead>
<tr>
<th>n</th>
<th>Operative findings</th>
<th>Surgical procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Free peritoneal fluid, multiple peritoneal and mesenteric implantations, strangling of the sigmoid colon. Ovaries were disease-free.</td>
<td>Bilateral salpingo-oophorectomy - Omentectomy - Low anterior resection of rectosigmoid</td>
</tr>
<tr>
<td>2</td>
<td>Multiple nodules of the peritoneum, mass encasing the sigmoid colon, small superficial lesions on both ovaries and fallopian tubes.</td>
<td>Total abdominal hysterectomy - Bilateral salpingo-oophorectomy - Omentectomy - Sigmoidectomy</td>
</tr>
<tr>
<td>3</td>
<td>A large omental tumor spreading to the sigmoid colon, multiple nodules scattered on the peritoneum and upon the surface of the ovaries.</td>
<td>Omentectomy - Sigmoidectomy - Bilateral salpingo-oophorectomy</td>
</tr>
<tr>
<td>4</td>
<td>Peritoneal seeding, a large omental tumor densely adherent to the sigmoid colon. No ovarian or uterine involvement.</td>
<td>Total abdominal hysterectomy and bilateral salpingo-oophorectomy - Omentectomy - Sigmoidectomy</td>
</tr>
<tr>
<td>5</td>
<td>An omental tumor obstructing the sigmoid colon, Multiple nodules on the peritoneum.</td>
<td>Omentectomy - Sigmoidectomy</td>
</tr>
</tbody>
</table>

revealed sigmoid colon stenosis. Finally, colonoscopy mainly demonstrated an external compression effect on the wall of the sigmoid colon.

Exploratory laparotomy revealed extensive abdominal carcinomatosis involving mainly the peritoneal surfaces and the large omentum, invasion of the sigmoid colon in all cases and only minimal involvement of the ovarian surface (Stage IIIC). Omentectomy and sigmoidectomy with end-to-end anastomosis of the descending colon with the rectum was carried out in all patients, while bilateral salpingo-oophorectomy was performed in four patients (cases # 1-4), with a total hysterectomy in those who had not had the uterus removed in the past (cases 2 & 4). A serous-type carcinoma indistinguishable from ovarian carcinoma moderately (FIGO grade 2) or poorly (grade 3) differentiated with the presence of psammoma bodies in four patients was the histological analysis of the surgical specimens (Figures 1, 2). Immunohistochemistry was positive for keratin, CEA andEMA in all cases with similar reactivity in the other antigens (B72.3, WT1 and Leu-M1) and negative in calretinin. The postoperative course was uneventful. Chemotherapy was received by every patient according to the protocol mentioned before. Four patients died after a survival time of 10-23 months (median 18 months), while one patient (case # 1) was asymptomatic and still alive one year after the initial diagnosis.

**Discussion**

EPSPCs are embryologically considered as mullerian-type tumors due to a common heritage of the ovary and the peritoneum [4], which can explain the similar aggressive clinical behavior and associated poor prognosis of EPSPC and ovarian carcinoma. EPSPC is found in 7-21% [5-10] of patients who undergo laparotomy for ovarian cancer. In several published series, age at the time of diagnosis was found to be greater in patients with EPSPC than in patients with ovarian cancer, without any differences regarding parity, menopausal status or age at menopause [11]. In other studies [12, 13] it has been demonstrated that in patients with EPSPC the concentrations of CA-125 were five times greater than in the group of patients with ovarian cancer.

Patients with EPSPC have similar clinical manifestations as with ovarian cancer, usually presenting with abdominal distension, pain or pressure often associated with ascites [14]. EPSPC tends to present in advanced stages where exploratory laparotomy reveals extensive...
peritoneal spread, and debulking surgery can be successful in approximately 38-41% of patients [7, 15]. However, Dubernard et al. [16] reported that in patients receiving neoadjuvant chemotherapy the percentage of patients who underwent debulking surgery was greater (63%) and optimal cytoreductive surgery (residual disease measuring < 2 cm) was obtained in 89%.

Adjuvant combination chemotherapy (paclitaxel plus platinum) seems to offer a survival advantage similar to ovarian cancer in advanced disease, especially when initial cytoreductive surgery is optimally performed [11]. Median survival in patients with EPSPC has been reported to range from 17 to 24 months [7, 8, 13, 17]. The median survival for our group of patients was 18 months.

**Conclusion**

EPSPCs are considered aggressive tumors, usually mimicking ovarian cancer and even rarely presenting with colonic obstruction. Optimal debulking surgery in combination with adjuvant chemotherapy seems to have a survival advantage for patients with advanced stages of the disease.

**References**


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Postoperative radiotherapy in intermediate and high-risk Stage I endometrial cancer: analysis of prognostic factors and survival

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3Department of Gynecology and Obstetrics, Ege University Faculty of Medicine, Izmir (Turkey)

Summary

Purpose: Patients with Stage IA Grade (G) III, Stage IB GII-III and Stage IC GI-II-III endometrial cancer who received postoperative adjuvant radiotherapy were evaluated in terms of local control, disease-free and overall survival rates and prognostic factors. Materials and Methods: Four hundred and three patients with Stage I endometrial cancer treated with radiotherapy from January 1990 to December 2003 at Ege University Faculty of Medicine Department of Radiation Oncology were reviewed retrospectively. According to our radiotherapy protocol patients with Stage IB G2 disease (149 patients) received only external radiotherapy and the remaining (254 patients) received both external radiotherapy and intracavitary brachytherapy. Results: Median age of the patients was 58 (range: 37-83). Nine patients (2.2%) had Stage IA, 196 (48.6%) had Stage IB and 198 (49.1%) had Stage IC disease. Histologic grade was 1 in 52 (12.9%) patients, 2 in 268 (66.5%) patients and 3 in 83 (20.6%) patients. Seventy-one (17.7%) patients had lymphovascular space invasion. Five-year locoregional relapse-free, distant-free, disease-free survival (DFS) and overall survival (OS) were 98.2%, 92.8%, 91.8% and 87.7%, respectively. In multivariate analysis, myometrial invasion and lymphovascular invasion were predictive factors for DFS and for OS prognostic factors were histologic type, myometrial invasion, and histologic grade. During radiotherapy 47.9% of the patients developed acute morbidity and 26.3% developed late morbidity, vaginal stenosis being the most frequent late morbidity. Conclusion: Postoperative adjuvant radiotherapy provides high locoregional control rates with acceptable toxicity in selected patients with Stage I endometrial carcinoma.

Key words: Intermediate and high risk stage I endometrial cancer; Radiotherapy; Prognostic factors.

Introduction

Endometrial cancer is the most common malignancy of the female genital tract, and it is the fourth most frequently diagnosed cancer in women. It was estimated that during 2006, 41,200 new cases and 7,350 deaths would be attributed to endometrial cancer in the United States [1]. The estimate was that 72% of women diagnosed with endometrial cancer would have locally confined disease [2]. Widely accepted management of endometrial cancer consists of total hysterectomy, removal of the remaining adnexal structures, and appropriate surgical staging in patients considered at risk for extrauterine disease [3-5]. In the adjuvant setting prognostic factors like FIGO stage, histopathology and grade, myometrial invasion, status of pelvic lymph nodes, lymphovascular invasion and performance status of the patients have an important role in the treatment choices [2-24]. If risk factors are present, that is, myometrial invasion is 50% or more of the myometrial width and/or grade 2 or 3 histology, pelvic radiotherapy is indicated to reduce the risk of pelvic failure [6]. In the recent published data, postoperative pelvic radiotherapy reduced vaginal and pelvic recurrences from 15% to 0-3%, however survival was not improved [5, 11, 12, 19]. Women with low-risk histopathologic features [grade 1 or 2, < 50% myometrial invasion] do not routinely receive adjuvant radiotherapy (RT) since the risk of recurrence is lower than 5% with or without surgical staging [6]. For “intermediate or high risk” subgroup patients, randomized studies have reported significantly less pelvic and vaginal recurrences with postoperative RT compared to surgery alone [7, 10, 16]. Based on these data, our institutions have developed and maintained a standardized policy for adjuvant RT based on certain disease characteristics. The intent of this retrospective analysis was to evaluate disease outcomes in women with early-stage (FIGO Stage IA to IC) endometrial cancer treated at Ege University. Clinical and pathologic factors were evaluated for potential prognostic significance.

Patients and Methods

Between October 1990 and December 2003 patients were included in this retrospective study at the Ege University Medical School Department of Radiation Oncology. The initial evaluation included chest radiography, CT scan of the abdomen, a complete blood count and biochemistry. All patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH/BSO) with (n = 148) or without (n = 255) bilateral pelvic lymph node dissection. In the patient group with lymph node dissection, the median number of lymph nodes removed was 19 with a range of three to 76. Patients were staged according to the 1988 FIGO staging system [25].
According to the protocol of Ege University, women with grade 1 or 2, and < 50% myometrial invasion do not receive adjuvant radiotherapy and all patients with Stage I, grade 2 and IA grade 3 disease are treated with postoperative external-beam RT alone. The rest of Stage I patients receive postoperative external-beam RT and vaginal brachytherapy. Adjuvant RT was administered as external-beam RT only in 149 (36.9%) patients or combined with vaginal brachytherapy in 254 (63.1%) patients. Patient characteristics are given in Table 1.

External-beam RT was delivered using a linear accelerator with 6 MV photons with a daily dose of irradiation of 1.8 Gy. Patients were treated with a combination of the 4-field box or AP-PA technique of irradiation and high-dose rate intracavitary brachytherapy. The irradiation volumes were designed to include the primary tumor and the locoregional lymph nodes. The superior border of the AP-PA fields was placed at the L5-S1 interspace. The inferior border of the AP-PA fields was placed at the bottom of the obturator foramina. The lateral margins of the AP-PA fields were placed 2 cm laterally to the bony pelvis. The anterior margin of the lateral fields was placed at the pubic symphysis. The lateral fields had the same cephalo-caudal extent as the AP-PA fields. The posterior margin of the lateral fields was individually designed using custom made blocks to cover the posterior part of the sacrum and posterior portion of the rectum. Vaginal brachytherapy was performed by using microselectron “high-dose rate” (HDR) Ir-192 equipment. Between 1990 and 2000, 9.25 Gy in one fraction was delivered to point 0.5-0.9 cm depth, from 2000 till 2003 a dose of 6.5 Gy in two fractions and after 2003, a dose of 3 x 6 Gy has been delivered. Median total radiation therapy duration was 49 days (range: 31-120 days).

After completion of the whole treatment, patients were evaluated every three months by history taking, gynecological examinations, and blood counts. Toxicity was assessed using by RTOG-EORTC early and late radiation morbidity criteria [26]. The French-Italian glossary was used for grading vaginal side-effects [27]. Toxicity was recorded at each visit.

### Statistical analysis

Overall survival (OS) was calculated from the time of diagnosis to death or date of last follow-up. Disease-free survival (DFS) was calculated from the time of diagnosis to relapse or death from any cause. All statistical analyses were performed by the software program SPSS 13.0. OS, DFS, and pelvic local control were calculated according to the actuarial method of Kaplan and Meier. Risk of failure was determined by Cox multiple regression analysis. Histologic type, histologic grade, myometrial invasion, patient age, extension to isthmus, body mass index (BMI), lymphovascular space invasion (LVSI), overall radiation treatment time, and operation type were the variable factors analyzed for actuarial overall survival and actuarial local control. Statistical significance was considered with p-values of less than 0.05.

### Results

#### Descriptive statistics

The median age of the 403 patients was 57 (range: 37-83) and median BMI was 29.6 kg/m² (range: 26.3-51.1 kg/m²). Two hundred and ninety-nine patients (77.7%) were postmenopausal. Comorbid conditions in the patients were as follows: 77 (19.1%) with hypertension, 94 (23.3%) with diabetes mellitus, 17 (4.1%) with secondary malignancies. Histologically 88.3% were adenocarcinoma, 5.2% adenosquamous carcinoma, 5% clear cell carcinoma and 1.5% serous papillary carcinoma. Six of the Stage IA patients’ histopathology was other than adenocarcinoma. Distribution of the patients according to stage and grade were as follows: nine patients IA grade 3, 149 patients IB grade 2, 47 patients IB grade 3, 52 patients IC grade 1, 116 patients IC grade 2, 30 patients IC grade 3. Lymphovascular invasion was positive in 71 patients (17.6%). Pathological characteristics of the tumors and surgical stages are shown in Table 2.

Median follow-up was 96 months (range: 12-262 months). We observed locoregional relapse in four patients, distant metastases in 26 patients and simultaneous recurrence at both locoregional relapse and distant metastases in four patients. Distribution of distant metastases was as follows: lung 13 (3.2%), bone two (0.5%), paraaortic lymph nodes six (1.5%), supraclavicular lymph nodes one (0.2%), liver one (0.2%), brain one (0.2%) and multiple six (1.5%). Five-year locoregional relapse-free, distant free, DFS and OS were 98.2%, 92.8%, 91.8% and 87.7%, respectively.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (No.)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
<td>240</td>
<td>59.5</td>
</tr>
<tr>
<td>≥ 60</td>
<td>163</td>
<td>40.5</td>
</tr>
<tr>
<td>Median age (yrs)</td>
<td>57 (range: 37-83)</td>
<td></td>
</tr>
<tr>
<td>Median BMI (kg/m²)</td>
<td>29.6 (range: 26.3-51.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Menopausal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>86</td>
<td>21.3</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>327</td>
<td>77.7</td>
</tr>
<tr>
<td>Hypertension</td>
<td>77</td>
<td>19.1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>94</td>
<td>23.3</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>51</td>
<td>12.7</td>
</tr>
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<td><strong>Operation</strong></td>
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<td></td>
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<td>TAH+BSO</td>
<td>255</td>
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</tr>
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<td>TAH+BSO+LD</td>
<td>148</td>
<td>36.7</td>
</tr>
<tr>
<td><strong>FIGO Stage I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>9</td>
<td>2.3</td>
</tr>
<tr>
<td>IB</td>
<td>196</td>
<td>48.6</td>
</tr>
<tr>
<td>IC</td>
<td>198</td>
<td>49.1</td>
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<td><strong>Histopathology</strong></td>
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<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>356</td>
<td>88.3</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>21</td>
<td>5.2</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Papillary serous carcinoma</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Histologic grade</strong></td>
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</tr>
<tr>
<td>1</td>
<td>52</td>
<td>12.9</td>
</tr>
<tr>
<td>2</td>
<td>265</td>
<td>65.8</td>
</tr>
<tr>
<td>3</td>
<td>86</td>
<td>21.3</td>
</tr>
<tr>
<td><strong>Lymphovascular invasion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>332</td>
<td>82.4</td>
</tr>
<tr>
<td>Yes</td>
<td>71</td>
<td>17.6</td>
</tr>
<tr>
<td><strong>Extension to isthmus</strong></td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>391</td>
<td>97</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td><strong>Length of radiotherapy</strong></td>
<td></td>
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</tr>
<tr>
<td>≥ 8 weeks</td>
<td>95</td>
<td>23.6</td>
</tr>
<tr>
<td>&lt; 8 weeks</td>
<td>308</td>
<td>76.4</td>
</tr>
</tbody>
</table>
Prognostic factors

According to univariate analyses prognostic factors influencing DFS were histologic type (p = 0.004), myometrial invasion (p = 0.001), LVSI (p = 0.04), while the prognostic factor affecting local recurrence-free survival was histologic type (p = 0.025). According to multivariate analyses the prognostic factor influencing DFS was myometrial invasion (p = 0.001) and LVSI (p = 0.033). Prognostic factors related with OS in multivariate analyses were: myometrial invasion (p = 0.011), histologic type (p = 0.026), and histologic grade (p = 0.005). Univariate and multivariate analyses for locoregional relapse-free, distant-free, DFS and OS are shown in Tables 2, 3, 4, 5, respectively.

Side-effects

Treatment was generally well tolerated. There were no treatment-related deaths. Only one patient experienced grade 3 diarrhea. The most frequently observed acute side-effects related to radiotherapy were diarrhea (22.2%), cystitis (21.3%) and radiodermatitis (16.1%). One hundred and ninety-three patients developed toxicity due to radiotherapy. According to the RTOG/EORTC late radiation morbidity scoring scheme guidelines, of the patients, 31 had vaginal stenosis, 26 cystitis, seven urinary incontinence, two proctitis, 23 grade 1-2 vaginal atrophy and two had fistulas. Treatment-related acute and late side-effects are listed in Table 6.

Discussion

Long-term life expectancy is very high in endometrial carcinoma because most patients are diagnosed in early stages. There are lots of retrospective studies which have evaluated the effect of postoperative radiotherapy in local and systemic disease control. In these studies, radiotherapy increases pelvic control but it has no effect on survival, especially in high-risk patients [18, 19]. In low-risk patients (Stage IA G1-2, Stage IB G1) the rates of vaginal recurrence were below 5% with surgery and additional therapy was not recommended. In intermediate-risk patients (Stage IB G2-3, Stage IC, Stage IIA-B) addition of radiotherapy decreased pelvic failure rates from 15% to 0-3% [7, 16]. In the last few years two large studies investigated the role of postoperative radiotherapy. In 1999, the GOG (Gynecological Oncology Group) randomized 390 endometrium cancer patients to 50.4 radiotherapy vs observation. Although most of the patients were in the low-risk group such as Stage IB and grade 1-2, progression-free survival rates were 96% in the radiotherapy arm versus 88% in the control arm (p = 0.004), however the survival difference did not reach significance [16]. In the PORTEC trial 715 patients in the intermediate-risk group were randomized to pelvic radiotherapy and control arms. Five-year locoregional recurrence rates were 4% in the radiotherapy arm and 14% in the control arm (p < 0.001), and 5-year survival rates were 81% and 84%, respectively (p = 0.31) [7]. In a third trial by Aalders et al. they randomized 540 clinically Stage I

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**Table 2.** Univariate and multivariate analyses for prognostic factors influencing disease-free survival.

<table>
<thead>
<tr>
<th>Factor</th>
<th>5 years</th>
<th>Univariate (p value)</th>
<th>Multivariate (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60</td>
<td>90.9</td>
<td>0.540</td>
<td></td>
</tr>
<tr>
<td>&gt; 60</td>
<td>93.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operation type</td>
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<tr>
<td>TAH+BSO</td>
<td>93</td>
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<td>TAH+BSO+LD</td>
<td>89.3</td>
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<td>FIGO stage</td>
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</tr>
<tr>
<td>IA</td>
<td>87.5</td>
<td></td>
<td></td>
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<tr>
<td>IB</td>
<td>97.9</td>
<td>0.001</td>
<td>0.001</td>
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<td>IC</td>
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<tr>
<td>Histopathology</td>
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<tr>
<td>Adenocarcinoma</td>
<td>92.9</td>
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<td>Other pathological types</td>
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<td>2</td>
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<td>No</td>
<td>93.2</td>
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<td>0.033</td>
</tr>
<tr>
<td>Yes</td>
<td>85.1</td>
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<tr>
<td>Overall radiation treatment time</td>
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</tr>
<tr>
<td>≤ 8 weeks</td>
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<td></td>
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<tr>
<td>&gt; 8 weeks</td>
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<tr>
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<tr>
<td>&gt; 30</td>
<td>95.6</td>
<td>0.540</td>
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<tr>
<td>Extension to isthmus</td>
<td></td>
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<tr>
<td>Yes</td>
<td>83.3</td>
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<tr>
<td>No</td>
<td>92.3</td>
<td>0.282</td>
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**Table 3.** Univariate and multivariate analyses for prognostic factors influencing overall survival.

