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Pregnancy-associated breast cancer - a review analysis

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Summary
The aim of the present review was to assess the relationship between pregnancy and/or lactation and breast cancer, the influence of pregnancy on mortality and prognosis of the disease, the consequences of breast cancer to the current pregnancy and also to discuss the future perspective for women’s fertility. Materials and Methods: Articles were obtained from Medline (1988-present) using as keywords breast cancer, pregnancy, breastfeeding, lactation, carcinoma and pregnancy. Results: Unfortunately, delays in diagnosis and treatment are common during pregnancy and the prognosis is thus worsened. Nulliparity, early menarche and late age at first pregnancy are associated with increased risk for breast cancer. Breastfeeding confers a protective effect on risk of breast cancer, which appears to be related to the duration of breastfeeding. In cases of advanced metastatic disease during the first 14 to 15 weeks of pregnancy when chemotherapy is necessary for prompt treatment, termination of pregnancy may be proposed, particularly if the patient is ER-positive. Modified radical mastectomy is probably the procedure most frequently used today. In general chemotherapy should be delayed until after 14 to 15 weeks of gestation and radiation should be reserved until post delivery. Several authorities generally advise that future pregnancy should be delayed for at least two years after breast cancer treatment. Conclusion: Breast cancer has an equivalent prognosis in pregnant and non pregnant patients when matched by age and stage at diagnosis. Women are invariably best treated by multidisciplinary teams.

Key words: Pregnancy; Breast cancer; Chemotherapy; Mammography; Breastfeeding and breast carcinoma.

Introduction
Breast cancer remains the most common cancer in women, with a lifetime risk of almost 11% [1]. It is expected to be increasingly common as childbearing is delayed until later in life. It is actually the most common cancer encountered in pregnant women, occurring from one in 3,000 to one in 10,000 pregnancies [2]. Most researchers define pregnancy associated breast cancer as one that is diagnosed during pregnancy or up to one year postpartum [3]. At least 10% of patients with breast cancer who are younger than 40 years will be pregnant at diagnosis [3]. It is estimated that if one includes the latent time period of breast cancer, then quite probably a lot more women with breast cancer have been pregnant at some time during the disease. When women delay their first pregnancy until the age of 35 years or more, the risk for developing breast cancer increases 3-fold compared to women who conceive at the age of 20 years [4]. Only about 3% of women diagnosed with breast cancer will be pregnant. Unfortunately, delays in diagnosis and treatment during pregnancy are common. Pregnancy induces both proliferation and differentiation of the mammary epithelium. The size, weight, vascularity and density are markedly increased. Thus, the engorgement of the breasts during pregnancy and lactation may hinder detection of masses. There is significant difficulty in detecting pathology within a breast with physiological changes in pregnancy. As a consequence of this delay in diagnosis and treatment prognosis is worse in pregnant than in non-pregnant women, with an increased risk of late-stage disease, particularly in women younger than 30 years old [5]. A delay of one month in primary tumor treatment increases the risk of axillary metastases by 0.9% and a six month delay by 5.1% [6].

This article reviews the currently available literature on diagnosis, risk factors, treatment and prognosis.

Methodology/Sources
Articles were obtained from Medline and PubMed (1988-today) using as keywords “breast cancer, pregnancy, breastfeeding, lactation, carcinoma and pregnancy, breast neoplasms”. We summarized the current literature regarding epidemiology, diagnosis, risk factors, treatment and prognosis. Only articles in English were used. Data extraction was performed by the first and corresponding authors.

Clinical presentation and differential diagnosis
Pregnancy-associated breast cancer usually presents as a painless thickening, lump or mass, which is firm, deep-seated and frequently accompanied by nipple discharge. During breastfeeding the woman might note that the infant refuses to breastfeed from the breast that contains the cancer (‘milk rejection sign’) [7]. Local infiltration may cause fixation of the tumor to the chest wall or edema. Bloody nipple discharge per se does not indicate
malignancy during pregnancy or lactation, unless it is accompanied by a palpable mass, or if it persists for more than two months. In many cases it may be associated with the initiation of breast-feeding [5]. In any case, cytological study of the bloody discharge is indicated, although the interpretation might be difficult due to physiological proliferative changes associated with pregnancy [2].

Mean breast weight normally doubles during pregnancy, resulting in breast firmness and increased density. All these facts make the clinical and imaging examinations more difficult to interpret. The differential diagnosis of a breast mass in a pregnant woman includes carcinoma, fibroadenoma, lactating adenoma, fibrocystic disease, lobular hyperplasia, lipoma, adenolipoma, galactocele, abscess, sebaceous cysts, fat necrosis, hamartoma and more rarely lymphoma, sarcoma, tuberculosis and neurofibroma [8]. Most of the benign masses diagnosed in pregnancy are the same as those found in non-pregnant women, although they may differ in size, consistency and histological characteristics, due to the hormonal stimulation of pregnancy and lactation. It is noted though that about 30% of these such as lactating adenomas, galactoceles, mastitis and infarcts are unique in pregnancy [9].

Genetic and reproductive risk factors for breast carcinoma

In general, the risk for breast cancer is directly related to the duration of ovarian function. Interruption of the normal cyclic ovarian function by pregnancy appears protective. Pregnancy itself does not appear to influence the outcome of an established breast cancer [8]. Paradoxically, women with a genetic predisposition to breast cancer may be at increased risk during pregnancy and lactation [1, 6]. Various studies in different centers in Japan [10] and Sweden [11] conclude that carriers of BRCA1 and BRCA2 mutations are more likely to develop breast cancer before the age of 40 and during pregnancy than carriers who are nulliparous, and each pregnancy is associated with an increased risk of cancer [1]. Among BRCA mutation carriers, high levels of circulating estrogens during pregnancy may accelerate a malignant transformation that has already begun. Giving birth at a young age does not appear to protect these women against breast cancer [12].

Nulliparity, early menarche and late age at first pregnancy are associated with increased risk for breast cancer [1, 13]. After a full-term delivery a short-term increase in the risk of breast cancer is observed, peaking at three to four years after delivery (relative risk 1.21, 95%CI 1.02-1.44), followed by a subsequent decrease [1]. The mechanism of hormonal influence during pregnancy and lactation is not clear and a pregnancy that ends with preterm delivery has less long term protection [13, 14]. Additionally, studies have concluded that complications of pregnancy like preeclampsia, are associated with a reduction in the risk for subsequent breast cancer [15, 16]. According to a meta-analysis from the Collaborative Group on Hormonal Factors in Breast Cancer breastfeeding confers a protective effect on risk of breast cancer (reduction of about 4%), which appears to be related to the duration of breastfeeding [17]. Termination of pregnancy during the first trimester will lead to a less protective effect against future breast cancer for the women and actually doubles the risk, in comparison with those women who carry a full-term pregnancy [7, 8].

Diagnostic evaluation

Since breast changes become more pronounced in later pregnancy, it is important to perform a thorough breast examination at the initial visit. Diagnostic delays are often attributed to physician reluctance to evaluate breast complaints or abnormal findings in pregnancy. Actually, delay in diagnosis of breast cancer in pregnancy is three to seven months and women are at increased risk of presenting with advanced disease [8]. The increased vascularity and lymphatic drainage of the breast during pregnancy and lactation could be an important factor for metastatic spread, leading to a rather late diagnosis, when the disease is at a more advanced stage. Over 75% of pregnant women diagnosed with breast cancer have nodal metastases when diagnosis is confirmed [8] and women with breast cancer during pregnancy are 2.5 times more likely to have distant metastases than non-pregnant patients.

Physical examination is rather difficult and inaccurate due to the anatomical differences of the breast during pregnancy. A mass might seem to be the same clinically due to the increasing enlargement of the breast, but in reality the mass is surrounded by and buried under the changing breast tissue and is enlarging. A laboratory test that could be useful for monitoring breast cancer in pregnancy is the serum tumor marker CA 15-3.

Mammographic evaluation is controversial with high false-negative rates during pregnancy owing to the high density of the breast. Most authors agree that radiation exposure of the fetus during the mammography is negligible and safety can be established with abdominal shielding [6, 17, 18]. A standard mammography subjects the fetus to only 0.4 mrad (0.004 Gy) [3, 19]. Congenital malformations and spontaneous abortion occur with exposure to more than five rads (0.05 Gy) during the first 24 weeks of pregnancy. There are no reports of untoward effects of mammography on the mother and/or the fetus [20]. The examination though is associated with a low sensitivity. At least 25% of mammograms in pregnancy may be negative in the presence of a cancer. Ishida et al. reported successful breast cancer diagnoses in 34 of 50 mammograms of pregnant women [10]. However limited in the evaluation of the pregnant patient, mammography can and must be used as a screening test during pregnancy and lactation when indicated.

Ultrasound is a useful and inexpensive diagnostic modality suitable for diagnosing pregnant women, showing an accuracy of almost 97% in distinguishing between a cystic and a solid lesion and increased accuracy in confirming the presence of palpable masses [18]. It can be used as an adjunct to mammography, increasing the accuracy of diagnosis [21].
Magnetic resonance (MRI) is another diagnostic modality potentially useful in distinguishing and delineating the soft tissue lesions of the breast. Although it does not involve any irradiation, heating and cavitation could be potential risks to the fetus [19, 20, 22]. There is no consensus regarding avoidance of MRI imaging in the first trimester, however it is known that gadolinium crosses the placenta and is associated with fetal abnormalities in rats (FDA category C) [19, 21]. For the diagnosis of metastasis in the brain, liver and bones MRI imaging is the preferred option with quite high sensitivity. Metastases to the placenta are very rare but possible, and usually are discovered post partum. Generally they have been reported in association with widespread metastatic disease. No metastases to the fetus have ever been detected though in cases of breast carcinoma [23], but there are reports for metastases in cases of melanoma, hepatoma, choriocarcinoma and hematopoietic malignancies.

Fine-needle aspiration (FNA) of the breast mass for cytologic study is a simple procedure. As a diagnostic method, FNA is very reliable for the diagnosis of carcinoma. It is the initial procedure of choice for evaluating a breast mass during pregnancy and lactation. In cases of solid masses excisional biopsy and histological examination of the tissue is recommended when FNA is non diagnostic. Biopsy is usually performed under local anesthesia which is not harmful for the fetus. Consistent to the fact that most patients during pregnancy are young, the majority of breast cancers diagnosed are estrogen receptor (ER) and progesterone receptor (PR) negative. Approximately, 20% of breast biopsies performed during pregnancy reveal breast malignancy [8, 9, 18]. It is very important though to remember the fact that the proliferation seen in normal breasts in pregnancy can be confused with malignant changes and that there are false negative results for ER status because of competitive inhibition by high levels of estrogen with the ligand-binding method. Immunologic assays using monoclonal antibodies which recognize both occupied and unoccupied receptors seem to more accurately reflect the receptor status.

Liquid crystal thermography is a type of brachythermometry where the breast is pressed against a thermosensitive plate lined with cholesteric crystals and a thermographic film representing the thermal pattern of the breast is obtained. Although some authors recommend thermography as an adjunct in diagnosing breast malignancies, others do not agree [24].

Staging studies should be performed and an individualized decision must be made regarding need for subsequent chemotherapy. Among blood tests, measurement of alkaline phosphatase levels is unreliable since they normally double or even quadruple during pregnancy. Chest X-ray is considered safe during pregnancy. Ultrasound imaging of the liver is preferable to CT scanning. MRI may be considered in cases of non diagnostic or hard to interpret ultrasound findings. If bone metastases are suspected a bone scan with $^{99m}$technetium is advisable.

Treatment of pregnancy associated breast cancer

Pregnancy itself does not appear to influence the outcome of an established breast cancer [8]. Carcinoma of the breast in pregnant women is histologically similar to the ones encountered in non-pregnant patients. There is a similar incidence of inflammatory breast cancer in both groups [6, 24]. Pregnant women tend to have more ER-negative cancers, possibly due to a physiological receptor downregulation in pregnancy [6, 24]. Various studies have reported almost two times higher incidence of ER-negative breast cancers, as many as 80% in pregnant women in comparison with non-pregnant patients [10, 25, 26]. Younger non-pregnant patients tend to have decreased estrogen receptor status when diagnosed with breast cancer. This fact gives little theoretical grounds for suggesting pregnancy termination and oophorectomy as an adjunct to therapy when relevant. There is no evidence that termination of pregnancy after diagnosis of breast cancer is necessary to improve prognosis [1]. A harmful effect of continuing pregnancy has not been demonstrated in most published series, and therapeutic abortion fails to improve survival rates [1, 24, 27]. Spontaneous abortions and premature deliveries are not increased in pregnant breast cancer patients. The only advantage of pregnancy termination seems to be that it removes the need to consider possible detrimental effects on the fetus and complete treatment with chemotherapy, radiotherapy and surgery can be instituted immediately. However, it is difficult to evaluate the potential bias on the published literature, concerning the impact of termination of pregnancy in patients with advanced disease. In general, delays of therapy should be avoided and the recommendation to terminate the pregnancy should be based only on whether the pregnancy itself is an obstacle to effective therapy and on whether the treatment is going to be harmful for the fetus. In cases of advanced metastatic disease diagnosed during the first 14 to 15 weeks of pregnancy when chemotherapy is necessary for prompt treatment, termination of pregnancy may be suggested and considered, particularly if the patient is ER-positive [1, 8, 24, 28]. Termination of pregnancy may also be considered for women with recurrent disease. Breastfeeding women who have completed their treatment can safely breastfeed from the unaffected breast, although radiotherapy may affect or even inhibit lactation due to subsequent fibrosis. Breastfeeding is however discouraged in patients receiving chemotherapy or radiotherapy [29, 30].

Although there are no standard protocols available, the data for immediate treatment are generally reassuring. On the other hand, delay or refusal to undergo appropriate treatment has serious consequences [31]. Because of potential risks to the developing fetus, decisions on therapeutic protocols carry an additional burden. Any treatment must therefore be individualized in accordance with current knowledge and stage of disease as well as the wishes of the patient. Breast surgery during pregnancy appears to be reasonably safe, particularly in the second and third trimester. The relative risk for spontaneous...
abortion is 1.58 to 2.0 [8, 24, 32]. There is an increase in infant mortality rate (RR 2.1) and low birth weights (RR 2.0-2.2), but no increase in congenital anomalies or stillbirths and no association between type of anesthesia and pregnancy outcome [8, 32].

Breast surgery is first-line treatment for pregnancy-associated breast cancer with mastectomy and axillary dissection being the preferred option [31, 32]. Modified radical mastectomy is probably the procedure most frequently used today and entails the preservation of the pectoralis major and minor muscles, providing better arm motion and thoracic outline and reducing the incidence of postoperative lymphoedema [24]. Mastectomy and axillary dissection are traditionally considered the best option for Stage I, II and III cancers for women who continue pregnancy. The operation eliminates the need for postoperative irradiation in early-stage disease. Because nodal metastases are common in pregnancy-associated breast cancer and the nodal status affects the choice of adjuvant chemotherapy, axillary dissection is essential. It is prudent to limit breast conserving therapy only for women with early-stage disease, who desire breast conservation and for women diagnosed in the late third trimester [24]. The modality of breast conserving surgery (lumpectomy or quadrantectomy) is discouraged because it generally involves postoperative radiotherapy, which is contraindicated during pregnancy. An external irradiation dose of 5000cGy to the breast exposes the fetus to at least 10-15 cGy with increased risk of teratogenicity, intrauterine growth restriction, mental retardation, childhood malignancies and hematological disorders [24, 32]. Irradiation during the first trimester is more likely to lead to fetal demise. The radiation required to complete therapy can be delayed safely as much as eight weeks from the diagnosis, allowing safe delivery of the infant [33].

For node-positive women or node-negative with a tumor greater than 1 cm, a four to six months course of chemotherapy is the standard care [34]. Generally speaking, all chemotherapeutic agents cross the placenta and are theoretically teratogenic and mutagenic. They may lead to intrauterine restriction, fetal malformation, spontaneous abortion, fetal intrauterine death, preterm delivery, hyaline membrane disease, transient leucopenia, and pancytopenia. However, the greatest teratogenic risk occurs in the first trimester. About 10-20% of infants exposed to cytotoxic agents during the first trimester, when organogenesis is taking place, have major malformations [35, 36]. The risk for malformations is rapidly decreased to 1.6% to 3% if chemotherapy is administered later in pregnancy [8, 35, 36]. In general chemotherapy administration should be delayed, if possible, until after the 14th to 15th week of gestation. Among chemotherapeutic agents used in breast cancer, methotrexate is strongly contraindicated during the first trimester, due to its high abortifacient and malformation action. Also, tamoxifen citrate, a selective ER modulator, is considered inappropriate due to concerns over associated fetal anomalies and lack of efficacy. Most authorities consider sufficiently safe for second and third trimester administration the combination of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF) and the combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) [31, 32, 34-36]. Breastfeeding women should not receive chemotherapy as these agents are secreted to the milk. Chemotherapy dosage is another important issue because of the increased plasma volume, increased hepatorenal function, decreased albumin concentration and decreased gastric motility. Palliative chemotherapy or radiotherapy may be considered for advanced and technically inoperable disease.

Prognosis, subsequent pregnancy and fertility

Breast cancer has an equivalent prognosis in pregnant and non pregnant patients when matched by age and stage of disease at diagnosis [3]. There is no statistically significant difference to the 5-year survival rates for patients matched for age and stage of disease [3, 6, 37]. Pregnancy-associated breast cancer might carry a worse prognosis since it tends to be diagnosed in more advanced stages. The nodal status is of prognostic significance, as well as the number of positive nodes. Pregnancy, probably due to the delay in diagnosis, appears to increase the risk for nodal metastatic disease, with 60 to 85% of women exhibiting auxiliary nodal disease and thus resulting in a worse prognosis [1, 2, 7, 8].

There are no prospective studies evaluating the impact of subsequent pregnancy on breast cancer and even in cases with metastatic disease no adverse effects of subsequent pregnancy have been reported. Unfortunately, most relevant studies are small and retrospective. According to the limited evidence, future pregnancies seem safe for women as long as they have an ER-negative cancer [38, 39], and no statistically significant difference was found in overall or disease-free survival rates for survivors of breast cancer who become pregnant [40-42]. Long-term survival after breast cancer does not appear to be affected by subsequent pregnancy [1]. Additionally, there is some evidence of a ‘healthy mother effect’, in the sense that a future pregnancy might have a protective effect with a trend towards improved prognosis when compared with women not becoming subsequently pregnant regarding the 5- and 10-year survival rate [1, 6, 39, 43]. However, when the interval is smaller than six months, the 5-year survival has been reported to be 54% in comparison to 78% for women with an interval between treatment of breast cancer and pregnancy of six months to two years [1]. Several authorities generally advise that future pregnancy should be delayed for at least two years after breast cancer treatment and this interval should be extended up to five years for women with metastatic disease [1, 6, 24, 37]. Most women have recurrences within two years and it is likely that this interval will contribute to the identification of those with a better prognosis than those with more aggressive disease. Since younger women have higher relapse rates and significantly lower survival rates, those under 33 years old should consider delaying pregnancy for at least three years to reduce the risk of relapse.
[44]. No firm conclusions can be drawn from the available body of evidence up to date. In the end the decision on when it is safe to become pregnant again is really difficult and should be based on a realistic assessment of the worst case scenario if recurrence of cancer occurs during the subsequent pregnancy.

Chemotherapy significantly affects subsequent fertility, mainly because of increased risk of ovarian failure. Alkylating agents (cyclophosphamide) might cause amenorrhea through ovarian depression [3, 45]. Other chemotherapeutic agents like methotrexate and 5-fluorouracil do not have the same negative effect. Women must be counseled for fertility preservation prior to chemotherapy about the new techniques of egg freezing and ovarian tissue cryopreservation [46]. High levels of circulating estrogens during ovarian stimulation treatments should be considered as a potential risk factor for recurrence especially for women with a history of ER-positive disease [45, 46].

Conclusion

Pregnancy-associated breast cancer incidence will increase as more women postpone childbearing until later in their reproductive life. No aspect of breast cancer is more challenging than diagnosing, counseling and treating pregnant women. The approach should be based on individual patient needs and can not be generalized. Women are invariably best treated by multidisciplinary teams involving surgeons, obstetricians, medical oncologists, neonatologists and psychologists.

References


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Concomitant expression of HER2 and HIF-1α is a predictor of poor prognosis in uterine cervical carcinoma treated with concurrent chemoradiotherapy: prospective analysis (KGROG0501)


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Summary

Background: In previously reported retrospective analyses of uterine cervical carcinoma cases, HER2 was correlated with poor radiation sensitivity and poor treatment outcomes and HIF-1α was found to be an indicator of poor prognosis. To date, no prospective studies have been performed to evaluate the radiation sensitivity and treatment outcomes of patients with uterine cervical carcinoma relative to HER2 and HIF-1α expressions. We conducted a prospective evaluation of HER2 and HIF-1α in cases of locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy. Methods: Between June 2005 and April 2008, 25 patients with locally advanced uterine cervical carcinoma were registered in this study, KGROG0501. Their clinical stages were Ib2/IIb/Iib/Va in 1/2/22/1 cases, respectively. Nineteen cases had squamous cell carcinoma and six had adenocarcinoma. HER2 expression and HIF-1α expression were analyzed using an immunohistochemical kit on pretreatment biopsied specimens. HIF-1α expression was studied using another commercial immunohistochemical kit on pretreatment biopsied specimens. The survival rates were compared between patients with and without positive HER2 and HIF-1α expressions. Results: The 20-month survival of HER2(-) and HIF-1α(-) cases (n = 6) was 100% and that of HER2(+) and HIF-1α(+) cases (n = 4) was 37.5% (p = 0.0032). Conclusions: In this first prospective analysis of patients with uterine cervical carcinoma treated with concurrent chemoradiotherapy, concomitant expression of HER2 and HIF-1α was suggested to be a strong indicator of poor prognosis. A novel therapy including molecular targeted therapy such as anti-HER2 and anti-HIF-1α may be worth considering in patients with concomitant expression of HER2 and HIF-1α.

Key words: Uterine cervical carcinoma; Chemoradiation; HER2, HIF-1α.

Introduction

HER2 is an epidermal growth factor receptor (EGFR)-like protein [1] considered to be related with tumor growth [2]. However, no ligands that bind directly to HER2 have been detected. On the other hand, in clinical practice, HER2 has been widely recognized to be a very significant poor prognostic factor of breast cancer [3, 4]. Moreover, the anti-HER2 molecular targeted drug trastuzumab has already been produced and used worldwide to counter the overexpression of HER2 in breast cancer, with successful results [5-7]. Furthermore, retrospective studies have reported that expression of HER2 might be correlated with poor prognosis in uterine cervical carcinoma [8-10].

Hypoxia-inducible factor 1 (HIF-1) is composed of the heterodimers HIF-1α and HIF-1β, which are bHLH/PAS proteins [11]. HIF-1α is an indicator of hypoxia in response to the cellular O2 concentration and is rapidly ubiquitinated and degenerated under aerobic conditions [11-13]. Recently, HIF-1α expression has been correlated with hypoxia in uterine cervical carcinoma [14, 15]. Moreover, a retrospective study has reported that the expression of HIF-1α in uterine cervical carcinoma is correlated with poor prognosis [16]. Activity of tumor proliferation and hypoxia are very important radioresistant factors. However, to date, no prospective studies have been performed to evaluate the radiation sensitivity and treatment outcomes of patients with uterine cervical carcinoma relative to HER2 and HIF-1α expressions. We are engaged in a prospective phase II study of concurrent chemoradiotherapy for locally advanced uterine cervical carcinoma using nedaplatin, a new platinum agent; this study has been designated as KGROG0501 (Kitasato Gynecologic Radi-
ation Oncology Group Study 0501) and its protocol has been reported elsewhere [17, 18]. In March 2009, we performed an interim analysis of the KGROG0501 results and the correlation between HER2 expression, HIF-1α expression, and treatment outcomes was analyzed prospectively.

**Patients and Methods**

Between June 2005 and April 2008, 25 patients with locally advanced uterine cervical carcinoma were enrolled in KGROG0501. Patient characteristics are listed in Table 1. The median age was 55.5 years (range, 31-75 years). The clinical stages based on the International Federation of Gynecology and Obstetrics (FIGO, 1994) were Ib2/IIb/ IIIb/IVa in 1/2/21/1 cases, respectively. Nineteen cases had squamous cell carcinoma and six had adenocarcinoma. The protocol therapy of KGROG0501 was completed in all patients.

HER2 expression was analyzed using an immunohistochemical kit for HER2 (Hercept Test II kit, DAKO, Denmark) on

<table>
<thead>
<tr>
<th>Table 1. — <strong>Patient characteristics.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>31-75 years (median: 55.5 years)</td>
</tr>
<tr>
<td><strong>PS</strong></td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td><strong>Clinical Stage</strong></td>
</tr>
<tr>
<td>Ib2</td>
</tr>
<tr>
<td>IIb</td>
</tr>
<tr>
<td>IIIb</td>
</tr>
<tr>
<td>IVa</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td><strong>MTD</strong>**: 30-100 mm (median: 65.0 mm)</td>
</tr>
</tbody>
</table>

*PS: Performance Status.
**MTD: maximal tumor diameter.
Concomitant expression of HER2 and HIF-1α is a predictor of poor prognosis in uterine cervical carcinoma treated with etc.

Biopsied sections according to the manufacturer’s instructions. HIF-1α expression was analyzed using immunohistochemical method (LSAB2 kit, DAKO, Denmark). Briefly, 5-μm thick sections were deparaffinized in xylene and rehydrated. They were treated with 0.3% hydrogen peroxide for 5 min. Then, the sections were incubated in 10 mM citrate buffer at 121°C for 15 min. After blocking with 10% normal swin serum (DAKO), the sections were incubated with anti HIF-1α mouse monoclonal antibody (BD Biosciences, USA, 1:1000 dilution) overnight at room temperature. As negative controls, normal mouse or rabbit serum at the same dilution was used. Detection was achieved with diaminobenzidine reaction for 10 min. Nuclei were lightly counterstained by Mayer’s hematoxylin.

Level of HER2 expression was ranked to 0 to 3+ according to the United States Food and Drug Administration (FDA) guidelines. For statistical analysis, 0 was assigned to HER2 (-) and 1+ to 3+ to HER2 (+).

Nuclear labeling indices of HIF-1α were calculated for all specimens. The cut-off value of HIF-1α (+) was set at ≥ 35% nuclear positivity. For statistical analysis, a survival curve was constructed by the Kaplan-Meier method. The log-rank test was performed to evaluate statistical significance. A p-value < 0.05 was defined as statistically significant. Comparisons among patients with and without HER2 and HIF-1α expressions and survival rates were performed.

Results

HER2 was expressed as 0 in ten cases, 1+ in nine cases, 2+ in five cases, and 3+ in one case. Figure 1a indicates the positive expression of HER2 in squamous cell carcinoma of the uterine cervix. Figure 1b indicates the positive expression of HER2 in adenocarcinoma. Only eight patients had positive expression of HIF-1α. Figure 2a and 2b indicate the positive expression of HIF-1α in squamous cell carcinoma and adenocarcinoma, respectively.

The 3-year overall survival of HER2-positive patients was 100% and the 3-year overall survival of HER2-negative patients was 56.7% (Figure 3). These differences were statistically significant (p = 0.035). The 3-year overall survival of HIF-1α-positive patients was 76.5% and the 3-year overall survival of HIF-1α-negative patients was 72.9% (Figure 4). These were almost the same (p = 0.69). However, in an analysis of the combination of HER2 and HIF-1α, the 20-month overall survival of HER2-negative and HIF-1α-negative patients was 100% and the 20-month overall survival of HER2-positive and HIF-1α-positive patients was 37.5% (Figure 5). This difference was statistically significant (p = 0.032) and the difference in the survival curves was quite large.
Discussion

Concurrent chemoradiotherapy is recognized to be standard treatment for locally advanced uterine cervical carcinoma. However, the 5-year overall survival of these patients was no more than 73% [19]. This indicated that he same disease status leads to different results. About 70% of the patients with locally advanced uterine cervical carcinoma who received concurrent chemoradiotherapy achieved complete recovery and about 30% of them failed to death. To date, many studies have been performed to overcome these problems from the era of radiation therapy alone as the standard therapy for locally advanced uterine cervical carcinoma.

Oka et al. investigated the pretreatment expressions of p27 and p53 in 77 patients with squamous cell carcinoma of the uterine cervix treated with radiation therapy alone and concluded that high expression of p27 and low expression of p53 were correlated with poor overall survival [20]. Manganese superoxide dismutase (Mn-SOD), which removes radiation-induced toxic superoxide radicals, was also found to be a prognostic factor for patients with uterine cervical carcinoma treated with radiation therapy alone [21]. The 5-year overall survival of Mn-SOD-positive patients was 42.5%, significantly poorer than the 77.0% of Mn-SOD-negative patients (p < 0.05). Other cell cycle- or tumor-proliferation-related proteins were investigated such as p63 [22], vascular endothelial growth factor (VEGR) [23], cyclooxygenase-2 expression (COX-2) [24, 25], and survivin [26]. All of these factors were correlated with clinical outcomes of radiation therapy alone. In addition to cell-cycle- or tumor-proliferation-related proteins, the genotype of human papillomavirus was investigated [27]. However, there was no evident correlation between overall survival and the genotype of human papillomavirus. On the other hand, imaging approaches to predict the clinical outcomes have been performed. Kodaira et al. reported that magnetic resonance imaging indicated the prognosis of Stage III uterine cervical carcinoma to predict distant metastasis [28]. Kidd et al. reported the usefulness of positron emission tomography to evaluate the pretreatment maximum standardized glucose uptake values of the cervical tumor, which was correlated with prognosis [29]. However, no prospective studies have been performed in conjunction with prospective clinical trials.

HER2 has been investigated previously. Nakano et al. reported that the 5-year overall survival rate of HER2-positive patients with locally advanced uterine cervical carcinoma treated with radiation therapy alone was 45.1% and that of HER2-negative patients was 75.6% [30]. This was statistically significant (p < 0.01). Furthermore, Niibe et al. investigated patients with advanced uterine cervical carcinoma with paraaortic lymph node metastasis treated with radiation therapy and concluded that the prognosis of HER2-positive patients is poorer than that of HER2-negative patients [9]. Recently, Yamashita et al. reported that the prognosis of HER2-positive patients with locally advanced uterine cervical carcinoma treated with chemoradiation is poorer, although the difference was only marginally statistically significant [10]. All of these findings suggested that the prognosis of HER2-positive patients with uterine cervical carcinoma treated with radiation therapy is poorer than that of HER2-negative patients. However, these studies were all retrospective analyses. No prospective studies concerning HER2 in cases of locally advanced uterine cervical carcinoma have been performed yet. The current study is the first prospective clinical study to evaluate the role of HER2 in locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy.

The current study indicated that the 3-year overall survival of HER2-positive patients was 100% and the 3-year overall survival of HER2-negative patients was 56.7%. These differences were statistically significant (p = 0.035). This result is the same as in the previous retrospective studies. Moreover, this is first confirmation prospectively that HER2 is the prognostic factor of uterine cervical carcinoma.

HIF-1α has also been investigated previously. Dellas et al. reported that the 5-year overall survival of HIF-1α-positive patients with locally advanced uterine cervical carcinoma was 45% and that of HIF-1α-negative patients was 92% (p < 0.02) [31]. Ishikawa et al. reported that expression of HIF-1α predicts frequent distant metastases (p = 0.03) [16]. Other studies of almost the same results have been reported already [32, 33]. However, the current study indicated that the 3-year overall survival of HIF-1α-positive patients was 76.5% and the 3-year overall survival of HIF-1α-negative patients was 72.9%. These survival rates were nearly the same (p = 0.69) and contrasted with the results of previous studies. Nonetheless, the interpretation of these prospective results must be made very cautiously.

The results of this prospective study are interim ones, not final results. The follow-up period was not especially long. The mechanism of the correlation between HIF-1α and the poor clinical prognosis of radiation therapy is that HIF-1α is strongly correlated with hypoxia and that hypoxic tumor cells are resistant to irradiation, so tumor cells left after radiation therapy lead to a poor prognosis [14, 15]. However, if radiation-resistant hypoxic cells are dormant cells, hypoxic cells have no strong proliferation potential, which does not result in imminent patient death but in patient survival for several more years. The results of the current study could indicate this situation. The evidence to support this hypothesis is that in the analysis of the combination of HER2 and HIF-1α expressions, the 20-month overall survival of HER2-negative and HIF-1α-negative patients was 100% and the 20-month overall survival of HER2-positive and HIF-1α-positive patients was 37.5%, which was statistically significant (p = 0.032) and the difference in the survival curves was quite large. Patients with HIF-1α-positive expression coincident with HER2-positive expression, indicative of a highly proliferative state of tumor cells, were predicted to have very bad results. On the other hand, patients with HIF1α-negative expression coincident with HER2-nega-
Concomitant expression of HER2 and HIF-1α is a predictor of poor prognosis in uterine cervical carcinoma treated with etc.

tive expression, achieved a 20-month survival of 100%. This is the first investigation of this combined expression of HER2 and HIF-1α, which is a clear and reasonable result. Further investigation is required to validate this combination pretreatment test as a promising predictor of the prognosis of locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy. Furthermore, applying molecular targeted therapy of both anti-HER2 and anti-HIF-1α will warrant a better prognosis of locally uterine cervical carcinoma treated with concurrent chemoradiotherapy.

In conclusion, this is the first prospective analysis to evaluate the prognostic role of the expressions of HIF-1α and HER2 in uterine cervical carcinoma treated with concurrent chemoradiotherapy. The concomitant expression of HER2 and HIF-1α in patients with locally advanced uterine cervical carcinoma treated with concurrent chemoradiotherapy was a strong predictor of poor prognosis. A novel therapy including molecular targeted therapy may be worth considering in patients with concomitant expression of HER2 and HIF-1α.

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References


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Follow-up in a long-term randomized trial with neoadjuvant chemotherapy for squamous cell cervical carcinoma

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Summary

Objective: To assess the role of neoadjuvant chemotherapy to achieve radical surgery in a larger number of patients with locally advanced/or bulky Stage IB cervical carcinoma. We conducted a trial to determine whether neoadjuvant chemotherapy would improve disease-free survival and overall survival in Stage IB-III cervical cancer. Design: Prospective randomized clinical study with long-term follow-up. Setting: Department of Gynecology, Perinatology and Child Health, II Faculty University of Rome “La Sapienza”. Methods: 288 patients with squamous cell carcinoma of the uterine cervix, FIGO Stage IB-IIIB were randomized to one of the following treatments: three courses of neoadjuvant chemotherapy with cisplatin, vincristine, bleomycin (NACT arm; n = 159); conventional surgery or exclusive radiotherapy (CONV arm; n = 129). There was no difference in age, FIGO stage, tumor size and lymph node involvement between the two groups (p = ns). Two hundred and thirty-four patients in Stage IB-IIb (n = 129 NACT arm and n = 105 CONV arm) and 24 patients in Stage III (NACT arm) who proved to be chemosensitive underwent radical hysterectomy. Six Stage III patients, non responders to chemotherapy, and 24 patients, Stage III of the CONV arm, underwent radiotherapy. Follow-up extended for seven years. Results: The study was performed on disease-free survival related to several prognostic factors: age, FIGO stage, tumor size, grading, parametrial involvement, lymph node status and surgical margins. Recurrence of disease occurred in 49 (32.1%) patients of the NACT arm (n = 153) and in 39 (37.1%) patients of the CONV arm (n = 105). Statistically significant differences in the recurrence of the disease were related to FIGO stage (p < .003), grading (p < .05), parametrial involvement (p < .002) lymph node status (p < .0001) and tumor size (p < .002). No statistical significance was related to age and surgical margins (p = ns). Disease-free and overall survival in the two groups were, respectively, 65.4% vs 53.5% (p = ns) and 70.4% 65.9% (p = ns)

Key words: Cervical cancer; Neoadjuvant chemotherapy; Radical hysterectomy.

Introduction

Although the early stages carcinoma of the uterine cervix have a high possibility of treatment aimed at a permanent cure, there is still no agreement on the best approach for locally advanced cervical cancer.

The prognosis of such disease has not improved despite the therapeutic advances since 1950 [1]. Several factors may contribute to treatment failure; the risk of recurrences is high in patients with unfavorable prognostic factors, such as lymph node involvement, tumor volume, positive parametrical specimens, high tumor-grading and invasion of the lymph-vascular spaces [2-4].

Neoadjuvant chemotherapy inducing elimination of micrometastasis and regression of cervical neoplasia made it possible to achieve radical operability. Chemotherapy was proposed during the eighties as a neoadjuvant regimen to increase the surgical operability of neoplasia classified unsuitable for surgery [5-14]. Whether the introduction of preoperative chemotherapy effectively improves the overall and/or disease-free survival is controversial [15-19]. In spite of the many therapeutic protocols for cervical carcinoma proposed during the past 50 years, survival has remained, substantially, almost unchanged [20]. Data on recurrence are available from three trials [21-23] in which the OR for recurrences is in favor of neoadjuvant chemotherapy, but the results are not statistically significant (p = .21). Neoadjuvant chemotherapy has, however, an undoubted biologic rationale because it represents an in vivo test of chemosensitivity that can influence subsequent therapeutic strategies [24].

Materials and Methods

Design

We conducted a prospective, randomized clinical study on 304 patients with histologically diagnosed squamous cell carcinoma of the uterine cervix in Stages IB-IIIB. The recruitment started January 1988 and ended December 2002 at the Institute of Gynecology and Obstetrics, University of Rome “La Sapienza”. Inclusion criteria were: age less than 65 and absence of severe systemic pathologies or other neoplastic pathologies; exclusion criteria were: pre-existing neuropathies, leucopenia (< 4,000 mm³), thrombocytopenia (< 100,000 mm³), abnormal renal (serum creatinine > 1.5 mg/dl, creatinina clearance < 60 ml/min) and hepatic (bilirubinemia > 1.2 mg/dl) functions. Two hundred and eighty-eight patients were enrolled in the study, with a median age of 48.5 years (range 32-65 yrs).

The clinical staging procedure was performed according to the International Federation of Gynecology and Obstetrics (FIGO, 1985): 189 patients were classified as Stage IB, 45 as Stage IIA and 54 as Stage IIIA-IIIB. To improve clinical staging, in addition to clinical examination, cystoscopy, rectosig-
the following sequence: cisplatin (P) 50 mg/m² (day +1), vincristine (V) 1 mg/m² (day +1) and bleomycin (B) 25 mg/m² (day +1 - +3). During treatment, patients were evaluated with hematological and instrumental tests, such as blood count, serum levels of creatinine, urea nitrogen, liver function, study of the respiratory function, and radiological pulmonary control. The toxicity of the chemotherapeutic drugs was evaluated according to the World Health Organization guidelines (WHO, 1979) [25].

Treatment was delayed one week if leukocytes were between 2,000 and 3,000 mm³ and platelets were between 50000 and 100000 mm³. If symptoms persisted for more than one week, vincristine was reduced to 50% of the normal dose, while the doses of bleomycin and cisplatin were not changed.

Clinical examination, colposcopy and pelvic ultrasound were used to evaluate the responsiveness to chemotherapy and therapeutic response was classified according to the WHO criteria [25].

Surgical procedure

Two hundred and thirty-four patients in Stage IB and IIB (n = 129 NACT arm and n=105 CONV arm) underwent type III-IV radical hysterectomy according to Piver [26] with systematic lymph node dissection of the lumbar-aortic area as well as pelvic lymphadenectomy (modified radical hysterectomy according to Wertheim-Valle [27]. Furthermore, the group of patients undergoing surgical treatment was increased by 24 Stage IIIA-IIIB patients (NACT arm) reassessed and classified as suitable for radical surgery. Surgery was performed within four weeks of neoadjuvant chemotherapy.

A macroscopic evaluation was systematically made of the residual tumor as well as a histological study of the uterine cervix, parametria, paracolpos and all nodal tissue removed. The tumor grading was also evaluated.

Radiotherapy

Surgical treatment could not be performed on the patients classified as Stage III, in either the control group (n = 24) or non respondent patients treated by neoadjuvant chemotherapy (n = 6). In these cases, patients were randomized to radiotherapy: patients were initially treated with external-beam radiotherapy on the whole pelvis (50 Gy) over five to six weeks. Subsequently, the patients were treated by intracavitary brachytherapy with a maximum dose of 30 Gy (low-dose-rate; LDR) to the recto-vaginal septum. According to International Commission Radiation Unit report 38, the dose was prescribed according to disease volume, without a fixed minimum dose at point A [28]. In some cases extended field radiotherapy was used to treat paraaortic lymph nodes involved in the neoplastic process.

External adjuvant radiotherapy (50 Gy) was given four to six weeks after surgical treatment to the patients who showed parametrial neoplastic involvement or lymph node positivity at the final histological examination. Postoperative LDR brachytherapy (30 Gy) was also given to patients with positive surgical resection margins.

Follow-up

After completing the treatment, patients were evaluated every four months for the first two years, every six months during the fourth and fifth years and, subsequently, one time a year (up to 84 months). Pelvic examination, cervical or vaginal Pap smear, colposcopy, histological examination of any biotpic samples, urography and radiological examinations such as CT and/or MRI were performed to assess disease status and degree of treatment-related toxic effects.

