european journal
of
gynaecological oncology
an International Journal

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European Journal of Gynaecological Oncology - Vol. XXXI, no. 1, 2010

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Referral and ascertainment bias in patients with synchronous and metachronous endometrial malignancy[*]

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Summary

The purpose of this study was to evaluate the frequency in patients with endometrial cancer of other malignancies and the influence of referral and ascertainment biases on these associations. Analysis of 1,028 local and referred patients who had a hysterectomy for endometrial cancer was based on residence at the time of diagnosis. Altogether, 208 patients had a history of another malignancy, most frequently breast, colon, and ovary. At the time of surgery for endometrial cancer, the prevalence of lymphoma and breast and ovarian cancers was greater than expected although the higher prevalence of lymphoma was limited to referred patients. During follow-up after hysterectomy, the incidence of lung cancer was lower than expected, whereas the incidence of lymphoma was higher. Breast, colorectal, and bladder cancers were more common than expected although this finding was limited to local patients. We concluded that results of epidemiologic studies from tertiary care centers may be misleading if they do not account for referral and ascertainment biases.

Key words: Ascertainment bias; Endometrial cancer; Epidemiology; Multiple malignancies; Referral bias.

Introduction

Endometrial cancer is the most common malignancy of the female reproductive tract in the United States [1]. Multiple primary cancers can occur in the same patient. Colon, ovarian, and breast cancers [2-6] have been reported previously to be the malignancies most commonly associated with endometrial cancer. Other associated malignancies are those arising in the bladder, small intestine, skin, and soft tissue [7]. However, these associations may be influenced by referral and ascertainment biases, which may be present in analyses of patients from tertiary care centers. In fact, there is a high likelihood that a greater proportion of patients with high-risk histologic features, in poor medical condition, or both are referred to tertiary care centers, resulting in an artificial increase in the number of patients with the above characteristics (referral bias). Patients with endometrial cancer who are referred to tertiary care centers are more likely to have advanced lesions, history of other malignancies, and more comorbid conditions than local patients [8]. Similarly, the rate of malignancies reported during follow-up is decreased among referred endometrial cancer patients [3]. This last finding demonstrated that less accurate follow-up, as may occur in patients who live far from tertiary care centers, may lead to ascertainment bias.

The aims of the present study were to evaluate the incidence and prevalence of other associated malignancies in a cohort of women with endometrial cancer and to assess the potential influence of referral and ascertainment biases on the above associations.

Patients and Methods

With approval by the Mayo Clinic Institutional Review Board, we identified 1,109 patients whose endometrial cancer was managed surgically at Mayo Clinic, Rochester, Minnesota, between 1984 and 1996. A portion of this cohort of patients has been described in detail in our previous analyses [9,10]. All patients had epithelial endometrial cancer, and their treatment included hysterectomy and removal of existing adnexal structures. Overall, 81 patients were excluded from the present analysis because they did not authorize use of their information for research [11], or follow-up information on associated malignancies was inadequate. The remaining 1,028 patients form the cohort of the current study.

As previously described [9], all hematoxylin-eosin–stained slides of the endometrial cancers were reviewed retrospectively to confirm the original diagnosis of adenocarcinoma and to determine histologic grade and subtype. Staging was also defined according to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system [12]. For patients who received treatment before 1988, stage was determined retrospectively on the basis of the surgical and pathologic assessments. Histologic classification was performed according to the World Health Organization classification [13]. Architectural grading was based on the degree of glandular differentiation in accordance with the FIGO guidelines [12].

The presence of other associated tumors was verified by histologic diagnosis in all patients undergoing surgery at Mayo Clinic, and the diagnosis was confirmed by pathology review. When

patients had surgery outside Mayo Clinic, the diagnosis of an associated cancer was abstracted from the medical records of the other medical facilities or from letters from outside physicians.

For distinguishing synchronous tumors of the ovary and endometrium from ovarian metastases, the criteria of Ulbright and Roth [14] were used. All endometrial cancers with associated ovarian involvement had been reviewed and appropriately classified as either synchronous primary or metastatic.

History of malignancy was defined as the diagnosis of another invasive malignant disease before or at the time of the operation, including also the immediate 30 days after the operation for endometrial cancer (prevalence) or during the subsequent follow-up (incidence). As previously described [9], if sufficient follow-up information about survival, recurrence, or presence of other malignancies was not available in the clinical records, death certificates were obtained, and letters were sent or telephone calls were made to patients and family physicians to obtain the information.

The expected number of other primary malignancies was estimated from Surveillance Epidemiology and End Results (SEER) data for the year 2000, using the age-adjusted rate of the female population [15]. The incidence and prevalence statistics were generated by the locally developed SAS "personyrs" procedure [16]. We determined the age-specific person-years of follow-up and compared expected to observed numbers of subsequent malignancies. For comparison with SEER data, the prevalence information was limited to the ten years before the endometrial cancer diagnosis. However, information about incidence was considered even beyond ten years after the diagnosis of endometrial cancer.

The standardized incidence ratio (SIR) and standardized prevalence ratio (SPR) were calculated according to the SEER statistics manual [15]. All the analyses were performed with SAS version 8 software (SAS Institute Inc, Cary, NC).

When we analyzed the incidence and prevalence of "all cancers" in the SEER database, we subtracted the rate of endometrial cancers. Moreover, according to the definition of "all cancers" in the SEER database [15], in our analysis we included in the definition of "other malignancy" all invasive cancers (i.e., no in situ malignancies, except for in situ cancer of the urinary bladder), excluding basal and squamous cell carcinomas of the skin (except when squamous cell carcinomas occurred on the vulva). Furthermore, for the analysis of incidence and prevalence of different tumors, we considered the following cancer sites altogether: colon together with rectum; kidney with renal pelvis; and lung with bronchus. For the definition of cancer of the urinary bladder, we included in situ carcinoma; for lymphoma, we grouped together both Hodgkin's and non-Hodgkin's lymphoma.

When a patient had multiple independent cancers (not recurrences) at the same site diagnosed at different time periods (for example, 2 different breast cancers diagnosed 4 years apart), for the purpose of the analysis of incidence and prevalence (and the count of the overall number of associated cancers), only the first appearance of the cancer at a given anatomic site was considered. For the evaluation of possible referral and ascertainment biases, we performed a stratified analysis subdividing the cohort by residency at initial diagnosis (i.e., coming from within or beyond a 50-mile radius from our institution), using residency information from the Mayo Clinic database. Constancy of the risk estimates from one geographic area to the next would be evidence for nearly complete ascertainment of subsequent malignancies throughout the study; but a decline from the level attained where follow-up is most reliable (i.e., locally) would suggest underascertainment among referred patients [3].

Results

Characteristics of the 1,028 patients with endometrial cancer are summarized in Table 1. The mean (SD) age at surgery for endometrial cancer was 64.7 (11.0) years (median, 65 years). Mean body mass index (BMI) was 30.3 (8.3) (median, 28.6).

Analysis of the referral pattern at the Mayo Clinic

Altogether, 218 patients (21%) were living within a 50-mile radius of our institution at the time of their surgery; 182 patients (18%) were living between 51 and 100 miles away; 197 (19%) were living between 101 and 200 miles away; and the residence area of 431 patients (42%) was beyond 200 miles from our institution (Table 1). Compared with the 218 patients living within a 50-mile radius, the 810 living beyond 50 miles did not differ significantly by age, stage of endometrial cancer, depth of myometrial invasion, histologic grade, or subtype.

Table 1. — *Characteristics of 1,028 patients at the time of diagnosis of epithelial endometrial cancer undergoing surgery at Mayo Clinic between 1984 and 1996.*

Characteristic	No. (%)[a]	Mean (SD)
Age at diagnosis (years)		64.7 (11.0)
Body mass index, diagnosis		30.3 (8.3)
Follow-up (months)[b]		74.1 (44.9)
Vital status at last follow-up		
Deceased	297 (30)	
Alive	708 (70)	
Missing information[c]	23	
Associated other tumors[d]		
Yes	208 (20)	
No	820 (80)	
Stage		
I	706 (69)	
II, III, IV	322 (31)	
Depth of myometrial invasion (%)		
≤ 50	773 (76)	
> 50	246 (24)	
Missing information	9	
Histologic grade		
1	430 (42)	
2	346 (34)	
3	250 (24)	
Missing information	2	
Histologic subtype		
Endometrioid	911 (89)	
Nonendometrioid	117 (11)	
Referral patterns, mi[e]		
≤ 50	218 (21)	
51-100	182 (18)	
101-200	197 (19)	
> 200	431 (42)	

[a] Percentage excludes missing patients.

[b] Months from diagnosis of endometrial cancer to last known.

[c] No available information after discharge from the hospital at the time of hysterectomy.

[d] Excluding skin cancers other than melanoma and in situ cancers other than bladder cancer. Some patients had multiple primary cancers.

[e] Distance in miles from Rochester, Minnesota.

Table 2. — *Prevalence of other cancers in women with endometrial cancer compared with the general population and stratified by residency (≤/> 50 mi from Rochester, Minnesota)[a].*

Cancer	All patients (N = 1,028)			≤ 50 mi (n = 218)			> 50 mi (n = 810)		
	O/E	SPR (95% CI)	*p* value	O/E	SPR (95% CI)	*p* value	O/E	SPR (95% CI)	*p* value
Breast	48/27.3	1.8 (1.3-2.3)	< .001	12/5.6	2.1 (1.1-3.7)	.007	36/21.7	1.7 (1.2-2.3)	.002
Colorectal	8/6.6	1.2 (0.5-2.4)	.60	1/1.4	0.7 (0.0-3.8)	.71	7/5.2	1.3 (0.5-2.8)	.42
Ovary	26/2.1	12.7 (8.3-18.6)	< .001	7/0.4	16.9 (6.8-34.9)	< .001	19/1.6	11.6 (7.0-18.1)	< .001
Lung	2/3.2	0.6 (0.1-2.2)	.50	0/0.6	0.0 (0.0-5.8)	.42	2/2.6	0.8 (0.1-2.8)	.72
Bladder	0/1.7	0.0 (0.0-2.1)	.19	0/0.4	0.0 (0.0-10.0)	.54	0/1.4	0.0 (0.0-2.7)	.24
Kidney	1/1.1	0.9 (0.0-5.2)	.95	1/0.2	4.6 (0.1-25.8)	.09	0/0.8	0.0 (1.5-4.3)	.36
Lymphoma	7/2.4	2.9 (1.2-6.1)	.003	0/0.5	0.0 (0.0-7.5)	.48	7/1.9	3.7 (1.5-7.7)	< .001
Melanoma	4/2.2	1.8 (0.5-4.6)	.24	1/0.5	2.1 (0.1-12.0)	.43	3/1.8	1.7 (0.3-4.9)	.36
Thyroid	1/1.1	0.9 (0.0-5.1)	.93	1/0.2	4.4 (0.1-24.7)	.10	0/0.9	0.0 (0.0-4.3)	.35

O/E, observed/expected; SPR, standardized prevalence ratio; CI, confidence interval.
[a] Shaded cells indicate significant values (*p* < 0.05). To compare our data with the SEER database (15), we included only patients from our series who had a diagnosis of another tumor either synchronous (including the immediate 30 days after hysterectomy) or during the previous ten years before hysterectomy (see note c of Table 1).

However, patients living within 50 miles of Mayo Clinic had a mean BMI of 30.1 (0.3) compared with 31.3 (0.6) for those living beyond 50 miles (*p* < 0.05).

Frequency of other tumors

In total, 242 patients (24%) had a history of another malignancy. In 34 patients, however, the additional malignancy was skin cancer other than melanoma (i.e., basal cell carcinoma or localized squamous cell carcinoma other than vulvar cancer). Because these 34 women would be considered as having no history of associated malignancy according to the SEER definition [15], they were excluded. Thus, 208 patients (20%) were categorized with a history of associated malignancy. Overall, 238 malignancies occurred in these 208 patients.

According to the pathology reports, the most frequent malignancies associated with endometrial cancer were carcinomas of the breast in 98 patients (10%), colon in 30 patients (3%), and ovary in 36 patients (4%). However, after the 36 patients listed as double primary ovarian and endometrial cancer were reviewed, nine were categorized as ovarian metastases of an endometrial tumor according to published criteria. Therefore, 27 patients (3%) were listed as double tumors of the ovary and endometrium.

Of the 73 patients who had breast cancer either before or at the time of the diagnosis of endometrial cancer, 20 (27%) had been (or still were) receiving tamoxifen treatment.

We observed ten lung tumors (< %). Overall, nine of the ten patients with lung cancer had a histologic diagnosis of adenocarcinoma, carcinoid, neuroendocrine, or small cell lung tumor. Only one patient had a diagnosis of squamous cell cancer (7 years before the diagnosis of endometrial cancer).

Of the five patients who developed bladder cancer after treatment for endometrial cancer, two had radiotherapy as part of their primary treatment for the uterine neoplasm.

Prevalence of other tumors associated with endometrial cancer

At the time of surgery for endometrial cancer, the prevalence of lymphoma (SPR = 2.9; *p* = 0.003), breast cancer (SPR = 1.8; *p* < 0.001), and ovarian cancer (SPR = 12.7; *p* < 0.001) was higher than expected for the general population (Table 2). All ovarian cancers had been diagnosed at the time of surgery for endometrial cancer. The higher prevalence of breast and ovarian cancers was confirmed both in local patients and referred patients, whereas the higher prevalence of lymphoma was observed only in referred patients (Table 2).

Incidence of other tumors associated with endometrial cancer

Median follow-up was 69 months after the diagnosis of endometrial cancer. During this period of follow-up, the incidence of lung cancer was significantly lower than expected (SIR = 0.4; *p* < 0.05), whereas the incidence of lymphoma was significantly higher (SIR = 2.4; *p* = 0.008) (Table 3).

Among the endometrial cancer patients living within a 50-mile radius, the incidence of breast (SIR = 1.9; *p* < 0.05), colorectal (SIR = 3.1; *p* < 0.001), and bladder (SIR = 5.4; *p* = 0.001) cancer and lymphoma (SIR = 4.7; *p* < 0.001) was significantly higher than that among the general US population. However, none of these risks were elevated among patients residing beyond the 50-mile radius (Table 3). The finding of a decreased incidence of lung cancer was consistent in all subgroups analyzed (i.e., within or beyond the 50-mile radius) (*p* = 0.08 after stratification for residence area within or beyond the 50-mile radius).

Discussion

Patients who have already had a malignancy present a high likelihood of having a second primary cancer diagnosed during their lifetime. The overall rate of other associated cancers in patients with endometrial cancer was 20% in our series. Similarly, the frequency of synchronous or metachronous tumors associated with corpus cancer has been previously reported to be between 10% and 23% [3, 6, 17-20]. However, these percentages are only crude rates. To determine a true figure of the epidemiology of multiple tumors, we described separately the prevalence and the incidence of different cancers, comparing the results with data for the general US population [15].

Table 3. — *Incidence of other cancers in women with endometrial cancer compared with the general population and stratified by residency (≤/> 50 mi from Rochester, Minnesota)[a].*

| Cancer | All Patients (N = 1,028) | | | ≤ 50 mi (n = 218) | | | > 50 mi (n = 810) | | |
	O/E	SIR (95% CI)	p value	O/E	SPR (95% CI)	p value	O/E	SIR (95% CI)	p value
Breast	25/24.9	1.0 (0.6-1.5)	.99	10/5.3	1.9 (0.9-3.5)	.04	15/19.6	0.8 (0.4-1.3)	.30
Colorectal	12/11.3	1.1 (0.5-1.8)	.84	8/2.6	3.1 (1.3-6.1)	< .001	4/8.7	0.5 (0.1-1.2)	.11
Ovary	1/3.2	0.3 (0.0-1.7)	.22	1/0.7	1.4 (0.0-8.0)	.71	0/2.5	0.0 (0.0-1.5)	.11
Lung	5/13.7	0.4 (0.1-0.8)	.02	0/2.9	0.0 (0.0-1.3)	.09	5/10.8	0.5 (0.1-1.1)	.08
Bladder	5/2.5	2.0 (0.6-4.6)	.12	3/0.6	5.4 (1.1-15.8)	.001	2/2.0	1.0 (0.1-3.7)	.98
Kidney	2/1.8	1.1 (0.1-4.1)	.85	1/0.4	2.6 (0.1-14.6)	.31	1/1.4	0.7 (0.0-4.0)	.75
Lymphoma	9/3.8	2.4 (1.1-4.5)	.008	4/0.8	4.7 (1.3-12.1)	< .001	5/3.0	1.7 (0.5-3.9)	.24
Melanoma	1/2.2	0.4 (0.0-2.5)	.42	0/0.5	0.0 (0.0-7.5)	.48	1/1.7	0.6 (0.0-3.2)	.59
Thyroid	1/0.8	1.2 (0.0-6.6)	.87	0/0.2	0.0 (0.0-19.7)	.67	1/0.7	1.5 (0.0-8.4)	.67

O/E, observed/expected; SIR, standardized incidence ratio; CI, confidence interval.
[a] Shaded cells indicate *p* < .10.

We observed that the prevalence of breast and ovarian cancers in patients with endometrial malignancy was higher than that in the general population (Table 2). This is probably due to shared risk factors [3, 5] or to familial clustering [7], and it agrees with previously reported findings [3, 7]. However, an interesting observation of this study, not reported in the previous analysis from Mayo Clinic [3], was the significantly lower incidence of lung cancer in patients with endometrial tumors than in the general US population (Table 3). Our new observation might be explained by the fact that some of the "lung cancers" may have been diagnosed as "lung recurrences" in our series. Alternatively, we must emphasize that patients with lung cancer have different epidemiologic characteristics than those with endometrial cancer [21]. In fact, smoking, which is the most important risk factor for lung cancer, has been previously reported to be negatively associated with endometrial cancer [22]. In accord with the above observations, we reported that most lung cancers in our series were adenocarcinomas or other types different from the squamous lung malignancy and not related to smoking. This finding is also consistent with the fact that, in North America, the incidence of adenocarcinoma of the lung now exceeds that of squamous cell cancer [23].

The higher incidence of colon and bladder cancers (Table 3) may be explained by a genetic predisposition, like the familial hereditary nonpolyposis colorectal cancer-related syndrome [2]. In a minority of patients, the postoperative administration of radiotherapy [24] may have contributed to the higher incidence of bladder cancer (Table 3). However, our data are insufficient to support or reject this hypothesis.

It is possible that the patients managed in a particular institution and included in a certain study may not be representative of all patients with endometrial cancer. In fact, epidemiologic analyses from tertiary care institutions that do not account for possible differences between referred and local patients may lead to inaccurate results [3,8]. For the above reasons, in the present study we planned to analyze the association of other malignancies with endometrial cancer, stratifying for residence area. The 50-mile radius, which we used for defining local patients, permitted us to focus on a stable cohort, including patients who tend to return to Mayo Clinic for subsequent treatment. This area excluded the Minneapolis-St. Paul metropolitan statistical area, which includes patients more likely to be referred to other metropolitan hospitals for postoperative management.

In our patients, when stratified for residence area, we observed significant variations in incidence and prevalence of the associated malignancies (Tables 2 and 3). These findings may be simple artifacts, due to the relatively low numbers observed in the stratified subgroups. Alternatively, referral bias may explain the finding that the observed higher prevalence of lymphoma was limited to referred patients (Table 2). In fact, it is possible that patients were more likely to be referred to our hospital if they had a history that was "complicated" by the presence of lymphoma. Similarly, the higher incidences of breast, colorectal, and bladder cancers and lymphoma observed only in local patients and not in referred patients may be attributable to ascertainment bias (Table 3). Moreover, it is possible that the follow-up information was less accurate in patients living far from Mayo Clinic than it was in those living nearby.

Compared with the general US population, endometrial cancer patients present a higher likelihood of developing various malignancies during their lifetime (i.e., breast, ovarian, colorectal, and bladder cancers and lymphoma). Moreover, due to different epidemiologic risk factors, patients with endometrial cancer present a low risk of developing lung cancer. Our stratified analysis allowed us to characterize separately local and referred patients, and some significant differences were observed. Thus, results of epidemiologic studies from tertiary care centers must be interpreted with caution and can be misleading if they do not account for referral and ascertainment biases. In particular, for the analysis of cancer associations, ascertainment bias may artificially decrease the incidence of patients with double tumors in referred patients. Conversely, referral bias may artificially increase the prevalence of double tumors in patients who are attended at tertiary care centers.

Acknowledgement

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Impact of three-dimensional (3D) ultrasonography and power Doppler angiography in the management of cervical cancer

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Summary

Purpose: To evaluate the potential role of three-dimensional (3D) ultrasound, and to assess its diagnostic performance and ability to predict therapeutic efficacy in cervical cancer. *Methods:* Thirty patients with cervical cancer and 35 normal controls were studied by transvaginal 3D power Doppler ultrasound before treatment. Eleven patients who received neoadjuvant chemotherapy ($n = 6$), radiation ($n = 3$), or chemoradiation ($n = 2$), had further measurements taken one month and two months after treatment. *Results:* From the receiving operating characteristics curve analysis, the best vascularization index (VI) cutoff value of 5.24 distinguished cervical cancer from the normal cervix, with a sensitivity of 73.3% and a specificity of 94.3%. Cervical tumor volume measured by magnetic resonance imaging was positively correlated with the tumor volume measured by 3D ultrasonography ($r = 0.91$, $p < 0.0001$). In six patients who received neoadjuvant chemotherapy, the percent change in tumor volume during the second month of treatment was positively correlated with the percent change in flow index (FI) during the first month of treatment ($r = 0.83$, $p < 0.05$). *Conclusions:* VI may be a diagnostic marker and FI may be a predictive marker of treatment response in cervical cancer.

Key words: Uterine cervical cancer; Three-dimensional imaging; Ultrasonography; Doppler ultrasound; Neoadjuvant chemotherapy.

Introduction

Cytological and histological methods are effective for the diagnosis of cervical cancer. However, imaging can be used to determine clinical stage from tumor size after cervical cancer has been diagnosed histopathologically.

Magnetic resonance imaging (MRI), which is valuable for superior soft-tissue contrast resolution, is the choice method for imaging to determine the extent of the primary tumor. However, MRI is expensive and we cannot usually perform MRI immediately. Therefore, there are problems with regard to economics and convenience.

It has been suggested that intratumoral blood flow gives a clinical index of malignancy of cervical cancer and an indication of the efficacy of treatment. Several methods have been developed to evaluate intratumoral blood flow, including intratumoral microvessel density [1], tumor intercapillary distance [2] and vascular endothelial growth factor determination [3]. However, these do not provide a global assessment of the tumor, cannot be performed immediately, and results cannot be accurately reproduced [4].

Recently, three-dimensional (3D) ultrasound has been developed and used to quantify vascularity and flow, providing additional information in the assessment of overall blood flow of a tumor [5-7].

In this study, we measured the tumor volume and 3D power Doppler indices for cervical cancer using 3D ultrasonography and angiography, and examined the diagnostic performance in distinguishing cervical cancers from normal cervical tissue, the ability to measure tumor volume, and the viability in prediction of therapeutic efficacy in cervical cancer.

Materials and Methods

Patients

We evaluated 65 patients, mean age 49.5 years (range: 19-86), who gave informed consent to their inclusion in the study. Only routine procedures were performed on the patients included in this study. Two groups were defined: Group A comprised 35 women with gynecological tumors but with a normal cervix; Group B comprised 30 women with a histological diagnosis of invasive cervical cancer. In group A, gynecological examination and a Pap test indicated that the uterine cervices in these subjects were normal.

In group B, cervical biopsy was performed in all patients to obtain a definitive histologic diagnosis. The clinical and pathological characteristics of the 30 patients with invasive cervical cancer are shown in Table 1. Clinical staging was performed according to the FIGO classification [8]. Pretreatment evaluation consisted of taking the patient's history, and a biopsy, gynecological examination, abdominal computed tomography (CT), pelvic MRI, pelvic sonography, chest X-ray, cystoscopy and sigmoidoscopy were performed. In all patients of group B, 3D ultrasound findings were evaluated before treatment.

Primary surgery was performed for 19 of the group B patients. In the 11 patients in Group B that did not undergo primary surgery, 3D ultrasound findings were also evaluated at one and two months after treatment. All patients underwent MRI at the same time. The cervical tumor volumes determined by MRI were calculated by the formula for the volume of an ellipse ($\pi(R_1 \times R_2 \times R_3)/6$), where R_1, R_2 and R_3 were the maximal transverse, anteroposterior, and longitudinal length of tumor, respectively [9].

The clinical and pathological characteristics of the 11 patients with invasive cervical cancer are given in Table 2. Six patients received neoadjuvant chemotherapy (platinum based), three patients received radiation alone and two patients received chemoradiation.

Equipment and volume acquisition

A single observer performed all B-mode and 3D ultrasound and Doppler examinations using a Voluson 730 Expert (GE Healthcare, Zipf, Austria) with a vaginal multi-frequency probe (5-9MHz) and a visualization angle of 146°. All women were examined in the lithotomy position. All patients underwent sonography the day before surgery, radiation or chemotherapy.

An initial B-mode exploration provided information about uterine and ovarian sizes. The power Doppler window was then placed over the longitudinal scan section of the uterus in order to include the whole of the uterine cervix. For every woman, Doppler settings were prefixed as follows: normal quality of color (normal resolution and intermediate photogram index); color gain –6; PRF (pulse repetition frequency) 0.9 kHz; and wall motion filter 'low1'. Once the 2D power Doppler examination was finished, the 3D volume box was placed at a prefixed 90° angle over the cervical area. Exploration was repeated if intestinal or respiratory movements of the patient appeared. The volumes for each patient were stored on a hard disk for further evaluation.

Volume, power Doppler indices and mean gray calculation

The stored volumes were further analyzed using the VOCAL program (Virtual Organ Computer Aided Analysis). The same investigator analyzed all the volumes. Using manual mode, cervical areas were traced using longitudinal views as the work pattern. The rotation steps were 30°, resulting in the definition of six contours of the cervix or cervical cancer. Once all contours had been drawn, the VOCAL program automatically calculated the volume in ml; 3D power Doppler indices were calculated using a histogram facility. The vascularization index (VI) measures the number of color voxels in the volume. It represents the mass of vessels in the tissue and is expressed as a percentage (%). The flow index (FI) corresponds to the mean color value in the color voxels, indicating the average intensity of blood flow. It is expressed as a whole number between 0 and 100. The vascularization flow index (VFI) is the mean color value in all the voxels in the volume; therefore, it represents both vascularization and blood flow or tissue perfusion. It is also expressed as a whole number between 0 and 100. With the histogram, the mean value of the gray-scale voxels can also be calculated and is expressed as the mean gray (MG, scale 0-100).

Statistical analysis

The statistical data were analyzed using the Statistical Package for the Social Sciences (SPSS version 11.0. Chicago, IL, USA). Data are represented as mean ± standard error (SEM). Comparisons between two groups were performed by Welch's *t*-test. A *p* value less than 0.05 was considered statistically significant.

Correlation between tumor volume and VI, FI and VFI before treatment, correlation between tumor size measured by MRI and tumor size measured by 3D ultrasound before and after treatment, and correlation between the percent change in tumor volume during the second month of treatment and the percent change in 3D parameters (VI, FI and VFI) and tumor volume during the first month of treatment were performed using linear regression.

Receiver operating characteristic (ROC) curves were obtained with their respective areas under the curve ± standard error and compared by Hanley and McNeil [10] using Analyse-It Software (Leeds, England) [11]. If the lower limit of the confidence interval (CI) for the area under the ROC curve was > 0.5, the diagnostic test was considered to have discriminatory poten-

tial. The ROC curves were also used to determine the mathematically best cut-off value corresponding to the point in the ROC curve situated farthest away from the reference line [12]. The sensitivity, false-positive rate and positive and negative likelihood ratios (LR) with regard to malignancy of the mathematically best cut-off values were also calculated with exact 95% CI. A diagnostic test is regarded as useful if LR+ is higher than 2 and LR- is lower than 0.5 (according to Khan *et al*. [13]).