<table>
<thead>
<tr>
<th>Factor</th>
<th>5 years</th>
<th>Univariate (p value)</th>
<th>Multivariate (p value)</th>
</tr>
</thead>
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<td>Age</td>
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<tr>
<td>≤ 60</td>
<td>85.3</td>
<td>1.85</td>
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<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>88.9</td>
<td>0.005</td>
<td>0.026</td>
</tr>
<tr>
<td>Other pathological types</td>
<td>76.6</td>
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<td></td>
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<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>89.2</td>
<td>0.02</td>
<td>0.005</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
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<td>Lymphovascular invasion</td>
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<tr>
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<td>88.5</td>
<td>0.012</td>
<td>0.170</td>
</tr>
<tr>
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<td>83.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall radiation treatment time</td>
<td></td>
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</tr>
<tr>
<td>≤ 8 weeks</td>
<td>89.7</td>
<td></td>
<td></td>
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<tr>
<td>&gt; 8 weeks</td>
<td>81.1</td>
<td>0.021</td>
<td>0.134</td>
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<td>BMI (kg/m²)</td>
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<td></td>
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<tr>
<td>≤ 30</td>
<td>85.3</td>
<td></td>
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<tr>
<td>&gt; 30</td>
<td>89.1</td>
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<td>Extension to isthmus</td>
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<tr>
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<td>83.3</td>
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<tr>
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<td>87.9</td>
<td>0.995</td>
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patients after hysterectomy and vaginal brachytherapy to receive pelvic radiotherapy or no further treatment. The local control rate was 2% with vaginal brachytherapy and pelvic radiotherapy versus 6.9% with vaginal brachytherapy alone [10]. All these results reveal that postoperative radiotherapy has an impact on locoregional control in intermediate-risk groups of patients but this benefit does not reflect overall survival. In the present study intermediate-risk patient locoregional recurrence rates were 2% and 5-year overall survival 87.7%, in accord with other reports [7, 10].

Radiotherapy is used in the treatment of endometrial carcinoma, especially in patients with poor prognostic factors, and many published series have tried to identify prognostic factors for local recurrence and distant metastases [9, 17, 24]. The degree of histopathologic differentiation is generally considered to be one of the most sensitive predicting factors of tumor spread [4]. Histological degree, myometrial invasion depth and presence of intraperitoneal metastases have been clearly defined as poor prognostics. In 1991, the GOG revealed that there was tendency for deep myometrial invasion and subsequently a higher rate of pelvic and paraaortic lymph node involvement [17]. Touboul et al. analyzed 437 operable endometrium carcinoma cases and concluded that there is a correlation between grade and myometrial invasion [4]. In multivariate analysis, myometrial invasion (p = 0.011) and grade (p = 0.005) were confirmed to be significant prognostic factors. Survival rates for Stage IA, IB, IC were 87.5%, 94.1%, 81.7%, respectively. In that study, lower survival rates for Stage I patients were due to poor histopathology (6 of the 9 Stage IA patients had serous papillary and clear cell carcinoma histopathology).

As in our series, many authors have also found that each histopathology subtype has a different biologic behavior and adenocarcinoma has a better prognosis than adenosquamous, clear cell, and serous papillary subtypes. Recurrence rates were higher in these groups [17, 23, 24]. After multivariate analyses, histopathological subtypes other than adenocarcinoma have an independent impact on locoregional relapse-free survival and OS (p = 0.004).

Lymphovascular space invasion is defined as an independent risk factor for locoregional or systemic recur-
rence and a common sign of tumors with deep myometrial penetration and cervical invasion. It is related to the high regional and intraabdominal failure rates. According to Alders et al., the death rate increases from 4% to 27% in the presence of LVSI (p = 0.01) [10]. In the current study the presence of LVSI had an adverse effect on overall, disease-free and distant metastasis-free survival. Extension to the isthmus and/or cervix has often been cited as a prognostic factor for outcome. In the literature, when univariate analysis is applied, extension to the isthmus and/or cervix effectively influences DFS. However, after multivariate analysis it is not independently significant because it is thought that these results are influenced mainly by tumor stage [4]. In the present study there was no statistical significance for extension to the isthmus and/or cervix on DFS.

The impact of age on survival remains uncertain. According to the PORTEC trial the most important prognostic factors that affected locoregional control were age and radiotherapy. The locoregional recurrence rate for patients under 60 years was 4%, and 10% for patients over 60 years (p = 0.02), and in multivariate analyses locoregional recurrence rates were three times higher in patients over 60 years (p = 0.003) [7], however, in several other series (including the present), age did not influence survival rate [4].

In past years it was shown that overall radiation time in patients with intact cancer is an important prognostic factor for OS [28, 29]. The impact of overall radiation time was questioned in two retrospective studies and no influence was found [30, 31]. Different from the literature, prolongation of overall radiotherapy treatment time in our study was found to be an adverse prognostic factor on OS in univariate analysis.

Obesity is a known risk factor for endometrial cancer due to the excess endogenous estrogen found in adipose tissue. Anderson et al. found that time to recurrence increased significantly (p = 0.014) and recurrence rates decreased (not significant) as BMI increased [32]. Also pathologically, patients with a BMI > 40 were more likely to have endometrioid histology, lower stage disease, expression of HER-2/neu and lower grade tumors than women with a BMI of < 30 [33-35]. Obesity is associated with OS in cancer of the uterus. However, because of the association of obesity with confounding variables like age, stage and grade, obesity does not appear to be an independent predictor of survival in women with endometrial carcinoma [33]. In the present study because of the low number of patients with a BMI of < 25 or > 40, the relationship between obesity and survival was not defined statistically.

Because of long-term life expectancy in endometrium carcinoma, the best tumor control with minimal morbidity should be obtained. The benefit of lymphadenectomy is unclear; firstly lymphadenectomy increases complication rates and the risk of lymph node metastases is below 10% for Stage I carcinomas except Stage IC grade 3 [7, 8, 24]. However the most important benefit of lymphadenectomy is to select patients with high-risk pelvic recurrence or accurate staging [36]. A significantly higher 5-year OS rate was seen in patients who had not had lymphadenectomy performed compared to those undergoing lymphadenectomy (88.5% vs 83.9%; p = 0.012). In the first group, more than 40% of the patients were Stage IB G2 and in the second group only 40% of the patients were Stage IB G2. This statistical significance on the OS could be associated with distribution of the patients with Stage IB G2.

In the reported literature severe complication rates were low. Grade 3 and 4 late toxicity rates were published after TAH-BSO with external radiotherapy from 2% to 6%, after surgery followed by ERT with VBT from 4% to 13% and after ERT and surgery including lymphadenectomy from 7% to 18% [37]. Grade 1-2 late toxicities were about 20% and the most frequent were diarrhea, increasing motility of intestines and abdominal cramps. Urinary complications such as polyuria, minor incontinence and cystitis episodes were seen less frequently [15]. In a GOG study, hematological, gastrointestinal, genitourinary and cutaneous toxicities rates were higher in the pelvic radiotherapy arm compared to the observation arm (p < 0.001); two patient deaths were related to gastrointestinal toxicity. In the radiotherapy arm GI and genitourinary toxicity were reported in 68% and 30% of patients, respectively [16]. In the PORTEC study, during radiotherapy 63% of the patients were treated with medication or dietary aids or both, for treatment-related symptoms. In the present study, 48% of the patients were treated for acute side-effects. Mild and moderate vaginal stenosis is the most commonly noted late side-effect after radiotherapy [38-40]. Sorbe et al. reported 15% of vaginal stenosis with only HDR brachytherapy [38]. In the present study, we observed 22.2% of gastrointestinal and 21.3% of genitourinary toxicity. The most common late side-effect was vaginal stenosis (7.7%). The overall late toxicity rate was 25.7% and no RTOG grade 4 complication was recorded.

In conclusion, our analysis showed a relatively good prognosis in endometrial cancer patients treated with external RT and vaginal brachytherapy. Postoperative treatment increases the risk of late radiation side effects. To reduce the side-effects, the role of vaginal brachytherapy alone or the addition of vaginal brachytherapy to external pelvic RT needs to be clarified through well designed randomized trials using modern radiotherapy techniques. Future work is needed to define the role of radiotherapy in a subgroup of Stage I patients. We conclude that postoperative adjuvant RT provides high locoregional control rates with acceptable toxicity in selected patients with Stage I endometrial carcinoma.

References


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Lysosphatidic acid: an ovarian cancer marker

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Summary

Objective: To determine whether lysosphatidic acid (LPA) can serve as an ovarian cancer marker, we compared plasma LPA levels in ovarian cancer patients, in women with no ovarian pathology, and in women with benign ovarian tumors. We determined the optimal plasma LPA level cutoff value and correlated clinico-pathological parameters with plasma LPA levels. Method: Capillary electrophoresis with indirect ultraviolet detection was used to analyze the plasma LPA levels of 133 patients (60 patients with ovarian cancer, 43 women without ovarian pathologies and 30 patients with benign ovarian tumors) during a three-year period. Results: Patients with ovarian cancer had a significantly higher plasma LPA level (n = 60, median (med) 16.99 μmol/l, range 4.53-43.21 μmol/l) compared with controls with no ovarian pathology (n = 43, med 2.92 μmol/l, range 0.94-22.93 μmol/l) and patients with benign ovarian tumor (n = 30, med 7.73 μmol/l, range 1.12-28.84 μmol/l) (p < 0.001). We found that plasma LPA levels were associated with the International Federation of Gynecology and Obstetrics (FIGO) stage and ovarian cancer histological type. Patients with endometrial ovarian cancer had significantly higher plasma LPA levels in comparison with other histological types of epithelial ovarian carcinoma. Conclusion: The plasma LPA level can be a useful marker for ovarian cancer, particularly in the early stages of disease.

Key words: Ovarian cancer; Lysosphatidic acid; Marker.

Introduction

Ovarian cancer has the worst prognosis of all gynecological malignancies. The poor prognosis results from the inability to detect ovarian tumors at an early, a curable stage, as well as from the lack of effective therapies for the disease in advanced stages. Current treatment, which consists of radical surgery and chemotherapy, has resulted in improved patient survival and quality of life; however, there has been no significant increase in the rate of curing ovarian cancer during the last 30 years. Currently, there are no proven biomarkers that can be used for early detection. The most common biomarker, cancer antigen 125 (CA 125), lacks specificity and is elevated in only around 50% of Stage I ovarian cancer cases [1]. Lysosphatidic acid (LPA, 1-acyl-2-hydroxy-sn-glycero-3-phosphate) is a phospholipid that is elevated in the ascites and serum in ovarian cancer patients [2]. It is produced by malignant ovarian epithelium and activates various biological responses by binding and activating G-protein-coupled receptors. The most prominent LPA effects include stimulation of cell proliferation, cell survival and tumor cell invasion [3]. LPA reduces the sensitivity of ovarian cancer cell lines to apoptosis induced by the chemotherapeutic agent cisplatinum and may also play a role in metastatic competence [4]. It increases the expression of several angiogenic factors, such as interleukin-8, vascular endothelial growth factor, and growth regulated oncogene (GRO), which promote angiogenesis and tumor metastasis [2, 5, 6]. The expression levels of the LPA1 and LPA3 receptors were significantly increased in ovarian cancer tissue, compared with benign tumors and normal ovarian tissue [7]. While ovarian cancer cells produce LPA, normal ovarian epithelial cells do not [8]. The objective of this prospective study was to determine whether LPA could serve as a new ovarian cancer marker. We measured the plasma levels of LPA in ovarian cancer patients, in woman without ovarian pathologies, and in women with benign ovarian tumors and used these data to correlate LPA plasma levels sensitively and specifically with clinicopathological parameters.

Materials and Methods

Instrumentation

A Beckman P/ACE 5510 System with System Gold software (Beckman Ins., USA) was used for capillary electrophoresis (CE) separation. On-line indirect UV detection was performed, and the separation temperature was maintained at 25°C. Samples were introduced into the capillary by pressure injection at 3.45 kPa for 10 sec. Separations were carried out under an applied potential of 25 kV at the normal polarity with the cathode placed at the capillary outlet. Between runs, the capillary was rinsed with the separation buffer for 2 min. Each new capillary was conditioned sequentially with 0.1 M NaOH for 1 hr, deionized water for 20 min, methanol for 20 min, and separation buffer for 30 min before use. Each day, the capillary was rinsed with 0.1 M NaOH for 5 min, deionized water for 2 min, and separation buffer for 15 min.

Chemicals and solutions

Lysosphatidic acid (synthetic, purity > 99%), was purchased from Avanti Polar Lipids, Inc. (USA). Adenosine 5’-monophosphate monohydrate (AMP, 99%), sodium hydroxide (> 99.99%), acetoniitrile (> 99%) and 8-amino-1-naphthalenesulfonic acid were obtained from Sigma-Aldrich (Germany). HPLC grade methanol (> 99.9%) and boric acid were purchased from
Lachema (Czech Republic). Thin layer chromatography (TLC POLY SIL) was purchased from Macherey-Nagel (Germany). Aqueous solutions were prepared with deionized water from Millipore-milli-Q plus (France). Stock buffer solutions of 50 mM AMP in various concentrations of boric acid were prepared by dissolving the appropriate amounts of AMP and boric acid in deionized water. The solutions were then adjusted to the desired pH values with 1 M NaOH. Prior to CE analysis, standard working solutions of lysophospholipids were freshly prepared by serial dilution of the standard stock solutions in a solvent of 5% separation buffer in methanol-water (9:1 v/v).

**Statistical Analysis**

Comparisons of patients groups were performed using the Kolmogorov-Smirnov and Mann-Whitney tests; p values less than 0.001 were considered statistically significant. To evaluate the diagnostic accuracy of LPA as a marker for ovarian cancer, receiver operating characteristic (ROC) analysis was used.

**Results**

The ages of the patients, the ovarian cancer stages, grades, and histological types are shown in Table 1. Patients with ovarian cancer had a significantly higher LPA plasma level (med 16.99 μmol/l, range 4.53-43.20 μmol/l) compared with the healthy control group with no ovarian pathologies (med 2.92 μmol/l, range 0.94-22.93 μmol/l) and patients with benign ovarian tumors (med 7.73 μmol/l, range 1.12-28.84 μmol/l) (Table 2, Figure 1). We detected plasma LPA elevation in early stages of ovarian cancer (Table 3). Plasma LPA levels were associated with the FIGO stage and ovarian carcinoma histology type. Patients with endometrial ovarian cancer had a significantly higher plasma LPA level (n = 14, med 22.89 μmol/l, range 4.50-42.58 μmol/l) compared with other histological types of epithelial ovarian carcinoma (serous ovarian cancer n = 26, med. 15.59 μmol/l, range 5.13-42.62 μmol/l, mucinous ovarian cancer n = 9, med. 12.52 μmol/l, range 6.83-27.12 μmol/l, clear cell ovarian cancer n = 3, med. 5.07 μmol/l, range 4.53-8.30 μmol/l). The histological grade did not influence the plasma LPA level in this study. We used a 10.4 μmol/l cutoff value, which had 85% sensitivity and 83.7% specificity using the ROC curve (Figure 2).

**Table 1. — Characteristics.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age median (range)</th>
<th>Stage I+II</th>
<th>Stage III+IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9</td>
<td>53 (20-62)</td>
<td>64 (40-82)</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>0</td>
<td>64 (40-82)</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>0</td>
<td>64 (40-82)</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>64 (40-82)</td>
</tr>
</tbody>
</table>

**Table 2. — LPA levels in plasma.**

| LPA | 
|------|------|------|------|------|------|------|
| Ovarian cancer | No ovarian pathology | Benign ovarian tumor |
| n = 60 | n = 43 | n = 30 | μmol/l |
| Med 16.99* | 2.92* | 7.73* | p < 0.001 |
| Range 4.53-43.20 | 0.94-22.93 | 1.12-28.84 | p < 0.001 |

*p < 0.001.

**Table 3. — LPA levels in ovarian cancer stages (FIGO).**

<table>
<thead>
<tr>
<th>Stage</th>
<th>LPA (μmol/l)</th>
<th>Range (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12.23</td>
<td>4.53 - 43.2</td>
</tr>
<tr>
<td>II</td>
<td>16.46</td>
<td>5.13 - 42.58</td>
</tr>
<tr>
<td>III</td>
<td>18.59</td>
<td>4.5 - 42.53</td>
</tr>
<tr>
<td>IV</td>
<td>35.03</td>
<td>15.05 - 36.05</td>
</tr>
</tbody>
</table>
Lysophosphatidic acid: an ovarian cancer marker

Discussion

Higher plasma LPA levels have been reported in ovarian cancer patients than in healthy women [11-14]. A study, which included 48 healthy controls and 48 women with ovarian cancer, showed that plasma LPA levels were elevated in patients with ovarian cancer. Importantly, elevated LPA levels were detected in early-stage ovarian cancer cases compared with the controls [13]. However, another study that measured the LPA plasma levels in 32 patients with ovarian cancer and 32 healthy controls using a liquid chromatography/mass spectroscopy assay found no significant elevation in plasma LPA in cancer patients [15]. The reason for the discrepancy between the findings is unclear, since there were many methodological differences between the two studies, including differences in sample collection, processing, and lipid analyses [11]. The plasma LPA assay offers the possibility of earlier diagnosis of ovarian cancer, a disease that has a poor prognosis, mainly because it is rarely detected in early stages. Identification of effective biomarkers for early cancer detection can improve survival rates [11]. Elevated plasma LPA levels were detected in 90% of patients with Stage I ovarian cancer [13]. Here, we identified that plasma LPA levels were slightly higher in patients with benign ovarian tumors compared with women with no ovarian pathology (Table 2). These results may be influenced by our inclusion of patients suspected of having ovarian cancer, but without histological diagnoses, in the group of women with benign ovarian tumors. Ovarian cancer cells produce LPA but normal ovarian epithelial cells do not [8]. LPA increases the expression of vascular endothelial growth factor (VEGF) in established ovarian cancer cell lines by binding to the LPA2 receptor, which is highly expressed in ovarian cancer cells but not in normal ovarian epithelium [16]. The expression levels of the LPA1 and LPA3 receptors were significantly increased in ovarian cancer samples (92.6%) compared with benign tumors (45.5%) and normal ovarian tissue (43.8%) [7]. This study demonstrates plasma LPA elevation in ovarian cancer patients. At present, it is necessary to determine the optimal cutoff value for plasma LPA levels.

Conclusion

Compared to healthy controls and to patients with benign ovarian tumors, elevated plasma LPA levels were detected in ovarian cancer patients. Our results suggest that plasma LPA levels can act as a biomarker for ovarian cancer, particularly in the early carcinoma stages.

Acknowledgement

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References


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Simultaneous diagnosis and treatment of bilateral breast carcinoma and endometrial adenocarcinoma. Implications for screening

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Summary

The association of breast and endometrial carcinoma has been demonstrated by many studies. Most of the cases are related to the previous use of tamoxifen for the treatment of breast carcinoma. However, triple primary malignancies on the same individual are very rare. This is an unusual case regarding simultaneous diagnosis and treatment of bilateral breast carcinoma and endometrial adenocarcinoma. The clinical aspects and treatment of this unique case are presented.

Key words: Endometrial carcinoma; Breast carcinoma; Triple primary malignancies.

Introduction

The mean age of patients with endometrial cancer is 61 years, with 75%-80% of women being postmenopausal and 3-5% being less than 40 years old [1]. The incidence of endometrial cancer varies widely, the lowest rates being in Asian populations and the highest among the North American whites. It is noteworthy, however, that when Asian populations migrate to the USA the rates rise very markedly to approach those seen in the local white population [2]. The aetiology of endometrial cancer is unknown but several factors are known to increase or decrease the likelihood of its development. The main risk factors identified for endometrial cancer relate to hormonal status and reproductive history. Obesity, nulliparity, late menopause, polycystic ovary syndrome, unopposed oestrogen therapy, functioning ovarian tumors, personal or family history of breast or colon carcinoma and also tamoxifen therapy for breast cancer increase the incidence for endometrial cancer [3]. In contrast, a decrease in risk for endometrial cancer has been observed both for cigarette smokers and for users of the combined oral contraceptive pill [1].