Randomization and statistical methods

Randomization was done by means of a computer generated algorithm that divided the patients in two homogeneous arms according to the parameters considered: age, tumor size, FIGO stage and radiological state of the lymph nodes (Table 1). In order to assign more patients to the presumably favorable treatment arm we decided to allocate 55% of the patients to the NACT arm and 45% to the CONV arm.

The chi-square test was used to make a statistical evaluation of the frequencies by nominal variables (patient age, tumor volume, FIGO stage, state of lymph nodes, chemosensitivity of the neoplasia); a value of $p < 0.05$ was considered significant (confidence interval, CI 95%). The Cox method was used for evaluating the prognostic factors independently with greater weight on recurrence of the disease [29]. Local relapses and distant metastases were defined as the recurrence of the disease after a disease-free period between the surgical operation and the last follow-up (limited to 84 months) at which the diagnosis of recurrence was made. Survival was calculated as the period between the initial diagnosis of neoplasia and the patient’s last follow-up (limited to 84 months).

<table>
<thead>
<tr>
<th>Table 1. — Baseline data of the 288 patients (%)</th>
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<tbody>
<tr>
<td>Patient age</td>
</tr>
<tr>
<td>&lt; 35 yrs</td>
</tr>
<tr>
<td>35-50 yrs</td>
</tr>
<tr>
<td>&gt; 50 yrs</td>
</tr>
<tr>
<td>FIGO stage</td>
</tr>
<tr>
<td>Ib-IIa</td>
</tr>
<tr>
<td>Iib</td>
</tr>
<tr>
<td>IIIa-IIib</td>
</tr>
<tr>
<td>Tumor size</td>
</tr>
<tr>
<td>&lt; 5 cm</td>
</tr>
<tr>
<td>&gt; 4 cm</td>
</tr>
<tr>
<td>Lymph nodes (radiol. status)</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
</tr>
</tbody>
</table>

* median age 48.5 years (range 32-65 yrs).
Follow-up in a long-term randomized trial with neoadjuvant chemotherapy for squamous cell cervical carcinoma

However, the incidence of complications observed after surgical or radiotherapy treatment and of the toxicity found after chemotherapy is not reported here.

Furthermore, the results are compared in terms of overall survival and recurrence of disease between patients that received adjuvant radiotherapy treatment and the group of patients that did not have adjuvant therapies. The SPSS 8.0 statistical software package was used for all analyses.

**Results**

**Response to chemotherapy**

Chemotherapy was delayed in 19 cases (12%) for moderate to severe transient, mostly hematologic toxicity, and dose-reduced in six cases (3.8%).

The data related to the response of the primary tumor of the 159 patients with locally advanced cervical carcinoma and assigned to the neoadjuvant chemotherapy arm are summarized in Table 2. The patient characteristics considered for the analysis were: patient age, clinical staging, size of the tumor and lymph node status (preoperative radiological evaluation). A complete response was obtained only in 36 patients (22.6%), a partial response in 90 patients (56.6%) and no response (stable-progressive disease) in 33 patients (20.8%).

No statistically significant differences were observed in responsiveness to chemotherapy in relation to age (< 35 yrs, 35-50 yrs, > 50 yrs) (chi-square = 3.08; p = 0.54) and disease Stage (Ib-IIa, IIb, IIIa-IIIb) (chi-square = 2.41; p = 0.66).

In patients with Stage Ib-IIa cervical carcinoma the complete responsiveness to chemotherapy was 72.2%, while in patients with carcinoma in Stage IIb and IIIa-IIIb a complete response of 16.7% and 11.1%, respectively, was found.

The tumor size (≥ 5, < 5 cm) was considered relevant to the responsiveness to chemotherapy (chi-square = 20.03; p = 0.001). In patients assigned to NACT there were 27 (28.1%) complete response for tumor size < 5 cm and nine (14.3%) partial response for tumor size ≥ 5 cm (14.3%).

Finally, response to chemotherapy on the primary tumor in the presence of radiologically ascertained (preoperative) lymph node positivity was evaluated; the difference found between the two groups was statistically significant (chi-square = 9.01; p = 0.01). In patients with lymph node positivity on radiological examinations, there was complete response in 18.2% (n = 5), partial in 44.5% (n = 20) and no response in 36.4% (n = 16); however, in patients with clinically negative lymph nodes the complete response was 24.3% (n = 28), the partial 60.9% (n = 70) and no response 14.8% (n = 17).

No surgical treatment was performed on six patients classified as Stage III non responsive to chemotherapy of the NACT arm and 24 patients of the CONV arm, at the same stage, and they were therefore excluded from the statistical evaluation.

**Histological results**

In the 258 responsive patients (Ib-IIb: 234 and IIIa-IIIb: 24), radical hysterectomy with systematic pelvic/aortic lymphadenectomy was performed with a median number of nodes removed of 44 (range 32-56): 27 pelvic (range 21-33) and 17 of the aortic area (range 11-23). Concerning tumor grading: 81 cases were well differentiated, 49 cases moderately differentiated and 128 cases poorly differentiated.

Pathologic examination of the surgical specimens revealed positive surgical margins in 28 (10.8%), parametrial involvement in 78 (30.2%), and lymph node positivity in 66 patients (25.6%).

**Adjuvant radiotherapy**

After surgical treatment, 84 patients who had at least one of the following risk factors were treated with additional postoperative radiation therapy: positive pelvic/aortic lymph nodes (n = 6), parametrial involvement (n = 4), parametrial involvement and nodes positivity (n = 45), parametrial involvement and/or positive margins (n = 14), parametrial neoplastic involvement, positive margins and lymph node positivity (n = 15).

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**Table 2. — Responsiveness of the primary tumor to neoadjuvant chemotherapy (NACT arm).**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Complete response</th>
<th>Partial response</th>
<th>No response*</th>
<th>Chi-square test</th>
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<tbody>
<tr>
<td></td>
<td>pts</td>
<td>pts</td>
<td>%</td>
<td>pts</td>
<td>%</td>
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<tr>
<td><strong>Patient age</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt; 35 yrs</td>
<td>42</td>
<td>9</td>
<td>(21.4)</td>
<td>27</td>
<td>(64.3)</td>
</tr>
<tr>
<td>35-50 yrs</td>
<td>36</td>
<td>9</td>
<td>(25.0)</td>
<td>21</td>
<td>(58.3)</td>
</tr>
<tr>
<td>&gt; 50 yrs</td>
<td>81</td>
<td>18</td>
<td>(22.2)</td>
<td>42</td>
<td>(51.9)</td>
</tr>
<tr>
<td><strong>FIGO stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ib-IIa</td>
<td>105</td>
<td>26</td>
<td>(24.8)</td>
<td>58</td>
<td>(55.2)</td>
</tr>
<tr>
<td>IIb</td>
<td>24</td>
<td>6</td>
<td>(25.0)</td>
<td>12</td>
<td>(50.0)</td>
</tr>
<tr>
<td>IIIa-IIIb</td>
<td>30</td>
<td>4</td>
<td>(13.3)</td>
<td>20</td>
<td>(66.7)</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 cm</td>
<td>96</td>
<td>27</td>
<td>(28.1)</td>
<td>61</td>
<td>(63.6)</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>63</td>
<td>9</td>
<td>(14.3)</td>
<td>29</td>
<td>(46.0)</td>
</tr>
<tr>
<td><strong>Lymph nodes (radiol. status)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>115</td>
<td>28</td>
<td>(24.3)</td>
<td>70</td>
<td>(60.9)</td>
</tr>
<tr>
<td>positive</td>
<td>44</td>
<td>8</td>
<td>(18.2)</td>
<td>20</td>
<td>(44.5)</td>
</tr>
</tbody>
</table>

Patients: pts; *stable-progressive disease.
Recurrences

Table 3 shows the main prognostic factors that influenced the recurrence of the disease* in the 258 patients on whom surgery was performed.

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>NACT arm</th>
<th>CONV arm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Disease recurrence</td>
</tr>
<tr>
<td></td>
<td>pts</td>
<td>pts</td>
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<tr>
<td>CLINICAL</td>
<td></td>
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<tr>
<td>Patient age</td>
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<td></td>
</tr>
<tr>
<td>&lt; 35 yrs</td>
<td>42</td>
<td>15</td>
</tr>
<tr>
<td>&gt; 35-50 yrs</td>
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<td>10</td>
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<tr>
<td>Ib-IIa</td>
<td>105</td>
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</tr>
<tr>
<td>IIb</td>
<td>24</td>
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<tr>
<td>G3</td>
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<td>30</td>
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<td>27</td>
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<tr>
<td>present***</td>
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<tr>
<td>Lymph node status</td>
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<tr>
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<td>25</td>
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<tr>
<td>positive***</td>
<td>38</td>
<td>24</td>
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<td>free</td>
<td>137</td>
<td>42</td>
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<tr>
<td>involvement***</td>
<td>16</td>
<td>7</td>
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<td>CHEMOTHERAPY</td>
<td></td>
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<tr>
<td>complete</td>
<td>36</td>
<td>6</td>
</tr>
<tr>
<td>partial</td>
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<td>28</td>
</tr>
<tr>
<td>absent**</td>
<td>27</td>
<td>15</td>
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</table>

*distal metastasis and local recurrences; **twenty-four patients (NACT arm) in Stage IIIa-IIIb, found sensitive to neoadjuvant chemotherapy, were able to undergo surgical treatment. Six patients in the same stage not responsive to chemotherapy treatment (NACT arm) and 24 patients in the control group (CONV arm) did not undergo surgery and were excluded from the statistical evaluation of recurrences; ***adjuvant radiotherapy treatment was given 4-6 weeks after surgical treatment to 84 patients who showed, at the final histological examination: parametrial neoplastic infiltration and/or lymph node positivity and/or parametrial neoplastic infiltration.

Recurrence of disease was documented in 49 patients of the NACT arm (n = 153) and in 39 patients of the CONV arm (n = 105). Disease-related deaths occurred in 47 patients of the NACT arm (29.6%) and 44 in the CONV arm (34.1%).

No statistically significant differences between the two arms were observed in relation to the age of the patients with regard to disease recurrences, while a significant difference in relation to the stage was noted in the NACT arm group (chi-square = 11.91; p = 0.003).

Patients in the NACT arm group showed a recurrence rate of 23.8% FIGO Stage Ib-Ila, 41.7% and 58.3% Stage IIb and III, respectively.

Statistically significant differences were observed in terms of recurrence of disease related to the size of the primary tumor. In patients with a tumor < 5 cm, recurrence of the disease was 22.2%, and in patients with larger tumor sizes ≥ 5 cm, 46.0% contributed to an increase in local and distance recurrences: in the NACT arm (chi-square = 9.65; p = 0.002) and in the CONV arm (chi-square = 17.96; p = 0.001). In the NACT arm group, the odds ratio (OR) was 2.985 (CI 95% = 1.480-6.022) indicating that patients with tumor size ≥ 5 cm are three times more likely to have recurrences, while in the CONV arm
group the result is higher with an OR = 6.471 (CI 95% = 2.617-16.000).

Statistically significant differences in the recurrence rate were observed in both study groups, for patients with histologically negative prognostic factors as: parametrial infiltration (NACT arm: chi-square = 9.16; p = 0.002; OR = 3.037; CI 95% = 1.458-6.326), (CONV arm: chi-square = 13.05; p = 0.001; OR = 4.756; CI 95% = 1.984-11.402) and lymph node status (NACT arm: chi-square = 22.50; p = 0.001; OR = 6.171; CI 95% = 2.789-13.656), (CONV arm: chi-square = 23.43; p = 0.001; OR = 9.833; CI 95% = 3.599-26.866); only in the NACT arm group was a low significant difference (chi-square = 5.74; p = 0.05) found related to grading.

Responsiveness to chemotherapy can also influence recurrence of the disease (chi-square = 10.80; p = 0.005). Recurrence of the disease was observed in 16.7% of the patients who had shown a complete response to neoadjuvant chemotherapy, in 31.1% of the patients with partial response and in 55.5% of the patients not responsive (stable-progressive disease).

**Overall and disease-free survival**

Overall and disease-free survival, subdivided for stage and total, are summarized in Tables 4 and 5.

Table 4. — Overall survival and deaths for stage in NACT and CONV arms (%).

<table>
<thead>
<tr>
<th>Status</th>
<th>NACT arm</th>
<th>CONV arm</th>
<th>Chi-square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ib-IIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall survival</td>
<td>83 (79.0)</td>
<td>62 (73.8)</td>
<td>.71</td>
<td>.39 (ns)</td>
</tr>
<tr>
<td>Deaths</td>
<td>22 (21.0)</td>
<td>22 (26.2)</td>
<td>.09</td>
<td>.76 (ns)</td>
</tr>
<tr>
<td>IIb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall survival</td>
<td>17 (70.8)</td>
<td>14 (66.7)</td>
<td>.14</td>
<td>.70 (ns)</td>
</tr>
<tr>
<td>Deaths</td>
<td>7 (29.2)</td>
<td>7 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa-IIIb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall survival</td>
<td>12 (40.0)</td>
<td>9 (37.5)</td>
<td>.09</td>
<td>.70 (ns)</td>
</tr>
<tr>
<td>Deaths</td>
<td>18 (60.0)</td>
<td>15 (62.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total overall survivals and deaths in NACT and CONV arms (%).</td>
<td>1.84</td>
<td>.17 (ns)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. — Disease-free survival and recurrences for stage in NACT and CONV groups (%).

<table>
<thead>
<tr>
<th>Status</th>
<th>NACT arm</th>
<th>CONV arm</th>
<th>Chi-square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ib-IIa</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Disease-free</td>
<td>80 (76.2)</td>
<td>54 (64.3)</td>
<td>.32</td>
<td>.07 (ns)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>25 (23.8)</td>
<td>30 (35.7)</td>
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<td></td>
</tr>
<tr>
<td>IIb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease-free</td>
<td>14 (58.3)</td>
<td>12 (57.1)</td>
<td>.007</td>
<td>.93 (ns)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>10 (41.7)</td>
<td>9 (42.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIIa-IIIb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease-free</td>
<td>10 (33.3)</td>
<td>3 (25.0)</td>
<td>.004</td>
<td>.93 (ns)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>20 (66.7)</td>
<td>6 (75.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total disease-free survivals and recurrences in NACT and CONV groups (%)</td>
<td>1.84</td>
<td>.17 (ns)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Of the patients classified as Stage Ib-IIa of the NACT arm (n = 105) and of the CONV arm (n = 84), even though no significant difference was noted, an increase of about 5% in overall 7-year survival was observed in patients classified as Stage Ib-IIa of the NACT arm (79.0%) compared to patients of the CONV arm classified as the same stages (73.8%). On the other hand, a comparison of the disease-free survival in relation to the same groups (Ib-IIa) documents an increase, although not statistically significant in (p: .07) in disease-free survival in the chemotherapy group (76.2%) compared to the control group (64.3%).

The same tables show the results of the follow-up after 84 months and the related overall and disease-free survival of the 45 patients classified as IIb (n = 24) NACT arm and (n = 21) CONV arm. No statistically significant differences between the two groups were found (p = ns). The overall survival was 70.8% in the NACT arm and 66.7% in the CONV arm (chi-square: 0.9; p = 0.76) and disease-free 58.3% vs 57.1% (chi-square: 0.07; p = 0.93).

In the group of 30 patients classified as Stage III and given neoadjuvant chemotherapy, 24 were found to be responsive to polychemotherapy, which enabled the surgical approach, and six were assigned to radiotherapy. In this group, overall survival was 40.0% (n = 12). Twenty-four patients of the control group (CONV arm) were assigned to radiotherapy: in this group the overall survival was 37.5% (n = 9); there was no statistical difference between the two groups (chi-square: 0.14 and p = 0.70).

A comparison between overall and disease-free survival in the NACT and CONV arms is summarized in the same tables. The overall survival in the NACT arm was 70.4% (n = 112) vs 65.9% (n = 85) in the CONV arm (chi-square: 1.86; p = 0.17). Disease-free in NACT was 65.4% (n = 104) vs 53.5% (n = 69) in CONV (chi-square: 4.22; p = 0.04). Increase in terms of overall and disease-free survival in the NACT arm is mainly linked to 36 patients in whom a complete response to chemotherapy was obtained.

The overall and disease-free survival curves with the Kaplan Meier method, and the determination of degree of significance with the log-rank test are now under evaluation.

**Discussion**

Several studies have shown the sensitivity to chemotherapy of squamous cell cervical carcinoma [30]. The main purpose of our study was to confirm [31] whether, in diagnosed cases of cervical carcinoma, preoperative chemotherapy could improve the overall survival and disease-free period, and what are the main prognostic factors that influence recurrences.

Concerning response to neoadjuvant chemotherapy, in our clinical experience, a complete response of tumor to chemotherapy was noted in 25% of patients classified as Stages Ib-IIa and in 28% of tumors < 5 cm. The partial and complete response to chemotherapy observed in our study is comparable to that reported by other authors. Chen et al. [14] in a study on 142 women with bulky cervical carcino-
ma (Ib2-IIb) had an overall clinical response of 69.4%. Our study registered in 63 cases with tumor size $\geq$ 5 cm, a total response in 14.3%, a partial in 46.0% and no response in 39.7% cases (chi-square 20.03; $p < 0.001$). If we see the responsiveness of 159 patients who underwent neoadjuvant chemotherapy, we find a total response in 22.6%, a partial response in 56.6% and no response in 20.8% cases. Neoadjuvant therapy enabled a significant increase in the operability of patients even in locally advanced stages [32, 33]. In our study the operable group was increased by 24 chemosensitive cases, which constituted 80% of the patients belonging to Stages IIIa-IIIb. This permitted an increase in survival compared to the group of patients classified as the same stage but not responsive to chemotherapy or to the patients of the control group.

Concerning the recurrences, several authors observed that lymph node positivity is the most important prognostic factor for risk of recurrence [34]. In our study, the presence of lymph node metastasis caused an increase in recurrence, which ranged between 63% ($p < 0.001$) and 75% ($p < 0.0001$) in the NACT and CONV arms, respectively. Recurrence rate from three trials [21-23] could improve an OR favorable to neoadjuvant chemotherapy (OR: 0.76; $p = 0.21$) but the results are not significant. Pathologic findings, in Chen et al.’s study [14] showed that pelvic lymph node metastasis and parametrial infiltration rates were significantly lower in the NACT group than in the primary surgery group ($p = 0.025$; $p = 0.038$). We found no significant difference between the two groups in relapses for involvement of surgical margins, respectively 43.7% and 50%. Relapses were significantly lower in parametrical involvement, respectively, 50% and 61.8% ($p = 0.002$). Chen et al. [14] found that NACT responders had a higher tumor-free survival and lower recurrence rate that non-NACT responders ($p = 0.000$; $p = 0.013$). With regard to recurrences Sardi et al. [21] observed a significant decrease in pelvic failure ($p < 0.001$). Our study confirms the same results ($p < 0.002$). Eddy and colleagues [23] reached opposite conclusions concerning bulk tumor size with similar recurrence rates (relative risk 0.998) and death rates (RR 1.008) when compared to the NACT group vs surgery alone. We have to note that as far as surgical approach to cervical carcinoma is concerned, it is directly linked to the concept of resectability and radicality, and is justified since this tumor remains localized in the pelvis for a fairly long time [30].

Concerning survival, several authors [35] have reported in randomized studies an increase of the overall survival in patients in Stage IB treated with neoadjuvant chemotherapy, documenting a significant reduction in the aggressivity of the neoplasia on involvement of the parametria and lymph node metastases. When we analyze our results, related to the patients classified Ib-IIa, we find an increase of about 5% in overall and 12% in disease-free 7-year survival, although not statistically significant in the group of patients given neoadjuvant chemotherapy compared to the control group; it was mainly due to the 26 patients completely responsive to the chemotherapy regimen (22.6%) who contributed to this improvement in the survival rate. With regard to bulky tumors ($> 4$ cm), Sardi et al. [21] found statistical significance in overall and disease-free survival of the neoadjuvant plus surgery group vs the control group. This was due, however, in our study, to increased operability of bulky responsive tumors after neoadjuvant chemotherapy ($p < 0.01$).

Data on progression-free survival are also available from three trials [14, 23, 36] with significant benefits from neoadjuvant chemotherapy (hazard ratio 0.76, $p = 0.01$). Disease-free survival in our study was 65.4% in the NACT group vs 53.5% in the CONV group (chi square: 4.22; $p = 0.04$).

The main result which documents total remission of the disease is represented by total regression of the primary focus. Moreover, evaluation of the size of the primary tumor after neoadjuvant treatment allowed us to hypothesize a subsequent treatment strategy: patients showing an excellent response to neoadjuvant chemotherapy could be treated after surgery with successive cycles of chemotherapy; on the other hand, a limited response should suggest withdrawal of the postsurgical chemotherapy protocol and redefining the treatment strategy. For these reasons we think that the combination of neoadjuvant chemotherapy plus surgery and adjuvant radiotherapy (or, if possible, adjuvant chemotherapy) is feasible and does not constitute “overtreatment” for most patients with cervical carcinoma at high risk of recurrence.

It is reasonable to think that the evolution of chemotherapeutic drugs could cause changes in future protocols to the benefit of increased survival results.

Conclusions

Despite the conclusions of Eddy et al. [23] who advise against adding NACT to radical surgery in Stage Ib2 cervical cancer cases, the results of several studies suggested that neoadjuvant chemotherapy prior to radical surgery is acceptable in locally advanced cervical cancer. This may reduce the bulky tumor, allowing surgery for patients otherwise inoperable and in which the concept of radicality could not be satisfied, thus improving the prognosis in patients with locally advanced cervical cancer. The prognosis for patients in the control group who could not undergo resection was significantly worse than for those in whom surgery could be performed. From this clinical experiment it can be concluded that cases of cervical carcinoma, with an almost complete responsiveness to the neoadjuvant chemotherapy treatment, benefit both in terms of overall survival and in terms of disease-free survival. The same group of patients could be treated after surgery with cycles of chemotherapy, avoiding adjuvant radiotherapy treatments which are subject to a rather high incidence of complications.

It is to be hoped that the chemotherapeutic agents already in use for so many years and others in the experimental phase can change the present therapeutic approach and, what is more, assign the greatest number of patients to treatment in order to obtain improved disease-free survival in a group of poor prognosis patients.
References


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Late toxicities in concurrent chemoradiotherapy using high-dose-rate intracavitary brachytherapy plus weekly cisplatin for locally advanced cervical cancer: a historical cohort comparison against two previous different treatment schemes

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Summary

Purpose: To determine the long-term toxicity of concurrent chemoradiotherapy (CCRT), using high-dose rate intracavitary brachytherapy (HDRICB) compared to radiation (RT) alone in patients with advanced cervical cancer using a control-cohort study.

Methods: A total of 332 cases of Stage IIB-III disease were included in this comparative study. Seventy-three patients were treated with a 3-insertion schedule and labeled group A, whereas the other 146 patients with a 4-insertion schedule became group B. One hundred and thirteen patients treated by a 4-insertion protocol with concurrent weekly cisplatin were labeled group C. Results: The cumulative rate of grade 2 or above rectal complication was 13.7% for group A, 9.6% for the group B and 15.9% for group C (p = 0.76), whereas the grade 3 to 4 non-rectal radiation-induced intestinal injury was 6.8% for group A, 6.2% for group B and 9.7% for group C (p = 0.20). Grade 2 to 4 late bladder toxicity was higher in group C, with the cumulative rate being 5.5% for group A, 4.8% for group B and 15.0% for group C (p = 0.004). The independent factor for a rectal complication was the occurrence of a bladder complication (p = 0.01, hazard ratio 3.06). The independent factors for bladder complications were the use of CCRT (p = 0.01, hazard ratio 2.08), and the occurrence of rectal complications (p = 0.02, hazard ratio 2.77). Conclusions: When treating advanced cervical cancer, HDRICB consisting of four 6 Gy insertions and weekly cisplatin shows a trend of increasing late bladder complications. The interval between drug administration and HDRICB should be kept long enough to avoid any synergistic effect of both regimens.

Key words: Carcinoma of the cervix; Radiotherapy; Concurrent chemoradiotherapy; High-dose rate brachytherapy; Complications.

Introduction

The routine use of high-dose rate intracavitary brachytherapy (HDRICB) has been questioned because of the presence of a narrow therapeutic window and a lack of consensus on fractionation [1]. Despite some skepticism, HDRICB has been widely used for the management of cervical cancer since it allows the application of brachytherapy during outpatient visits. Orton et al. has suggested that an increase in the fraction number accompanied by a decreasing fraction size reduces the incidence of complications [2]. However, there is no consensus as to the optimum fractionation regimen that should be used; such a consensus is available with the low-dose-rate (LDR) regimen. The American Brachytherapy Society (ABS) dose recommendation for the radiation treatment of advanced cervical cancer is 45 Gy of external beam radiotherapy (EBRT) to the entire pelvis in combination with a prescribed dose of 6.5 Gy to point A in five fractions or 5.8 Gy in six fractions. Other alternatives that have been proposed and these include an EBRT of 50.4 Gy to the pelvis together with 7 Gy to point A in four fractions or 6 Gy in five fractions or 5.3 Gy in six fractions [3]. The prescribed doses that make up these schedules, when calculated, range from 90.5 to 99 Gy of the LDR equivalent when using the LDR/HDR conversion factor [2, 4]. More clinical datasets are required to compare the outcomes of the different fractionation schedules because these schedules have not been thoroughly tested in a clinical situation.

The American National Cancer Institute made a strong recommendation that those patients with invasive cervical cancer who require RT should be treated concurrently with cisplatin-based chemotherapy. To avoid prolongation of the overall treatment time, HDRICB should be initiated after tumor regression. Thus, HDRICB is always interspersed with EBRT when weekly cisplatin is given. The potential risk of increased late toxicity when combining chemotherapy and HDRICB also needs to be further investigated. While HDRICB treatment allows better custom-tailoring of the dose distributions compared to LDR, it also requires more attention in order to achieve a precise and accurate dose distribution calculation and treatment delivery because there is a loss in the biological therapeutic ratio. Although retrospective studies of HDR and concurrent chemotherapy have demonstrated toxicity rates similar to those with LDR [5-10], these investigations have involved only limited numbers of patients and there is also a lack of long-term follow-up.
In this study, we compared the late complications among patients with advanced cervical cancer who had been treated with HDRICB over three distinct treatment systems through a historical cohort control. Since this study was retrospective and CCRT has become an established treatment policy for locally advanced cervical cancer, the aim was to analyze the late toxicities rather than to compare the survival curves.

### Materials and Methods

#### Patient characteristics

Between January 1993 and December 2006, a total of 451 patients with previously untreated cervical cancer completed curative-intent RT at the China Medical University Hospital. Before January 2000, most of our patients were treated with RT alone using two different ICB schedules. After January 2000, the routine use of CCRT for advanced tumors became the standard pattern of care and a total of 153 patients were treated in this later period. The inclusion criteria were:

1. Stage IIB-III disease with a homogeneous EBRT dose to the pelvis and brachytherapy protocol. Patients with Stage IB-IIA disease were excluded because the optimal RT policy for bulky IB-IIA tumors was not consistently reproducible across the different treatment periods.
2. The patients had three sessions of HDRICB with a prescribed dose of 7.2 Gy per fraction to point A (before December 1995) or four sessions of HDRICB with 6.0 Gy to point A (after January 1996).
3. The patients completed at least two years of regular follow-up and laboratory studies.

A total of 332 cases were included in this comparative study. No studied subjects received extended field irradiation and were labeled as group A. Another 146 patients underwent a 4-insertion schedule and were labeled group B. Finally, the remaining 113 patients were treated with a 4-insertion ICB protocol and concurrent weekly cisplatin and these were labeled as group C. No patient in either group A or B had been treated with combination chemotherapy. All the patients were treated by the same radiation oncology team. The patient characteristics of the three groups are summarized in Table 1.

### Radiotherapy

Irradiation treatment consisted of EBRT followed by HDRICB. Initially, the whole pelvis was treated with 10 MV X-rays via anterior and posterior parallel fields or box variants where the AP diameter was over 18 cm. The standard prescribed dose was 44 to 45 Gy, which consisted of 22 to 25 fractions four to five weeks apart. The radiation dose for patients diagnosed as FIGO Stage IIB-III bilateral parametrial disease was boosted to 50.4 to 59.4 Gy with 4-cm wide mid-line shielding.

After adequate tumor regression, HDRICB was performed using an Ir-192 remote after-loading technique at 1-week intervals and this was carried out concurrently with parametrial boosting. The total prescribed point A doses (EBRT + HDRICB) ranged from 65.6 to 69 Gy (median, 68 Gy). The details of the radiotherapy techniques are listed in Table 2.

### Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>group A no = 73</th>
<th>group B no = 146</th>
<th>group C no = 113</th>
<th>χ² test</th>
<th>p value</th>
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</thead>
<tbody>
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<td>Age (years)</td>
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<td>35 ~ 82 (median 60)</td>
<td>33 ~ 83 (median 58)</td>
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<tr>
<td>&lt; 45</td>
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<td>15 (13.3%)</td>
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<tr>
<td>45-65</td>
<td>44 (60.3%)</td>
<td>84 (57.5%)</td>
<td>71 (62.8%)</td>
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<tr>
<td>&gt; 65</td>
<td>23 (31.5%)</td>
<td>40 (27.4%)</td>
<td>27 (23.9%)</td>
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<td>Stage</td>
<td></td>
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<tr>
<td>IIB</td>
<td>43 (58.9%)</td>
<td>106 (72.6%)</td>
<td>80 (70.8%)</td>
<td></td>
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<tr>
<td>III</td>
<td>30 (41.1%)</td>
<td>40 (27.4%)</td>
<td>33 (29.2%)</td>
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<td>Tumor size</td>
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<td>&lt; 4 cm</td>
<td>13 (17.8%)</td>
<td>27 (18.5%)</td>
<td>21 (18.6%)</td>
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<tr>
<td>≥ 4 cm</td>
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<td>119 (81.5%)</td>
<td>92 (81.4%)</td>
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<tr>
<td>Negative</td>
<td>64 (87.7%)</td>
<td>130 (89.0%)</td>
<td>100 (88.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (12.3%)</td>
<td>16 (11.0%)</td>
<td>13 (11.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>squamous cell ca.</td>
<td>67 (91.8%)</td>
<td>136 (93.2%)</td>
<td>102 (90.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adenocarcinoma/adenosquamous</td>
<td>6 (8.2%)</td>
<td>10 (6.8%)</td>
<td>11 (9.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT duration (days)</td>
<td>50 ~ 121 (median 63)</td>
<td>49 ~ 115 (median 59)</td>
<td>39 ~ 108 (median 55)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Whole pelvis dose (Gy)</td>
<td>44 ~ 46 (median 44)</td>
<td>40 ~ 50 (median 44)</td>
<td>39.6 ~ 50.4 (median 45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parametrial dose (Gy)</td>
<td>58 Gy</td>
<td>58 Gy</td>
<td>57.6 Gy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of insertion</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Point A dose (Gy)</td>
<td>7.2</td>
<td>6.0</td>
<td>6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>11 ~ 110 (median 62)</td>
<td>18 ~ 101 (median 58)</td>
<td>19 ~ 90 (median 54)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The definition of a parametral dose is the summation of external beam irradiation dose before and after central shielding. It represents the cumulative dosage to the bilateral parametra; ns = non significant.

### Table 2. — Outcome and survival across the treatment groups.

<table>
<thead>
<tr>
<th>Survival</th>
<th>group A</th>
<th>group B</th>
<th>group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-year CSS</td>
<td>IIB</td>
<td>77%</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>55%</td>
<td>68%</td>
</tr>
<tr>
<td>5-year PRFS</td>
<td>IIB</td>
<td>89%</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>71%</td>
<td>79%</td>
</tr>
<tr>
<td>5-year DMFS</td>
<td>IIB</td>
<td>74%</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>64%</td>
<td>70%</td>
</tr>
</tbody>
</table>

The values in parentheses represent patient number.

CSS = cause-specific survival; PRFS = pelvis relapse-free survival; DMFS = distant metastasis-free survival.
Table 3. — Chronic complications across the three treatment groups.

<table>
<thead>
<tr>
<th>Category of complication</th>
<th>group A (no = 73)</th>
<th>group B (no = 146)</th>
<th>group C (no = 113)</th>
<th>χ² test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation proctitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2-4</td>
<td>10 (13.7%)</td>
<td>14 (9.6%)</td>
<td>18 (15.9%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>4 (5.8%)</td>
<td>3 (2.1%)</td>
<td>3 (2.7%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Median duration (range)</td>
<td>14 months (5-28)</td>
<td>12 months (6-26)</td>
<td>11 months (7-22)</td>
<td></td>
</tr>
<tr>
<td>NRRIII Grade 3-4</td>
<td>5 (6.8%)</td>
<td>9 (6.2%)</td>
<td>11 (9.7%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Median duration (range)</td>
<td>17 months (9-23)</td>
<td>18 months (6-39)</td>
<td>13 months (7-28)</td>
<td></td>
</tr>
<tr>
<td>Radiation cystitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2-4</td>
<td>4 (5.5%)</td>
<td>7 (4.8%)</td>
<td>17 (15.0%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>2 (2.7%)</td>
<td>3 (2.1%)</td>
<td>6 (5.3%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Median duration (range)</td>
<td>22 months (11-43)</td>
<td>26 months (8-41)</td>
<td>15 months (3-27)</td>
<td></td>
</tr>
<tr>
<td>Lower leg edema</td>
<td>2 (2.7%)</td>
<td>5 (3.4%)</td>
<td>4 (3.5%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>0</td>
<td>1 (0.7%)</td>
<td>5 (4.4%)</td>
<td>–</td>
</tr>
<tr>
<td>Renal failure (need dialysis)</td>
<td>0</td>
<td>1 (0.7%)</td>
<td>1 (0.9%)</td>
<td>–</td>
</tr>
<tr>
<td>Electrolyte imbalance</td>
<td>0</td>
<td>0</td>
<td>4 (3.5%)</td>
<td>–</td>
</tr>
<tr>
<td>Ureteral stenosis</td>
<td>1 (1.3%)</td>
<td>3 (2.1%)</td>
<td>2 (1.7%)</td>
<td>–</td>
</tr>
<tr>
<td>Bone marrow failure</td>
<td>0</td>
<td>0</td>
<td>1 (0.9%)</td>
<td>–</td>
</tr>
</tbody>
</table>

The values in parentheses represent the percentage of patients with chronic complications.

NRRIII = non-rectal radiation-induced intestinal injury.

Table 4. — Interval between brachytherapy and weekly cisplatin in group C.

<table>
<thead>
<tr>
<th>Grade 2 to 4 bladder complication</th>
<th>Negative (No = 85)</th>
<th>Positive (No = 28)</th>
<th>χ² test p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No course with the interval</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interval less than 24 hours</td>
<td>60</td>
<td>15</td>
<td>0.07</td>
</tr>
<tr>
<td>At least one course with the</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interval &lt; 24 hours</td>
<td>25</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>one course</td>
<td>18</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>two courses</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>three courses</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>four courses</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

After January 2000, most patients with advanced disease were treated with concurrent chemotherapy. The chemotherapy consisted of cisplatin delivered weekly at a dose of 40 mg/m² intravenously to give a total dose of up to 60 mg. The first cycle of cisplatin was initiated at the first RT treatment. In accordance with the duration of the RT, the treatment plan thus included a total of five to six cycles of cisplatin. The details of the drug administration protocol have been described in our previous study [11].

During the RT course, weekly monitoring of hemoglobin levels was required. Blood transfusion was mandatory if the hemoglobin level fell below 1000/dl. In addition, to reduce the risk of aspiration when conscious sedation was used, HDRICB was delivered before the administration of chemotherapy when both modalities were given simultaneously.

Treatment planning and the rules of the source dwell

For patients treated with the two-field technique, the EBRT dose was calculated at the midplane, while the dosimetry of the box field was calculated using computer-based software and the doses were prescribed to the isocenter. The HDRICB dosimetry was calculated using orthogonal films exposed during each insertion. The HDRICB isodose curves were reviewed by physicians to ensure that the residual tumors were fully irradiated within the high-dose area. The applicator for the brachytherapy was a Henochke’s type. The detailed method of modulating the weight of the dwell time has been reported in the study of Wang et al. [12].

During each insertion, the posterior and anterior vagina was packed with radio-opaque gauze to reduce rectal and bladder exposure and to visualize the posterior vaginal septum. The detailed method used to calculate the rectal and bladder reference doses has been described elsewhere [13].

Follow-up and complication analysis

We assessed the treatment response four weeks after completion of treatment. If residual disease was suspected, a biopsy was performed. Patients underwent regular follow-up examinations every one to two months for the first year and then every three months thereafter. A pelvic examination was performed during each follow-up visit. Tumor markers (squamous cell and carcinoembryonic antigens) were checked every three to six months and radiographical examinations (a chest X-ray and abdominopelvic computed tomography (CT) scanning) were conducted yearly. Pelvic recurrence was confirmed if the disease was detected in the irradiated field. Distant metastases were confirmed if tumors occurred in the paraaortic lymph nodes or elsewhere outside the pelvis. Once central recurrence was noted at follow-up, a salvage operation would be performed if possible. Otherwise, palliative RT with or without chemotherapy would be administered to treat the metastatic paraaortic lymph nodes or painful recurrent tumors.

Patients who had bloody stools or hematuria underwent endoscopy to identify the site of the bleeding and a blood count every two to four weeks for surveillance of the severity of complications. Rectal and bladder complications and non-rectal gastrointestinal sequelae (small bowel complications) were scored according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer (RTOG/EORTC) grading scale [14]. Non-rectal radiation-induced intestinal injury (NRRIII) was defined as RT-induced gastrointestinal sequelae other than rectal complications and has been described in our previous study [15]. Due to a concern that less than comprehensive history-taking might not give a correct score for low-grade NRRIII, only grade three or above complications were entered into our analysis.

Statistical analysis

Patient survival was measured from the date of initiation of therapy to the date of the last follow-up examination. Survival
Late toxicities in concurrent chemoradiotherapy using high-dose-rate intracavitary brachytherapy plus weekly cisplatin for locally etc.  507

Table 5. — Results of various HDRICB studies with concurrent chemotherapy in locally advanced cervical cancer.

<table>
<thead>
<tr>
<th>First author</th>
<th>Patient no.</th>
<th>Stage</th>
<th>EBRT schedule</th>
<th>Drug regimen</th>
<th>End point/Outcome</th>
<th>Late complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Souhami [7]</td>
<td>50</td>
<td>IIA-IVA 30 Gy/3Fr</td>
<td>EBRT 46 Gy</td>
<td>cisplatin 30 mg/m² weekly</td>
<td>complete remission 88%</td>
<td>rectal ulcer 20% rectovaginal fistula 4% small bowel obstruction 4% grade 2-4 all toxicity 5%</td>
</tr>
<tr>
<td>Sood [6]</td>
<td>49</td>
<td>IB-IIIB</td>
<td>EBRT 45 Gy 18 Gy/2Fr</td>
<td>cisplatin 20 mg/m²/day x 5 days week 1 and 5</td>
<td>5-year OS 78%</td>
<td>grade 3-4 all toxicity 6%</td>
</tr>
<tr>
<td>Novetsky [9]</td>
<td>77</td>
<td>IB2-IV</td>
<td>EBRT 45 Gy 18 Gy/2Fr</td>
<td>cisplatin 40 mg/m²/day or cisplatin 20 mg/m²/day every 3 weeks</td>
<td>5-year LCR IB2/II 88%; III/IV 68%</td>
<td>5-year DFS IB2/II 83%; III-IV 61%</td>
</tr>
<tr>
<td>Toita [10]</td>
<td>40</td>
<td>IB2-IVA</td>
<td>EBRT 40 Gy 18 Gy/3Fr</td>
<td>cisplatin 20 mg/m²/day x 5 days every 3 weeks</td>
<td>3-year PCR 91%</td>
<td>proctitis (all grade) 9% enterocolitis (all grade) 15%</td>
</tr>
</tbody>
</table>
| Tseng [5]    | 60          | IIB-IIIB | EBRT 44 Gy 25.8 Gy/6Fr | cisplatin + vincristine + bleomycin every 3 weeks for 4 courses | 3-year DFS 50% | proctitis (no grading) 10% cystitis (no grading) 3.3% intestinal obstruction 3.3%
| Present study | 113        | IIB-IIIB | EBRT 45 Gy 24 Gy/4Fr | cisplatin 40 mg/m² weekly | 5-year CSS IIB 78%; III 72% | proctitis (grade 2-4) 15.6% cystitis (grade 2-4) 15% cystitis (grade 3-4) 5.3% NRRIII (grade 3-4) 9.7% |

EBRT = external beam radiotherapy; OS = overall survival; LCR = local control rate; DFS = disease-free survival; CSS = cause-specific survival; PRFS = pelvic relapse-free survival; DMFS= distant metastasis-free survival; NRRIII = non-rectal radiation-induced intestinal injury.

was calculated using the Kaplan-Meier method. Comparison of the categorical variables was performed using the chi square test. The logistic regression test was utilized for assessment of the patient and treatment factors associated with the occurrence of late complications. Statistical significance was considered to be present when the p value was less than 0.05. All calculations were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, Ill).

Results

The median duration of follow-up for all groups was 57 months (group A: 62 months; group B: 58 months; group C: 53 months). The outcomes and survival for the three groups of patients are listed in Table 2.