Results

In all patients with cervical cancer, the tumor was identified in the gray-scale analysis as a hypo-high lesion. In the 3D power Doppler analysis, all patients with cervical cancers showed detectable vessels.

A total of 35 patients with cervical cancer were eligible for analysis. The demographic characteristics of all the patients are shown in Table 1. Figure 1 shows an example of invasive cervical cancer analyzed by 3D power Doppler angiography (a) and VOCAL histogram analysis (b). This is in constant with the sparse power Doppler signals that were occasionally detected in the cervices of the 30 controls with a normal cervix.

The mean age of the 35 patients with cervical cancer was 58.1 ± 2.6 (range, 34-86) years. Their mean tumor volume, MG, VI, FI and VFI values were 33.3 ± 6.8 ml, 34.7 ± 1.3%, 16.5 ± 2.7, 34.9 ± 1.2, and 7.1 ± 1.3, respectively. The mean tumor volume, MG, VI, FI and VFI values in patients with Stage IB cervical cancer were 14.0 ±

Table 1. — *General characteristics of group B patients.*

Characteristic	Patients n (%)
Total	30
Age - years, mean (range)	58 (34-86)
FIGO stage	
IB	11 (36.7)
II	9 (30)
III	5 (16.7)
IV	5 (16.7)
Histotype	
squamous cell carcinoma	22 (73.3)
adenocarcinoma	5 (16.7)
adenosquamouscarcinoma	2 (0.7)
small cell carcinoma	1 (0.3)
Tumor diameter	
< 4 cm	18 (60)
≥ 4 cm	12 (40)

Table 2. — *General characteristics of group B patients that did not undergo primary surgery.*

Characteristic	Patients n (%)
Total	11
Age - years, mean (range)	60 (35-80)
FIGO stage	
IB	1 (9.1)
II	1 (9.1)
III	4 (36.4)
IV	5 (45.6)
Histotype	
squamous cell carcinoma	9 (81.8)
adenocarcinoma	1 (9.1)
small cell carcinoma	1 (9.1)
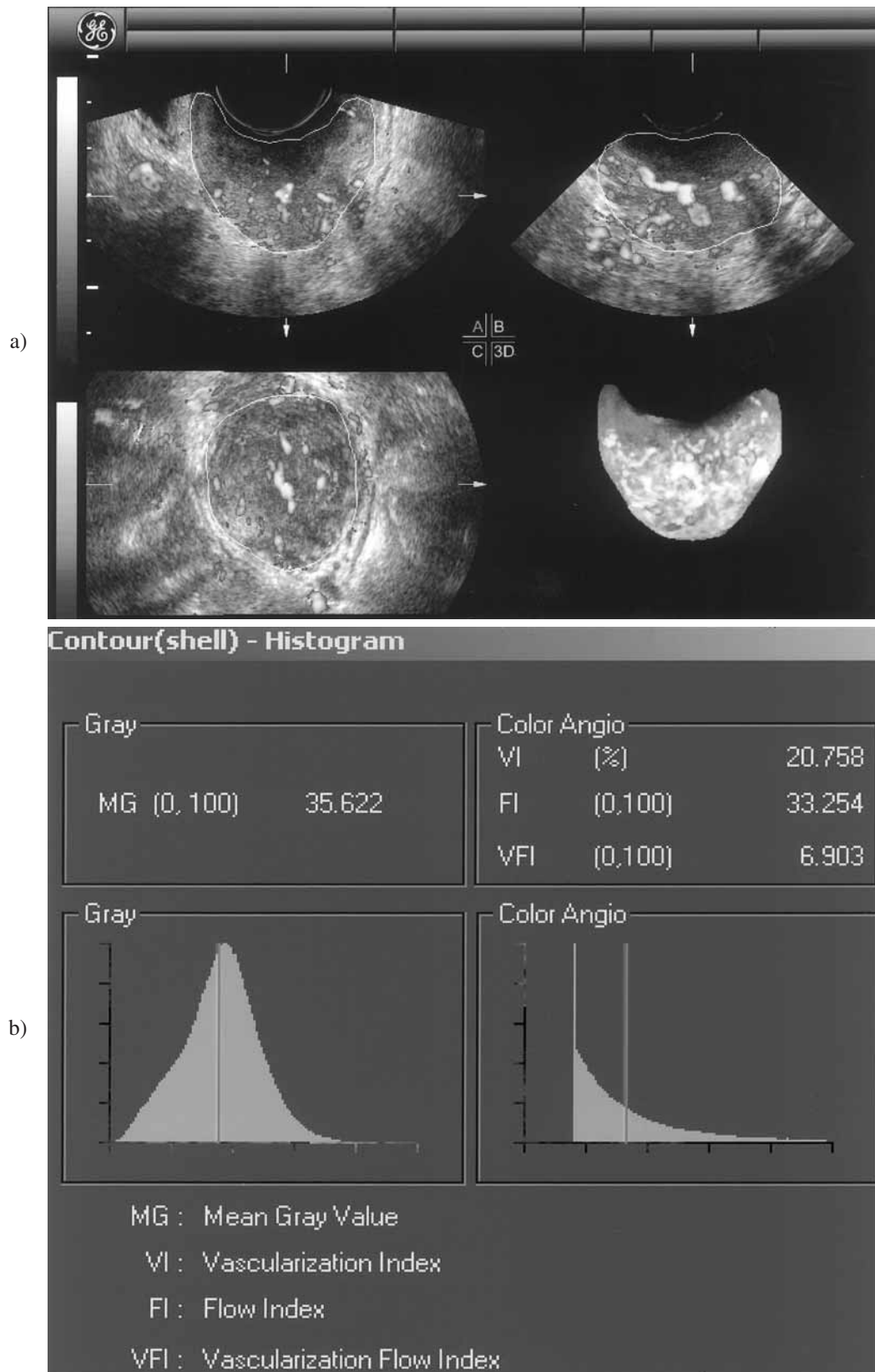
Figure 1. — An example of invasive cervical cancer analyzed by three-dimensional (3D) color power Doppler ultrasound angiography: a) longitudinal view, upper left; frontal view, lower left; transverse view, upper right; reconstructed tumor mass, lower right; b) VOCAL histogram analysis of 3D power Doppler sonography in a cervical cancer patient.
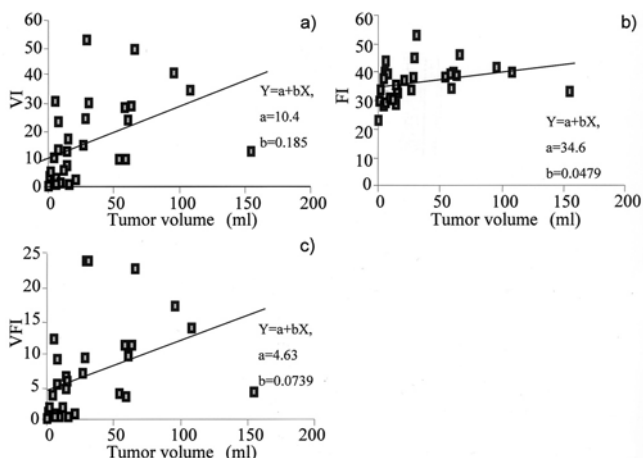
Figure 2. — Correlation of cervical cancer volume with 3D parameters (VI, FI and VFI) in 30 patients with cervical cancer.
(a) Vascularization index (VI; $r = 0.46$, $p < 0.05$);
(b) Flow index (FI; $r = 0.21$, $p = 0.13$);
(c) Vascularization flow index (VFI; $r = 0.38$, $p < 0.05$).
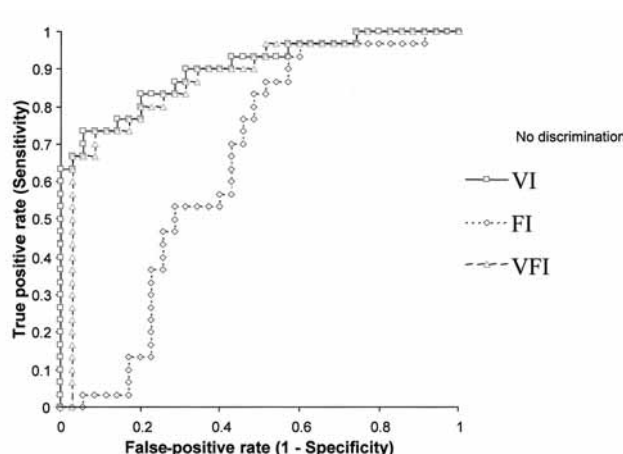
Figure 3. — Receiver operating characteristic (ROC) curve of 3D parameters (VI, FI and VFI) for differential diagnosis between cervical cancer and the normal cervix.
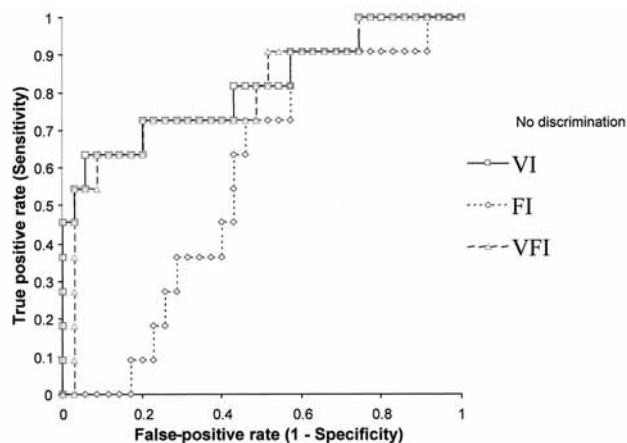
Figure 4. — Receiver operating characteristic curve of 3D parameters (VI, FI and VFI) for the differential diagnosis between Stage IB cervical cancer and the normal cervix.
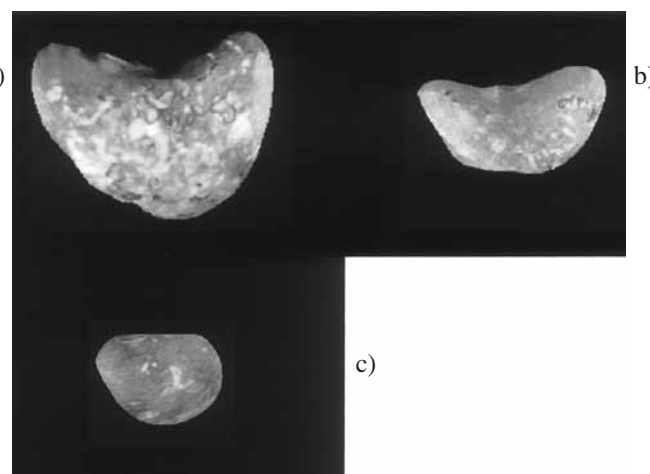
Figure 5. — An example of invasive cervical cancer analyzed by 3D color power Doppler ultrasound angiography: a) before treatment, b) 1 month after treatment, and c) 2 months after treatment.

5.3, 35.2 ± 1.7, 8.96 ± 2.63, 33.9 ± 1.8, and 3.58 ± 1.04, respectively. The mean tumor volume, MG, VI, FI and VFI values in patients with Stage IIB-IVB cervical cancer were 44.5 ± 9.4, 34.5 ± 1.8, 20.9 ± 3.7, 37.5 ± 1.4, and 9.13 ± 1.8, respectively.

The mean age of the 30 control subjects was 42.9 ± 2.0 (range, 20-70) years. Their diagnoses were uterine leiomyoma ($n = 14$), ovarian endometrioma ($n = 18$), serous cyst adenoma of ovary ($n = 2$), and serous cyst adenofibroma of ovary ($n = 1$).

The mean VI and VFI values were significantly higher in patients with cervical cancer compared with those of controls with a normal cervix (VI; $p < 0.05$, VFI; $p < 0.0005$, Welch's *t*-test). The mean VI value was significantly higher in patients with Stage IIA-VIB cervical can-

cer compared with that of patients with Stage IB cervical cancer ($p < 0.001$, Welch's *t*-test). The mean VI value was significantly higher in patients with Stage IB cervical cancer compared with that of controls with a normal cervix ($p < 0.05$, Welch's *t*-test) (Table 3).

The correlations between tumor volume and VI, FI and VFI before treatment are plotted in Figure 2. Linear regression analysis showed a modest correlation between tumor volume and VI ($r = 0.46$, $p < 0.05$) and VFI ($r = 0.38$, $p < 0.05$), but not FI ($r = 0.21$, $p = 0.13$).

Figure 3 shows the ROC curves of 3D parameters (VI, FI and VFI) for the diagnosis of cervical cancer compared with the normal cervix. ROC curve analysis showed that the best cutoff values were 5.24 for VI, 27.7 for FI and 1.69 for VFI.

VI, with an area under the ROC curve = 0.90 ± 0.039

Table 3. — *Three-dimensional parameters in 35 controls with a normal cervix and 30 patients with cervical cancer.*

	n	Volume (ml)	MG	VI (mean ± SEM)	FI	VFI
Normal cervix	35	19.8± 1.5	33.5 ± 1.5	1.39 ± 0.34	33.2 ± 2.1	1.12 ± 0.70
Cervical cancer	30	33.3 ± 6.8*	34.7 ± 1.3	16.5 ± 2.7**	34.9 ± 1.2	7.1 ± 1.3**
Stage Ib	11	14.0 ± 5.3	35.2 ± 1.7	8.96 ± 2.63*	33.9 ± 1.8	3.58 ± 1.04
Stage IIa -IVb	19	44.5 ± 9.4*†	34.5 ± 1,8	20.9 ± 3.7**†	37.5 ± 1.4	9.13 ± 1.8*†

The parameters were measured before treatment.
Data represent the mean ± SEM. Mean Gray index (MG) is a quantification of the echogenicity.
Vascularization index (VI) expresses the percent vessels in mass. Flow index (FI) express the average intensity of flow in the vessels. Vascularization flow index (VFI) is a feature of vascularization and flow.
** $p < 0.001$ compared with parameters of normal cervix.
* $p < 0.05$ compared with parameters of normal cervix.
† $p < 0.01$ compared with parameters of Stage IB cervical cancer.

Table 4. — *Area under the ROC curves, sensitivity, specificity, accuracy, positive predictive value, negative predictive value, likelihood ratios, and cutoff value for 3D parameters (VI, FI, VFI) in the diagnosis of cervical cancer (n = 30) compared with normal cervices.*

	VI	FI	VFI
Area under the ROC curve	0.90 ± 0.039*	0.64 ± 0.071	0.87 ± 0.045*
Sensitivity (%)	73.3	96.7	73.3
Specificity (%)	94.3	40.0	91.4
Accuracy (%)	84.6	66.2	8.1
Positive predictive value (%)	91.7	58.0	88.0
Negative predictive value (%)	80.5	93.3	80.0
Likelihood ratio (+)	12.83	1.61	8.56
Likelihood ratio (–)	0.28	0.08	0.29
Cut-off value	5.24	27.7	1.69

ROC, receiver operating characteristic.
Likelihood ratios were used to select cutoff values, thus maximizing the area under ther eceiver operating characteristic curves for VI, FI and VFI.
* $p < 0.0001$ compared with area under the ROC curve of FI.

(95% CI, 0.82-0.98) was more effective than FI = 0.64 ± 0.071 (95% CI, 0.51-0.78) in discriminating the women with cervical cancer ($p < 0.0001$, Table 4). VFI, with an area under the ROC curve = 0.87 ± 0.045 (95% CI, 0.79-0.96) was more effective than FI in discriminating the women with cervical cancer ($p < 0.0001$, Table 4).

The sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratios, and cutoff value for 3D parameters (VI, FI, VFI) are shown in Table 4. VI had sensitivity of 73.3% (95% CI, 62.5-78.0) and specificity of 94.3% (95% CI, 85.0-98.3). The two cases with a false-positive result included two uterine myomas. The eight cases with false-negative results included five squamous cell carcinomas (Stage IB1: four, Stage IIIB: one), two adenosquamous carcinomas (Stage IIB) and one mucinous carcinoma (Stage IIB).

VFI had a sensitivity of 73.3% (95% CI, 62.0-79.5) and specificity of 91.4% (95% CI, 81.7-96.7). The three cases with a false-positive result included three uterine myomas. The eight cases with false-negative results were the same as the cases of VI.

Figure 4 shows the ROC curves of 3D parameters (VI, FI and VFI) for the diagnosis of Stage IB cervical cancer compared with the normal cervix. ROC curve analysis

Table 5. — *Area under the ROC curves, sensitivity, specificity, accuracy, positive predictive value, negative predictive value, likelihood ratios, and cutoff value for 3D parameters (VI, FI, VFI) in the diagnosis of Stage IB cervical cancer (n = 11) compared with normal cervices.*

	VI	FI	VFI
Area under the ROC curve	0.82 ± 0.086*	0.57 ± 0.09	0.80 ± 0.085*
Sensitivity (%)	63.6	90.9	63.6
Specificity (%)	94.3	42.9	91.4
Accuracy (%)	87.0	54.3	84.8
Positive predictive value (%)	77.8	33.3	70
Negative predictive value (%)	89.2	93.8	88.9
Likelihood ratio (+)	11.1	1.59	7.42
Likelihood ratio (–)	0.39	0.21	0.4
Cut-off value	5.24	28.3	1.76

ROC, receiver operating characteristic.
Likelihood ratios were used to select cutoff values, thus maximizing the area under the receiver operating characteristic curves for VI, FI and VFI.
* $p < 0.0001$ compared with area under the ROC curve of FI.

Table 6. — *Changes in parameters in 11 patients with cervical cancer.*

	before treatment	aftertreatment	
		1M	2M
Volume (ml)	64.5 ± 12.7	26.1 ± 5.7**	14.2 ± 3.8**
VI	27.1 ± 4.8	12.4 ± 1.6*	14.0 ± 3.7*
FI	39.1 ± 1.4	34.9 ± 1.7	38.0 ± 2.0
VFI	11.8 ± 2.1	4.9 ± 1.4**	6.1 ± 1.7*

The parameters were measured before treatment, 1 and 2 months after treatment.
Data represent the mean ± SEM.
* $p < 0.05$ compared with baseline.
** $p < 0.01$ compared with baseline.

showed that best cutoff values were 5.24 for VI, 28.3 for FI and 1.76 for VFI.

VI, with an area under the ROC curve = 0.82 ± 0.086 (95% CI, 0.65-0.98) was more effective than FI = 0.57 ± 0.09 (95% CI, 0.40-0.75) in discriminating the women with Stage IB cervical cancer ($p < 0.0001$, Table 5). VFI, with an area under the ROC curve = 0.80 ± 0.085 (95% CI, 0.63-0.97) was also more effective than FI in discriminating the women with cervical cancer ($p < 0.01$, Table 5).

The sensitivity, specificity, PPV, NPV, likelihood ratios, and cutoff value for 3D parameters (VI, FI and VFI) are shown in Table 5. VI had a sensitivity of 63.6% (95% CI, 41.2-76.0) and specificity of 94.3% (95% CI, 87.2-98.2). VFI had a sensitivity of 63.6% (95% CI, 40.5-79.0) and specificity of 91.4% (95% CI, 84.1-96.3).

In 11 patients of group B who received neoadjuvant chemotherapy, radiation or chemoradiation, the tumor volume, VI FI and VFI before treatment, one and two months after treatment are shown in Table 6. Tumor volume, VI and VFI one and two months after treatment were significantly lower compared with baseline ($p < 0.05$).

Figure 5 shows an example of invasive cervical cancer analyzed by 3D color power Doppler ultrasound angiography: (a) before treatment, (b) one month after treatment, and (c) two months after treatment.

The correlations between tumor volume measured by MRI and tumor volume measured by 3D ultrasound
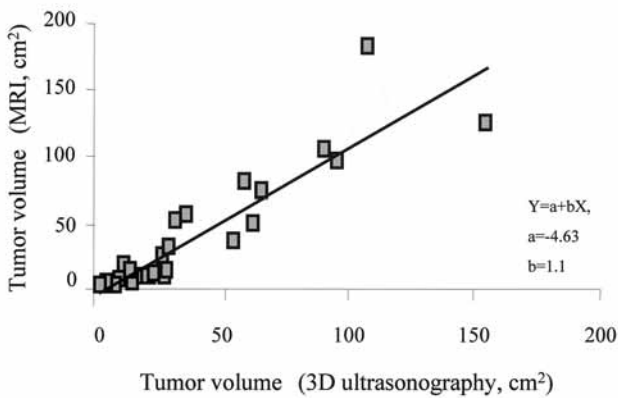
Figure 6. — Correlation of the tumor volume MRI with the tumor volume (3D ultrasonography) ($r = 0.91$, $p < 0.0001$) X: Tumor volume (3D ultrasonography, cm^2), Y: Tumor volume (MRI, cm^2).

Figure 7. — Correlation of the percent change in tumor volume during the second month of treatment with the percent change in 3D parameters (VI, FI and VFI) and tumor volume during the first month of treatment.
(a) VI ($r = 0.62$, $p < 0.05$);
(b) FI ($r = 0.88$, $p < 0.0005$);
(c) VFI ($r = 0.67$, $p < 0.05$);
(d) Tumor volume($r = 0.34$, $p = 0.30$).
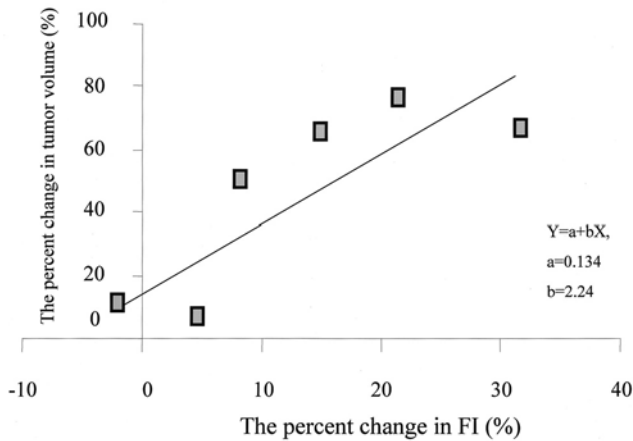
Figure 8. — Correlation of the percent change in tumor volume during the second month of neoadjuvant chemotherapy with the percent change in FI during the first month of neoadjuvant chemotherapy ($r = 0.83$, $p < 0.05$).

before and after treatment are plotted in Figure 6. Linear regression analysis showed a strong correlation ($r = 0.91$, $p < 0.0001$).

The correlations between the percent change in tumor volume during the second month of treatment and the percent change in 3D parameters (VI, FI and VFI) and tumor volume during the first month of treatment are plotted in Figure 7. Linear regression analysis showed a correlation between the percent change in tumor volume during the second month of treatment and the percent change in 3D parameters (VI, FI and VFI) during the first month of treatment (VI; $r = 0.62$, $p < 0.05$, FI; $r = 0.88$, $p < 0.0005$, VFI; $r = 0.67$, $p < 0.05$) but not the percent change in tumor volume during the first month of treatment.

In the six patients who received neoadjuvant chemotherapy, the correlations between the percent change in tumor volume during the second month of treatment and the percent change in FI during the first

month of treatment are plotted in Figure 8. Linear regression analysis showed a correlation between the percent change in tumor volume during the second month of treatment and the percent change in FI during the first month of treatment ($r = 0.83$, $p < 0.05$) but not the percent change in VI, VFI or tumor volume during the first month of treatment.

Discussion

In this study we clarified whether data acquired by 3D ultrasonography and 3D power Doppler could potentially contribute to the differentiation of a normal cervix and a cancerous cervix.

We found that 3D power Doppler analysis of cervical cancer was able to detect intratumoral vessels in all lesions. The improvement in the resolution of ultrasound equipment could explain the high detection rate of intratumoral vessels in our series as well as that of other authors [14-16].

This preliminary study of 3D quantitative analysis of vascularization in cervical cancers was focused on 3D power Doppler indices. The mean VI value was significantly higher in patients with Stage IB cervical cancer compared with that in control patients with a normal cervix, and the mean VI value was significantly higher in patients with Stage IIA-VIB cervical cancer compared with that in patients with Stage IB cervical cancer.

This might indicate that VI detected by 3D ultrasonography was elevated in early stage cervical cancer compared with that in the normal cervix, and increased as the clinical stage advanced. The positive correlations between tumor volume and VI and VFI before treatment might show that VI and VFI increased as tumor volume increased.

To our knowledge, no one has previously demonstrated the area under the ROC curve of the 3D power Doppler indices (VI, FI and VFI), as assessed by 3D ultrasound in cervical cancers and normal cervices. In this study, VI showed a specificity of 73.3%, with a sensitivity of 94.3% (Table 3) and was the best parameter of the 3D power Doppler indices for distinguishing cervical cancer from the normal cervix.

It is interesting to note that the density of vessels likely to be reflected in the total color content of the tumor scan was the single best vascular predictor of malignancy. This was because in cervical carcinoma, intratumoral vascularity index assessment by 2D power Doppler ultrasound is well correlated with the conventional indicator of tumor angiogenic activity (microvessel density) [17].

A previous study reported that abundant intratumoral power Doppler signals could be detected, and that VI, FI, and VFI were significantly increased in cervical cancer patients compared with women with a normal cervix [16]. However, in our study the VI and VFI were significantly increased in cervical cancer patients compared with that in women with a normal cervix, but FI was not increased. The region of interest (ROI) defined by 3D ultrasonography as a normal cervix included the branches of the uterine arteries. This could explain why the FI values were not elevated and might result in false-positive results for VI and VFI.

On the other hand, cancer is associated with increased angiogenesis, and when the cervical tumor outgrows the vessel support, tumor necrosis occurs. This could explain the false-negative result on VI and VFI in the case of Stage IIIB squamous cell carcinoma.

Because the strong correlations between tumor volumes measured by MRI and tumor volumes measured by 3D ultrasound before and after treatment were shown by 1inear regression analysis, we might show that the ROI defined by 3D ultrasonography as cervical cancer was similar to the ROI defined by MRI as cervical cancer.

We then investigated its potential for predicting therapeutic efficacy in cervical cancer. The percent change in tumor volume during treatment was not correlated with the tumor volume and 3D parameters (VI, FI and VFI) before treatment (data not shown). However, the percent change in tumor volume during the second month of treatment was positively correlated with the percent change in 3D parameters (VI, FI and VFI) during the first month of treatment, therefore 3D parameters have the potential to predict therapeutic efficacy in cervical cancer.

In the six patients who received neoadjuvant chemotherapy, the percent change in tumor volume during the second month of treatment positively correlated with the percent change in only FI during the first month of treatment, therefore FI has the potential to predict therapeutic efficacy in cervical cancer treated with neoadjuvant chemotherapy.

Since 3D power Doppler ultrasound provides functional, objective and quantitative evaluation of vascularity of the whole tumor, it can be used to monitor the response to neoadjuvant chemotherapy in the treatment of bulky cervical cancer [18-20]. In addition, this non-invasive tool may be used to evaluate vascular changes in the tumor after antiangiogenesis therapy for the treatment of cancer. However, this novel technique is not without its limitations. As with any ultrasound technique, results may vary depending on the operator. Power Doppler ultrasound is also subject to motion artifacts from the transducer and the patient.

Conclusions

Tumor volume and 3D parameters can be noninvasively measured and easily obtained by 3D ultrasound sonography. VI may be useful for diagnosis of cervical cancer and FI may be useful for prediction of treatment response. Further research will be undertaken in a larger series of patients for analyses in which the additional value of the measurement of 3D parameters will be estimated.

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Out-of-protocol concurrent use of cisplatin and radiation therapy in locally advanced cervical cancer: feasibility and survival

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Summary

Purpose of investigation: We assessed the feasibility, response rates, and overall survival of patients with locally advanced cervical cancer treated with cisplatin-based chemotherapy during radiation therapy on an out-of-protocol basis. *Methods:* Sixty-nine consecutive newly diagnosed untreated patients with locally advanced cervical cancer who received chemoradiation between 1999 and 2003 were retrospectively reviewed. Treatment consisted in external beam radiation followed by one 137-cessium intracavitary application. Cisplatin was administered for six weeks during external beam radiation. *Results:* Treatment was well tolerated, although 52 patients presented some degree of acute adverse toxicity (gastrointestinal 65%, hematological 48%, genitourinary 10%). The 3-year survival rate was 61.8% (95% CI 54.5-69.0), with a mean 41.8 months (95% CI 35.7-48.3). Overall survival after adjusting by FIGO Stage IB_2-IIA and IIB-IVA was 73.9% and 50%, respectively ($p = 0.1839$). Overall survival according to Stages IB_2-IIb and III-IVA was 74.8% and 34.9%, respectively ($p = 0.0376$). *Conclusion:* In patients with locally advanced cervical cancer, adding a weekly regimen of cisplatin to standard pelvic radiation in an out-of-protocol basis is feasible, effective, and showed no unexpected toxicity.