Breast cancer is the most common malignancy affecting women in North America and in most European countries. It is the commonest single cause of all deaths in women aged 35-55 years, but is most commonly seen in women between 55 and 85. There is no single identifiable factor or group of factors which can identify the majority of women who will develop breast cancer [4]. The practitioner must recognise that only 12% of breast cancer patients have an identifiable risk factor. In 88% of female patients no risk factor can be pointed out, indicating that all women should be considered at risk, which will enhance the thoroughness of the examination and the history. The most recognisable epidemiologic risk factors are breast cancer in a first degree relative or cancer in the opposite breast, or women who have undergone prolonged exposure to endogenous oestrogens such as early menarche, late menopause, nulliparity, and obesity. The incidence of breast malignancy in the contralateral breast is approximately 1% annually. If there is a family history of breast cancer, the risk is increased by two or three times and can be as high as nine times when there has been bilateral pre-menopausal breast cancer in the relatives [5]. Women with endometrial cancer are at higher risk of developing breast cancer. They should be offered mammography and taught self-palpation [1].

Occurrence of multiple primary cancers is a rare phenomenon. We describe an unusual case regarding simultaneous diagnosis and treatment of three primary malignancies on the same individual that, to our knowledge, has never been reported.

Case Report

A 68-year-old female patient presented at the gynaecology clinic complaining of postmenopausal bleeding. Her medical history was unremarkable. She was a non smoker and had a body mass index within the normal range. She had been post-menopausal since the age of 50. She had delivered vaginally two daughters, unfortunately one died at a young age because of leukaemia. Transvaginal ultrasound examination revealed atrophic ovaries and uterus, and a thick 11 mm endometrium. On the left breast, a suspicious mass was palpated on the upper inner segment and the right breast appeared normal. The patient was fully informed about the need to investigate the bleeding and the clinical breast findings. Therefore dilatation and curettage of the uterus and mammography of the breasts were carried out urgently. Two weeks later, histology of the sharp curettage of the uterus revealed a moderately differentiated endometroid adenocarcinoma (grade 2) expanding from the base of a hyperplastic endometrial polyp. On mammography, a mass with
microcalcifications appeared on the inner upper quadrant of the left breast and also a suspicious mass on the outer upper right breast. The patient was fully counselled and consented to proceed to total abdominal hysterectomy and bilateral salpingo-oophorectomy including pelvic lymph node sampling, and also bilateral breast lump excision and bilateral axilla lymph node sampling contemporaneously. The patient opted for radiotherapy if it was possible to keep her breasts.

Admission followed one week later. Preoperative assessment tests were all normal, including a full blood count, urea, glucose, blood group and crossmatch, clotting screen, hepatitis B status, CA 15-3, urinalysis, electrocardiogram and cardiology examination, chest X ray, technetium bone scan, and finally upper and lower abdominal computed tomography scan. Total abdominal hysterectomy and bilateral salpingo-oophorectomy, including peritoneal washings, right-sided external and common iliac as well as left external iliac lymph node sampling were uneventful. The operation then continued with wide excision of the bilateral tumors of the breasts, which were both positive for malignancy at urgent biopsy. Therefore, lymph node sampling of both axillae was carried out. The operation lasted for more than four hours. All specimens were sent for histological and histochemical examination. The histology of the left breast tumor was a moderately differentiated ductal invasive adenocarcinoma with the margins free of disease and metastasis in two axillary lymph nodes out of the 21 excised. The hormonal receptors of the left-sided tumor were negative for oestrogen receptors (ER), negative for progesterone receptors (PR), p53 positive, EGFR positive, Ki67: 90%, HercepTest: +1 (negative). The tumor on the right breast was a moderately differentiated ductal invasive adenocarcinoma with margins free of disease. The 12 right-sided axillary lymph nodes excised were normal. The hormonal receptors of the right-sided tumor were positive for ER, positive for PR, p53 positive, EGFR negative, Ki67: 35%, HercepTest: +1 (negative). At histology the uterus appeared to have no further neoplastic disease and apparently the curettage contributed to that. Both ovaries, iliac lymph node biopsies and peritoneal washing cytology were also normal.

The patient received six cycles of adjuvant chemotherapy with epirubicin (100 mg), cyclophosphamide (800 mg) and 5 fluouracil (800 mg) and subsequently she would undergo radiotherapy.

**Discussion**

The occurrence of uterine carcinoma and breast carcinoma in the same patient is a very rare incident. The following described studies include patients with breast carcinoma who subsequently developed endometrial carcinoma. Ewertz et al. [6] reported that among 51,638 women diagnosed with primary breast cancer in Denmark between 1943-1977, 115 cases of endometrial cancer were identified after more than three months and confirmed histologically. The study indicates that breast and endometrial cancer share several common aetiological factors and that studies of second primary cancers have the potential to provide information on the risk factors other than those associated with therapy.

The association between cancers of the breast, endometrium and ovary was reviewed by Ewertz and Storm [7] in another study. Breast cancer patients had an approximately three-fold increased risk of developing a cancer in the contralateral breast. The risk of breast cancer was also elevated following cancers of the uterine corpus and ovary, with relative risk (RR) estimates of about 1.5 from one to four years after the diagnosis of the first primary cancer. An increased risk of cancer of the uterine corpus subsequent to breast cancer was also found. After an ovarian cancer, the risk of cancer of the uterine corpus was elevated (RR = 1.6-2.3). An increased risk of ovarian cancer was observed subsequent to breast cancer (RR = 1.3-1.7), whereas ovarian cancer risk decreased with time after the diagnosis of cancer of the uterine corpus, probably reflecting treatment involving oophorectomy.

A survey by Shunemann and Jourdain [8] included 1,503 endometrial carcinomas and concluded that 163 cases were multiple tumors (10.8%). Double malignancies that occurred within six months were termed simultaneous and all others were termed successive cases. Of these 100 (6.6%) occurred together with breast cancer and 63 (4.2%) with other primary malignancies. The average latency period from the first to the second malignancy was 4.5 years. Patients with endometrial carcinoma run higher risks of secondary tumors. One out of ten develops a double and one out of 100 a triple malignancy. The combination of endometrial and breast cancer occurs in 60% of cases and the combination of genital and breast cancer in nearly 80% of cases. Aftercare of endometrial carcinoma must also include early detection of potential double and triple malignancies.

In our case the diagnosis and treatment of the carcinoma of the uterus and bilateral breasts were synchronous. The patient was affected with the three most common gynaecological malignancies. The worse tumor was the invasive bilateral breast carcinoma.

Endometrial carcinoma with Stage pTa and grade 2, according to FIGO, is curable. Total abdominal hysterectomy and bilateral salpingo-oophorectomy, including peritoneal washings, right-sided external and common iliac as well as left external iliac lymph node sampling was a sufficient operative procedure.

Carcinoma of the left breast was poorly differentiated (grade 3), Stage pT1a/b N1pos M0 (IIA) and the right breast was a moderately differentiated (gradeII) stage pT1a/b N1neg M0(I) by the TNM system based on the UICC criteria. Histological and histochemical examinations of the breast tumors as well as lymphadenectomy were important in carrying out the treatment plan. The receptors of the left sided breast tumor were negative to ER, negative to PR and in contrast with the hormonal receptors of the right-sided breast tumor which were positive to ER, and also positive to PR, indicating that the tumors were possibly different from an aetiology point of view.

To the best of our knowledge, this is a unique case to be reported in the literature, because the diagnosis and treatment of bilateral breast carcinoma and endometrial adenocarcinoma were synchronous. Therefore, the patient did not have to go through three different operations. At the same time, she was entitled to prompt initiation of chemotherapy and radiotherapy for the breast cancers, which were apparently of different type as described.
Based on the case presented, we realise the importance of screening women with endometrial cancer with mammography. Also, women diagnosed with breast cancer should be examined with transvaginal ultrasonography for endometrial or ovarian carcinoma (a good clinical practice point).

References


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A case of extremely chemoresistant pure pleomorphic rhabdomyosarcoma of the uterus associated with a high serum LDH level


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Summary

Background: Pleomorphic rhabdomyosarcoma (RMS) of gynecologic origin is an exceedingly rare, highly malignant tumor. Only a few cases have been reported in the last decades. Case report: A 60-year-old postmenopausal woman presented with a high LDH level of unknown origin. Ultimately, she was diagnosed with pleomorphic RMS. She underwent total hysterectomy, bilateral salpingo-oophorectomy, left pelvic and paraaortic lymphadenectomy and partial omentectomy. Surgery was followed by systemic chemotherapy and pelvic irradiation. Unfortunately, the patient did not respond to treatment. Her disease course correlated with the fluctuation of plasma LDH levels. Despite the evaluation of a high LDH level, her disease course paralleled the fluctuation in blood LDH levels. Ultimately she died within 20 months of the diagnosis. Conclusion: It is important to have better insight and to set a standard multimodal treatment for adult RMS. In addition, plasma LDH levels can be considered as a prognostic marker for RMS, particularly in advanced stage.

Key words: Uterine sarcoma; Uterine rhabdomyosarcoma; Lactate dehydrogenase.

Introduction

Pleomorphic rhabdomyosarcoma (RMS) has clinicopathological characteristics that set it apart from other types of RMS. It presents as an aggressive, predominantly spindle-cell tumor expressing myogenic differentiation. It is defined by the presence of large, pleomorphic tumor cells, which show, at least focally, immunophenotypic or ultrastructural sarcomeric muscle differentiation. This subtype of RMS occurs almost exclusively in adults while the embryonal and alveolar varieties are more common in children and adolescents. It is highly malignant, and patients frequently present with extraterine spread. Chemoresistance, coupled with early metastatic recurrence make this tumor highly lethal [1]. In adults, RMS usually occurs as a heterologous component of a malignant mixed mesodermal tumor [2]. In the last decades, however, few pure RMS cases have been reported [2, 3]. Here, we describe a rare case of pleomorphic RMS in an elderly, asymptomatic postmenopausal woman whose diagnosis was incidental to the evaluation of a high LDH level. Her disease course paralleled the fluctuation in blood LDH levels. Despite multimodal treatment, the patient never entered remission and died 20 months after diagnosis. The rarity of this histologic variant of RMS, its uncommon presentation and extremely chemoresistant behavior make this case notable.

Case Report

The patient, a 60-year-old postmenopausal woman, was noted to have a high LDH level at a routine physical exam in December 2004. A pelvic ultrasound (US) exam demonstrated no abnormalities, and other causes of a high LDH level were ruled out. Her LDH level continued to rise rapidly, and eventually, the patient developed abdominal discomfort secondary to a rapidly growing abdominal mass. She had no prior history of vaginal bleeding or pain. Physical examination and repeat US revealed a large abdominal mass which appeared to be arising from the pelvis and extending towards the umbilicus. On computed tomography (CT) scan, the mass measured 7 x 10 x 12 cm on the posterior wall of the uterus and enlarged paraaortic lymph nodes were noted (Figures 1A/B). There was no increase in endometrial thickness on US. In addition, endometrial biopsy ruled out the possibility of endometrial carcinoma. T2-weighted magnetic resonance imaging (MRI) showed a heterogeneous hyperdense abdominal mass with slightly high signal intensity (Figures 1C/D). This suggested uterine sarcoma. Tumor marker blood levels were CA19-9 29 U/ml, CA125 10 U/ml, and CEA 2.6 ng/ml. Exploratory laparotomy demonstrated a large, irregularly shaped uterine mass extending beyond the pelvis, and enlarged pelvic and left paraaortic lymph nodes (Figure 2). There was no invasion either into the bladder or intestine. A small amount of serous ascites was noted. Total hysterectomy, bilateral salpingo-oophorectomy, left pelvic and paraaortic lymphadenectomy and partial omentectomy were performed on March 3, 2005. The resected specimen weighed 690 g and measured 12 x 7 x 12 cm. The final histopathological diagnosis was pure pleomorphic RMS originating from the uterus.

On April 11, 2005, the patient was readmitted for back pain. A repeat CT scan revealed multiple lung and liver metastases along with cervical, paraaortic and pelvic lymph node enlargement. We planned to begin treatment with combined chemoradiation using the Intergroup Rhabdomyosarcoma Study regimen. Chemotherapy began with four courses of an IE regimen consisting of ifosfamide (1.8 g/body weight (wgt) daily...
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for 5 days) and etoposide (75 mg/body wgt daily for 5 days). Initially, chemotherapy was effective in decreasing the sizes of the metastatic nodules in the lung, liver and lymph nodes on CT scan and lowering the blood LDH level. IE therapy was followed by two courses of a VAC regimen containing vincristine (2 mg/body wgt once weekly for 3 weeks), actinomycin D (0.015 mg/day/body wgt for 5 days), and cyclophosphamide (2.29 g/body wgt). The CT scan at this time suggested metastasis. Positron emission tomography confirmed the presence of residual disease in the lungs, lymph nodes and pelvis. Prior to the next course of chemotherapy the patient received radiotherapy, a total of 45 Gy. Despite these measures, her LDH level began to rise. Chemotherapy was continued with VAC (vincristine 2.0 mg/body wgt 1/wk x 3), Actinomycin D (0.015 mg/body wgt x 5 days) and cyclophosphamide (2.2 g/body wgt x 75% = 1.6 g/body wgt). Unfortunately, her disease pro-

Figure 1. — A/B) CT showing a homogeneous hyperdense mass in the abdomen arising from the pelvis and enlarged paraaortic lymph nodes. C) MRI, a hypodense mass on T1-weighted images D) MRI, a hyperdense mass on T2-weighted images.
gressed, as evidenced by further elevation of her LDH level (Figure 3) and multiple lung and lymph node metastases were detected by CT scan. Chemotherapy was changed to the CAP regimen containing carboplatin (75 mg/body wgt), adriamycine (30 mg/body wgt) and cyclophosphamide (500 mg/body wgt).

Despite a variety of employed chemotherapy regimens, the patient failed to respond further and had widespread tumor dissemination in the lungs, liver and bone marrow.

Over the course of treatment the patient suffered from several chemotherapy-induced side-effects including grade 3 and 4 neutropenia, severe anemia and infection. These were treated with G-CSF and antibiotics. Her bone metastases caused severe back pain that was managed with opioid analgesics. Ultimately, she had permeation of her kidneys by the tumor and died of renal failure on September 13, 2006.

**Histology and immunohistochemistry**

The tumor was composed of poorly differentiated spindle-shaped cells with abundant eosinophilic cytoplasm and eccentric nuclei (Figures 4A/B). Phosphotungstic hematoxylin acid (PTHA) staining showed cross striation of the rhabdomyoblasts (Figure 4C). Immunohistochemistry revealed positive immunoreactivity for myosin and desmin (Figures 5A/B) but no reactivity for p53. The tumor was negative for estrogen and progesterone receptors (Figures 5D/E), indicating that it was hormone independent. The proliferation index was very high, around 25 to 30 mitoses per 10 high power fields. This was confirmed by a high Ki-67 labeling index (Ki-67 LI: 60) (Figure 5C).

**Discussion**

Soft tissue sarcomas are a group of tumors comprised of a wide variety of subtypes. Sarcomas account for 6% of pediatric and 1% of all adult malignancies. The most common subtypes originating in the female genital tract are uterine leiomyosarcomas and endometrial stromal sarcomas. RMS is one of the rarest subtypes of gynecological sarcomas and accounts for only 3% of all adult malignancies [4]. It is more frequent among children and adolescents. Therefore, most of the studies regarding treatment and outcome are from the pediatric literature.

Sarcomas of the uterus were originally classified into pure, mixed and malignant mixed Müllerian tumors. Recently, however, mixed Müllerian tumors have been reclassified as carcinomas [5]. Pure sarcomas are of two categories: pure homologous and pure heterologous. Pure homologous uterine sarcomas originate from tissues that are normally present in the uterus. The pure heterologous variety includes those derived from tissues not normally present in the uterus. Examples include rhabdomyosarcomas, liposarcomas, chondrosarcomas and osteosarcomas.

Rhabdomyosarcomas are divided into three major morphologic categories: embryonal, alveolar and pleomorphic. The most common is the embryonal subtype which accounts for 70-75% of all RMS cases. It is followed by the alveolar (20-25%) and pleomorphic types (5%). The embryonal and alveolar subtypes are most frequently seen in children. In contrast, the pleomorphic category is found almost exclusively in adults and is currently not used for pediatric RMS subtyping [6]. RMS occurs throughout the body including in the head and neck, orbita, extremities, trunk, genitourinary and retroperitoneal regions. Although RMS in the genital tract most frequently occurs in children, it is extremely rare in adults [7]. All three types of RMS have been reported in the uterus. The most common site for uterine RMS is the cervix followed by the corpus [8]. RMS originating from the female genital tract is mostly either embryonal or botryoid type occurring more frequently in children and adolescents.

Pleomorphic RMS, first described by Stout in 1946, is a rare tumor. Whether it was a distinct entity was controversial until immunohistochemistry confirmed its myogenic potential. It is a high-grade rare malignant neoplasm occurring most frequently in adults over 45 years of age. Most patients present with a rapidly growing, painful mass or unusual vaginal bleeding. Imaging reveals lesions which are isodense to skeletal muscle on T1 weighted images, heterogeneous on T2 images, and may or may not have necrotic foci. Grossly the tumor is
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Figure 4. — Uterine pleomorphic rhabdomyosarcoma: A) low magnification image and B) high magnification image showing a highly cellular tumor composed of cells with abundant eosinophilic cytoplasm and eccentric nuclei; C) phosphotungstic acid hematoxylin (PTHA) staining showing cross striations of the rhabdomyoblasts.

Figure 5. — Tumor cells showing immunoreactivity of A) myoglobin, B) cytoplasmic desmin, C) Ki67 and no immunoreactivity for D) estrogen and E) progesterone receptors.
quite large and occasionally surrounded by a pseudocapsule. Cut surfaces are whitish and firm with variable hemorrhagic necrosis. Diagnosis is made on the basis of hematoxylin-eosin staining of the pathological slide. The presence of undifferentiated, round to spindle shaped cells with eosinophilic cytoplasm and eccentric nuclei, expressing at least one skeletal muscle-specific marker (myoglobin, MyoD1, fast skeletal muscle myosin, or myf4) by immunohistochemistry, is required for diagnosis. In addition nonspecific muscle markers (desmin, MSA, SMA, myf3) may also be present [6]. For diagnostic purposes, the most widely used antibodies are against desmin, muscle-specific actin, and myoglobin. RMS is uniformly aggressive, and prognosis is affected by age and stage at diagnosis, anatomic site of disease and the histologic variant of the tumor. Among all histologic varieties of adult RMS, the nonembryonic variant is the rarest. It is particularly aggressive, has often spread beyond the uterus at the time of diagnosis, and has the worst prognosis [9].

Currently there is no standard treatment regimen for RMS in adults. To date, clinical trials have focused on pediatric patients. However, the cytogentic, outcome and response to chemotherapy differ between adult and pediatric populations. The alveolar and pleomorphic varieties are more common in adults, are inherently more aggressive and progress more rapidly than embryonal RMS [10]. In adults, the overall survival is 40% at five years compared to 85% in children with RMS in the reproductive tract [8]. There is significant controversy regarding the disparity in outcome. One viewpoint is that adult RMS is a distinct biologic entity, that behaves more like other adult soft tissue sarcomas that are inherently insensitive to chemotherapy [11]. Others argue that there is no fundamental difference between adult and pediatric patients and that similar treatment modalities are suitable for both groups [4].

According to the intergroup rhabdomyosarcoma study, an IE (ifosfamide, etoposide) containing regimen showed a better response rate [12]. Patients who responded well to IE therapy were then switched to 12 weeks of VAC therapy. This study was conducted in patients younger than 21 years of age, and this IE regimen has not been trialed in adults. There have been reports of response to other regimens. For example, there is one case report of uterine RMS that demonstrated a good response to doxorubicine-based chemotherapy and was still in complete remission one year later [3]. Though our patient initially responded to IE therapy, the effect was short-lived and she failed to respond to any subsequent regimens.