Table 3 summarizes the late complications and latency for the three treatment groups. Forty-two patients (12.7%) had grade 2 to 4 rectal complications and ten patients (3.0%) had grade 3 to grade 4. The cumulative rate of grade 2 or above rectal complications was 13.7% for group A, 9.6% for the group B and 15.9% for group C (p = 0.01, hazard ratio 2.77, 95% CI 1.07~6.29). No patient or treatment-related factor was associated with grade 3 or above rectal complications.

Some further irreversible adverse effects were also noted in the group C. Five patients (4.4%) developed renal insufficiency and one needed hemodialysis. Four patients developed persistent electrolyte imbalance and one patient developed irreversible bone marrow failure.

From the logistic-regression analysis, the independent factor for grade 2 or above rectal complications was the occurrence of bladder complications (p = 0.01, hazard ratio 3.06, 95% CI 1.36~12.71). The independent factors for grade 2 or above bladder complications were the use of CCRT (p = 0.01, hazard ratio 2.08, 95% CI 1.02~5.43), and the occurrence of rectal complications (p = 0.02, hazard ratio 2.77, 95% CI 1.07~6.29). No patient or treatment-related factor was associated with grade 3 or above NRRIII.

To clarify the risk factors of bladder complications, further analysis of the interval between the HDRICB and weekly cisplatin was carried out (Table 4). The result showed that the interval was less than 24 hours in 13 of the 28 patients with grade 2 or above complications, compared to 25 of the 85 patients without obvious complications (p = 0.07). Furthermore, the cumulative bladder biologically effective dose (CBBED) from the different treatment periods was calculated as the formula reported in our previous study [14]. There was no statistical difference of the mean bladder CBBEDs in the three periods (group A: 109.2 Gy; group B: 113.2 Gy; group C: 111.7 Gy).
Discussion

It is interesting to compare the data from the patients treated with HDRICB and LDRICB, with or without addition of cisplatin-based chemotherapy; this is because a small gain in local control through CCRT might be counteracted by the possibility of increased morbidity through the combination of HDRICB and chemotherapy. Furthermore, there might be dilution due to the unpredictable biological effects of the two different dose rates. To clarify these possibilities it would be necessary to conduct a phase III randomized trial. However, such a study is difficult to conduct since combination treatment has become the standard pattern of care. Thus, the utilization of control-cohort analysis from different treatment periods would seem to be a feasible way to examine the concept that late toxicities with CCRT plus HDRICB are equivalent to that of RT alone. The studies available for a combination of CCRT with HDRICB are summarized in Table 5. The outcomes for our CCRT patients are also comparable to other similar investigations. However, considering the prescribed doses of HDRICB for the three studies using three or more brachytherapy fractions are obviously lower than those suggested by the ABS, the current ABS recommendations needed to be tested clinically.

HDRICB treatment allows better custom-tailored dose distributions compared to LDR. However, irreparable mistakes can happen very quickly and quality assurance of the treatment plan has proved to be much more important than with LDR. For those institutions performing CCRT, one of the questions that remains unanswered includes whether the addition of concurrent cisplatin with HDRICB increases or not the complication rate; this is because there is paucity in reporting late adverse effects due to a lack of long-term follow-up with some patients. This is especially true for late urological sequelae, which may occur regularly up to 20 years later [16, 17]. Although four LDR trials reported no significant difference in the incidence of long-term toxicity [18-21], one HDR trial [5] reported that treatment-related late toxicity did appear to be higher with CCRT compared with RT alone (23.4% vs 12.9%, \( p = 0.13 \)). Souhami et al. [7] also reported a higher late gastrointestinal complication rate with CCRT when compared to other non-CCRT HDR series. In contrast, Sood et al. [6] found no evidence of an increase in bladder or rectum toxicity when applying two courses of HDRICB (9 Gy to point A per fraction) plus two cycles of cisplatin (20 mg/m²/days for five days). As summarized in Table 5, the late complications of our study were clearly classified and the major sequelae appear to be higher than with the other series. In addition, there is a trend toward a higher incidence and a shorter latent period for bladder complications compared to non-CCRT patients. Specifically, the reduction in latency might imply an increased severity of tissue damage and, as a consequence, a subsequent increase in the incidence of late complications might thus be anticipated. Further optimization of the EBRT protocol and/or the HDRICB fractionation scheme for CCRT patients needs to be performed in order to obtain an increase in the therapeutic gain.

In this study, the finding of close association between rectal and bladder complications was addressed in our previous report [13]. The reason for the higher incidence of bladder complications in our group C might be attributable to the possibility of a concurrent rapid decrease of both the tumor and the thickness of the uterus after CCRT; this may contribute to an increase in the irradiated volume during HDRICB. Thus, for patients receiving higher ICRU bladder doses, modification of the ICB fraction size should be done to reduce the risk of bladder sequelae [13]. Furthermore, the use of 3D image-based dosimetry for HDRICB is becoming increasingly more common and it may increase the feasibility of further optimizing ICB planning [8]. Finally, the interval between drug administration and HDRICB should be kept long enough to avoid any synergistic effect of both regimens as there was a trend that the short interval might be associated with grade 2 or above complications, which was demonstrated in the current study.

On the other hand, Ferrigno et al. [22] reported the incidence of grade 3 to 4 small bowel complications with RT alone was 7.2%, which is similar to our NRRIII incidence across all group patients. They also recommended limiting the total parametrial dose to 54 Gy (45 Gy to the whole pelvis with a 9 Gy boost to the parametrium). In this study, the majority of pelvic failures originated from recurrence of the central disease. Therefore, it should be possible to reduce the parametrial dose in order to decrease the risk of NRRIII.

Conclusion

HDRICB consisting of four 6 Gy insertions and concurrent weekly cisplatin has similar efficacy when compared to the other HDRICB series. Nonetheless, this regimen did demonstrate an increase in late bladder complications. The best results from HDRICB plus CCRT treatment are probably achieved by two approaches. The interval between drug administration and HDRICB should be kept long enough. Furthermore, more prospective trials of the ICB scheme in the CCRT era would seem to be essential if a better overall treatment outcome is to be achieved.

References

Late toxicities in concurrent chemoradiotherapy using high-dose-rate intracavitary brachytherapy plus weekly cisplatin for locally etc.


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Isolated groin recurrence in vulval squamous cell cancer (VSCC). The importance of node count


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Summary

Objective: To determine whether there is a node count which can define an adequate inguinofemoral lymphadenectomy (IFL) in primary VSCC. Methods: A retrospective and prospective review of patients with node negative VSCC who had a full staging IFL. Detection of isolated groin recurrences (IGR) would allow groins with higher risk of groin recurrence to be identified. Results: The median node count of 228 IFLs in 139 patients was eight (0-24). There were six IGR (4.3%). Increased rate of IGR was present in patients with increased age, tumour diameter and depth of invasion, lymphovascular space invasion, unilateral IFL, and moderate/poor tumour grade. In the 138 groins with node counts of eight or greater there were no IGRs compared to six in the patients with either undissected groins or groin node counts less than eight (p = 0.030). Interval to IGR was significantly shorter than other sites of recurrence. Both disease-specific and overall survival were significantly reduced in IGR. Conclusions: An inadequate IFL is a nodal count of less than eight per groin; both these groins and undissected groins are at increased risk of IGR and should have close surveillance.

Key words: Vulval Cancer; Node count; Lymphadenectomy.

Introduction

Vulval squamous cell cancer (VSCC) is a rare tumour of the female genital tract and most studies have involved small numbers of patients. There have been no studies to address in detail the prognostic importance of the number of nodes removed from the groins. The goals of surgery in VSCC are to resect the primary tumour and to remove the regional lymph nodes that may harbour metastatic disease. The standard management of VSCC is a tailored radical wide local excision of the vulval tumour and inguinofemoral lymphadenectomy (IFL) usually through separate incisions [1]. Alternative management of the lymph nodes includes in selected cases sentinel lymph node biopsies, high resolution ultrasound with fine needle aspiration, and inguinofemoral radiation without IFL [2-4]. Superficial lymphadenectomy is not safe because of the risk of involved lymph nodes being missed by the dissection and the poor prognosis of progressive undetected groin node disease [5].

Prognostic value of the number of nodes removed

The extent of metastatic disease in the groin lymph nodes is an important prognostic factor and determines the need for adjuvant treatment [6, 7]. In some tumour sites (e.g., colorectal, pancreas, breast, and bladder) the number of lymph nodes excised has been shown to have prognostic importance as a low nodal harvest may indicate residual lymph nodes with an unknown disease status [8-11]. There have been limited studies to determine the adequacy of IFL in VSCC, and previous reports include patients who had LNM and received radiotherapy [12]. We set out to determine whether groin node count is of clinical relevance in VSCC.

Low nodal counts following IFL may be because: (1) There are few nodes in the groin; (2) The lymphadenectomy was inadequate leaving residual nodes that may contain metastases [5]. The histopathological count of the lymph nodes was incomplete. A low node count would only be relevant to management and prognosis if the unresected nodes did contain tumour.

Approximately a third of patients with VSCC will have groin node metastases at the time of diagnosis and these will usually, but not always, receive adjuvant radiotherapy. In a series of 40 patients, Homesley showed that those with a single node involved without extracapsular spread did not derive a significant survival benefit from radiotherapy and were adequately treated with surgery alone [13]. It has subsequently been common practice not to offer radiotherapy to these women. A residual groin node containing disease may present later as a recurrence in the groin, or pelvis.

Previous studies assessing the adequacy of lymphadenectomy have used the total count of lymph nodes regardless of whether a unilateral or bilateral lymphadenectomy was performed; whether debulking or full lymphadenectomy was the objective; or whether adjuvant radiotherapy was given. It is important therefore to study women who have undergone a full lymphadenectomy and who have not had radiotherapy. The end point used in these studies was either death or recurrence but this would include patients with local recurrence and those whose death was not related to groin node disease. A more appropriate end point is groin recurrence. Groin recurrence may present in isolation or in combination
Isolated groin recurrence in vulval squamous cell cancer (VSCC). The importance of node count

with recurrence on the vulva. In the latter case, the groin node disease may be previously undetected tumour in an unresected node; new metastasis to the groin from the vulval recurrence; or tumour growth in the connective tissue of the resected nodal basin. For that reason, it is essential to consider separately isolated groin recurrences and those that occur in conjunction with recurrence in the vulva.

**Aims**

This study seeks to determine the rates of isolated groin recurrence (IGR) in the dissected and undissected groins of a low-risk group of vulval cancer patients. Assessment of node count will help determine whether there is a nodal count that can be considered to represent an adequate staging lymphadenectomy.

**Patients and Methods**

All patients who were managed for primary or recurrent VSCC from 1980 to 2008 in three London gynaecological cancer centres were identified and a clinicopathological database was constructed retrospectively and prospectively. Histopathology records were retrieved and follow-up data obtained. Patients who had a full IFL were identified. Patients were excluded if they had lymph node metastases; if they received adjunctive groin radiotherapy; if the procedure was palliative; if full central pathology review was not available; or if follow up data were not available. Assessment of nodal disease was performed using standard haematoxylin and eosin staining and microscopy by gynaecological oncology pathologists. Tumour depth of invasion, grade, and diameter were recorded. At follow-up, the site and interval to recurrence was recorded and, in those with groin recurrence, whether there was co-existent or previous local recurrence.

The end point of the study was cytologically confirmed isolated groin recurrence, VSCC recurrence (local, groin, or distant), VSCC death, death from other causes and overall survival. Local recurrence was defined as biopsy confirmed recurrence on the vulva, and suspected groin recurrences were confirmed by cytology or histology. Survival was compared using Kaplan Meier survival curves and Fisher’s exact test for recurrence groups. Statistical analysis was performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com).

**Results**

In the study period (1980-2008) 139 patients with primary VSCC met the criteria for analysis. Age at diagnosis ranged from 26 to 95 years (mean 67, median 71), and follow-up from three to 209 months (mean 54 months, median 44 months).
In the 139 patients there were 266 IFLs (89 bilateral IFLs and 50 unilateral IFLs). In the 133 patients with no IGR there were 219 IFLs (47 unilateral IFL, 86 bilateral IFL) the negative node count ranged from two to 23 (mean = 8.7, median = 8). First recurrence was IGR in six patients, and other sites in 41 patients. Three IGRs were in contralateral undissected groins of patients who had lateral tumours and a unilateral IFL, and three patients recurred in dissected groins where initial node counts were 0, 7 and 7 (Figure 1).

Four of the IGR patients died of recurrent disease, and two are alive with disease. There were 18 deaths of recurrent disease in other sites and 31 of other causes.

Rates of IGR per patient increased with age greater than 70 (p = 0.209), tumour diameter greater than 20 mm (p = 0.230), presence of lymphovascular space invasion (p = 0.079), unilateral IFL (p = 0.667), moderate/poor tumour grade (p = 0.663), however only depth of invasion greater than 5 mm was significant (p = 0.007).

IGR rates were increased in groins with no IFL (6%) compared to any IFL (1.3%) regardless of node count (p = 0.074). In the 138 groins with node counts of eight or greater there were no isolated groin recurrences compared to six in the patients with either undissected groins or groin node counts less than eight (p = 0.030) (Table 1).

The median interval to recurrence for IGR was 8.5 months (6 to 35 months) compared to 25 months for other sites of recurrence (p = 0.0487) (Figure 2). Overall and disease specific survival were significantly lower in the IGR vs non IGR patients (p = 0.0216, p < 0.0001) (Figures 3 and 4).

### Discussion

Patients who develop an isolated groin recurrence may be considered to have had an inadequate initial lymphadenectomy at which nodal disease was not resected. Although this is a rare outcome rates have been observed between from 0 to 8.7% [5, 14-16] and our rate of 4.3% is consistent with previous studies.

In the absence of lymph node metastases there were no isolated groin recurrences where eight or greater lymph nodes were resected. It would therefore seem reasonable to define an inadequate lymphadenectomy as less than eight nodes per groin. It would not necessarily follow that an adequate lymphadenectomy is a node count of eight or greater as in the study period the 23 patients with single nodal metastases had groin node counts ranging from two to 16. Of these groins 43% had a nodal harvest of greater than eight and contained a solitary nodal metastasis that may have been missed if the node count was only eight. Furthermore previous studies have shown a range of node counts from IFL ranging from one to 36, therefore a count alone is unlikely to determine a full IFL. Interestingly, one of our patients recurred in a groin where no nodes were identified on histology despite dissection to the femoral vessels. Despite radiotherapy the patient developed widespread metastatic disease.

In the 50 patients where a unilateral lymphadenectomy was performed, there were three (6%) isolated groin node recurrences in the undissected side where metastatic disease may have been resected and identified if a bilateral IFL had been performed. There were no isolated groin recurrences in the dissected side if the nodes were negative. This increased risk of node failure questions the safety of unilateral lymphadenectomy for lateral tumours and the need for close groin node surveillance of undissected groins.

It is unlikely that clinical examination alone is adequate to detect early nodal progression or recurrence which may be amenable to curative treatment. High resolution ultrasound combined with fine needle aspiration has been shown to be successful in the detection of early nodal disease and this would have a useful role in the surveillance of this high-risk group of patients [4]. Close follow up of the groin should probably continue for at least 36 months as isolated groin node metastases without local recurrence are unlikely after this time period.

### Table 1. — Risk factors for groin node recurrence.

<table>
<thead>
<tr>
<th>Risk factors (per patient)</th>
<th>Isolated groin recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>≤ 70</td>
<td>1/68 (1.5%)</td>
</tr>
<tr>
<td>&gt; 70</td>
<td>5/71 (7.0%)</td>
</tr>
<tr>
<td>Unilateral/bilateral IFL</td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>3/89 (3.5%)</td>
</tr>
<tr>
<td>Tumour diameter (mm)</td>
<td></td>
</tr>
<tr>
<td>≤ 20</td>
<td>1/6 (1.6%)</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>5/78 (6.4%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>Well differentiated</td>
<td>1/46 (2.2%)</td>
</tr>
<tr>
<td>Moderate/poor</td>
<td>5/93 (5.4%)</td>
</tr>
<tr>
<td>Depth of invasion (mm)</td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>0/69 (0%)</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>6/54 (11.1%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0/16 (0%)</td>
</tr>
<tr>
<td>LVSI</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3/26 (11.5%)</td>
</tr>
<tr>
<td>No</td>
<td>3/113 (2.7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factors (per groin)</th>
<th>Isolated groin recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFL</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3/228 (1.3%)</td>
</tr>
<tr>
<td>No</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Node count</td>
<td></td>
</tr>
<tr>
<td>&lt; 8 or No IFL</td>
<td>6/140 (4.3%)</td>
</tr>
<tr>
<td>&gt; 8</td>
<td>0/138 (0%)</td>
</tr>
</tbody>
</table>
Isolated groin recurrence in vulval squamous cell cancer (VSCC). The importance of node count

References


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The association of preoperative thrombocytosis with prognostic factors in malign ovarian tumor

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Dicle University, Faculty of Medicine, Diyarbakir (Turkey)

Summary

Purpose: We assessed the association of preoperative thrombocytosis with prognostic factors in malign ovarian tumor. Methods: Over a five-year period, cases treated for ovarian cancer were randomly assigned. The data were collected from gynecological oncology, radiation oncology, medical oncology and pathology departments. Statistical analyses were carried out by using the statistical packages for SPSS 12.0 for Windows (Chicago, IL, USA). Survival was analyzed by the method of Kaplan and Meier, using log-rank (Mantel-Cox) analysis. Results: 51 cases with ovarian cancer were evaluated. Cases with thrombocytosis were found to have greater CA-125 levels, more advanced stage disease, more ascites and shorter periods of survival. Conclusion: Thrombocytosis is a poor prognostic factor in ovarian cancer. As reported previously, it is associated with aggressive tumor biology. Thus, preoperative thrombocytosis can be a used as a marker of poor outcomes.

Key words: Thrombocytosis; Ovarian cancer; Prognosis.

Introduction

Thrombocytosis in cancer was first reported by Reiss in 1872. Thrombocytosis has been reported in a variety of solid tumors including lung, renal, gastric, breast, pancreatic, and colon. In gynecological malign tumors such as endometrial, vulvar, and cervical cancers thrombocytosis has also been reported, and it is considered to be a poor prognostic factor in ovarian cancer [1, 2].

In the literature there are several reports on prognosis of ovarian cancer in patients with thrombocytosis [3-5]. In rat models and human beings, in malignant situations thrombocytosis has been related to the activation of megakaryocytes by granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), interleukin 1 (IL 1), IL 3, IL 4, IL 11, leukemia inhibitor factor (LIF), erythropoietin and thrombopoietin [6].

Platelets release thrombospondin (adhesive glycoprotein) and platelet-derived growth factor (PDGF) (potent mitogen for different cell types). PDGF is related with tumor growth and thrombospondin with metastases [7-9].

In this study, we evaluated the association between preoperative thrombocytosis and prognostic factors in malign ovarian tumors.

Material and Methods

This retrospective study was conducted at Dicle University, School of Medicine, Department of Obstetrics and Gynecology between June 2005 and June 2009. A total of 51 cases with a malign ovarian tumor diagnosis were treated. The cases were divided into two groups according to their platelet count. Group 1 included cases with thrombocytosis and Group 2 cases with normal platelets. Thrombocytosis was considered when platelet count was greater than 400 x 10⁹/l [5]. The data of the cases were collected from gynecological oncology, radiation oncology, medical oncology and pathology departments. For each woman, categoric data were collected concerning age, gravidity, parity, preoperative thrombocytosis, tumor markers, stage, grade, ascites, operation types, chemotherapy, radiotherapy, and overall and disease-free survival.

The mean and standard deviation (SD) were calculated for continuous variables. The normality of the variables was analyzed by the Kolmogorov-Smirnov test. The chi-square test and Student’s t-test evaluated associations between the categorical and continuous variables. Two-sided p values were considered statistically significant at p < 0.05. Statistical analyses were carried out by using the statistical packages for SPSS 12.0 for Windows (Chicago, IL, USA). Survival analysis of two distributions (according to platelet count) were analyzed by the method of Kaplan and Meier, using log-rank (Mantel-Cox) analysis.

Results

In this study, we evaluated 51 cases with malign ovarian tumors. The cases were divided into two groups according to platelet count. Group 1: cases with platelets ≥ 400 x 10⁹/l (n = 26), group 2: platelet < 400 x 10⁹/l (n = 25). The mean platelet counts in group 1 and 2 were; 311.56 ± 38.45 10⁹/l (211 to 399), and 488.07 ± 42.26 10⁹/l (416 to 610), respectively.

The mean age of the cases in group 1 and 2 were 50.07 ± 14.42 and 47.44 ± 19.19 (p = 0.581). The cases had cytoreductive surgery and staging including total abdominal hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing fluid samples, infracolic omentectomy, pelvic and paraaortic lymph node sampling, and appendectomy. A total of 27 cases underwent adjuvant
chemotherapy (47.05% had carboplatin/paclitaxel for 6 courses, and 0.6% bleomycin/etoposide/paclitaxel for 3 courses). The majority of the cases were found to have advanced stage disease. Fifteen (29.42%) were diagnosed with Stage I or II, and 36 (70.58%) with Stage III or IV disease. The most common histopathological type was papillary serous in 33 (64.7%) cases followed by borderline in five (9.80%), dysgerminoma in four (7.84%), endometroid in four (7.84%), granulosa cell in three (5.88%), clear cell in one (1.96%), and yolk sac tumor in one (1.96%), respectively.

The association between platelet count and clinical characteristics of the cases are shown in Table 1. Cases in group 1 were detected to have higher levels of CA-125 levels, more advanced stage disease, and greater volumes of ascites. We did not find any association of thrombocytosis with age, gravidity, parity, and other tumor markers.

To examine survival analysis, we focused on advanced stage (Stage III/IV). Disease-free survival and overall survival of the cases with advanced stage are shown in Figure 1. Patients with thrombocytosis had a statistically shorter disease-free interval.

Table 1. — Correlation of platelet counts with clinical and characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 26)</th>
<th>Group 2 (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.07 ± 14.42</td>
<td>47.44 ± 19.19</td>
<td>0.581</td>
</tr>
<tr>
<td>CA 125</td>
<td>70.73 ± 67.12</td>
<td>469.04 ± 84.33</td>
<td>0.014</td>
</tr>
<tr>
<td>CA 15-3</td>
<td>64.734 ± 21.54</td>
<td>36.40 ± 4.23</td>
<td>0.980</td>
</tr>
<tr>
<td>Tumor Stage III or IV</td>
<td>22</td>
<td>11</td>
<td>0.046</td>
</tr>
<tr>
<td>Ascites</td>
<td>12</td>
<td>4</td>
<td>0.021</td>
</tr>
<tr>
<td>Disease-free survival (months)</td>
<td>20.977 ± 1.21</td>
<td>27.465 ± 4.82</td>
<td>0.034</td>
</tr>
<tr>
<td>Overall survival (months)</td>
<td>29.077 ± 3.11</td>
<td>36.587 ± 6.39</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*p < 0.05 is accepted to be statistically significant.

Discussion

In spite of new chemotherapeutic, operative techniques and other auxiliary treatment, the 5-year survival is still low in malignant ovarian tumors. Thus, early diagnosis and therapy are important to prolong the life and improve the life quality of epithelial ovarian cancer cases. Therefore, investigators are always searching for new markers for early detection of malignant ovarian tumors.

Thrombocytosis is a frequent preoperative finding in many solid tumors. Gynecologic malignancies shown to be associated with thrombocytosis are ovarian cancer, vulvar cancer, cervical cancer, and endometrial cancer [1, 2]. Levin and Conley [10], reported in 1964 that approximately 40% of cases with inoperable cancers had thrombocytosis (platelets ≥ 400 x 10^9/L).

Ziemet et al. [11] studied the prognostic factors of thrombocytosis in 130 women with ovarian cancer and reported that the cases that had thrombocytosis (n = 48) had advanced stage disease, higher serum levels of CA-125, and greater volumes of ascites, but they did not detect any differences in survival.

Li et al. [12] carried out a study to determine if thrombocytosis is a negative prognostic factor in ovarian cancer. Of 183 women evaluated, 41 (22.4%) had thrombocytosis. They reported the cases with thrombocytosis had greater elevations of CA-125, more advanced stage disease, higher grade tumors, more frequent lymph node metastases, greater volume of ascites, and shorter overall and disease-free survival.

Menczer et al. [13] evaluated a series of 70 cases with invasive epithelial ovarian carcinoma in a retrospective study. They also reported thrombocytosis as a poor prognostic factor, such as advanced stage disease and shorter survival periods. Similarly, in a retrospective study in Turkey a total of 292 cases with epithelial ovarian cancer were evaluated, and 42.5% of the cases with thrombocytosis were found to have statistically higher levels of preoperative CA-125 levels, more advanced stage disease, higher grade tumors, and shorter periods of survival [14].

In the present study, we evaluated 51 cases with malignant ovarian tumors, 26 of which (50.98%) had thrombocytosis. We did not observe any differences in age of the cases. The cases with thrombocytosis had higher levels of CA-125, more advanced stage, and more ascites. Cases with thrombocytosis had shorter survival periods than patients with platelets < 400 x 10^9/L.

In conclusion, preoperative thrombocytosis is a poor prognostic factor in ovarian cancer. It is associated with aggressive tumor biology. Thus, preoperative thrombocytosis can be used as a marker of poor outcome.

References


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Initial analysis of relationship between plasma platinum concentration and hematological adverse reaction associated with weekly chemotherapy using nedaplatin in combination with radiotherapy for cervical carcinoma

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Summary

Purpose: Established therapeutic guidelines for cervical carcinoma recommend concurrent chemo- and radiotherapy as standard treatment for locally advanced cervical carcinoma. Nedaplatin (CDGP) is a platinum agent developed in Japan that is less nephrotoxic than cisplatin (CDDP), but with equivalent antitumor potency. In the standard dosage regimen for cervical carcinoma, CDGP is administered once every four weeks (monthly regimen). We investigated the efficacy and safety of a new dosage regimen, in which CDGP was administered once weekly for five weeks (weekly regimen). Methods: We measured plasma platinum concentration of patients after administration of CDGP, and analyzed the relationship between plasma platinum concentration and hematological adverse reactions such as thrombocytopenia and leucopenia. Results: The relative rates of change in platelet and white blood cell counts tended to increase as the plasma concentration of platinum increased. Furthermore, the rate of change in platelet counts in relation to the area under the curve was greater for the monthly regimen as compared to weekly. On the other hand, the relative rates of change in WBC were nearly the same between the regimens. Conclusions: These findings indicate that when using chemotherapy with CDGP for a patient with a cervical carcinoma, a weekly regimen might reduce the severity of thrombocytopenia, while still exhibiting the same therapeutic efficacy as the monthly regimen.

Key words: Cervical carcinoma; Nedaplatin; Chemotherapy; Adverse reaction; Thrombocytopenia; Leucopenia.

Introduction

Nedaplatin (CDGP) is a platinum agent that was developed in Japan to reduce the severity of nephrotoxicity-related adverse reactions associated with cisplatin (CDDP) use. According to the package insert, CDGP should be administered once every four weeks (monthly regimen) and is indicated for various carcinomas, including cervical carcinoma. Severe bone marrow suppression is one of its important adverse effects, thus thorough analyses of efficacy and safety are needed for rational clinical usage. When treating cervical carcinoma with CDGP in combination with radiotherapy, a new dosage regimen of once weekly for five weeks (weekly regimen) was investigated [1, 2]. Past reports found no significant differences in efficacy between monthly and weekly regimens [1-3], though comparisons of safety between these regimens have not been reported. As for bone marrow suppression associated with CDGP, it was shown that the plasma concentration of platinum is correlated with the severity of hematological adverse reactions, such as thrombocytopenia and leucopenia, in patients receiving the monthly regimen [4]. In the present study, to assess the safety of a new dosage regimen of CDGP for cervical carcinoma, we investigated the pharmacokinetics of platinum after administration of CDGP, and the relationships between plasma platinum concentration and severity of thrombocytopenia or leukopenia. The present investigation accompanied the Kitasato Gynecologic Radiation Oncology Group 0501.

Study (KGROG0501) conducted at Kitasato University Hospital [5, 6] and was approved by the ethics review board of that institution. The expected 3-year overall survival rate is 40% and the threshold value is defined as 20%. The sample size was calculated as 45 (α 0.05 and β 0.1, non-compliance 10%). All patients will be followed up for three years. This study is an initial analysis.

Materials and Methods

Plasma platinum concentration after administration of CDGP

1) Measurement of plasma platinum concentration

Based on previously reported methods [7], the concentration of platinum in plasma was measured using graphite furnace atomic absorption spectrophotometry with the quantification procedure shown in Figure 1. Total platinum concentration was measured using a standard addition technique, in which the amount of extraction solvent (methyl isobutyl ketone) was adjusted to bring the final concentration of platinum to near 0.1 µg/ml.
Among patients enrolled in KGROG0501, the present subjects comprised those who consented to blood sampling. The subjects received chemotherapy with CDGP (single dose, 30 mg/m²) with radiotherapy once weekly for 5 weeks. During one of those five courses, blood samples were collected at 1, 2, 3, 4, and five hours after the start of injection of CDGP, while they were collected at few points after the start of injection during the other four courses. The AUC\(_{0-\infty}\) was calculated using the moment method based on five sampling points, with the moment analysis (MOMENT) macro in Microsoft Excel® (Microsoft Visual basic for application) used for analysis [8]. Since CDGP is a drug that is excreted renally, the influence of multiple injections on renal function was assessed by one-way analysis of variance based on the pre-administration values of serum creatinine (Scr) and blood urea nitrogen (BUN). The StatView® 5.0 (SAS Institute, Inc. Japan) software package was used for this analysis.

Relationship between renal function and plasma platinum concentration

None of the five patients showed significant increases in the values for Scr or BUN among the courses, and repeated administrations did not cause marked influences on renal function (Figure 3). In the figure, the dotted lines indicate the upper and lower limits of normal. All values for Scr and BUN in the patients were within a normal range.

Results

Plasma platinum concentration after administration of CDGP

1) Measurement of plasma platinum concentration

The accuracy and reproducibility of the present method were investigated. In the present method, the relative error regarding the predicted value was about 5% with a RSD of 4.12%.

2) Measurement of plasma platinum concentration following CDGP and calculation of AUC\(_{0-\infty}\)

Patient characteristics

Five female patients served as subjects. Table 1 summarizes the number of plasma samples and number of courses with blood sampling, as well as values for dosage, age, body weight, Scr, BUN, AST, and ALT.

<table>
<thead>
<tr>
<th>Total number of patients 5</th>
<th>Gender (male/female) 0/5</th>
<th>Number of plasma samples 40</th>
<th>Number of plasma course 13</th>
<th>Dose (mg/m²) 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old) 52.6 ± 15.3 [30-69]</td>
<td>Body Weight (kg) 52.4 ± 9.8 [49.7-69.5]</td>
<td>Scr (mg/dl) 0.67 ± 0.15 [0.5-0.87]</td>
<td>BUN (mg/dl) 12.2 ± 2.4 [9-15]</td>
<td>AST (IU/l) 15.6 ± 6.0 [10-25]</td>
</tr>
</tbody>
</table>

Time-course of plasma platinum concentration in each patient

Figure 2-1 shows the time-courses of plasma platinum concentration changes in the patients from whom blood samples were collected during multiple courses, while Figure 2-2 shows those in patients from whom blood samples were collected during only one course. In case 1, blood samples were collected during all five courses, while they were collected during three courses in cases 2 and 3. None of the patients displayed marked changes in plasma platinum concentration during any of the courses after the start of CDGP administration.

Relationship between renal function and plasma platinum concentration

None of the five patients showed significant increases in the values for Scr or BUN among the courses, and repeated administrations did not cause marked influences on renal function (Figure 3). In the figure, the dotted lines indicate the upper and lower limits of normal. All values for Scr and BUN in the patients were within a normal range.
Initial analysis of relationship between plasma platinum concentration and hematological adverse reaction associated with etc.

Figures 2-1. — Time courses of changes in plasma platinum concentrations in patients from whom blood samples were collected during multiple courses.

Figures 2-2. — Time courses of changes in plasma platinum concentrations in patients from whom blood samples were collected from only 1 course.
Relationships between plasma platinum concentration and severity of thrombocytopenia and leukopenia.

Tables 2 and 3 show relative rates of change in total AUC_{0-\infty} and PLT and WBC counts in the five patients. Total AUC_{0-\infty} in all patients ranged from 24.35 to 50.40 g h/ml. The relative rates of change ranged from -42% to -81% for platelet counts and from -36% to -93% for WBC counts.

Table 2.

<table>
<thead>
<tr>
<th>Case</th>
<th>Total AUC (μg h/ml)</th>
<th>PLT_{pre} (10^4/μl)</th>
<th>PLT_{nadir} (10^4/μl)</th>
<th>Relative change in PLT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50.4</td>
<td>22.9</td>
<td>4.4</td>
<td>-81</td>
</tr>
<tr>
<td>2</td>
<td>36.4</td>
<td>21.8</td>
<td>6.9</td>
<td>-68</td>
</tr>
<tr>
<td>3</td>
<td>24.35</td>
<td>27.1</td>
<td>7.7</td>
<td>-72</td>
</tr>
<tr>
<td>4</td>
<td>26.05</td>
<td>35.2</td>
<td>7.3</td>
<td>-79</td>
</tr>
<tr>
<td>5</td>
<td>25.25</td>
<td>20.4</td>
<td>11.8</td>
<td>-42</td>
</tr>
</tbody>
</table>

Next, the weekly and monthly regimens were compared. Figure 4 shows the relationships between the AUC_{0-\infty} and severity of thrombocytopenia and leukopenia with the regimens. Our results showed that the relative rates of change in PLT and WBC counts tended to increase as AUC increased. Furthermore, the rate of change in PLT count in relation to AUC was greater for the monthly regimen as compared to the weekly regimen. On the other hand, the relative rates of change in WBC were nearly the same between the regimens.

Discussion

Since the subjects satisfied the inclusion criteria for KGROG0501, the present study displayed minimal bias in regard to patient characteristics. However, all subjects were women with cervical carcinoma.

In the results of the population analysis for the monthly regimen, it was shown that creatinine clearance was a significant variable for clearance, while body weight was a significant variable for the distribution volume of CDGP [9]. In the present study, the dosage of CDGP was based on body surface area. Since individual differences in distribution volume were taken into account and individual differences in clearance were not, individual differences in the pharmacokinetics of CDGP were observed. When a dosage of 30 mg/m² of CDGP per course was administered in the present study, slight individual differences were seen in the AUC_{0-\infty} values (range, 4.87-10.08 μg h/ml), which has been reported previously. Idei et al. [1] noted that the average value of AUC after administration of 30 mg/m² of CDGP was about 5 μg h/ml, while Oguma et al. [10] reported that the value of AUC_{0-\infty} after administration of 20-40 mg/m² of CDGP was 9.49-16.22 μg · h/ml. In the present study, CDGP was administered once weekly at 30 mg/m² for five weeks. Since the half-life of CDGP was reported to range from 2-13 hours [10], it was considered to be completely eliminated after one week. No marked differences were found in regard to the time-course of plasma platinum concentration among the five courses in each patient, suggesting that those concentrations were nearly the same among all the courses. This was also supported by the finding of no changes in Scr and BUN values among all five courses. Based on these findings, it was considered reasonable to calculate the total AUC in the weekly regimen by multiplying the AUC for a single administration by five. Two points should be considered when comparing the degrees of thrombocytopenia and leukopenia in relation to the AUC between the weekly and monthly regimens. First, CDGP therapy was combined with radiotherapy in the weekly regimen, but was not combined
with radiotherapy in the monthly regimen. Bone marrow suppression is one of the adverse reactions caused by radiotherapy [11].

However, since combining CDGP with radiotherapy does not exacerbate adverse reactions [3], the effects of radiotherapy were not taken into account in the present study. Second, the AUC was calculated based on total plasma platinum concentration in the weekly regimen, whereas it was calculated based on the plasma unbound platinum concentration in the monthly regimen. In general, plasma unbound platinum is responsible for antitumor action and adverse reactions, however, since CDGP exists mostly as unbound platinum in blood [10], we considered that calculation of the AUC based on total plasma platinum concentration did not impact the results of this study.

When compared to the monthly regimen, the slope of the regression line for the relative rates of change in thrombocytopenia in relation to the AUC was less than that for the weekly regimen, suggesting that a divided administration of CDGP may lessen its effects on platelets. Regarding leukopenia, no marked differences were observed, suggesting that the influences of the monthly and weekly regimens on WBC are comparable.

Together, our findings indicate that when using CDGP for cervical carcinoma, a weekly regimen might reduce the severity of thrombocytopenia, while exhibiting the same therapeutic efficacy as a monthly regimen [1-3]. Due to the relatively few cases considered in the population pharamokinetis study, we recommend analysis based on a larger statistical sample.

References


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Expression of cytokines in cervical stroma in patients with high-grade cervical intraepithelial neoplasia after treatment with intrasional interferon α-2b

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Summary

Conservative treatment with interferons (IFNs) has the advantage of preserving reproductive capacity in patients with grade 2 or 3 cervical intraepithelial neoplasia (CIN). The objective of this work was to study patients with high-grade CIN treated with intrasional IFN α-2b and to analyze the expression of Th1, Th2 and Th3 cytokines in cervical stroma. We observed that patients with a satisfactory response (60%) to treatment with IFN α-2b expressed more Th1 (IFN-γ, TNF-α, IL-2) cytokines, with a significant reduction in the viral load of high-risk human papillomavirus (p = 0.0313). All patients with therapeutic failure were smokers and had higher expression of cytokines Th2 (IL-4) or Th3 (TGF-β2 and TGF-β3).

Key words: Cervical stroma; Cytokines; HPV; Interferon α-2b; Cervical intraepithelial neoplasia; CIN 2-3.

Introduction

Infection with the human papillomavirus (HPV) is considered to be one of the most common infections within the sexually active population, and more than 100 different genotypes of the virus have been identified to date, of which 40 infect the human genital tract; and of these, 15 are associated with cervical carcinogenesis [1, 2]. This infection resolves spontaneously in 70% to 90% of cases, being eliminated between 12 and 24 months after the initial diagnosis. Cervical cancer develops in patients who have had oncogenic HPV for years or decades without an immune response effective enough to eliminate the infection [3, 4].

HPV effectively evades the inactive immunology response and delays the activation of adaptive immunological response. The infection cycle of HPV itself is a mechanism of immunological avoidance that inhibits the host’s detection of the virus. HPV infection is not accompanied by inflammation, and replication of the virus does not cause cell death since the differentiated keratinocytes are already programmed to die, and this “death” by natural causes does not represent a danger to the immunological system. These characteristics allow HPV to develop a chronic and persistent infection [5].

In the host, depending on the viral type, HPV can remain in an episomal form or be incorporated into DNA. In its episomal form, HPV activity follows cell differentiation in the epithelial beds, resulting in surface cells with copies of HPV that are ready to be transmitted. When the viral genome is incorporated into DNA, encoded proteins are produced that modify cell activity by means of a sequence of reactions that result in cell proliferation and the inhibition of apoptosis [6, 7].

The E6 and E7 viral oncoproteins are integrated with tumor suppressor proteins (p53 and pRB), and they alter the cycle and functioning [8], although evidence suggests that, in HPV infection, additional factors are involved in the progression to precursor lesions, such as early onset of sexual activity, the use of oral contraceptives, parity, smoking, multiple sexual partners, infection with Chlamydia trachomatis, and immune system deficiency. Thus, even though in the majority of cases of CIN 2 or cervical cancer, high-risk oncogenic HPV is present, in order for this virus to cause some kind of lesion, various co-factors seem to need to be involved [9-11]. Most studies suggest that infection with genital HPV is very common among young, sexually active women, with a prevalence of up to 80% among certain adolescent populations [12].

Records show that IFN-α is the cytokine with the longest use in clinical oncology [13], and various studies carried out on administering interferon in the treatment of cervical intraepithelial neoplasias (CINs) that have shown promising results. IFN antitumor effects result in a direct action on antigenic proliferation or composition, inhibiting the induction of specific cell growth factors; IFNs can also have indirect effects such as immunomodulation and the inhibition of tumor angiogenesis [14]. Studies [15, 16] using IFN-α in the treatment of CINs have achieved good results. The treatment of a patient with invasive epidermal vaginal carcinoma using intrasional IFN α-2b yielded a complete regression of the vaginal lesion [17]. In the treatment of cervical cancer, interferon-α retinoic...
Materials and Methods

A prospective transverse study was carried out in the Federal University of the Triângulo Mineiro, by the Discipline of Gynecology and Obstetrics, Immunology, and Surgical Pathology, and by the Oncology Research Institute (Instituto de Pesquisa em Oncologia, IPON). The study was analyzed and approved by the Research Ethics Committee of the Federal University of the Triângulo Mineiro (UFTM).

The study group consisted of ten patients, between 18 and 50 years of age, with a diagnosis of grade 2 or grade 3 CIN who had not received any prior treatment. We obtained information about age, habits and lifestyle (smoking, drug use, number of sexual partners, and contraceptive methods used), and advised them to use condoms throughout the treatment period. Patients were identified by number, with the first patient to participate in the study identified as “1”, the second “2”, the third “3”, and so on.