Key words: Locally advanced cervical cancer; Cisplatin; Pelvis radiation therapy.

Introduction

Cytological screening has significantly reduced the rates of cervical cancer in many developed countries. However, cervical cancer remains a leading type of cancer among women, particularly those living in low-income regions of the world [1]. In Spain, due to preventive screening programs, invasive cervical cancer only represents 10.3 cases among 100,000 women/year, with a mortality rate of 3.6 cases among 100,000 women/year. However, up to 25% of these cases are diagnosed in FIGO locally advanced stages [1]. Therefore mortality in these cases rises to 50% compared with the 10% observed in early cervical cancer patients [2].

For many years, the current standard treatment of locally advanced cervical cancer has been exclusively based on radiation therapy. Although chemotherapy has been used in neoadjuvant and in a concomitant modality with radiation therapy to maximize the response to it, until 1999 all studies failed to show a significant advantage of using chemotherapy as a part of the treatment of cervical cancer [3-7]. In 1999 and 2000, five randomized studies using a concurrent chemotherapy schedule showed that the survival rate of patients with cervical cancer treated with radiotherapy alone was lower than with concomitant chemotherapy, and that mortality declined from 50% to

30% [8-12]. Faced with these data, the National Cancer Institute issued a clinical announcement stating, "Based on these results, strong consideration should be given to the incorporation of concurrent cisplatin-based chemotherapy, with radiation therapy in women who require radiation therapy for treatment of cervical cancer" [13]. Although the search for the ideal "radiation sensitizer" in ongoing, cisplatin chemoradiation therapy is standard treatment for locally advanced cervical cancer [2, 14].

According to the results of the randomized trials supporting the use of cisplatin-based chemoradiotherapy in cervical cancer, we adopted the treatment with cisplatin chemoradiation as routine management of women with locally advanced cervical cancer. The aim of this study was to evaluate feasibility, response rates, and overall survival of patients with locally advanced cervical cancer treated with cisplatin-based chemotherapy during radiation on an out-of-protocol basis.

Methods and Patients

We conducted a retrospective review of 69 consecutive newly diagnosed and previously untreated patients with locally advanced cervical cancer, who received radiotherapy and concurrent cisplatin at the Gynecologic Oncology Unit of the Materno-infàntil Vall d'Hebron Hospital in Barcelona, Spain between June 1999 and July 2003. All patients had a histologic diagnosis of cervical carcinoma and were staged according to the FIGO classification using standard pretreatment workup

studies including magnetic resonance imaging (MRI). Before starting treatment one patient died, five patients were treated in other oncological centers because of breakdown of the lineal accelerator equipment and two more were excluded for concomitant treatment because of renal failure. The baseline characteristics of the patients are shown in Table 1. As this was a retrospective review of patients treated on a routine basis, approval of the institutional review board was not required.

Table 1. — *Patient characteristics*.

Mean follow-up, months (range)	25 (4-63)
Median age, years (range)	56 (24-81)
Menopausal status	63%
Histologic diagnosis, no. (%)	
Squamous cell carcinoma	51 (75)
Adenocarcinoma	11 (16)
Others	6 (9)
FIGO Stage	
IB$_2$-IIA	37 (54.4)
IIB-IVA	31 (45.5)
Tumor size	
Maximum diameter (range)	5.87 (4–9)
Tumor volume (cm^3) (range)	49.25 (9–191)

A total of 60 patients received external beam radiation using a megavoltage machine (lineal accelerator) with a photon-beam energy of 2.25 MV and isocenter technique to the whole pelvis. Patients were treated with the conventional 4-field box technique. Total dose planned was 45 Gy in 25 fractions (5 weeks, 1.8-2 Gy fractions from Monday to Friday) followed by one 137-cessium intracavitary application within two weeks of finishing external radiation, an isodose of 25 Gy referred to the cervix. The planned total dose to point A was at least 85 Gy. Total treatment duration had to be eight weeks. Treated pelvic volume had to include the whole uterus, paracervical, parametrial and uterosacral regions, as well as the external iliac, hypogastric and obturator lymph nodes. If there was clinical or radiological suspicion of paraaortic lymph node infiltration, we planned an extended field until the T12-L1 vertebral body to include them.

Cisplatin was administered for six weeks during external beam radiation, beginning on the first day of treatment in an outpatient setting. Cisplatin infusion was administered within 2 h before radiation. A dose of 40 mg/m^2 (maximum dose 70 mg) was administered via a peripheral vein to patients in an outpatient setting.

Response to treatment was evaluated clinically, colpocytologically, and histologically when necessary at three months after the end of treatment. Response was also evaluated radiologically by MRI at three and six months. Complete response was defined as no clinical or cytological evidence of disease. Partial response was defined if there where a decrease > 50% in initial lesion size and no new lesions. Persistent disease was considered with any less-than-50% response. Finally, progression was defined if there was an increase > 25% in initial lesion size or new lesions appeared [15].

Upon treatment completion and response evaluation, patients were evaluated every three months for the first two years and every six months thereafter until five years of follow-up were completed. At each control, a physical, pelvic and colpocytological examination, blood counts and clinical chemistry were performed. Annually chest X-rays and MRI were conducted. If persistent or recurrent disease was suspected, it was confirmed by biopsy whenever possible.

Acute and chronic toxicity to chemoradiation were evaluated according to EORTC/RTOG common toxicity criteria [16]. The acute side-effects were defined as those occurring during or within 90 days of completing radiotherapy. During treatment, blood counts and chemistry profiles were performed prior to each cisplatin administration. Late reactions were defined as those occurring 90 days or more from completing radiotherapy.

Statistical analysis

Overall and progression-free survival times were analyzed and registered from date of diagnosis to date of death or date at last visit, and from date of diagnosis to date of progression or relapse, respectively. Curves were constructed using the Kaplan-Meier method [17]. Due to the low number of patients, they were grouped depending on FIGO stage. We named bulky stages: IB$_2$-IIA and locally advanced Stages: IIB-IVA. Univariate and multivariate analyses were carried out to examine the relationship between the most frequent variables considered in the literature as prognostic factors for treatment response and occurrence of response or not [18-25]. The SPSS (Windows version 11.0) statistical program (SPSS, Inc., Chicago) was used for the analysis of data.

Results

A total of 60 patients who received chemoradiation were analyzed. The mean age was 56 years (range 24-81 years). A total of 25% of cases were adenocarcinomas, and the distribution according to FIGO Stage was IB2-II$_a$: 54.4% and IIb-IV$_a$: 45.5%. The mean tumor diameter at diagnosis was 5.87 cm (range 4-9 cm).

Table 2 shows treatment data. Overall treatment time was eight weeks (range 6-11.7 weeks). The mean dose of external beam radiation was 45 Gy (26-65 Gy). Overall 68.3% of patients completed external beam and intracavitary therapy. Nineteen patients (31.7%) did no receive brachytherapy because of anatomic problems that conditioned technical difficulties for insertions (n = 11), progression (n = 2), fistula (n = 2), no indication (n = 3), and discontinuation of treatment after external beam (n = 1). With regard to chemotherapy, the majority of patients (71.7%) received the six planned cycles; furthermore 91.8% of patients received at least four cycles.

Table 2. — *Treatment data*.

Interval between diagnosis and treatment, weeks (range)	7 (2.7-16.8)
Median external radiation duration, weeks (range)	6 (2-24)
Median total treatment duration, weeks (range)	9.8 (9.1-11.2)
Mean dose of external radiotherapy (range)	45 (26-65)
CDDP mean cycles	5 (1-7)
Intracavitary radiation	
N (%)	41 (68.3)
Mean (SD) dose (Gy)	26 (15-35)

Overall, treatment was well tolerated, although 52 patients (83%) presented some degree of acute adverse toxicity. The most common acute adverse effects were gastrointestinal (n = 39, 65%), hematological (n = 29; 48%), and genitourinary (n = 6, 10%). The most frequent hematological toxicity was anemia (n = 10; 10%). The
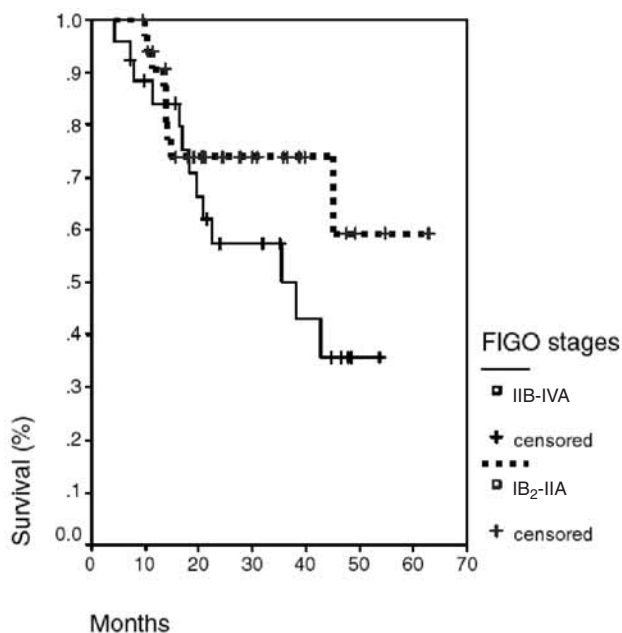
Figure 1. — Overall survival after adjusting by FIGO stage.

Figure 2. — Overall survival after grouping IIb with bulky stages (IB2-IIa).

most common acute grade 4 toxicity was gastrointestinal (n = 2) with peritonitis and perforation of the sigmoid colon that obliged putting off the treatment until patients recovered. A total of 26.7% of patients referred some gastrointestinal toxicity (G1-2) after 90 days of follow-up. Only one patient presented a fatal late complication occurring six months after the first day of radiotherapy because of an intestinal occlusion secondary to enteritis.

Treatment response was evaluated by intention-to-treat basis. After finishing chemoradiation therapy, complete response was achieved in only 16 patients (26.7%), partial responses in 30 cases (50%), whereas 13 patients had either persistent (n = 11) or progressive (n = 3) disease. Among patients who received brachytherapy (n = 41), overall complete response was achieved in 85.4% of patients. Therefore, 91.7% of partial responses became complete after intracavitary therapy.

At a median follow-up time of 25.4 months (range 4-62 months), 38 patients (63.3%) were alive, 35 with no evidence of disease. According to FIGO stage, 73% of these patients were FIGO IB$_2$-IIA, and 50% FIGO IIB-IVA. Overall survival at three years was 61.78% (95% CI 54.5-69.0), with a mean of 41.8 months (95% CI 35.3-48.3). Overall, survival after adjusting by FIGO stage was 73.9% and 50.05%, respectively (Figure 1). However, differences were not statistically significant (long rank, p = 0.1839). Overall survival according to Stages IB$_2$-IIB and III-IVA significantly changed to 74.8% (95% CI 67.9-81.7) and 34.9% (95% CI 21.7-48.1), respectively (p = 0.0376) (Figure 2).

Prognostic factors for treatment response identified after carrying out the univariate analysis were tumor volume lower than 50 cm³, tumor diameter < 6 cm, non-suspicious lymph nodes in MRI and intracavitary radiation
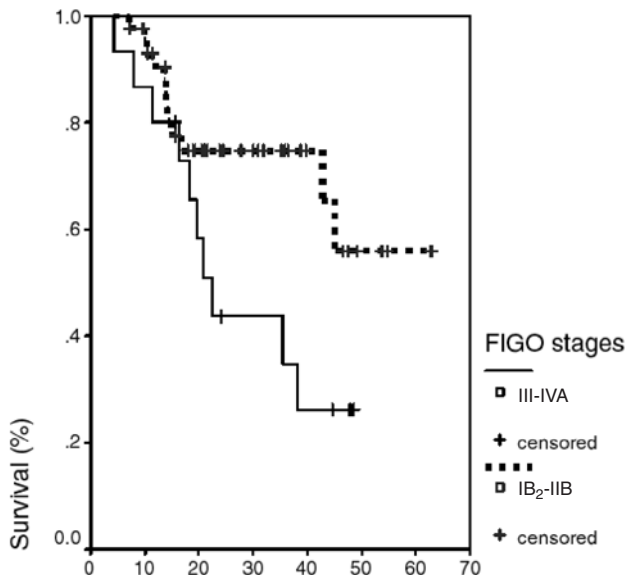
($p < 0.05$) (Table 3). After performing the multivariate analysis only the application of endocavitary radiation was identified as an independent prognostic factor to treatment response. No association was found between treatment response and increasing FIGO stage, age, prolonged overall treatment time or waiting time to start treatment.

Table 3. — *Prognostic factors for treatment response. Univariate analysis.*

| | Treatment Response | |
	Odds ratio	95% CI
Intracavitary radiation	11.375	2.079-62.232
Tumor diameter ≤ 6 cm	6.643	1.189-37.107
Tumor volume ≤ 50 cm³	6.458	1.100-37.918
Non suspicious lymph nodes (MRI)	6.417	1.204-34.193

Table 4. — *Comparison of overall survival of large randomized trials.*

Study	Reference	FIGO stage	Treatment	Survival at 3 years	RR Control group
RTOG 9001	Morris [11]	IB2-IV$_a$ ± Pelvic LN +	CDDP + 5FU –	75 63	0.52 –
GOG 85	Whitney [8]	IIb-IV$_a$	CDDP + 5FU H-Urea	67 57	0.72 –
GOG 120	Rose [9]	IIb-IV$_a$	CDDP CDDP+5FU+ H-U H-Urea	65 65 47	0.58 0.61 –
NCIC	Pearcey [32]	Ib > 5 cm IVa	CDDP –	69 66	0.9 –
Vall d'Hebrón	Mancebo	Ib2-IV$_a$	CDDP	61	–
Vall d'Hebrón	Mancebo	Ib2-II$_a$ IIb-IV$_a$	CDDP CDDP	74 50	Reference 1.9

Discussion

Until recently, the greatest strides in reducing cervical cancer mortality have occurred with the advent and implementation of screening programs. However, locally advanced cervical cancer remains a significant health problem. Prognosis of patients with locally advanced cervical cancer has remained unalterable during 20-30 years, when therapeutic options were basically surgery or radiation therapy. However, great advances have also been made in the treatment of locally advanced cervical cancer after the results of several important clinical trials [8-12]. A subsequent meta-analysis of 19 randomized controlled trials with a total of 4,580 patients confirmed that the addition of chemotherapy to radiation therapy improved progression-free and overall-survival of these patients [26-28], which represents an 11-12% absolute benefit in survival.

Notwithstanding these results, there are not a lot of data about feasibility and results of concurrent chemotherapy and radiotherapy in the routine management of locally advanced cervical cancer in an outside research setting [29-31]. However, considering differences in the chemotherapeutic regimens and patients included, our data show comparable overall survival to chemoradiotherapy arms of large randomized trials and others (Table 4). We found a 36.6% mortality that is near the 45% at five years reported by Whitney *et al*. [8]. A total of 61.7% of our patients had a median survival of 45 months, similar to that in the NCIC study [32]. It should be noted that 50% of our patients with FIGO Stages III-IVA survived three years compared with 65-67% of survival reported by Rose and Whitney [8, 9]. Although it has been reported that distant metastasis to lymph nodes is one of the most important prognostic factors of survival in locally advanced cervical cancer, we did not exclude women with proven or suspicious spread to the paraaortic lymph nodes. This could be the main reason for survival differences with data reported by these authors.

We did not conduct a randomized comparison to radiotherapy-only because of ethical implications; however, a comparison could be made to previous results published by Denton *et al*. [33] in an English national audit of the management and outcome of carcinoma of the cervix treated with radiotherapy in 1993. This group showed overall survival at five years of 47% (Stages Ib [62%], IIb [47%], IIIb [23%]). A more recent single-center audit by Taylor and Powell [34] identified 69 women with cervical carcinoma treated with radical chemoradiation. After medial follow-up of 15 months they found a 40% relapses. It is important to mention that doses administered were lower than those in US randomized trials or in our study.

Overall, 68.3% of patients completed external beam and intracavitary therapy. This is lower than Addenbrooke *et al's* experience [35]. They reported that successful brachytherapy was possible in 84.7% of women. In our study like others [36-39], successful brachytherapy was the only independent prognostic factor associated with response to treatment. Thus, it is important to remark that efforts should be made to improve successful brachytherapy rates in order to improve overall response and overall survival.

It should be emphasized that when we included patients with FIGO Stage IIB, overall survival in this group did not change significantly; these results are similar to those reported by Morris *et al*. [11]. This finding has important prognostic implications that should be deeply explored in order to offer most accurate information to patients.

Acute toxicity of chemoradiation for LAC has been reported in several phase II and III studies [8, 9, 11, 31, 40]. A comparison of the results is difficult because of the differences in the chemotherapeutic regimens, the radiotherapy delivered and whether or not surgery was performed. In general, as in our study, the main toxicity encountered during combined chemoradiation is hematological or gastrointestinal, which is well tolerated and rarely obliges putting off treatment.

In conclusion, in patients with locally advanced cervical cancer, adding a weekly regimen of cisplatin to standard pelvic radiation in an out-of-protocol basis is feasible, effective, and showed no unexpected toxicity.

Acknowledgments

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Shoulder mobility after axillary sentinel node biopsy for early infiltrating breast cancer treatment

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Summary

It is known that complete axillary lymph node dissection for breast cancer treatment causes more frequent sensitive and motor alterations in the homolateral shoulder and upper limb than sentinel lymph node (SLN) biopsy. However, it is not clear how often patients treated by SLN biopsy suffer from shoulder mobility (SM) restriction, as well as its severity and duration. This study was done aiming to evaluate SM in 38 patients with early infiltrating breast cancer treated by SLN biopsy in whom shoulder movements were assessed before surgery and repeated at one, two and three months later. Shoulder-arm mobility was evaluated by goniometry considering flexion, abduction, aduction, extension, internal rotation and external rotation. An abnormal result for each movement was defined by restriction greater than ten degrees compared to preoperative findings. Significant abnormal results for flexion and abduction were found in all of the patients at the first month evaluation. At the third month assessment no women showed any kind of SM impairment. The average restriction evolution for each of the parameters is presented. It is concluded that there is frequently a slight and transient SM limitation in patients undergoing SLN biopsy. Early postoperative physiotherapeutical assistance should thus be advisable to relieve and shorten disability symptomatology.

Key words: Breast cancer; Sentinel node biopsy; Morbidity.

Introduction

The lifetime risk of developing breast cancer is estimated at 13% for women living in the USA and 8% in Europe [1, 2]. The number of breast cancer survivors is progressively increasing worldwide due to the high neoplasia incidence, early diagnosis and more accurate therapy. Nevertheless some hazardous physical morbidity, transitory or definitive, secondary to axillary lymph node surgical management are very common in these women as limitations of shoulder mobility (SM), paresthesias and arm swelling [3-5].

The severity of these disturbances is related to the extent of axillary lymph nodes dissection (ALND). Sentinel lymph node (SLN) biopsy is a minimally invasive staging procedure that reduces the frequency and severity of the complications observed after full axillary clearance [5-7]. SLN biopsy is currently the gold-standard procedure for managing early infiltrating breast carcinomas up to 3 cm in diameter that should also be employed in combination with radioguided occult lesion localization [8-11].

While the side-effects of ALND have been extensively described in the literature, the short and long-term morbidity after SLB biopsy are not well established. Some studies have compared shoulder arm mobility in patients who underwent ALND and SLN biopsy and all of them pointed out the benefits of the less extensive maneuver, but to the best of our knowledge, they all were carried out without assessing arm function previously. Theoretically someone about to initiate an accurate research on arm function after SLN biopsy should first evaluate the mobility parameters before surgery and repeat them afterwards.

The aim of this study was to investigate if there is SM restriction after axillary SLN biopsy for breast cancer treatment comparing the surrogate arm movements in the same patients before and after the surgery.

Patients and Methods

Thirty-eight patients with palpable T1-2, N0 breast carcinomas were prospectively enrolled in the study. They underwent radioisotopic lymphatic mapping, breast segmental resection and SLN biopsy under probe guidance. Average patient age was 48.3 years (35-65).

The Research Protocol Review Committee of our institution approved the investigation and a written informed consent was obtained from each patient.

On the day before surgery a solution containing dextran labeled with 15 MBq of 99mTc was injected in the peritumoral area. Lymphoscintigraphy was performed preoperatively to identify lymphatic pathways and hot spots were marked on the skin. Detailed nuclear medicine methodology was published elsewhere [12].

Immediately after breast segmental resection SLN was biopsied with gama probe monitoring. The mean number of excised lymph nodes was 1.9 [1-4] for each patient.

Regardless of the different breast tumor locations, SLN harvesting was always performed through a unique breast incision. SLN was intraoperatively cut at 1 mm intervals for fresh imprint cytological testing. In this casuistic the definitive analysis always confirmed the intraoperative cytology findings. All the patients showed uninvolved SLN and received no further axillary treatment.

SM extent was measured by a goniometer, which consists of a plastic circle with two rulers, graduated in degrees (0-360°). With the patient in the orthostatic position the following movements were evaluated in the homolateral shoulder to the axillary biopsy: flexion, extension, abduction, aduction, internal and external rotation. Illustrative flexion, extension and abduction measurements are presented in Figure 1.
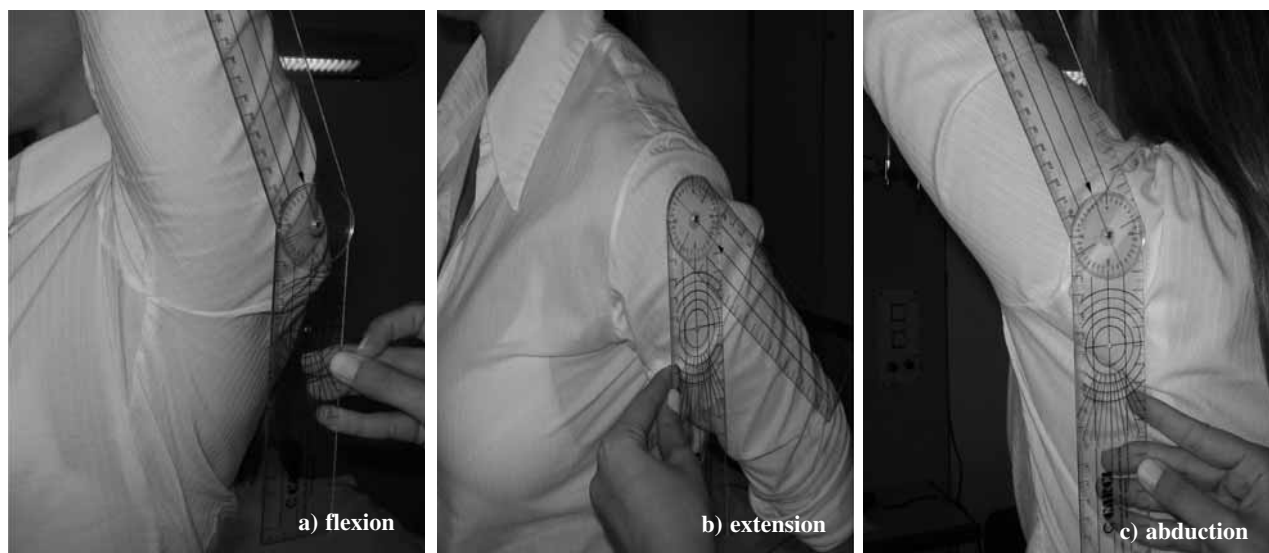
Figure 1. — Shoulder mobility measurements: a)flexion, b) extension, c) abduction.

No postoperative physioterapeutical intervention was offered to these women to avoid interference in the results.

All patients were examined the day before the operation and one, two and three months thereafter by a physiotherapist. The range of the different pre- and postoperative shoulder movements were compared. Results were registered as abnormal when outcome measures compared to preoperative evaluations found a restriction diversion greater than 10 degrees.

Results

The most frequent affected shoulder movements are flexion and abduction, however the impairments were without exception transitory. The range of shoulder flexion and abduction was abnormally restricted in all of the patients at one month evaluation compared to preoperative measurement (100%). In addition nine patients (23.6%) suffered from aduction disturbance. In a single case (2.6%) shoulder extension deficiency occurred. There was not any case of abnormality in shoulder internal rotation, external rotation and extension.

Table 1 shows the evolution of the abnormal results for each of the shoulder movements in the three postoperative assessments.

It is possible to observe that aduction and extension hazards disappeared at the second assessment. On the other hand the number of patients with deficient flexion and abduction was reduced at two months and at the last evaluation (3 months after the surgery), all patients had fully recovered SM.

Figures 2 and 3 show the evolution of the average degree values of the extension of shoulder flexion and abduction movements.

Discussion

Undoubtly with the less extensive lymph node dissection required for SLN staging there is less morbidity than after ALND [13-16, 18-21]. Nevertheless the prevalence, severity and duration of SM restriction in patients undergoing SLN biopsy clearance are still a point of concern.

Table 1. — *Number of abnormal results for measures of mobility of the shoulder (reduction greater than 10°).*

	1 month		2 months		3 months	
	n	%	n	%	n	%
Flexion	38	100	33	86.4	0	-
Abduction	38	100	29	76.3	0	-
Extension	1	2.6	0	-	0	-
Aduction	9	23.7	0	-	0	-
Internal Rotation	0	-	0	-	0	-
External Rotation	0	-	0	-	0	-

The major finding in this study was the demonstration that most of the patients presented slight and transient shoulder-arm movement impairment after SLN biopsy, mainly flexion, abduction and aduction. The movement modifications were short-term restricted. Three months after the operation full range of shoulder motion, compared with preoperative measurements, was always observed, with no residual signs of shoulder limitation.

Our study corroborates previous work by Leidenius *et al*., who found that a large subset of the patients (75%) after SLN biopsy experienced limited and ephemeral SM restriction [4].

The exact etiology of the SM transitory limitations is not well understood, but probably they are caused by pain and/or strain in the wound and muscles, as result of the inhibitory effects of tissue injury and fibroses [17, 18].

Schrenk *et al*., in 2000, pioneerly stressed that SLN biopsy is associated with less postoperative SM limitation compared with conventional ALND [19]. Currently it is the consensus that axillary staging by SLN biopsy, without complete clearance, decreases the interference with daily life caused by SM limitation. In the literature there are only three randomized controlled clinical trials comparing SLN biopsy versus primary ALND [3, 20, 21] and all these studies have confirmed the best performance in the former group of patients regarding physical postoperative morbidity.
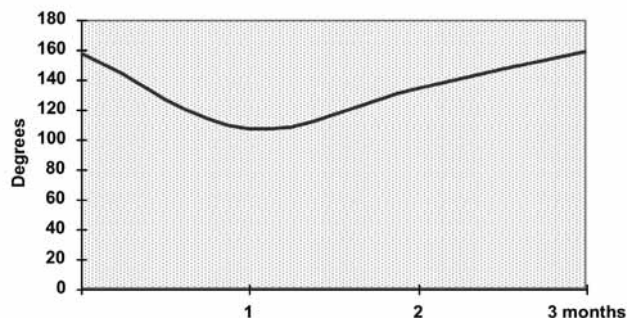
Figure 2. — Average values of shoulder flexion movement evolution after sentinel lymph node biopsy.

Figure 3. — Average values of shoulder abduction movement evolution after sentinel lymph node biopsy.

It is reasonable to suppose that the rather high mean number of excised lymph nodes [1-9] is associated with the shoulder functional symptomatology. However it is worthwhile to point out the importance of removing all radioactive and suspicious nodes on palpation after SLN harvest to avoid false-negative results [24].