A number of tumor markers and specific antibodies have been reported in RMS. Philip et al. reported one case of RMS with a high CA125 level [12]. However, to our knowledge, there is no report suggesting the association between the elevated LDH and RMS in adults. However, Moritake et al. described such a correlation in three children with advanced alveolar RMS [13]. Our patient’s clinical course coincided completely with the fluctuation of serum LDH levels. This finding suggests that LDH may be a potential marker for diagnosis, prognosis and follow-up for RMS, particularly in advanced stage.

Since RMS in adults is very rare, no large, randomized, double-blinded multicenter studies have been undertaken to compare different treatment modalities and outcome of RMS in the adult age group. Therefore, results concerning treatment, long-term outcome and prognosis of adult RMS are conflicting. The aggressive nature of this tumor in adults calls for more intensive treatment comprising surgical excision with wide margins, appropriate chemotherapy and adequate radiotherapy. Because of the rarity of adult gynecological RMS and the complexity of its clinicopathological features and treatment, the management of patients with gynecological sarcomas should be handled by multidisciplinary teams experienced in the treatment of this entity. Thus, collaboration is needed to establish a standard protocol for adult RMS to optimize patient survival.

References


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Metastatic spread of gynaecological neoplasms to the adrenal gland: case reports with a review of the literature

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Summary

Metastatic involvement of the adrenal glands due to gynaecological neoplasms is a relatively rare condition. The aim of our study was to present four cases of metastases to the adrenal gland due to endometrial adenocarcinoma, ovarian and cervical cancer. These cases are correlated with a review of the literature. CT scan and MRI have been previously used in an attempt to define the nature of the adrenal mass but this approach is of limited value in diagnosis. Image-guided pathological confirmation of an adrenal lesion may significantly change the staging or management of the primary neoplasm. The authors suggest that isolated adrenal metastasis should be routinely considered for surgical management.

Key words: Adrenal metastasis; Endometrial adenocarcinoma; Ovarian cancer; Cervical cancer; Vulvar cancer.

Introduction

The most common tumours to metastasise to the adrenal gland, in order of frequency, are lung cancer, breast cancer, gastrointestinal tumours, followed by malignant melanoma and thyroid neoplasms [1]. The metastatic involvement of the adrenal glands due to gynaecologic neoplasms is a relatively rare condition. Autopsy reports indicate an incidence of 25% for all malignancies [2]. However, the clinically antemortem observed ranges are difficult to estimate.

The aim of our study was to present four cases of metastases to the adrenal gland due to gynaecological neoplasms and to correlate our findings with a review of the literature.

Clinical and pathologic factors were assessed in four patients with a metastasis of the adrenal gland from primitive gynaecological cancer.

In addition, we reviewed the English literature using PubMed over the past 30 years using the following key words: “adrenal metastasis”, “endometrial adenocarcinoma”, “ovarian cancer”, “cervical cancer”, “vulvar cancer”. We present the most representative cases reported.

Case Reports

Case 1

A 76-year-old woman was referred to our cancer centre with a diagnosis of FIGO Stage IV endometrial carcinoma in 1996. Pathological right acetabular fracture and bone biopsy suggested the diagnosis.

The patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic lymph node dissection. Histopathological results revealed an endometrial adenocarcinoma, well differentiated, with depth of myometrial invasion in the inner one-third, with no invasion of the cervix or adnexa and no pelvic node involvement. The patient underwent postoperative pelvic radiation (40 Gy) with a booster dose on the right ischiopubic bone.

The patient was asymptomatic and physical examination was normal. Surveillance computed tomography (CT) scan performed nine months later showed a 50 mm suspicious right adrenal mass. Laparotomy was performed and only partial resection was carried out due to the direct extension to adjacent organs (liver, inferior cavous vein). Pathological examination was consistent with a metastasis of an endometrial adenocarcinoma. The patient was administered medroxyprogesterone (500 mg per os daily). She subsequently developed recurrence with lung metastases and died two years after initial surgery.

Case 2

A 62-year-old woman, gravida 2, para 2, presented in 1992 with postmenopausal bleeding. She underwent a hysteroscopy which revealed an irregular endometrium. A biopsy during the procedure revealed a well differentiated endometrial adenocarcinoma. CT scan of the abdomen and pelvis was considered normal. After uterovaginal brachtherapy (60 Gy), the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic lymph node dissection. Histopathology revealed no residual tumour and 15 pelvic lymph nodes without metastases.

At follow-up seven years after initial surgery, the patient presented with dyspnea. Lung metastases from endometrial carcinoma were detected on the chest CT scan, and confirmed by bronchoscopy and biopsies, with no other evidence of disease. The patient received six courses of adriamycin and cisplatin. She also underwent a second-line treatment with medroxyprogesterone (500 mg per os daily). After a two year period of remission, she was admitted due to dyspnea. CT revealed marked disease recurrence with lung metastases and bilateral inhomogeneous solid masses of the adrenal glands (right 40 x 18 mm, left 44 x 21 mm) as well as a collection of ascites resembling peritoneal carcinosis. The patient was treated with analgesic medication and died two months later, nine years after the initial diagnosis.

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Table 1. — Summary of reports on metastases of the adrenal gland from primitive gynaecologic cancers.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Ref</th>
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<td>Gross BH</td>
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<td>Vulva, squamous cell carcinoma</td>
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<td>[8]</td>
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<td>[13]</td>
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<td>Uterus, clear cell adenocarcinoma</td>
</tr>
<tr>
<td>Einat S</td>
<td>[10]</td>
<td>1</td>
<td>Ovary, papillary adenocarcinoma</td>
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<td>Author et al.</td>
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Case 3

A 62-year-old woman was admitted to our cancer centre in 1997 due to abdominal pain and swelling. On physical examination an abdominal mass was found. Ultrasound and CT scan of the abdomen and pelvis revealed a heterogeneous pelvic mass, which developed from the right ovary. CT scan showed a right upper lobar bronchus mass and three suspicious brain lesions. A bronchoscopy with biopsies confirmed the diagnosis of metastasis due to ovarian cancer. The patient underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and appendicectomy. During the procedure, a left adrenal mass was removed. Pathological examination revealed a bilateral, poorly differentiated, papillary ovarian adenocarcinoma.

The patient was subsequently treated with a combination of cyclophosphamide and paraplatine, and whole brain radiotherapy. The patient died six months later.

Case 4

In 1996, a 61-year-old woman gravida 7, para 7, with a previous history of subtotal hysterectomy for adenomyosis, presented with squamous cell carcinoma of the cervix. The lesion was Stage FIGO IB1 based on physical examination including the chest X-ray, abdomen and pelvic CT scan as well as cystoscopy.

The patient underwent an abdominal radical cervicectomy and pelvic lymph node dissection. Histopathology revealed a 20 x 15 mm well differentiated squamous cell carcinoma with vascular lymph space invasion and healthy margins, and 19 pelvic lymph nodes without metastases. Postoperative pelvic radiation was subsequently administered (48 Gy).

Surveillance CT performed six months after initial surgery showed a new 27 x 20 mm nodule in the left adrenal gland. A CT scan obtained six months later showed that the adrenal nodule had increased in size to 50 x 40 mm. Laparatomy was performed to remove the mass. Pathological findings revealed a 20 x 15 mm well differentiated squamous cell carcinoma with vascular lymph space invasion and healthy margins, and 19 pelvic lymph nodes without metastases. Postoperative pelvic radiation was subsequently administered (48 Gy).

Discussions

We report two cases of metachronous metastases to the adrenal gland from endometrial adenocarcinomas (cases 1 and 2) and two cases of synchronous metastases from ovarian cancer (case 3) and from cervical cancer (case 4). As a short disease-free interval (≤ 6 months) is an indicator of biological aggressiveness of the tumour, we considered case 4, where the adrenal metastasis was discovered within six months of diagnosis of the primary tumour, as M1 (synchronous). All cases, except case 2, were metastases confirmed by surgical pathology evaluation.

Most asymptomatic adrenal lesions are found on CT scan, with a high degree of accuracy in detecting adrenal masses as small as 5 mm [3, 4]. An incidentally discovered adrenal mass, in a patient with no previous history of cancer, is rarely malignant or metastatic, corresponding to a benign non-functioning nodule (including nodular hyperplasia and adenoma). In contrast, as many as 36% of adrenal masses in patients with a history of malignant disease are metastatic [2]. This suggests that over 50% of adrenal masses are benign [4]. All patients had a previous medical history of malignancy and all were asymptomatic (i.e. without clinically apparent adenral disease). Symptoms such as asthenia, lassitude, weight loss or nausea are common in patients with widespread malignancy and were absent in all cases. Seidenwurm et al. recommend that any patient found to have enlarged adrenal glands on CT scan and known cancer, should undergo endocrine testing to detect adrenal insufficiency by an ACTH stimulation test [5].

Various imaging techniques have previously been employed in an attempt to define the nature of the adrenal mass. CT scan cannot reliably characterise an adrenal lesion as benign or metastatic. Size above 50 mm, invasion into adjacent tissue, and a growing mass at follow-up CT scan are helpful criteria in distinguishing malignant lesions from adenomas. Attenuation values of less than 10 Hounsfield units at unenhanced CT are practically diagnostic for adenomas, while attenuation values of greater than 10 HU are not diagnostic of metastatic disease since non-metastatic disease is also a possibility. Magnetic resonance imaging (MRI) is of limited value. Nevertheless, an adrenal lesion with decreased signal intensity in out-of-phase MRI acquisition may be regarded as benign [18]. F-FDG PET could possibly be useful in differentiating benign from metastatic adrenal lesions detected on CT or MRI, with a sensitivity of 100%, a specificity of 94%, and an accuracy of 96% (patients with lung cancer). In addition, PET scan has the advantage of revealing the primary cancer sites and detecting other metastases.

Pathological confirmation of an adrenal lesion is of prime importance for subsequent management [4, 7]. Although percutaneous fine-needle aspirations or core biopsies are sensitive, specific and less invasive techniques than surgery, we decided to perform three laparotomies for two patients in order to assess the diagnosis (cases 1 and 4). For case 3, the adrenal metastasis was diagnosed during the surgical debulking procedure of an
ovarian cancer. Complete resection rendering the patient free of disease was possible in only one case (case 4), for a two-stage procedure with an interval of six months. No major perioperative complications were found. With evidence of metastatic disease (brain, lungs), case 3 should not have been selected for surgical management. Patients whose adrenal metastases are discovered synchronously may be less amenable to cure from metastasectomy because other “occult” sites may rapidly be revealed after surgery. According to Kim et al., adrenalectomy should be strongly considered for surgical management if complete resection of an isolated metastasis can be achieved, given the relatively poor results reported with radiation and chemotherapy. A disease-free interval > 6 months is a significant predictor of improved survival [1, 8]. Adrenalectomy can be performed safely and could prolong survival in selected patients. The oncological outcome appears similar with no difference in the incidence of positive resection-margins or survival between patients with laparoscopic adrenalectomy or open adrenalectomy [8].

Cervical carcinoma

In a retrospective review of autopsy findings and comparison of the pattern of metastatic spread of squamous cell carcinoma and adenocarcinoma of the cervix, adrenal gland involvement was observed in seven of 21 patients with adenocarcinoma and in none of 21 patients with squamous cell carcinoma. This may indicate a propensity toward hematogenous spread, possibly along the ovarian vessels. Additionally, the adrenal gland may provide a local hormonal factor that is favourable for the growth of metastatic implants in adenocarcinoma. [9]. To our knowledge, we report the first case (case 4) of bilateral adrenal metastasis from squamous cell carcinoma of the cervix. Gross et al. previously reported this type of histological lesion from a vulvar carcinoma [3].

Ovarian carcinoma

Ovarian carcinoma can spread via peritoneal implantation, lymphatic invasion, and haematogenous dissemination. Hematogenous metastases from ovarian carcinoma may become more commonly recognised as new treatments are implemented, resulting in improved survival rates. The reported prevalence of adrenal metastases in patients with ovarian cancer at autopsy is 15% [10]. Einat et al. reported the case of successful laparoscopic removal of a solitary adrenal metastasis from an ovarian papillary adenocarcinoma [11]. The patient was clinically free of disease after two years of follow-up. A case of simultaneous adrenal and pancreatic metastases in a patient with serous ovarian carcinoma has also been reported, with no further treatment following the diagnosis of metastasis [12]. The third case was a CT scan description of a bilateral adrenal gland enlargement consistent with metastatic disease from an ovarian carcinoma in a prospective evaluation of adrenal insufficiency in 15 consecutive patients with adrenal metastasis [13]. No data are available on histology, adrenal insufficiency, treatment or follow-up for this particular patient. According to these authors, one third of the patients in this series presented with an adrenal insufficiency.

Endometrial carcinoma

The sole reported case of adrenal metastasis from endometrial carcinoma was an advanced stage of a particular subtype [14]. Clear-cell carcinoma of the endometrium is a highly malignant neoplasm that accounts for less than 5% of endometrial carcinomas. Cases 1 and 2 were more common and well differentiated endometrial carcinomas, nevertheless these patients subsequently experienced widespread malignancy after a disease-free interval of nine months and seven years. In case 1, an approach other than an “aggressive” and incomplete surgery should have been considered due to an initial Stage IV synchronous bone metastasis.

Conclusion

An incidentally discovered adrenal mass in a patient with a history of cancer should suggest histological confirmation, which avoids a mistaken diagnosis of metastasis in patients with benign adrenal masses or detects an unusual adrenal metastasis. Adrenal metastasis most commonly occurs due to multiple synchronous metastases in other sites. Palliative management is then considered the treatment option. Resection of solitary adrenal metastasis may offer the best alternative to cure the patient and can be achieved both by laparoscopy or laparotomy.

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Primary squamous cell carcinoma of the endometrium: a case report

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Summary

Purpose of investigation: Primary squamous cell carcinoma of the endometrium (PSCCE) is an extremely rare entity. Methods: We present the clinical and pathological findings of a 90-year-old patient with International Federation of Gynecologists and Obstetricians Stage 1C primary squamous cell carcinoma of the endometrium who was treated with hysterectomy and bilateral salpingo-oophorectomy. Results: The patient declined adjuvant therapy and continues on progestin therapy. She was free of disease at a one-year follow-up visit. In addition, the current literature is discussed in this report. Conclusions: Since primary squamous cell carcinoma of the endometrium is so infrequent, it is difficult to evaluate the efficacy of adjuvant therapy. Although the prognosis historically has been reported as poor compared to endometrial adenocarcinoma, the prognosis does seem to be dependent on the surgical stage at diagnosis rather than on the adjuvant treatment component.

Key words: Endometrial cancer; Squamous epithelium; Primary squamous cell carcinoma of the endometrium; HPV; Treatment; Follow-up.

Introduction

Squamous epithelium in the uterine cavity has been reported in association with benign uterine pathologies, but occasionally it may be found in relation to malignancy of the endometrium, appearing as a benign component in adenosquamous carcinoma or as a malignant component of an adenosquamous carcinoma [1]. Although up to 30% of endometrial carcinomas present with squamous cell differentiation [2], primary squamous cell carcinoma of the endometrium (PSCCE) is extremely rare and represents less than 1% of endometrial carcinoma cases [3]. Gebhard described the first substantiated case in 1892 [4]. Furthermore, in 1928, Fluhmann [5] proposed three criteria to determine with precision that a squamous carcinoma is a primary neoplasia of the endometrium: 1) A coexistent adenocarcinoma of the endometrium must not be present, 2) There should be no connection between the tumor and the squamous epithelium of the cervix, 3) Absence of primary squamous cell carcinoma of the cervix. In 1975, the WHO extended Fluhmann’s criteria and added a fourth criterion: There must be clear evidence of squamous differentiation in the tumor such as intercellular bridges and/or keratin [3, 6]. The diagnosis demands meticulous histological examination, especially with regard to the microscopic appearance of the cervical area [7]. Since Gebhard’s initial report in 1892 [4], the literature contains less than 100 cases fulfilling the previously cited four criteria.

The management of PSCCE is mainly surgical with abdominal hysterectomy and bilateral salpingo-oophorectomy as the most frequently used procedures [8, 9]. Radical hysterectomy with pelvic lymphadenectomy has also been reported as the treatment of choice in some cases [8]. Postoperative adjuvant chemoradiation has been used in selected cases. However, since the condition occurs so infrequently, it is difficult to evaluate the efficacy of this additional therapy [9]. Although historically the prognosis of PSCCE has been reported as poor compared to endometrial adenocarcinoma [9], the prognosis does seem to be dependent on tumor stage emphasizing that early diagnosis and treatment are imperative.

The purpose of this case study is to report the clinicopathological features of a recent case of PSCCE and to discuss the current literature.

Case Report

A 90-year-old multiparous 60 kg Caucasian female presented with a 5-day history of postmenopausal bleeding. She did not have a history of abnormal pap smears or hormone replacement therapy use. A CT of the abdomen/pelvis was performed showing the endometrial cavity to be fluid-filled with thickened uterine walls. No focal uterine or adnexal masses or lymphadenopathy were apparent. A pelvic ultrasound measured the uterus to be 10.5 cm x 5.4 cm x 6 cm with an endometrial stripe of 2.2 cm and atrophic adnexa. An endometrial biopsy was reported as poorly differentiated squamous cell carcinoma.

The patient was referred to our institution for additional evaluation and management. The patient was counseled on her options in view of her age and associated comorbidities including definitive surgical management, radiation therapy or continued observation with progestin therapy. She underwent exploratory laparotomy, total abdominal hysterectomy, and bilateral salpingo-oophorectomy without complications. Intraoperative findings showed a normal upper abdomen and no palpable lymph nodes. Cytologic examination from pelvic washings was negative. Grossly, the uterine cavity showed a 5 cm x 4.5 cm exophytic...
mass filling the uterine cavity (Figure 1). Histological examination showed an invasive poorly differentiated squamous cell carcinoma of the endometrium with 98% myometrial thickness invasion (13.8/14mm) and extension to the lower uterine segment (Figure 2). There were frequent keratin pearls throughout the tumor (Figure 3). Extensive tumoral neutrophilic infiltrates were also present. The cervical mucosa was unremarkable. Both adnexes were unremarkable. Immunohistochemistry studies for estrogen and progesterone receptors were negative and tumor cells were focally positive for p16.

The patient was discharged home on the seventh postoperative day. Further treatment options were reviewed with the patient and she declined radiation or chemotherapy treatment at that time. She continues on oral progestin therapy and was doing well at her 12-month follow-up visit.

Discussion

The pathogenesis of squamous cell carcinoma of the endometrium continues to be controversial. Two theories have been offered to explain the pathogenesis of PSCCE: the vertical field theory and the squamous metaplasia theory [6, 11]. Indeed, some investigators claim that squamous metaplasia is always a pathological process, and may be a precursor to the development of squamous cell carcinoma [11]. Factors implicated in the development of squamous metaplasia include various infectious and foreign irritants including pyometra and intrauterine devices, fibromyoma, vitamin A deficiency, hormonal disturbances (deficiency as well as excess of estrogen hormones), uterine prolapse or inversion, pelvic irradiation, and chemical agents [11]. However, in a review of 34 cases of PSCCE, squamous metaplasia was documented clearly in only 11 cases, suggesting that endometrial squamous metaplasia is not a definitive precursor of PSCCE [12]. Several other factors have been discussed as being influential in the development of PSCCE. HPV has been implicated strongly in squamous neoplasms of the lower genital tract and has also been identified in the squamous component of endometrial adenocarcinoma [6, 10]. In case reports using DNA primers for HPV and in situ hybridization, HPV was not detected and appears to be an unlikely carcinogenic factor in the development of PSCCE [2, 6, 10, 12-13]. The expression of p16, p14, p53, cyclin D1, and steroid hormone receptors (estrogen, progesterone, and androgen) has also been examined immunohistochemically in eight PSCCE cases [14]. All
but one case was negative for HPV analysis. One case was positive for estrogen receptors, four cases were positive for progesterone receptors, and none of the cases showed androgen receptor immunostaining. The results of that study suggest that alterations of the p16 pathway may play an etiologic role in at least a proportion of PSCCE, but without any association to HPV infection [14]. In our case, p16 was focally positive and estrogen/progesterone receptors were negative.