Criteria for inclusion were lesion bigger than 1 cm² and satisfactory colposcopy. Criteria for exclusion were: previous history of treatment for CIN; bleeding during the exam; patients carrying immunodpressing diseases, severe cardiopathies or with alteration of the hepatic or renal function; pregnant patients; patients with history for intolerance to interferon; patients whose use of anti-inflammatories or immunodepressors could not be paused during the treatment and sexual activity two days before the day of the colposcopy. Criteria for exclusion were: previous histology of grade 2 or grade 3 CIN who had not received any prior treatment. We obtained information about age, habits, and lifestyle (smoking, drug use, number of sexual partners, and contraceptive methods used), and advised them to use condoms throughout the treatment period. Patients were identified by number, with the first patient to participate in the study identified as “1”, the second “2”, the third “3”, and so on.

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IFN application

Patients with high-grade CIN gave their free and clarified consent and underwent a cervical biopsy before treatment with intralesional IFN α-2b. The criterion for response to treatment was the regression of the lesion based on colposcopic exam and, if necessary, biopsy (histological analysis). After the first and last application, the patients underwent a colposcopic exam, and photographic images were captured using a video colposcope (Video Diagnose® Program Software). Follow-up of patients consisted in one year after treatment, but we consider the response to treatment until one month after the last IFN application.

Human recombinant IFN α-2b (Blauferon B. Blausiegel) was used in this study. The treatments were administered on alternate days (Mondays, Wednesdays, and Fridays) over a period of six consecutive weeks, making a total of 18 applications, with each application involving the administration of a 3,000,000 U dose.

A vaginal speculum was used to expose the cervix, with antiseptic of the cervix and vaginal walls being conducted with gauze soaked in topical polyvidine, using Cherron tweezers. In cases that involved multiple lesions or lesions that occupied more than one quadrant of the canal, applications were made on each lesion (in the case of isolated lesions) or in each quadrant (in the case of continuous lesions).

RNA Reserve Transcription for complementary DNA (RT-PCR)

The collected fragments were immersed in 1.0 ml of TRIZOL (Invitrogen™ life technologies, Carlsbad, CA, USA), according to the manufacturer’s protocol and stored in a freezer at -20°C for later extraction of the RNA. The TRIZOL-immersed samples were subjected to sonication for approximately 30 min at 50 W at room temperature to homogenize the tissue in preparation for RNA extraction. RNA extraction was carried out using the Invitrogen™ life technologies protocol. RNA extracted from the samples was subjected to complementary DNA (cDNA) RT-PCR synthesis, which consists of transforming an RNA strand into cDNA, using Invitrogen™ reagents, according to the manufacturer’s protocol.

Dried RNA was suspended in 11.0 μl of MilliQ water treated with diethylpyrocarbonate (DEPC) and mixed with a mixture of reagents including 1.0 μl oligo dT primers and 1.0 μl dNTPs. Reverse transcription took place in an Eppendorff thermocycler; the program consisted of 1 5-min denaturation cycle at 65°C, followed by incubation at 4°C. Following this procedure, 4.0 ml of 5x buffer, 1.0 μl of 0.1 M DTT, 1.0 μl of Superscript IIIrt Invitrogen™enzyme, and 1.0 μl of DEPC-treated Milli Q water were added to each sample. The samples were incubated for 5 min at 25°C, followed by a cycle of incubation at 50°C for 60 min. The reaction was inactivated at 70°C for 15 min. The cDNA obtained was subjected to RT-PCR containing the following components for amplification: 2.5 μl 10x buffer; 0.15 μl 10 mM dNTP; 0.75 μl 50 mM MgCl₂; 0.2 μl Taq DNA polymerase Invitrogen™; 1.0 μl each of 10 μm primer, 100 or 200 ng cDNA, and Milli Q water treated with qsp 25.0 μl DEPC.

RNA expression analysis of the cytokines for PCR

Expression mRNA of cytokines (IFN-γ, TNF-α, IL-2, IL-12, IL-4, IL-10, TGF-β1, TGF-β2 and TGF-β3) was analyzed using PCR methodology, which involves the enzymatic amplification of a specific sequence of DNA or cDNA. For reaction control beta-actin gene was analyzed. Tubes containing the mix solution were homogenized and placed in an Eppendorf-thermocycler with 64 ports. The equipment was programmed in the following manner: 40 cycles at 94°C for 5 min and 40 sec (denaturation); 45 sec at a specified temperature for each primer (annealing); 72°C for 10 min and 45 sec (polymerization). At the end of the amplification cycles, the reaction was interrupted by cooling to 16°C and the amplified products were removed from the thermocycler. The primers were preserved according to an established protocol [19] and as described in Table 1. The temperatures for annealing, as well as the mixes for each primer and the steps and number of cycles for the PCRs, were optimized according to previous standards developed by the IPON at UFTM. Products amplified underwent a gel electrophoresis of polyacrylamide at 10% and were stained in a silver nitrate solution.

HPV DNA research by hybrid capture

HPV viral load was evaluated using the Hybrid Capture II (Digene) technique, which is able to detect the presence of 18 viral types, including high and low risk types. In this technique, DNA-containing specimens are hybridized with a specific RNA-HPV probe cocktail and are captured on the surface of a micropore sensitized with specific antibodies for RNA/DNA hybrids. Immobilized hybrids react with alkaline phosphatase together with specific antibodies for RNA/DNA hybrids and are detected by a chemiluminescent substrate.
patients, 50% responded to treatment with IFN-α. Of the CIN 2 patients, 66.6% responded to treatment with (1,4,5,6,8 and 9) and 40% were CIN 3 (2, 3, 7 and 10). Of the ten patients in this study, 60% of the patients were CIN 2 (1,2,3,4,6 and 6) were smokers, and 50% (n = 5) had already had three or more sexual partners, while the median age of sexarche was 16.6 years old (with a minimum age of sexarche was 16.6 years old (with a minimum years old). At the initial diagnosis, 60% of the patients were CIN 2 (1,2,3,4,6 and 9) and 40% were CIN 3 (2, 3, 7 and 10). Of the CIN 2 patients, 66.6% responded to treatment with IFN-α-2b, and 33.4% failed therapy. Of the CIN 3 patients, 50% responded to treatment with IFN-α-2b, and 50% failed therapy.

Sixty percent of the patients in our study (1,2,3,4,6 and 9) exhibited a reduction in a high-grade lesion after treatment with IFN-α-2b, while treatment failure was observed in 40% of the patients (5,7,8 and 10). Patients who responded to treatment were passed on for trimestral follow-ups by colposcopy in the outpatient unit, while of those whose therapy failed (n = 4), three underwent conization and one underwent diathermic excision.

We observed an absence of the expression of Th1 cytokines (IFN-γ, TNF-α, IL-2 and IL-12) before treatment with IFN-α-2b in patients who responded as well as those whose treatment failed. However, after treatment with IFN-α-2b, we observed expression of IFN-γ (6f) and IL-2/TNF-α (1f) (Figure 1) selectively in patients with a satisfactory response, suggesting that Th1 may potentially be related to cure.

The expression of IL-10 (Th2) was absent in all patients prior to treatment with IFN-α-2b. IL-4 (Th2) was expressed before treatment with IFN-α-2b in two patients who responded (4i and 6i, Figure 1), as well as in two patients whose treatment failed. In addition, concomitant expression of IL-4 and TGF-β3 was observed in patients with failed therapy (8i to 10i, Figure 1) after treatment with IFN-α-2b.

It was not possible to correlate the results of mRNA expression of the cytokines TGF-β1, TGF-β2 and TGF-β3 with a clinical response to treatment with IFN-α-2b, since one patient with a satisfactory response (4f, Figure 1) had concomitant expression of TGF-β1 and TGF-β2 after treatment with IFN-α-2b, while two other patients who did not respond to treatment (5f and 7f, Figure 1) expressed TGF-β2 and TGF-β3 after treatment with IFN-α-2b. We also observed that those patients whose therapy had concomitant expression of IL-12/TGF-β2 and IL-4/TGF-β3, suggesting that a Th1 and Th2 immune response during treatment with IFN may be the reason for therapeutic failure. Expression of the β-actin reaction control gene was positive in the 20 samples analyzed Figure 1.

Figure 2 compares the tendencies of the immune response profiles (Th1, Th2 and Th3) between the groups with therapeutic response and failure. We were able to observe greater expression of Th2 and Th3 cytokines in the group of patients with therapeutic failure.

In patients with a satisfactory response, there was a significant drop in HPV viral load after treatment with IFN-α-2b, while in the group of patients with failed therapy, there was an increase in viral load. The average and range of expressed values, before and after treatment in the groups with good and poor response to treatment were, respectively, 19,501 (62-279,971) versus 490.5 (54-6,302), p = 0.0313, and 3,370 (110-16,682) versus 7,754 (264-251,482), p = 0.37 (Wilcoxon test).

**Discussion**

Many studies have previously described the benefits of IFN immunotherapy, which has among its advantages preservation of the cervix and future reproductive capacity of patients with high-grade CIN [20-22]. In our study, 60% of the patients responded to treatment with IFN-α-2b with a reduction in the size of the high-grade lesion. One limiting factor was the overall number of patients, though this did not hinder the interpretation of the results.

Various studies have shown that many factors can increase the risk of HPV infection and development of CIN [23, 24]. Among those cited are parity and smoking [25, 26], precocious sexual relations and the number of sexual partners [27]. Our study observed all these factors in the patients: 70% (n = 7) were multiparous, 60% (n = 6) were smokers, and 50% (n = 5) had already had three or more sexual partners, while the median age of sexarche was 16.6 years old.
Expression of cytokines in cervical stroma in patients with high-grade cervical intraepithelial neoplasia after treatment with etc.

(A) **IFN-γ**

(B) **TNF-α**

(C) **IL-2**

(D) **IL-12**

(E) **IL-4**

(F) **IL-10**

(G) **TGF-β1**

(H) **TGF-β2**
Smoking causes changes in the immune system, including an increase in T suppressor lymphocytes, a decrease in natural killer cells, and low blood levels of immunoglobulins. Smoking also suppresses the chemotaxic and phagocytic functions of polymorphonuclear leukocytes in tissues, increasing susceptibility to pathogens [28, 29]. An important observation in our study was that all patients who did not respond to treatment with IFN-α2b were smokers (n = 4). Smoking is considered one of the principal factors associated with the persistence of viral activity, increasing the risk of progression or recidivism of lesions in patients with HPV infection-associated CIN [30]. Using RT-PCR, various studies have analyzed the expression of the Th1, Th2 and Th3 cytokines in the cervical stroma of patients with CIN [31-33]. Regression of high-risk HPV has been observed in patients with low or moderate dysplasia who expressed IFN-γ [34]. HPV infection raises T cell immunity against the E6 protein expressed during infection. IFN-γ-producing T cells circulating in the peripheral blood play an important role in fighting against the persistence of HPV infection associated with the development of malignancies. Studies have observed that defects in the production of IFN-γ are associated with persistent HPV infection and the development of neoplasias [35].

TNF-α plays an important role in inflammatory reactions and can be involved in the regulation of the growth and differentiation of keratinocytes infected by HPV, inhibiting the expression of the E6 and E7 genes [36]. TNF-α expression may favor the recruitment of natural killer cells, promoting mechanisms for the elimination of tumor cells [37]. However, one study found that TNF-α promoted the progression of the cell cycle by increasing mRNA expression of E6/E7 HPV-16 and the consequent immortalization of keratinocytes infected by HPV [38].

The absence of IL-2 mRNA in lesions caused by HPV might explain the weak response of TCD8+ memory lymphocytes in patients with CIN and cervical carcinoma. In addition, the fact that circulating lymphocytes produce IL-2 that specifically acts against HPV antigens suggests that the absence of IL-2 expression in the cervical canal is a local phenomenon [39].

The association between the in situ expression of IL-12 mRNA and the regression of grade 3 CIN lesions suggests that the Th1 immune response mediated by IL-12 is related to curing HPV infection [40]. Production by dendritic cells depends on other factors such as GM-CSF, TNF-α and IL-1β, which in turn have been found to be diminished in cervical carcinoma. The diminished activity of dendritic cells due to low production of TNF and IL-1 may be responsible for the reduced expression of IL-12 mRNA in patients with cervical carcinoma associated with HPV infection [41].
Expression of cytokines in cervical stroma in patients with high-grade cervical intraepithelial neoplasia after treatment with etc.

Type Th1 immune response predominates in premalignant lesions associated with or unassociated with HPV infection, and studies have observed a decrease in these cytokines in relation to an increase in the grade of the CIN [42]. The expression of Th1 cytokines in the cervical stroma activates cytolytic T lymphocytes, contributing to the regression of lesions and to the elimination of HPV [43]. In our study, there was expression of Th1 cytokines after treatment with IFN-α-2b in patients who responded to treatment. Before treatment with IFN-α-2b, these patients presented with high grade (2 and 3) CIN and after treatment CIN 1 or HPV infection, suggesting that these cytokines may have blocked the growth of keratinocytes infected by HPV, inhibiting the expression of viral oncoproteins, which promoted the disappearance of high-grade lesions.

Cytokines produced by Th2 cells can inhibit the activation of the macrophages of T cytotoxic lymphocytes and suppress immunity mediated by Th1 cells [44]. IL-10 inhibits the functions of activated macrophages, and IL-4 can antagonize the activating effects of IFN-γ on macrophages, inhibiting cell mediated immune reactions. It is known that increased production of cytokines Th2 can be a mechanism used by tumor cells to escape immune recognition, with this increase being associated with the persistence and progression of premalignant lesions [45]. However, one study has reported that IL-4 can play an important role in viral elimination and in the control of HPV infection, as well as in the control of the development of carcinomas associated with HPV [46].

A reduction in IL-10 expression and an increase in FoxP3 expression have been observed in patients with CIN 2 and CIN 3 [47]. A polymorphism of IL-10 in the 1082-position is associated with the elimination of HPV infection [48]. An analysis of IL-10 expression in a group of patients with high-grade CIN did not show a significant difference when compared to a control group of patients [49].

In our study, IL-4 expression before treatment with IFN-α-2b in patients with therapeutic failure suggests that the Th2 immune response pattern may have contributed to the persistence of the high-grade lesion and to HPV evasion of immunological vigilance. In addition, the concomitant expression of IL-4 and TGF-β3 in patients with therapeutic failure after treatment with IFN-α-2b suggests that the Th3 immune response may have modulated the Th2 immune response, impeding a clinically satisfactory response.

Regulator cells produce cytokines, such as TGF-β and IL-10, which block the activation of lymphocytes and macrophages and can also suppress other lymphocytes or antigen presenting cells, through unknown mechanisms that do not involve cytokines [50]. TGF-β inhibits epithelial cell proliferation and transcription of the E6/E7 genes of HPV. Moreover, mRNA levels of TGF-β1, TGF-β2 and TGF-β3 were reduced in samples of CIN positive for HPV 16, in comparison to normal cervical samples [51]. One study has shown that the expression of TGF-β1 and TGF-β2 in CIN biopsies is not clearly associated with lesion grade or clinical course of HPV infection [52]. However, other studies have demonstrated that mRNA expression of TGF-β1 and of TGFβR1, its receptor in the uterine tract, shows a malignant transformation in patients with CIN [53]. Regulatory T cells are powerful inhibitors of antitumor immune responses and are associated with the persistence of high-grade CIN [54].

In our study, it was not possible to correlate the mRNA expression of TGF-β1, TGF-β2 and TGF-β3 with a clinical response to IFN-α-2b treatment, since one patient with a satisfactory response had concomitant expression of TGF-β1 and TGF-β2 after treatment with IFN-α-2b, while two other patients who did not respond to treatment expressed TGF-β2 and TGF-β3 after treatment with IFN-α-2b. The post-treatment expression of Th3 cytokines in one patient who did respond to treatment suggests an inhibition of proliferation of transformed epithelial cells, which resulted in a satisfactory clinical response.

Patients with failed therapy were smokers and had concomitant expression of IL-12/TGF-β2 and IL-4/TGF-β3, suggesting that the Th3 immune response may have modulated Th1 and Th2 immune responses during IFN treatment and thereby caused the therapy to fail. In addition, toxic substances present in tobacco cause changes to the immune system that harm the organism’s defense, impeding the production of proinflammatory cytokines that might act in the regression of lesions caused by HPV. The expression of Th1 cytokines in patients with a satisfactory response after treatment with IFN-α-2b suggests that this pattern of immune response is related to the regression of high-grade lesions.

Studies have shown that HPV viral load increases with the progression in grade of CIN [55, 56]. Our study demonstrated a significant fall in HPV viral load in patients who responded to IFN treatment. These data suggest that treatment with intralesional IFN-α-2b can reduce HPV viral load in patients with cervical intraepithelial neoplasia, facilitating the regression of high-grade lesions.

More patients are needed to better understand the pattern of immune response that accompanies treatment with IFN-α-2b so that new therapeutic strategies to help improve the treatment of premalignant cervical lesions can be developed.

Conclusions

Treatment with intralesional IFN-α-2b in patients with high-level CIN had satisfactory clinical response in 60% of the patients. Th1 immune response (IFN-γ, TNF-α, IL-2) seems to be related to the decrease of the level of CIN after treatment with IFN-α-2b in responsive patients. TGF-β expression after treatment with IFN-α-2b in patients with therapeutic failure suggests an immunomodulatory role of this cytokine inhibiting the Th1 protector response. There was a significant decrease in the HPV viral load of high-risk patients who responded to treatment with IFN-α-2b.
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References


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Immunohistochemical evaluation and lymph node metastasis in surgically staged endometrial carcinoma

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Summary

Objective: To assess the expression of immunohistochemical markers in surgically staged endometrial cancer patients. Methods: We studied 107 cases of primary untreated endometrial carcinoma in which the p53, bcl-2, her-2/neu, Ki-67, estrogen receptor (ER) and progesterone receptor (PR) antigens were investigated by an immunohistochemical method. In the last 50 consecutive patients immunoreactivity for MMP-7 and MMP-26 was assessed as well. We evaluated the correlations among the immunohistochemical staining assessed by histoscore, and the age, grading, depth of invasion, stage of the neoplasm and extraterine disease. Results: Mean age was 65 years (range 34-88). All patients were submitted to total abdominal or modified radical vaginal hysterectomy plus bilateral salpingo-oophorectomy and systematic pelvic lymphadenectomy; p53, bcl-2, her-2/neu, Ki-67, MMP-7, MMP-26, estrogen and progesterone receptors were positive in 36 (43%), 71 (86%), 13 (16%), 80 (96%), 65 (78%), 80 (96%), 61 (73%) and 71 (86%) patients, respectively. p53 overexpression was found to be related to poor grade of differentiation and deep myometrial invasion. Immunostaining for ER was inversely related to the histopathological differentiation of the tumors. Decreased expression of PR was related to advanced stage, poor histopathologic differentiation and extraterine spread of disease. Conclusion: The overexpression of p53 seems to indicate more malignant phenotype, while PR expression correlates with parameters of better clinical outcome.

Key words: Endometrial cancer; Immunohistochemistry; Prognostic factor.

Introduction

Adenocarcinoma of the endometrium is the most common malignancy of the female genital tract in developed countries and represents the seventh leading cause of death for cancer in women [1]. The prognostic impact of traditional clinicopathological variables in endometrial cancer patients, such as International Federation of Gynecology and Obstetrics (FIGO) stage, histologic type, depth of invasion, histologic grade and lymph node metastasis, is well established [2-5]. Correlations between steroid hormone receptor status (estrogen receptor – ER, progesterone receptor – PR) of endometrial carcinoma and known prognostic parameters such as tumor stage, tumor grade, and depth of myometrial infiltration have been documented [6-9]. Endometrial cancers expressing ER and PR seem to characterize clinically less aggressive tumors with a better chance of response to endocrine treatment [10, 11]. Recently, some other factors have been proven to offer additional data about the biologic behavior of the endometrial carcinoma. The mutation of the p53 tumor suppressor gene has been documented in endometrial cancer, although the prognostic significance of its overexpression is still conflicting [12-14]. Likewise, the oncogene her-2/neu, the apoptosis indicator bcl-2, and the cell proliferation indicator Ki-67 have also been shown to be prognostic factors of endometrial cancer [15-20]. However, the majority of patients in these studies did not undergo surgical staging with lymphadenectomy. Increased expression of certain matrix metalloproteinases (MMPs) in advanced tumors and the ability of these enzymes to degrade extracellular matrix barriers suggest a role for them not only in the initial steps of tumor development, but also in tumor metastasis [21]. MMP-7 and MMP-26 are two members of the matrilysin subfamily, containing the minimal domain organization. and belonging to the few MMPs with epithelial expression. Both have been reported to be expressed in endometrial tumors [22-24]. However, the prognostic value of MMP-7 and MMP-26 expression in endometrial carcinoma is controversial [25, 26].

The aim of this study was to evaluate the prognostic significance of p53, bcl-2, her-2/neu, Ki-67, MMP-7, MMP-26, ER and PR antigens in patients with endometrial carcinoma who underwent surgical staging including lymphadenectomy.

Materials and Methods

Tissue samples were collected from women ranging in age from 34-88 years undergoing surgery for endometrial cancer between September 2001 and March 2009 at the Department of Gynecology and Obstetrics, Olomouc University Hospital, Czech Republic. None of the patients received chemotherapy or radiotherapy before surgery, which consisted of total hysterectomy, bilateral salpingo-oophorectomy, peritoneal cytology, and pelvic and paraaortic sampling as necessary. Two pathologists reviewed all the tumors to confirm the diagnosis and to determine histologic characteristics.

The 107 formalin-fixed, paraffin-embedded tumor samples were from patients with endometrioid carcinoma. The histologic grade, the depth of myometrial invasion, and the clinical stage were classified as recommended by the FIGO [27]. Epidemiologic and histologic features of the 107 patients are shown in Table 1.
Immunohistochemical evaluation and lymph node metastasis in surgically staged endometrial carcinoma

Statistical analysis

Fisher’s exact test of chi-square test was used to analyze the distribution of cases considered positive for the biological parameters examined according to several clinicopathological features; p values below 0.05 were considered significant. Statistical analysis was carried out using SPSS version 15 statistical software (SPSS Inc., Chicago, USA).

Results

Correlation with clinicopathological parameters

In Table 2 we present the distribution of positive immunohistochemical staining in relation to individual clinicopathological parameters in 107 cases of endometrial cancer. MMP-7 and MMP-26 were evaluated in only 50 patients with endometrial cancer. A statistically significantly dependence between p53, grading, and myometrial invasion was observed. In the G3 group, there was significantly greater p53 positivity compared to groups G1-G2 (42.3% vs 22.2%, \( p = 0.045 \)). Similarly, p53 expression was higher when myoinvasion exceeded 50% compared to myoinvasion less than 50% of myometrial thickness (38.6% vs 19.0%), \( p = 0.025 \). In the group of ER and PR positive tumors, correlation with grading was noted; in group G3 a significantly lower ER and PR positivity was seen compared with groups G1-G2 (53.8% vs 80.2% and 69.2% vs 88.9%, respectively, \( p = 0.008 \) and 0.028, respectively). The only marker correlating with FIGO stage was PR, where in the group of patients FIGO Stage I-II a significantly higher positivity of this marker was observed in comparison to patients FIGO Stage III-IV (87.9% vs 62.5%, \( p = 0.02 \)). Furthermore, PR positivity correlated inversely with lymph node status; PR positivity was higher in patients with negative lymph nodes (87.2% vs 61.5%, \( p = 0.032 \)).

Although no statistically significant dependence between bcl-2, c-erbB-2, Ki-67, MMP-7, MMP-26 and any clinicopathological parameter was observed, we noted a trend of increased c-erbB-2 and Ki-67 expression when myoinvasion exceeded 50% of myometrial thickness, as well as a trend of increased MMP-7 expression in the group of patients aged less than 65 years. Contrarily, with bcl-2 a trend of decreased expression was observed in clinically advanced tumors (FIGO III-IV).

As seen in Table 3, no significant difference in staining positivity of p53, c-erbB-2, Ki-67, MMP-7 and MMP-26 dependent on ER or PR positivity was observed. Contrarily, the percentage of bcl-2 positive endometrial tumors was significantly higher in the ER positive group than in the ER negative group (77.2% vs 46.4%, \( p = 0.002 \)). Also, significantly greater bcl-2 positivity was seen in the PR positive group than the PR negative group (75.6% vs 35.3%, \( p = 0.001 \)).
Table 2. — Distribution of tumor positivity for several biological markers according to clinicopathological characteristics in endometrial cancer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of cases</th>
<th>p53 positive no. (%)</th>
<th>bc-2 positive no. (%)</th>
<th>c-erb/neu positive no. (%)</th>
<th>Ki-67 positive no. (%)</th>
<th>ER positive no. (%)</th>
<th>PR positive no. (%)</th>
<th>No. of cases</th>
<th>MMP-7 positive no. (%)</th>
<th>MMP-26 positive no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>107</td>
<td>29 (27.1)</td>
<td>74 (69.2)</td>
<td>32 (29.9)</td>
<td>50 (46.7)</td>
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<td>3 (23.1)</td>
<td>8 (61.5)</td>
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**Statistically significant.**

Table 3. — Distribution of tumor positivity for several biological parameters according to ER and PR status.

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<th>Parameter</th>
<th>No. of cases</th>
<th>p53 positive no. (%)</th>
<th>bc-2 positive no. (%)</th>
<th>c-erb/neu positive no. (%)</th>
<th>Ki-67 positive no. (%)</th>
<th>ER positive no. (%)</th>
<th>PR positive no. (%)</th>
<th>No. of cases</th>
<th>MMP-7 positive no. (%)</th>
<th>MMP-26 positive no. (%)</th>
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<td>74 (69.2)</td>
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<td>13 (46.4)</td>
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**Statistically significant.**

Discussion

In endometrial carcinoma, conventional clinicopathological factors such as FIGO grade and stage, histologic type, lymph-vascular invasion, or depth of myometrial invasion are well-known predictors of prognosis. However, identification of molecular genetic alterations that could add relevant prognostic information to the traditional factors has become one of the goals of recent research. Our results show a correlation between certain biological markers and clinicopathological prognostic factors in surgically staged primary endometrial cancer.

Elevated p53 expression significantly correlated with poor differentiation and deep myometrial invasion in endometrial tumors. A number of works confirm association between elevated p53 expression and unfavorable prognostic factors in women with primary endometrial cancer [28-31]. This is in accordance with our finding of p53 overexpression in endometrial tumors with deep endometrial invasion. Contrary to the present series where p53 positivity was similar in lymph node positive and lymph node negative tumors, Mariani et al. describe p53 as the only molecular marker able to predict distant metastases independent of other histopathological, molecular and cytokinetic parameters [18]. Furthermore, various authors confirm a much higher p53 expression in serous tumors and clear cell tumors than in endometrial cancer, which supports the hypothesis of mutation of gene p53 as a late event in endometrial cancer and contrarily an early event during the development of rare endometrial tumors [32-34]. The results of Halperin et al. regarding p53 immunoreactivity in G3 tumors and papillary serous tumors demonstrate the uniqueness of G3 tumors, which are histopathologically more similar to papillary serous tumors than to endometrial cancer. G3 tumors probably follow a different path of carcinogenesis than G1 and G2 tumors [32, 35]. Our results assume the same conclusion.

The antiapoptotic gene bcl-2 regulates programmed cell death and thus lengthens cell survival which aids in...
the spreading of the tumor process. A number of studies have confirmed that bcl-2 expression increases in endometrial hyperplasia and is decreased in endometrial cancer. This loss of expression correlates with worse prognosis, worse clinical stage, depth of myometrial invasion and lymph node involvement [16, 28, 29, 36, 37]. The relation between loss of bcl-2 expression and biological aggressiveness of endometrial cancer seems paradox; the mechanism is not yet fully understood. Similarly to Appel et al., we did not observe a correlation between bcl-2 expression and degree of tumor differentiation, depth of myometrial invasion and lymph node involvement [38]. In accordance with works by other authors, we demonstrated a significant positive correlation between bcl-2 expression and positivity of hormonal representation of ER and PR [16, 32, 36], which could be an important prognostic factor for a negative prognosis.

Elevated expression of Ki-67 indicates increased cellular mitotic activity and proliferation. A number of studies have shown that Ki-67 is an independent prognostic indicator of survival [30, 39-41]. Contrarily, Pansare et al. did not show any correlation between Ki-67, histological type, grade or clinical stage of the disease [42]. We observed a trend of elevated Ki-67 expression in tumors with deep invasion. Our results are partly in accordance with works by Laxe et al. and Salvesen et al., who demonstrated correlations between elevated expression of Ki-67 with grading, depth of myometrial invasion and risk of recurrence [13, 43].

The fact that increased expression of oncogene c-erbB-2 correlates with worse prognosis has already been confirmed in various malignant tumors. According to certain authors, increased expression correlates with grading, depth of myometrial invasion and advanced disease stage [44-47]. Morrison et al. recently in their extensive study (483 cases) demonstrated that increased expression of c-erbB-2 as an independent prognostic factor correlated with worse survival [48]. Contrarily, Coronado et al. and Czerwenka et al. did not confirm a significant dependence with traditional prognostic factors of endometrial cancer [49, 50]. A trend of increased expression in patients with deep invasion was observed in our series, which correlates with the above-mentioned study results. Due to often opposing study results, the utilization of this factor remains ambiguous.

ER and PR are present in both normal endometrial tissue and endometrial cancer. Based on the results of various authors, the presence and amount of steroid receptors correlate with the clinical stage of the disease, histological grade and survival. The absence of steroid receptors is considered a negative prognostic factor of aggressive growth and poor prognosis [51-54]. Expression of ER in our work reached statistical significance in dependence on histopathological grading. PR expression correlated inversely with clinical Stage III-IV, poor tumor differentiation and extrauterine spread of tumor. This result is in accordance with the above-mentioned works; in addition, it seems that PR may be a stronger prognostic factor than ER, as supported by other authors [54-56].

An important member of the family of metalloproteinases with epithelial expression is MMP-7 (matrilysin-1), whose expression was captured in both normal and malignant epithelial cells. There is a limited number of published studies involving expression of MMP-7 in endometrial cancer. Ueno et al. demonstrated that increased MMP-7 expression correlated with a worse clinical disease stage and with presence of lymphatic metastases [24]. A similar trend was described by Graesslin et al. and Wang et al. [24, 57]. Contrary to these results, our work showed a trend of higher MMP-7 expression in patients under age 65.

Another member of the subfamily of matrilysin enzymes was described as MMP-26 (matrilysin-2). MMP-26 is also expressed in various tissues, including endometrial cancer. It has been established that MMP-26 expression specifically fluctuates during the menstrual cycle. Findings of elevated mid-cycle levels and in hyperplastic endometrium and contrarily low levels in the late phase of the cycle and in endometrial cancer point to an association with estrogen receptors. Isaka et al. and Pilka et al. demonstrated a significantly decreased MMP-26 expression in endometrial cancer, which is in discordance with the results of Tunuguntla et al., who describe increased immunohistochemical expression of MMP-26 in poorly differentiated endometrial cancer [22, 23, 26]. Our study did not show dependence on classical prognostic factors of endometrial cancer.

Conclusion

In conclusion, our findings suggest that there is no simple relationship between the determination of various immunohistochemical parameters and the biological aggressiveness of endometrial cancer. The results of our work show that besides clinicopathological factors, molecular biological prognostic factors may contribute to better tumor characterization and thus more precisely determine its clinical behavior. For the eventual practical diagnostically therapeutic use of molecular biological factors, additional studies are needed.

Acknowledgement

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References

Immunohistochemical evaluation and lymph node metastasis in surgically staged endometrial carcinoma


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The relationship between Bcl-2 oncogene expression and clinicopathological criteria in various stages of cervical neoplasia in Egyptian women

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2Department of Obstetrics and Gynaecology, Ain Shams University, Cairo (Egypt)

Summary

Purpose: To assess the degree of bcl-2 expression in the various stages of cervical neoplasia in a sample population of Egyptian women and relate the findings to clinicopathological criteria of invasive cervical cancer. Methods: Bcl-2 protein expression was assessed by immuno-histochemistry in 40 patients with cervical neoplasia (intraepithelial and invasive) in comparison to 20 patients with benign changes. Patients with invasive disease were followed up 2 years later and the outcome was correlated to the bcl-2 status at the time of diagnosis. Results: Bcl-2 expression increased from 20% in normal cervical tissue to 42.9% in cervical intraepithelial neoplasia grade II then dropped to 33% in invasive disease. Bcl-2 was not expressed (0%) in patients with advanced disease stage and grade nor in patients with lympho-vascular space invasion. Conclusion: Bcl-2 expression is reduced along the spectrum from benign towards invasive disease of the cervix. The maximum expression found in CIN II may suggest increased potential of progression to CIN III.

Key words: Bcl-2 protein; Cervical intraepithelial neoplasia; Apoptosis; Cancer of uterine cervix; Cervix squamous cell carcinoma.

Introduction

The growth of malignant cells is ultimately determined by their genetically induced proliferative capacity and their ability to escape host-induced programmed cell death (apoptosis). Bcl-2 oncogene may play an important role in keeping the transformed cells alive at the early stage of multi-step carcinogenesis in squamous tissue of the cervix. The highest percentage of bcl-2 positive neoplastic cells has been found in high-grade cervical intraepithelial neoplasia (CIN) grade 3 with reduction in expression as invasive squamous cell carcinoma (SCC) develops [1-3]. In this study, bcl-2 oncogene expression was studied in various degrees of cervical neoplasia in a homogeneous population sample.

Materials and Methods

In this cross-sectional, case-control study, we examined cervical tissue specimens of all patients diagnosed with squamous cell carcinoma of the uterine cervix during the 3-year study period (1997-1999) at the Gynaecology Department of Ain Shams University Hospital, Cairo (a large tertiary referral cancer centre). Ethical approval was obtained from the local scientific committee. Cervical tissue specimens of the invasive SCC group were obtained by cervical biopsy or during radical hysterectomy. Tumour stage, grade and lymphovascular space involvement were recorded. Follow-up included clinical examination (with vaginal smears) every three months as well as an annual chest X-ray. The median survival rate until 1999 was recorded and, accordingly, patients were classified into three subgroups: alive with no recurrence, alive with recurrence and deceased. Cervical tissue specimens for the CIN and the non-neoplastic control group were obtained during the years 1998-99. Tissue samples were obtained by cervical biopsy of suspicious lesions or during hysterectomy (indicated either for CIN or for benign conditions such as menorrhagia). The intensity of Bcl-2 staining was classified according to Joensuu et al. [4].

Statistical analysis

Group comparisons were performed using the chi-square test. The two-sided Kruskal-Wallis test was used for group comparisons of unpaired continuous data where normality could not be assumed due to small case numbers; p values < 0.05 were considered significant. All calculations were carried out using SPSS for Windows, version 9 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA).

Results

Twenty patients were diagnosed with invasive SCC of the uterine cervix in our hospital during the study period and the bcl-2 immunoreactivity of these tissue specimens was compared to a similar number of CIN and non-neoplastic tissues. Figure 1 shows the distribution of bcl-2 expression in the study population. Demographics are shown in Table 1.

Cervical specimens for the control group were obtained by a colposcopy-guided punch biopsy (n = 4) and during hysterectomy for benign uterine conditions such as leiomyoma and menorrhagia (n = 16). Bcl-2 protein was

<table>
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<th>Table 1. — Age and parity of patients in the 3 groups.</th>
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<td>Parity</td>
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*The late Prof. M. Hassan and the late Prof. I. Abou-Senna passed away on October 18, 1998.

Revised manuscript accepted for publication February 11, 2010
expressed in 17 (85%) normal squamous epithelia. Of the 17 specimens, 13 (65%) showed moderate positivity and four (20%) specimens were strongly positive. Staining was mainly patchy and in the cytoplasm of the basal cell compartment with occasional suprabasal staining.

Tissue samples of the CIN group were obtained by colposcopy-guided punch biopsy (n = 11), large loop excision (n = 4), cone biopsy (n = 1), and hysterectomy (n = 4). Seven specimens showed CIN 1, seven had CIN 2, and six showed CIN 3 changes. Bcl-2 protein was detected in 16 (80%) of CIN lesions; six (30%) of which were strongly positive and ten (50%) were moderately positive. CIN specimens showed an increase in the number of positive cells with increasing severity from CIN 1 to CIN 2. All the seven CIN 2 samples were positive; three (42.9%) strongly positive and four (57.1%) moderately positive. Of the six specimens showing CIN 3, only four (66.7%) were bcl-2 positive. However, this difference between the three grades of CIN was not significant.

Cervical tissue specimens of the invasive SCC group were obtained by colposcopy-guided cervical punch or cone biopsies (n = 6), radical abdominal hysterectomy (n = 11), or radical vaginal hysterectomy (n = 3). Subsequent staging showed that eight patients were Stage I, six were Stage II, five were Stage III, and one was Stage IV. Six specimens showed grade 1 ISCC, eight grade 2, and six grade 3. Seven patients had lymphovascular space invasion.

Only six (30%) ISCC specimens showed bcl-2 reactivity; all being moderately positive. A highly significant difference in bcl-2 expression grade was found between the cancer group and the control group ($p = 0.0005$); as well as between the cancer group and the CIN group ($p = 0.001$) with more in the latter. The six (30%) positive ISCC specimens showed a decrease in the number of positive cells with increasing severity from G 1 to G 3. Four (67%) specimens with G 1 and two (25%) specimens with G 2 were bcl-2 positive. All six specimens with G 3 ISCC were negative for bcl-2 protein. The difference between the three cancer grades was statistically significant ($p = 0.03$). It was found that FIGO clinical cancer stage did not alter the bcl-2 expression grade ($p = 0.440$). However, a significant relation was found between the bcl-2 expression grade and lymphovascular status ($p = 0.032$). All seven (35%) patients with lymphovascular invasion were negative for bcl-2. Three of the 20 patients in the cancer group had a central vault recurrence and five died. The causes of death were mainly attributed to renal failure and/or generalized toxaemia.

**Discussion**

Bcl-2 over-expression is well documented in various malignancies and has been associated with more [5, 6] or less [7-9] aggressive tumour features. Our study shows that overall bcl-2 expression was present in up to 85% of non-neoplastic cervical tissue increasing to 100% in CIN 2. After transformation to invasive disease, bcl-2 expression was markedly reduced in our study population. Moreover, lack of bcl-2 expression correlated significantly with lymphovascular invasion and worse clinical prognosis. Our observation of increased bcl-2 expression in CIN, compared to normal cervical tissue has been supported by other authors [10]; however, in contrast to our findings most studies show the peak expression of bcl-2 in CIN 3 and not in CIN 2 [1, 9-12].

Our observations regarding bcl-2 expression and squamous cell carcinoma are in accordance with other reports on this topic [8, 9, 13]. A study of tissue specimens from 22 patients with carcinoma in situ (82% positive) and 137 with invasive cancer concluded that expression of bcl-2 is lost during tumour progression and is a strong prognostic parameter [14]. Crawford *et al.* in 1998 examined the prognostic significance of p53 and bcl-2 expression in primary and recurrent cervical cancer [15]. Positive bcl-2 staining defined a group of patients with primary disease with a good prognosis. On the other hand, p53, an activator of the bax promoter, identified a group with a worse outcome. In recurrent disease, none of these markers reflected prognosis.
However, reduced bcl-2 expression does not always equate to increased apoptosis, as an association between reduced bcl-2 expression and decreased apoptotic activity was shown in cervical cancer compared to benign cervical tissue [16]. It is possible that the whole apoptotic pathway in increasingly less differentiated cells ceases to exist; a dysfunctional apoptotic machinery could convey a survival advantage to cancer cells in the hypoxic microenvironment of a rapidly expanding tumour [17].

It is well known that over a two-year period only less than 2% of CIN 3 progresses to invasive cancer, whereas less than a third regress spontaneously to normal cervical tissue [18]. It is therefore conceivable that CIN 3 consists of two distinctly different histological subspecies - those with the potential for persistence and invasion, and those without. Bcl-2 expression is perhaps one marker for the cell subgroup that will not proceed to invasive disease. In fact, a study comparing bcl-2 expression and hormone receptor status in 29 patients with early stage (Ia1) cervical cancer with those from 25 patients with CIN 3 identified co-expression with hormone receptor status and bcl-2 as an independent factor in defining low risk of CIN progression [12].

Our current study lacks power due to the relatively small number of cancer patients included. On the other hand, it is well known that due to demographic and socioeconomic factors, the reported incidence of cervical cancer in Egypt is comparatively low with only 0.07/100,000 women reported to be affected in 1999 [19]. Therefore, the number of patients included in our study represents more than 30% of all newly diagnosed women in that 3-year period. This is largely due to the centre being a tertiary referral one and, to our knowledge, this is probably the largest study to date to investigate bcl-2 expression in such a homogeneous population of women with SCC in Egypt.

Further research is needed to answer the question whether decreased bcl-2 expression in micro-invasive and invasive cervical cancer is caused by an abandonment of the normal apoptotic pathways or if it marks the presence of a different subgroup of dysplastic cells with an increased aggressive potential.

Conclusion

Incorporation of bcl-2 staining into routine immunohistochemical investigations for diagnosing cervical neoplasia could prove a useful tool in the determination of the aggressive potential. Before treating CIN 2 more aggressively, further studies are required to determine other factors influencing progression to CIN 3. Testing for bcl-2 expression may lead to an increasingly tailor-made approach in treatment of both pre-invasive and invasive disease.