The frequency of short-term SM restriction is not negligible and a substantial number of women undergoing axillary SLN biopsy suffer from transient SM limitation after surgery [25-27]. As a consequence, surgeons consider the SLN biopsy risk-benefit relationship for each case, avoiding the procedure in situations in which there is very low involvement probability, for instance, in prophylactic mastectomy for women at high risk of breast cancer and segmental mastectomy for low-grade ductal carcinoma in situ.

After axillary SLN biopsy, physical therapy combining specific arm exercises and massages performed in the setting of a tailored program under the guidance of a trained therapist is very useful. Physiotherapeutic measures should reduce symptoms and shorten the duration of mobility limitations. Rehabilitation care should begin in the first 24 hours of the postoperative period to preserve muscle strength and maintain SM. Health providers involved with breast cancer patients need to be aware of SLN biopsy repercussions and available preventive physical therapy options to render optimal assistance in these patients, allowing them, as early as possible, to follow a normal lifestyle.

In conclusion, breast cancer patients undergoing SLN biopsy suffer from transitory debilitating SM restriction, mainly due to flexion and abduction limitations, lasting up to three months after the operation. It is a self-limited condition that should be potentially relieved by early physiotherapeutic interventions.

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The significance of HPV in the follow-up period after treatment for CIN

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Summary

Purpose of investigation: High-risk anogenital human papillomavirus (HPV) infections are causally related to cervical cancer. Successful treatment of cervical intraepithelial neoplasia (CIN) results in complete eradication of HPV in most cases. There is an increasing interest regarding the role of HPV testing in the follow-up period after treatment for CIN. *Patients and Methods*: This retrospective study includes 107 women who underwent conization for histologically verified CIN. All of them had HPV testing pre- and postoperatively. HPV testing was carried out using a hybrid capture assay (HC2). The mean follow-up period was 21.4 months (range 2-76 months). The data were analyzed with respect to success of conization, HPV persistence/recurrence and CIN recurrence. Sensitivity, specificity and negative predictive value (NPV) of HPV testing were assessed and compared to the cytological results. *Results*: Preoperatively, 97 of 107 women were HPV positive. Ninety-seven conizations showed negative resection margins with 86 women becoming HPV negative. In the following months, nine of these HPV negative women became HPV positive again. Out of ten conizations with positive resection margins, six women became HPV negative. Recurrent CIN 2/3 lesions were observed in 11 women, nine of whom had persistent positive HPV testing throughout the entire study period. Regarding CIN recurrence HPV testing showed a sensitivity of 93%, a specificity of 85% and a NPV of 99%. *Conclusions:* The sensitivity of HPV testing concerning persistent or recurrent CIN as well as the NPV are high. The present data suggest that HPV testing should be integrated in a follow-up algorithm after treatment for CIN by conization.

Key words: CIN; Conization; LEEP; Follow-up.

Introduction

Approximately 500.000 women worldwide are annually diagnosed with invasive cervical carcinoma (ICC) and about 230,000 women die from the disease [1]. Although the incidence of ICC has declined over the last decade, the incidence of cervical intraepithelial neoplasia (CIN) has increased, especially in younger women. If untreated, 15-20% of these women will develop severe dysplasia and 5-10% invasive carcinoma [1-3]. About 15 of more than 40 genital mucosal types of HPV are known to be oncogenic, causing almost all ICC and cervical precancerous lesions, including CIN 3 [4-6]. Therefore, it appears reasonable that HPV-DNA detection in cervical samples would improve the performance of existing screening methods. In fact, it has been shown that HPV testing in combination with Pap tests are 96% to 100% sensitive for the detection of CIN [4, 6, 7]. Furthermore, it has been shown that HPV is eliminated after successful treatment of CIN whereas it persists in recurrent disease [6-8]. This implies a potential role of HPV testing in the follow-up period after treatment of CIN. Several studies suggested that HPV testing is useful in predicting the presence of residual CIN while others indicated that the presence of HPV after treatment resembles only a risk factor for residual CIN and that additional diagnostic procedures are indispensable [9-13].

We studied the value of HPV-DNA testing in the follow-up period after treatment of CIN. In particular we evaluated:

– If conization eradicates HPV.
– The sensitivity of HPV testing in the detection of persistent or recurrent CIN.
– If HPV testing should be combined with Pap tests in the follow-up.

Patients and Methods

Over a period of six years 385 women were admitted to the Department of Gynecology and Obstetrics, University of Munich for conization of histologically verified CIN 2/3 or because of a cervical smear showing Pap III to Pap IV dysplasia. One hundred and seven of these patients who underwent HPV testing pre- and postoperatively were included in this retrospective study.

The gynecological examinations were carried out at the Colposcopy Clinic of the Department of Gynecology and Obstetrics, University of Munich, and followed a specific sequence: two Pap smears were obtained, one from the ectocervix (cotton tip swab) and one from the endocervix (cytobrush). A HPV DNA sample was obtained from the cervix with a cytobrush. Standard colposcopy was performed with acetic acid (3%). Directed biopsies were taken from acetic acid positive areas. Cervical smears were classified according to the revised Munich classification which is the most widely used in Germany (Münchner Nomenklatur II): °I, normal cytology; °II, mild to moderate inflammatory, metaplastic or degenerative changes; °III, squamous or glandular cells of defined significance; °IIID, mild to moderate dysplasia; °IVa, severe dysplasia or carcinoma *in situ;* °IVb, carcinoma *in situ*, invasion cannot be ruled out; °V, invasive carcinoma. Histology was classified as follows: CIN 1, mild dysplasia; CIN 2, moderate dysplasia; CIN 3, severe dysplasia/carcinoma *in situ*.

HPV testing was carried out using the Hybrid Capture System 2 (HC2) (Digene, Gaithersburg, MA, USA). This

test detects 13 different high-risk HPV types (16,18,31,33,35,39,45,51,52,56,58,59,68), and is approved by the FDA. It was run in accordance to the manufacturer's protocol. HPV-DNA analysis was quantitative and women with samples producing readings higher than the positive controls (1 pg/ml HPV DNA) were regarded as being HPV test positive.

Statistical analyses were performed using SPSS version 8.0 (SPSS Inc. Chicago IL, USA). Significant differences in proportions were assessed using the chi-square test.

Results

The 107 women who were included in this study had a mean age of 34.5 years (range 22-68 years). Electrosurgical loop conization (LEEP) was carried in 87 women (81%) while 20 women had cold-knife conization. The mean follow-up period was 21.4 months (range 2-76 months) with the first postoperative control after a median of four months (range 1 to 54 months). The women had between one and nine follow-up investigations (mean 2.7 investigations). The first follow-up HPV testing was carried out at 4.8 months (range 1 to 10.7 months) after conization.

Preoperative HPV testing showed that 97 women (91%) were high-risk HPV positive and ten women (9%) HPV negative. Preoperative cervical biopsies were available for 104 women revealing CIN 1 in 17 cases CIN 2 in 30 cases, CIN 3 in 55 cases as well as two negative findings. In these women as well as in those with CIN 1 conization was carried out because of persistent Pap IIID dysplasia in cervical cytology. Among the ten women with negative HPV testing preoperatively we observed two CIN 2 lesions, six CIN 1 lesions and two negative findings . However, all these women showed severe dysplasia in cervical cytology. Eighty-seven women (81%) underwent electrosurgical loop conization. An in sano resection was achieved in 78 women (89%). Seventy-seven women (88%) became HPV negative. Cold-knife conization was performed in 20 women (19%). Here, free resection margins were achieved in 19 patients (95%) and a negative HPV status in 17 patients (85%). There were no statistically significant differences between either group.

Operative histology revealed one negative finding, CIN 1 in 16 cases, CIN 2 in ten cases, and CIN 3 in 61 cases. A comparison between preoperative and postoperative histological findings is shown in Table 1.

Among the 97 women in whom free resection margins were achieved, 86 (87%) had a negative postoperative HPV test. In contrast, among the ten women that were considered as treatment failures, only six (60%) had a negative postoperative HPV test whereas four (40%) were positive for HPV (Table 2).

Regarding the follow-up, a permanent HPV eradication or a persistent negative HPV test were seen in 83 women. One of these women developed recurrent CIN 2/3. In 24 women a permanent eradication of CIN/HPV was not achieved. Fifteen women remained HPV positive throughout the course, whereas nine women became

Table 1. — *Comparison of preoperative cervical biopsies and final operative histology.*

Operative Histology	Preoperative				Histology Total
	Negative	CIN1	CIN2	CIN3	
Negative	1	4	6	3	14
CIN1	0	9	5	2	16
CIN2	0	1	7	2	10
CIN3	1	3	11	46	61
Microinvasive carcinoma	0	0	1	2	3
Total	2	17	30	55	104

HPV positive again after having been negative in the initial follow-up period. Ten (42%) of these 24 women developed recurrent CIN 2/3.

The difference between both groups was highly significant ($p < 0.001$). The sensitivity of HPV testing in detecting treatment failures was 93% with a specifity of 85%. The negative predictive value (NPV) of persistent negative HPV to predict recurrent/residual disease was 99% and the positive predictive value (PPV) 42%.

Postoperative cervical cytology showed inconspicious results in 61 women whereas 46 women developed a positive cytology (Pap IIID or higher). Recurrent CIN 2/3 was observed in ten patients with positive cytology and in one woman with negative cytology. Accordingly, in the present series cervical cytology reached a sensitivity of 91% and a specifity of 63%. The NPV to predict recurrent/residual disease was 98% and the PPV 22%.

Discussion

In recent years, several national societies have established specific guidelines for the follow-up after treatment of CIN. Most of these protocols include cytology, colposcopy and HPV testing at various intervals. However, they are difficult to compare as the approaches are different and have not been evaluated in randomized clinical trials.

The efficacy of cytology screening in detecting recurrent disease is controversial as various studies show inconsistent results. Its specifity lies above 95% with most recurrences being associated with pathological findings, though its main disadvantage is the dissatisfying sensitivity between 20 and 85% [10, 11, 14-16]. This degree of false negative follow-up cytology accounts for the interest in finding additional diagnostic tools such as HPV testing that either alone or in combination would increase the predictive value in detecting recurrent disease.

Systematic reviews by Paraskevaidis *et al*. [10] and Zielinski *et al*. [11] have found that the pooled sensitivity of HPV testing for detecting recurrent or persistent disease reaches 90% six months after treatment and remains at this level for at least 24 months. Some studies showed that the combination of HPV testing and cytology resulted in increased sensitivity [10].

In the present study ten women (9%), six of them with a CIN 1 lesion, had a negative HPV status initially. It is known that the prevalence of HPV rises with increasing

Table 2. — *HPV status and clinical course after conization for CIN in 107 women (hr: high risk).*

PATIENTS | n = 107 (97 hr HPV pos, 10 hr HPV neg)

CONIZATION | 97 free resection margins | 10 involved resection margins

POSTOPERATIVE STATUS | 86 HPV neg | 11 persistent HPV pos | 6 HPV neg | 4 persistent HPV pos

FOLLOW-UP | 9 recurrent HPV pos | no recurrent HPV

CIN RECURRENCE | 1 CIN 2/3 | 1 CIN 2/3 | 8 CIN 2/3 | 1 CIN 2/3
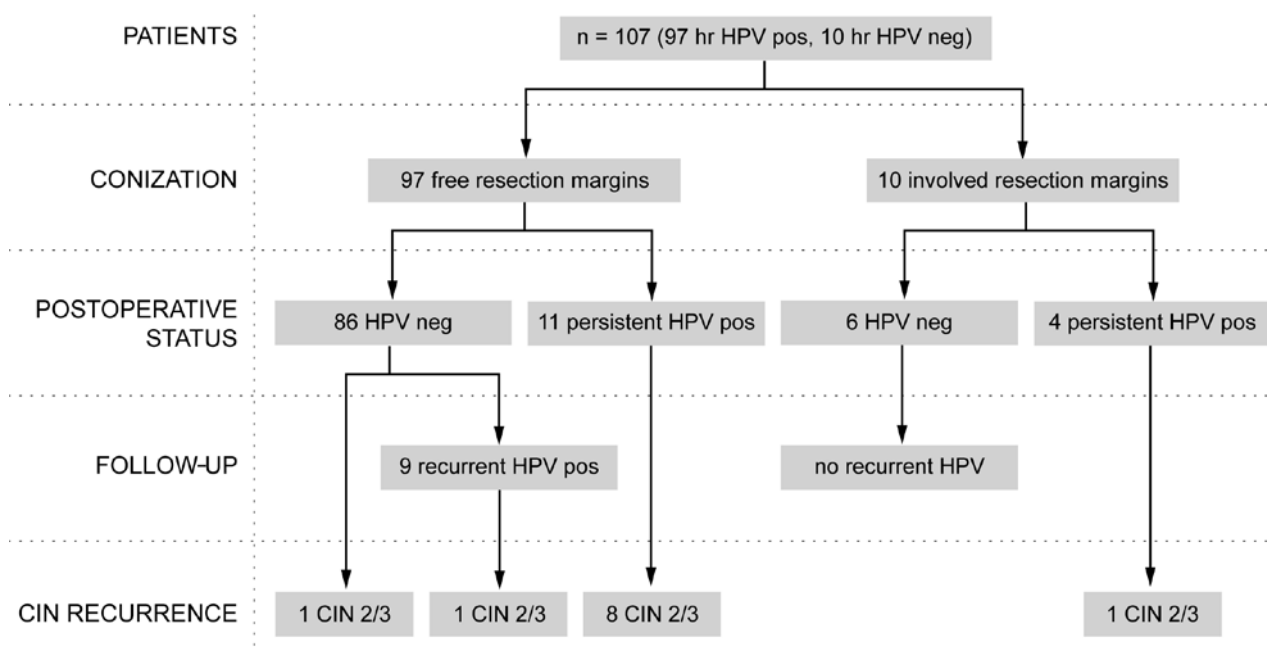
severity of the CIN lesion. By applying the PCR technique HPV was shown to be present in 78% of women with CIN 1, 86% of women with CIN 2 and 88% of women with CIN3 [6, 17]. Concerning the different tests available today, the clinical accuracy of hybridization tests such as the HC2 used in this study is at least equal to PCR-based assays [18].

The issue of whether HPV DNA becomes negative after conization is of relevance for the question of the usefulness of HPV testing in the follow-up period. In their review of the literature, subsuming 11 studies, Paraskevaidis *et al.* report, that among 672 women in whom CIN was treated successfully 566 (84%) showed negative postoperative HPV testing, whereas 106 (16%) remained HPV positive. Among the 204 women that were considered as treatment failures only 35 (17%) showed negative HPV testing, whereas 169 women (83%) were positive [10]. In accordance with these studies the present data indicate that conization to a high extent eradicates HPV. However, there exists a significant difference between negative and positive resection margins.

CIN positive excisional margins (non in sano resection) are accepted as a risk factor for recurrent disease, and it is more likely that these women redevelop abnormal cervical cytology. However, CIN-positive margins are not a reliable predictor of treatment failure as residual or recurrent disease can develop with both involved and clear margins [9-11]. This has also been shown in the present study, where most women with involved margins remained disease-free on follow-up although HPV eradication was significantly lower than after in sano excision ($p < 0.05$) .

In subsuming 11 studies, Zielinski *et al.* found in their meta-analysis of combined testing for cytology and free resection margins a low HPV (92%, range 85-96%) when compared to that of combined testing for HPV and cytology (99%, range 98-100%) or HPV and resection margins (99%, range 95-100%) [11]. Although the sensitivities of combined testing for HPV and resection margins or cytology were comparable, the specifity of combined HPV testing and cytology (81%, range 77-84%) was much higher than that of HPV testing and resection margins (54%, range 47-61%). The authors therefore concluded that HPV testing in combination with cervical cytology represents the best combination to monitor women in the follow up period [11].

The ongoing European multicenter study has set the goal to reach a conclusion regarding the optimal follow-up algorithm in order to define a strategy that would ultimately diminish the incidence of post-treatment cervical carcinoma. Based on our experience and the available studies, the implementation of HPV testing in post-treatment screening programmes might lead to a decrease in the rate of false-negative results and to an extension of the screening intervals. Open questions remain in setting the length of optimal screening intervals and the combination or sequence of cytology and HPV testing.

In conclusion, involvement of the surgical margins and the presence of HPV are associated with a higher risk of recurrence. HPV testing does not seem to be obviously superior to cervical cytology screening but the combined tests increase the sensitivity of detecting persistent or recurrent CIN and seem to be more effective than either test alone or the resection margin status. Furthermore, the combination of both tests increases the NPV identifying those women with minimal risk for persistent or recurrent disease.

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Possible role of palliative surgery for bowel obstruction in advanced ovarian cancer patients

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Summary

Objectives: Bowel obstruction is a relatively common event (30-40%) in advanced or recurrent ovarian cancer patients. No definitive data are available on the optimal management of this serious complication and treatment is generally limited to adoption of palliative measures. These modalities include both surgical and medical procedures. The aim of this study was to define selection criteria for subjects who would benefit from palliative surgery. *Study design:* Out of 270 epithelial ovarian cancer patients treated in the period 1984-2005, 75 (28%) developed bowel obstruction related to progression/recurrence of the disease. Palliative treatment – both medical and surgical – was applied on an individual basis. A new score developed by these authors was retrospectively applied to this group of patients with the aim of defining a subgroup that could benefit from surgical treatment. *Results:* Fifty cases (66.7%) were medically treated whereas 25 patients (33.3%) underwent surgery. Mean and median survival rates were 34 and 28 weeks in the surgical group versus 12 and four weeks in the medical group. Distribution according to score showed 53 cases (71%) in the low score group (< 14) and 22 (29%) in the high score group (> 14). A significantly better survival was observed in the low-score group ($p < 0.0001$) and in the surgically treated patients ($p < 0.001$). According to the risk score variables patients treated surgically for obstruction with low scores had a longer survival ($p < 0.005$) compared to medical treatment but this difference was not found in the high-risk group ($p < 0.05$). *Conclusions:* The prognosis of patients with bowel obstruction in relation to advanced ovarian cancer is best determined by comprehensive assessment of all prognostic parameters to define a subgroup of patients in a low-risk group that may benefit from surgical treatment.

Key words: Ovarian cancer; Palliative surgery; Prognostic factors.

Introduction

Bowel obstruction is a relatively common event in advanced or recurrent ovarian cancer patients. Different authors have shown an incidence of bowel obstruction of up to 30-40% in these patients [1, 2]. This serious and often life-threatening complication is mainly caused by progressive intraabdominal tumor growth leading to extrinsic occlusion of the bowel lumen, intraluminal occlusion due to pelvic, mesenteric or omental disease, and/or intestinal motility disorders due to infiltration of the mesentery or bowel muscle and nerves [2].

No definitive data are available on the optimal management of this serious complication and treatment of ovarian cancer-related bowel occlusion is still generally limited to the adoption of palliative measures.

Different modalities of treatment have been proposed in these patients, including both surgical (bypass procedures, colostomy, ileostomy, percuteneous endoscopic gastrostomy (PEG), and, rarely, bowel resection) and medical procedures (nasogastric tube decompression, intravenous fluid hydration, and drug administration) [2]. Specifically, PEG consists of the construction of a tube stoma, and it has shown to be superior to both nasogastric suction and operative gastrostomy for palliation of small bowel obstruction in terminal patients [2-4].

Significant morbidity and mortality are related to the surgical treatment of ovarian cancer related-bowel obstruction and different studies have shown major complications in 31% to 43% of these patients [3, 5-7]. Mortality rates within 30 days of surgery range from 10% to 25% [2, 8-12] and median postoperative survival ranging from 10-20 weeks [1-3, 13, 14] are reported in the scientific literature (Table 1). Although successful surgical relief of bowel obstruction can often be achieved, selection of those subjects who will benefit from palliative surgery should be carefully evaluated.

The aim of this study was to evaluate the possible clinical benefit deriving from a surgical approach in advanced ovarian cancer patients affected by cancer-related bowel obstruction.

Material and Methods

From 1984 to 2005, 270 patients with epithelial ovarian cancer were diagnosed and treated at the Department of Obstetrics and Gynecology, University of Brescia. Seventy-five (28%) cases developed bowel obstruction related to progression/recurrence of disease.

All clinical records of ovarian cancer-related bowel obstruction were retrospectively reviewed and the necessary clinical data obtained. All the histopathological sections were reviewed. All patients underwent surgery as the primary treatment. Tumor stage was assigned according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). Criteria defined by the World Health Organization (WHO) were employed for histologic diagnoses.

Table 1. — *Comparison of data in the literature and results of the present study: perioperative deaths and survival of surgically treated ovarian cancer patients with bowel obstruction.*

Authors	Total patients n°	Surgically-treated Patients n°	Perioperative death n° (%)	Mean survival (weeks)
Tunca (1981)	127	90	13 (14)	28
Piver (1982)	60	49	11 (22)	10
Krebs (1983)	98	92	26 (18)	12
Clarke-P. (1987)	49	49	7 (14)	18
Redman (1988)	38	24	4 (17)	11
Rubin (1989)	52	43	9 (21)	23
Larson (1989)	33	19	3 (16)	14
Lund (1989)	41	19	8 (42)	11
Bais (1995)	31	19	0	16
Jong (1995)	53	53	?	13
Gadducci (1998)	34	22	2 (9)	17
Present study	75	25	2 (25)	34

Table 2. — *Risk score prognostic variables in bowel obstruction.*

Parameters	Risk score	Parameters	Risk score
Age		*Previous RT*	
< 45	0	None	0
45 - 65	1	RT to pelvis	1
> 65	2	RT to abdomen	2
*Free-interval (yrs)**		*Previous CT*	
> 2	0	None	0
1-2	1	Single drug	1
< 1	2	Multiple drugs	2
Hematocrit (%)		*Tumor status*	
> 30	0	No palpable intrabdominal masses	0
25-30	1	Palpable masses	1
< 25	2	Distant metastases	2
Albumin (g/dl)		*Ascites (l)*	
> 3.06	0	0.1-1	0
2.55-3.06	1	1.1-3	1
< 2.55	2	> 3	2
Lymphocytes (cell/mm³)		*Site of obstruction*	
< 1350	0	Large bowel	0
< 1125	1	Small bowel	1
< 900	2	Both	2
PSK (%)		*Vomiting*	
> 80	0	No	0
60-70	1	Occasional	1
< 60	2	Persistent	2
Previous operations		*Pain*	
Standard	0	No	0
Others	1	Yes	2
None	2		

* From diagnosis to onset of obstruction.

The median age of patients was 56 years (range: 30-80 years). Four patients (5.3%) were initially diagnosed with FIGO surgical Stage I disease, 50 (67%) were Stage III, and 15 (20%) Stage IV. Six cases were referred from other institutions as advanced stage but without adequate staging procedures. The study included 50 serous carcinomas, nine mucinous, nine endometrioid, six undifferentiated and one mixed form. All 75 patients received platinum-based first-line chemotherapy.

Bowel obstruction (BO) was diagnosed on the basis of clinical symptoms and/or physical findings, and it was confirmed by a supine and upright abdominal X-ray showing dilated loops of small bowel and/or air fluid levels. A few patients (9/75, 12%) underwent other investigations such as radiographic contrast evaluation of the small and/or large intestine, abdominal computed tomography (CT) scan or ultrasound (US).

The choice of surgical or medical treatment of the BO was not based on a clinical protocol, but rather the type of therapy was individually tailored. The conservative medical approach treatment included nasogastric suction, intravenous fluid hydration, and/or drug administration, mainly consisting of hyoscine butyl-bromide, haloperidol, corticosteroids, somatostatin, and morphine. Parental nutrition was seldom administered in the perioperative period (3/25, 12%). The surgical treatment consisted of bowel resection and anastomosis, bypass procedures, explorative laparotomy, colostomy, ileostomy, and explorative laparotomy with resection of the tumor mass. In the group of surgically treated patients, the surgical team included a gynaecologic oncologist and a general surgeon.

An updated proposed risk score [15] was retrospectively applied to our patients to better define the characteristics of patients who could benefit from surgical treatment and to identify any possible correlation with their prognosis (Table 2).

The Pearson chi-square test was used to compare different groups of patients. Survival from relapse curves were plotted using the Kaplan-Meier method and analyzed by the log-rank test.

Results

In this study 75 advanced ovarian cancer patients experienced bowel obstruction related to their disease. The site of obstruction was the large bowel in 13 cases (17.3%), small bowel in 13 cases (17.3%), and both in the remaining 49 patients (65.4%).

Mean and median survival since obstruction was 19.6 weeks and eight weeks, respectively.

Fifty cases (66.7%) were medically treated, whereas the remaining 25 patients (33.3%) underwent surgery. In the latter group the sites of obstruction were large bowel in nine cases (36%), small bowel in seven (28%), and both in the remaining nine patients (36%).

The types of surgical procedure performed to cure bowel obstruction included: bowel resection and anastomosis in 14 cases (56%), bypass procedures in four cases (16%), explorative colostomy in one case (4%), ileostomy in another two cases (8%) and one explorative laparotomy with resection of the tumor mass (4%); three cases underwent exploratory laparotomy and were intraoperatively considered as inoperable (16%) (Table 3).

Median operative time was 180 minutes (range: 120-480 min). The peri-operative mortality rate, expressed as death within four weeks of surgery, was 8% (2/25); one patient died within a week after surgery and her death was directly related to the surgical procedure, while the second one died after three weeks because of a pulmonary complication in a different institution.

Twelve out of 25 patients submitted to surgery (48%) received further chemotherapy, whereas only six (12%) of the 50 cases that were medically treated underwent further antineoplastic treatment.

Table 3. — *Surgical procedure, score and survival for surgically treated patients*.

	Surgical procedure	Score	Survival (weeks)
1	Ileal-cecal bypass, permanent colostomy, multiple biopsies	13	36
2	T AH, left salpingo-oophorectomy, recto-sigmo id resection, low colonic end-to-anastomosis	12	54
3	Ileal-traverse colon anastomosis	9	42
4	Ileal-traverse colon by-pass, multiple biopsies	9	6
5	I leal resection, end-to-end anastomosis	9	6
6	Recto-sigmoid resection, low colonic end-to-end anastomosis, mesenteric lymphadenectomy	4	91
7	Esploratory laparotomy (in a different institution)	9	< 1
8	Traverse colostomy	9	33
9	Esploratory laparotomy, multiple biopsies	13	20
10	Adhesionlysis, ileal resection, ileal colonic bypass, permanent colostomy	14	8
11	Adhesionlysis, resection of ileal metastasis, ileal-traverse colon anastomosis, colostomy	12	5
12	Adhesionlysis, resection of tumor mass	11	64
13	Ileal-colonic anastomosis	11	14
14	Esploratory laparotomy	16	10
15	Ileal-traverse colon anastomosis, permanent colostomy	14	11
16	Ileostomy	9	46
17	Resection of descending colon, latero-lateral anastomosis	13	11
18	Esploratory laparotomy, resection of tumor mass	11	5
19	Ileal-colonic anastomosis	17	3
20	Adhesionlysis, resection of bowel with end-to-end anastomosis	10	72
21	Omenthectomy, resection of sigma, left salpingo-oophorectomy	12	56
22	Ileo-traverse colostomy	10	28
23	Ileal resection, latero-lateral anastomosis adhesionlysis	8	76
24	Ileal resection, terminal ileostomy	12	44
25	Ileal-ascendent colon bypass, latero-lateral anastomosis, multiple biopsies	16	15

Table 4. — *Survival of low score (≤ 14) and high score (> 14) patients by type of treatment*.

Score	Treatment	Patients n°	Mean survival (weeks)	Range (weeks)
> 14	Surgical	3	10	3-15
	Medical	19	4	
≤ 14	Surgical	22	37	< 1-94
	Medical	31	17	< 1-102

Mean and median survival were 34 and 28 weeks, respectively (range: 0-94 weeks) in the group of patients who underwent surgery and 12 and four weeks, respectively (range: 0-102 weeks) in the medically treated group. A significant difference was observed in terms of survival for the obstruction in the medically and surgically treated patients, with a prognostic advantage in the surgical group ($p < 0.001$) (Figure 1).