In a comprehensive review of the literature, Goodman et al. described the clinical characteristics of 64 patients with PSCCE [9]. As described in the case above, most cases of PSCCE present in postmenopausal women with an average age of 67 years (older than most patients with corpus carcinoma). Eighty-eight percent (37/42) of the cases reviewed were recorded as Caucasian and the average weight in their six cases was 136 pounds. Of the 48 patients whose parity was known, 17 (35%) were nuliparous. Nulliparity, presumed to be secondary to chronic anovulation, has been cited as a risk factor for carcinoma of the endometrium in general, with frequencies ranging from 18 to 40% [11]. In seven of 41 cases in which information was available, the patient was recorded to have taken estrogens (17%); in one additional case tamoxifen was used. Six patients had previous cancers: three breast carcinomas, two colon carcinomas, and one concurrent ovarian carcinoma. In addition, a review of these 64 cases for predisposing factors revealed that 20 patients presented with pyometra [9]. The etiology of a pyometra has been described by Yamashina and Robara and attributed to the tendency of PSCCE to undergo necrosis; a relatively late discovery of PSCCEs; and poor drainage due to polyplody growth of tumors and a nulligravida state of the patient [15]. It also has been suggested that pyometra and chronic infection may be the result rather than the cause of the disease [2, 9]. In addition, three patients had uterine inversion or prolapse and five had a history of pelvic radiation. Cervical stenosis was stated to be present in four patients [9]. In our case, we could not identify any risk factor. The presenting signs and symptoms in these 64 cases were postmenopausal bleeding (68%), vaginal discharge (28%), pain (17%), weight loss (6%), pelvic mass (6%), brain metastases (1.5%), and more than one sign/symptom (39%) [9]. Cervical or vaginal smear status was known in 32 cases of which 15 were abnormal [9].

The diagnosis of PSCCE should be considered in postmenopausal women whose smears show abnormal squamous cells and in whom there is a negative cervical biopsy [1, 9]. It is suggested that cytology permits earlier and more accurate diagnosis [1, 9]. However, the final diagnosis of PSCCE should be made on the hysterectomy specimen. Hysterectomy with bilateral salpingo-oophorectomy was the primary treatment in 62 of the 64 cases [9]. Although it is difficult to evaluate the efficacy of additional therapy, postoperative adjuvant chemoradiation has been used in selected cases [9, 10].

Although historically the prognosis has been reported as worse than for the more common adenocarcinoma, adenocanthoma, and adenosquamous carcinoma, Goodman et al. reported the evaluable outcome data in 42 of their 64 cases presented [9]. All the patients with surgical Stage I tumors (8/8) survived with a median survival of 45 months (range 14-114 months), suggesting that a tumor that is confined to the uterus, regardless of the grade and depth of myometrial invasion, has a good prognosis [9]. Similar to other types of endometrial cancer, vascular space invasion appears to be a risk factor for recurrence. In contrast, all six patients with Stage IV disease (median time to death 17 months, range 5-18 months), and 80% (8/10) with Stage III disease, died despite adjuvant therapy [9]. Our patient had Stage I disease and we can speculate a good survival rate despite her decline to undergo adjuvant therapy.

Conclusion

Vaginal bleeding and discharge, pyometra, abnormal squamous cytologic findings, or combinations thereof that are unexplained after thorough evaluation of the cervix require further investigation. Cytology seems to permit earlier and more accurate diagnosis. Therefore, abundant benign-appearing squamous epithelium on a curettage specimen obtained from the endometrial cavity in a postmenopausal patient usually warrants a hysterectomy because of the possibility of carcinoma [9]. After definitive surgical therapy, postoperative adjuvant chemoradiation has been used in selected cases. However, since PSCCE is so infrequent, it is difficult to evaluate the efficacy of this additional therapy [9, 10] Although the prognosis of PSCCE historically has been reported as poor compared to endometrial adenocarcinoma, the prognosis does seem to be dependent on the surgical stage at diagnosis rather than on the adjuvant treatment component [8, 9, 11].

References


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Carcinosarcoma of the uterus:
a case report and review of the literature

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Introduction

Carcinosarcoma, previously defined as malignant mixed Mullerian tumor, is a rare neoplasm, accounting for 2-5% of all malignant tumors of the uterus [1-4]. It is characteristically composed of malignant epithelial (carcinomatous) and mesodermal (sarcomatous) cells. Carcinosarcoma is classified into homologous and heterologous types. In homologous tumors, both the carcinomatous and sarcomatous elements are normal components of the Mullerian system. In heterologous tumors, sarcomatous elements that have no benign counterpart in the uterus, such as skeletal muscle, bone and cartilage, are present [5]. Homologous and heterologous carcinosarcomas occur with approximately equal frequency [6].

We present a case of homologous carcinosarcoma and review the clinicopathological features, treatment options and prognosis of this aggressive neoplasm.

Case Report

A 82-year-old woman was admitted with the complaint of postmenopausal bleeding during the previous ten months.

The patient attained menarche at the age of 14 years and had a regular cycle with 6-day flow every 29 days until she was 50 years old when spontaneous menopause supervened. She was never submitted to Pap smear tests and she had never used any contraceptive device or pills. She was not sexually active. Obstetric history showed four full-term spontaneous vaginal deliveries.

At physical examination she was cachectic and pale. Her blood pressure was 130/80 mmHg. Abdominal examination did not reveal any abnormal mass or ascites. Per vaginal examination revealed ongoing bleeding with the presence of a cervical hematoma. The adnexa and the Douglas pouch were normal.

Blood cell count revealed signs of anemia (red blood count = 3,050,000/mm³, hemoglobin = 8.8 g/dl, hematocrit = 28.5%). Anemia was treated with transfusion of two units of packed red cells (hemoglobin = 11.4 g/dl).

Transvaginal ultrasonographic (TVS) scan showed an enlarged uterus (8 x 7.5 x 4 cm) with an intrauterine mass measuring 5 x 5.5 cm. The adnexa were normal and no evidence of intraabdominal fluid collection was detected. Abdominopelvic nuclear magnetic resonance (NMR) imaging confirmed the presence of the intrauterine mass infiltrating the myometrium and reaching the cervix. The adnexa and other abdominal organs were normal (Figure 1).

The patient was submitted to total abdominal hysterectomy with bilateral salpingo-oophorectomy. There was no macroscopic evidence of intraabdominal dissemination and/or pelvic and paraaortic lymph node involvement. Her postoperative course was uneventful.

Gross examination of the surgical specimen showed a grayish exophitic mass, arising from the lumen of the uterus. Histological examination revealed the presence of mixed malignant epithelial and stromal cells, compatible with the diagnosis of carcinosarcoma (Figure 2). The neoplasm infiltrated the inner one-third of the myometrial layer (pT1b, pNx, pMx; FIGO Stage 1B). A CT of the total body performed six months after surgery showed no signs of recurrent and/or metastatic tumor.

Summary

Carcinosarcoma is a rare tumor of the uterus with a poor prognosis. We present a case of uterine carcinosarcoma in an 82-year-old woman who suffered from pervaginal bleeding for ten months duration with progressive anemia. Abdominopelvic nuclear magnetic resonance (NMR) imaging showed the presence of an intrauterine mass, infiltrating the myometrium and reaching the cervix. The patient was submitted to total abdominal hysterectomy with bilateral salpingo-oophorectomy. The carcinosarcoma, arising from the lumen of the uterus, infiltrated the inner one-third of the myometrial layer (pT1b, pNx, pMx; FIGO Stage 1B). A CT of the total body performed six months after surgery showed no signs of recurrent and/or metastatic tumor.

The clinicopathological features, treatment options and prognosis of this aggressive neoplasm are reviewed.

Key words: Carcinosarcoma, Uterus.
Carcinosarcoma generally occurs in postmenopausal women at a median interval from menopause ranging from 15 to 17 years [3, 7-10]. Recognized risk factors, similar to those reported for endometrial carcinoma, are obesity, nulliparity, exogenous estrogen use and tamoxifen therapy [11-18].

Our patient presented with postmenopausal bleeding for the previous ten months. Bleeding, combined with signs of uterine enlargement, is the commonest symptom [5]. In some cases abdominal pain is also present [19]. Endometrial curetting can be diagnostic in 50-70% of cases [20-22]. Recognized limiting factors are the small amount of tissue obtained, frequent necrosis, and inflammation of the tumor surface. Moreover, uterine curetting can be misleading in that only one type of tissue may be obtained, i.e. either the epithelial or stromal component only, so that the true biphasic nature of the neoplasm becomes apparent only when the entire specimen is available for study [6, 23].

Carcinosarcomas are characterized by an aggressive clinical course and an extremely poor prognosis. Seventy to 90% of tumor-related deaths occurred within 18 months after diagnosis [2, 24, 25]. Advanced stage at diagnosis has been postulated to account for much of the clinical aggressiveness of this tumor type, the prognosis being very poor when the neoplasm has extended beyond the uterus [2, 6, 7, 20, 26-31]. However, even patients with disease confined to the uterus have 5-year survival rates of less than 50% [21, 32-37]. An important prognostic factor is the depth of myometrial invasion [6, 23, 28, 29, 38-45]. However, given the high rate of microscopic metastases in patients with disease clinically confined to the uterus, this pathologic risk factor may simply be a surrogate marker for metastatic disease [46]. The homologous and heterologous subtypes do not seem to influence the prognosis [5].

Although the early literature is conflicting, recent studies have found that behavior and overall prognosis of carcinosarcoma is much more dependent on the characteristics of the epithelial than the stromal elements [43, 47]. Metastases invariably consist of the carcinomatous elements [43, 48, 49]. These characteristics, associated with evidence derived from immunohistochemical and molecular studies, suggested that carcinosarcoma is, in reality, derived from a single stem cell, in which the sarcomatous component is a metaplastic transformation of the epithelial component [50-56]. However, a small proportion of carcinosarcomas may originate from independent carcinomas and sarcomas [48].

NMR imaging has showed high accuracy in the local-regional staging of endometrial tumors, while the assessment of pelvic and lumbo-aortic lymph nodes seems more difficult [57].

Surgery in the form of abdominal hysterectomy and bilateral salpingo-oophorectomy including a visual inspection of the pelvic and paraaortic lymph nodes with removal of any and all suspicious lymph nodes, is the mainstay of treatment, according to the 1988 FIGO surgical staging system. About 20-60% of patients with disease confined to the uterus preoperatively will be upstaged after proper surgical staging [21, 33-37, 58]. Approximately, 20% of patients will be surgically upstaged because of metastases to regional lymph nodes [9, 34-37, 43, 58, 59]. The therapeutic importance of lymphoadenectomy still remains unclear, although an improved outcome may be expected to be similar to what was found in patients with endometrial carcinomas [46].

Due to its relative rarity, optimal adjuvant treatment of carcinosarcoma has remained poorly defined. Part of the difficulty with determining the “best therapy” stems from...
the question of whether this entity should be treated as a “carcinoma” or a “sarcoma”. While traditional treatment strategies have focused on both local and extended field radiation therapy and whole abdominal radiation techniques, a number of investigators have argued in favor of chemotherapy, because of the substantial activity observed with chemotherapy in endometrial adenocarcinomas. A randomized phase 3 trial reported by Wolfson and colleagues [60] has provided strong support for the superiority of chemotherapy compared with radiation in this difficult disease entity. This trial compared whole abdominal irradiation with a combination regimen of cisplatin plus ifosfamide in 224 women with optimally resected Stages I to IV carcinosarcoma. Adjusting for stage, the trial revealed a 28.5% reduction in the risk of recurrence associated with chemotherapy, and, most importantly, a 33% decrease in the death rate (hazard ratio 0.672; p = .042).

In conclusion, carcinosarcoma is a rare uterine neoplasm. Vaginal bleeding is the most common symptom. A correct diagnosis can often be achieved after histological examination of the entire surgical specimen. It is a highly aggressive tumor with poor prognosis. Surgery is the mainstay of treatment. The optimal adjuvant treatment remains to be established.

References


Small cell carcinoma of the ovary successfully treated with radiotherapy only after surgery: case report

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Summary

Small cell ovarian tumors are rare and highly malignant, occurring mainly in young patients. Early mortality is high due to the lack of an effective treatment. The first adjuvant therapy is usually chemotherapy. Case: During laparotomy for renal transplant in a 17-year-old girl, the right ovary exhibited a suspicious mass, whose pathological diagnosis was Stage 1A small cell ovarian tumor. Prognosis was poor (young age, hypercalcemia, tumor > 10 cm, and presence of large cells). Since chemotherapy is contraindicated for dialysed patients, only radiotherapy was given. The patient is still alive and disease-free ten years after diagnosis. Conclusion: This is the first case with a poor prognosis reported in the literature that has been successfully cured by surgery plus adjuvant radiotherapy only.

Introduction

Small cell carcinoma of the ovary, hypercalcemic type (SCCOHT), is an extremely malignant tumor [2]. The treatment is often ineffective, aggressive surgery is recommended as the primary treatment with further adjuvant chemotherapy [1-3]. Though this tumor seems to be both chemo- as well as radiosensitive, the long-term survival of patients who receive both adjuvant chemo- and radiotherapy is generally disappointing [1]: recurrence occurs on average 18 months after diagnosis [2]. We report a case of SCCOHT in a 17-year-old girl with several poor prognostic factors, who, after surgery, received radiotherapy as the only adjuvant therapy. She is still alive and has been disease-free for more than ten years.

Case Report

The patient, a 17-year-old female, suffered from terminal chronic renal insufficiency, secondary to primary mesangial glomerulonephritis, which had become corticoid-resistant. She had been under hemodialysis since the age of 13. In August 1994, serum calcium was 11.2 mg/dl and phosphorus 7.0 mg/dl; the levels of PTHi (intact parathyroid hormone) and alkaline phosphatases were normal. Since the patient did not receive any vitamin D, the diagnosis of adynamic osteomalacia was considered. Treatment with calcium kayexalate was initiated. Despite treatment, calcium levels remained high, reaching up to 12.8 mg/dl in September 1994. Even the non-compliance of the patient to this treatment was considered.

In October 1996 the patient underwent laparotomy for renal transplantation. She had experienced abdominal pain for several months before. During laparotomy a right ovarian mass (17 cm in diameter), showing a smooth wall with focal nodules, was discovered. No ascites or adenopathies were found. The right ovary was removed; pathological examination on frozen section revealed an undifferentiated malignant tumor. Debulking was optimal with no macroscopic residual disease. Biopsies were taken from the left ovary, iliac lymph nodes, peritoneal and epiploic tissues. Uterine curettage was also performed. Because of the presence of malignant disease, the renal transplantation was not performed.

The tumor weighed 850 g on sectioning; the tissue was partially solid and white with cysts containing coagulated blood. No ovarian tissue could be recognized. Microscopically the lesion was composed of small non-differentiated cells with scanty amphophil cytoplasm, arranged in clusters, cords or follicle-like structures; there were numerous atypical mitoses. Some tumor cells had abundant eosinophilic cytoplasm. Areas of hemorrhage and necrosis were observed. The histological diagnosis considered was that of a granulosa cell tumor (juvenile type). Because of high calcium levels, this diagnosis was however reconsidered since granulosa and germ-cell tumors can often be confused with small cell ovarian tumors. The histological slides were therefore sent to Prof. Robert E. Scully (Harvard Medical School, Boston, USA), who established the diagnosis of small cell ovarian tumor containing large cells. Immunohistological staining detected parathyroid hormone-related protein (PTHrP) in the tumoral tissue. The contralateral ovary, multiple peritoneal biopsies, the lymphatic ganglia and peritoneal cytology showed no evidence of malignancy. The tumor was thus classified as Stage 1A according to the FIGO classification.

Serum calcium levels returned rapidly to normal postoperatively. Though there is no consensus concerning the adjuvant therapy, chemotherapy (usually the first choice) was not considered because of renal insufficiency. Since several poor prognostic factors were present (patient age < 20 year old, a large tumor > 10 cm, hypercalcemia and presence of large cells in the tumor [5]), whole abdomen radiotherapy was administered and started in December 1996. A dose of 30 Gy was delivered to the whole abdomen with the liver shielded at 22 Gy with a daily fraction.

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Histological diagnosis relies on the presence of nests, cords, clusters of small hyperchromatic round or oval cells diffusely or closely packed. The various patterns of growth are interrupted by follicle-like spaces. The neoplastic cells typically exhibit important mitotic activity. Young et al. [4] reported that 50% of the tumors had a variable component of cells with moderate to abundant amounts of eosinophilic cytoplasm, which sometimes contained large hyaline globules and large nuclei that are typically paler and have more prominent nucleoli than the small cells.

The genesis of small cell carcinoma remains obscure [5, 6]. Some evidence has been provided that small cell carcinoma of the ovary could be an inhomogeneous tumor which is either related to a germ cell tumor (co-expression of vimentine and cytokeratins, positivity for alpha 1 antitrypsine) or to an epithelial ovarian cancer (thin basal lamina, numerous desmosomes) [5]; it could even represent a distinct tumor entity [7]. The differential diagnosis with various tumors can be difficult and confused with granulosa cell tumors (because of the pseudo follicular structures, as in our case) [4], with sex-cord tumors, dysgerminoma, ovarian metastases of lymphomas, alveolar rhabdomyosarcoma or melanoma.

Hypercalcemia is often evidenced in patients having this kind of tumor and calcium levels usually return to normal after tumorectomy [1, 2]. The mechanism of such hypercalcemia is not fully understood. PTHrP produced by the tumor itself, has been implicated in the pathogenesis of tumor hypercalcemia. PTHrP binds to PTH receptors in bone and kidney with equal affinity as PTH [1], leading to increased osteoclastic bone resorption and renal calcium reabsorption. The stimulation of increased bone resorption and renal tubular reabsorption of calcium results in persistent elevation of serum calcium levels. This hypothesis remains, however, not totally satisfactory. Postoperatively, these serum calcium levels are a marker of recurrence [2, 6].

Small cell carcinoma of the ovary is highly malignant and has a poor prognosis. The overall survival rate after five years is approximately 10%. Only 33% of the patients with a Stage 1A tumor, as in this case, were alive without evidence of recurrence 1-13 years post surgery, 54% died within two years and recurrence occurred in 13% of the cases [4]. Since there is no consensus on the optimal adjuvant therapy, this makes the report of our case quite interesting. In 99% of the cases the tumor is unilateral and therefore unilateral salpingo-oophorectomy was usually performed; it is possible that bilateral salpingo-oophorectomy might be somewhat more curative, especially since recurrence in the contralateral ovary has been reported [1]. Young et al. recommended the latter procedure, despite no significant difference between the two modalities. A more aggressive therapy is even recommended in young women: hysterectomy and bilateral salpingo-oophorectomy followed by chemotherapy [2]. Some authors recommend, similarly to advanced ovary cancer, using immediately intensive polychemotherapy, with or without external treatment.
radiotherapy and bone marrow graft [8]. Due to the fact that SCCOHT is a rare tumor with a poor prognosis, no consensus is available about adjuvant therapy. The best results in the literature have been obtained with surgery followed by polychemotherapy plus radiotherapy [4, 5, 9]. In their review of 150 cases, Young et al. [4] reported that, among the five patients who received postoperative radiotherapy, four were still alive. It is only recently that Harrison et al. [5], from data collected in Australia, Canada and Europe, have advocated a multimodality treatment approach including surgery, chemotherapy and the addition of radiotherapy. It would suggest that radiotherapy could be more effective to cure this kind of tumor. Whole abdomen radiotherapy for treatment of ovarian cancer has also been stressed by Firat et al. [10], who reported ileal occlusion to be a classic complication, as seen in our patient. The case of our patient with a poor prognostic tumor, who received only radiotherapy and is still alive ten years later without recurrence, reinforces and strongly supports our hypothesis. Thus, we cannot agree with another recent review of SCCOHT in children and adolescents by Distelmaier et al. [3] who propose only multi-agent chemotherapy as adjuvant therapy.