Acknowledgement

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References

Introduction

Breast cancer is a complex disease with numerous genetic and environmental factors contributing to its development. It is well known that a great number of genes influencing protein function and/or expression are involved in the control of key cell functions like the control of the cell cycle and proliferation, apoptosis, DNA repair etc. [1, 2]. Among them, the TP53 gene is declared as being a “genome gatekeeper” due to its important functions in preserving the genome integrity. Numerous mutations within the TP53 gene are the most frequently reported genetic alterations in human cancers [3, 4], but there is growing evidence that some polymorphisms can also contribute to a predisposition for disease occurrence [5]. According to the IARC (International Agency for Research on Cancer) TP53 database, the majority of polymorphisms are localised in introns of the gene [6]. The two most frequently studied polymorphisms are the 16 bp duplication polymorphism in intron 3 (PIN3 (+16bp)) and the codon 72 polymorphism (Arg72Pro) in exon 4 of TP53 [7-11]. Despite extensive studies performed especially for the codon 72 polymorphism, the role of these two polymorphic sites and their coaction as susceptibility factors for breast cancer development is not yet strongly and decidedly determined.

Haplotype construction encompasses coding and non-coding polymorphic regions equally and provides greater statistical power for identification of risk alleles of low penetration, being thus more helpful in revealing the genetic risk factors for the development of complex diseases such as sporadic breast cancer [12]. As the relative contribution of genetic factors in the occurrence of breast cancer differs among populations, the purpose of the present case-control study was to investigate the possible association of genotypes and haplotypes of Arg72Pro and PIN3 (+16bp) polymorphisms in the TP53 gene with sporadic breast cancer in Croatian women.

Patients and Methods

Patients and study design

Ninety-five women with histologically confirmed primary breast carcinoma were retrospectively enrolled in the study. They were randomly chosen among the women treated for breast carcinoma at the Department of Oncology, Clinical Hospital Centre Zagreb, in the period 1996 to 2000. The majority of them, i.e., 80 women, were treated during 1998 and 1999. In 2006 the following data were obtained from medical records: age at the onset of the disease (mean age 54.4 yrs, range 26-77 yrs, median 55 yrs), tumour histology, stage according to TNM classification [13], grade, oestrogen and progesterone receptor status. The control group was extracted from a broader sample of 10,074 participants that was collected from 1995 to 1997 within the subproject “Health promotion” included in the First Croatian Health Project [14]. Participants were adult volunteers aged 18 to 80 years from 30 randomly selected settlements belonging to all major geographical regions of Croatia. The control group consisted of 108 women matched to cases with regard to age (mean age 51.6 yrs, range 22-88 yrs, median 48.5 yrs) and place of birth. The study was approved by the Ethics Committee of the Institute for Anthropological Research Zagreb, Croatia.
Table 1. — Distributions of genotype and allele frequencies of the TP53 gene codon 72 and intron 3 16bp duplication polymorphisms in breast cancer patients and controls.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Alleles</th>
<th>Genotypes</th>
<th>p*</th>
<th>p**</th>
<th>HW (p**)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg72Pro</td>
<td>Arg (%)</td>
<td>Pro (%)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>164 (75.9)</td>
<td>52 (24.2)</td>
<td>61 (56.5)</td>
<td>42 (38.9)</td>
<td>5 (4.6)</td>
</tr>
<tr>
<td>Cases</td>
<td>145 (76.3)</td>
<td>45 (23.7)</td>
<td>61 (64.2)</td>
<td>23 (24.2)</td>
<td>11 (11.6)</td>
</tr>
<tr>
<td>PIN3 (+16bp)</td>
<td>A1 (%)</td>
<td>A2 (%)</td>
<td>0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>185 (85.7)</td>
<td>31 (14.3)</td>
<td>77 (71.3)</td>
<td>31 (28.7)</td>
<td>0</td>
</tr>
<tr>
<td>Cases</td>
<td>155 (81.6)</td>
<td>35 (18.4)</td>
<td>67 (70.5)</td>
<td>21 (22.1)</td>
<td>7 (7.4)</td>
</tr>
</tbody>
</table>

*Fisher’s exact test; **χ² test; HW – Hardy-Weinberg equilibrium.

Specimen characteristics and assay methods

For women with breast carcinoma, genomic DNA extraction from paraffin-embedded tissue samples was performed with Nucleon Genomic DNA Extraction Kit (Nucleon Biosciences). For the control group, genomic DNA was isolated from leucocytes of peripheral blood using a macromethod [15]. The TP53 gene Pro72Arg polymorphism was detected using ordered TaqMan single nucleotide polymorphism (SNP) assay (Applied Biosystems):

Primer 1 – CCAGATGAGCTCCCCAGATGCG
Primer 2 – GCCGCCGGTGTGTAAGGA
Reporter 1 VIC –TGCTCCCCCCTGTCGC
Reporter 2 FAM – CTCGCCGCGTGGGC

The polymerase chain reaction (PCR) amplification and allele detection were performed on a 7500 real-time PCR System (Applied Biosystems) according to the manufacturer’s instructions. Each PCR reaction mixture (25 μl) contained TaqMan Universal PCR Master Mix (Applied Biosystems) 12.5 μl, 40X TaqMan SNP assay for genotyping (0.625 μl), the genomic DNA (10-100 ng) and H₂O (depending on the quantity of DNA).

For detection of TP53 gene PIN3 (+16bp) polymorphism, DNA amplification was performed with a Gene Amp System 9600 (Applied Biosystems). The primers were:

Primer 1 (MWG-Biotech) - 5’ FAM – CTG AAA ACA ACG TTC TGG TA-3’
Primer 2 (MWG-Biotech) - 5’-AAG GGG GAC TGT AGA TGG GTG-3’.

Each PCR reaction mixture (15 μl) contained 0.2 μM of each primer, 1X puffer, 2.5 mM of MgCl₂, 0.1 mM of deoxynucleotide triphosphate, 0.05 U/μl of Taq polymerase and 2 ng/μl of DNA. The PCR conditions were 95°C for 5 min, 15 cycles at 94°C for 1 min, 53°C for 1 min and 72°C for 1 min, then 15 cycles at 94°C for 1 min, 50°C for 1 min and 72°C for 1 min. Final extension was at 72°C for 10 min.

The PCR products were detected on an ABI Prism 310 DNA Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instructions using Rox Size Standard. Detection of allele length was done with the GeneScan Analysis 3.7 programme. Wild type allele with no duplication is labelled as A1 and allele with 16 bp duplication is labelled as A2.

Statistical analysis methods

For each polymorphism, allele frequencies were calculated from the genotype frequencies in cases and controls. Deviation from the Hardy-Weinberg equilibrium was assessed by the chi-square test with 1 df. Differences in genotype and allele distributions and haplotype distributions between the cases and controls as well as differences between clinical and pathological features of patients with different genotypes were tested by the chi-square test and Fisher’s exact test when appropriate. The odds ratios (OR) for breast cancer related to genotypes and their combinations and their 95% confidence intervals (CI) were calculated performing the chi-square test in the crosstabs procedure for a 2 x 2 table. Logistic regression analysis was not applied due to the calculation problems created by zero cell count. All statistical analyses were performed using SPSS version 10.0 for Windows (SPSS, Inc., Chicago, IL) software.

Linkage disequilibrium was calculated on the basis of estimated haplotype frequencies using the Arlequin software [16]. The D’ value (D/Dmax) between polymorphic loci was calculated as suggested by Lewontin [17].

Results

The genotype distributions of the studied polymorphisms within the TP53 gene were compatible with Hardy-Weinberg expectations in controls proving that the study population was genetically stable and thus appropriate for this analysis (Table 1). In cases a lower prevalence of heterozygous and a higher prevalence of homozygous genotypes relative to expectations were found. Differences between cases and controls in genotype distributions were observed for both Arg72Pro and PIN3 (+16bp) polymorphisms (p = 0.03 and p = 0.01, respectively). The A2A2 genotype was found exclusively in cases (7.4%). The allele distributions of the studied polymorphisms did not differ between cases and controls.

Additionally, the clinical and pathological features of breast cancer patients were compared among genotypes of Arg72Pro and PIN3 (+16bp) polymorphisms (Table 2). No significant differences were found among the codon 72 genotypes, while a significant difference emerged for progesterone receptor status (p = 0.049) and age at diagnosis (p = 0.018) among genotypes in intron 3. Positive progesterone receptors prevailed in patients carrying the A2A2 genotype (85.7%), and negative receptors in those carrying the A1A2 genotype (66.7%). Individuals with the A1A1 genotype had almost the same proportion of positive and negative progesterone receptors (52.2% and 46.3%, respectively).

To test the association of the two studied polymorphisms with sporadic breast cancer, genotypic ORs were
calculated. Taking the ArgArg genotype of the Arg72Pro polymorphism as a reference, the OR for sporadic breast cancer associated with the ProPro genotype was 2.20 (95% CI 0.72-6.71) and the OR associated with the ArgPro genotype was 0.55 (95% CI 0.30-1.02) (Table 3). Taking A1A1 genotype of the PIN3 (+16bp) polymorphism as a reference, the OR for sporadic breast cancer associated with the A2A2 genotype was 2.15 (95% CI 1.80-2.56) and that associated with the A1A2 genotype was 0.78 (95% CI 0.41-1.48).

Arg72Pro and PIN3 (+16bp) polymorphisms were found to be in linkage disequilibrium in cases and controls (D’ = 0.64 and D’ = 0.57 respectively; p = 10^-5) (Table 4). In both groups, the haplotypes ProA2 and ArgA1 were found more frequently than expected; the haplotype ProA2 that was three-fold more frequent than expected in both samples. No difference in haplotype frequencies was found between cases and controls.

The OR for the disease associated with the combination of ProPro and A2A2 genotypes versus all other combinations was 2.20 (95% CI 1.89-2.56), that is, very similar to the one observed in the separate analysis of PIN3 (+16bp) polymorphism (Table 3). This combination was chosen due to its frequency in cases, i.e., five out of seven patients with A2A2 genotype had also ProPro genotype.
Discussion

In this study, the association of Arg72Pro and PIN3 (+16bp) polymorphisms of the TP53 gene with sporadic breast cancer was investigated in a case-control sample from Croatia. The case and control groups were matched for age and place of residence to minimise the biases. We found the association of both studied polymorphisms with sporadic breast cancer development in Croatian women.

The studied polymorphisms showed departure from the Hardy-Weinberg equilibrium in the patient group due to a lower prevalence of heterozygous and higher prevalence of both homozygous genotypes. A decreased prevalence of the codon 72 heterozygote genotype among women affected by sporadic breast cancer was detected in numerous studies [3, 7-9], while some others did not report such findings [2, 10, 11, 18]. Data for the PIN3 (+16bp) polymorphism are less abundant and also inconsistent. A decreased prevalence of heterozygotes among affected women was also found in Sweden [19], but this was not observed in the majority of available studies [2, 10, 11].

In the present study, the homozygous genotype A2A2 was found exclusively in patients with a similarly low frequency to that reported by Costa et al. [2]. These authors observed a frequency of 1.9% A2A2 among 216 healthy women. As our control group was half the size of the Portuguese group, we suppose that the small sample size and the overall low genotype frequency are the reasons for finding no A2A2 genotype in the healthy Croatian women. The allele frequencies of both studied polymorphisms did not differ significantly between cases and controls and were in accordance with the findings from other European populations [2, 11, 19].

Several studies examined associations of alleles and genotypes on codon 72 of the TP53 gene with clinical and pathological features of breast cancer patients. We found no significant differences in these features among patients carrying different genotypes like Vieira et al. [20]. In contrast, Själander et al. [19] observed more well-differentiated breast cancers in patients with the Pro allele. In the study of Noma et al. [18], the frequency of estrogen positive breast cancers was greater in the homozygous genotype ProPro on codon 72. In another study [21], the Arg allele was associated with estrogen receptor positive tumours. In the study of Han et al. [22] ArgPro and ProPro genotypes were associated with negative axillary lymph nodes status. The homozygous genotypes of minor alleles were found to be predictor factors for the presence of lymph node metastases in a Portuguese population [2].

Studies dealing with the association of clinical and pathological features of breast cancer patients with different genotypes of PIN3 (+16bp) polymorphism of the TP53 gene are scarce [23]. However, the present study revealed a significant difference for progesterone receptor positivity and age at diagnosis between patients carrying different genotypes in intron 3. Positive progesterone receptors were found frequently in the A2A2 individuals, while negative progesterone receptors were more frequent in heterozygotes. Older age at diagnosis was found to be associated with A2A2 genotype.

This study revealed the association of A2A2 genotype of PIN3 (+16bp) polymorphism with sporadic breast cancer suggesting the minor allele effect on the risk. However, the results for Arg72Pro were more complex. The heterozygous genotype indicated a protective effect on sporadic breast cancer development that was in accordance with the findings of Singh et al. [24]. Although OR for ProPro genotype was 2.2, its association with sporadic breast cancer was not significant. It can be explained with increased frequency of both homozygous genotypes in cases.

The literature on the association of codon 72 polymorphism with breast cancer is abundant and highly controversial. The association of the Pro allele and/or the ProPro genotype with increased risk of breast cancer has been observed in numerous studies [19, 25, 26]. In contrast, many studies presented the association of the Arg allele and/or the ArgArg genotype with the risk for the disease [3, 7, 9, 27]. Finally, there are also studies that found no association of the Arg72Pro alleles and/or genotypes with the increased risk of breast cancer development [8, 11, 28-32].

The findings of the association of PIN3 (+16bp) polymorphisms with breast cancer development are also inconsistent. Costa et al. [2] reported that women carrying the A2A2 genotype have an increased risk of developing breast cancer, both those with and without familial history. The A2 allele was more frequent in patients with breast cancer than in healthy controls in the study of Wang-Gohre et al. [11], and it was estimated that the odds ratio for breast cancer was increased in women carrying the A2 allele. In contrast, Mahasneh and Abdel-Hafiz [29] observed an increased risk of developing breast cancer in carriers of the A1 allele in a Jordanian population. The study of Wei et al. in a Chinese Han population [32] reported no association of PIN3 (+16bp) polymorphisms with susceptibility to breast cancer.

The two analysed polymorphic sites of the TP53 gene were in moderate linkage disequilibrium (LD) in our study. The result for the breast cancer group should be taken with reserve because both polymorphisms deviated from the Hardy-Weinberg equilibrium needed for LD test statistics [33]. Linkage disequilibrium among the TP53 gene polymorphisms can be an important factor influencing breast cancer incidence [19].

It has been suggested that specific TP53 haplotypes as well as genotype combinations may modify breast cancer risk. Our findings do not support any significant association of four possible haplotypes with sporadic breast cancer development. However, the genotype combination ProPro/A2A2 was found to be frequent in cases with OR of 2.2 (95% CI 1.89-2.56) which was, similar to the one observed for A2A2 genotype itself. Presence of the Pro allele in the combination did not decrease the OR. As these two polymorphisms were in moderate LD, the assoc-
cation observed for each genotype separately possibly reflects different mechanisms.

Eight studies were found in the literature investigating the TP53 gene polymorphisms and their risk haplotypes or genotype combinations for the development of breast cancer [2, 10, 11, 19, 25, 29, 34, 35]. The genotype combinations were investigated in the Swedish study [19] but the combinations that were associated with breast cancer differed from those revealed in the Croatian population. The studies of risk haplotypes offered diverse but significant results as opposed to the present study. Weston et al. [34] revealed an association of minor TP53 gene haplotype Pro, A2 (intron 3), MspI A1 (intron 6) with breast cancer risk in postmenopausal women and women older than 50 years. Costa et al. [2] reported a significant difference in the ArgA2 haplotype frequencies between women with breast cancer with familial history and the control group among a Portuguese population. The haplotype containing A2 allele was associated with an increased risk of breast cancer in a Turkish population [10]. Haplotype analysis identified the A2 allele (intron 3) as the risk allele for breast cancer development in a German population [11]. In Pakistani breast cancer patients, the absence of the 16 bp duplication in the intron 3 with the Pro allele and the MspI restriction site in the intron 6 were the most frequent [35].

The existence of diverse results can be explained not only by the different frequency of alleles and genotypes of the studied polymorphisms in different populations but also by their different biochemical and biological features [36]. The Arg allele of codon 72 polymorphism is more efficient in inducing apoptosis, while the Pro allele is a better inducer of cell cycle arrest and induces transcription activation more efficiently than the Arg allele [36-38]. The allele without 16 bp duplication in codon 3 is more efficient in inducing apoptosis, and DNA repair is more efficient in its presence [39]. A lower level of the mRNA of the TP53 gene was found in the presence of A2 allele in intron 3 [40].

In conclusion, our findings suggest the association of both studied TP53 gene polymorphisms with sporadic breast cancer in the Croatian population. For the PIN3 (+16 bp) polymorphism, the association was revealed for the homozygous genotype of the minor allele. Hypothetically, that could be the consequence of decreased apoptotic efficiency. On the contrary, results for the Arg72Pro polymorphism were complex suggesting the association of heterozygous genotype with a protective effect for sporadic breast cancer development. However, these findings need to be substantiated with additional studies and on greater sample size taking into consideration numerous other genetic and environmental factors that can influence sporadic breast cancer risk.

Acknowledgment

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References


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Adjuvant chemotherapy versus radiation therapy after radical surgery in high-risk positive node Stage IB/IIA cervical cancer

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Summary

Objective: The aim of this study was to evaluate whether the addition of adjuvant chemotherapy will improve the outcomes of high-risk patients with Stage IB, IIA squamous cervical carcinoma with positive pelvic and/or aortic nodes. Materials and Methods: 127 patients with Stage IB and IIA cervical carcinoma treated with radical hysterectomy and systematic pelvic/aortic lymphadenectomy (RS) and who had lymph node involvement, confirmed at the final histological examination were enrolled from January 1987 to December 2001. All the patients received three cycles of adjuvant chemotherapy (AC) with cisplatin, bleomycin and vinblastine. The median patient age was 47.3. Seventy-seven patients had FIGO Stage IB1, 26 IB2 and 24 IIA. The results were compared with those obtained from a group of 136 patients with comparable age, stage and lymph node involvement, on whom radical surgery, systematic pelvic/aortic lymphadenectomy (RS) and adjuvant radiotherapy (RT) was performed on period 1971-1984. The follow-up period ranged from 7-13 years. Results: Overall survival rate of the two groups (RS+AC) vs (RS+RT) at seven years was 69.3% and 59.5%, respectively ($\chi^2 = 2.70; p = .10$). Progression-free survival was 59.8% vs 50.0% ($\chi^2 = 2.56; p = .10$). The best results were however obtained with the common iliac and over two lymph node metastases. Conclusions: Adjuvant chemotherapy in high-risk patients for lymph node positivity did not produce statistically significant results in terms of overall and disease-free survival vs adjuvant radiotherapy; however, a group of these patients, approximately 10%, could receive benefit from the treatment.

Key words: Cervical cancer; Adjuvant chemotherapy; Lymph node metastasis.

Introduction

Cervical cancer is the second most common cancer among women. Both radical hysterectomy with pelvic lymphadenectomy and radical radiation therapy are the primary therapy for Stage IB/IIA cervical cancer of the cervix. Several studies suggest that the overall 5-year survival rates are similar [1, 2].

Disease control is difficult for patients at high risk for recurrence. Different clinical and histopathologic factors have been shown to be related to local recurrence and long-term survival of patients with cervical carcinoma [3-8]. These include: lymph node metastasis [8-11], lesion size [11], depth invasion more than 10 mm [8, 11], lymphovascular space invasion [12], microscopic parametrial invasion [13], and tumor grade [14]. However, the most unfavorable prognostic factor remains lymph node involvement: the 5-year survival is 91% to 88% for Stage IB and IIA versus 68% and 52% in case of positive lymph nodes. Furthermore, the risk of recurrence is related to the number and site of the lymph nodes affected [15-17]. Recurrences are much more frequent in patients with lymph node involvement. In lymph node-negative patients the disease relapses only in 15% of cases, whereas in the same stage, the percentage of recurrence varies between 20% and 75% if there is lymph node involvement [18, 12].

For many years adjuvant therapies have been introduced after radical hysterectomy in an attempt to eradicate micrometastasis in patients at high risk of recurrence due to lymph node positivity [19]. Furthermore, evidence of the efficacy of polychemotherapy in advanced neoplasias [20], as well as the reduction of positive lymph nodes in patients treated with neoadjuvant polychemotherapy [21] and, lastly, the failure of radiotherapy in the control of extra-pelvic recurrences [22, 23] have constituted the rationale for the approach with adjuvant chemotherapy in patients on whom radical surgery is performed and where histology reveals lymph nodes involved in the neoplastic disease. A report of the American Gynecologic Oncologists Society concludes that survival after radical surgical treatment is not influenced by adjuvant therapy when the number of positive lymph nodes is greater than three [22]; similar results are reported by other authors [23-25]. Some non-randomized studies suggest benefit from the use of chemotherapy in Stages IB/IIA in the presence of several risk factors, including lymph node metastasis in 16/28% of the cases [26-28].

Tattersall M. et al., on the other hand [29], in a randomized trial on women treated by radical hysterectomy and pelvic lymphadenectomy, reported no significant difference in the disease-free interval and overall survival in women receiving adjuvant chemotherapy.

The purpose of this study was to verify with long-term follow-up the efficacy of adjuvant chemotherapy in women at high risk of recurrence due to histological diagnoses of lymph node positivity, FIGO Stages IB/IIA from 1987-2009.
The data were compared and statistically evaluated in relation to a similar group of patients on whom radical surgery and postoperative radiation therapy were performed.

Materials and Methods

The study was carried out on patients treated for squamous cell carcinoma of the cervix in Stages IB/IIA with pelvic and/or aortic lymph node metastasis.

Surgical recruitment was opened on January 1987 and closed on December 2001 at the Department of Gynecology and Obstetrics University “Sapienza” of Rome. Follow-up was completed and evaluated on December 2009. The patients were staged according to the FIGO 1985 system. The study was reviewed and approved by the Institutional Review Board. All patients participating in the study gave their informed consent. Patient characteristics are shown in Table 1. There were no significant differences between the groups (p: NS).

### Table 1. — Clinical-pathological characteristics of the patients.

<table>
<thead>
<tr>
<th></th>
<th>RS+AC</th>
<th>RS+RT</th>
<th>χ² test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>127</td>
<td>136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age</td>
<td>47.3</td>
<td>48.5</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB1</td>
<td>77 (60.5)</td>
<td>79 (58.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>26 (20.5)</td>
<td>27 (20.0)</td>
<td></td>
<td>.40 .81 (ns)</td>
</tr>
<tr>
<td>IB2</td>
<td>24 (19.0)</td>
<td>30 (22.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node n.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>involvement</td>
<td>&lt; 3</td>
<td>69 (58.0)</td>
<td>79 (58.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 3</td>
<td>58 (42.0)</td>
<td>57 (42.0)</td>
<td>.37 .53 (ns)</td>
</tr>
<tr>
<td>pelvic</td>
<td>95 (74.8)</td>
<td>98 (72.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>common iliac</td>
<td>20 (15.7)</td>
<td>23 (16.9)</td>
<td>.28 .86 (ns)</td>
<td></td>
</tr>
<tr>
<td>aortic</td>
<td>12 (9.5)</td>
<td>15 (11.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eligibility criteria

Inclusion criteria were: patients with squamous cell carcinoma Stages IB/IIA who underwent a radical hysterectomy (type 3 Piver); histologically confirmed pelvic and/or aortic lymph node metastasis; free margins of tumor; age less than 65 years (mean age 47.3 years); absence of severe systemic diseases; good general condition (performance status < 2); and absence of other malignant neoplasias.

Initially, 145 patients were taken into consideration, of which 18 were excluded from the study: ten (6%) did not complete the chemotherapy cycles, four deaths for causes unrelated to the diseases, and four were lost to follow-up. The final analysis was therefore based on 127 patients. The average follow-up was ten years (range 7-13).

Surgery

All the patients underwent type 3 radical surgery and systematic pelvic/aortic lymphadenectomy (RS) [30-35]. The lymphadenectomy therefore included the removal of all the lymphatic tissue surrounding the pelvic vessels bearing anterior, lateral and posterior to the common, external and internal iliac vessels, and anterior, lateral and inferior to the obturator nerve. Aortic lymphadenectomy was performed at least up to the level of the inferior mesenteric artery. An average of 45 lymph nodes were removed from each patient (range 15-65), of which on average, 25 were pelvic, six common iliac and 14 aortic. The number and site of the positive lymph nodes and related percentage of recurrence and survival were defined.

Chemotherapy

Adjuvant chemotherapy (AC) [36, 37] was started two weeks after the operation according to the PVB scheme: cisplatin 50 mg/m² on day 1, vinblastine 4 mg/m² on days 1 and 2, and bleomycin 15 mg on days 1-8-15 at intervals of three weeks for a maximum of three cycles. The cycles were delayed by one week for leukocytes between 2000 and 3000/mm³ and platelet counts lower than 50000/mm³. Persistence of the situation for more than seven days led to a reduction of vinblastine to 50% of the normal dose while the doses of bleomycin and cisplatin were not changed. Chemotherapy was suspended if the value of the white blood cells was less than 2000 and platelets less than 50000. The toxicity of the chemotherapy drugs was evaluated according to the WHO guidelines (1979). Ten patients (6%) were unable to complete the chemotherapy because of the seriousness of the side-effects.

Control Group

Control was performed on a group of patients who were similar in relation to admission criteria, stage (reclassified according to FIGO 1985) and lymph node involvement, on whom radical surgery (RS) and radiation therapy (RT) [38] was performed during the period 1971-1985. One hundred and thirty-six cases were selected out of 812 treated with complete systematic pelvic/aortic lymphadenectomy. Adjuvant radiotherapy was performed; the first time with 60CO and then with high energy. The patients were initially treated with external-beam postoperative radiotherapy on the whole pelvis (5000 rad or 50 Gy) for five or six weeks. Subsequently, the patients were treated by brachytherapy 3000 rad or 35 Gy (low dose rate) to the rectovaginal septum.

Surgery and follow-up were performed by the same surgical-oncological team.

The overall survival, disease-free survival, recurrences and sites of recurrence were taken into consideration for each group.

The relations between FIGO Stage (IB1, IB2, IIA), site (pelvic, common iliac, aorto-caval) and number (1, 2, > 2) of the positive lymph nodes and related recovery indexes were shown. Finally, overall survival was evaluated.

Descriptive follow-up

After completion therapy, each patient underwent regular follow-up every four months in the first two years, then every six months subsequently. After the fifth year, one time per year, a pelvic examination, Pap smear and colposcopy were performed. Computerized axial tomography (CT) or magnetic resonance imaging (MRI) were carried out every year. The mean follow-up of the study group was 120 months (range 84-156 months). Four patients (2.7%) were lost to follow-up, and four (2.7%) died due to causes unrelated to the disease.
The distribution of the prognostic variables does not change whether the patients lost to follow-up are included or excluded ($p = NS$).

The follow-up control group was limited to the same period of observation (120 months).

**Statistical analysis**

The chi-square test was used for statistical evaluation of the frequencies of the nominal variables or by category: a value of $p < .05$ was considered significant.

Survival was calculated as the period between the initial diagnosis of neoplasia with surgical staging and the last follow-up. Recurrences and/or metastases were defined as recurrence of the disease after a disease-free period between the surgical operation and the last follow-up. The SPSS 8.0 statistical software package was used for all analyses.

**Results**

Table 2 shows the overall result in terms of recurrences and related deaths, disease-free survival and overall survival.

Table 2. — *Results of the treatment.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patients</th>
<th>Overall survival</th>
<th>Mortality</th>
<th>Disease-free survival</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS+AC</td>
<td>127</td>
<td>88 (69.3)</td>
<td>39 (30.7)</td>
<td>76 (59.8)</td>
<td>51 (40.2)</td>
</tr>
<tr>
<td>RS+RT</td>
<td>136</td>
<td>81 (59.5)</td>
<td>55 (40.5)</td>
<td>68 (50.0)</td>
<td>68 (50.0)</td>
</tr>
</tbody>
</table>

In 127 patients RS+AC no evidence of disease appeared at follow-up in 76/127 cases (59.8%) while recurrences occurred in 51 (40.2%) cases. The overall survival was 69.3% (88/127) with 39 deaths related to the disease (30.7%).

Overall survival in the control group was 59.6% (81/136) with 55 deaths (40.4%) related to disease. Disease-free 5-year survival was 50.0% (68/136). Odds-ratio = 1.532 (CI 95% = 0.921 - 2.550) in relation to death events, and odds-ratio = 1.490 (CI 95% = 0.914 - 2.429) in relation to recurrence, indicate that patients RS+RT were about 1.5 times more likely to have relapse and death than patients RS+AC.

Table 3 summarizes the incidence and site of recurrence in the two groups. Pelvic and distant recurrence were 22.8 and 17.4% vs 28.7 and 21.3% in the two groups, respectively. The site of recurrence shows a higher incidence of recurrence for pelvic and distant sites for the control group but with chi-square .05 and $p$: NS.

Table 4 shows the frequencies of recurrences related to FIGO staging in the two groups. For Stage IB1 the percentage was 36.4% vs 44.3%, for Stage Ib2 42.3% vs 55.6% and 50% vs 60% for Stage IIA. However, the better results obtained in group RS+AC had no statistical significance [$\chi^2$ = .16 (NS)].

In Table 5 the recurrence rate related to number of involved nodes is summarized. The recurrence rate was respectively 30.4% vs 40.5% in the two groups with positive nodes < 3 and 51.7% vs 63.2% in the node positive groups $\geq$ 3 [$\chi^2$ = .40 (NS)].

In Table 6 the data of recurrences in relation to the site of positive lymph nodes are summarized. The recurrence rate for positivity of pelvic lymph nodes was 30.5% vs 39.8% in the two groups (- 9.3% in the the RS+AC group compared to the RS+RT group). The differences in positivity of the common iliac lymph nodes were considerable -65% vs 78.3% (- 13%), while the only case of inversion of frequency of recurrences in the ratio between the two groups occurred in the aortic lymph nodes (+1.7% in the RS+AC group). However the $\chi^2$ = .04 was not statistically significant.

**Analysis of recurrences**

The site of the recurrences shows a higher incidence of recurrence in the control group for both local and distant (28.7% vs 22.8% and 21.3% vs 17.4%).

In the RS+AC group, the 22 patients with extrapelvic recurrence were subsequently given chemotherapy with a non cross-resistant scheme [39] with the precedent: epirubicin alone or ifosfamide plus MESNA. Instead, the 29 patients with pelvic recurrences had radio/surgical treatment: eight patients with pelvic recurrences and four with...
extrapelvic recurrences obtained remission of disease with the treatment (23.5%).

In the control group (RT+RS), the 68 recurrences received surgical treatment. Nine patients with pelvic recurrences and four with distant recurrences (19.1%) had disease remission. The statistical comparison between deaths in the two groups in relation to metastases was not significant (chi-square .05 p = NS).

Analysis of the survival curves of the groups studied using the log-rank test was estimated.

Side-effects and complications

Both surgical treatment and chemotherapy were well tolerated. No treatment-related deaths occurred.

In particular, there were no severe intraoperative surgical complications. Blood transfusion was given in 8% of the cases; postoperative bladder dysfunction occurred in 20% of the cases and lymphorrhage in 18.6%, without any lymphohost since systematic drainage of the pelvic cavity was done, with an average of 7.5 days.

Five cases (2%) of urethral fistula occurred which were subsequently repaired: two surgically and three by inserting a double J, and five cases of bladder lesions were repaired intraoperatively.

Concerning chemotherapy, it was not possible to complete treatment in ten patients of 145 (6.9%) who were in any case excluded from the protocol. Toxicity existed in 20% of the cases of leucopenia, 10% of thrombocytopenia and 25% of mucositis. Neurotoxic effects (paresthesia, tremor) were observed in 30% of the cases and regressed after temporary suspension of the treatment. Adverse effects of postoperative radiotherapy consisted mainly of acute cystitis, diarrhea, symptomatic cutaneous edema, and myelodepression, but they were rarely severe.

Discussion

In the study we investigated the effectiveness of adjuvant chemotherapy on the prognostic impact of lymph node metastasis. Despite the fact that the majority of women with Stage IB/IIA cervical cancer can be treated with radical hysterectomy and pelvic lymphadenectomy, there has been no reduction over the last two decades in the 20% mortality rate of these patients [40]. The lower incidence of positive lymph nodes in patients affected by squamous cell carcinoma of the cervix treated with neoadjuvant therapy [41], the poor efficacy of radiotherapy in the control of extrapelvic recurrences [22-37] and evidence of the progressive increase in efficacy of the new antiblastic drugs [19-21] lead to the hypothesis of adjuvant chemotherapy in patients at high risk of recurrence for lymph nodal positivity. A study by Lai et al. [42] on a group of women with positive lymph nodes demonstrated a statistically significant higher incidence of survival in patients treated with adjuvant chemotherapy (survival 75% vs 47%; p = .05). However, in a randomized trial, Tattersall et al. [29] did not confirm the evidence of a beneficial effect in patients with positive lymph nodes treated with adjuvant chemotherapy. However the potential effect of chemotherapy has been observed in some phase II trials [43]. Cisplatin is the most effective single agent [44].

The data reported in the literature has led to verification of the operative case history followed for over 30 years of oncological activity [30-34]. The homogeneity of treatment and selection of the patients have made the cases more comparable statistically, whereas some institutes [29] did not make a statistical evaluation of the different therapeutic strategies adopted in relation to the prognostic state.

One study ended in a sufficient time period to guarantee a satisfactory survival analysis (with a minimum period of observation of 7 years up to 13 years) compared to other data and other analyses reported in the literature [39]; survival was therefore evaluated after at least seven years so that the percentage of controls would not be concluded too soon.

The decision to select cases on the basis of surgical and not clinical staging is linked to the diagnostic accuracy of the anatomicopathologic control. As early as 1972 Napolitano et al. [45] reported an error incidence of 24% on the clinical diagnosis of staging. Today, diagnostic procedure have reduced this margin but it can in any case be estimated at around 16% for under- or over-staging.

Most analyses of prognostic factors identified lymph node metastasis and size of the neoplasia as the most important survival factors [42]; but the age of the patients was found to be important in some studies: this parameter was therefore limited to 65 years to avoid excessive interferences linked with senile pathology.

Radical surgery and lymphadenectomy [46] have been suggested by an analysis of the results linked to the surgical technique. In fact, the aim of selective lymphadenectomy is basically diagnostic.

A study [47] carried out by means of multivariate analysis revealed that several independent risk factors exist; we therefore selected patients on the basis of histologic type of the neoplasia and number and site of positive lymph nodes, parameters evaluated to define the efficacy of the adjuvant chemotherapy.

Related to the type of chemotherapy used, it should be pointed out that all the therapeutic protocols demonstrate that cisplatin, vincristine and bleomycin can be safely administered and there is no evidence of excessive toxicity on the mucosa or bone narrow [29-34].

The selection criterion of the choice of control group, related to the period 1971-85, was homogeneous with the group being studied.

Even if the relative limitation of the number of cases treated did not permit optimum statistical reliability of the results obtained, the results are consistent with the data found in the literature in reporting an improvement in survival ranging between 3% and 28% [15, 29, 42].

On a comparative statistical analysis, the overall data in terms of survival and disease-free interval show evidence of worsening of the prognosis as staging advances, without significant differences between the two groups (p =
An examination of global survival also shows a similar pattern to the foregoing ($p = NS$), but a constant better performance of the study group compared to the control group can be seen. This constant approaches statistical significance when the graph related to lymph node positivity greater than two is examined. Tattersall et al. [29] noted no significant advantages with adjuvant chemotherapy but they only studied patients with lymph node positivity limited to the pelvis and not to the lumboaortic nodes.

On the other hand, Sivanesaratnam et al. [26] reported a survival of 76% in cases with positive pelvic lymph nodes given adjuvant treatment, compared to 60.3% in cases without adjuvant treatment.

Although the prognostic improvement is not significant at statistical examination, it can also be seen in the analysis of the disease-free interval, according with the findings of Lahousen et al. [15] in the only similar randomized work. However, in that study the percentage of local recurrences suggested that chemotherapy does not have relevant effects on pelvic recurrences. Nonetheless these studies include patients with positive lymph nodes and with invasion of the lymphovascular spaces in order not to limit the number of cases involved. Furthermore, the lumbo-aortic site of the lymph nodes is not taken into consideration.

Conclusions

For patients with high-risk Stage IB/IIA cervical cancer who underwent radical surgery and pelvic lymphadenectomy, adjuvant chemotherapy resulted in better survival [4]. However, as reported in the literature, an improvement in survival and disease-free interval has been observed, as well as a reduction in the incidence of metastasis.

The overall statistical comparison of survival in the group given adjuvant treatment shows no significant differences from the control group as in our data published in 2003 [36].

These results were found to be better when the number of positive lymph nodes was greater than two, when the common iliac stations were affected, and Stage was Ib.

Lastly, it should be considered that patients in whom recurrences occur after surgery and chemotherapy can be treated with radiation treatment, where chemotherapy is found to be inefficacious in patients already treated with surgery and adjuvant radiotherapy.

The limits imposed by radiotherapy in the prophylaxis of repetitive distant processes and the progressive increase in efficacy of the new chemotherapy drugs appear to constitute the basis for increasingly widespread pharmacological experimentation.

References


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Vulvar cancer: prognostic significance of the clinicopathological characteristics

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Summary
Vulvar cancer is a relatively uncommon neoplasm of the female reproductive system. The aim of this retrospective study was the analysis of the pathologic and clinical characteristics of vulvar cancer and their prognostic significance. During the period January 1996 to December 2005, 82 patients were treated for a vulvar malignancy. The management was surgical.

Key words: Vulva; Cancer; Carcinoma; Melanoma; Sarcoma.

Introduction
Primary vulvar carcinoma is an uncommon disease which usually presents in elderly women with comorbidity such as hypertension, diabetes or obesity. Vulvar carcinoma is a skin tumor presenting with different histological types. The vast majority of vulvar carcinomas are of squamous cell type and more rare histological types such as melanoma (5%), basal cell carcinoma (1-2%), adenocarcinoma or sarcoma. The incidence of such tumors accounts for 3-5% of all gynecological cancers [1]. Its detection is possible in an early stage due to the characteristic signs and symptoms. Biopsy in an outpatient clinic is very important for the diagnosis. Decreased survival rates have been reported according to tumor size, localization of the primary tumor and lymph node involvement. The major prognostic factors of overall and disease-free survival are the stage, grade of differentiation, tumor diameter, node involvement, depth of invasion and tumor-free surgical margins.

Method
This was a retrospective analysis of women diagnosed with vulvar carcinoma treated between January 1996 and December 2005 in the 2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, Athens, Greece. The necessary data were collected by reviewing patients records or hospital electronic bases and contacting the physicians and patients regarding the age at diagnosis, stage, histological type of the tumor, treatment, relapse rate and 5-year survival. The staging of disease was performed according to FIGO criteria. Ethical review approval was achieved by the ethical committee of our hospital. Statistical analysis was made by using SPSS with statistical significance in all cases defined as \( p < 0.05 \).

Management
The first diagnosis is usually made by vulvar biopsy. Further investigation following the diagnosis includes chest X-ray, abdominal computed tomography (CT) scan and bone scanning. The management is the same regardless of tumor type, but different according to the stage of the disease. All patients were operated within 15 days after diagnosis. Radical wide excision, radical vulvectomy, radical vulvectomy with unilateral lymphadenectomy and radical vulvectomy with bilateral lymphadenectomy were the surgical options. A redovac slow suction drain was used for ten days. Foley’s catheter was used to avoid wound soiling. Twice daily dressing of the wound was necessary and the stitches were usually removed on the tenth postoperative day. Radiotherapy was given when inguinofemoral lymph nodes were involved. Follow-up was scheduled for every three months the first year and then every six months for five years. The postoperative radiotherapy was directed at the bilateral groin nodes and the perineum. Patients with Stage III or IV were additionally irradiated at the pelvic nodes. The doses ranged from 32.4 Gy to 54 Gy (median 50 Gy) with a 6-mega electron volt linear accelerator and 1.8 Gy daily tumor dose.

Results
Eighty-two patients were included in our retrospective study. The mean age of them was 72.33 years (range 52-84 years). The mean body mass index (BMI) was 26.3 ± 3.2 (range 20.5-34.2). Regarding obstetric history 6/82, 12/82, 64/82 patients were nulliparas, primiparas and multiparas, respectively. There was no significant relationship with smoking. HPV infection, low-grade squamous intraepithelial lesion (LGSIL) and high-grade squamous intraepithelial lesion (HGSIL) in the Pap smear were detected in 14/82, 3/82 and 1/82 patients, respectively. Of the patients 12/82 and 7/82 had a previous diagnosis of VIN I and VIN II or III, respectively. The main initial symptom in 59/82 patients was pruritus, followed by presence of the tumor 48/82, pain 7/82 and bleeding 6/82. Eight of 82 patients reported no symptoms. The vast majority of vulvar carcinoma were squamous cell carcinoma (74/82), then basal cell carcinoma 4/82, melanomas (3/82) and sarcoma (1/82). Preoperatively 19/82, 10/82, 46/82 and 7/82 had Stage I, II, III and IV, respectively. Clitoral involvement was found in 20/82 patients. The mean tumor size was 47.2 ± 12.7 mm
Surgical treatment consisted of radical wide excision in 15/82, radical vulvectomy in 10/82, radical vulvectomy with unilateral lymphadenectomy in 14/82 and radical vulvectomy with bilateral lymphadenectomy in 43/82 patients. The most common complication was wound breakdown (19/82) followed by lymphedema (12/82). Thirty-eight of 82 patients received adjuvant chemotherapy and 57/82 patients received adjuvant radiotherapy. The mean follow-up was 48 months (range 36-60 months). All patients had tumor recurrence from three months up to 54 months (median, 20.4 months). Of the patients 31/82, 11/82, and 40/82 had a groin recurrence, a recurrence in the former location of the vulva, and a distant metastasis, respectively.