A significantly higher percentage of cases (80%, 20/25 patients) survived longer than eight weeks after the surgical procedure, compared to an 8-week survival rate of 34% (17/50 cases) in the conservative treatment group ($p < 0.0001$). Two out of three patients who survived less that eight weeks had been intraoperatively evaluated as inoperable. After having excluded the patients that were
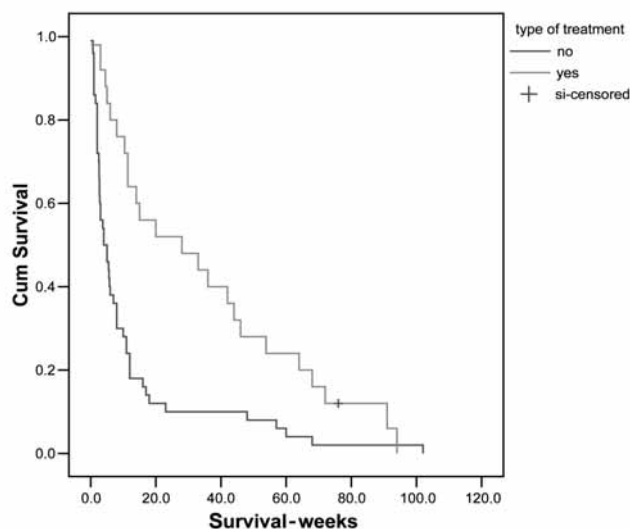
no: no surgery; yes: surgery

Figure 1. — Survival by type of treatment.

not candidates for surgical therapy, a survival higher than eight weeks was observed in 18/22 cases (81%).

Patient distribution according to a recently defined score (Table 3) showed that 53 cases (71%) had a low score (≤ 14) and the remaining 22 cases (29%) had a high score (> 14).

Median survival was 23 weeks (range: 0-102) in the low-score group and four weeks (range: 0-15) in the high-score group. Significantly better survival was observed in the low-score group ($p < 0.0001$).

The correlation between the new prognostic score and the type of treatment of ovarian cancer-related bowel obstruction (surgical versus medical) was evaluated. Twenty-two out of the 25 (88%) surgically treated patients had a low score, and 3/25 (12%) had a high score ($p < 0.05$), while 31/50 (62%) and 19/50 (38%) medically treated patients had low and high scores respectively ($p < 0.05$) (Table 3). Among the 22 surgically treated patients with a low score, two (9%) were intraoperatively defined as inoperable and 18 (81.8%) showed survival longer than eight weeks. Conversely, among the three surgically treated patients with a high score, one (33.3%) was intraoperatively defined as inoperable and two (66.7%) survived longer than eight weeks.

In the surgically treated group of patients, mean and median survival were 38 and 33 weeks, respectively (range: 0-94) when the score was 14 (22 cases), compared to nine and ten weeks, respectively (range: 3-15) in the high-score group (3 cases). In the medically treated group, mean and median survival of patients with a low score was 17 and eight weeks, respectively (range: 0-102) compared to four and three weeks (range: 0-12) in the high-score group, respectively (Table 3). A significant difference in survival for obstruction both in the surgically and medically treated groups of patients according to

2a "surgery"

2b "conservative therapy"
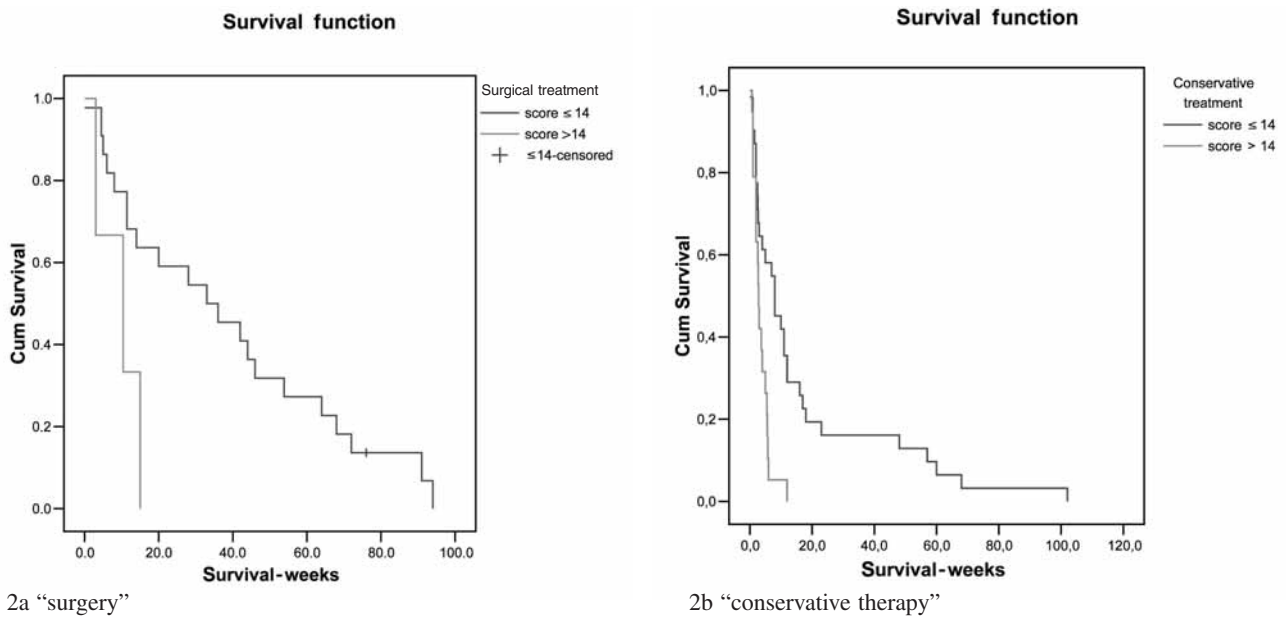
Figure 2. — Survival of patients treated with (a) surgery and with (b) conservative therapy by different scores.

3a "low score"
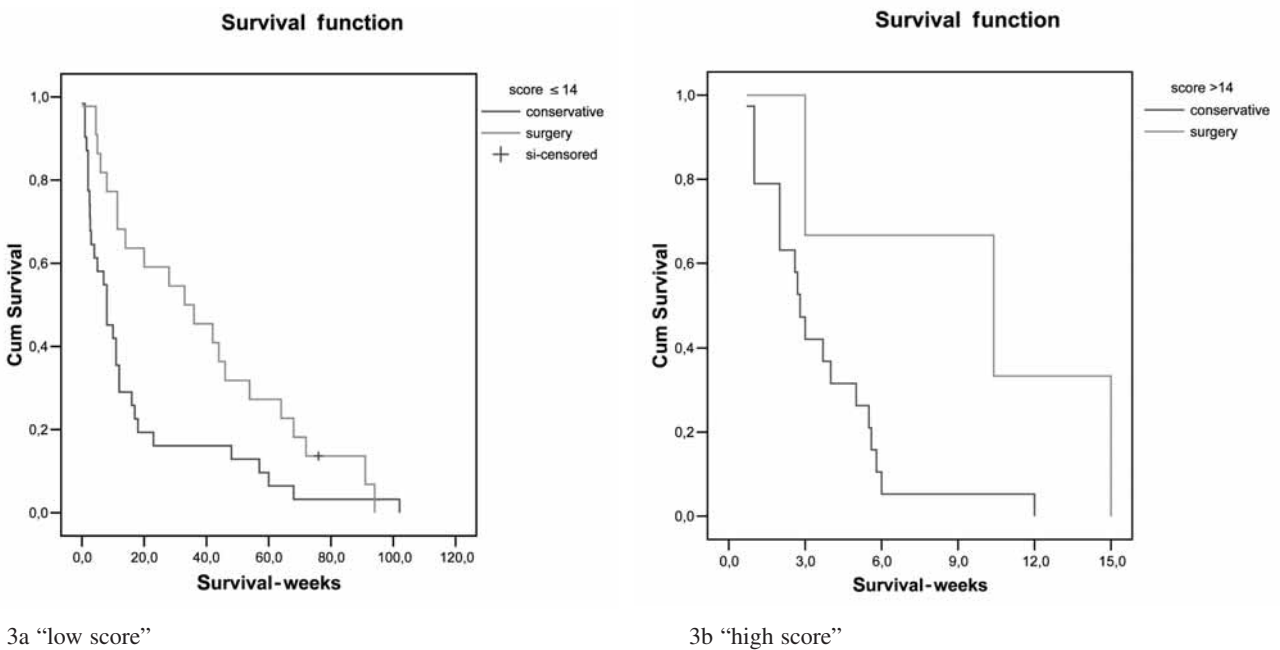
3b "high score"

Figure 3. — Survival of patients with (a) low score (≤ 14) and with (b) high score (> 14) by modality of treatment.

the score was observed ($p < 0.05$ and $p < 0.001$ respectively) (Figure 2a and 2b).

Among the patients with low scores, survival according to the medical versus surgical treatment of obstruction is shown in Figure 3a; significant differences were observed in the different groups ($p < 0.05$). Conversely, patients with a high score did not show a marginally significant difference in survival since onset of obstruction in the medically and surgically treated groups ($p = 0.06$) (Figure 3b).

Discussion

The main goal of treatment of ovarian cancer-related bowel obstruction is to obtain some benefit of the quality of life for these patients in terms of both palliation of symptoms and relief of obstruction.

Different studies showed that survival of ovarian cancer patients with bowel obstruction receiving conservative management is shorter when compared to subjects who undergo surgery, and mean survival time ranged from

four to nine weeks and from 12 to 30 weeks, respectively [1, 2, 13, 16].

The selection of the ideal candidate for surgery is still under debate. Palliative surgery should be considered when relief from symptoms is not obtained within three to seven days after one of the medical procedures mentioned above [2]. It has been suggested by the scientific literature [2, 3] that surgical intervention is of unlikely benefit in advanced ovarian cancer patients with bowel obstruction whose life expectancy in less than two months, and/or radiographic contrast of the bowel shows a free passageway with prolonged passage indicative of intestinal motility problems with functional obstruction due to extensive intraperitoneal carcinomatosis [2, 5, 14, 17].

In our study, according to data from the scientific literature, 28% of patients (75/270) with ovarian cancer experienced cancer-related bowel obstruction.

The operative mortality rate was 8% (2/25), lower than that observed in other studies (10 to 25%): moreover, only one death was directly related to the surgical procedure (4%) (Table 1).

It has commonly been experienced that palliative surgery sometimes cannot be performed because of the unexpected intraoperative finding of extensive tumor involvement at exploratory laparotomy. Piver *et al*. [7] reported that 18% of the 60 subjects who underwent surgery for bowel obstruction were actually inoperable. In the series of Krebs and Goplerud [14], 12% of the ovarian cancer patients with bowel obstruction who underwent surgical intervention could not be operated on. In our series, three patients (16%) underwent exploratory laparotomy and were intraoperatively considered as inoperable; still, a significantly better survival was observed in the surgically treated patients compared to the medically treated group ($p < 0.001$). A relatively long mean and median survival in the surgically treated patients was observed (35 and 28 weeks, respectively, compared to data from other studies, ranging from 12 to 30 weeks and from 10 to 20 weeks, respectively). Eighty percent of surgically treated cases survived longer that eight weeks.

The prognostic role of several clinical variables has been evaluated in the scientific literature [1, 2, 5, 6, 14] that could help the clinician to tailor the proper management of advanced ovarian cancer patients with bowel obstruction. Krebs and Goplerud [14] proposed a prognostic score that seemed to offer reliable eligibility criteria for those patients who would be optimal candidates for surgery. A recent study evaluating more prognostic variables than those included in the Krebs and Goplerud score seems to show interesting results in terms of both a better prognostic definition of cases with bowel obstruction and a better selection of the cases that could undergo successful palliation or benefit from surgery [15]. A significantly different survival rate was observed according to this newer score, and the difference remained significant when it was analyzed in both surgically and medically treated groups of patients.

When the comparison between surgical and medical treatment of bowel obstruction was performed in different groups of patients according to the new score value, it was interesting to observe that the prognostic advantage deriving from a surgical approach to bowel obstruction remains significant in the low-score group of patients, while subjects with a high score did not show a significant benefit from surgery.

In conclusion, from both data in the literature and our results, surgical palliation of ovarian cancer patients with bowel obstruction seems to have an important role to achieve significant relief of symptoms that cannot be expected from non-operative modalities of treatment. Surgical procedures show longer survival from the time of diagnosis of bowel obstruction compared to medical treatment. The prognosis of patients with bowel obstruction related to advanced ovarian cancer is best determined by comprehensive assessment of all prognostic parameters that can be synthesized in different risk scores. One which has been fairly recently defined shows to be highly predictive of the outcome and, therefore, to be very helpful in the selection of patients who are most likely to benefit from surgical intervention. This assessment seems to be supported by the observation that patients with a good prognostic value of this score significantly benefit from surgery, while no significant differences on survival can be observed in the medically treated group of patients with a negative prognostic score.

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The incidence, treatment and prognosis of cervical carcinoma in young women: a retrospective analysis of 4,975 cases in Japan

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Summary

Objective: To determine the clinical characteristics of patients (young women) with cervical carcinoma aged less than 35 years. *Methods:* Data from patients who were treated for cervical carcinomas from 1990 to 2000 in the Kinki District were retrospectively investigated for clinical stage, histologic type, treatment procedure and prognosis. *Results:* Of a total of 4,975 cases, 441 patients were aged less than 35 years old. The incidence of cervical carcinoma in these women was 7.9% from 1990 to 1995, 9.1% from 1996 to 2000, and 9.5% from 2001 to 2005. FIGO Stage I included 374 cases, followed by, 49 in Stage II, 11 in Stage III, and seven in Stage IV. Squamous cell carcinoma incidence was 80.7% and non-squamous cell carcinoma incidence was 19.3%. Several types of surgery were performed in patients with Stage I and II, while patients with Stage III and IV were treated with radiotherapy and/or chemotherapy without any type of surgery. In patients who underwent lymphadenectomy, 21.1% cases had nodal involvement. The 5-year survival rate was 95% for Stage I disease, 73% for Stage II, 68% for Stage III, and 19% for Stage IV. *Conclusion:* The incidence of cervical carcinoma in young women slightly increased from 1990 to 2005. The prognosis of cervical carcinoma tends to be better in young women than in older patients, especially in Stage III disease.

Key words: Young women; Cervical carcinoma; Incidence, prognosis.

Introduction

Cervical cancer is the second most common cancer among women worldwide (GLOBOCAN 2002, http://www.depdb.iarc.fr/blobocan/GLOBOframe.htm). In Japan, the crude mortality rate of cervical cancer is 21.3 per 100,000 women and it was the third most common cause of female deaths in 1960 [1]. The age-standardized uterine cervical cancer incidence based on the world population was 13.4 in 1975 as stated on the website of the Center for Cancer Control and Information Services National Cancer Center in Japan (http://ganjoho.ncc.go.jp/professional/statistics/statistics.html). Widespread use of cervical cancer mass screening was started in Japan by the Japanese Ministry of Health, Labor and Welfare in 1982. The mortality and incidence of cervical cancer decreased up to the mid-1990s. However, the incidence of cervical carcinoma has tended to increase since the late 1990s, especially among young women [2-4].

Several reports have evaluated the effect of cervical cancer screening since national screening programs were initiated. Although the overall incidence of invasive cervical carcinoma has decreased during the last few decades, several studies have demonstrated that cervical carcinoma has been steadily increasing [5-7] or has remained stable in younger women [8]. Histologic analysis revealed that the incidence of adenocarcinoma (AC) has risen in young women, whereas squamous cell carcinoma (SCC) has been reduced [7-10]. In addition, we often have seen unanticipated rapid disease progression in young women despite treatment. Many earlier reports demonstrated poorer prognosis in young women [11-14], while some studies indicated no difference or a better prognosis in young patients [13, 16]. Therefore, it remains controversial whether young patients with invasive cervical carcinoma have a poorer prognosis than older women.

This retrospective study was conducted to examine the trends in the incidence, histologic type, and treatment procedure of invasive cervical carcinoma in Japanese women less than 35 years old since 1990. The purpose of our study was to assess survival rates and to evaluate prognostic factors for cervical carcinoma in young women.

Patients and Methods

The present study was designed by the tumor sectional meeting of the Obstetrical Gynecological Society of the Kinki District of Japan. The medical records of patients with cervical carcinomas treated from 1990 to 2005 were retrospectively reviewed.

Patients were included in the study if primary treatments were carried out in the Kinki District in Japan. The number of institutions included in the study was five from 1990 to 1995, and increased to ten after 1996. Carcinoma in situ of the cervix was not registered, and only cases of invasive cervical cancer were included in the study. The clinical staging and histologic criteria were based on the International Federation of Gynecology and Obstetrics (FIGO). The time of diagnosis was considered to be the date of the primary treatment. Time to recurrence and death or last contact was calculated. Trends in incidence and distribution of clinical stage and histologic type were examined in all ages of women. The treatment types, pathologic risk factors, and prognosis were submitted to date by patients aged less than 35. Informed consent was not deemed necessary for this chart review. We did not request institutional review board approval for this study because of its retrospective nature.

Treatment procedures

Most of the patients were subjected to one or more types of treatment including surgery, radiotherapy, and chemotherapy. Only a few patients had photodynamic therapy or immunotherapy performed. Types of hysterectomy treated with cervical carcinoma were classified into five types by Piver [17]. In Japan, three types of hysterectomy were performed as follows: simple hysterectomy, which corresponds with type I hysterectomy by Piver's classification, extended hysterectomy, which is equivalent to type II, and radical hysterectomy consisting of type III and IV hysterectomy. If women with Stage I disease desire to preserve their fertility, cervical conization is chosen. In Japan, typical primary radiation procedures have consisted of 45-50 Gy with external beam irradiation, followed by a high-dose-rate intracavitary brachytherapy (20-25 Gy), for a total dose of 60-75 Gy to point A. The radiation method used with postoperative cases is 45-50 Gy to the whole pelvis with an external beam. Chemotherapy consists of adjuvant and neoadjuvant treatments, but does not include a maintenance procedure with oral fluoropyrimidines. In this study, concurrent chemoradiotherapy was classified as radiation plus chemotherapy. If women were treated with cervical conization alone, their treatment type was surgery alone. If women underwent cone biopsy without other types of surgery, followed by radiation or chemotherapy, their treatment type was radiation or chemotherapy, not surgery.

Statistical analysis

Trends of incidence, clinical stage, and histologic type among three-year periods were examined for all eligible cases.

Descriptive analysis for women aged less than 35 is presented for treatment type, surgical procedure, and pathological prognostic factors. The Fisher exact test was used to estimate the disease-free interval and overall follow-up period. Survival curves were generated using the Kaplan-Meier Method. Univariate analysis of potential prognosis and predictive factors for women aged less than 35 related to clinical stage, histologic type, lymph node metastases, and lymph vascular involvement at the primary treatment was performed using the log-rank test to determine statistical significance. Cox's proportional hazards regression was employed to model the multivariate association of survival. A *p* value of less than 0.05 was considered to reflect a significant difference.

Results

Patient demographics

Between 1990 and 2005, 4,975 women with invasive cervical carcinoma were registered by the tumor sectional meeting of the Obstetrical Gynecological Society of the Kinki District of Japan. Time trends in the distribution of FIGO stage and histologic type are shown in Table 1. The number of all cases of cervical carcinoma has increased since the late 1990s, especially in the population of women aged less than 35. The distribution of FIGO stage did not change in this period. The incidence of adenocarcinoma significantly increased; in the most recent period, there were 426 of 1,306 (24%) cases in all patients and 45 of 164 (27%) cases in the group of young women. Out of a total of 4,975 women, 441 women aged less than 35 were identified and had clinical pathological data analyzed. The characteristics of patients in each age group are given in Table 2. Most of the patients were young women 30-34 years old (69%). Twenty-five out of 441 (5.7%) patients were aged less than 25, and none of the patients was diagnosed with Stage III or IV disease. Patients with FIGO Stage IIIA and more advanced disease were detected in the groups aged over 25. In patients

Table 1. — *Characteristics of cervical carcinoma in the Kinki District in Japan (1990-2005).*

Year periods	1990-95		1996-00		2001-05	
Age group	< 35	Overall	< 35	Overall	< 35	Overall
Stage of FIGO						
I	113	772	129	932	132	965
II	6	367	20	383	23	393
III	1	316	5	234	5	233
IV	0	67	3	82	4	141
Histologic type						
Squamous cell carcinoma	108	1313	129	1420	119	1306
Non-squamous carcinoma	12	209	28	301	45	426

Table 2. — *Details of characteristics of cervical carcinoma in age groups.*

Age group	< 25	25-29	30-34	All ages
No. of patients	25	111	305	4975
Stage of FIGO				
IA	9 (36)	52 (47)	130 (43)	1027 (21)
IB	14 (56)	42 (38)	127 (42)	1642 (33)
IIA	0	1 (1)	7 (2)	267 (5)
IIB	2 (8)	11 (10)	28 (9)	876 (18)
III	0	2 (2)	9 (3)	873 (18)
IV	0	3 (3)	4 (1)	290 (6)
Histologic type				
Squamous cell carcinoma	20 (80)	92 (83)	244 (80)	4000 (80)
Adenocarcinoma	4 (16)	10 (9)	44 (14)	739 (15)
Adenosquamous carcinoma	0	5 (5)	10 (3)	157 (3)
Other types of carcinoma	1 (4)	4 (4)	7 (2)	79 (2)

Table 3. — *Distribution of surgical procedures for FIGO stage.*

Stage of FIGO	IA	IB	II	III	IV
No. of patients	191	183	49	11	7
Types of treatment					
Surgery alone	182 (95)	116 (63)	5 (10)	1 (9)	1 (14)
Surgery + chemotherapy	2 (1)	18 (10)	15 (31)	2 (18)	0
Surgery + radiotherapy	1 (0.5)	34 (19)	9 (18)	0	0
Surgery + chemo + rad	0	14 (8)	15 (31)	3 (27)	0
Radiotherapy alone	0	1 (0.5)	4 (8)	4 (36)	0
Rad + chemo	0	0	0	0	4 (57)
Chemotherapy alone	1 (0.5)	0	1 (2)	0	1 (14)
Others	5 (3)	0	0	1 (9)	1 (14)

Table 4. — *Stage distribution of surgical procedures.*

Stage of FIGO	IA	IB	II
No. of patients	191	183	49
Surgical procedure			
Conization alone	75 (39)	14 (8)	0
Simple hysterectomy	61 (32)	5 (3)	1 (2)
Extended hysterectomy	26 (14)	18 (10)	3 (6)
Radical hysterectomy	23 (12)	145 (79)	40 (82)
No surgery	6 (3)	1 (0.5)	5 (10)

aged 25-29 years old, there were two of 111 cases (1.8%) with Stage IIIB, two (1.8%) cases with Stage IVA, and one (0.9%) case with Stage IVB. In women aged over 30, there was one of 305 (0.3%) cases with Stage IIIA, eight (2.6%) cases with Stage IIIB, three (1.0%) cases with Stage IVA, and one (0.3%) with Stage IVB. The distribution of histologic types among the three age groups was not identified.

Treatment procedures for women aged less than 35

The type of treatment was divided in four types including surgery, chemotherapy, radiotherapy, and other types of treatment. The concurrent chemoradiotherapy (CCRT) was classified as combined treatment with radiotherapy plus chemotherapy. Details of the treatment procedure based on FIGO stage are shown in Table 3. The stage distribution in surgical procedures is addressed in Table 4. In patients with Stage Ia, most of the cases were treated with surgery alone in which conization only was carried out in 39% and simple hysterectomy in 32%. None of the patients underwent radiation therapy alone. Only three patients had combined therapy. Two of them with AC underwent radical hysterectomy followed by systemic chemotherapy. One patient with SCC Stage IA_2 was treated with a simple hysterectomy followed by radiotherapy. One patient with squamous cell carcinoma Stage IA_1 had conization performed followed by chemotherapy. In patients with Stages IB and II disease, 185 of 232 (80%) cases received radical hysterectomy. In patients with Stage III disease, six of 11 (55%) patients underwent radical hysterectomy, while four of 11 patients were treated with radiotherapy alone. Four patients received neoadjuvant chemotherapy (NAC) before surgery. In patients with Stage IV disease, four of seven (57%) cases received CCRT.

Univariate analysis

Among 441 young patients, 53 (12%) patients had tumor recurrence and 36 (8%) patients died of the disease (Table 5). The incidence of recurrent disease was one out of 191 (0.5%) for Stage IA, 27/183 (14.7%) for Stage IB, 17/49 (34.7%) for Stage II, 3/11 (27.3%) for Stage III and 5/7 (71%) for Stage IV. The incidence of tumor death was zero in Stage IA patients, 16/183 (8.7%) for Stage IB, 12/49 (24.5%) for Stage II, 3/11 (27.3%) for Stage III and 5/7 (71%) for Stage IV. The incidence of recurrence and death was 7.9% and 5.1% in squamous cell carcinoma, and 29.4% and 21.2% in non-squamous cancer, respectively. The median follow-up period in young women was

Table 5. — *Univariate analysis related variables with cervical carcinoma in young adult women.*

Variable	No. of patients	No. of recurrences	No. of deaths	Median OAS (months)	5-year survival (%)	p value
Stage of FIGO						
I	374	28	16	65 (1-201)	95	
II	49	17	12	43 (1-139)	73	< 0.0001
III	11	3	3	36 (1-101)	68	
IV	7	5	5	12 (1-57)	19	
Histologic type						
Squamous cell carcinoma	356	28	18	65 (1-201)	95	< 0.0001
Non-squamous carcinoma	85	25	18	41 (1-176)	75	
Lymph node metastasis						
Negative	146	16	9	55 (3-187)	93	< 0.0001
Positive	39	17	9	39 (1-139)	74	
Lymph vascular involvement						
Negative	207	8	6	62 (3-187)	97	< 0.0005
Positive	99	26	14	41 (1-145)	83	

OAS: overall survival.

Table 6. — *Specific factors on survival as determined by multivariate analysis.*

Variable	Risk ratio	95%CI	*p* value
Early stage (Stage I)	0.298	0.106-0.842	0.022
Non-squamous carcinoma	0.201	0.076-0.527	0.0011
Lymph node metastasis	0.358	0.132-972	0.044

63 months (range 1-201). Kaplan-Meier survival curves and log-rank tests were generated to evaluate the influence of individual prognostic factors on overall survival (Table 5). The 5-year survival rate was also estimated in young women using the Kaplan-Meier method. Earlier stage (Stage I), histologic type (squamous cell carcinoma), the absence of lymph vascular involvement, and no evidence of lymph node metastasis were all associated with significantly improved overall survival rates (Figures 1, 2, 3 and 4). Subset analysis of treatment methods in Stages IB_2 and II disease revealed that patients treated with combined methods including surgery had a better prognosis (5-year survival, 78.9%) than those without surgery (Figure 5). In all patients with Stages IB_2, II, III and IVA, the 5-year survival showed 75.8% with neoadjuvant chemotherapy (NAC) and 68.5% without NAC. NAC showed the potential of improved survival, but there was no significant difference with or without NAC.

Multivariate analysis

The influence of specific factors on survival as determined by univariate analysis may have resulted from selection bias rather than from the variable itself. Therefore, multivariate analysis was performed to account for the potential influence of confounding factors (Table 6). A Cox proportional hazards model was employed. The following variables were considered: early stage (Stage I), histologic type (squamous cell carcinoma), and lymph node metastasis. All factors showed relative risks of less than 1, indicating a favorable effect on survival. These factors were found to have an independent influence on cause-specific survival.