Conclusion

When unexplained hypercalcemia is diagnosed in a young woman, the presence of an ovarian tumor must immediately be excluded. This case report illustrates how difficult a correct diagnosis is, but the presence of hypercalcemia can be helpful. Small cell carcinoma of the ovary, hypercalcemic type, is of poor prognosis and the survival rate after five years is usually around 10%. The type of optimal adjuvant therapy to recommend is unclear but, according to our case together with the literature, it appears that radiation therapy could be of prime importance.

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Fallopian tube malignant mixed müllerian tumor (carcinosarcoma): a case report with immunohistochemical profiling

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Summary

We report a case of malignant mixed müllerian tumor (MMMT) (carcinosarcoma) of the right fallopian tube in a 69-year-old woman presenting with abdominal pain and an adnexal mass. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, received adjuvant chemotherapy and is without evidence of disease 12 months postoperatively. The tumor involved the fallopian tube and was composed of in situ and invasive high-grade serous and undifferentiated carcinoma, leiomyosarcoma, rhabdomyosarcoma and undifferentiated sarcoma. Immunohistochemically, the epithelial and mesenchymal cells expressed CD56, Leu-7 and p53. The epithelial elements expressed nuclear WT1 and calretinin while the mesenchymal cells showed negative nuclear and strong cytoplasmic staining. HBME was observed focally in carcinoma. The expression of mesothelial-associated antigens WT1, calretinin and HBME in MMMT likely reflects the common embryologic derivation of the mesothelium and urogenital ridge. Loss of nuclear WT1 expression in the mesenchymal component may be involved in MMMT tumorigenesis.

Key words: Fallopian tube; Carcinosarcoma; Malignant mixed müllerian tumor (MMMT); Immunohistochemistry; WT1.

Introduction

Malignant mixed müllerian tumors (MMMT)/carcinosarcomas of the female genital tract are uncommon, but clinically highly aggressive neoplasms with biphasic histology of carcinomatous and sarcomatous elements [1]. Fallopian tube carcinosarcoma is extremely rare, with only about 70 cases previously reported in the international literature [2]. We present a case of fallopian tube MMMT, describe its immunophenotypical characteristics with respect to tumor genesis, and review the literature.

Case Report

A 69-year old, gravida 4, para 2, postmenopausal Greek woman presented with right lower quadrant abdominal pain. Her past medical, surgical and family history was unremarkable. Gynecologic examination revealed a right adnexal mass, but was otherwise unremarkable, findings confirmed by computed tomography (CT) and ultrasound (US) examination. Pre-operative chest X-ray and CT, intravenous urography, colonoscopy and urethrocystoscopy were normal. On exploratory laparotomy, the right fallopian tube was markedly distended. Frozen section examination confirmed malignancy and the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy, and omentectomy.

Pathology

On gross examination, a solid and cystic tumor mass measuring 12.4 x 8.5 x 4 cm, and covered by purple serosa bearing adhesions was identified in a progressively distended tortuous fallopian tube measuring 0.8-2.2 cm in diameter. The cut surface was solid and cystic, tannish-white with necrotic foci and the consistency elastic to soft-friable. The right ovary and uterus with attached left adnexa showed no macroscopic abnormalities.

The specimen was examined histologically and the tumor involved the fallopian tube and was composed of in situ and invasive high-grade serous and undifferentiated carcinoma, leiomyosarcoma, rhabdomyosarcoma and undifferentiated sarcoma. Immunohistochemically, the epithelial and mesenchymal cells expressed CD56, Leu-7 and p53. The epithelial elements expressed nuclear WT1 and calretinin while the mesenchymal cells showed negative nuclear and strong cytoplasmic staining. HBME was observed focally in carcinoma. The expression of mesothelial-associated antigens WT1, calretinin and HBME in MMMT likely reflects the common embryologic derivation of the mesothelium and urogenital ridge. Loss of nuclear WT1 expression in the mesenchymal component may be involved in MMMT tumorigenesis.

Key words: Fallopian tube; Carcinosarcoma; Malignant mixed müllerian tumor (MMMT); Immunohistochemistry; WT1.
desmin, myogenin, myoD1, WT1 (cytoplasmic only) and calretinin. Both components were positive for p53, CD56 and Leu-7, and negative for synaptophysin, chromogranin, S-100 protein, HMB-45, MART-1, CD31, CD34, ER, PR, c-erb-B2 and c-erbB2.

Histologic examination of the uterus, ovaries, contralateral fallopian tube and omentum was negative for tumor. A diagnosis of primary fallopian tube heterologous MMTT with serous carcinoma, rhabdomyosarcoma and leiomyosarcoma was rendered. The peritoneal washings were negative for malignant cells. The patient, staged as pT1c NX M0, underwent postoperative chemotherapy consisting of six courses of adriablastine (40 mg/m²), paclitaxel (175 mg/m²) and cisplatin (75 mg/m²) and remains well with no evidence of disease as evidenced by CT scans of the chest, abdomen and pelvis, and abdominal US 12 months postoperatively.

Discussion

A case of MMTT of the fallopian tube with leiomyosarcomatous and rhabdomyosarcomatous differentiation has been described. The expression of the neuroendocrine markers CD56 and Leu-7 in both the epithelial and mesenchymal components of the tumor is suggestive but not certain evidence of neuroendocrine/neuroectodermal differentiation, since the more definitive neuroendocrine markers, synaptophysin and chromogranin were negative. CD56 is expressed in several neuroendocrine/neuroectodermal derived tumors, and various carcinomas [3], but to the best of our knowledge, it has not been identified in MMTT. Leu-7 expression has been reported in MMTT and neuroendocrine differentiation, identified in 17% of MMTTs, has been associated with more aggressive behavior [4]. The absence of the melanocytic markers (S-100 protein, HMB-45, MART-1) excluded melanocytic differentiation [5].

The mesenchymal elements consisted of undifferentiated medium sized ovoid to spindle mesenchymal cells among which aggregates of large rounded or tadpole-shaped eosinophilic cells resembling rhabdomyoblasts and large multivacuolated cells resembling lipoblasts were noted. The mesenchymal elements including eosinophilic and multivacuolated cells expressed vimentin and HHF-35 and various carcinomas [3], but to the best of our knowledge, it has not been identified in MMTT. Leu-7 expression has been reported in MMTT and neuroendocrine differentiation, identified in 17% of MMTTs, has been associated with more aggressive behavior [4]. The absence of the melanocytic markers (S-100 protein, HMB-45, MART-1) excluded melanocytic differentiation [5].

The histogenesis of carcinosarcomas has been a matter of speculation and debate. Uterine MMTTs are currently considered as metaplastic carcinomas [7] because of the expression of epithelial markers such as keratins and EMA by the mesenchymal component. In line with this concept is the coexpression of keratins CK8/18, EMA and CEA along with vimentin, SMA, HHF-35 and desmin observed in the multivacuolated cells of this case. Furthermore, the identification of the same mutations in the TP53 tumor suppressor gene in both epithelial and mesenchymal elements implies a common derivation from a multipotent stem cell of the müllerian system differentiating towards the epithelium and mesenchyme [8].

### Table 1. Results of immunohistochemical reactions in the tumor elements.

<table>
<thead>
<tr>
<th>Ab</th>
<th>Epithelial element</th>
<th>Spindle cells</th>
<th>Mesenchymal element</th>
<th>Multivacuolated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK8/18</td>
<td>Diffuse, 3+</td>
<td>–</td>
<td>–</td>
<td>Rare cells, 3+</td>
</tr>
<tr>
<td>AE1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>AE3</td>
<td>Diffuse, 3+</td>
<td>Variable, cy 1+</td>
<td>Variable, cy 1+</td>
<td>Variable, cy 1+</td>
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<tr>
<td></td>
<td>Variable, nu 3+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EMA</td>
<td>Variable, 3+</td>
<td>–</td>
<td>–</td>
<td>Variable, 2+</td>
</tr>
<tr>
<td>CEA</td>
<td>Focal, 2+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Variable, 3+, (nu+cy)</td>
<td>Diffuse, 3+</td>
<td>Diffuse, 3+</td>
<td>Variable, 3+</td>
</tr>
<tr>
<td>SMA</td>
<td>–</td>
<td>Variable, 3+</td>
<td>Diffuse, 3+</td>
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Bn: benign, Cy: cytoplasmic, me: membranous, Mg: malignant, nu: nuclear.
Figure 1. — A) In situ and invasive carcinoma of the fallopian tube. B) C) spindle cells and large pleomorphic neoplastic cells with abundant rounded or elongated eosinophilic cytoplasm resembling rhabdomyoblasts B) or multivacuolated cytoplasm resembling lipoblasts C).

Figure 2. — A) Strong SMA staining of the eosinophilic and multivacuolated cells. B) Strong desmin staining of the eosinophilic and multivacuolated cells. C) Weak to moderate myogenin staining of the spindle cells.
Strong overexpression of p53 protein was observed in both mesenchymal and epithelial cells in this case, including carcinoma in situ, suggesting involvement of p53 mutations early in carcinogenesis.

Given the common embryologic origin of the urogenital ridge and mesothelium from coelomic epithelium we investigated the expression of mesothelial markers such as WT1 (Wilms’ tumor gene), calretinin and HBME in this neoplasm. WT1 protein, the product of WTI suppressor gene, is important in the development of the organs of the genitourinary system and mesothelium [10]. WT1 protein has been localized in the nuclei of malignant mesothelioma [11], and carcinoma of the ovaries, fallopian tube and uterus, mostly of serous type [12]. Cytoplasmic localization of WT1 has been reported and is thought to represent cross reactivity with an epitope unrelated to WT1 [13]. We noted diffuse, nuclear only, expression of WT1 in the epithelial component of the tumor in striking contrast to diffuse, cytoplasmic only, expression in the sarcomatous component. An analogous observation was made for the p27 tumor suppressor protein in malignant peripheral nerve sheath tumors [14], where the cytoplasmic accumulation and nuclear absence of p27 was later related to disruption of p27 nuclear transport [15]. Whether disruption of nuclear transport of WT1 is responsible for the cytoplasmic localization of the protein needs further investigation. If this is true, then WT1 involvement in MMMT carcinogenesis is a likely event following p53 mutations. Involvement of c-erbB2 and KIT in carcinogenesis of this tumor is less likely, since these proteins were not detected immunohistochemically.

Conclusion

We have presented a case of early-stage fallopian tube MMMT, consisting of high-grade serous and undifferentiated carcinoma, rhabdomyosarcoma, leiomyosarcoma and undifferentiated sarcoma. The expression of mesothelial-associated markers is in line with derivation of this tumor type from the coelomic epithelium although the distinct nuclear loss and cytoplasmic accumulation of WT1 in the sarcomatous component suggests possible involvement of this tumor suppressor gene in MMMT tumorigenesis.
References


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Malignant transformation of uterine leiomyomata

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Summary

The malignant transformation of a uterine leiomyoma is still debated and, if it occurs, it is very rare. The case of a patient affected by three small leiomyomas, monitored by the same gynecologist over the years is described. Two of these leiomyomas were transformed into leiomyosarcoma after menopause and the patient died despite receiving therapy. The case reported here is meant to underline the need to keep all uterine myomas in check since the transition into leiomyosarcomas may occur with an evolution over a time period which has not been established so far.

Key words: Leiomyosarcoma; Uterus; Leiomyoma.

Introduction

Uterine leiomyosarcoma is an unusual neoplasia. It usually crop ups in a fibromatous uterus, although it has been debated whether leiomyosarcoma may develop from leiomyoma. However, such event seems to be very infrequent [1]. In this case report we would like to highlight that malignant transformation of uterine leiomyoma can occur over the years.

Case Report

Since the age of 41 the patient had been submitted to transabdominal and transvaginal pelvic and ultrasound (US) examinations carried out by the same gynecologist. The first US examination showed a small (0.7 cm in diameter) intramural leiomyoma of the posterior wall of the uterine body. Over the years, no alteration visible through US scanning was detected in the lesion. When the patient was 46 years old, the dimensions appeared increased (1.3 cm in diameter), and at the same time another subserous leiomyoma (1.2 cm in diameter) became visible on the fundus of the uterus. When she was 48, these leiomyomata did not show any visible alterations on US, while the presence of a third intramural leiomyoma (1.4 cm in diameter) became visible on the fundus of the uterus. When she was 49, the patient entered menopause. When she was 53, a transvaginal US examination highlighted the presence of a mass 4 cm wide inside the posterior wall of the uterine body along with another mass 3.5 cm wide inside the anterior wall of the uterine body, together with the presence of a small subserous leiomyoma on the fundus, unchanged in size with US features a comparable to the previous checks. Both masses, hypogenic on US scan, showed a low-resistance blood flow. Nuclear magnetic resonance confirmed the US findings (Figures 1 and 2). Consequently, the patient was urgently submitted to hysterectomy with bilateral salpingo-oophorectomy.

The microscopic features of the masses were compatible with leiomyosarcoma (more than 10 mitoses per 10 HPF), with severe nuclear atypias and vascular space involvement. The tumor was limited to the uterus and situated in the site where the previous US scans had detected the two intramural leiomyomata. Moreover, the presence of a small subserous myoma on the fundus of the uterus was confirmed, while no other leiomyomata were found. Just a few months after the surgery, a central pelvic relapse was found so the patient was submitted to chemotherapy and debulking surgery. She died the following year.

Discussion

Analyzing the evolution of the case under examination, and in light of the several US scanning checks carried out over the years on the patient, it is likely that the two – very small-sized – intramural myomas may have undergone malignant transformation. However, it is reported that leiomyosarcoma is usually a newly appearing tumor [1] having a genetic structure different from that of leiomyomata [2]. Only a few authors [3, 4] have provided evidence supporting the transition of a leiomyoma into a leiomyosarcoma, which is in any case a very sporadic event. In our opinion, it does not seem possible to verify the transition of a leiomyoma into a leiomyosarcoma in clinical practice so it cannot be established how unusual such event is and within what lapse of time it can appear. The case reported here is meant to underline the need to keep all uterine myomas, including when small in size, under control. Cases leaning towards a suspicion of malignancy after menopause when even minimum growth is noticed should be followed closely since the transition into a leiomyosarcoma may occur, although – as we have already stated – this is a very unusual case, with a progression over time which cannot be established.

Key words: Leiomyosarcoma; Uterus; Leiomyoma.
References


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Solid variant of a pure intracystic papillary carcinoma of the breast: case report

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Summary

Intracystic papillary carcinoma (IPC) of the breast is an uncommon malignant breast neoplasm and usually occurs in advanced age. It is characterized by a more benign behavior and a subsequent higher survival rate. We describe such a case of a 58-year-old female, who displayed a gradually growing tumor of the right breast. The lesion was well circumscribed and had a hard consistency with a cystic appearance. Mammography, breast ultrasonography and fine needle aspiration cytology failed to obtain a definite diagnosis. Based on the preoperative clinical identification of right axillary lymphadenopathy, the patient eventually underwent segmental resection of the right breast and right axillary nodal dissection. As regards the histological findings, the neoplasm corresponded to a pure intracystic papillary carcinoma of the solid variant. IPC represents a breast tumor with papillary differentiation growing inside a cyst, and excisional biopsy is often necessary to confirm the disease. Careful pathological examination is essential, to exclude the presence of coexistent ductal carcinoma in situ or invasive carcinoma.

Key words: Intracystic papillary carcinoma, Breast cancer, Neoplasm.

Introduction

Intracystic papillary carcinoma (IPC) is an uncommon malignant breast neoplasm generally characterized by slow growth and a better prognosis than ductal carcinomas not otherwise specified. IPC can occur in a pure form, or it may be associated with ductal carcinoma in situ or invasive carcinoma. To our knowledge, this is the second reported case of pure IPC of the solid cellular variant [1].

This paper describes in detail the case history and discusses thoroughly the diagnostic and therapeutic implications of this unusual entity.

Case Report

A 58-year-old woman became aware of a large lump, located in the right lower medial quadrant. She had first noticed this mass three months before, and since then it slowly grew and hindered her daily activity. Her family history was unremarkable, as well as her medical history. She had never undergone any radiation and there was no reported history of breast trauma.

On physical examination, the patient had a hard “cystlike” mass. It was well-circumscribed, non-tender, approximately 8 cm in diameter, and was detected in the right lower medial quadrant at 4 o’clock. The lesion seemed to have clear borders and a flat surface, mimicking a phyllodes tumor (Figure 1). There was evidence of clinical right axillary lymphadenopathy, but there were no abnormal findings in the left breast or in either axilla.

A mammogram, first performed to investigate this lump, revealed a dense, well-defined mass, 8 cm at the longest dimension in the lower medial quadrant, which corresponded to the palpable mass (Figure 2), while breast ultrasonography (US) confirmed the presence of a large complex lesion, with solid and cystic components, occupying the lower medial quadrant of the right breast (Figure 3a). Doppler US interrogation demonstrated flow within the septa of the mass (Figure 3b). US of the right axilla showed enlarged axillary nodes that were homogeneously hypoechoic. A puncture aspiration provided 60 ml of bloody fluid content. Fine-needle aspiration (FNA) cytology from the breast cystic lesion raised the suspicion of a papillary carcinoma, but yielded no definite diagnosis.

Considering the patient’s age, clinical examination, the bloody feature of the fluid and the presence of residual mass after inspiration, we decided to perform excisional biopsy, under general anesthesia. Excisional biopsy and frozen section analysis confirmed the malignant nature of the lump. The patient eventually underwent a segmental resection of the right breast, including axillary lymph-node dissection.

The macroscopic examination showed a pink, friable, cystic mass of 7 cm in diameter, containing 25 ml of hemorrhagic fluid (Figure 4). The histological essay revealed a cystic lesion with solid structures lined by layers of epithelial malignant cells and surrounded by a thick fibrous wall. Large atypical cells, with irregular nuclei and low mitotic index were observed within the cyst wall and were arranged in a solid pattern. No epithelial neoplastic tissue was present in the adjacent mammary tissue, outside the fibrous wall. Cut sections documented the presence of nine axillary lymph nodes, with no metastatic deposits noted within the nodes. On immunostaining, the neoplastic cells expressed progesterone and estrogen receptors, but they did not express HER 2/neu and p53 protein. The confirmed final diagnosis was pure intracystic papillary carcinoma of the solid variant (Figure 5). Currently, the patient is being treated with 2.5 mg/day of letrozole at the outpatient clinic, and no signs of recurrence have been recognized.

Discussion

IPC encompasses a small distinctive subgroup of non-invasive breast cancer, which accounts for less than 0.5% of breast malignancies. The mean patient age at diagnosis is 63-67 years. Approximately half of IPCs arise in the retroareolar region of the breast and the usual clinical manifestation is a palpable mass or nipple discharge [2].
Figure 1. — Clinical appearance of the large “cystlike” mass in the right lower medial quadrant.

Figure 2. — Craniocaudal mammography showing a large mass with well-defined margins and high density.

Figure 3. — a) Targeted breast ultrasonography revealing complex cystic masses in the lower medial right breast. b) Doppler sonography showing intratumoral blood flow.

Figure 4. — Photograph of gross specimen showing the pinkish appearance of spherical tumor (arrows) within the hemorrhagic cystic space.