Overall five-year survival was 27/82. The five-year survival was 12/19, 6/10, 9/53 for Stage I, II (III and IV), respectively. Patients with recurrences 12 months or less after surgery had a significantly decreased median overall survival compared with those with a later recurrence.

Discussion

Although vulvar cancer yields early symptoms, its prognosis can not be characterized as favorable. Significant prognostic factors include tumor size and location, lymph node involvement, location of recurrence, and disease-free survival. Smoking and obesity seem to increase the risk of vulvar carcinoma. Nola et al. revealed that the depth of tumor invasion represents the most important prognostic parameter in the group of patients with invasive squamous vulvar carcinoma [2]. Lymph node status is an important prognostic factor and for this reason, complete inguinofemoral lymph node dissection is proposed. Stage and nodal involvement are predictors of survival, and stage is a predictor of disease-free survival [3]. Clinical significance of DNA ploidy and proliferative activity has not been found [2]. Moreover, bilateral involvement of the inguinal lymph nodes carries a worse prognosis [4]. Narayansingh et al. showed a 20-fold higher recurrence rate in patients with lymph node micrometastases [5]. HPV is proposed to be a possible risk factor in the etiology of vulvar carcinoma. Epithelial disorders are found adjacent to vulvar carcinoma in 70-80% of patients and could serve to separate patients that differ in prognosis [6]. According to Eva et al. vulvar carcinoma arising in a background of differentiated VIN appears more likely to recur than vulvar carcinoma arising from undifferentiated VIN [7].
Landrum et al. found a mean age of 59.9 years in a similar study to ours with Stage I 51%, Stage II 30%, Stage III 17% and Stage IV 4.2% [8]. According to Cheng et al. age and lymph node metastasis were the most significant prognostic factors of disease-free survival [9]. In the same study, recurrence was mentioned locally (58.8%), in the groins (5.9%) and distant metastases (14.7%) [9]. de Giorgi et al. in a retrospective study showed the different characteristics of vulvar basal cell carcinoma (average size was 2.1 cm and 28% were ulcerated at presentation) and proposed that basal cell carcinoma should be suspected whenever inflammatory vulvar lesions do not respond to usual treatment [10].

The excision margins also have an important role in the prognosis of women with vulvar cancer. Heaps et al. have already reported a sharp rise of local recurrence when the resection margins were less than 10 mm [11]. Gonzalez Bosquet et al. in a large retrospective study found that ipsilateral lymphadenectomy is suitable for patients with unilateral lesions distant from the midline, and either negative ipsilateral lymph nodes or positive ipsilateral lymph nodes with lesions smaller than two cm [12]. Landrum et al. compared the outcome in patients with advanced squamous vulvar carcinoma treated with surgery or primary chemoradiation and found no differences regarding overall survival, progression-free survival or recurrence rates (2 = 19). However, older patients with smaller lesions and positive lymph nodes tend to be surgically managed whereas younger patients with larger volume disease and fewer lymph node metastases are treated with primary chemoradiation [13]. Radiotherapy and conservative surgery could be an alternative to radical surgery with less morbidity in elderly patients [14]. According to Katz et al. radiotherapy alone or in combination with lymph node dissection is highly effective in preventing disease recurrence in patients with vulvar carcinoma [15]. Woolderink et al. showed that wide local excision and superficial inguinal lymphadenectomy with separate incisions result in a high groin recurrence rate. For this reason, deep inguinofemoral lymphadenectomy is proposed [16].

Gaavenstroom et al. [17] found that the main complications after vulvectomy and inguinofemoral lymphadenectomy using separable groin incisions are wound breakdown (17%) and/or infection (39%) of the groin, lymphocyst formation (40%) and lymphedema (28%). In a similar study of Hanprasertpong et al. the most common complication was wound infection, wound dehiscence, lymphosis and leg edema [3]. Magrina et al. showed that modified radical vulvar surgery is associated with decreased complications and five-year overall and disease-free survival and recurrence rates similar to those of radical vulvar surgery [18, 19]. Woolderink et al. showed that the 5-year local relapse-free survival is 70% [20]. After a local recurrence, 72% of the patients developed a second local recurrence [20].

Gonzalez Bosquet et al. stated that most recurrences usually occur within the first two years of follow-up, while 35% of their patients showed a recurrence five years or more after diagnosis [21]. Thus, long-term follow-up is proposed.

Landrum et al. compared data from 1990-2005 with data of the Gynecologic Oncology Group from 1977-1984 and found that the survival rates are similar in the minimal and low risk groups in spite of less radical surgery [22]. Furthermore, they found that five year survival rates for intermediate- and high-risk groups have improved due to the advancement in adjuvant chemoradiation and a younger patient population that presents with less advanced disease [22].

Conclusion

Vulvar carcinoma is a disease of elderly women. As life expectancy increases, more women are going to face this a problem in the near future. Clinicians should be aware of such an entity as early diagnosis and treatment are critical for the patient. Individualization of the treatment is proposed. A close follow-up is necessary.

References


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Clinical evaluation of vulvar lichen sclerosus: case series

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Summary

Introduction: Vulvar lichen sclerosus is a chronic dermatitis which is located in labial, perineal and perianal areas. The etiology of lichen sclerosus is multifactorial including genetic, autoimmune, hormonal and infectious aspects. Materials and Methods: A retrospective analysis was carried out of the medical records of 82 patients who were suffering from pruritus vulva. All patients had vulvar biopsy-proven diagnosis of lichen sclerosus. Results: Sixty-six of patients (80.4%) were in the postmenopausal period and 16 patients (19.5%) were in the premenopausal phase. Fifteen patients (18.2%) had thyroid disease, six had (7.3%) diabetes mellitus, five had (6.0%) asthma and five patients had (6.0%) other autoimmune diseases. Lichen sclerosus was most commonly located on the labia majora – 58 cases (70.7%). Sixty-four patients (78.04%) had used only potent corticosteroid therapy as the sole treatment. Conclusion: The first-line treatment is topical-potent or ultra-potent corticosteroids in the treatment of lichen sclerosis. Vulvar lichen sclerosis may be associated with autoimmune and thyroid diseases.

Key words: Vulval lichen sclerosus; Vulvar pruritis; Vulvar biopsy.

Introduction

Vulval lichen sclerosus is a chronic dermatitis which is located in the labial, perineal and perianal areas. A study by Lorenz et al. [1] found lichen sclerosus to be located on the labia (100%), clitoris (70.4%), perineum (67.9%), buttocks (32.3%), perianal area (32.1%), crural area (8.6%), and urethra (3.7%). Vulval lichen sclerosus is most commonly present in peri- or postmenopausal women. The etiology of lichen sclerosus is uncertain and probably multifactorial including a genetic, autoimmune, hormonal and infectious aspects.

A variety of symptoms may occur in patients with vulval lichen sclerosus. The most important symptom is pruritus, while others include soreness, burning, dyspareunia, pruritus and dysuria, and pain with defecation. Typical lesions of vulval lichen sclerosus are white, atrophic papules and secondary features such as hemorrhagic, purpuric, hyperkeratotic, edema, eroded or ulcerated lesions may be discovered [2]. Vulval lichen sclerosus may be a precursor for squamous hyperplasia [3] and may convert to invasive vulvar carcinoma in 4-6% of cases [4].

Materials and Methods

We retrospectively analyzed the medical records of 82 patients who were suffering from vulvar pruritus and admitted to our clinic (Department of Gynecology, Ege University Hospital) between 2004 and 2009. All the patients who had histopathologic biopsy results of lichen sclerosus were included in the study. All patients had vulvar biopsies and the toluidine blue test was used occasionally to identify areas of vulvar abnormalities. Blue staining areas that might represent abnormal skin were biopsied liberally. If there was no abnormal staining, multiple biopsies were taken from the most pruritic areas of the vulva.

Results

The age of the patients ranged from 37 to 77 years with a median of 56.48 years. Sixty-six patients (80.4%) were in the postmenopausal period and 16 patients (19.5%) were in the premenopausal phase. The median postmenopausal period was ten years (SD ± 10.38) (Table 1).

In our study 67 patients (81%) had a first biopsy, 14 patients (17%) a second biopsy and one (1%) patient a third biopsy. Seventy-two patients (87.8%) were non-smokers and ten (12.1%) patients were smokers. Medical histories of the patients revealed that 30 patients (36.5%) had hypertension, 15 patients (18.2%) had thyroid disease, six patients (7.3%) had diabetes mellitus, five patients (6%) had asthma and five patients (6%) had other autoimmune diseases including vitiligo, psoriasis and rheumatoid arthritis. Sixty-four patients (78%) had used only potent corticosteroid therapy for treatment, 11 patients (13.41%) had used a combination therapy of corticosteroids and topical testosterone and seven patients (8.53%) had used only topical testosterone therapy. The treatments continued at least eight weeks. Clobetasol propionate, halcinonide and fluocortolone are the potent corticosteroids that were administered for the therapy.

The frequency of lichen sclerosus varies depending on the location. In our study we found lichen sclerosus located on the labia majora in 58 cases (70.7%), clitoris 16 (19.5%), labia minora 26 (31.7%), posterior fourchette 35 (42.6%), periurethral area four (4.8%), mons pubis one (1.2%) and perineum one (1.2%) (Table 2).

Table 1. — Clinical characteristics of patients.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean 36.48</th>
<th>SD ± 8.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (n = 82)</td>
<td>66 (80.4%)</td>
<td>16 (19.5%)</td>
</tr>
<tr>
<td>Menopause</td>
<td>Mean 10.25</td>
<td>SD ± 10.3</td>
</tr>
<tr>
<td>Menopause time</td>
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could be related to Borellia
Borrelia burgdorferi which may explain the lack of efficacy of testosterone treatment [17]. Some studies have shown that nuclear androgen receptors in lichen sclerosus disease. This infection is associated with lar histologic features with acrodermatitis chronica atrophicans disease. This infection is associated with B. burgdorferi.

In one study, the expression of IL-4, TGF-β, IL-6 and interferon-γ have been determined in patients with lichen sclerosus [23]. Therefore topical corticosteroids might be effective because they impede the action of prostaglandins and leukotrienes by blocking local inflammatory processes.

Pruritis is the most frequent symptom in lichen sclerosus. Other symptoms are external dysuria, dyspareunia and a burning sensation in some patients. The lesions mostly appear in the perineum, labia majora, labia minora, fourchette and clitoris but most are found in the labia majora. We found the same findings in our study. Perianal involvement can create the classic “figure of eight” appearance. Extragential lesions are rare and have been reported in about 6% of all women with lichen sclerosus [24] the most common lesions involve the inner thigh, submammary area, neck, shoulders and wrists. Lesions are white, polygonal and flat-topped with papules or plaques. Lesions can also be hemorrhagic, purpuric, and hyperkeratotic. Atrophy can lead to loss of the labia minora, burying of the clitoris, and obstruction of urinary outflow.

The disease occurs mostly in the elderly population. One study found that 9% of cases occurred in prepubertal children, 49% occurred in women over 50 years of age and 42% occurred in the reproductive years [25]. In our study we found that 80.4% patients were in the postmenopausal period and the mean age was 56.4 years. Other diseases of the genitalia (lichen planus, vitiligo, cicatricial pemphigoid, and vulvar intraepithelial neoplasia) can look like lichen sclerosus in the absence of true skin biopsy of the lichen sclerosus.

Today the first-line treatment is topical corticosteroids in lichen sclerosus. Potent or ultra-potent corticosteroids are preferred. Clobetasol propionate (0.05%) or halobetasol propionate are the most commonly recommended regimens [36]. One study reported a remission rate of 77% [1]. There are different regimens for corticosteroid

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of patients (n = 82)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labia majora</td>
<td>58</td>
<td>70.7</td>
</tr>
<tr>
<td>Labia minora</td>
<td>26</td>
<td>31.7</td>
</tr>
<tr>
<td>Clitoris</td>
<td>16</td>
<td>19.5</td>
</tr>
<tr>
<td>Posterior fourchete</td>
<td>35</td>
<td>42.6</td>
</tr>
<tr>
<td>Periurethra</td>
<td>4</td>
<td>4.8</td>
</tr>
<tr>
<td>Perineum</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Mons pubis</td>
<td>1</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Discussion
The etiology of lichen sclerosus is still unclear. Lichen sclerosus has an important association with HLA type 2 antigens, particularly DQ7, DQ8 and DQ9. In one study, association with DQ7, DQ8 or DQ9 alone or combined was found in 78% of studied patients compared to 40% of controls [5]. Other studies have also shown that interleukin-1 (IL-1) receptors and antagonists could be related to disease severity [6, 7]. Autoimmune disorders have been found in patients with lichen sclerosus. One study showed that 60% of women with lichen sclerosus were reported to have one or more associated autoimmune disorders, particularly vitiligo, alopecia areata, and thyroid disease [8, 9]. We found 31.7% (26/82) of autoimmune disorders with lichen sclerosus in our study patients and the most common coexisting disease was hypertension, which is commonly prevalent in the elderly population.

Many studies showed an infectious organism as a causative factor in lichen sclerosus pathogenesis. These infectious organisms are acid-fast bacteria, human papillomavirus (HPV), hepatitis C virus and spirochete B. burgdorferi [10-12]. Lichen sclerosus has similar histologic features with acrodermatitis chronica atrophicans disease. This infection is associated with B. burgdorferi. In some studies Borellia has been identified in skin biopsy samples of lichen sclerosus [13, 14].

Lichen sclerosus is associated with low-estrogen levels like the prepuberty and postmenopausal period. Genital hormones might play a role in lichen sclerosus pathogenesis. One hypothesis is a reduction of the enzyme 5α-reductase in the vulva [15]. In this study serum levels of dihydrotestosterone and androstenedione were significantly decreased and the level of free testosterone was significantly increased in patients. After these studies topical testosterone treatment became popular [15]. However this treatment not so successful. Other studies have shown a decrease in nuclear androgen receptors in lichen sclerosus [16-38] which may explain the lack of efficacy of testosterone treatment [17]. Some studies have shown that nuclear estrogen receptor and progesterone receptor expression is normal. Therefore use of topical estrogen and oral contraceptives is not so effective [18-38].

In one study the percentage of peripheral T-lymphocytes was decreased in patients with lichen sclerosus compared with controls. Studies of vulvar lichen sclerosus have identified patterns of immune activation at the dermis [19]. In a recent study, the inflammatory infiltrate of lichen sclerosus contained epidermotropic CD3+, CD8+, CD57+ cells, increased intraepidermal HLA-DR+ cells and a dermal infiltrate with CD8+, CD57+, HLA-DR+ and CD68+ inflammatory cells [20]. However vulvar samples had a significantly lower number of CD2, CD3, CD4 and CD8 cells in the dermis and epidermis of patients with lichen sclerosus [21] and showed that lichen sclerosus is not caused by a cell-mediated response. In one study, TGFβ-1 and TGFβ-2 were increased in the upper and mid-dermis in lichen sclerosus [22]. In addition, the expression of IL-4, TGF-β, IL-6 and interferon-γ have been determined in patients with lichen sclerosus [23]. Therefore topical corticosteroids might be effective because they impede the action of prostaglandins and leukotrienes by blocking local inflammatory processes.
therapy. One regimen is clobetasol (0.05%) applied to the vulva twice daily for one month, then once daily for two months, and then twice weekly for a further three months. Another regimen uses clobetasol (0.05%) twice daily for one month, then daily for two months (not to exceed 30 g in 3 months) topically [35]. Long-term corticosteroid use is tapered by switching to a less potent corticosteroid and decreasing the frequency of application [28, 29]. Scarring is not reversible by any medical therapy. Intralesional injection of corticosteroids (triamcinolone acetonide) has been helpful for steroid-resistant patients [6]. Severe itching and steroid-resistant patients may respond to the addition of a topical 5% lidocaine and/or a low-dose tricyclic antidepressant. There is no place for surgery in treatment [30, 31]. Surgery should be preferred exclusively in patients with malignancy and to correct scarring secondary to the disease.

Vulvar lichen sclerosus is associated with a 4-6% risk of malignant transformation [32]. Studies have shown this risk to be between 0 and 20% [25-33]. Vulvectomy specimens of patients with squamous cell carcinoma showed lichen sclerosis in up to 61% of cases [34]. Squamous cell carcinoma is the primary malignancy associated with lichen sclerosis. In our study none of the patients had malignant transformation. All patients with lichen sclerosis should have long-term follow-up. Self-examination is important and any growth, erosion, ulcer, or any persistent lesion should be biopsied [37].

References


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Comparative findings of oncogenic cervical risk and its follow-up in two different periods 1982-1999 and 2000-2007

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National Association Against Cancer (ANCEC), Social Security Foundation (CSS), Panama City (Panama)

Summary

During the 2000-2007 period, my group and I reviewed the cases of 3,036 patients undergoing routine colposcopies, cytology and cervical biopsies for those cases that merited it, and a 20% increase was found in lesions defined as dysplasias as compared with those found in the studies of the previous century. This is something that reaffirms the association with HPV by 96.5% versus 80% in the last century. In relation to HPV infection, we found that the “pure” form was not only reached but rather surpassed by the findings associated with cancer and dysplasia in the years 2005-2006, a behavior considered irregular as compared to the usual.

The majority of the patients were guided toward conservative local treatments, among which cryosurgery and cauterization prevailed as choices, and their evolution resulted in an 81% cure (considered as destruction of the lesion and negativity of the cytology and eventual biopsy). It can be concluded that the high vulnerability of the virus, in spite of its great aggressiveness as an oncogenic risk (OR) to a cervical-uterine lesion, can be supported by the high degree of lesions diagnosed by OR.

Key words: Oncogenic risk; Incidence; Degree of pathology; Evolution.

Introduction

From 1982 to 2007 we reached coverage of 11,112 patients, performing 23,033 colposcopies, 22,504 colpocytologies and 5,587 directed biopsies. Of these patients, we selected those who attended in the 21st century, examined in the capital city and coming from all the provinces of the Republic of Panama, and those selected during a medical tour to the Pocri community in Los Santos Province in order to compare findings with the patients who attended between 1982 and 1999 (Table 1).

Between the years 1982 and 1999, our group took care of 8,076 patients and 3,737 patients in the 21st century, to whom we applied direct colposcopy, sample collection for cytology, extended colposcopy with sequential application of acetic acid, Lugol solution and the application of sodium bisulphite, followed by an eventual biopsy directed immediately or programmed for a later date [1-4].

Materials and Method

During the 25 years of study we evaluated 11,112 patients, of which 3,036 were evaluated between 2000 and 2007; 7,695 cytologies were carried out and 1,558 histological studies or directed biopsies were performed.

It should be pointed out that more than 50% of the population that attended came out of health management or as a result of health controls. In these cases we applied routine colposcopies and risk diagnosis was reached, with negative cytology in a significant number of them.

In other cases, the study was carried out applying selective colposcopy, as a consequence of cytologies that were clearly pathological or from referrals of clinical cervical lesions at risk and lastly, to those patients with three or more repetitive cytologies due to inflammatory alterations.

The colposcopy study was carried out respecting the usual steps of direct and extended colposcopy, to which the application of sodium bisulphite was added as a last step. Cytology was taken as a first step, before carrying out extended colposcopy in the cases of routine colposcopy, and an immediate biopsy when faced with an image or pathological picture, whereas on selective cases the colposcopy and the directed biopsy were directly and immediately carried out [5].

Once the diagnosis of oncogenic risk (OR) was reached, the patients were guided to conservative local treatments, among which cryosurgery and cauterization prevailed as choices, and their evolution resulted in an 81% cure (considered as destruction of the lesion and negativity of the cytology and eventual biopsy). It can be concluded that the high vulnerability of the virus, in spite of its great aggressiveness as an oncogenic risk (OR) to a cervical-uterine lesion, can be supported by the high degree of lesions diagnosed by OR.

Table 1. — Periods of study.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>8,076</td>
<td>3,036</td>
<td>11,112</td>
</tr>
<tr>
<td>Colposcopies</td>
<td>15,338</td>
<td>7,695</td>
<td>23,033</td>
</tr>
<tr>
<td>Cytologies</td>
<td>14,897</td>
<td>7,607</td>
<td>22,504</td>
</tr>
<tr>
<td>Biopsies</td>
<td>4,029</td>
<td>1,558</td>
<td>5,587</td>
</tr>
<tr>
<td>OR</td>
<td>4,439</td>
<td>1,567</td>
<td>6,006</td>
</tr>
<tr>
<td>OR %</td>
<td>56%</td>
<td>52%</td>
<td>54%</td>
</tr>
</tbody>
</table>

OR: oncologic risk.

Results

From the 3,773 patients who attended in the 21st century, 3,036 patients were new cases and the rest had already been visited.

Even though the total OR incidence was 56% and 52%, respectively for the period 1982-2000 and 2000-2007 it was observed that the breakdown of the different pathologies underwent significant changes, with a marked decrease of HPV infection in its “pure” form of 18%, and...
of cancer by 2%, whereas dysplasias increased by 19.5%, and in colposcopic lesions only there were no changes (Table 1). HPV diagnosis in totality (pure or associated to dysplasia or cancer) comparatively maintained its incidence for the three evaluated cycles I/II/III (85.5% - 84.4% - 85.3%, respectively) (Table 2).

Likewise, when we reviewed the influence of HPV on OR, we realized that for the 21st century it had increased by 20%, for those cases associated with dysplasia or cancer, and its influence in this association was almost exclusively in the genesis of the dysplasias or in association with them by 96% (Table 3).

HPV infection, in spite of a decreased incidence with 834 cases, had a significant 20% increase in associated cases, which we demonstrated in our annual analysis that in the years 2005 and 2006 the associated HPV cases surpassed HPV only, and these are considered as "crossroads between these two HPV forms" [6].

We dedicated special attention to OR in the 21st century, as its behavior is most particular. The greatest contribution came from the new patients (86.3%), followed by the patients already in the study and without pathology up to now (8.4%), and lastly from those patients who progressed to a greater degree of pathology (5.2%).

The pathologic diagnosis of studied cases between 2000-2007 was: HPV only 53.2%, dysplasias 31.9%, cancer 2%, the colposcopic pathology (OR) 12.5% and finally herpes 0.4%. These numbers indicate that dysplasias increased in such a marked way to the point of surpassing the cases of HPV only in the years 2005 and 2006, but keeping the proportions with reference to the mild, moderate and severe cases, respectively with 313, 153 and 39 cases (Table 4) [7, 8].

These results compared with those published in 1993 demonstrate an elevated increase in pathological lesions. The majority of patients underwent conservative local treatments, 59% of them corresponding to cryotherapy, 32% to cauterizations and 9% to others, for a total of 814 patients, equivalent to 52% of the patients with OR in this century (Table 5).

OR: oncologic risk.

Table 2. — Comparative findings of oncogenic risk (OR) from 1982-2007.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV</td>
<td>3,160 71%</td>
<td>834 53%</td>
<td>3,994 66.5%</td>
</tr>
<tr>
<td>Dysplasias</td>
<td>550 12.4%</td>
<td>500 31.8%</td>
<td>1,050 17.4%</td>
</tr>
<tr>
<td>Cancers</td>
<td>175 3.9%</td>
<td>31 1.9%</td>
<td>206 3.4%</td>
</tr>
<tr>
<td>Pure OR</td>
<td>548 12.3%</td>
<td>196 12.5%</td>
<td>744 12.5%</td>
</tr>
<tr>
<td>Herpes virus</td>
<td>6 0.1%</td>
<td>6 0.2%</td>
<td>12 0.2%</td>
</tr>
<tr>
<td>Total OR</td>
<td>4,439 55%</td>
<td>1,567 51.6%</td>
<td>6,006 50.1%</td>
</tr>
<tr>
<td>Total HPV</td>
<td>3,799 85.5%</td>
<td>1,330 84.4%</td>
<td>5,129 85.3%</td>
</tr>
</tbody>
</table>

Table 3. — Behavior of HPV and/or associated disease.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV</td>
<td>3,160 83%</td>
<td>834 63%</td>
<td>3,994 78%</td>
</tr>
<tr>
<td>HPV assoc.</td>
<td>639 17%</td>
<td>496 37%</td>
<td>1,135 22%</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>514 80%</td>
<td>476 96.2%</td>
<td>990 87%</td>
</tr>
<tr>
<td>Cancer</td>
<td>125 20%</td>
<td>20 3.8%</td>
<td>145 13%</td>
</tr>
<tr>
<td>Total HPV</td>
<td>3,799 130</td>
<td>1,330 84.4%</td>
<td>5,129 85.3%</td>
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</tbody>
</table>


<table>
<thead>
<tr>
<th>Pathology</th>
<th>00</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>Total</th>
<th>%</th>
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<tr>
<td>Colposcopic pathology (OR)</td>
<td>41</td>
<td>41</td>
<td>45</td>
<td>20</td>
<td>17</td>
<td>15</td>
<td>20</td>
<td>239</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Herpes</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0.4</td>
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<tr>
<td>HPV</td>
<td>93</td>
<td>58</td>
<td>140</td>
<td>219</td>
<td>111</td>
<td>112</td>
<td>66</td>
<td>68</td>
<td>867</td>
<td>53.2</td>
</tr>
<tr>
<td>Mild dysplasia</td>
<td>19</td>
<td>11</td>
<td>31</td>
<td>53</td>
<td>11</td>
<td>70</td>
<td>75</td>
<td>43</td>
<td>313</td>
<td></td>
</tr>
<tr>
<td>Moderate dysplasia</td>
<td>6</td>
<td>1</td>
<td>14</td>
<td>25</td>
<td>17</td>
<td>34</td>
<td>34</td>
<td>22</td>
<td>153</td>
<td>31.9</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>In situ carcinoma</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Adeno carcinoma</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>120</td>
<td>241</td>
<td>354</td>
<td>165</td>
<td>244</td>
<td>200</td>
<td>156</td>
<td>1,648</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. — Local conservative treatments.

<table>
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<tr>
<th>Types of treatments</th>
<th>00</th>
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<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryotherapy</td>
<td>18</td>
<td>28</td>
<td>62</td>
<td>83</td>
<td>47</td>
<td>110</td>
<td>70</td>
<td>60</td>
<td>478</td>
<td>59</td>
</tr>
<tr>
<td>Cauterization</td>
<td>58</td>
<td>4</td>
<td>62</td>
<td>75</td>
<td>18</td>
<td>17</td>
<td>11</td>
<td>13</td>
<td>258</td>
<td>32</td>
</tr>
<tr>
<td>Others</td>
<td>27</td>
<td>17</td>
<td>12</td>
<td>5</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>78</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>32</td>
<td>124</td>
<td>158</td>
<td>65</td>
<td>127</td>
<td>81</td>
<td>73</td>
<td>814</td>
<td>100</td>
</tr>
</tbody>
</table>

Patients with OR whose follow-up was carried out to the year 2007.

The follow-up of patients with known evolution allows us to know and compare their responses to local treatments, which in 2007 corresponded to a cure in 81% of the cases, considered as clinical disappearance of the lesion, negativity of the cytology and/or histology to improvement in 6% of the cases, persistence in 6.1% and progression in only 1.6%. Such numbers are the ones found in patients evaluated in the year 2007 and correspond to all patients with OR in the 25 years of the study (Table 6).

Table 6. — Evolution of patients with OR.

<table>
<thead>
<tr>
<th>Evolution</th>
<th>00</th>
<th>01</th>
<th>02</th>
<th>03</th>
<th>04</th>
<th>05</th>
<th>06</th>
<th>07</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cure</td>
<td>214</td>
<td>243</td>
<td>238</td>
<td>273</td>
<td>223</td>
<td>262</td>
<td>424</td>
<td>402</td>
<td>81</td>
</tr>
<tr>
<td>Improvement</td>
<td>24</td>
<td>44</td>
<td>30</td>
<td>26</td>
<td>46</td>
<td>47</td>
<td>40</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>Persistence</td>
<td>15</td>
<td>18</td>
<td>8</td>
<td>33</td>
<td>35</td>
<td>25</td>
<td>46</td>
<td>33</td>
<td>6.1</td>
</tr>
<tr>
<td>Recurrence</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>26</td>
<td>29</td>
<td>17</td>
<td>21</td>
<td>23</td>
<td>4.6</td>
</tr>
<tr>
<td>Progression</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>15</td>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td>Total cures</td>
<td>261</td>
<td>320</td>
<td>292</td>
<td>373</td>
<td>348</td>
<td>365</td>
<td>546</td>
<td>496</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>82</td>
<td>76</td>
<td>81.5</td>
<td>73</td>
<td>64</td>
<td>72</td>
<td>72</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The natural history of cervical-uterine cancer, as well as that of HPV infection, has experienced significant changes in the last 50 years, including the precociousness of appearance and the degree of the pathological lesion to its fast evolution. Cures, represented by eradication of clinical lesions in association with negative cytologies and/or histologies, reach excellent numbers because of strict follow-up colposcopic pathology, indicating and justifying that the preventive measures applied in our population are valid [10]. In spite of findings of a greater degree of dysplastic pathology, we accomplished destruction of the lesions with accurate local conservative treatments demonstrated by the follow-up of these patients, which could translate into a greater vulnerability of HPV and its effect on the uterine cervix. The national policy regarding cervical-uterine cancer should be updated and its application periodically monitored to either confirm or make the necessary changes to avoid inertia and thus save lives [11-15].

Conclusions

In spite of findings of a greater degree of dysplastic pathology, we accomplished destruction of the lesions with accurate local conservative treatments demonstrated by the follow-up of these patients, which could translate into greater vulnerability of HPV and its effect on the uterine cervix.

References


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Evaluation of LOOP electrosurgical excisional procedure: case series

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¹Department of Obstetrics and Gynecology, ²Department of Pathology, Ege University Faculty of Medicine, Izmir (Turkey)

Summary

Objective: To evaluate the loop electrosurgical excisional procedure (LEEP) which is performed to treat high-grade cervical intraepithelial lesions (HGSIL). Material and Methods: Twenty-one cases diagnosed as LGSIL and HGSIL after histopathological examination were included in the study among patients who had cervical colposcopy-directed biopsies after an abnormal cytology report at Ege University School of Medicine, Obstetrics and Gynecology Department between the years of 2007 and 2009. The patients underwent LEEP or LEEP-cone procedures. Results: The patients with cervical smear results of ten ASCUS, eight LGSIL, and three HGSIL underwent colposcopy-guided punch biopsies. Those with the result of CIN 1 and CIN 2 underwent LEEP or LEEP-cone procedures. Pathologic examination correlated with biopsy results and surgical margins were all negative. All patients were followed-up with cervical cytology. Conclusion: LEEP and LEEP-cone procedures are therapeutic procedures in cervical intraepithelial lesions.

Key words: LEEP, HGSIL.

Introduction

The loop electrosurgical excisional procedure (LEEP) is both a diagnostic and therapeutic procedure for high-grade cervical intraepithelial lesions. It permits complete excision of transformation zone which includes preinvasive cervical lesions. The LEEP was mostly popularized in the 1980s by the studies of Cartier et al. and Prendiville et al. [1, 2]. The important parameters that determine the residual lesion in a LEEP specimen are histologic type, grade, glandular involvement and exocervical and endocervical margin status. In order not to have a residual cervical lesion, the entire transformation zone should be removed. LEEP-cone is the term used when the traditional LEEP is followed by a second pass or top hat. The aim of the top hat is to remove the cervical tissue in the endocervical canal and this procedure is equivalent to a cold knife conization [3]. The indications for LEEP-cone is a two-grade difference between cytology and cervical biopsy, positive endocervical curettage and unsatisfactory colposcopy [4].

In the present study, we reviewed the LEEP and LEEP-cone procedures in our clinic and compared preoperative factors with the pathologic results.

Material and Method

All the LEEP and LEEP-cone procedures at the University of Ege, Faculty of Medicine, Department of Obstetrics and Gynecology from 2004 to 2009 were included. At the time of the patient’s initial visit due to an abnormal cytology report, colposcopic evaluation was performed with 5% acetic acid with both green filter and normal lighting to delineate cervical lesions and the borders of the transformation zone. Multiple cervical biopsies were performed and the LEEP or LEEP-cone was scheduled.

The LEEP-cone was performed with a variable sized loop electrode attached to an electrosurgical unit (Valleylab®) with a cut setting of 50W and coagulation setting of 50W.LEEP dimensions were typically 15-20 mm in diameter and the excisional procedure was performed to a depth of 7 mm. A second pass was performed using a smaller loop electrode with the same settings. Coagulation was performed at the cervical bed using 50W coagulation. All specimens were evaluated at the University of Ege, Department of Pathology. Margin status was reported as positive if dysplasia was present histologically at the deep (endocervical) resection margin. Margin status was reported for both the first and second pass. All patients were instructed to be followed-up every three to six months for cervical cytology for a minimum of one year after the procedure. Patients were considered to be positive for persistent disease if both cytology and biopsy were positive for dysplasia within 12 months after the procedure. Patients who were positive for dysplasia after 12 months were considered to be recurrent cases.

Results

The ages of patients were between 24-44 years. All 21 patients with the cervical smear results of ten ASCUS, eight LGSIL, and three HGSIL underwent colposcopy-guided punch biopsies. Seven were diagnosed as koilocytosis with negative HPV, eight were diagnosed as CIN 1 and seven were diagnosed as CIN 2. Four patients with the diagnosis of ASCUS in the cervical cytology were diagnosed as koilocytosis. Three patients with the diagnosis of LGSIL in the cervical cytology were diagnosed as koilocytosis. Four patients diagnosed as LGSIL in the cervical cytology were diagnosed as koilocytosis. Four patients diagnosed as LGSIL in the cervical cytology were diagnosed as CIN 1 and one patient diagnosed as LGSIL was diagnosed as CIN 2, respectively (Table). All CIN 1 and CIN 2 patients underwent LEEP or LEEP-cone procedures. Pathologic examination correlated with biopsy results and surgical margins were all negative. All patients were followed-up with cervical cytology.
Discussion

In young women the main goal is to avoid the use of the LEEP-cone procedure in order not to shorten the cervical length. Approximately 60% of CIN-1 lesions will regress spontaneously when followed over a 1- to 2-year period. If there is no treatment, regression rate of CIN-2 will be 43% and persistence rate 35%, and progression rate 22%. CIN-3 regresses in 32%, persists in 56% and progresses to cancer in over 12% [5]. In our study all the LEEP procedures performed to CIN 1 patients were according to the patients’ desire.

Tillmanns et al. [6] in a retrospective analysis defined the indications of LEEP-cone. Women under 21 years of age should have a single pass LEEP technique. Age over 35 was the greatest percentile predictor of dysplasia in the top hat, and 91.5% of women under 21 years had normal top hat pathology. Women 35 and older may require a second pass or LEEP-cone to include the whole squamocolumnar junction, which tends to be higher in the endocervical canal. Moreover, there is a risk of deeper dysplastic invasion as a result of persistent disease in older women.

The incomplete excision of neoplastic epithelium is noted in a considerably high proportion of women and is mainly at the endocervical cone margin [7]. Extensive endocervical cone margin involvement is a significant predictor for residual lesion after LEEP [8].

Ayhan et al. [9] evaluated the effectiveness of repeat LEEP procedure in patients with CIN-3 and positive ectocervical margins. Final diagnosis after the repeat LEEP procedure revealed six women (10.7%) with microinvasive squamous cell carcinoma and 27 (37.5%) with CIN-3. Repeat LEEP may reveal microinvasive cervical carcinoma in women with positive ectocervical margins.

In a meta-analysis including 35,109 women Ghaem-Maghami et al. [10] found that in women with complete excision the rate of recurrence of low-grade or high-grade abnormality was 4% and of high-grade alone 3% compared to 20% (low- or high-grade) and 18% (high-grade) where the margins were not reported as being clear of the dysplastic process.

Conclusion

Cervical intraepithelial lesion incidence is increasing worldwide and LEEP and LEEP-cone procedures can be performed safely in patients who cannot undergo regular examination; moreover they are one-step therapeutic procedures for cervical intreptithelial lesions.

References


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Systemic chemotherapy - before or after radical surgery in treatment of patients with advanced ovarian carcinoma?

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Summary

Purpose of investigation: The aim of the study was to analyze whether application of systemic chemotherapy prior to radical surgery in patients with advanced ovarian carcinoma influences the length of the progression-free period and overall survival rate.

Methods: This study analyzes two groups of patients in the period 2006-2009. The first group received systemic chemotherapy prior to radical surgery. The second group first had radical surgery after which systemic chemotherapy was administered. The therapeutic response to systemic chemotherapy was analyzed as well as progression-free survival and overall survival which was calculated according to the Kaplan-Meier method and compared using the log rank test.

Results: Statistical analysis showed that patients who were administered systemic chemotherapy prior to radical surgery have significantly better progression-free survival as well as overall survival. Progression-free survival in patients who were first treated with systemic chemotherapy after which radical surgery followed was equal to 28 months and was significantly longer \( p = 0.001 \) than progression-free survival in patients who were first subjected to radical surgery; it equaled 13 months, while the overall survival equaled 43 and 36 months, respectively.

Conclusion: Application of systemic chemotherapy prior to radical surgery has a significant influence on the length of the progression-free period and on the length of overall survival of patients affected by advanced ovarian cancer.

Key words: Ovarian carcinoma; Radical surgery; Chemotherapy; Overall survival; Progression-free survival.

Introduction

Ovarian carcinoma is the sixth most frequent malignant tumor in the world. Every year a total of approximately 204,000 new cases of ovarian carcinoma are registered while 125,000 women die of this disease [1]. References often refer to ovarian carcinoma as the gynecological killer “number one” in the modern world [2]. Therefore a very large number of scientists across the world are working on the issues of treatment and prolongation of patient life-span.

When treatment of ovarian carcinoma is concerned, surgery has a significant role. It is the first therapeutic procedure which facilitates histopathological diagnostics, gives an insight into the outspread of the disease, makes it possible to remove tumor tissue, and determines further treatment of the patient [2].

Nevertheless, in patients with advanced stage of ovarian carcinoma it is sometimes very difficult to perform radical surgery. Most frequent complications of extensive cytoreduction include: prolonged surgery duration, increased blood loss, possible anastomotic complications as well as potential vascular damage, frequent pulmonary embolisms in postoperative period, prolonged postoperative period which causes delays in application of systemic chemotherapy or other types of oncological treatments until complete postoperative recovery [3, 4].

Systemic chemotherapy has been proven effective in treatment of ovarian carcinoma. It has significantly prolonged the progression-free survival period as well as the overall survival of patients with ovarian malignant tumours [5].

It has been noted that execution of radical surgery is made much easier by previous application of systemic chemotherapy [6]. There is a demarcation line of the tumor tissue. Tumor neovascularization is reduced. Intraoperative bleeding is reduced as well as need for blood and blood derivatives. Surgery duration is shorter and there are less intraoperative and postoperative complications. Postoperative recovery is faster which, in turn, ensures faster continuation of treatment by systemic chemotherapy.

The aim of the study was to analyze whether application of systemic chemotherapy prior to radical surgery in patients with advanced ovarian carcinoma influences the length of the progression-free period and overall survival of patients.

Material and Methods

This prospective and study analyzed 46 patients with advanced ovarian carcinoma diagnosed and treated in the Obstetrical and Gynecological Clinic “Narodni Front” in the time period 2006-2009. Patient average age was 57 and they were all affected by histopathologically diagnosed serous ovarian carcinoma. All patients were subjected to explorative laparotomy which included abdominal exploration and tumor biopsy as means of histopathologic diagnostics. The decision whether to start treatment by radical surgery, i.e., whether to continue with a radical surgical procedure or to start treatment by application of systemic chemotherapy after histological diagnostics done during biopsy depended on the assessment of intraoperative findings performed by the surgeon. In these cases, the surgeon was a gynecologist with vast experience in oncological surgery and the assessment was based on prediction of intraoperative and postoperative complications which could prolong the postoperative recovery period and postpone systemic chemotherapy until complete recovery. Such complica-
tions include extensive blood loss, vast amounts of free fluid, highly vascularized tumor, risks of thromboembolism-related complications, need to place a stoma onto the anterior abdominal wall and so on.

In this way, patients were divided in two groups based on the primary therapy applied, i.e., whether systemic chemotherapy or radical surgery was used first. The first group consisted of 27 patients who were first administered systemic chemotherapy which was followed by radical surgery. The second group consisted of 19 patients who underwent radical surgery (which included classic hysterectomy with bilateral salpingo-oophorectomy and complete omentectomy) followed by systemic chemotherapy.

All patients were administered systemic chemotherapy with taxol and carboplatin. Therapeutic response to applied chemotherapy was analyzed using the chi-square test.

After completion of chemotherapy (about 12 to 14 months after the first surgery), the surgery was performed.

In the first group of patients it was a radical surgery, i.e., classic hysterectomy with bilateral salpingo-oophorectomy and complete omentectomy were performed – maximum cytoreduction was performed. In the second group, it was a second-look surgery, i.e., reexploration of the abdomen and cytoreduction.