Discussion

Many reports have demonstrated that the incidence of cervical carcinoma has decreased over the past 40 years [6-10]. However, an increased incidence of cervical carcinoma in young women has been well documented. Histologic examination has shown that the incidence of SCC has obviously decreased, while the population of AC has significantly increased. This retrospective study was designed to evaluate the recent trends in cervical carcinoma in Japan. From 1990 to 2005, 7,472 cases with cervical neoplasms were reported in the Kinki District in Japan. Two thousand four hundred and ninety-seven of these were diagnosed as carcinoma in situ, and 4,975 cases were invasive carcinoma. Time trends in the total incidence did not decrease during this period, whereas an increased number of cases in young women aged less than 35 was detected. In Japan, other investigators have reported that the age-standardized incidence of invasive cervical cancer decreased from 13.4 to 7.2 per 100,000 women from 1975 to 1998 [3]. In young women aged less than 30, invasive cervical cancer decreased until 1984, but increased thereafter. Carcinoma in situ (CIS) has rapidly increased by approximately seven times [3]. In our investigation, the rate of CIS was 26.8% from 1990-1995, and was elevated by 36.4% during the next five years and by 36.7% in the most recent five years (data not shown). The incidence of non-SCC has significantly increased since 1990, especially in young women. In our series, the frequency of non-SCC has increased by 1.7 times in all patients and 2.7 times in young women during the past 15 years. This time trend in incidence and histologic type are consistent with many another countries.

Several epidemiological studies have demonstrated that cervical carcinoma in young women has increased steadily in the last few decades [5-7]. Using medical records, we examined the characteristics of clinical status, treatment procedure, and prognosis in young women aged less than 35 since 1990. In our series, the youngest patient was 18 years old, diagnosed with mucinous adenocarcinoma with FIGO Stage IB_2. She underwent radical hysterectomy followed by irradiation with external beam at pole pelvis because multiple lymph node metastases had been observed. Recurrent disease was detected 22 months after primary treatment and she received chemotherapy. It is generally accepted that patients in the younger age group tend to have earlier stage disease. The FIGO annual report indicated that the portion of patients for Stages I, II, III and IV, treated from 1993-1995, was 42.7, 32.3, 20.5, and 3.9%, respectively [20]. In our study, the population of all women treated in 1990-2005 was 53.6% for Stage I, 23.0% for Stage II, 17.5% for Stage III, and 5.8% for Stage IV. Of a total of 441 cases in young women, there were 374 (84.8%) with FIGO Stage I, 49 (11.1%) with Stage II, 11 (2.5%) with Stage III, and seven (1.6%) with Stage IV. No cases with Stages III and IV were observed in the youngest group aged less than 25. Only two patients were diagnosed with FIGO Stage IVB. One was 25 years old with mucinous adenocarcinoma. She has undergone CCRT and is alive without any recurrent diseases. The other patient was 31 years old with other types of carcinoma. Although she had received systemic chemotherapy, she died of primary disease after one month. The population of advanced stages was 4.1% in young women and 23.3% in older women aged over 35; there was a significant increase in the older group. On the other hand, the rate of non-SCC was 19.3% in young women and 18.8% in older women. While the population of non-SCC has been elevated the last 15 years, there is no difference between women aged under 35 and those over 35.

Earlier reports demonstrated that young patients with cervical cancer have a poorer prognosis than older patients [11-13]. Rutledge reported that women aged less than 35 had a poor prognosis as compared with patients
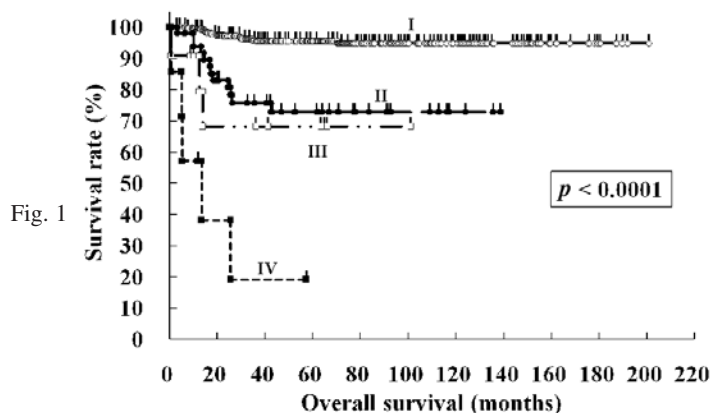
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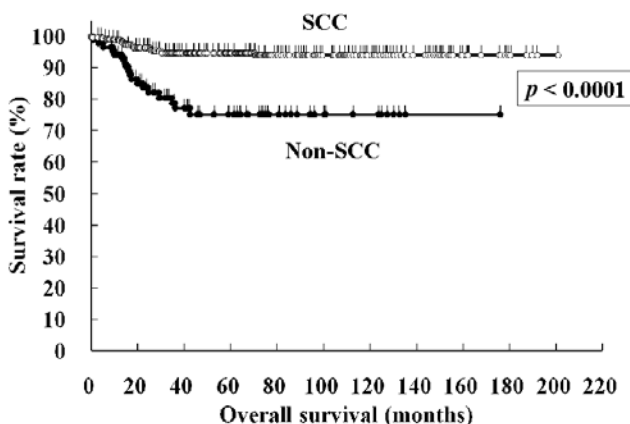
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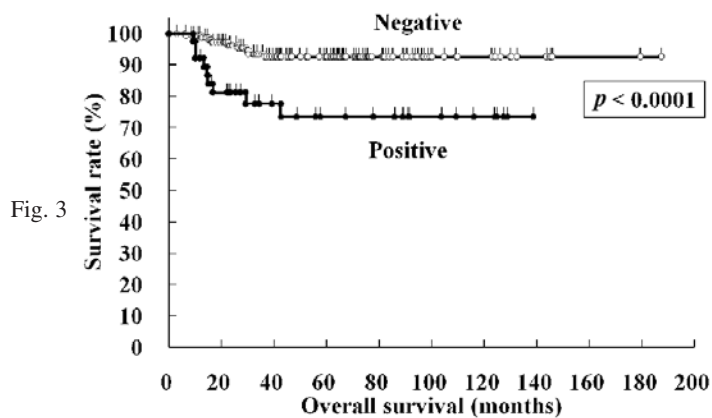
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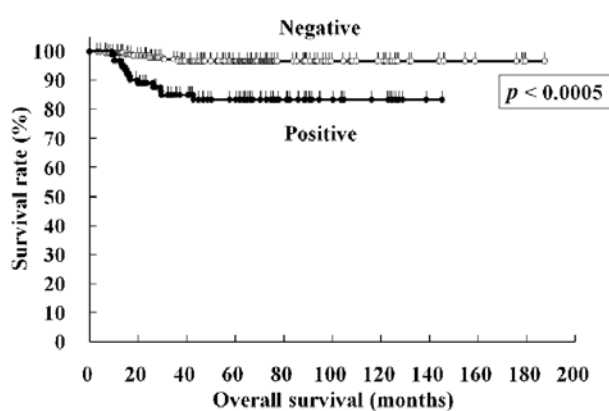
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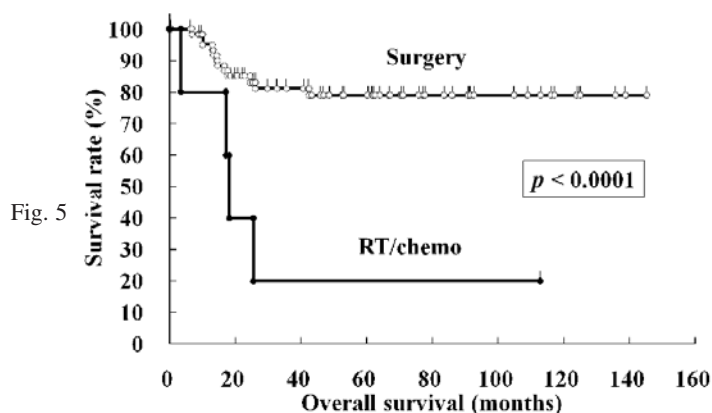
Fig. 5

Figure 1. — Kaplan-Meier analysis of overall survival related to FIGO stage in young women. The x-axis indicates overall survival in months after primary treatment. The y-axis indicates the proportion of patients surviving with uncensored data.
I; FIGO Stage I, II; FIGO Stage II, III; FIGO Stage III and IV; FIGO Stage IV.
Figure 2. — Kaplan-Meier analysis of overall survival related to histologic types in young women. The x-axis indicates overall survival in months after primary treatment. The y-axis indicates the proportion of patients surviving with uncensored data.
SCC; squamous cell carcinoma, non-SCC; non-squamous carcinoma.
Figure 3. — Kaplan-Meier analysis of overall survival related to lymph node metastasis in young women. The x-axis indicates overall survival in months after primary treatment. The y-axis indicates the proportion of patients surviving with uncensored data.
Negative; absence of lymph node metastasis, Positive; presence of lymph node metastasis.
Figure 4. — Kaplan-Meier analysis of overall survival related to lymph vascular involvement in young women. The x-axis indicates overall survival in months after primary treatment. The y-axis indicates the proportion of patients surviving with uncensored data.
Negative; absence of lymph vascular involvement, Positive; presence of lymph vascular involvement.
Figure 5. — Kaplan-Meier analysis of overall survival related to treatment procedure for FIGO Stages IB_2-IIB in young women. The x-axis indicates overall survival in months after primary treatment. The y-axis indicates the proportion of patients surviving with uncensored data.
Surgery; patients treated with surgery alone and in combination, RT/chemo; patients treated without surgery.

over 35 in a stage- and treatment-matched analysis [12]. Serur *et al.* indicated that women with FIGO Stages IIB and III had a poorer prognosis in patients aged less than 50 than in older patients [14]. In addition, we have often seen unanticipated rapid disease progression in young women, although aggressive and combined treatments had been received. However, some reports indicated that the prognosis by FIGO stage at diagnosis was poorer in older aged women than that in young women. Kosary demonstrated that the 5-year survival rate was highest in women aged less than 30 and declined steadily as age increased in patients both overall and within stage [17]. Another study indicated that the stage-matched 5-year survival rates were better for young women aged less than 40 than older patients with FIGO Stages I and II [16]. In Japan, the 5-year survival rates for FIGO Stages I, II, III, and IV were 84.7, 60.6, 36.3, and 11.1%, respectively. In our study, patients aged less than 35 had a better prognosis within each stage. The FIGO annual report showed that 5-year survival rates for Stages Ib, IIb, IIIb, and IVa patients treated from 1993-1996 were 80.7, 73.3, 46.4 and 29.6%, respectively [19]. In our analysis, 5-year survival rates for Stages IB, IIB, IIIB, and IVA were 90.3, 73.4, 64.3, and 20.0, respectively. Therefore, the present retrospective study indicates that the younger the patient is, the better their prognosis. However, it is doubtful whether younger patients have a better prognosis than older patients. In our cases, young patients tended to be more willing to undergo surgery and combined aggressive treatment. It is generally accepted that radical hysterectomy with lymph node dissection or CCRT should be considered for patients with Stage IB or IIA. It is recommended that patients with Stage IIb and more advanced disease should be treated with CCRT, not radical surgery [20]. In Japan, a report in 2005 demonstrated that the population of primary treatment including surgery was 1,026 of 1,490 (68.9%) for Stages IB_2 and II disease, and 79 of 719 (11%) for Stage III. In our cases aged less than 35, primary treatment including surgery was 64 of 69 (92.8%) patients with Stages IB_2 and II, and six of 11 (54.5%) women for Stage III [21]. While primary treatment without surgery for Stages IB_2 to IVA was 1,156 of 2,366 (48.8%) cases in Japan in 2005, 14 of 87 (16.1%) women underwent radiotherapy or chemotherapy without surgery in our young patients. The 5-year survival rate for Stages IB_2 and II was 78.9% with surgery, but 20.0% without surgery. Therefore, it is possible that the distribution of treatment methods affects the better prognosis observed in our young patients.

Since the US National Cancer Institute alert in 1999 stated that concurrent chemoradiotherapy (CCRT) should be considered for locally advanced cervical carcinoma, it is recommended that patients with Stage IB_2 and greater should be treated with CCRT [20, 22, 23]. Although CCRT has tended to increase, the population of CCRT is only 5-10% for Stage IIB-IVA [20]. On the other hand, in Stage Ib disease, the rate of CCRT has decreased, and NAC plus surgery was significantly increased from 1993-95 compared to that from 1990-92 [20]. Several studies have indicated that NAC has been useful in the control of locally advanced cervical carcinoma [24, 25]. It is thought that the major theoretical advantages of NAC may be to promote the efficacy of surgery on local control by down-staging the disease, and to contribute to micro-metastasis control. We have shown that CDDP-based chemotherapy results in transient increases in apoptosis in locally advanced cervical cancer [26]. We also demonstrated that patients with FIGO IIIB stage cervical carcinoma treated with CPT-11 plus MMC showed remarkable reduction of tumor size, and could successfully undergo radical hysterectomy [27]. In general, the response rate has been reported to be approximately 70-80% for NAC with a CDDP-based regimen [28-30]. Sardi et al. reported that NAC followed by surgery was associated with a significant improvement in 8-year survival rates compared with surgery alone in patients with FIGO Ib disease [28]. Benedetti-Panici et al. demonstrated that FIGO IB_2-IIB patients treated with NAC followed by surgery showed a better prognosis than those with RT alone [29]. Our previous study demonstrated that 5-year survival rates for Stages IIA, IIB, III disease treated with NAC were 100%, 74%, and 75%, respectively [30]. In this study, the 5-year survival for Stages IB_2-IVA was 75.8% in patients treated with NAC and 68.5% without NAC. Other reports, however, have noted that NAC followed by RT for advanced disease did not result in an improvement in the overall survival compared with RT alone [31, 32]. Therefore, the effectiveness of NAC for prolonged survival in patients with locally advanced cervical carcinoma is controversial.

In conclusion, the incidence of invasive cervical carcinoma in young women aged less than 35 has steadily increased from 1990 to 2005, especially in patients with non-SCC. Most of the patients tended to choose radical surgery or combined treatment including surgery. The prognosis was better in young women than in the overall population. The reasons for this better survival may be associated with an earlier diagnosis and aggressive combined treatment with surgery, but additional studies are required to confirm this possibility.

Acknowledgments

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Prediction of suboptimal cytoreduction of epithelial ovarian carcinoma by preoperative computed tomography

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Summary

In an aim to evaluate the diagnostic efficacy of preoperative abdominal-pelvic CT for the prediction of suboptimal cytoreduction of epithelial ovarian carcinoma (EOC) at primary surgery, CT scans of 48 patients who underwent primary surgery for EOC were retrospectively analyzed. The presence of at least one of the following CT findings: multiple implants > 1 cm in maximum diameter in the mesenteria of the small or large intestines, porta hepatis or intersegmental fissure or on the hepatic surface, diaphragmatic peritoneum, gastrohepatic or gastrosplenic ligaments or the extension of tumor infiltration > 2 cm on the omentum towards the spleen or stomach or the intestines encased by the tumor > 2 cm, diffuse peritoneal thickening or invasion of the lateral pelvic wall > 1 cm or multiple lymph nodes > 1 cm at the cardiophrenic and suprarenal levels were accepted as the critical markers for predicting suboptimal cytoreduction. Suboptimal surgery, defined as leaving a residual tumor mass > 1 cm, was determined in 18 (37.5%) patients. CT predicted suboptimal cytoreduction with 83.3% (15/18) sensitivity, 90% (27/30) specificity and 87.5% (42/48) accuracy. PPV and NPV values were 83.3% (15/18) and 90% (27/30), respectively. These results suggested that preoperative CT could successfully predict suboptimal surgery in patients with EOC.

Key words: Ovarian carcinoma; Cytoreduction; Computed tomography.

Introduction

Epithelial ovarian carcinoma (EOC) is the leading cause of death among gynecological malignancies. Although great advances have been achieved in the treatment of human cancer, the mortality rate of ovarian cancer has not improved significantly; 75% of patients are still diagnosed in advanced stages [1-3]. The current mode of treatment of advanced stage ovarian carcinoma includes optimal cytoreduction and surgical staging followed by combined chemotherapy of platinum and taxanes. It is well known that cytoreduction of the tumor is the key factor for an optimal response to chemotherapy and the maximal diameter of residual tumor prior to intiation of chemotherapy is an important determinant of prognosis [4, 5]. Although various sizes have been reported, it is believed that more favorable responses to chemotherapy and prolonged survival are achieved by reduction of the maximal tumor diameter to < 1 cm during surgery and this result is defined as optimal cytoreduction [6-10].

Optimal cytoreduction can not be achieved in all attempts due to disseminated tumor metastasis to life-threatening critical regions, and personal or institutional experiences [7]. Despite aggressive surgical efforts, it has been reported that optimal surgical outcome was not obtained in 10-67% of patients with advanced stage disease [11-13]. It is believed that surgical complications and delayed chemotherapy can result in decreased survival and quality of life in these patients. Neoadjuvant chemotherapy, given prior to aggressive surgery, has been

shown to prevent serious surgical complications and increase the chance of optimal cytoreduction by eliminating, or at least decreasing, the size of the tumor in some critical areas of this subgroup of patients [14, 15]. However, the diagnosis of this subgroup of patients who will benefit from neoadjuvant chemotherapy, before any subobtimal surgical attemts, is anothor challenge. Although, numerous studies have evaluated the efficacy of pelvic-abdominal computed tomography (CT) imaging for the differential diagnosis of pelvic tumors and the extent and localization of metastasis, relatively little data exist regarding its use in predicting the outcome of primary cytoreductive surgery for advanced ovarian carcinoma [7, 8, 16-19].

This study was designed to evaluate the reliability of CT for predicting the optimal cytoreduction of EOC at primary surgery with the collaboration of the Departments of Gynecologic Oncology and Radiology.

Materials and Methods

This retrospective study was carried out collaboratively by the Departments of Radiology and Gynecologic Oncology after approval of the Ethics Board of the Medical Faculty. Forty-eight patients referred to the Gynecologic Oncology Clinic with the finding of a pelvic mass suspicious for ovarian cancer between November 2003 and September 2008 were included in the study. All of the patients were evaluated by abdominal and pelvic CT preoperatively and underwent surgery for staging and cytoreduction.

Preoperative CT scans were obtained with a helical unit (X Vision, Toshiba, Japan), with the imaging parameters of 7 mm collimation, 1:1 pitch, 120 kVp, and 150-300 mA. The imaging fields of all patients covered the total area between the dome of the diaphragm, including the base of the lungs superiorly and

Table 1. — *Fifteen specific areas comprising the CT criteria used for prediction of optimal cytoreduction.*

Peritoneal sites	Nodal sites	Others
1. Hepatic surface	11. Suprarenal	13. Hepatic metastasis
2. Diaphragmatic peritoneum	12. Pericardiac	14. Pulmonary or pleural
3. Porta hepatis		15. Abdominal wall invasion
4. Intersegmental fissure		
5. Extension of infiltration on omentum towards spleen and stomach		
6. Gastrohepatic ligament		
7. Gastrosplenic ligament		
8. Diffuse peritoneal thickening		
9. Mesentery		
10. Intestines encased by tumor		

Table 2. — *Distribution of the patients with regard to histology, grade and stage.*

Parameters	Number of patients	%
Histological type		
Serous	37	77
Mucinous	3	6
Clear cell	2	4
Endometroid	5	10
Undifferentiated	1	2
Tumor Grade		
1	6	12
2	26	54
3	16	33
FIGO Stage		
I	11	23
II	2	7
III	30	62
IV	4	8

the pubic symphysis inferiorly. Oral (50 cc urografin 76% diluted in 1.5 l of water) and intravenous contrast agents (non-ionic contrast agent, either omnipaque 350, iomeron 350, ultravist 370, or xenetix 350, 100 ml) were used in all cases. Intravenous contrast medium was administered after a 70-sec delay via the right or left antecubital vein at a rate of 3 ml/sec using an automatic injector.

Preoperative CT images were evaluated by two radiologists who were blinded to the surgical outcome and the stage of disease. Imaging criteria were derived from the surgical and imaging literature [7, 8, 16-19] and supplemented by personal communication with a gynecologic oncologist. The CT findings accepted as the criteria for prediction of suboptimal cytoreduction are presented in Table 1. According to these criteria the CT findings of multiple implants > 1 cm in maximum diameter in the mesenteria of the small and large intestines, hepatic surface, diaphragmatic peritoneum, porta hepatis, intersegmental fissure, gastrohepatic ligament and gastrosplenic ligament, extension of the tumor infiltration on the omentum towards the spleen and the stomach, multiple intestinal segments encased by the tumor, diffuse peritoneal thickening more than 4 mm of thickness in at least two of the five peritoneal fields including the lateral colic gutter, lateral conal fascia, anterior abdominal wall, diaphragm and pelvic peritoneal surfaces, multiple tumor invasion of the lateral pelvic wall, and multiple lymph nodes > 1 cm at the cardiophrenic and suprarenal levels were all defined as markers for suboptimal surgery. In addition to these findings, tumor involvement of the hepatic parenchyma, tumor nodules in

the lung or pleura and multiple tumor invasion of the abdominal wall were also defined as other markers (Figures 1a, 1b, 1c). Obtaining any one of these defined criteria by CT was accepted as a predictor of suboptimal surgical outcome.

All patients underwent explorative laparotomy within two weeks after CT scan, and surgical staging was performed including total abdominal hysterectomy, unilateral or bilateral oophorectomy, total or partial omentectomy or omental biopsy, paraaortic and pelvic lymph node dissection or sampling, multiple biopsies of suspicious nodules, peritoneal cytology by one of the two gynecologic oncology attending surgeons with maximum effort for excision of the total tumor mass or to decrease to a minimum diameter of < 1 cm. Excision of all visible tumors or the maximal diameter of a residual tumor mass < 1 cm in any critical site were accepted as optimal cytoreduction.

The results of CT findings and the outcome of surgery for each patient were compared, and the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the results of the CT findings for prediction of suboptimal surgery were calculated. Kappa analysis and Fisher's exact test were used to define the correlation between surgical outcome and CT findings.

Results

The median age of 48 patients was 54 years (range 24-78 years). Surgical staging revealed that 13 patients had early-stage disease (surgical stage I or II), while 35 had advanced stage disease (surgical stage III or IV). The histology, grade and surgical stage of the tumors are presented in Table 2. Suboptimal and optimal cytoreduction was achieved in 18 (37.5%) and 30 (62.5%) patients, respectively. When surgical stage and surgical outcome of the patients were compared, it was seen that optimal cytoreduction was achieved in all patients with early-stage disease, while 18 of the patients with advanced stage disease had suboptimal cytoreduction. The sites of residual tumor > 1 cm in patients with suboptimal cytoreductive surgery are shown in Table 3.

Based on the defined imaging criteria obtained by CT scan findings, it was presumed that 18 (37.5%) and 30 (62.5%) of the 48 patients would have optimal and suboptimal cytoreductive surgery, respectively. All 13

Table 3. — *Sites of residual tumor > 1 cm after suboptimal cytoreductive surgery.*

Sites of residual tumor	No. of patients
Hepatic surface	4
Diaphragmatic peritoneum	2
Porta hepatis	1
Intersegmental fissure	4
Omentum	4
Gastrohepatic ligament	3
Gastrosplenic ligament	4
Peritoneal surfaces	5
Mesentery of the small intestines	8
Lymph node at suprarenal level	4
Metastasis to the hepatic parenchyma	1
Pleural and pulmonary fields	2
Serosa of the intestines	2

Fig. 1a

Fig. 1b

Fig. 1c

Figure 1. — Contrast-enhanced CT findings: liver surface and diaphragmatic implants (arrows). (a); omental tumor extension to spleen and implants in the porta hepatis (arrows). (b); implants in the intersegmental fissure (arrow) (c).

patients with early-stage disease who had optimal cytoreduction were correctly predicted by CT criteria. However, all of the six incorrect predictions were observed in the patients with advanced stage disease, including three false-positive and three false-negative results. When compared to outcome of the surgery, it was found that defined imaging criteria of CT findings could predicte optimal cytoreduction with 83.3% (15/18) sensitivity, 90% (27/30) specificity and 87.5% (42/48) accuracy. PPV and NPV values were 83.3% (15/18) and 90% (27/30), respectively. Kappa analysis revealed that there were very high correlations between CT imaging criteria and outcome of the surgery in terms of predicting suboptimal cytoreduction ($\kappa = 0.726$, $Z = 4.925$, $p < 0.001$).

Each of the CT imaging criteria used for predicting suboptimal surgical outcome was tested according the surgical findings of the patients by Fisher's exact test and the results are given in Table 4. The mesenteric tumors observed by CT scan had a statistically significant association with the surgical findings ($p < 0.001$), while intersegmental fissures, gastrosplenic ligaments, diffuse peritoneal thickening, and lymph nodes at the suprarenal level were found to have moderately significant correlations ($p < 0.05$).

Discussion

Ovarian cancers present a great challenge to clinicians in terms of treatment, as most of them are diagnosed in advanced stage disease with a high mortality rate.

Table 4. — *Univariate analysis of CT criteria used for predicting suboptimal cytoreduction.*

CT criteria	No. of patients	p
Implant > 1 cm on the mesentery	9	0.001
Implant > 1 cm within the intersegmental fissure	4	0.019
Implant > 1 cm on the gastrosplenic ligament	4	0.019
Diffuse peritoneal thickening	6	0.019
Lymph node > 1 cm at the suprarenal level	4	0.019
Implant > 1 cm on the gastrohepatic ligament	3	0.054
Pulmonary-pleural nodule > 1 cm	2	0.054
Implant > 1 cm on the hepatic surface	5	0.069
Encasement of the intestines by the tumor and involvement of the serosa	2	0.148
Extension of the omentum towards the spleen-stomach	4	0.284
Implant > 1 cm within the porta hepatis	1	0.391
Hepatic metastasis	1	0.391
Invasion of the abdominal wall	1	0.391
Implant > 1 cm on the diaphragmatic peritoneum	3	0.552
Pericardiac lymph node > 1 cm	2	1

Optimal cytoreduction during primary surgery, which evidently determines the success of adjuvant chemotherapy and survival, is the mainstay in the management of these patients [15]. Suboptimal cytoreduction can not only increase the mortality and morbidity of the patients due to surgery, but also delay the chemotherapy and decrease its effectivenes. Moreover, it was reported that chemotherapy given after suboptimal cytoreduction was no more effective than the chemotherapy given alone without any surgical attempt, as a neoadjuvant agent for palliation [20]. Meantime, it has been emphasized that

Fig. 2
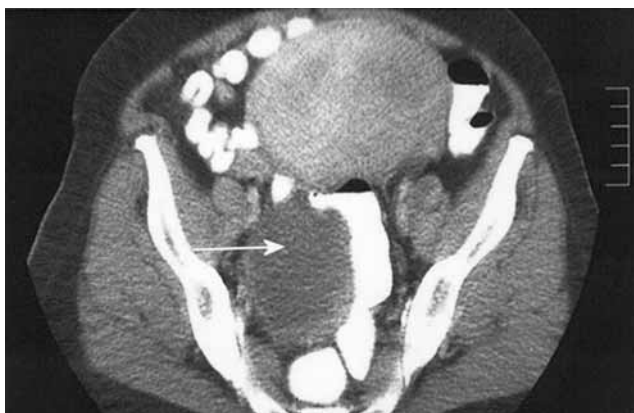
Fig. 3

Fig. 4
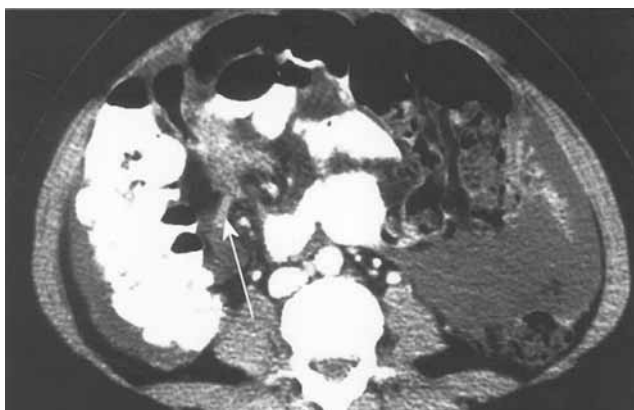
Figure 2. — Contrast-enhanced CT shows pelvic mass surrounding sigmoid colon (arrow). Optimal resection was not achieved due to involvement of the sigmoid colon serosa at surgery.

Figure 3. — Contrast-enhanced CT shows ovarian mass including massive calcification (arrow). Optimal resection was not achieved due to invasion of the lateral wall of the pelvis at surgery.