Figure 5. — Papillary proliferation composed of solid neoplastic cells with low mitotic index (hematoxylin & eosin staining x 200).
According to Carter et al., IPC is divided in three subtypes: i) pure IPC, ii) IPC with associated DCIS, and iii) IPC with associated invasive cancer. In most series, the frequency of each subtype is 33%, respectively. The majority of patients with IPC will have associated DCIS or invasive cancer, or both, and should be treated on the basis of this associated pathology [3]. The nature of the associated lesions to IPC is essential for prognostic reasons and for assessment of the margins. Moreover, it has been reported that IPC accompanied by DCIS is an important precursor to invasive carcinoma and in this occasion further treatment is always indicated [4].

Pathologically, IPCs may show four different cellular patterns: i) cribriform, ii) solid columnar epithelial, iii) stratified spindle cell, or iv) a transitional cell form resembling urethromel, or a combination of two or more of these patterns may be seen [5]. IPCs usually contain fibrous and vascular elements, but the existence of necrosis is often associated with the presence of an invasive component. Most tumors are pink to tan, have a soft or friable consistency, and have a spherical, well-defined contour. Hemorrhagic areas within the solid components of the tumor and bloody content within the cystic spaces are often identified [6]. As regards our patient, the macroscopic appearance and clinicopathologic findings were in accordance with the features of a pure IPC of the solid cellular pattern.

The mammographic findings of IPC are usually well circumscribed high-density masses, because of the hemosiderin hemorrhage deposits. Sometimes, satellite nodules or microlcifications or both are present. Targeted breast ultrasonography confirms the presence of solid or complex cystic masses with posterior acoustic enhancement, while Doppler sonogram often demonstrates vascularity of the solid portion or large feeding vessels [7]. Although it is a rare manifestation, some investigators have reported an uncommon sonographic appearance of IPCs, without any solid component, thus mimicking a breast cyst [8]. On the other hand, contrast-enhanced breast magnetic resonance imaging (MRI) may detect marked enhancement of cyst walls, septations and mural nodules, but the imaging findings cannot establish a definitive diagnosis [6, 7].

It is noteworthy that recent studies were undertaken to evaluate the diagnostic value of FNA and core needle biopsy (CNB), in order to approach an accurate preoperative diagnosis, if possible. FNA cannot always differentiate between benign and malignant papillary breast tumors. It is strongly postulated that the difficulty in obtaining a definite diagnosis of malignancy by FNA can be attributed to the cystic and hemorrhagic nature of these lesions, sparse cellularity and necrotic debris [7]. In our case, a FNA cytologic study revealed cells with minimal atypia and papillary morphology, but the result was interpreted as a borderline lesion. Matsuo et al., have suggested measuring the CEA value of aspirated fluid, using anti-CEA monoclonal antibody, as this is an easy, safe and valuable procedure for the accurate diagnosis of IPC [9]. Some studies have proposed that CNB has been proven to be more effective in distinguishing papillary neoplasms from other diseases and benign papillomas from papillary carcinoma [10, 11]. Although CNB is a useful diagnostic modality for IPCs, it may be unable to distinguish between in situ and invasive lesions, because it cannot obtain adequate tissue by the periphery of the mass [7].

Today, there is still no clear consensus regarding the optimal treatment of IPC. The treatment of choice for pure IPC is simple local excision without axillary lymphadenectomy, while long-term follow-up is necessary after surgical treatment [11, 12]. Local excision is therapeutic and any adjuvant therapy is needed following appropriate surgery. “Appropriate surgery” means that clear margins should be achieved, in order to reduce the possibility of tumor recurrence. Although recurrence seems to be extremely rare following complete tumor resection, there are no collected data regarding recurrence rates because of the limited number of reported cases in the medical literature and the short follow-up of these patients.

As regards our patient, the diagnosis of IPC required an excisional biopsy. The patient eventually underwent segmental excision of the right breast with axillary lymph node dissection because of the prominent clinical regional lymphadenopathy.

References


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Synchronous primary cancers in a woman with scleroderma: a case report

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Introduction

Scleroderma is a chronic, multisystem, autoimmune disease. Previous studies have shown an increased risk of malignancy in scleroderma; the most common cancers were lung cancer and breast cancer. Case: The patient, a 43-year-old nulliparous premenopausal Greek woman with scleroderma, presented with a history of abdominal pain and atypical vaginal bleeding. She underwent total hysterectomy with bilateral salpingo-oophorectomy, total omentectomy, appendectomy and pelvic lymph node dissection. The histopathology revealed synchronous primary cancers of the endometrium and left ovary. She underwent postoperative chemotherapy and remains well without evidence of disease 25 months after surgery. Conclusion: Synchronous primary cancers of the endometrium and ovary are relatively uncommon in the general population. Only a few cases of cancer of the female genital tract in women with scleroderma have been reported in the English literature.

Key words: Synchronous primary cancers; Scleroderma; Endometrial cancer; Ovarian cancer.

Summary

Background: Scleroderma is a chronic, multisystem, autoimmune disease. Previous studies have shown an increased risk of malignancy in scleroderma; the most common cancers were lung cancer and breast cancer. Case: The patient, a 43-year-old nulliparous premenopausal Greek woman with scleroderma, presented with a history of abdominal pain and atypical vaginal bleeding. She underwent total hysterectomy with bilateral salpingo-oophorectomy, total omentectomy, appendectomy and pelvic lymph node dissection. The histopathology revealed synchronous primary cancers of the endometrium and left ovary. She underwent postoperative chemotherapy and remains well without evidence of disease 25 months after surgery. Conclusion: Synchronous primary cancers of the endometrium and ovary are relatively uncommon in the general population. Only a few cases of cancer of the female genital tract in women with scleroderma have been reported in the English literature.

Key words: Synchronous primary cancers; Scleroderma; Endometrial cancer; Ovarian cancer.

Case Report

A 43-year-old, nulliparous premenopausal Greek woman presented with a history of abdominal pain and atypical vaginal bleeding. Her past surgical history was unremarkable. She had had scleroderma for the last 21 years. Her family history revealed no evidence of cancer among the first-degree relatives.

On vaginal examination a pelvic mass was palpated. There were no palpable inguinal lymph nodes, and the rest of pelvic examination was normal.

Computed tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (US) revealed an intraabdominal mass of 15.6 x 15 x 12 cm. Preoperative CT of the chest, chest X-ray, intravenous pyelography (IVP), colonoscopy and urethrocystoscopy were normal. Preoperative CA125 was elevated to 426 U/ml.

On exploratory laparotomy, the left ovary was markedly distended, measuring 20 x 15 x 10 cm. Frozen section showed malignancy and the patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy, total omentectomy, appendectomy and pelvic lymph node dissection.

The histopathology revealed synchronous primary cancers of the endometrium and left ovary. The uterine tumor consisted of glandular and villoglandular structures lined by simple to pseudostratified columnar cells, and invaded less than half of the myometrium. The ovarian tumor consisted of glandular and villoglandular structures lined by simple to pseudostratified columnar cells and ruptured the capsule, invaded the left fallopian tube and extended to the omentum. The right ovary was normal. The two carcinomas were positive for vimentin, cytokeratin, epithelial membrane antigen, estrogen receptor, progesterone receptor, CA-125, CA19-9 and B72.3 but CEA negative. The peritoneal washing smear was negative for malignant cells.

The final diagnosis was Stage Ib endometrial carcinoma endometrioid type and Stage IIIc ovarian carcinoma endometrioid type.

The patient underwent postoperative chemotherapy. She received six courses of carboplatinum (AUC 5) and paclitaxel (175 mg/m²).

She remains well without evidence of disease 25 months after operation.

Discussion

Scleroderma is a chronic, multisystem, autoimmune disease characterized by the presence of excessive deposits of conjunctive tissue components, expressed as fibrosis and structural alterations of the vascular bed. Three factors intervene in the pathogenesis of the disease:
alterations in collagen synthesis, vascular alterations and immunologic anomalies. Previous studies have shown an increased risk of malignancy in scleroderma [1-3].

All subtypes of scleroderma are associated with an increased risk of malignancy, but differences in risk have been found between them. Patients with diffuse scleroderma had the highest relative risk of malignancy. In contrast, patients with limited or other forms of scleroderma had similar increased relative risks [1].

The most common cancers were lung cancer and breast cancer [1-3]. Other types of cancers were non-melanoma skin cancer, esophageal cancer, liver cancer and hematopoietic cancers [1, 3]. Only a few cases of cancer of the female genital tract in women with scleroderma have been reported in the English literature [1, 3].

One disease may increase the risk of the other, either as a direct complication or as a result of the treatment given. Alternatively, the two disorders may share common risk factors [2].

Perhaps patients with scleroderma have a more fragile genome, and prior genetic damage may predispose to both scleroderma and cancer [1].

It has been hypothesized that the immunologic alterations produced in the pathogenesis of scleroderma could be related to the development of cancer [1].

It is possible that the use of immunosuppressive agents in patients with scleroderma might have predisposed them to develop cancer [1, 2].

Perhaps the response of the uterine corpus, fallopian tubes, and the ovarian epithelium as a morphologic unit could explain the development of synchronous endometrioid tumors in different components of the müllerian system, when simultaneously subjected to carcinogens [4].

Endometrial and ovarian cancer have several risk factors in common, and on this basis they could occur together in the same woman [5]. Hormonal causes may be involved in the pathogenesis. Future studies are needed to further evaluate the role of estrogen in these synchronous primary cancers of the endometrium and ovary [6].

The empirical criteria for identification of synchronous primary cancers include either different histologic types (major criterion) or all of the following minor criteria: 1. both tumors confined to primary sites, 2. no direct extension between tumors, 3. no lymphovascular tumor emboli, 4. no or only superficial myometrial invasion, and 5. no distant metastasis [4]. According to this criteria, the present case was synchronous primary cancers.

Patients with synchronous primary cancers tended to be 10-20 years younger than their counterparts with endometrial or ovarian carcinoma [8]. The median age at diagnosis is 50 years [6, 7]. The women had distinct clinical characteristics including young age, obesity, premenopausal status and nulliparity [6].

Independent prognostic factors for synchronous primary cancers seem to be age, stage of ovarian cancer, grade of endometrial cancer, and adjuvant therapy [9].

Treatment of choice for early-stage synchronous primary cancers is total abdominal hysterectomy with bilateral salpingo-oophorectomy, total omentectomy, appendectomy and pelvic lymph node dissection. In advanced stage, patients require more aggressive management with adjuvant chemotherapy or radiotherapy after surgery [4, 10].

The two tumors may have a similar appearance (usually endometrioid but sometimes papillary, clear cell, or mucinous) or be of different histologic types [7, 8].

Patients with synchronous primary endometrioid tumors of the endometrium and ovary (endometrioid/endometrioid) had a better median overall survival than those with non-endometrioid or mixed histologic subtypes [6, 4]. The Gynecologic Oncology Group (GOG) found that patients had an overall good prognosis with a 5-year survival of 86% and 10-year survival of 80% [7].
The reason for the better median overall survival for these patients is not intuitively obvious [7]. Perhaps this may be due to the detection of patients at earlier clinical stage and lower graded disease state [10].

**Conclusion**

The reason for the better median overall survival for our patient is not intuitively obvious, and may be due to the detection of the cancer in early-stage and low-grade disease state. We have no information on tumor behavior, response rate to standard therapy, or natural history of synchronous primary cancers in scleroderma patients.

**References**


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A case with three primary tumors of the ovary, endometrium and gallbladder

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Summary

A case with three synchronous tumors is presented. A 52-year-old patient underwent a total abdominal hysterectomy with bilateral salpingo-oophorectomy, bilateral pelvic and paraaortic lymph node dissection, and partial omentectomy for endometrial carcinoma accompanied by an adnexal mass. She further underwent cholecystectomy for a perioperative incidental suspicious nodule on the serosal surface of the gallbladder. Histopathology revealed a uterine endometrioid adenocarcinoma, a mucinous adenocarcinoma of the gallbladder, and an ovarian endometrioid carcinoma with a clear cell component. The progress of the patient until the time of death is discussed.

Key words: Endometrial carcinoma; Ovarian carcinoma; Gallbladder carcinoma; Synchronous tumor.

Introduction

The synchronized association of ovarian and endometrial tumors is not a rare occurrence among female genital cancers. While this may be due to metastasis, it can also be a primary neoplasm originating from two different tissues. These two events present with different clinical profiles and prognoses. The combination of different primary cancers is observed more rarely than metastatic cancers; however, they generally have a better prognosis.

The etiology of synchronous cancers is yet unknown. Tissues with the same embryological origins are believed to respond in a similar fashion to carcinogens [1, 2]. However, in contrast, synchronous cancers may be observed in separate tissues, as observed in the present case.

In the present report, we discuss a patient with three synchronously occurring tumors, i.e., a uterine endometrioid adenocarcinoma, a gallbladder mucinous adenocarcinoma, and an ovarian endometrioid carcinoma with a clear cell component.

Case Report

A 52-year-old patient (gravida, 5, parity, 3, abortion, 2) was admitted to an outpatient clinic in December 2004 due to menometrorrhagia. She had a past history of appendectomy performed 20 years ago. Endometrial biopsy revealed endometrioid adenocarcinoma, and a heterogeneous mass measuring 20 × 11 cm was observed in the right adnexal space. She was referred to the Department of Obstetrics and Gynecology, Uludag University Medical Faculty. She had been menstruating at intervals of 30 to 90 days with excessive bleeding lasting for ten days during the previous six months. The patient had no significant family history.

Gynecological examination revealed a semi-mobile mass originating from the right side, occupying the pelvis, and extending to the umbilicus that made the uterus nonpalpable. Ultrasonography showed a mass with cystic components that pushed the bladder anteriorly. Localized ascitis was observed lateral to the adnexal mass and uterus on the right side. Upper abdominal and thoracic tomographies and mammography were normal. Cystoscopy was performed and a biopsy was taken from the suspicious area at the bladder neck. Histopathological examination revealed squamous metaplasia. The serum CA125 level was 170.3 U/ml; CA15-3, 51.6 U/ml; CA19-9, 33.7 U/ml; and CEA, 1.76 ng/ml.

Laparotomy was performed with the diagnosis of endometrial carcinoma. In the right adnexal area, a smooth-surfaced cystic mass measuring 18 × 13 cm was observed. Adhesions were present between the mass and the omentum. The left adnexal area and the uterine size were normal. During the examination of the upper abdomen, the gallbladder was observed to be completely filled with multiple stones. Moreover, there was a grayish-pink nodule measuring 1.5 × 1.0 cm on the peritoneum covering the neck of the gallbladder. Total abdominal hysterectomy with bilateral salpingo-oophorectomy, bilateral pelvic and paraaortic lymph node dissection, partial omentectomy, and cholecystectomy were performed.

Macroscopically, the right ovarian cystic mass measured 15 × 11 × 7 cm. Dissection of the cyst showed that it had a multicystic pattern with solid areas of a maximum of 4 cm in size. Microscopically, the atypical cells were polygonal or oval shaped with vesicular nuclei, prominent nucleoli, and eosinophilic cytoplasm, and were intermingled with solid areas of atypical epithelial cells with large vesicular nuclei (Figure 1). Tumor cells were strongly and diffusely positive for CK7 and were negative for CK20, CA125, and CA19-9 in immunohistochemical staining. Examination of the right ovary, which had an intact surface, revealed endometrioid carcinoma with a clear cell component and no tumor cells were observed in the left ovary.

Macroscopically, the endometrial cavity was occupied with a grayish-pinkish-brownish fragile tumor tissue measuring 3.0 × 8.6 cm, with polypoid extensions into the endocervical canal. The maximum depth of invasion was 1.3 cm of the whole wall thickness that measured 3.0 cm. Microscopically, the tumor tissue had grown with polypoid extensions into the luminal

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surface and had invaded subepithelial stroma with glandular cribriform structures. The tumor was comprised of atypical oval or columnar epithelial cells with large vesicular nuclei and eosinophilic cytoplasm (Figure 2). Lower uterine segment involvement and superficial invasion of the cervical stroma were observed. The histopathological examination reported grade 1 endometrioid adenocarcinoma.

Macroscopically, a grayish-pinkish area measuring 1.5 × 1.2 cm was present on the peritoneal surface of the gallbladder neck. Dissection revealed nodular thickening on the corresponding side along with the presence of multiple stones. Microscopically, the tumor was observed to infiltrate the whole thickness of the gallbladder wall and was comprised of atypical oval or columnar epithelial cells with vesicular nuclei, prominent nucleoli, and eosinophilic cytoplasm; these cells formed adenoid and cribriform structures with extracellular mucin accumulations. Adjacent to the tumor tissue, atypical changes and metaplasia were observed within the surface epithelium. There was no perineural, venous vascular, or lymphatic invasion (Figure 3). Mucin was observed to stain with PAS and mucin stains. Immunohistochemical staining demonstrated that tumor tissue was strongly and diffusely positive for CK7, focally positive for CK20, and negative for CA19-9 and CH25. Histopathological diagnosis was mucinous adenocarcinoma of the gallbladder.

Peritoneal washing samples, the omentum, and pelvic and paraaortic lymph nodes were tumor-free in cytopathologic and histopathologic examination. The patient was diagnosed with ovarian endometrioid carcinoma with a clear cell component, endometrial endometrioid adenocarcinoma, and mucinous adenocarcinoma of the gallbladder.

There were no postoperative complications, and she was administered six courses of PAC (cisplatin, 50 mg/m²; Adriamycin, 50 mg/m²; cyclophosphamide, 750 mg/m²) chemotherapy. She responded to the chemotherapy; follow-up examinations included abdominopelvic and thoracic computerized tomography, whole body scintigraphy, and serum tumor marker levels. Pelvic computerized tomography in November 2005 revealed thickening of the rectal wall. At that time, the serum CA125 was 419.7 U/ml; CA15-3, 15.6 U/ml; CA19-9, 14.4 U/ml; CEA, 59.2 ng/ml; and AFP, 1.97 ng/ml. The patient refused any further investigations and did not attend follow-up

Figure 1. — Hematoxylin-eosin stained section of the ovarian tumor (x 100).

Figure 2. — Hematoxylin-eosin stained section of the endometrial tumor (x 40).

Figure 3. — Hematoxylin-eosin stained section of the gallbladder tumor (x 40).
examinations for 16 months. In March 2007, she was admitted to our outpatient clinic with the complaint of vaginal bleeding for the previous three months. Gynecological examination revealed an ulcerated, bleeding, infiltrative mass on the vaginal cuff measuring $3 \times 4$ cm. The result of the biopsy reported endometrioid adenocarcinoma. Computerized tomography revealed a single suspicious metastatic nodule in the right lung parenchyma, a $5 \times 5$ cm metastasis in the spleen, conglomerated lymph nodes around the head of the pancreas, and urinary bladder invasion with a tumor mass measuring $3 \times 6$ cm in the pelvis. Radiotherapy was planned as further treatment; however, the patient died.

**Discussion**

The synchronous formation of tumors originating from different tissues is rather rare. While the incidence of synchronous endometrial tumors for patients with ovarian tumor varies between 1.6% and 67%, the incidence of ovarian tumors in patients with endometrial cancer varies between 0.7% and 10% [3-5]. Synchronous ovarian and endometrial cancers are generally of metastatic character; moreover, the endometrium is a common site of metastasis for ovarian carcinomas.

The difficulty in dealing with synchronous tumors is differentiating the origin of the tumor, i.e., whether they are of primary or metastatic character. Synchronous cancers are most commonly metastatic in nature. The ovary often harbors metastatic gastrointestinal tract (GIT) tumors. The most common GIT tumor metastases originate from the stomach and the colon; those from the gallbladder are rare [6]. Differences between the histological patterns are the most important criteria in differentiating the primary or metastatic properties of tumors. Restriction of the tumors to their primary site, absence of any direct relation between the two tumors, absence of lymphovascular invasion, either absence or superficial presence of myometrial invasion, and absence of metastasis can all be used as minor criteria [7-10].