Progression-free survival as well as overall survival of patients has been calculated according to the Kaplan-Meier method while comparison of survival rates of the two groups was done according to the log rank test.

Results

Forty-six patients whose average age equaled 57 participated in the study. All patients were affected by histopathologically verified serous ovarian carcinoma. Patients were divided in two groups, based on the primary treatment type, i.e., whether systemic chemotherapy or radical surgery was administered first.

Group 1: The first group consisted of 27 patients who were administered systemic chemotherapy prior to radical surgery. Group 2: The second group consisted of 19 patients who were administered systemic chemotherapy after radical surgery. All patients were administered taxol carboplatin systemic chemotherapy.

Systemic chemotherapy response assessment was performed based on clinical, ultrasonographic (US) and computed tomography (CT) findings (before the surgery) as well as based on intraoperative findings during the surgery.

Analysis of the therapeutic response to chemotherapy yielded the following results.

Good response to chemotherapy was obtained from as much as 93.6% of patients from the first group; complete disease regression affected 51.9% of the patients while incomplete disease regression was found in 40.7% of the patients. Only 7.4% of the patients reacted with no response or poor response to therapy.

Good response to chemotherapy was obtained from 78.9% from the second group while 21.1% of patients belonging to this group responded poorly to therapy.

As far as primary chemotherapy response is concerned, a statistically highly significant difference between the two groups of patients has been ascertained ($X^2 = 10.631; p < 0.01$) i.e., patients who received chemotherapy prior to radical surgery responded to chemotherapy much better.

After the surgery, patients were subjected to regular quarterly checkups.

Disease relapse has been diagnosed by US and CT diagnostics.

It was ascertained that relapse occurred in all patients who were first subjected to radical surgery after which they were administered systemic chemotherapy (100%) and only in half of the patients who were administered systemic chemotherapy first and later had radical surgery (51.9%). Statistical analysis showed that a highly statistically significant difference exists between the two patient groups regarding relapse occurrence ($X^2 = 12.752; p < 0.0001$), i.e., relapse occurrence was much more frequent in patients who were first subjected to a radical surgical procedure and then received chemotherapy than in patients who received systemic chemotherapy first and had radical surgery afterwards.
The Kaplan-Meier method was used to calculate initial patient progression-free survival which equaled 28 months for patients from the first group, i.e., for patients who received systemic chemotherapy prior to radical surgery as opposed to 13 months for patients from the second group, i.e., patients who were first treated by radical surgery and by systemic chemotherapy afterwards. Statistical analysis showed the existence of a highly statistically significant difference in progression-free survival between the observed groups of subjects (X² = 16,260; p < 0.0001) meaning that progression-free survival was significantly longer in the group of patients who were first administered systemic chemotherapy after which radical surgery was performed (Figure 1). Overall survival of patients was also calculated by the Kaplan-Meier method. It was ascertained that average overall survival for patients from the first group equaled 43 months compared to 36 months for patients from the second group (Figure 2).

Discussion

In recent years various references include discussions about when radical surgery should be applied and when systemic chemotherapy should be used, i.e., which is the optimal moment for radical surgical treatment of patients with advanced ovarian carcinoma [7].

Even though primary cytoreduction still represents the primary method of treatment of ovarian carcinoma, the role of chemotherapy is obvious. Application of new therapy modalities with platinum (taxol carboplatin, etc.) has been proven to be very efficient in treatment and control of ovarian carcinoma [8]. It has been noted that ovarian carcinoma patients respond very well to systemic chemotherapy, which can lead to “transformation” from inoperable into operable state. Several papers describe the role of cytoreductive surgery after application of systemic chemotherapy. The research performed by Matsumoto and collaborators in 2006 [9], for example, has shown that survival of patients first treated with systemic chemotherapy after which radical surgery was performed, equaled 41.7 months. This survival was significantly longer (p < 0.01) than survival of patients who first underwent radical surgery after which systemic chemotherapy was administered – in this case survival equaled only 18.8 months.

The role of systemic chemotherapy prior to radical surgery becomes even more important when taking into account that it facilitates subsequent performance of optimal radical cytoreduction, which is still the key factor influencing the survival data, coupled with the possibility of intraoperative intraperitoneal application of chemotherapy drugs. It has been determined that intraperitoneal application of chemotherapy drugs during intraoperative treatment doubles survival length [10].

This research has shown that patients with advanced ovarian carcinoma respond well to systemic chemotherapy; in cases where chemotherapy was the primary treatment method, it was noted that patients react even better. Progression-free survival was significantly longer in patients who were first treated by systemic chemotherapy after which radical surgery was performed. When this fact is considered from the aspect of quality of life of patients with advanced malignant disease, a completely new dimension of malignant ovarian disease treatment is reached [11, 12]. There was also a difference in the overall three-year survival of patients [13, 14]. Patients first treated with systemic chemotherapy followed by radical surgery had an average survival of 43 months, while patients who were primarily treated with radical surgery followed by systemic chemotherapy had an average survival of 36 months.

Performed research has shown that application of systemic chemotherapy prior to radical surgery has a significant influence on disease remission duration as well as on the overall survival of patients.

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Normal-sized ovarian papillary serous carcinoma: a case report

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Summary

A normal-sized ovarian papillary serous carcinoma is rare. We present the case of a 46-year-old woman with progressive abdominal fullness of one week's duration. The medical evaluation revealed abdominal carcinomatosis with normal-sized ovaries and an elevated serum CA-125 level of 147,365.8 U/ml. Cytoreductive surgery (hysterectomy, bilateral salpingo-oophorectomy, omentectomy, lymphadenectomy, infracolic omentectomy, peritoneal biopsy, washing cytology, and appendectomy) was performed. The histologic examination revealed an ovarian serous papillary carcinoma. Adjuvant chemotherapy was administered. The serum CA-125 level decreased after completion of treatment. Normal-sized ovarian serous surface papillary carcinomas should be kept in mind as an origin of disease in patients who have peritoneal carcinomatosis, which sometimes is a diagnostic dilemma of the disease source. We report this case to emphasize the clinical symptoms and importance of the early and accurate diagnosis of a normal-sized ovarian papillary serous carcinoma.

Key words: Papillary serous carcinoma; Normal sized ovary; Primary ovarian carcinoma.

Introduction

Epithelial ovarian carcinoma is the most lethal gynecologic cancer among women, and accounts for 4% of all malignant tumors in Western countries [1]. In Taiwan, an estimated 600 new cases of epithelial ovarian carcinoma are diagnosed annually, and approximately 350 women will die from the disease [2]. The high mortality rate of epithelial ovarian carcinoma may be partly explained by the difficulty in early diagnosis and the lack of a reliable screening test. The common presentation of epithelial ovarian carcinomas includes an enlarged ovarian tumor and ascites. However, an ovarian carcinoma which presents with a massive amount of ascites without ovarian enlargement is rarely encountered.

In 1989 Feuer [3] first introduced the concept of normal-sized ovarian carcinoma syndrome. A clinical situation occasionally presents in which diffuse metastatic disease of the peritoneal cavity is noted, but the ovaries are of normal size, with or without a fine granularity on the external surface. Normal-sized ovarian carcinoma syndrome has been subdivided into the following categories: mesotheliomas, primary peritoneal carcinoma (PPC), metastatic tumor from another primary origin, and primary ovarian carcinoma.

Among the different subtypes, PPC should be considered first in female patients [4, 5]. Primary epithelial ovarian cancer should be considered next among the differential diagnoses in normal-sized ovarian carcinoma syndrome [5]. Primary epithelial ovarian carcinoma of normal-sized ovary syndrome is a rare neoplasm and is characterized histologically by an exophytic papillary tumor originating from the surface epithelium. Those patients with primary epithelial ovarian cancers and ovaries of normal size may receive a late diagnosis due to the absence of enlarged ovaries and the resulting poor prognosis. In addition, the biological behavior of normal-sized ovary syndrome might be different from common epithelial ovarian cancer with ovarian enlargement.

We present a case of a papillary serous carcinoma in an ovary of normal size with peritoneal cavity spread in a 46-year-old Taiwanese woman.

Case Report

A 46-year-old gravida 1, para 1, presented with progressive abdominal fullness of one week's duration. The physical examination revealed abdominal distension and shifting dullness; no palpable mass was noted. A bimanual vaginal examination revealed a normal uterus without any adnexal enlargement. Ultrasonography (US) revealed massive ascites and both ovaries were confirmed to be of normal size (Figure 1). Computed tomography (CT) indicated massive ascites with an omental caking (Figure 2). The serum CA-125 value was 147,365.8 U/ml (normal level, < 35 U/ml). The other laboratory tests were normal. On the basis of the clinical examination findings, there was no suspicion of a primary malignancy, such as gastric, pancreatic tail, colon, or chest. An exploratory laparotomy was performed and grossly revealed gray-tan irregular nodules with involvement of the right ovarian surface. Additionally, massive ascites and omental caking were noted. The histopathologic examination of a frozen section showed serous papillary carcinoma of the ovary. The left ovary appeared normal.

Cytoreductive surgery, including a total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, and appendectomy was performed. In addition, pelvic and paraaortic lymphadenectomy and biopsy of the peritoneum were performed. The final histopathologic examination revealed serous papillary carcinoma of the ovary and extra-ovarian metastasis. The final diagnosis was Stage IIIC ovarian serous papillary adenocarcinoma.
The patient received adjuvant chemotherapy consisting of carboplatin (area under the concentration curve = 5) and taxol (175 mg/m²). The serum CA-125 level decreased after completion of treatment.

**Discussion**

Epithelial ovarian carcinomas are believed to originate from the surface epithelium covering the ovary. The most common symptoms of epithelial ovarian carcinomas include abdominal distention and pain. Some patients are asymptomatic. Therefore, the most common presentation is a large ovarian mass at the time of diagnosis. However, normal-sized ovarian carcinomas with disseminated peritoneal spread are rare and this primary epithelial ovarian cancer has been classified as a normal-sized ovarian carcinoma syndrome.

In our case, the tumor was histologically identical to serous papillary carcinomas originating from the surface epithelium of normal-sized ovaries. Embryologically, serous surface papillary carcinoma of the ovary is assumed to originate from the ovarian epithelium and the peritoneal mesoderm that potentially could became Mullerian ducts or from the malignant degeneration of nests of ovarian tissue remnants in the peritoneum [6]. Serous surface papillary carcinoma of the ovary is a rare neoplasm found in women that is characterized histologically by an exophytic papillary tumor originating from the surface epithelium of the ovary. The characteristic growth pattern of this carcinoma of the ovary is characterized by peritoneal seeding; with only surface involvement of the ovaries, both ovaries are likely to be of normal size [7-9]. However, the clinical presentation and histologic appearance of this disease are quite similar to other malignant ovarian neoplasms and it has a great tendency to spread externally [10].

The peak incidence of epithelial ovarian carcinoma is 50-60 years of age. A previous study reported that the mean age of patients with PCC was 64 years (range, 56-69 years), and the median age of patients with ovarian serous carcinoma was 52 years [5]. Additionally, ovarian cancer has been associated with nulliparas, low parity, and infertility. A reduction in risk by as much as 40%-60% among multiparas has been reported consistently [11, 12]. However, Choi et al. [5] described all the patients in both groups (PPC and papillary serous carcinoma in ovaries of normal size) to be multiparas [5].
Serum CA-125 level is useful in distinguishing benign from malignant adnexal masses, following the response to treatment of ovarian cancer, and monitoring tumor recurrence. In one report, when the serum CA-125 level was > 1000 U/ml, 89% of patients had gynecologic cancer, 7% had non-gynecologic cancers, and 3% had benign conditions [13]. In a case series report, the serum CA-125 level was reported to be elevated in all patients with normal-sized ovarian papillary serous carcinoma; moreover, in most patients, the level was > 200 U/ml [14]. We believe that a highly elevated serum CA-125 level is a critical indicator that suggests the possibility of serous surface papillary carcinoma of the ovary. Therefore, CA-125 levels are useful for estimating the response of cytoreductive surgery or chemotherapy and monitoring tumor recurrence.

Several reports have described the most characteristic CT features of serous surface papillary carcinomas of the ovary as mesenteric or omental involvement, ascites, peritoneal thickening, and normal-appearing ovaries [15-17]. The MRI findings of papillary serous carcinomas in ovaries of normal size in the current study were similar [5]. Therefore, primary ovarian carcinoma should be included in the differential diagnosis of patients with diffuse peritoneal seeding and ovaries of normal size. In assessing cases of serous surface papillary carcinoma of the ovary it is important to distinguish between this carcinoma and peritoneal carcinomatosis from another primary malignancy. On serial imaging, the small foci of malignancy may not be identified, and some cases of gastrointestinal malignancy can be overlooked. Therefore, the absence of a visible primary tumor cannot always suggest the possibility of serous surface papillary carcinomas of the ovary or exclude the possibility of peritoneal carcinomatosis from another primary malignancy.

The treatment results and prognosis for patients with serous surface papillary carcinoma of the ovary remains controversial. A recent report showed that serous surface papillary carcinoma of the ovary responded completely to initial cytoreductive surgery followed by platinum-based chemotherapy [8]. Another report presented evidence of long-term survival of patients with serous surface papillary carcinomas of the ovary [18]. In patients treated with adjuvant paclitaxel and platinum for Stage III/IV epithelial ovarian carcinoma, the clinical complete response rate and progression-free survival have been reported to be 54% and 18 months, respectively [5]. Our patient was treated aggressively with cytoreductive surgery and adjuvant chemotherapy.

In conclusion, it is difficult to establish a preoperative diagnosis in patients with normal sized ovarian carcinoma syndrome. Therefore, serous surface papillary carcinoma of the ovary should be kept in mind as a possible diagnosis in patients with extensive peritoneal carcinomatosis, relatively normal-sized ovaries, and a highly elevated serum CA-125 level. In the case of patients with serous surface papillary carcinoma of the ovary, cytoreductive surgery followed by adjuvant chemotherapy is recommended. We emphasize the importance for clinical gynecologists to be familiar with normal-sized ovarian carcinoma syndrome in order to make an early and correct diagnosis.

References


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Case Report

A 38-year-old gravida 3, parity 3 female presented to the emergency department after three weeks of abnormal vaginal bleeding. Additional symptoms included mid-abdominal cramping, nausea, vomiting, decreased appetite, episodic palpitations and dyspnea on exertion. A pap smear five months prior was benign. Her last delivery was 46 months ago (cesarean section); her last menses was one month ago. Family history was negative for obstetric or gynecologic cancers. On physical exam, the uterus was 8 cm in size, midline, and mobile with minimal discomfort. Speculum exam revealed a 5 cm necrotic, friable mass protruding through the cervical os without active bleeding. Significant laboratory results included hemoglobin of 4.2 g/dl and beta-hCG of 13 mIU/ml. Transvaginal ultrasound revealed a 97 x 46 x 61 mm uterus, antverted with normal contours and endometrial cavity mass (22 x 9 x 17 mm). The cervical mass (52 x 29 x 27 mm) was primarily solid with cystic lucencies and high vascularity. Significant abdominal computed tomography (CT) findings included irregular hypodensities on the right endometrial wall and a 5.6 cm heterogenous cervical mass adjacent to the base of the bladder and inferior wall of the rectum (Figure 1). There was no evidence of significant lymphadenopathy or metastasis. The CT of the neck/chest were unremarkable. Endometrial biopsy showed no evidence of malignany. Cervical mass biopsy showed medium to large atypical cells with eosinophilic cytoplasm and marked nuclear pleomorphism arranged in sheets with patchy necrosis but no syncytiotrophoblasts. Immunohistochemical stains were positive for cytokeratin AE1/AE3, E-cadherin, human placental lactogen (hPL), and alpha inhibin. There was only focal positivity for beta-hCG and placental alkaline phosphatase (PLAP). Tissue was negative for CK5/6 and smooth muscle actin, ruling out squamous cell carcinoma and leiomyosarcoma. The MIB-1 (Ki-67) proliferative index was approximately 15-20%. The differential diagnosis included PSTT versus epithelioid trophoblastic tumor (ETT), but PSTT was favored based on marked nuclear atypia and extensive positivity for hPL. The patient underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy as there was no desire for future fertility. Exploratory laparotomy was unrevealing. Pathologic evaluation revealed a 4.5 cm mass attached to the endometrial surface by a stalk and confined to the uterus. Upon sectioning, the mass was tan and soft with focal areas of hemorrhage and necrosis. Surgical margins were negative. Following surgical, pathologic, and radiological evaluation, the patient was assigned Stage T1N0M0. The patient is currently followed with monthly beta-hCG levels and has no evidence of recurrent disease.

Discussion

The most common presenting symptom of PSTT is vaginal bleeding [4-6]. Amenorrhea and abdominal pain are other presenting symptoms. In addition to vaginal bleeding, our patient had a friable, fungating cervical mass with an enlarged uterus. In a review of 55 cases of PSTT, only 12.7% of tumors extended into the cervix [7].
Likewise, endometrial curettage is usually required for diagnosis [2]. In this unusual case, biopsy of the cervical mass (which was in fact a prolapsed uterine lesion) confirmed the diagnosis of PSTT. Adverse prognostic factors in this case are age (> 35 years) and interval since the last pregnancy (> 2 years) [7].

Mao and colleagues recently proposed that choriocarcinoma is a primitive form of GTN and consists of primitive cytotrophoblastic cells [8]. PSTT and ETT represent greater differentiation of cytotrophoblasts into intermediate trophoblasts (IT) that resemble implantation-site IT in PSTT or chorionic-type IT in ETT. Likewise, PSTT and ETT can be categorized as intermediate trophoblastic tumors (ITT). Interestingly, mixed tumors with morphologic and immunohistochemical features of choriocarcinoma, ETT, and PSTT are frequently reported [9]. The model of variable differentiation helps explain the variability.

Several immunohistochemical stains have been proposed to evaluate PSTT. Human placental lactogen (HPL) is positive in 87-100% of IT [5, 9, 10]. Immunoreactivity to hCG is present in the majority of cases (92% in one study), albeit weakly. Pregnancy-associated major basic protein (pMBP) is expressed in 78% of IT [10]. MUC-4 is reported to represent implantation-site IT cells, which was demonstrated by the differential expression in PSTT and ETT cases of 100% and 29%, respectively [8]. However, choriocarcinoma is also strongly reactive to MUC-4. Clearly, a combination of the above stains is imperative.

The absence of a single stain with high sensitivity and specificity for PSTT raises the question of clinical relevance. It is prudent for clinicians to differentiate PSTT from other forms of GTN because it responds poorly to chemotherapy. An algorithm based on an immunohistologic panel may differentiate choriocarcinoma, placental site nodule, exaggerated placental site, PSTT, and ETT [11]. The panel includes cytokeratin 18, HLA-G, p63, hPL, beta-hCG (highlighting syncytiotrophoblasts), and Ki-67%. Importantly, p63 is added to the analysis of trophoblastic tumors.

Kalhor et al. suggested a panel of HLA-G, CD10, and hCG to rule-in ITT and CK5/6 as a marker to rule out ITT [12]. It is well recognized that PSTT demonstrates sparse hCG staining and high immunoreactivity to hPL [1, 7]. However, considering the similar clinical behavior treatment, and outcome of PSTT and ETT there is small clinical value in differentiating between the two. [9, 13]. Therefore, the panel is designed primarily to distinguish ITT (i.e., PSTT and ETT) from squamous cell carcinoma of the cervix, which becomes a diagnostic challenge when ITT presents in the lower uterine segment or as a cervical mass.

Combination chemotherapy regimens do show promise.

Figure 1. — Computed tomography of the abdomen/pelvis obtained on the day of presentation.
A) Irregular endometrium with hypodensities on the right wall. 
B) The lower uterine segment has a heterogeneous appearance on the right, immediately above the cervix. 
C) The cervix is expanded and heterogeneous with a 6 cm transverse diameter.
Suggested first-line chemotherapy includes EMA/CO (etoposides, cisplatin, methotrexate, actinomycin D, cyclophosphamide, and vincristine) and EMA/EP (etoposide and cisplatin alternating with etoposide, methotrexate, and actinomycin D). EMA/EP, BEP (bleomycin, etoposide, cisplatin) and VIP (etoposide, ifosfamide, cisplatin) have been recommended for metastatic PSTT and recurrent disease [1, 6].

The patient represented in this case had the unusual finding of an endometrial tumor, which protruded from the external os of the cervix. Biopsies and immunohistochemical staining led to the correct diagnosis of PSTT. The pathologic diagnosis was T1N0M0. Follow-up CT did not reveal residual mass and no tumor marker was available to follow chemotherapy response, therefore, treatment was limited to surgical resection.

Conclusion

PSTT presenting as a friable cervical mass is uncommon. Evaluation of tissue biopsies is an important tool for diagnosis of PSTT and several immunohistochemical stains are suggested in the literature. Clinically, it is prudent for clinicians to differentiate PSTT from other forms of GTN because of the poor response of PSTT to chemotherapy.

References


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Villoglandular papillary adenocarcinoma of the uterine cervix diagnosed during pregnancy

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Introduction

The incidence of cervical adenocarcinoma has been on the rise over the last decades. Villoglandular papillary adenocarcinoma (VPA) is a very rare subtype of adenocarcinoma of the uterine cervix. The true incidence of this form of adenocarcinoma is unknown. The classical histologic appearance of this entity is a surface papillary component of variable thickness with papillae that are usually tall and thin, but occasionally short and broad, with a fibrous stromal core. The tumor cells should have no more than mild-to-moderate nuclear atypicality and scattered mitotic figures. VPA affects a younger age group and has an excellent prognosis as compared to other endocervical adenocarcinomas [1, 2]. To our knowledge, only five cases of VPA associated with pregnancies have been reported in the literature [3-7]. We report here a successful pregnancy with Stage IB2 VPA of the cervix.

Case Report

A 32-year-old woman, gravida 0, para 0, was referred to our hospital at 33 weeks’ gestation due to the positive cytological finding of adenocarcinoma. Physical examination revealed a 5-cm bleeding exophytic cervical tumor with no evidence of vaginal fornices or parametrial involvement (FIGO Stage IB2). We performed a biopsy of the uterine cervix, and verified cervical VPA (Figure 1). The patient underwent cesarean section (CS) at 34 weeks, and delivered a healthy 2,282 g newborn. Semi-radical hysterectomy with pelvic lymphadenectomy was performed two weeks after CS. Although VPA rarely has lymph node metastasis, this case had metastasis to the obturator lymph nodes. Following five courses of adjuvant chemotherapy (paclitaxel 180 mg/m2, carboplatin AUC 6), the patient has shown no evidence of disease recurrence for 30 months.

Discussion

We report an extremely rare case that was diagnosed with VPA during pregnancy with successful results both for the mother and baby. VPA of the uterine cervix is a rare form of cervical adenocarcinoma first described by Young and Scully in 1989 [1]. They found that this tumor has an excellent prognosis and suggested conization as a potential treatment for patients of childbearing age [1]. Conservative management of cervical VPA is considered to be a significant challenge; however, the English literature concerning treatment of VPA diagnosed during pregnancy is sparse. So far, over 115 cases of cervical VPAs have been reported worldwide; of these only nine metastases and two deaths were reported [7-11]. These few cases show an apparent discrepancy from the excellent prognosis of VPA described originally by Young, Scully and others [1, 12]. In 30% of cases, VPA is associated with other forms of invasive cancer [1-4, 7] which may have an important impact on the prognosis. Young and Scully therefore reserve the term VPA for tumors in which the villoglandular pattern is the exclusive or almost exclusive one. It has been suggested that in cases of superficial VPA diagnosed in young patients, unassociated with another type of cervical tumor and without lymph vascular invasion, less radical treatment may be suitable since these cases present a favorable outcome [12]. However, since the knowledge of the biologic spectrum of VPA appears to be evolving, a close follow-up should be pursued in VPA patients managed conservatively [13].

Pregnancy associated with VPA of the cervix has been reported in only five cases. In three cases, successful pregnancies were achieved following conservative treatment for VPA [3-5]. Two additional cases were diagnosed during pregnancy; one case ended with an early induced abortion (8 weeks of gestation) followed by a radical hysterectomy [7], and the second case, which was diagnosed during the 20th week of gestation, was conservatively...
followed until the 32nd week of gestation, when a cesarean radical hysterectomy was performed [6]. In the case whose pregnancy was terminated (8 weeks of gestation) followed by a radical hysterectomy and pelvic lymphadenectomy, the patient died of a tumor recurrence [7], suggesting that some VPAs are malicious, especially when other histological features are present. These authors recommend the attitude, “Beware of a wolf in sheep’s clothing”, in relation to VPA [14].

In conclusion, despite the limited experience of cervical VPA diagnosed during pregnancy, conservative treatment can be successfully achieved in selected patients after a thorough evaluation of the depth of invasion, the lymph vascular involvement and the association of other carcinoma histologies in conjunction with the VPA (i.e., adenocarcinoma or squamous cell carcinoma).

References


Granulosa cell tumor and endometrial cancer: a case report and review of the literature

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Summary
Granulosa cell tumors (GCTs) of the ovary represent an estimated 2-5% of ovarian cancers. This tumor is the most common of the ovarian sex cord-stromal tumors and its cells present many morphological, biochemical and hormonal features of granulosa cells, including both estrogen and inhibin biosynthesis [1]. Estrogen production is responsible for endocrine presentation of GCTs. The low incidence of this tumor creates difficulties in management and prognosis. It is generally diagnosed at an early stage, and is characterized by slow growth and late recurrence.

Key words: Granulosa cell tumor; Endometrial cancer; Ovarian cancer; Postmenopausal bleeding.

Introduction
Granulosa cell tumors (GCTs) of the ovary represent an estimated 2-5% of ovarian cancers. This tumor is the most common of the ovarian sex cord-stromal tumors and its cells present many morphological, biochemical and hormonal features of granulosa cells, including both estrogen and inhibin biosynthesis [1]. Estrogen production is responsible for endocrine presentation of GCTs. The low incidence of this tumor creates difficulties in management and prognosis. It is generally diagnosed at an early stage, and is characterized by slow growth and late recurrence.

Case Report
A 51-year-old patient was referred to our service for postmenopausal bleeding. She had no relevant history of pathology, and one normal delivery 30 years earlier.

She reported one year of intermittent bleeding. The ultrasound scan performed by her physician showed only a thickened endometrium measuring 11 mm.

A diagnostic hysteroscopy was scheduled to assess the uterine cavity and perform a guided biopsy. The examination revealed an endometrium consistent with malignancy, not only because of its irregular appearance, but also for the presence of increased vascularization. The result of the endometrial biopsy confirmed the suspected diagnosis of complex endometrial hyperplasia, with a focus of endometrioid adenocarcinoma.

In order to stage the endometrial neoplasm, magnetic resonance imaging (MRI) was performed prior to surgery. The images showed neither myometrial infiltration nor pelvic adenopathies, but they did reveal the presence of a previously unidentified pelvic solid cystic multicellular tumor. The tumor, which measured 14 x 8 cm, seemed to originate in the left ovary, and the MRI results were consistent with ovarian malignancy (Figure 1). Minimal ascetic fluid was seen. At this point, the patient was again examined using transvaginal ultrasound (US), which showed a thickened endometrium (8 mm) and a solid left adnexal tumor measuring 10 x 9 cm with a regular surface and minimal vascular flow in its central zone.

The patient thus had a diagnosis of synchronous gynecologic neoplasia: endometrial cancer Stage IA, and ovarian cancer.

Diagnostic laparoscopy was performed prior to laparotomy to determine the stage of the ovarian cancer. A giant solid pelvic tumor was seen on the left ovary with intact capsula. No other abdominal implants were detected in the laparoscopy. Consequently, a median infraumbilical laparotomy was performed. Minimal free abdominal fluid was aspirated and sent for cytologic study. The left adnexa was excised and examined by our pathologist to establish a perioperative diagnosis. A total hysterectomy, right salpingo-oophorectomy, pelvic bilateral lymphadenectomy and omentectomy were performed. There were no complications during surgery. The result of the perioperative biopsy revealed a granulosa cell tumor of the left ovary.

The postoperative course was without complications. The patient presented low levels of estradiol (< 18 pg/ml) and inhibin A (3.4 pg/ml).

The definitive result of the pathologic study confirmed both neoplasms (Figures 2 and 3). The patient had an adult type granulosa cell tumor of the left ovary with intact capsula, measuring 14.5 cm (Stage IA). As a result of its hormonal synthesis, she developed a well-differentiated endometrioid adenocarcinoma measuring 1.5 cm in diameter with a depth of myometrial invasion of < 50%. No vascular, adnexal, cervical or parametrial invasion was found. The surgical margins were free of disease, as were the epiploon and all 14 lymphatic pelvic nodes excised.

Because of the low stage of both GCT and endometrial neoplasia, the patient received no adjuvant therapy. She continues to be followed at our hospital and shows no signs of recurrent disease.
GCT is an uncommon type of ovarian cancer. Like thecomas and fibromas, GCTs are included in the subgroup of granulosa-stromal cell tumors of the ovary, which represent 70% of ovarian sex cord-stromal tumors. GCTs represent only 5-8% [2] of all primary ovarian neoplasms, and most have the distinguishing feature of hormonal production.

GCTs are classified into two subtypes, juvenile and adult. The juvenile form (5%) tends to appear in prepubertal girls and women younger than 30 years of age [3]. The adult type generally affects perimenopausal or early menopausal women with a mean age of 51 years [4]. Although both are caused by estrogen secretion, they are generally classified separately because their clinical manifestations and prognoses differ. In the juvenile type, girls may experience isosexual precocious pseudopuberty, whereas the adult type is associated with several endometrial pathologies as a consequence of estradiol production. Juvenile GCTs commonly have less differentiated cells, but a lower risk of recurrence than the adult form [3].

Like other types of ovarian cancer, its clinical presentations include abdominal pain, especially because tumor size at time of diagnosis is often large, with a mean tumor size of 11 cm [4]. As a result, most women have a palpable pelvic mass. The ovarian tumor can also cause acute abdominal pain if ovarian torsion or hemorrhagic processes (inside or outside the mass) occur [5].

Prolonged exposure of the endometrium to the high levels of estradiol produced by granulosa cells is a factor in the development of many types of endometrial pathology. Most of these pathologies involve postmenopausal bleeding (or abnormal bleeding in premenopausal women) as a first clinical manifestation, as in this case. Endometrial pathologies associated with GCT include glandular hyperplasia, atypical hyperplasia, adenocarcinoma in situ, and invasive adenocarcinoma [6, 7]. In a study of 63 cases of sex cord-stromal tumors by Zanagnolo et al. [8], endometrial hyperplasia appeared in 26.5% of cases, while 8.8% developed adenocarcinoma. Studies of other series report similar percentages, concluding that 25-50% of GCTs induce endometrial hyperplasia, and 5-10% of tumors induce endometrial carcinoma [5, 7]. If endometrial cancer develops, it typically appears as a well-differentiated early-stage tumor with a good prognosis [7], as in our case.

If the initial symptoms are abnormal bleeding and/or abdominal pain, the first step is to evaluate the patient with US scan. In most cases, GCTs are large multilocular-solid masses with a high number of locules, or solid tumors with heterogeneous echogenicity. Hemorrhages are common and increased vascularity is demonstrated by color/power Doppler US examination [9]. MRI findings of GCT include low signal intensity on T2WI for solid components, and also a sponge-like multilocular cystic mass [10].

As in this case, when abnormal bleeding is the main symptom, an endometrial biopsy must be obtained, either by hysteroscopy or endometrial aspiration biopsy. In fact, because of the high incidence of uterine pathology, an endometrial sample should always be obtained if GCT is suspected.

Thus, GCTs should be included in the differential diagnosis of a pelvic mass typically appearing as a large adnexal unilateral tumor associated with postmenopausal bleeding. Other possible diagnoses, which were also considered in our case, include ovarian metastasis of a primary endometrial carcinoma, endometrial metastasis of a primary ovarian cancer or synchronous endometrial cancer and epithelial ovarian cancer.

Surgery is the next step in the management of suspected ovarian cancer, in order to stage the tumor, perform a definitive histopathologic study and debulk as much disease as possible. Staging laparotomy must include inspection of the contralateral ovary, pelvic and paraaortic lymph nodes, omentum, diaphragm and bowel serosa, as well as cytologic examination of lavage fluid after peritoneal washing. GCTs are staged using the epithelial ovarian cancer FIGO classification. In postmenopausal and perimenopausal women, total hysterec-
Granulosa cell tumor and endometrial cancer: a case report and review of the literature

Toxmy with bilateral salpingo-oophorectomy is recommended. In premenopausal women with Stage IA to IC disease who wish to preserve their fertility, conservative treatment with unilateral salpingo-oophorectomy can be performed provided two conditions are met: the contralateral ovary must be inspected during the surgery (although only 2-8% of GCTs are bilateral) [11, 12]; and the endometrial biopsy must be negative. In a study supporting uterine-sparing surgery, Zhang et al. compared 5- and 10-year survival rates in 134 young patients (≤ 50 years) with Stage I GCT, concluding that there were no differences between women who underwent hysterectomy and those who had conservative surgery [4].

At present there is insufficient evidence in the literature to assess the role of adjuvant therapy in GCT because the low incidence of these tumors is an obstacle to studying them through randomized controlled trials. However, most patients are diagnosed at Stage IA and have a good prognosis, with long-term survival rates around 90% [4]. These patients do not require postoperative adjuvant therapy, and both chemotherapy and radiotherapy are therefore reserved for advanced stages or recurrent disease. In the review published by Schumer et al., patients with Stages II, III and IV, as well as Stage I with risk conditions (large tumor size, high mitotic index or tumor rupture) are considered candidates for adjuvant chemotherapy [5]. The protocols used are platinum-based, generally in combination with bleomycin and etoposide. Patients with low residual disease may be treated with either chemotherapy or radiotherapy, although the former is normally preferred because platinum-based chemotherapy is better tolerated and easier to administer. For gross residual disease, the optimal treatment is chemotherapy [5].

Prognostic factors for GCT have been analyzed in many studies, with variable results. Recently Zhang et al. [4] reported the largest series of sex cord-stromal tumors, 376 cases of which 339 were GCT. They concluded that age influences prognosis, since women ≤ 50 years at diagnosis had 10% longer survival times than older patients, although the reason for this difference remains unclear. As most studies have found, the most important prognostic factor is disease stage [7, 11]. Tumor size greater than 10 cm, which was associated with poor prognosis in other studies [6, 7, 11] had no effect on survival rates in Zhang et al’s cohort. Other prognostic factors include tumor rupture [13] and histologic features, including mitotic index [13, 14].

Granulosa cell tumors typically have well-differentiated cells that produce hormones, some of which have been studied as markers for GCT. Most of these markers, as in this case, are obtained postoperatively, once the histopathologic diagnosis is reached. Estradiol has demonstrated low sensitivity as a marker not only because it is uncorrelated with clinical progression [15] but also because approximately 30% of GCTs do not produce this hormone. Nevertheless, estradiol can be used to monitor possible recurrences postoperatively, but it is not a reliable marker [5]. Inhibin is a hormone secreted by normal granulosa cells in premenopausal women, and consists of two subunits, A and B. Both may be used for detection of recurrent disease, but inhibin B appears to be more sensitive for this purpose [16]. Elevated inhibin levels in postmenopausal women are not specific to GCT because mucinous epithelial ovarian carcinomas can also produce this hormone [17]. Mullerian inhibitory substance (MIS) has been identified as a hormone produced by the granulosa cells in the developing follicle of the ovary and for this reason it has been studied as a possible tumor marker. Although at present MIS levels are not used in clinical practice to detect GCT recurrences, further studies may demonstrate its usefulness, especially because it is generally not found in postmenopausal women and seems to be highly specific to GCT [15].

As noted above, GCT typically has a good prognosis, with high long-term survival rates, but the median time estimated for recurrences is four to six years following
diagnosis [5], and late recurrences as much as 30 years after the initial diagnosis have been documented [7, 14, 18]. Therefore, prolonged follow-up of these patients is of clinical value and should include periodic physical examination and determination of inhibin and estradiol levels, as well as image studies if recurrence is suspected.

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References


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Hysteroscopic diagnosis of a high-grade endometrial sarcoma in a 41-year-old woman

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Summary

Endometrial stromal sarcomas originate in the endometrial cavity. These tumors represent between 15-27.9% of uterine sarcomas. We present the case of a 41-year-old woman who underwent surgical hysteroscopy for metrorrhagia over a period of one month who had an intrauterine polypoid mass detected by ultrasonography. Histologic analysis of the polypoid mass removed by hysteroscopy was a high-grade endometrial stromal sarcoma of the uterus. The description of this case provides an opportunity to review the literature on uterine sarcomas diagnosed by surgical hysteroscopy.

Key words: Uterine sarcoma; Surgical hysteroscopy; High-grade endometrial stromal sarcoma.

Introduction

Endometrial stromal sarcoma represents between 15 to 27.9% of the uterine sarcomas [1-3]. Histologically the cells that compose endometrial stromal tumors are similar to those of the proliferative phase of endometrial stroma during the menstrual cycle. Low-grade endometrial stromal sarcoma is a tumor composed of uniform, oval cells of endometrial stromal-type; by definition significant atypia and pleomorphism are absent. Undifferentiated endometrial sarcoma is a high-grade sarcoma composed of marked cellular atypia and brisk mitotic activity. Traditionally, endometrial stromal sarcomas have been divided into low and high grade based on mitotic count [2]. However, the undifferentiated endometrial sarcoma lacks any histological resemblance to endometrial stroma, so the classification of low-grade endometrial sarcoma and undifferentiated endometrial sarcoma is not made on the basis of mitotic count but on features such as pleomorphism and necrosis.

This type of sarcoma appears more in younger patients than in others and the most common symptomatology is vaginal bleeding [4].

Preoperative diagnosis of uterine sarcoma is difficult, and hysteroscopy makes an accurate diagnosis of malignant intrauterine pathology, and could play a role in the diagnosis of intrauterine sarcomas.

We present below the case of a woman with endometrial stromal sarcoma diagnosed by surgical hysteroscopy.

Case report

A 41-year-old woman presented at our hospital for metrorrhagia over a period of one month, with bleeding in quantities superior to her menstrual period and mild asthenia. Her medical history included tuberculosis, discopathy (C5-C6), tendinitis, and a smoker of eight cigars per day.

Her gynecological and obstetric history revealed menarche at age 14, regular menstrual cycles with heavy bleeding, two normal deliveries and regular gynecological controls. Three years before, a polyp was detected by ultrasonography (US); hysteroscopy was indicated and a benign polyp was removed.

The gynecological examination was anodine. US showed an intrauterine polypoid mass measuring 34 x 17 mm. Hysteroscopy was scheduled. It revealed an intrauterine polyp suspicious of malignancy, occupying almost the entire uterine cavity with a broad attachment to the posterior wall which was removed almost entirely with scissors. Histologic analysis of the surgical specimen showed malignant mesenchymal proliferation consistent with high-grade uterine sarcoma (Figure 1).

Preoperative magnetic resonance imaging (MRI) was performed to evaluate the possible extraterine extension of the tumor. MRI revealed an intrauterine tumor which was not invading the myometrium and was limited to the uterine cavity. In consequence the patient underwent hysterectomy with bilateral salpingo-oophorectomy, bilateral pelvis lymphadenectomy and peritoneal lavage. Analysis of the surgical specimen was carried out by the pathology laboratory. Macroscopically the tumor was a polypoid gray mass occupying the endometrial cavity, infiltrating the superficial myometrium (Figure 2). Histologically, a high-grade tumor composed of undifferentiated cells with marked cytological atypia and brisk mitotic activity was shown. The final diagnosis of the surgical specimen was undifferentiated endometrial sarcoma measuring 4.5 cm, and infiltrating only 0.5 cm of the myometrium (Figure 3). The tumor did not invade the lymph nodes or any other structures analyzed, and peritoneal lavage was negative for malignant cells.

The patient’s postoperative course was normal and the hospital tumor board decided to do adjuvant treatment with vaginal radiotherapy, total dose of 25.2 GY.

At present the patient is being followed at our hospital and is free of disease. After 18 months of follow-up, her physical examination and vaginal cytology results were negative.

Discussion

Sarcoma of the uterus is rare, representing between 2% and 6% of all uterine tumors [5-7]. Endometrial stromal sarcoma is not a frequent type of uterine sarcoma (15-27.9%) that originates in the endometrial cavity [1, 2].
These tumors appear at younger age than other uterine sarcomas, usually in premenopausal women [4]. The average age at presentation is between 42 to 55 years old. This is similar to our case in which the patient was a 41-year-old premenopausal woman.

The most common initial symptom of endometrial sarcoma is metrorrhagia as in our case [3]. Ultrasound plays a fundamental role in diagnosing endometrial pathology [8, 9], as demonstrated by our case which showed an intrauterine polypoid mass measuring 34 x 17 mm.

Recently an increasing number of sarcomas diagnosed by hysteroscopy can be found in the literature [10-14]. The risk of malignancy is higher in postmenopausal women although in the literature there are some reports of cases of uterine sarcomas diagnosed by hysteroscopy in premenopausal women [12, 15, 16], as in our case.

We can also find reports of uterine sarcomas described as a polyp by hysteroscopy [13, 17, 18], but only in two cases [18] was it suspicious of malignity like in our case.