Figure 4. — Contrast-enhanced CT shows multiple implants within small bowel mesentery (arrow). At surgery mesenteric involvement could not be confirmed. Again examination demonstrated that the implants were inadequate for small bowel opacification.

success of a surgical effort for achieving optimal cytoreduction could increase when performed after neoadjuvant chemotherapy as an interval surgery [21, 22].

Several studies in the literature have attempted to define a preoperative imaging method which accurately predicted the surgical outcome of patients with advanced stage ovarian cancer and prevented suboptimal cytoreduction [8]. Nelson *et al*. first investigated the efficacy of preoperative CT for prediction of the surgical outcome of patients with epithelial ovarian cancer by using eight radiographic criteria of "inability to perform optimal cytoreduction" to < 2 cm residual disease in 1993 [17]. Meyer *et al*. employed a simple scoring system of five anatomic disease sites to predict surgical outcome and reported a diagnostic efficacy ranging from 58% to 100% in a retrospective study including 28 patients with ovarian carcinoma [16]. Bristow *et al*. reported that suboptimal surgery with residual tumor > 1 cm could be predicted with a sensitivity, specificity and accuracy of 100%, 85% and 92.7%, respectively, by using a very complex CT scoring system [8]. Byrom *et al*. proposed a simple method that could be used in clinical practice whereby residual disease < 0.5 cm was considered optimal cytoreduction and found a sensitivity and PPV of 88% and 85%, respectively [19]. However, most patients included in this study had benign or early-stage diseases. Other studies, using similar imaging methods also reported a diagnostic efficacy ranging from 52% to 99% for prediction of residual disease ranging from > 1 cm to > 2 cm [7, 18].

In this study, a simple method was defined by evaluating preoperative abdominal-pelvic CT images and observing multiple tumor nodules > 1 cm in the 15 critical areas including peritoneal and nodal areas, and hepatic, pulmonary and abdominal wall metastasis were used for prediction of suboptimal cytoreduction with residual tumor > 1 cm. Fifteen of the 18 patients who had suboptimal surgical cytoreduction were successfully predicted by CT with a sensitivity of 83.3%. There were three false-negative results in which complete resection was not possible at surgery while CT had predicted complete resectability. One of these patients had invasion of the lateral wall of the pelvis (Figure 2), another had residual tumor > 1 cm due to involvement of the sigmoid colon serosa (Figure 3), one had tumor deposits > 1 cm on the mesentery and involvement of the abdominal and pelvic parietal peritoneum thicker than 4 mm. Among the 30 patients who achieved optimal surgical cytoreduction, 27 were correctly predicted by CT with a specificity of 90%. The accuracy rate of the defined method was calculated to be 87.5%. There were three false-positive CT imaging results including one patient with omental infiltration to the spleen and mesenteric tumor nodules > 1 cm (Figure 4), two patients with tumor nodules > 1 cm on the diaphragmatic peritoneum and hepatic surface or pericardial lymph node > 1 cm. Omental infiltration to the spleen was resected completely by total omentectomy and splenectomy, however, mesenteric tumor nodules could not be verified surgically. Two of the false-negative

results with mesenteric nodules were due to inadequate small bowel opacification, as these patients with Stage IV disease and terminal health status could not take oral contrast agent before the CT scan. For the other false-negative results with the peritoneum, hepatic surface or pericardiac nodal metastasis, pelvic and serosal involvement were believed to be due to the CT technique used in the study and could be eliminated by a thin-section CT scan.

Considering that all patients with early-stage disease achieved optimal cytoreduction, the potential of CT to predict suboptimal surgery has practical importance only in patients with advanced stage disease. Of the 35 patients with advanced-stage disease, 18 had suboptimal and 17 had optimal surgical cytoreduction. Although, all false-negative results were obtained in advanced stage disease, CT was able to predict the outcome correctly in 15 of the 18 patients with suboptimal and 14 of the 17 patients with optimal cytoreduction. Based on these data, sensitivity, specificity and accuracy rates were calculated as 83.3%, 82.3%, and 82.8% respectively. Kappa analysis revealed a very high level of agreement between the success of CT to predict surgical and prognostic outcomes in patients with advanced-stage disease ($\kappa = 0.749$, $Z = 4.237$, $p < 0.001$).

When the significance of the predictors of suboptimal disease were explored, omental and mesenteric diseases, diffuse peritoneal thickening, and diaphragmatic disease and large intestine mesenteric implants were defined as the most significant predictors of suboptimal disease [7, 19, 24]. This study also revealed that the mesenteric disease > 1 cm was the most significant parameter for predicting suboptimal surgery and when obtained preoperatively, the risk of suboptimal cytoreduction increased by 12-fold.

One of the main difficulties in improving any one of the acceptable models that predict surgical outcome in patients with advanced-stage ovarian cancer is the personal philosophy and diligence of the surgeon in achieving maximal cytoreduction and his/her ability to perform advanced surgical techniques [23]. The success rate of primary cytoreduction in advanced-stage ovarian cancer is considerably variable and depends on personal and institutional treatment philosophy and experience. The optimal cytoreduction ratio is in the range of 33% to 90% in centers with expertise in cytoreductive surgery [11, 12]. The rate of optimal cytoreduction in this series of patients (48.5%) was lower than that mentioned in the literature, as most of the patients with advanced disease were reluctant to have organ resection and colostomy or urinary diversion, and did not give informed consent for these procedures due to their cultural and religious beliefs [7, 24].

Conclusion

The results of this study suggest that CT could be used successfully for predicting suboptimal cytoreduction in primary ovarian cancer patients. However, preoperative CT criteria used for predicting surgical outcome might be rearranged for different institutions, as the result of surgical effort mostly depends on the surgical experience and philosophy of each institution and the desire of the patients of that society.

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Analysis of odds ratio of increased relative risk of developing breast cancer in different groups of women

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Summary

Taking into account the large number and variety of factors of breast cancer there is constant need and necessity to monitor the risk of developing the disease. It is important to take preventive actions - health education concerning lifestyle and possible ways to modify unhealthy aspects. Quantitive assessment of risk of developing invasive breast carcinoma can be performed using the Gail model (GM). This method is designed to estimate relative and cumulative risk during the entire lifetime or at a certain age of a patient, considering risk factors. It is possible to identify women with increased risk of breast carcinoma and to choose a proper diagnostic path. The purpose of this study was to estimate the relative risk (RR) and to analyze the odds ratio (OR) of increased risk of developing breast cancer. The participants in the study were healthy women with no focal changes in mammary glands and women with diagnosed malignant or benign breast neoplasms. The total number of participants was 555 females aged 35-70 years. The study was carried on in the Great Poland and Lubuskie provinces between 2005 and 2006. High 5-year relative risk of developing breast cancer assessed by the Gail method, proved that this method was a useful tool in confronting reality. In classification of women to a group of increased risk of breast carcinoma, apart from assessment by the Gail method, factors like: BMI, education, medical interventions in puerperium and number of cases of familial invasive cancers should be taken into account.

Key words: Breast cancer; Risk factors.

Introduction

Taking into account a large number and variety of factors related to breast cancer there is constant need and necessity to monitor the risk of developing the disease.

According to Colditz *et al*. [1] identification and elimination of risk factors can significantly (up to 50%) reduce the morbidity of breast cancer.

It is necessary to take preventive action - health education concerning lifestyle and possible ways to modify it. Cancer preventive programs are also very crucial. Their aim is early diagnosis and as a result more effective and less deforming treatment can be achieved. Early diagnosis including regular self-examination, breast examination performed by general practitioners or specialists, and easy access to mammography is of great importance. A critical issue is to make patients conscious of the real threat of the disease [2].

Unfortunately in the Polish literature neither data concerning the number of women with increased risk of breast cancer nor epidemiological data are available.

Quantitive assessment of risk of developing invasive breast carcinoma can be performed using the Gail model (GM). This method is designed to estimate relative and cumulative risk throughout life or at a certain age of a patient, considering risk factors. It makes it possible to identify women with increased risk of breast carcinoma and to choose the proper diagnostic path.

Material and Methods

The participants of the study were healthy women with no focal changes in mammary glands and women with diagnosed malignant or benign breast neoplasms. The total number of participants was 555 females aged 35-70 years. The study was carried on in Great Poland and Lubuskie province between 2005 and 2006.

The inclusion criteria for the first group (healthy; BZ) (n = 292) was an examination performed by a specialist which revealed no pathological changes and normal mammography and/or ultrasound (US) examination.

The second and third group consisted of patients who were according to histo-pathological examination of material gained by breast biopsy or operation: benign changes (D; n = 184) and malignant lesions (CA; n = 79).

Every patient voluntarily filled out an anonymous questionnaire consisting of 43 questions about socioeconomic conditions, menstrual and obstetric history, breastfeeding, puerperium and hereditary transmission. They also answered questions concerning their life-style and healthy behaviour (breast self-examination, attitude towards these exams, dietary habits, alcohol consumption and physical activity).

For every patient we estimated the risk of breast cancer using the Gail method. Two aspects of risk were considered: relative risk (RR) and prediction of absolute risk. RR is the ratio of risk of developing breast cancer in relation to age in women with risk factors compared to the risk of women of the same age but without risk factors.

Absolute risk is the probability that women with specified risk factors will develop breast cancer in a specified age range.

The Gail model takes into account factors like patient's current age, age at menarche, age of first live birth, number of previous breast biopsies, atypical hyperplasia, the number of first-degree relatives with breast cancer and race. On the basis

of these risk factors it is possible to calculate risk of developing breast cancer. To calculate relative and absolute risk an interactive computer program was used. A Gail score > 1 was estimated as the increased risk of developing breast cancer.

For each risk factor the odds the ratio (OR) was calculated.

Risk factor	Present	Absent	Total
Study group	a	b	a+b
Control group	c	d	c+d
Total	a+c	b+d	a+b+c+d

We assessed odds of developing breast cancer in cases with presence of a risk factor:

$$\text{Odds}^{yes} = \frac{\dfrac{a}{a+c}}{1 - \dfrac{a}{a+c}}$$

and when a risk factor was not present:

$$\text{Odds}^{no} = \frac{\dfrac{b}{b+d}}{1 - \dfrac{b}{b+d}}$$

We calculated OR with a 95% confidence interval (CI).

$$OR = \frac{a*d}{c*b}$$

Statistical analysis was performed using StatSoft, Inc. (2005), STATISTICA (data analysis software system), v 7.1 and Cytel Studio v 7.0.0 (2005).

For the study approval of the Bioethical Commision of K. Marcinkowski University in Poznan was obtained.

Results

Relative risk of developing breast cancer assessed by the Gail method

On the basis of the Gail method, taking into account risk factors, we estimated individual five-year risk of developing breast cancer for each patient as well as five-year risk for a population of the same age but without risk factors. The results of both groups (D - with benign changes and BZ - no changes in breast) were as follows: mean value of individual 5-year risk in group D was 1.2 and in group BZ - 0.7. The differences between these groups were statistically significant ($p < 0.001$). Estimated mean value of individual five-year risk for patients with malignant breast lesions (CA) was the highest of the three studied groups - 3.28. Mean values of five-year risk for the healthy population were: group D - 0.6 and group BZ - 0.5. The differences between groups were statistically significant ($p < 0.001$).

The above-mentioned results together with minimum and maximum values and median are presented in Tables 1 and 2.

OR of an increase in RR of development of breast carcinoma.

Among patients in the BZ group, 22.6% of women were at increased risk of developing breast cancer (RR

Table 1. — *Mean values of 5-year relative risk for studied groups.*

Groups	Mean ± SD	Range (min-max)	ANOVA $p < 0.05$
CA - group with breast cancer	3.28 ± 1.88	0.60-10.8	CA vs BZ
D - group with benign breast tumors	1.2 ± 0.9	0.1-7.4	D vs BZ
BZ - group with no changes	0.7 ± 0.5	0.1-4.7	

Table 2. — *Mean values of 5-year relative risk for the healthy population.*

Groups	Mean ± SD	Range (min-max)	ANOVA $p < 0.05$
D - group with benign breast tumors	0.6 ± 0.2	0.1-1.4	D vs BZ
BZ - group with no changes	0.5 ± 0.2	0.1-1.1	

> 1 estimated according to the Gail method); for the remaining 77.4% in this group the risk was decreased (RR ≤ 1). We analyzed risk factors in both subgroups, and parameters with statistically significant differences are shown in Table 3.

Table 3. — *Risk factors with statistically significant differences in the BZ group in both subgroups with increased and decreased risks estimated by the Gail method.*

Parameter	p value
Age	0.001
BMI	0.03
Age at menarche	0.009
Age of first pregnancy	0.001
Number of deliveries	0.003
Age at first delivery	0.001
Fears concerning cancer	0.04
Familial cancer history	0.0003

BMI: Body mass index.

Among patients with neoplastic changes in the breast, 97.37% were at increased risk (RR > 1 estimated according to the Gail method) while in one patient (2.67%) risk was decreased.

In the BZ group we also assessed the OR of increased risk of developing breast cancer for chosen risk factors.

OR according to BMI

Odds ratio of an increased RR of developing breast cancer for women with a BMI ≥ 25 was OR = 2.094; 95% CI 1.16-3.77 in comparison to patients with a BMI within normal ranges (18.5-24.9).

OR according to menarche

Odds ratio of an increased RR of developing breast cancer for women who had menarche at the age of 12 was OR = 0.20; in comparison to those who experienced menarche at the age of 11; and at the age of 13, 14 and > 14 - OR = 0.16, OR = 0.13 and OR = 0.12, respectively (Table 4).

Table 4. — *OR according to age at menarche in patients with increased and decreased risk in the BZ group.*

Age at menarche	OR	CI 95%
12 years	0.20	0.05 - 0.83
13 years	0.16	0.04 - 0.62
14 years	0.13	0.03 - 0.50
> 14 years	0.12	0.02 - 0.49

OR according to education

Odds ratio of an increased relative risk of developing breast cancer for women with a secondary education was OR = 1.09; and for those with a university degree it was OR = 0.49, in comparison to women with only a technical education (Table 5).

Table 5. — *OR according to education for women with increased and decreased risk in BZ group.*

Education	OR	CI 95%
Secondary	1.09	0.57 - 2.06
University	0.49	0.24 - 1.00

OR for medical interventions in puerperium

Odds ratio of increased relative risk of developing breast cancer for women who had problems with lactation during puerperium requiring medical intervention was OR = 2.16 (95% CI 1.02-4.55), in comparison to those who did not experience problems with breastfeeding.

OR for positive familial cancer history

Odds ratio of increased relative risk of developing breast cancer for women who had one first-degree relative with cancer was OR = 1.47. When cancer occurred in more than one first-degree relative OR was 6.0 in comparison to women who had no cancer history in first-degree family members (Table 6).

Table 6. — *OR according to the number of cancers for women with increased and decreased risk in the BZ group.*

Number of familial cancers	OR	CI 95%
1	1.47	0.78 - 2.77
> 1	6.0	2.49 - 14.44

Discussion

In spite of the fact that breast cancer often seems to attack at random, for many years factors influencing this situation have been discovered. The majority of cases of breast cancer are probably connected with environmental factors and lifestyle. Studies carried out in the last three decades have made it possible to estimate the risk of developing breast cancer [3]. According to current knowledge, modification of risk factors may contribute to a reduction in breast cancer. Individual assessment performed by selecting a group of women with increased risk can help reduce mortality [4, 5].

Numerous clinical control and cohort studies have shown that obesity may increase the risk of developing breast cancer [6-8].

It has been confirmed in our studies that the majority of women suffering from breast carcinoma were characterized by increased BMI. We also observed a relation between increased BMI and risk of developing breast cancer in patients who were, according to the Gail method, classified to a group of patients with decreased risk (RR < 1).

In retrospective studies a strong positive correlation between BMI and development of breast cancer in postmenopausal women has been reported [9, 10]. According to epidemiological data concerning the Italian population, 20% of breast cancer in postmenopausal women and 27% in those older than 70 is due to overweight and obesity [11].

Time of exposure of breasts to ovarian hormones is considered as one of the more important and maybe even the most crucial factor among all known risk factors of breast cancer. A longer time of breast exposure to estrogens could be natural and result from early menarche and late menopause or could be caused by using contraceptive drugs or hormonal replacement therapy in the postmenopausal period.

According to Mazurkiewicz [12] the risk of breast cancer is three times greater in women who had menarche before they were 11 in comparison to those who experienced late onset – after the age of 16. Our results show that the OR of an increased RR of breast cancer significantly decreased in patients who had menarche after age 14 in comparison to patients who had menarche at the age of 11.

According to Budner *et al*. [13] relative risk for the age of menarche was RR = 1.2-1.5 for women with menarche before 12 years of age in comparison to women who experienced it after 12 years of age. The period between menarche and the first delivery also plays a crucial role. The shorter this time is the risk of breast cancer decreases. It is connected with the number of menstrual cycles within that period and shorter or longer exposure to carcinogens [14-16].

Godlewski [15], Tavani *et al*. [16] and Becher *et al*. [17], are of the opinion that the first delivery at earlier age and higher number of deliveries are protective factors against breast cancer. They also consider long-term breastfeeding to be protective.

Jernstromi *et al*. [18] revealed that the length of breastfeeding was connected with risk reduction and that for each month OR was 0.98 (95% CI 0.97-0.99). They also found that breastfeeding was protective and decreased risk among patients – carriers of mutated BRCA1 genes. In this group OR decreased in patients who breastfed longer that 12 months in comparison to those who did not breastfeed (OR = 0.55; 95% CI 0.38 - 0.80).

In our study we also calculated OR of breast cancer in women who during lactation experienced problems in which medical intervention was necessary. In this case OR was 2.25 (95% CI 1.20-4.19) in comparison to

women who had no such problems. OR concerning this parameter was significantly increased (more than two times greater) in patients who were classified to a group of decreased risk according to the Gail method (OR = 2.16; 95% CI 1.02-4.55). Although Gail did not take this parameter into account, our results suggest that medical intervention may be of great significance in selecting a group with increased risk, at least for breastfeeding women.

Women's education as well plays an important role. In the study of Graj and Grodecka-Gazdecka [19], among women suffering from breast cancer, the most numerous group had a secondary education (48.4%) while a university degree and technical education were 25.2% and 26.5%, respectively. In our study we observed a similar finding: 55.7% of patients had a secondary education, 21.52% a university degree and 22.78% a technical education. Nevertheless, after analyzing the OR of increased RR of breast cancer we found an opposite tendency in women with a university degree (OR = 0.49; 95% CI 0.24-1) in comparison to women with a technical education – which shows that better educated patients are less likely to have increased risk of breast cancer.

Studies carried out in highly developed countries reveal that breast cancer occurs more frequently in well-educated women – inhabitants of big cities. It could be connected with lifestyle which predisposes to breast cancer development (late first delivery, earlier menarche, childlessness, fewer pregnancies) or with higher health awareness and responsiveness to control examinations resulting in earlier diagnosis when the cancer is in early stage [15].

Hereditary transmission, especially when the mother and sister suffered from breast cancer, is a very crucial risk factor. In this group risk increases almost 14 times [20].

According to Budner and Przybylski [21] RR of developing breast cancer for women with first degree hereditary transmission is RR = 1.4-13.6 and second degree – RR = 1.5-2.0. Familial occurrence of breast cancer may be connected with similar lifestyle or have a genetic basis; 5-10% of all breast neoplasms are hereditarily predisposed. They are the result of impairment in BRCA1 and BRCA2 genes, which are also responsible for hereditary occurrence of ovarian cancer.

The Gail model is based on non-genetic factors and the majority of these result in development of invasive breast cancer, whose basis is endocrinological. Although in the Gail model the number of first-degree relatives suffering from cancer is taken into account, it considers neither the age of diagnosis nor concomitant cancers (e.g., ovarian cancer – its presence increases the possibility of discovering mutations in BRCA1 and BRCA2 genes). In our study we analyzed OR of increased RR of developing breast cancer in relation to familial cancer history (not only breast cancer) in a group of patients classified by the Gail method to a group at decreased risk (RR < 1). Odds ratio for women with one first-degree relative who suffered from cancer (breast, ovarian, colorectal or other) was: OR = 1.47 (95% CI 0.78-2.77) and if more than one

first-degree relative – OR = 6.0 (95%CI 2,49-14,14). This would make it necessary to add to the Gail model questions concerning the number and type of cancers in a patient's family to assess relative risk more precisely.

Ostrowska *et al*. [22] revealed similar results – occurrence of breast, ovarian and other cancers together significantly differentiated compared groups. When analyzing only breast cancers no statistically significant difference among study groups was observed in their study.

It seems that recognition of intensity of individual risk factors of developing breast cancer makes it possible to prepare more effective educational and preventive programs as well as to spend money for prevention in a more rational way.

Conclusions

– High 5-year relative risk of developing breast cancer assessed by the Gail method proved that this method is a useful tool in confronting reality.

– In the classification of women to a group at increased risk of breast carcinoma, apart from assessment by the Gail method, factors like BMI, education, medical interventions in puerperium and number of cases of invasive familial cancers should be taken into account.

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Analysis of protein profiles in human epithelial ovarian cancer tissues by proteomic technology

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Summary

Background: Screening in ovarian cancer is progressively finding out candidate genes and proteins which may work as screening biomarkers and play a role in tumor progression. We examined the protein expression patterns of ovarian cancer tissues using two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization-time of fight mass spectrometry (MALDI-TOF MS). *Methods:* Tissues from 36 ovarian cancers and 20 normal ovaries were examined by 2-DE. The images of silver stained gels were analyzed by ImageMaster 2D Elite. The peptide mixtures, after in-gel digestion, were determined by MALDI-TOF MS for fingerprinting. The de-isotope tryptic peptide profiles were matched by using the Mascot search engine based on the entire NCBI and Swiss-Prot protein databases. Western/dot blots were then applied to verify the findings. *Results:* In ovarian cancer, 12 proteins that showed differential expressions were identified unequivocally. Among these proteins, five proteins (galectin-1, cathepsin B, ubiquitin carboxy-terminal hydrolase L1, HLA class II antigen DRB1-11 and heat shock protein 27) were up-regulated and seven proteins (cellular retinol-binding protein, transthyretin, SH3 binding glutamic-rich-like protein, tubulin-specific chaperone A, DJ-1, gamma-actin and tropomyosin 4) were down-regulated. *Conclusion:* The present study is the first to report the up-regulation of ubiquitin carboxy-terminal hydrolase L1 and the down-regulation of SH3 binding glutamic-rich-like protein, tubulin-specific chaperone A, and tropomyosin 4 in human ovarian cancer tissues. Further cloning and functional analysis of these salient proteins will provide more information on their pathophysiologic roles in ovarian cancer.

Key words: Ovarian cancer; Proteomics; Two-dimensional gel electrophoresis; Matrix-assisted laser desorption/ionization-time of fight mass spectrometry.

Introduction

Ovarian cancer is the most lethal gynecological malignancy with a 5-year survival rate of about 30% [1]. The high death rate is due to late stage of presentation and lack of reliable biomarkers for detection of early-stage cases. CA-125, the currently best characterized serum marker for epithelial ovarian cancer, lacks the sensitivity for detecting early-stage disease, as only 50% of early-stage cases have elevated serum levels [2, 3].

Based on advances in automation and bioinformatics, a new discipline of biology, proteomics, has emerged. Proteomics refers to the study of all the protein forms expressed within an organism as a function of time, age, state, external factors, etc. [4, 5]. From the biomedical standpoint, the field of proteomics has a greater potential because the identified proteins are the biological end-products [6]. Recently, proteomic approaches to identify tumor markers of ovarian cancer are undergoing. Alaiya and co-workers reported the protein expression patterns in primary carcinoma of the ovary [7]. Another published study

reported the proteins that were differentially expressed between benign, malignant, and normal ovaries [8].

Despite enormous efforts, relevant markers useful for screening have not been established in ovarian cancer. In this study, by using two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization-time of fight mass spectrometry (MALDI-TOF MS), we examined the protein expression profile difference in human ovarian cancer tissues and normal ovaries.

Materials and Methods

Collection of tissue samples

Tissue specimens were obtained during the surgery from the National Taiwan University Hospital (NTUH) according to the standard procedures. Ovarian tissues from cases of uterine myoma were used as normal controls. The Ethics committee of the NTUH approved the study protocol and all subjects gave informed consent before participation in the study. Each case of ovarian cancer was also evaluated by H&E stain for pathological parameters including histologic subtype, grade, lymph node metastatic status, and clinical stage. Histologic grades of ovarian cancer included well, moderately, and poorly differentiated. The tissue specimens obtained at surgery were put in liquid nitrogen and stored at –70°C until use.

2-DE analysis

Ovarian tissue fragments were ground to powder in liquid nitrogen. Sample powder was extracted by PBS buffer containing protease inhibitor. After centrifuge the supernatant was precipitated by adding TCA to final 5%. The pellet was resolved by 8 M urea and 0.1 M DTT. Total 450 µg protein sample was applied to the 2-DE assay, then was rehydrated using Immobiline DryStrip pH 4-7, 13 cm (Amersham Pharmacia Biotech) in strip holder for the first-dimension isoelectric focusing (IEF) overnight at room temperature (RT). IEF was performed using the Multiphor II electrophoresis system. Following rehydration, the strips were focused at: 400V, 1 h; 400V~3500V (gradient) 1.5 h, final 3500V for a total of 70 kVh. Then, strips were equilibrated for 15 min in 1% (w/v) DTT buffer containing 0.05 M Tris-HCl (pH 8.8), 6 M urea, 2% (w/v) SDS, and 30% (v/v) glycerol, and then re-equilibrated for 15 min in the same equilibration buffer containing 2.5% (w/v) iodoacetamide in place of DTT. In the second-dimension separation, the proteins were separated in 12.5% polyacrylamide gel by 20 mA constant current and running buffer containing 0.025 M Tris pH 8.8, 0.192 M glycine and 0.1% SDS, and stained with silver staining [9].

The image from Silver stained gel was scanned with ScanMaker-8700 (Microtek, Hsinchu, Taiwan) and analyzed with the ImageMaster™ 2D Elite (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

MALDI-TOF MS analysis

The spots of interest were cut from the gel and washed with ddH2O before de-staining with 0.025 M ammonium bicarbonate/50% acetonitrile (ACN). The protein in the gel spot was digested overnight by trypsin at 37°C, and the proteolytic peptide fragments were extracted with 1% trifluoroacetic acid (TFA)/50% ACN. After lyophilized, the extracted peptides were dissolved in 30% ACN.

The digests were mixed with α-cyano-4-hydroxycinnamic acid solution (concentration: 50 nmol/µl) in acetonitrile/H_2O and spotted onto a MALDI sample plate. The MALDI-TOF MS analysis was performed on an Autoflex® workstation (Bruker Daltonics, Bremen, Germany) equipped with a 337 nm nitrogen laser. The peptide spectra, acquired in reflectron mode at an accelerating voltage of 20 kV, were the sum of 50 laser shots. The mass spectra were externally calibrated using low mass peptide standards. This procedure typically results in mass accuracies of 50-100 ppm. The de-isotope tryptic peptide fragments were used for protein identification through the Mascot search engine based on the peptide mass fingerprinting of SwissProt protein databases.

SDS-PAGE and Western/Dot blotting analysis

For SDS-PAGE, 30 µg protein was applied to each lane. All samples were heated for 5 min at 95°C before loading to the 15% acrylamide gel. After electrophoresis proteins were electroblotted onto polyvinylidene difluoride (PVDF) membranes. For dot blotting, 5 µg protein was loaded onto PVDF membranes. The membranes were treated with blocking reagent (5% nonfat dried milk, 2% Tween 20, 1 x PBS) for 1 h at RT. The membranes were then probed with anti-retinol binding protein antibody (USBiological, Cat# R1701-16), anti-cathepsin B antibody (USBiological, Cat# C2097-03D), and anti-galectin-1 antibody (Novocastra, Cat# NCL-GAL1) in blocking solution for 2 h at RT. After washing with PBST (0.05% Tween 20, 1 x PBS), the membranes were incubated with horseradish peroxidase-conjugated anti-immunoglobulin antibody in blocking

reagent for 1 h at RT. After additional washing with PBST, signals were developed by Western Lightning Chemiluminescence Reagent Plus (PerkinElmer). Signal intensities were scanned with UMAX Astra 4000U. The images were analyzed by GenePix 6.0 for dot blotting, and Fujifilm Science Lab 98 (Image Gauge V3.12) for Western blotting.