In our case, three different histological patterns, namely, endometrioid with a clear cell component, endometrioid, and mucinous pattern were observed. In the gallbladder, the surgical margins were tumor free, there was no serosal involvement of the ovary, and the invasion of the endometrium corresponded to less than half of the myometrium. There was no lymphovascular invasion or lymph node metastasis; moreover, the peritoneal cytology was negative. The patient was diagnosed as having three synchronously occurring tumors in the uterus, ovary, and gallbladder.

The prognosis in synchronous primary cancers is known to vary considerably [11]. Some authors have suggested that women diagnosed with synchronous primary cancers have a better overall prognosis than if their disease is classified as single-organ disease with metastases. This may be due to the increased chance of early diagnosis since the patient may have more than a single symptom and thereby seek medical help. Our patient would have been diagnosed in the advanced stages of gallbladder or ovarian cancer if she had not experienced bleeding due to endometrial cancer.

Our experience with this case shows that synchronous tumors of ovary, endometrium and gallbladder can be managed with appropriate chemotherapy.

**References**


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Primary insular carcinoid of the ovary

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Summary

Primary ovarian carcinoids are very rare tumors that account for less than 5% of all carcinoids and 0.1% of all ovarian malignancies. We present a rare case of a primary, non-functioning, insular carcinoid of the left ovary in a 44-year-old woman originating from the outer surface of a mature cystic teratoma. After an uneventful unilateral salpingo-oophorectomy, the patient had no sign of recurrence with computed tomography and 5-HIAA evaluation at 3-year follow-up. Although rare, primary ovarian insular carcinoid tumors that are confined to the ovary and treated with surgery are expected to have an excellent overall outcome.

Key words: Primary insular ovarian carcinoid; Mature cystic ovarian teratoma; Ovarian neoplasms; Salpingo-oophorectomy; Non-functioning carcinoma.

Introduction

Primary ovarian carcinoids are very infrequent tumors. They account for less than 5% of all carcinoids and 0.1% of all ovarian malignancies [1, 2]. The clinical carcinoid syndrome has been described in 43% of the insular type and 25% of the mature teratoma-associated insular carcinoids [3, 4]. There is a strong correlation with the size of the neoplasm to the manifestation of the carcinoid syndrome [3]. Although rare, primary ovarian carcinoid tumors treated with surgery alone and found to be confined to the ovary can be expected to have an excellent overall outcome [4]. We present a very rare case of a primary, non-functioning, insular carcinoid of the left ovary originating from the outer surface of a mature cystic teratoma in a 44-year-old woman.

Case Report

A 44-year-old woman with an unremarkable medical history presented to our department with a six-month history of lower abdominal pain and distension. On clinical examination, a nontender, well-circumscribed mass was noted in the left lower abdomen. Blood tests including neoplastic markers such as carbohydrate antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), and carbohydrate antigen 125 (CA 125) were normal. Abdominal ultrasound (US) revealed a 10 cm cystic mass in the left ovary and a 4 cm solid mass adjacent to the cyst; these findings were confirmed by contrast-enhanced abdominal computed tomography (CT) and, subsequently, laparotomy. The solid lesion was firmly attached to the cyst. Unilateral salpingo-oophorectomy was performed.

The solid, yellow-tan, lesion measured 4 x 3.5 x 0.4 cm and the cyst 10 x 10 cm. Histopathological examination showed the lesion to be a primary ovarian carcinoid characterized by a well defined insular pattern of uniform cells with round nuclei and abundant chromatin (Figure 1). There was no atypia and no mitosis noted. Fibrous acellular stroma separated the islets of carcinoid cells. The cystic structure was a mature cystic teratoma lined by epidermis with islets of carcinoid cells. The tumor cells were stained strongly positive to chromogranin A (Figure 2A). In addition, immunopositivity to neuron specific enolase (NSE) and synaptophysin was identified (Figures 2B and C). Consequently, diagnosis of a primary insular ovarian carcinoid originating from the outer surface of a mature cystic teratoma was made.

After an uneventful recovery, the patient was discharged on the fifth postoperative day. After a follow-up period of three years, our patient has had no sign of recurrence with CT and 5-HIAA evaluation.

Discussion

Primary ovarian carcinoids are slow growing malignant neoplasms. They are classified as insular, trabecular, strumal, mucinoc, and mixed. Insular carcinoids represent the most common type. They rarely metastasize while they are commonly associated with carcinoid syndrome. Size over 10 cm is well associated with carcinoid syndrome [3]. The differential diagnosis should include several other ovarian tumors such as granulosa cell tumor, Sertoli-Leydig tumor, and Brenner tumor. Immunopositivity to chromogranin A documents the diagnosis [5]. In case the tumor is incidentally found, it should be treated as a germ cell tumor with bilateral salpingo-oophorectomy and hysterectomy or unilateral salpingo-oophorectomy if preservation of fertility is desired [5].

A metastatic carcinoid to the ovary should also be excluded. Thorous investigation for synchronous gastrointestinal or mesenteric masses should be carried out pre- and intraoperatively. A metastatic carcinoid is likely if liver or peritoneal dissemination is identified. Additional clues are the presence of bilateral disease and multiple nodules in contrast to a single solid mass in the primary ovarian carcinoid [6]. The presence of teratoma-
Primary insular carcinoid of the ovary

After a follow-up of three years, our patient has no sign of recurrence of the disease. Survival for patients with primary insular carcinoid of the ovary is excellent with 10-year survival of nearly 100% if the disease is confined to the ovary and treated with surgery [4].

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A case of hepatoid carcinoma of the ovary

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Summary

A 50-year-old female was admitted with abdominal distention. Her serum CA125 level was elevated. Ultrasonography and computerized tomography showed adnexal tumoral masses with intraperitoneal metastases but no hepatic parenchymal involvement. She was operated on and histopathological and immunohistochemistry findings indicated ovarian hepatoid tumor. We present this case of ovarian hepatoid tumor and discuss the two-year disease progression from diagnosis to death.

Key words: Hepatoid tumor; Ovary.

Introduction

Hepatoid carcinoma is a rare malignant tumor defined as a primary extrahepatic tumor that morphologically mimics hepatocellular carcinoma. Its occurrence has been described in several organs, including the ovary. Differentiating the tumor is not only challenging but also critical, because treatment modalities and operative strategies depend on the exact nature of the hepatoid cancer. Here, we present a rare case of hepatoid carcinoma of the ovary.

Case Report

A 50-year-old, regularly menstruating woman, gravida 11 and parity 7, was admitted to Uludag University Medical Faculty Department of Obstetrics and Gynecology with complaints of abdominal distention and pain for 15-20 days. She had undergone appendectomy 23 years before and coronary by-pass surgery three months previously; she was on coumadin treatment. A left adnexal pelvic mass was palpated, however the margins were obscure due to abdominal distention. The external genitalia, vagina, cervix, and uterus were normal. The cervical smear was benign. Her hemoglobin level was 9.1 g/dl, leukocyte count 8000 K/μl, and the platelet count was 512,000 K/μl. The levels of other blood laboratory parameters were as follows: serum fasting glucose, 84 mg/dl; urea, 48 mg/dl; creatinine, 1.1 mg/dl; AST, 27 U/l; ALT, 16 U/l; total protein, 6.8 g/dl; and albumin, 4.0 g/dl. The serum levels of tumor markers were as follows: CA 15.3, 11.5U/ml; CA 19.9, 2.5 U/ml; CA 125, 538 U/ml; CEA, 2.1 ng/ml; and AFP, 1.7 ng/ml. The tumor marker levels were obtained again after three days, and the values were 10.5 U/ml, 2.5 U/ml, 638 U/ml, 2.2 ng/ml, and 1.5 ng/ml, respectively.

Transvaginal ultrasonography (TVS) revealed a right adnexal mass measuring 85 x 41 mm with solid and cystic components, which measured a maximum of 21 x 15mm, occupying the Douglas pouch. The uterus and endometrium were normal but pelvic ascites was present. The liver, gallbladder with the entire biliary system, intestines, spleen, bilateral kidneys, and suprarenal glands were normal; however, abdominal ultrasonography (US) revealed the presence of 2 cm perihilar fluid and 8 mm fluid in the Morrison pouch.

Abdominopelvic computerized tomography (CT) was performed 20 days after US, and it revealed a 15 x 10 cm septated mass with solid components. Massive ascites and widespread peritoneal implants were detected. The liver, gallbladder with the entire biliary system, pancreas, intestines, spleen, bilateral kidneys, and suprarenal glands were normal. Thoracic CT revealed bilateral 3 cm pleural effusion and atelectasis in the left lung. Pleurocentesis was performed and cytological findings were benign.

The patient was diagnosed with ovarian carcinoma and consequently underwent surgery. Three liters clear serous of ascitic fluid were aspirated.left ovarian (10 x 8 x cm) and right ovarian (7 x 6 cm) multilobulated cystic masses were found to obliterate the Douglas pouch. The omental cake and 7 x 8 cm tumoral masses invading the wall of the rectum, the hepatic and splenic flexures of the transverse colon and cecum were observed. The tumoral mass on the hepatic flexure was found to invade the adjacent liver capsule superficially. Three separate tumoral masses measuring 2 x 3 cm were detected on the ileum. In addition, a 5 x 6 cm tumoral mass was noted on the greater curvature of the stomach, but it did not invade the lumen. The entire peritoneum, diaphragm, and mesentery harbored tumoral implantations, measuring from a few millimeters to a few centimeters. The surgical treatment comprised total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, tumor excision from the pelvis and ileostomy. The tumoral masses on the gastrointestinal tract were planned to be removed with interval debulking.

Peritoneal cytology was positive for adenocarcinoma. Histopathology revealed solid groups and papillary structures of polygonal atypical epithelial cells that exhibited course and fine chromatin and prominent nucleoli in the nucleus and large cytoplasm. Histopathological diagnosis was: (1) hepatoid carcinoma of the right and left ovaries with invasion into the uterine serosal surface and focal involvement of the myometrium and (2) metastatic carcinoma of the omentum. Microscopy of the tumors showed oval or polygonal atypical epithelial cells with large eosinophilic cytoplasm and a hyperchromatic and pleomorphic nucleus with numerous atypical mitotic figures in fibrous stroma (Figure 1). Tumor cells were arranged in cords; there were solid areas with intermingling necrotic areas. Intracytoplasmic PAS-positive diastase-resistant globules were noted. Bile stains did not show pigments. Immunohistochemical staining revealed that the tumoral cells were positive for CK, EMA, AFP, CK-7 and CA125, and were focally and weakly positive for α-1 antitrypsin and negative for P1AP, HCG, GCDFP, chromogranin-A, S-100, calretinin, CK20, CEA, CD10, and 19-9.

The final diagnosis was Stage IIIc ovarian hepatoid carcinoma, and chemotherapy with 75 mg/m² cisplatin and 135 mg/m² paclitaxel was started. Although the patient’s serum
CA125 level was normal by the third course of chemotherapy, there were 3 cm tumoral masses at the caecum, transverse colon, rectum, greater curvature of the stomach, ileum, and mesentery. There was no suspicious lesion in the hepatic parenchyma, but tumoral implants were observed over the hepatic capsule and around the spleen. Ascites persisted but the amount had decreased. Positron-emission tomography (PET) confirmed the findings of abdominopelvic US and CT. The patient was considered unsuitable for cytoreduction by the general surgeons, and the remaining courses of chemotherapy were administered. After the completion of six courses by the chemotherapy, no tumoral mass, except that on the rectum, was detected. There was a 2.5 cm segmentary thickening of the sigmoid colon, which constricted the lumen. Her serum CA125 level was 17.3 U/ml. Surgery for the constricting lesion on the sigmoid wall was decided on. Laparotomy was performed, and following tumoral masses were observed: 4 × 3 cm mass on the rectosigmoid junction and a 3 cm mass on the sigmoid colon, both completely invading the wall and entering the lumen, a 4 × 3 cm mass on the splenic flexure, a 5 × 4 cm mass on the hepatic flexure of the transverse colon, and a 3 × 3 cm mass on the greater curvature of the stomach. There were multiple peritoneal implantations. The general surgeons agreed that it was an inoperable case, and biopsy samples were obtained from the appendices epiploicae. Histopathology findings revealed metastatic carcinoma with the same histological pattern of the primary. Her serum CA125 level was 95.6 U/ml, and she was administered six courses of 50 mg/m² liposomal doxorubicin as the third-line chemotherapy. Three months after the last course of chemotherapy, she was admitted for nausea, vomiting, cough, and dyspnea. Based on physical examination findings and complete blood count and chest X-ray reports, she was diagnosed with pneumonia. Abdominopelvic CT revealed multiple metastases within the abdominal cavity, with a maximum size of 4 × 3 cm. Despite antibiotic therapy, she developed sepsis and renal failure. She died 24 months after the diagnosis of hepatoid carcinoma.

Discussion

The term “hepatoid carcinoma” is used for tumors arising in extrahepatic tissues but resembling hepatocellular carcinoma both histologically and immunohistochemically in its staining for AFP. It was first defined as a separate entity by Ishikura and Scully in 1987 [1]. In the literature, hepatoid carcinoma has been reported as ovarian cancer in 17 cases, uterine cancer in four cases, and follopian tube cancer in one case [2-4]. It has a poor prognosis with a maximum one-year survival of 40% [5]. The tumor cells express AFP and therefore patients have a high serum AFP level [6, 7]. Although our patient was positive for AFP in immunohistochemical staining, her serum AFP levels were never high.

In the differential diagnosis, hepatic parenchymal involvement was not detected either at diagnosis or during disease progression until death by US, CT, and PET. Therefore, primary hepatocellular carcinoma of the liver was excluded. An increased serum CA125 level in our case was another finding supporting the histopathological diagnosis of the ovarian surface epithelial origin of the hepatoid tumor. Yolk sac tumor of the ovary, which is usually observed in females in their early reproductive age, is an AFP-related tumor that should be considered in the differential diagnosis. In our case, the tumor did not appear as an endodermal sinus or yolk sac tumor either macroscopically or histopathologically.

Immunohistochemical staining is a rather helpful technique for determining the primaries of some tumors that have obscure behavior and spread, and is useful in the differentiation of hepatoid tumors. However, because the tumor is rare, there may be some variations in the immunohistochemical findings of hepatoid ovarian carcinoma. Some authors have reported positivity of AFP and CEA and negativity for CK7, CK20, and chromogranin (2); nevertheless, CEA was negative and CK7 was positive in our case.

A limited number of cases are reported in the literature and our case shows that the behavior of an ovarian hepatoid tumor is rather aggressive and that its prognosis is poor.

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Primary malignant melanoma of the vagina


Abstract

Malignant melanoma originating in the vagina is considered extremely rare and has a very poor prognosis. We report a case of a 70-year-old woman with primary malignant melanoma of the vagina, and discuss the importance of prognostic factors and the efficacy of adjuvant chemotherapy.

Key words: Malignant melanoma; Vagina; Prognostic factors; Adjuvant chemotherapy.

Introduction

Primary malignant melanoma of the vagina is a rare neoplasm, which accounts for less than 3% of all vaginal malignancies and between 0.3 and 0.8% of malignant melanomas [1]. The majority of patients are older than 50 years; the commonest presenting symptoms are vaginal bleeding, discharge and palpable mass [1]. Despite the use of surgery and adjuvant therapy, vaginal melanoma still has an ominous prognosis.

Case Report

A 70-year-old, gravida 3, para 3, postmenopausal Japanese woman was admitted to our hospital because of abnormal vaginal bleeding for two months. Neither surgical nor medical history data were remarkable. Inspection showed a brown-pigmented nodular and irregular lesion, measuring 3 cm x 2 cm x 2 cm, on the right side of upper third of the vagina; the uterus and ovaries were normal. The satellite lesion, measuring 3 cm x 1.5 cm, was seen on the right side of the urethra. There was no palpable inguinal node. Computed tomography (CT) scanning of the brain, chest, abdomen and pelvis, and abdominal magnetic resonance imaging (MRI) did not disclose metastases. Colonoscopy and urotricystoscopy were normal. The serum level of 5S-cysteyltdopa, a tumor marker for malignant melanoma, was 5.0 nmol/l (normal 1.5-8.0 nmol/l). Modified radical hysterectomy, total vaginectomy, pelvic lymph node dissection, bilateral salpingo-oophorectomy, urethrectomy and cystostomy were performed (Figure 1). The postoperative course was unremarkable. Histopathological study of the specimen revealed a nodular malignant melanoma (the histologic diagnosis was confirmed by positive immunostaining for S100, vimentin and HMB 45) and free surgical margins, which were at least 10 mm laterally and 2 mm in depth (Figure 2). There was no pelvic node metastasis. On microstaging, Breslow depth was 11 mm and Chung level was IV; the case was allocated American Joint Committee on Cancer classification Stage IIB and FIGO Stage I. A regimen of postoperative adjuvant chemotherapy was immediately started. The regimen consisted of dacarbazine, nimustine, cisplatin, and tamoxifen. The patient underwent one course of this treatment but developed a local relapse and multiple metastases (liver, lung, adrenal gland, rib, mediastinal lymph nodes, paraaortic lymph nodes and presacral lymph nodes. She died nine months postoperatively. Her family did not permit an autopsy.

Discussion

Vaginal malignant melanoma is a very rare and highly aggressive tumor which overall 5-year survival ranges from 5 to 21% [2]. Prognostic factors have been difficult to identify; previous reviews [3, 4] have found tumor size as one of the most important. However, tumor thickness did not affect survival. Chung et al. [5] pointed out that this could be explained because vaginal melanoma usually consists of level III-IV lesions. In the case exposed, tumor size was 3 cm and Breslow depth 11 mm. The patient’s survival was nine months, which is consistent with those reported by Buchanan et al. [1]. There does not seem to be a relationship between overall outcome and age, parity, FIGO stage or location. Histological features such as cell type, mitotic count, ulceration, vessel involvement or amelanosis neither seem to correlate with patient survival [4].

Prognosis is poor regardless of the treatment delivered. Surgical management has been considered the most important potential curative treatment. There is no consensus, though, as to which would be the best approach, especially when there is no evidence of metastases. Some authors have recommended radical surgery [5]. The poor prognosis expected in our patient induced us to apply modified radical surgery in order to achieve local control and avoid metastatic disease; as the tumor was located in the upper vagina, pelvic lymphadenectomy was performed, though some authors recommend adding inguinal node dissection as well [5]. Assessment of histopathological nodal status by radiopharmaceutical-directed mapping could be used to decide which patients have to undergo lymph node dissection [6].

McClay et al. [7] reported that the joint administration of cisplatin, dacarbazine, carmustine, and tamoxifen was effective for 30% to more than 50% of metastatic melanomas. The regimen of dacarbazine, nimustine, cisplatin, and tamoxifen was not effective in the present case. High-dose interferon-alpha-2b has recently been
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reported to be an effective neoadjuvant treatment [8]. Clinical trials have focused on molecular targeting therapy with various multikinase inhibitors, including sorafenb [9]. Molecular targeting therapy includes drugs that target platelet-derived growth factor or c-kit, which are activated to high levels in melanoma cells. The data from recent published studies suggest that improvement of molecular targeting drugs as neoadjuvant treatment for advanced malignant melanoma might result in improved prognosis for this disease.

Conclusion

Vaginal melanoma is rare and associated with a dismal prognosis. Tumor size has been identified to be the strongest predictor of survival. Since it has been demonstrated that radical surgery has no significant advantage over conservative surgery, it is difficult to support the use of radical surgery as the primary surgical treatment for vaginal melanoma. The data from recent published studies suggest that improvement of molecular targeting drugs as neoadjuvant treatment for advanced malignant melanoma might result in improved prognosis for this disease.

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