Endometrial stromal sarcoma is one of the more frequent uterine sarcomas diagnosed by hysteroscopy, probably because it originates in the endometrial cavity. At least four endometrial stromal sarcomas diagnosed by hysteroscopy are described in the literature [12, 13, 15, 16]. All are low-grade type but in our case it was a high grade type. This is relevant because the five-year survival rate of the tumor is significantly lower in high-grade (25%) versus in low-grade type (60%) [19], and also because in these high-grade cases lymphadenectomy and adjuvant therapy are indicated [19] as was done in our case. Our patient is free of disease after two years of follow-up.

In conclusion, hysteroscopy is useful in facilitating an early diagnosis of sarcomas and could improve the patient’s prognosis in which diagnosis was made in early stage.

References

Hysteroscopic diagnosis of a high-grade endometrial sarcoma in a 41-year-old woman


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Use of surgical sealant in debulking surgery for advanced ovarian carcinoma - case report

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Summary

Haemostatic sealants are frequently used in gynaecological surgery. Several commercial products are available with similar mechanisms of action and chemical structure. We report the use of Floseal in a laparotomy for ovarian cancer to achieve haemostasis. This is the first reported case with the successful use of Floseal in gynaecological oncology.

Key words: Bleeding; Haemostasis; Surgical sealant.

Introduction

Use of haemostatic sealants in operative procedures across different specialties has steadily increased during recent years. There are several commercial products available with similar composition and mechanisms of action. Uncontrolled intraoperative haemorrhage significantly influences postoperative mortality and morbidity. Surgery for gynaecological malignancy is characterised by significant blood loss due to increased radicality of surgery (node dissection, parametrial dissection) and the presence of malignancy. This may further increase the risk of bleeding due to poor tissue characteristics and by causing haematomatous derangements. Ovarian cancer expresses serum and ascitic angiogenic factors including basic fibroblastic growth factor (bFGF), angiogenin (ANG) and vascular endothelial growth factor (VEGF) [1] which can lead to increased tissue vascularity and neovascularisation [2].

We report a case of a large intraoperative haemorrhage in a 75-year-old with advanced ovarian malignancy. Haemostasis was successfully achieved with Floseal sealant following failure of conventional techniques. This is the first reported case following successful use of Floseal® intraoperatively for advanced stage ovarian cancer.

Case Report

A 75-year-old woman was admitted to the Accident and Emergency Department with abdominal distension and pain. She reported a four-week history of gradual loss of appetite and weight. Her significant medical history included type 2 diabetes, hypertension and hyperlipidemia.

Examination revealed tense ascites and CA-125 grossly elevated to 4559kU/l (normal range 0-35). Computed tomography (CT) scan revealed marked ascites, extensive omental caking with widespread peritoneal thickening and a 12 cm complex pelvic mass. Paracentesis was performed to relieve symptoms with widespread peritoneal thickening and a 12 cm complex pelvic mass. The ascites was 24 (normal range 34-48).

Her liver function tests were normal except for albumin which was 24 (normal range 34-48).

Following discussion at the gynae-oncology multi-disciplinary team meeting, she was offered neo-adjuvant chemotherapy with carboplatin and paclitaxel with the possibility of interval debulking surgery based on the response to chemotherapy. After the second cycle, she developed grade 2 neuropathy of her left foot but continued with single agent carboplatin chemotherapy only and was scheduled for interval debulking surgery 22 days after her third cycle. Her CA-125 had dropped to 458 and the interval CT scan confirmed a good radiological response with no evidence of ascites, decrease in size of the omental caking and pelvic mass. Pre-assessment a week prior to surgery revealed pancytopenia with severe neutropenia. She was therefore admitted and transfused with two units of packed cells and once daily granulocyte colony stimulating factor for three days. Her preoperative haemoglobin (Hb) was 9.2 g/dl (normal range 11.5-16.5 g/dl) and platelets 115 (normal range 150-600 x 109).

A staging laparotomy was performed through a midline incision under general anaesthetic and thoracic epidural. This confirmed the omentum was completely replaced by tumour, a large 14 cm complex multiloculated pelvic mass with widespread peritoneal infiltration in the upper right quadrant and peritoneal surfaces of the right hemi-diaphragm, under the surface and anterior surface of the liver. The omentum was mobilised with difficulty, following which extensive bleeding from the under surface of the liver ensued which was packed as no specific bleeding points were identified. Supracolic omentectomy was performed. Since the pack from the hepatic under surface was soaked, an attempt was made to stop the ooze. The patient’s Hb on blood gas analysis was 7 g/dl and she was transfused two units of packed cells.

The ureters were identified through a retroperitoneal approach and the colon was mobilised from the frozen pelvis. A retrograde radical hysterectomy was performed to remove the tumour which was involving the uterus and parametria as well as both ovaries. Bleeding continued diffusely in the pelvis and was largely due to ooze from the diseased peritoneum and underlying soft tissues. The estimated blood loss (EBL) at this stage was 2.5 l. Attempts to cauterise and suture bleeding points were unsuccessful and analysis of blood counts revealed: Hb 6g/dl and platelet count of 45. Clotting profile at this stage showed an international normalized ratio (INR) of 4 and activated partial thromboplastin time (APTT) of 55 sec. She received two pools of platelets, two units of fresh frozen plasma (FFP) and two units of packed cells. She was transfused with four units of cryoprecipitate, recombinant factor VII and a further two units of packed cells following the haematologist’s...
advice. Floseal matrix haemostatic sealant was then used. Two vials were applied to the liver under the surface and one in the pelvis at sites where bleeding was most obvious. Haemostasis was instantly achieved and all bleeding seemed to settle in less than 4 min. The abdomen and pelvis were carefully checked and the abdominal wall was closed with two Robinson drains (28 F) placed in the pelvis and abdomen. Total blood loss was estimated at 6.5 l. The patient was transferred to intensive care and transfused with a further two units of packed cells and FFP. The pelvic drain collected 100 ml, 2 h following the operation and investigations revealed an Hb of 9.5 with a platelet count of 80. The clotting profile showed an INR of 2.5 and APTT of 35 sec. Overnight the drains collected a further 500 ml in total. The patient’s blood count steadily improved following two further units of packed cells. Following electrolyte correction, she was moved to the high dependency unit on day 2 and both drains were removed on day 4.

She was discharged on day 10 with no wound complications and normal blood counts. Chemotherapy was resumed three weeks following discharge.

Discussion

The National Confidential Enquiry into Patient Outcome and Death [3] observed that nearly 50% of cases of death primarily due to haemorrhage were associated with operations for gynaecological malignancies [3]; 70% of epithelial ovarian cancers are Stage 3 at presentation.

Intraoperatively, bleeding is a common yet potentially life threatening complication associated with major surgery. Pre-existing malignancy may cause changes in tissue characteristics; presence of coagulopathy, inadequate heparin reversal and inaccessibility of bleeding sites intraoperatively may further complicate this [4]. In our patient, this was further complicated by the chemotherapy which led to bone marrow suppression and suboptimal preoperative blood parameters.

Bleeding may not always be controlled by conventional procedures including cauterization, sutures or manual pressure and can lead to prolonged operating time and multiple procedures [5]. Prior to the advent of surgical sealants such as Floseal, intraabdominal packing and second look laparotomy after correction of clotting parameters and acidosis was the management of choice in cases of massive haemorrhage due to a gynaecological malignancy [6]. Embolisation has been recommended for bleeding with emphasis being laid on post-partum haemorrhage where mothers are more likely to be otherwise medically well - unlike women with ovarian malignancies [7]. In turn this significantly increases patient morbidity and mortality, costs, and delayed patient turnover with negative impact on operating and hospital stay times [5].

Topical agents of various types have been developed to promote haemostasis. These include Surgicel made of oxidized cellulose, microfibrillar collagen, and newer fibrin and thrombin sealants [8]. Agents may be divided depending on the composition, lack or use of animal components (proteins) or mechanism of action. They are commonly classified depending on the presence or absence of fibrin co-existing with the thrombin. The body’s endogenous fibrin is relied on to form a clot in thrombin-only agents. Agents containing both – such as Floseal – may be applied directly after mixing to a wound bed and act on the final common step in the coagulation cascade [9].

Ideal haemostatic agents should have both haemostatic and sealant properties [10]. This helps with immediate haemostasis and also to weld tissues together creating a tamponade effect and decreasing the risk of seroma formation [9]. Floseal appeared to fulfil both these criteria in our patient and the success of this may be due to the gelatin granular matrix which fills the wound conforming to its shape which then swell and create a tamponade effect. The wound site is then exposed to high concentrations of thrombin which activates the clotting cascade converting fibrinogen into fibrin [4].

A randomised control trial was carried out by vascular surgeons using Floseal and gelfoam demonstrating the haemostatic effect of Floseal with positive results while acknowledging the presence of bovine protein in both the thrombin and fibrin which may carry a theoretical risk of disorder of coagulation due to antibody formation and the risk of transmitting diseases caused by prions [4].

Conclusion

Our preliminary experience suggests that Floseal appears safe for use in gynaecological oncology with excellent efficacy and ease of use and it may be of value in cases of major haemorrhage. We are currently conducting a larger prospective study to assess it further.

References


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Endometroid adenocarcinoma of the uterus, borderline tumor of the ovary and Brenner tumor of the contralateral ovary in a 63-year-old woman

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Summary
Synchronous primary cancers of the endometrium and ovary occur in approximately 10% of all women with ovarian cancer and 5% of all women with endometrial cancer. The pathogenesis of synchronous endometrial and ovarian cancer is unclear. Synchronous tumors tend to be low grade and early stage. The prognosis is much better with survival approaching ten years than if the disease was classified as a single organ disease with metastasis. We report a case of unusual co-existence of endometroid adenocarcinoma of the uterus, serous borderline tumor of the ovary and Brenner tumor of the contralateral ovary in a 63-year-old woman. The patient received a surgical treatment and postoperative irradiation.

Key words: Synchronous tumor; Endometrial cancer; Brenner tumor.

Introduction
We report a case of unusual co-existence of multiple primary neoplasms of the female genital tract in a 63-year-old woman. The patient was admitted to our center for endometrial carcinoma diagnosed from the curette; the ovarian pathology was an incidental histological finding.

Case Report
A 63-year-old woman presented to our department to be treated for endometroid adenocarcinoma of the uterus. There was no family history of gynaecological disease and her personal history was unremarkable.

Hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy were performed. Histology of the surgical specimen showed a moderately differentiated endometroid endometrial adenocarcinoma, infiltrating more than half of the myometrium (Figure 1), serous borderline tumor of the ovary with microinvasion of the tumor cells (Figures 2, 3) and benign Brenner tumor of the contralateral ovary (Figure 4). A total of 36 lymph nodes were examined with no evidence of metastatic disease.

Postoperative irradiation was performed and the patient received a complete course of whole pelvic radiation.

One year after the treatment she was well with no evidence of recurrent disease.

Discussion
Synchronous primary cancers of the endometrium and ovary occur in approximately 10% of all women with ovarian cancer and 5% of all women with endometrial cancer [1] These patients can be classified into three groups: endometrial cancer with metastasis to the adnexa, ovarian cancer with metastasis to the endometrium [2], or synchronous primary cancers of both endometrium and ovary [3].

The pathogenesis of synchronous endometrial and ovarian cancer is unclear. The theory of a “secondary Müllerian system” proposes that the epithelia of the cervix, uterus, fallopian tubes, ovaries, and peritoneal surface share molecular receptors responding to carcinogenic stimulus leading to the development of multiple primary malignancies synchronously [4]. However, it may not be the case in synchronous cancers of dissimilar histology.

The most common presenting sign or symptom is abnormal bleeding, abdominal pain, abdominal fullness, elevated CA 125, abdominal mass, body weight loss, and constipation [5].

Most of the women are obese, premenopausal and nulliparous.

Patients with synchronous endometroid tumours of the endometrium and ovary are younger than reported for either endometrial or ovarian adenocarcinomas. Median age at diagnosis is 50 years [6].

Women diagnosed with synchronous primary cancers have a much better overall prognosis with survival approaching ten years than if their disease is classified as single organ disease with metastasis.

Synchronous tumors tend to be low grade and early stage [1].

Earlier detection of ovarian cancer is likely due to early symptoms (vaginal bleeding) related to concurrent endometrial cancer.
Endometroid adenocarcinoma of the uterus, borderline tumor of the ovary and Brenner tumor of the contralateral ovary in a 63 et c.

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References

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Exaggerated placental site mimicking placental site trophoblastic tumor: case report and literature review

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Summary
Exaggerated placental site is defined as a non-neoplastic trophoblastic lesion featuring exuberant infiltration into the endometrium and myometrium by intermediate trophoblasts and syncytiotrophoblasts. Exaggerated placental site can occur following normal or ectopic pregnancy, abortion, or hydatidiform mole. We encountered a case of reactive exaggerated placental site seven months following normal pregnancy that clinically mimicked placental site trophoblastic tumor. Few reports have described the clinical course, histopathology and differential diagnosis of exaggerated placental site; we present our patient’s case together with histopathological observations and review of related literature.

Key words: EPS, PSTT.

Introduction
Exaggerated placental site (EPS) is a non-neoplastic trophoblastic lesion featuring endometrial and myometrial invasion by intermediate trophoblasts and syncytiotrophoblasts. Morphology and biological behavior differ from those of other trophoblastic lesions [1]. Reports have noted development of EPS following a normal or ectopic pregnancy, abortion, or hydatidiform mole [2-6]. We report our experience with a 33-year-old woman whose normal delivery was followed seven months afterward by irregular vaginal bleeding and rising beta-hCG titer. Initial clinical diagnosis was exaggerated placental site trophoblastic tumor (PSTT), for which chemotherapy regimens were tried. Finally, histopathology of the hysterectomy specimen confirmed exaggerated placental site. We reviewed other reported cases of EPS and their clinical course. The literature shows that EPS represents an extreme end of a physiological process and simple uterine curettage or endometrial ablation is curative [1, 7]. Reporting such cases can increase physician awareness and help prevent unnecessary hazards of surgery and chemotherapy, as well as unnecessary loss of fertility.

Case Report
A 33-year-old woman presented with irregular vaginal bleeding that began seven months after a normal vaginal delivery. On examination, the uterus was bulky. Transvaginal ultrasonography showed an echogenic lesion on the posterior wall of the uterus involving both the endometrium and myometrium. Serum beta hCG-CTP was 3.3 mIU/ml (normal < 0.7 mIU/ml). Endometrial cytology revealed trophoblastic cells but no chorionic villi. Immunohistochemistry revealed a weakly positive beta hCG, PLAP (placental alkaline phosphatase) and Ki-67 (7.6% of aspirated cells were positive for Ki-67). Whole-body computed tomography revealed no evidence of metastasis. Magnetic resonance imaging (MRI) identified an enlarged uterus with a slightly high-signal-intensity mass at the periphery of the posterior wall that showed mild gadolinium enhancement. There was also disruption of the integrity of the junctional zone (Figure 1). The findings suggested possible uterine trophoblastic disease. Relatively low levels of serum hCG and Ki-67 index and weak hCG expression by trophoblastic cells ruled out choriocarcinoma. Close monitoring of serum beta hCG showed continued elevation over the following three months. Finally, the diagnosis of placental site trophoblastic tumor (PSTT) was considered based on high beta hCG titer and the presence of trophoblastic cells with a relatively high Ki-67 index.

Figure 1. — MRI of abdomen and pelvis showing enlarged uterus with a slightly high-signal-intensity mass at the periphery of the posterior wall that showed mild gadolinium enhancement. There was also disruption of the integrity of the junctional zone.
Because the patient desired to preserve her fertility, three courses of systemic MAC chemotherapy were given (e.g., 15 mg methotrexate intramuscularly days 1-5, 10 μg/kg actinomycin D intravenously days 1-5, and 100 mg cyclophosphamide intravenously days 1-5 every two weeks). However, serum hCG remained high and bleeding persisted, leading to the conclusion the patient had chemoresistant PSTT. Hysterectomy was performed. Grossly, the uterus had normal appearance, weighing 66.8 g and measuring 9 cm x 7.3 cm x 2.5 cm. In the myometrium, microscopically there was extensive trophoblastic infiltration of the endometrium and myometrium by mononuclear intermediate trophoblastic cells with intense eosinophilic cytoplasm (Figure 2). There were aggregates of trophoblastic cells around blood vessels, but no vessel invasion or necrosis. Also, no chorionic villi could be detected. Immunohistochemistry of trophoblastic cells showed weakly positive staining for hCG, PLAP and Ki-67 (Figure 3). The final histopathologic diagnosis was EPS. Immediately after surgery, serum and urinary beta hCG became undetectable.

**Discussion**

Physiologically, intermediate trophoblast invades only the inner third of the myometrium in the first trimester of pregnancy and undergoes progressive regression thereafter. In the extremely rare condition called EPS, implantation site intermediate trophoblasts infiltrate the myometrium exuberantly, although there are no data to quantify amount and extent of pathological trophoblastic infiltration [1]. Histologically, there are more implantation site intermediate trophoblastic cells than normally present in the implantation site. The few reported cases have developed following normal or ectopic pregnancy, abortion, or hydatidiform mole [2-6] (Table 1). Although EPS is a non-neoplastic lesion, it can be confused with various neoplastic and non-neoplastic trophoblastic and non-trophoblastic lesions. Because of its rarity of occurrence, gynecologists and pathologists often miss the diagnosis, at least during initial workup. Appropriate diagnosis is important for specific therapeutic approaches and for avoidance of unnecessary hazards such as chemotherapy and surgery, as well as unnecessary loss of fertility.

The most important diagnosis in the differential is PSTT. Unlike EPS, PSTT usually forms infiltrating lesions that are associated with chorionic villi and are confined within a third of the myometrium. In EPS,
mitosis is frequently absent and the Ki-67 labeling index is usually 0-1%; in contrast, the labeling index is higher, 14% ± 6.9%, in cases of PSTT [1]. Our patient was initially misdiagnosed with PSTT on the basis of a relatively high labeling index and the presence of a few mitotic figures.

Although a diagnosis of PSTT is recommended if the Ki-67 index in implantation site intermediate trophoblastic cells exceeds 5%, certain features are important in estimating the labeling index. Implantation site intermediate trophoblastic cells can closely resemble natural killer cells and activated T lymphocytes, both of which can be found in the placental site and are highly positive for Ki-67. Some pathologists believe a double staining index is usually 0-1%; in contrast, the labeling index is higher, 14% ± 6.9%, in cases of PSTT [1]. Our patient was initially misdiagnosed with PSTT on the basis of a relatively high labeling index and the presence of a few mitotic figures. Keeping these points of distinction in mind can minimize the possibility of a diagnostic dilemma. Another point of confusion with our patient was the absence of chorionic villi on cytologic aspiration. Some published cases of EPS reported an absence of coexisting chorionic villi [8], so an absence of chorionic villi in a histopathological sample does not rule out the diagnosis of EPS.

In EPS, infiltrating intermediate trophoblasts in the myometrium are a bit different from normal placental site trophoblasts. If we consider different immunologic markers, hCG is usually focally positive whereas human placental lactogen (hPL) and cytokeratin are diffusely positive in intermediate trophoblasts associated with normal pregnancy. However in EPS, intermediate trophoblasts show a lower level of production of hPL. On the other hand, hPL is expressed in most intermediate trophoblastic cells in PSTT, and thus hPL can be used as a diagnostic and monitoring marker [9]. In Table 2 the different histopathologic findings for reported cases of EPS are shown.

Approximately 10-15% of PSTTs are clinically malignant, and the mortality rate for patients with PSTT is about 15-20% [1]. Surgery is the cornerstone of treatment. In contrast, EPS is a benign condition that can be cured by simple curettage or endometrial ablation. However, there are times where a differential diagnosis of EPS versus PSTT is quite difficult and a definitive diagnosis is made only after hysterectomy. For example, one patient reported in the literature was diagnosed directly with PSTT [10]. As a consequence, one group has concluded that patients diagnosed with EPS, especially those patients who want to preserve fertility, should have serial monitoring of beta hCG [5]. Another point of clinical importance is the possible coexistence of EPS and PSTT, a situation in which PSTT remains potentially dangerous. Although EPS can be treated successfully by curettage, the hypervascular variety of PSTT can cause profound post-procedure bleeding. Pre-procedure imaging is helpful in determining lesion vascularity.

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors/ ref number</th>
<th>Age of patient</th>
<th>Antecedent pregnancy</th>
<th>Interval before diagnosis</th>
<th>Initial beta hCG</th>
<th>Initial diagnosis</th>
<th>Treatment</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>Stolnicu et al. [2]</td>
<td>55</td>
<td>normal delivery</td>
<td>15 years</td>
<td>NM</td>
<td>NM</td>
<td>TAH</td>
<td>NED</td>
</tr>
<tr>
<td>2008</td>
<td>Hasegawa et al. [3]</td>
<td>39</td>
<td>following curettage</td>
<td>15 years</td>
<td>20 mIU/ml (serum)</td>
<td>Gestational trophoblastic disease</td>
<td>TAH</td>
<td>NED</td>
</tr>
<tr>
<td>1996</td>
<td>Kase et al. [4]</td>
<td>44</td>
<td>cervical pregnancy</td>
<td>1 month</td>
<td>NM</td>
<td>Gestational trophoblastic disease</td>
<td>TAH</td>
<td>NED</td>
</tr>
<tr>
<td>1999</td>
<td>Menczer et al. [5]</td>
<td>48</td>
<td>molar pregnancy</td>
<td>1 month</td>
<td>1300 IU/ml (serum)</td>
<td>Postmolar trophoblastic disease choriocarcinoma</td>
<td>TAH</td>
<td>NED</td>
</tr>
<tr>
<td>2009</td>
<td>present case</td>
<td>33</td>
<td>normal delivery</td>
<td>7 months</td>
<td>3.3 mIU/ml (serum)</td>
<td>PSTT</td>
<td>TAH</td>
<td>NED</td>
</tr>
</tbody>
</table>

EPS, exaggerated placental site; NM, not mentioned; NED, no evidence of disease; TAH, total abdominal hysterectomy; PSTT, placental site trophoblastic tumor.

Table 2. — Histopathological findings of patients with EPS.

<table>
<thead>
<tr>
<th>Authors/ ref number</th>
<th>Site of EPS</th>
<th>Chorionic villi</th>
<th>Ki-67 index</th>
<th>Staining for beta hCG</th>
<th>hPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stolnicu et al. [2]</td>
<td>cervix</td>
<td>not found</td>
<td>1%</td>
<td>only focally positive</td>
<td>NM</td>
</tr>
<tr>
<td>Hasegawa et al. [3]</td>
<td>uterus</td>
<td>present</td>
<td>2%</td>
<td>positive</td>
<td>NM</td>
</tr>
<tr>
<td>Kase et al. [4]</td>
<td>uterus</td>
<td>present</td>
<td>NM</td>
<td>weakly positive</td>
<td>NM</td>
</tr>
<tr>
<td>Menczer et al. [5]</td>
<td>uterus</td>
<td>not found</td>
<td>insignificant</td>
<td>strongly positive</td>
<td>NM</td>
</tr>
<tr>
<td>Nigam et al. [6]</td>
<td>uterus</td>
<td>present</td>
<td>insignificant</td>
<td>positive</td>
<td>NM</td>
</tr>
<tr>
<td>present case</td>
<td>uterus</td>
<td>not found</td>
<td>7.6%</td>
<td>only focally positive</td>
<td>NM</td>
</tr>
</tbody>
</table>

EPS, exaggerated placental site; NM, not mentioned.
Although over-diagnosis may cause an unnecessary therapeutic hazard, under-estimating the risk of PSTT can complicate the clinical picture through severe hemorrhage after conservative treatment or by spreading malignant cells.

In reviewing published cases, we found that a long interval from previous pregnancy does not rule out the possibility of EPS (Table 1). Our patient developed bleeding seven months after a normal vaginal delivery. A similar case was described by Stolnicu et al. [2], in which diagnosis was made 15 years after the last pregnancy. These cases indicate that EPS can remain asymptomatic for a long time, with symptoms presenting weeks to years after the preceding pregnancy.

Due to advances in cytologic diagnosis with different immunohistological markers, it has become easier to diagnose different placental lesions. Continued reporting of cases of EPS should be encouraged because analysis of a larger number of cases may provide important information to help establish standard diagnostic criteria and treatment regimens.

References

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Late recurrence of malignant melanoma mimicking ovarian malignancy

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Introduction

Malignant melanoma is an extremely malignant tumor with an unpredictable profile of spread and variable periods of remission. Primary cutaneous melanoma lesions may precede gastrointestinal (GI) sites of metastasis by many years and may be difficult to diagnose. Between 1% and 4% of all patients with malignant melanoma will have clinically apparent GI involvement diagnosed ante mortem, with up to 60% of patients with melanoma found to have metastases at autopsy [1].

Metastases of malignant melanoma may be discovered many years after the diagnosis of the primary lesion. We describe an unusual case of malignant melanoma metastatic to the omentum occurring seven years after diagnosis and treatment of cutaneous malignant melanoma in the patient’s arm.

We believe that this case of multinodal omental metastasis arising from malignant melanoma deserves some comment from the point of view of practical management and appropriate choice of the imaging technique.

Case Report

A 56-year-old postmenopausal woman was admitted to our department because of lower abdominal pain and palpable left pelvic mass. She gave no significant gynecologic history. At the age of 49, a nodular-type cutaneous malignant melanoma originating in a giant pigmented nevus had been detected on the patient’s left arm and treated by wide local excision. Histopathologic examination had demonstrated tumor-free surgical margins and microstaging had revealed Breslow depth of 0.78 mm and Clark level of III. The patient had not received adjuvant therapy and her course had been uneventful for the follow-

Summary

Background: Malignant melanoma (MM) is an extremely malignant tumor with an unpredictable profile of spread and variable periods of remission. Case: We describe an unusual case of malignant melanoma metastatic to the omentum occurring seven years after diagnosis and treatment of cutaneous malignant melanoma in the patient’s arm. She received surgery and chemoimmunotherapy. To date, nine months after detection of malignant melanoma metastatic to the omentum, the patient is alive with no clinical and radiological metastatic disease. Conclusions: The diagnosis of omentum malignant melanoma in a living patient is uncommon, thus very few individuals and referral centers can build up an adequate experience of handling this disease. Optimal management has been a challenge and a subject of debate and has not yet been established.

Key words: Malignant melanoma; Ovarian malignancy; Omentum.

Figure 1. — Omentum with multinodal tumors - melanoma metastasis.
Late recurrence of malignant melanoma mimicking ovarian malignancy

After the surgical treatment the patient received one cycle of chemoimmunotherapy according to our protocol for malignant melanoma consisting of three days of chemotherapy (BCNU, day 1; DTIC, days 1-3; and cisplatin, days 1-3) followed by five days of immunotherapy (interleukin-2 [IL-2] and interferon [IFN], days 4-8).

To date, nine months after detection of malignant melanoma metastatic to the omentum, the patient is alive with no clinical and radiological metastatic disease.

Discussion

The vast majority of omental neoplasms are metastatic carcinomas arising from the ovary, gastrointestinal tract, or pancreas and these are often associated with abdominal ascites. Primary and secondary tumors of the omentum, when sufficiently large, present with a palpable abdominal mass or diffuse distention requiring surgical excision.

Malignant primary and secondary omental tumors are highly invasive and often present late with involvement of adjacent organs. Radical surgical excision of both the omentum and the involved organs may be required but often palliative surgery is the only treatment option [2].

Analysis of the case notes show that the cutaneous lesion was microstaged with Breslow depth of 0.78 mm and Clark level of III with no evidence of invasion, according to the staging prevalent prior to the current American Joint Committee on Cancer (AJCC) staging [3].

Review of the case notes shows that the clinical decision was to treat with wide excision of the lesion and not to proceed to regional lymph node dissection. There were no clinically enlarged lymph nodes and an elective lymph node dissection was not performed. Elective lymph node dissection is controversial in tumors between 1 and 4 mm thick, as a result of increased morbidity and no documented increased survival.

When a patient has a past history of malignant melanoma with an abdominal mass, a thorough physical examination and radiologic surveillance is essential to exclude multiorgan involvement.

As part of metastatic localizations, a total body computed tomography (CT) scan and positron emission tomography (PET) are recommended. Unfortunately we are not in the position to perform PET scan imaging. Postoperative MRI of the abdomen and pelvis demonstrated no metastatic lesion in this case.

In our case, the diagnosis of malignant melanoma metastatic to the omentum was established only after the explorative laparotomy. It is noteworthy that although the primary lesion on the patient’s back invaded to Clark’s level III, metastatic disease was found after seven years, much later than usually expected.

As the period of remission is unpredictable and possibly long, an adequate history is essential to arrive at the correct differential diagnosis. Ultrasound was unable to characterize the lesion in our patient.

Ben-David et al. [4] have described a rare case of malignant melanoma metastatic to the ovary and to the omentum 25 years after enucleation of one eye for malignant melanoma of the choroid.

Metastatic melanoma is associated with poor prognosis, Stage III with 45% 5-year survival rate and Stage IV disease with 11% 5-year survival [5]. Postoperative adjuvant radiotherapy could improve long-term disease control.

In recent years, adjuvant chemoimmunotherapy has been applied for patients with malignant melanoma metastatic to the ovary. However, there is no definitive evidence that adjuvant chemoimmunotherapy is beneficial.

Biochemotherapy uses chemotherapeutic agents in combination with interferon alpha and interleukin-2, and shows an improved response rate compared to chemotherapy alone [5].

Since the diagnosis of omentum malignant melanoma in a living patient is uncommon and since, consequently, very few individuals or even referral centers can build up an adequate experience of handling this disease, its optimal management has been a challenge and a subject of debate and has not yet been established.
References


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Bone metastasis arising from a polyp of the cervix as the first symptom in generalized multi-organ adenocarcinoma

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Summary
Our patient was a 40-year-old female with a positive familial history for malignancies but no chronic diseases. After two vaginal deliveries without any reported difficulties, the patient had no intermenstrual bleeding, postcoital bleeding, leucorrhea or hypermenorrhea, abnormal vaginal bleeding, or postmenstrual bleeding, except during the past five-year period when a polyp-like change in the cervix was found. There was no indication for polypectomy, considering the fact that the patient had no symptoms, had an iodine-positive Schiller test, as well as regular cytological smears on Papanicolaou testing. It is noteworthy that the patient had no symptoms until changes in the stool and painful sensation in the hip area. The patient was subjected to extensive surgery by a team composed of a gynecologist, surgeon and orthopedist. During Werthaim-Meigs surgery, four positive glandules and cervical adenocarcinoma Stage II were found. The colon was removed, as a right hemicolectomy, as well as the iliac bone upper segment. Unfortunately, considering the changes in the tissue of the colon and cervix, we considered the condition to be “generalized” adenocarcinoma.

Key words: Generalized adenocarcinoma; Polyps; Cervical adenocarcinoma.

Introduction
A diagnosis of “generalized” adenocarcinoma is significant because it indicates the presence of an unknown cancerous process. However, it is difficult to make treatment decisions and prognostic evaluation because it is determined by the tissue from which the tumor cells arise - the tissue of origin [1].

Adenocarcinoma is a cancer that originates in glandular tissue. Well differentiated adenocarcinomas tend to resemble the glandular tissue which they are derived from. Adenocarcinomas can arise in many tissues of the body due to the ubiquitous nature of glands within the body. While each gland may not be secreting the same substance, as long as there is exocrine function to the cell, it is considered glandular and its malignant form is therefore called adenocarcinoma [2]. Endocrine gland tumors, such as a vipoma, an insulinoma or pheochromocytoma, are typically not referred to as adenocarcinomas, and they are often called neuroendocrine tumors [3]. If the glandular tissue is abnormal, but benign, it is said to be an adenoma [4].

According to its incidence, cervical adenocarcinoma is the second most common malignant tumor in women after breast cancer [5]. Adenocarcinoma occurs less frequently compared to planocellular carcinoma, and appears in 12% to 20% of the cases. However, all available data show an evident increase in its frequency despite available and modern methods of diagnosis. Histological detection is more difficult than in planocellular carcinoma [6].

Although a cervical polyp may be very small and at first sight has no symptomatic cervical change, it is necessary to observe and examine the polyp in detail although it is known that the frequency of malignant changes is less than 1% [7]. The most common metastases of cervical adenocarcinoma are in liver 30%, bones 30%, lungs 21%, pleura 21%, kidneys 12%, ovaries 9%, and skull 3.3% [8]. Available data indicate that the coincidence of polyps in other tissues is almost 30%. In gastroenterology, the risk of cancer in villous adenoma, tubulovillous adenoma or tubular adenoma in polyps greater than 2 cm is 53%, 46% and 35%, respectively [9].

Case Report
Our patient was 40 years old, with a positive family history of malignancies but no chronic diseases, who had had two vaginal deliveries without any reported difficulties (7 and 13 years earlier). The patient had no intermenstrual bleeding, postcoital bleeding, leucorrhea or hypermenorrhea, abnormal vaginal bleeding, or postmenstrual bleeding, but was subjected to regular gynecological examinations during the past five-year period when a polyp-like change in the cervix was found. There was no indication for polypectomy, considering the fact that the patient had no symptoms, had an iodine-positive Schiller test, as well as regular cytological smears on Papanicolaou testing. The diameter of the polyp was approximately 0.5 mm. Colposcopy did not describe other changes, except scarring from cervical lacerations during deliveries.

However in May 2008 the patient had sudden changes in frequency and consistency of the stool – which she did not relate to her gynecological situation. Because of the mucous change in the stool and addition of blood she went to a gastroenterologist and the Adler test was positive. There was no weight loss, even though her body mass index was significantly low. The...
The patient had been a smoker for the past ten years. The procedure of colonoscopy that was performed diagnosed colon polyposis - multiple polyps, from 0.5 to 1 cm. The polyps were extracted, then analyzed at histopathology and tubulovillous adenomas were confirmed. After the first diagnosis in gastroenterology, based on the gynecological finding of a polyp, loop diathermy of polyp changes and routine fraxtionary explorative curettage were carried out. Histopathological analysis confirmed cervical adenocarcinoma. The patient was put on the protocol of complete diagnosis. In X-ray imaging secondary degenerative changes in the iliac bone were found. By MRI and PET scan imaging the diagnosis was more precise for metastatic changes in this area. Lung X-ray was without pathological changes and the patient’s cardiological condition was unremarkable, as well as the neurological and ophthalmological ultrasound.

It is remarkable that the patient had no symptoms until the change in stool and painful sensation in the hip area. She was subjected to extensive surgery by a team composed of a gynecologist, surgeon, and orthopedist. During the Werthaim-Meigs procedure, four positive glandules and cervical adenocarcinoma Stage II were found. A right hemicolectomy was performed to remove the colon as well as the iliac bone upper segment. The hip joint, pubic area and ischiatic bone did not show changes during the diagnostic workup.

Unfortunately, considering the changes in the tissue of the colon, and cervix, we presumed that the condition called “generalized adenocarcinoma” was established in this patient. Generalized adenocarcinoma is often a preliminary diagnosis and can be clarified by a pathologist using immunohistochemistry.

Even though it is rare in medical practice, this condition has a silent and mean development. The basis of the disease is in the appearance of adenocarcinomatous changes on the most vulnerable glandular tissues.

In these cases family history is extremely important. A more precise family history proved a positive history for adenomatous polyps. Colon carcinoma develops 100% more frequently in patients with a positive family history compared to those without a family history for adenomatous polyps [9].

**Conclusion**

Although cervical polyps can be very small and at first sight have no symptomatic cervical changes, it is necessary to observe and analyze in detail each and every polyp, even when available data show the incidence of malignant change to be less than 1%.

In our opinion the best solution is to perform a more precise diagnosis, polypectomy, and biopsy, even when benign results are expected.

Nature often gives us signals which, if not carefully observed, may progress into conditions where medicine has limited possibilities. The quality of this patient’s life...
was significantly reduced but she was informed about the medical board recommendations. Thus, with the radical procedure and expertise of the gynecology, surgery and orthopedic team, the life of our patient was prolonged.

References


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The clinical significance of HPV screening in premalignant cervical lesions

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Summary

Purpose: We evaluated the clinical significance of human papilloma virus (HPV) screening in premalignant cervical lesions. Methods: This prospective study was performed at Dicle University, School of Medicine, Department of Obstetrics and Gynecology, from January 2009 to June 2009. A total of 60 cases were evaluated. Thirty cases had premalignant cervical lesions. The prevalence of HPV was analyzed by polymerase chain reaction and types determined by Hybrid Capture II. The cases that had premalignant cervical lesions were evaluated with colposcopy. Statistical analyses were carried out by using the statistical packages for SPSS version 12.0 for Windows (Chicago, IL, USA). Results: Of all the cases, those with premalignant cervical lesions had higher prevalence of HPV DNA. The cases that had high oncogenic HPV type had more abnormal colposcopic findings. Conclusion: Premalignant cervical lesions should be evaluated by cervical cytology, colposcopy, HPV DNA screening and cervical tissue sampling. In this way, development of cervical cancer can be prevented.

Key words: Premalignant; Cervical; Lesion; HPV; Clinical significance.

Introduction

Premalignant cervical lesions (atypical squamous cells of undetermined significance – ASCUS, high-grade squamous intraepithelial lesions – HSIL, and low-grade squamous intraepithelial lesions – LSIL) have been considered to be preventable lesions of the cervix, caused by persistent human papilloma virus (HPV) infection [1, 2]. Exfoliative cytology, used to detect invasive and in-situ carcinomas of the uterine cervix, was first demonstrated by Papanicolaou and Traut [3]. When premalignant lesions have been detected in exfoliative cytology, HPV testing is used in the secondary assay with good sensitivity and a high negative predictive value (NPV), and colposcopy directed biopsies of suspicious areas should be taken for final diagnosis [4, 5]. HPV testing parameters include polymerase chain reaction (PCR), and Hybrid Capture II (HCH) assay [6]. In a recent study, 6.7% of cases of HSIL or cancer were found by histology, and 39.5% of patients had high-risk HPV. The sensitivity of HPV testing for high-grade dysplasia was 89.2%, and specificity was 64.1% [7].

In the present study the prevalence and types of HPV and colposcopic findings of premalignant cervical lesions were evaluated.

Material and Methods

This prospective study was performed at Dicle University, School of Medicine, Department of Obstetrics and Gynecology, from January 2009 to June 2009. A total of 60 cases were evaluated. The cases had normal cytology (n = 30, 50%) and premalignant cervical lesions (n = 30, 50%). Cervical exfoliative cytology was made by the conventional method and samples were taken for HPV DNA testing by using reverse hybridization-based (GenID HPV) screening apparatus. The cases that had premalignant cervical lesions assessed by colposcopy (Leisegang® OptiK™ light-emitting diode (LED) colposcopes) and of the cases that had abnormal colposcopy findings, colposcopy directed biopsies were taken.

The mean and standard deviation (SD) were calculated for continuous variables. Normality of variables was analyzed by the Kolmogorov-Smirnov test. The chi-square test and Student’s t-test evaluated associations between the categorical and continuous variables. Two-sided p values were considered statistically significant at p < 0.05. Statistical analyses were carried out by using the statistical package for SPSS 12.0 for Windows (Chicago, IL, USA).

Results

Sixty women were evaluated in this study. The demographic and clinical characteristics of the cases are shown in Table 1. Mean ages were 38.90 ± 9.58 and 37.70 ± 7.52 in the study and control group, respectively. Sixteen patients (26.66%) were smokers and 40 (66.6%) women used hormonal contraceptives. Mean ages at sexual debut were 19.46 ± 3.03 and 20.75 ± 5.03 years, respectively, in the study and control groups which were not statistically different (p = 0.237).

In the study group, 50% of the cases had high-risk (HR) HPV, 20% had low-risk (LR) HPV, and in 18% of the cases HPV was not detected. The cases that had high-risk HPV type had more abnormal colposcopic findings. Mean ages at sexual debut were 19.46 ± 3.03 and 20.75 ± 5.03 years, respectively, in the study and control groups which were not statistically different (p = 0.237).

In the study group, 50% of the cases had high-risk (HR) HPV, 20% had low-risk (LR) HPV, and in 18% of the cases HPV was not detected. The incidence of HPV DNA increased by advancing age, and 38 (63.33%) of the cases were patients aged 35 and older. Abnormal colposcopy findings (acetowhite epithelium,
The clinical significance of HPV screening in premalignant cervical lesions

Table 1. — Demographic and clinical characteristics of the cases.

<table>
<thead>
<tr>
<th></th>
<th>Group with normal Pap smear (n = 30)</th>
<th>Group with premalignant Pap smear (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.70 ± 7.52</td>
<td>38.90 ± 9.58</td>
<td>0.592</td>
</tr>
<tr>
<td>Age at sexual debut (years)</td>
<td>20.75 ± 5.03</td>
<td>19.46 ± 3.03</td>
<td>0.237</td>
</tr>
<tr>
<td>HR-HPV (%)</td>
<td>10 (33.3%)</td>
<td>21 (70%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR-HPV (%)</td>
<td>9 (30%)</td>
<td>15 (50%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

HR-HPV: High-risk human papillomavirus, p < 0.05 is accepted as statistically significant.

In conclusion, we found high-risk HPV infection to be highly and positively associated with the severity of premalignant cervical lesions. It should never be forgotten that exfoliative cytology is not a diagnostic method, but when combined with colposcopy and HPV DNA testing, will bring about early detection of premalignant cervical lesions and thus prevention of invasive cancer.

References


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