Biochemical function and pathway analysis

The PathwayAssist™ software (Strategene, La Jolla, CA, USA) was used to identify functional interrelationships among the protein analyzed in the present study. This software uses the KEGG, DIP and BIND database and natural language scans of Medline to define functional related genes or protein.

Results

A total of 56 tissue specimens were analyzed, which comprised 36 epithelial ovarian cancers and 20 normal ovaries. The clinical and histologic characteristics of the 36 ovarian cancers are summarized in Table 1. There

Table 1. — *Clinical and histologic characteristics of ovarian cancer tissue samples.*

Serial number	Age	Histologic type	Stage	Grade of differentiation*
1	43	Clear cell carcinoma	Ia	3
2	45	Clear cell carcinoma	Ia	3
3	48	Clear cell carcinoma	Ia	3
4	43	Clear cell carcinoma	Ib	3
5	52	Clear cell carcinoma	Ic	3
6	48	Endometrioid adenocarcinoma	Ia	1
7	81	Endometrioid adenocarcinoma	Ia	1
8	46	Endometrioid adenocarcinoma	Ic	1
9	48	Mucinous cystadenocarcinoma	Ia	1
10	36	Mucinous cystadenocarcinoma	Ia	2
11	65	Serous papillary adenoarcinoma	II	2
12	41	Serous cyadenocarcinoma	IIa	3
13	56	Serous adenocarcinoma	IIa	3
14	56	Serous carcinoma	IIa	2
15	70	Serous cystadenocarcinoma	IIb	2
16	59	Endometrioid adenocarcinoma	IIa	1
17	62	Serous cystadenocarcinoma	III	3
18	54	Serous papillary adenoarcinoma	III	3
19	61	Squamous cell carcinoma	IIIa	3
20	58	Clear cell carcinoma	IIIc	3
21	60	Clear cell carcinoma	IIIc	3
22	46	Endometrioid adenocarcinoma	IIIb	1
23	44	Endometrioid adenocarcinoma	IIIc	3
24	56	Serous carcinoma	IIIb	3
25	61	Serous cystadenocarcinoma	IIIc	2
26	70	Serous papillary adenoarcinoma	IIIb	2
27	78	Serous papillary adenoarcinoma	IIIb	3
28	42	Serous papillary adenoarcinoma	IIIc	2
29	70	Serous papillary adenoarcinoma	IIIc	2
30	46	Serous papillary adenoarcinoma	IIIc	2
31	82	Serous papillary adenoarcinoma	IIIc	3
32	72	Serous papillary adenoarcinoma	IIIc	3
33	71	Serous surface papillary adenocarcinoma	IIIb	2
34	59	Serous surface papillary adenocarcinoma	IIIc	3
35	47	Mixed adenocarcinoma (Endometrioid & Serous)	IV	3
36	51	Serous cystadenocarcinoma	IV	3

* Differentiation: Grade 1, well differentiated; Grade 2, moderate differentiation; Grade 3, poor differentiation.

(A) Nov-15
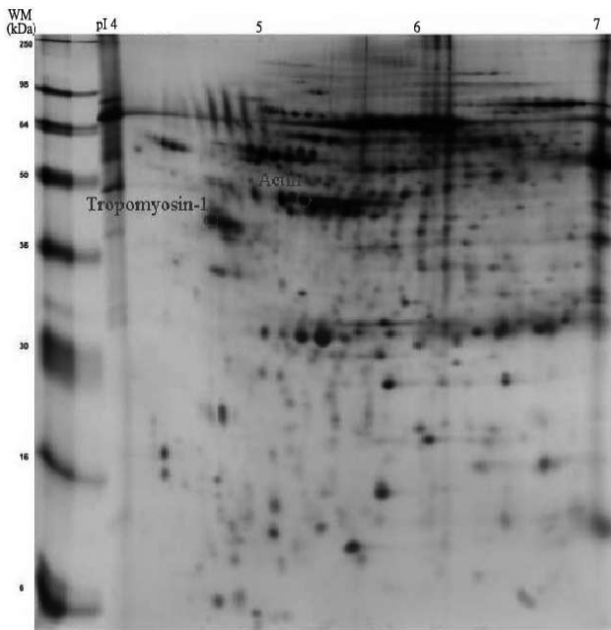
(B) Ov-ca 119
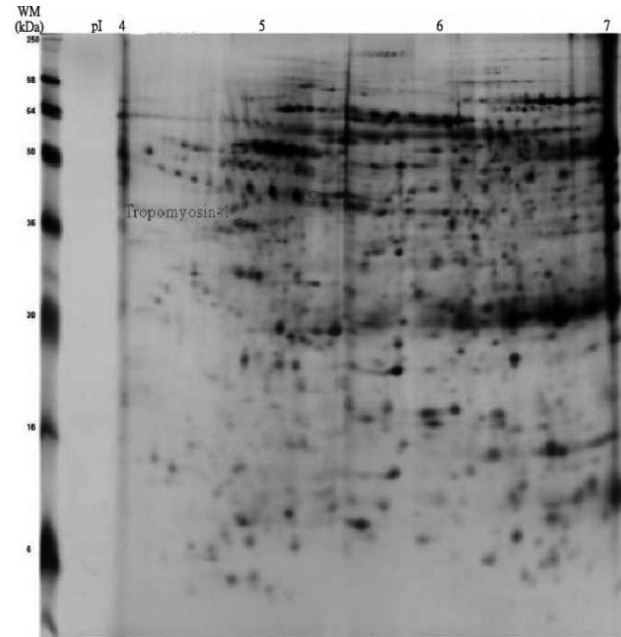
Figure 1. — Representative 2-DE maps.
(A) Protein profile from normal ovarian tissue.

(B) Protein profile from ovarian cancer tissue.
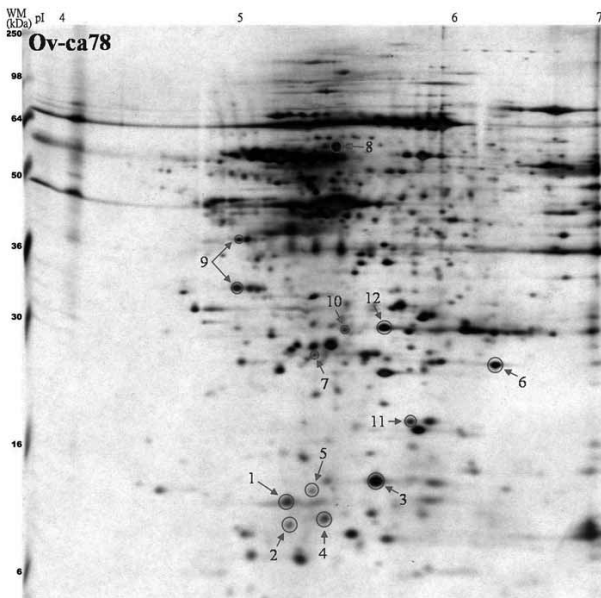
Figure 2. — Locations of several interesting protein spots showing differential expression.
1. CRBP; 2. Gal-1; 3. TTR; 4. SH3BGRL; 5. TCA; 6. DJ-1; 7. CathB; 8. GA; 9. TPM4; 10. UCH-L1; 11. HLA-DRB; 12. HSP27.

were ten cancer tissue samples in Stage I, six in Stage II, 18 in Stage III, and two in Stage IV. Protein extracts from normal ovaries and ovarian cancers were separated on SDS-PAGE before 2-DE. The total amount and quality of protein extracts are further verified by Western blot with

mouse anti-actin monoclonal antibody. Representative 2-DE maps from normal and malignant ovarian tissues are shown in Figure 1.

Down- or up-regulated protein expression between normal ovaries and ovarian cancer were evaluated using ImageMaster™ 2D Elite. In comparison with the 2D Elite software quantification, the spots which showed more than a 20% increase or decrease in intensity as compared with a normal ovary were defined as up- or down-regulated spots. According to this definition, more than 30 protein spots showing differential expression were observed in 2-DE maps. Among the protein candidates, 12 protein spots were selected and identified unequivocally. Locations of the 12 interesting protein spots are shown in Figure 2. Characterization of these protein spots is listed in Table 2. Representative expression of protein spots in 2-DE among normal and malignant ovarian tissues are demonstrated in Figure 3. Signal intensities of differential expression of protein spots in 2-DE maps analyzed by ImageMaster™ 2D Elite are shown in Figure 4. In ovarian cancer, up-regulated spots are galectin-1 (Gal-1), cathepsin B (CathB), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), HLA class II antigen DRB1-11 (HLA-DRB), and heat shock protein 27 (HSP27). Down-regulated spots were cellular retinol-binding protein (CRBP), transthyretin (TTR), SH3 binding glutamic-rich-like protein (SH3BGRL), tubulin-specific chaperone A (TCA), protein DJ-1, gamma-actin (GA) and tropomyosin 4 (TPM4). The differences in signal intensities were statistically significant (p value < 0.05) between cancerous and normal ovaries in the

Table 2. — *Identification of protein spots demonstrating differential expression in 2-DE among normal and malignant ovarian tissues.*

Spot no.	Accession number	Protein description	Score	Coverage (%)	MV / PI*
1	P09455	Cellular retinol-binding protein	577	58	15709/4.99
2	P09382	Galectin-1	157	34	14575/5.34
3	P02766	Transthyretin	854	73	15877/5.52
4	O75368	SH3 binding glutamic-rich-like protein	205	34	12766/5.22
5	O75347	Tubulin-specific chaperone A	264	43	12716/5.25
6	Q99497	Protein DJ-1	447	57	19834/6.33
7	P07858	Cathepsin B	110	13	37797/5.88
8	Q5U032	Gamma-actin	117	5	41766/5.31
9	P67936	Tropomyosin 4	808	59	28373/4.67
10	P09936	Ubiquitin carboxyl-terminal hydrolase L1	416	46	24808/5.33
11	P20039	HLA class II antigen, DRB1-11 beta chain precursor	54	3	30141/6.71
12	P04792	Heat shock protein 27	497	71	22768/5.98

Peptide profiles of the protein spots were analyzed by MALDI-TOF MS and by using the Mascot program.
*The MW (molecular weight)/PI (isoelectric point) of proteins were retrieved from the database of Swiss-Prot/TrEMBL, USA.

Table 3. — *Dot blot analysis of expression of proteins in different stages of ovarian cancer compared with normal ovarian tissues.*

Spot no.	Protein name	Tissue types and stage*		Signal intensity (mean ± SD)	Ratio
1	CRBP	Ov-ca	I+II	4813.14 ± 3298.03	0.38†
			III	10771.54 ± 7667.56	0.84
			I~III	8744.68 ± 6806.33	0.69†
		Normal		12748.84 ± 4763.91	
2	Gal-1	Ov-ca	I+II	10276 ± 4532.66	1.09
			III	15073.8 ± 4235.92	1.60†
			I~III	12941.44 ± 4896.63	1.38†
		Normal		9401 ± 4230.29	
6	DJ-1	Ov-ca	I+II	16127.62 ± 4705.28	0.81†
			III	15510.54 ± 3127.45	0.78†
			I~III	15770.36 ± 3760.55	0.79†
		Normal		19857.90 ± 4073.97	
7	CathB	Ov-ca	I+II	374300.75 ± 108412.76	2.99†
			III	378754.60 ± 125816.21	3.03†
			I~III	376775.11 ± 115001.00	3.01†
		Normal		125072.27 ± 79980.63	

*Stage I + II (n = 8); Stage III (n = 11); Normal ovary (n = 20).
†$p < 0.05$, cancer vs normal (Student's unpaired t-test).

expressions of TTR, SH3BGRL, TCA, DJ-1, CathB, GA, TPM4, HLA-DRB and HSP27.

To verify and confirm the signal intensities of protein spots in 2-DE maps, SDS-PAGE and Western/Dot blotting analysis were further performed on four proteins: CRBP, Gal-1, DJ-1 and CathB. Western/Dot blotting analyses of these four proteins are shown in Figure 5 and Table 3, respectively. As shown again in Figure 5 and Table 3, Gal-1 and CathB were up-regulated, whereas CRBP and DJ-1 were down-regulated in ovarian cancer tissues. A similar trend of regulation was also observed in the analyses of 2-DE maps (Figure 4). These results strengthen the validation of 2-DE analysis in this report.

The functional interrelationship networks built by using PathwayAssist™ software between CathB and Gal-1, as well as between CRBP and SH3BGR, are shown in Figures 6 and 7, respectively.
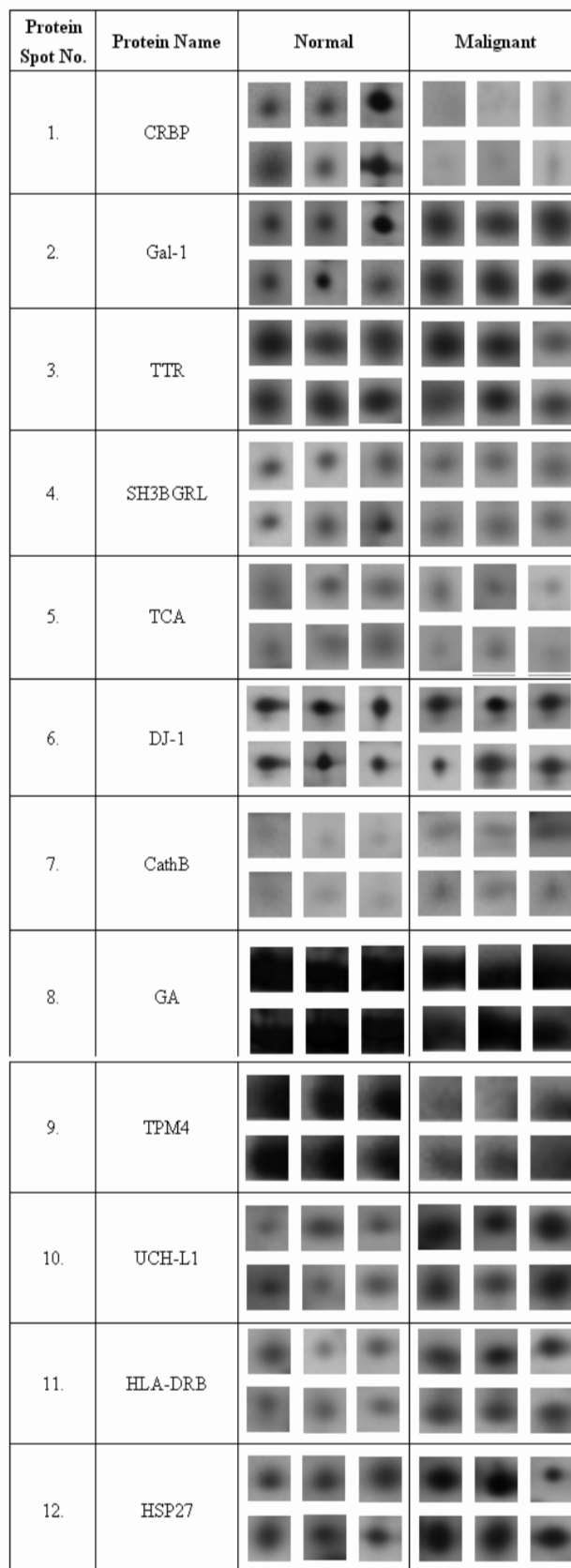
Figure 3. — Representative expression of protein spots in 2-DE among normal and malignant ovarian tissues.
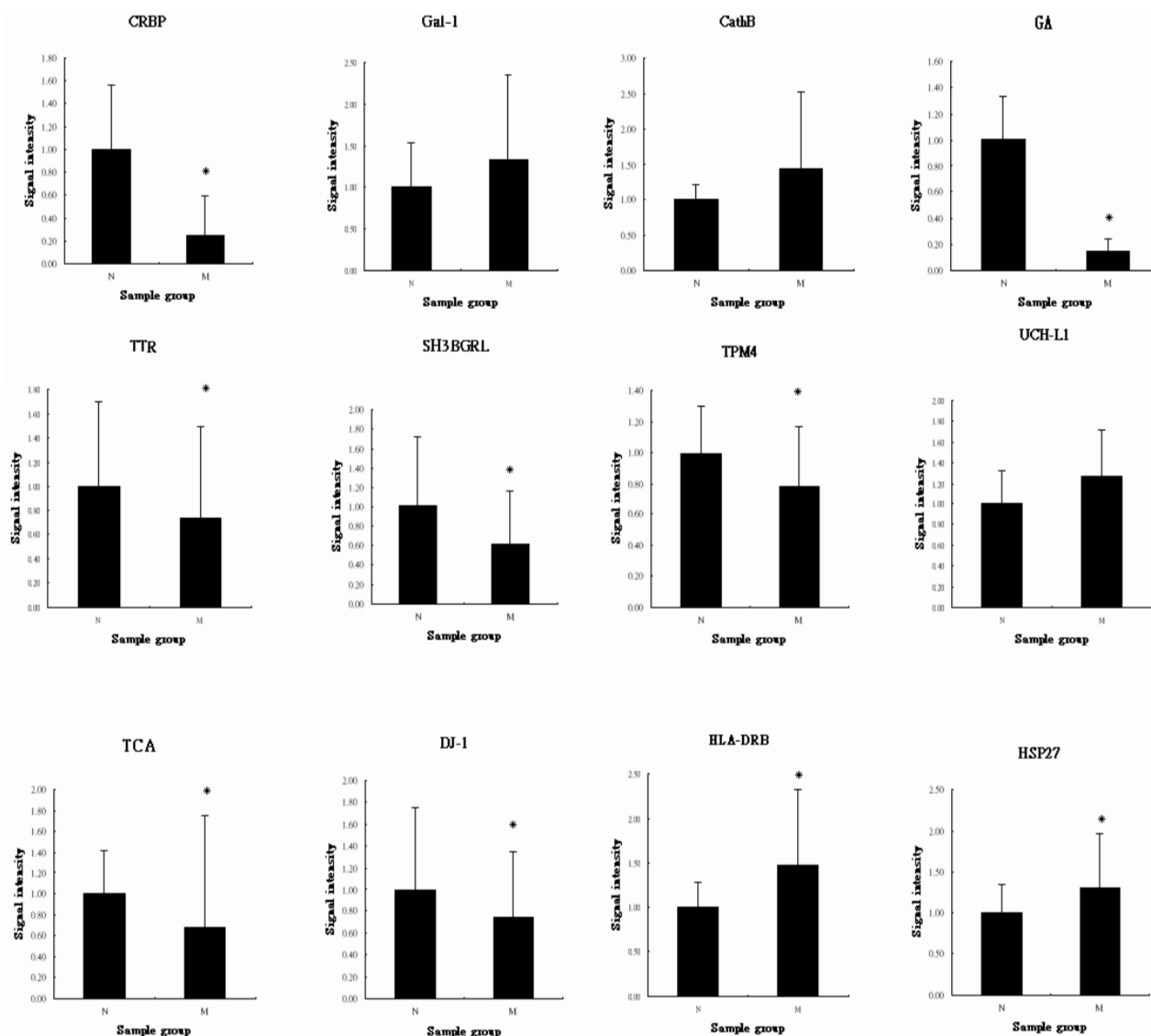
Figure 4. — Histogram of differential expression of protein spots separated in 2-DE maps among normal and malignant ovarian tissues. The mean signal intensity (± S.D.) is shown for each protein.
N: normal; M: malignant.
*: cancer vs normal, $p < 0.05$

Discussion

In this study, we used the tissue specimens of malignant ovarian tumors and normal ovarian tissue to study the protein profiles. Upon comparing the profile, we could get 12 candidate proteins. Among the proteins identified, five proteins (CRBP, Gal-1, TTR, CathB, and HSP27) were previously known proteins involved in tumor progression or differentially expressed in ovarian tumor, while seven proteins (SH3BRL, TCA, DJ-1, GA, TPM4, UCH-L1, and HLA-DRB) were newly identified in our study.

CRBP is essential for vitamin A homeostasis. Cvetkovic *et al*. [10] have reported that there was no detectable CRBP gene expression in 35% of the ovarian cancer samples studied. In addition, down-regulation of the CRBP was also noted in breast [11] and other human

cancers [12]. Gal-1, a member of the mammalian beta-galactoside-binding proteins, is involved in several biologic events including regulation of cancer cell proliferation and adhesion to the matrix. Expression of Gal-1 has been documented in many tumor types including the ovary, colon, bladder, melanoma and prostate [13]. TTR plays an important physiologic role in vitamin A homeostasis by its binding to the specific transport protein for retinol [14]. Down-regulation of TTR has been identified in ovarian carcinoma patients using serum proteomic analysis by Zhang *et al*. in 2004 [15]. CathB is a lysosomal cysteine protease whose expression and trafficking are frequently altered in cancers. Nishikawa *et al*. reported that CathB was evident in cancer cells and associated stromal tissues and may contribute to the mechanisms of invasion of ovarian cancer [16]. HSP27 is
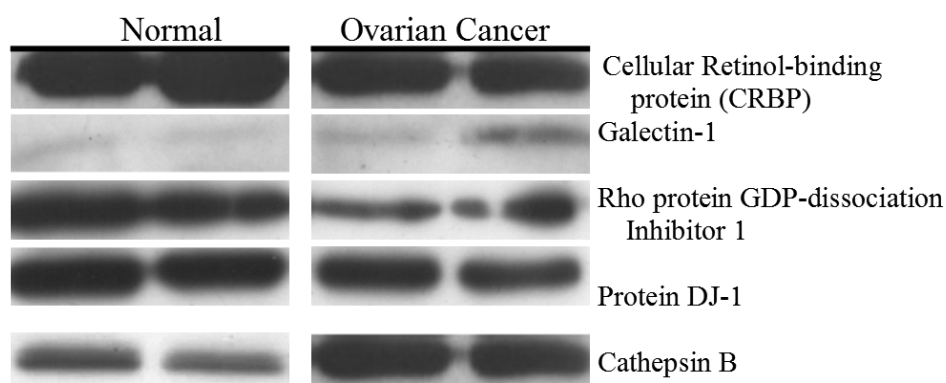
Figure 5. — Western blot analysis of CRBP, Gal-1, DJ-1 and CathB in the tissue homogenate of a normal ovary and ovarian cancer. The expression of Gal-1 and CathB showed up-regulation, but DJ-1 and CRBP showed down-regulation in ovarian cancer tissues.

related to cell growth, tumor invasion, and resistance to chemotherapeutic drugs [17]. In ovarian carcinomas, HSP27 levels are significantly higher in malignant than in benign or borderline tumors, and in Stage II-IV tumors than in Stage I tumors [18]. In addition, there may be a relation between HSP27 expression and worse prognosis in higher stage ovarian carcinoma [19]. All of the above five proteins have been reported to be involved in ovarian tumorigenesis, and our results are consistent with those of previous reports.

SH3BGRL is a member of the SH3BGR family of proteins. The SH3BGR family encodes for a protein that is characterized by the presence of a proline-rich region containing the consensus sequence for a SH3-binding domain and by an acidic carboxyl-terminal region containing a glutamic acid-rich domain. Protein interactions involving SH3-domains have been implicated in signal transduction, cytoskeletal rearrangements, membrane trafficking, and other key cellular processes [20]. Recently, Majid *et al*. [21] reported the down-regulation of SH3BGRL as an important step for v-Rel-mediated transformation. In our study, the SH3BGRL levels were found to be down-regulated in ovarian cancerous samples. The role played by the SH3BGRL protein in ovarian cancer deserves to be elucidated.

The folding pathway of tubulins includes highly specific interactions with a series of cofactors (A, B, C, D and E). Both cofactors A and D function by capturing and stabilizing beta-tubulin in a quasi-native conformation [22]. Our study demonstrated down-regulation of TCA in ovarian cancer. This observation indicates that TCA was critical for the maintenance of microtubules in normal ovarian cells, and suggests that altered function of tubulin cofactors might be implicated in human cancer.

Protein DJ-1 has been suggested to be a novel mitogen-dependent oncogene product involved in a ras-related signal transduction pathway [23]. DJ-1 affects cell survival, in part, by modulating cellular signaling cascades such as PTEN/phosphatidylinositol 3-kinase/Akt [24] and altering p53 activity [25]. The expression of DJ-1 correlates negatively with clinical outcomes in non-small

cell lung carcinoma patients [24]. In our study, DJ-1 was significantly down-regulated in ovarian cancer tissue. The discrepancy among the different types of cancers needs further investigation.

Both GA and TPM4 are essential components of cytoskeleton. Actin is the major component of the thin filaments of muscle cells and of the cytoskeletal system of nonmuscle cells. TPM4 is a member of the TPM family. TPM is normally found inside the cell and is associated with the actin cytoskeleton, where it plays a critical role in stabilizing actin filaments in a variety of cell types [26]. Down-regulation of GA and TPM can result in the decrease of cell adhesiveness, hence enhancement of cell motility and metastasis. Previous studies have demonstrated that specific TPM isoforms are down-regulated in human breast carcinoma cell lines [27, 28]. In our study, we found that GA and TPM4 were significantly down-regulated in malignant tissues in comparison to normal ovaries, indicating that GA and TPM4 may play an important role in ovarian cancer metastasis.

UCH-L1 plays an important role in protein degradation through recycling free ubiquitin by cleaving ubiquitylated peptides [29]. Expression of UCH-L1 is limited to neuronal tissue, testes and ovaries [29]. However, up-regulation of UCH-L1 in various tumors including leukemia, medullary thyroid carcinoma, colorectal cancer and breast cancer have been reported, indicating the involvement of UCH-L1 up-regulation in the pathogenesis of these tumors [29-32]. Until now, no report has been available on the expression status of UCH-L1 in ovarian cancer.

The MHC comprises a family of highly polymorphic genes encoding a set of transmembrane proteins that present peptide epitopes to specific antigen receptors on T cells. The HLA system is the human version of the MHC. There are several sorts of hypotheses about the involvement of MHC-antigens in the development of cancer. One hypothesis describes mechanisms by which the tumor itself evades immune surveillance (immune escape) [33]. Workers from our institution recently discovered disruptions of the HLA genotype and down-reg-
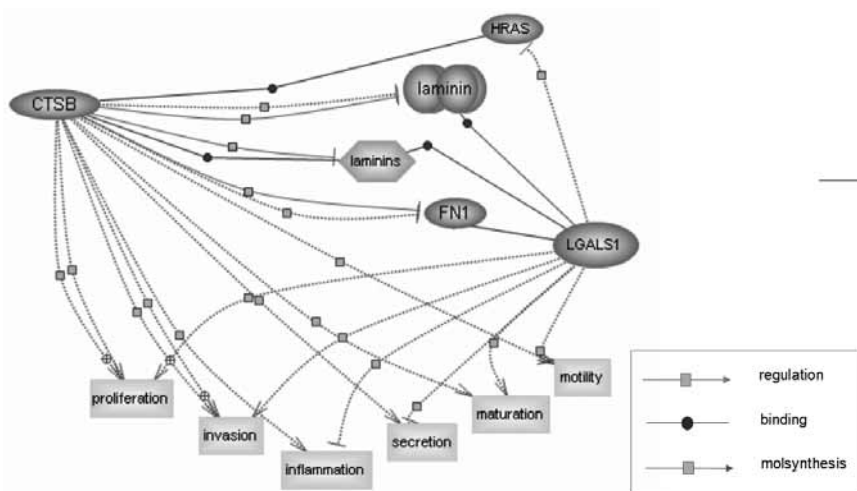
Figure 6. — A pathway built by PathwayAssist™ between CathB and Gal-1. This pathway showed the functional interrelationships of these two proteins. CTSB represents CathB and LGALS1 is the same protein as Gal-1. When using the PathwayAssistTM software program, each connecting line is a "clickable" link that displays the underlying text that supports the interaction.
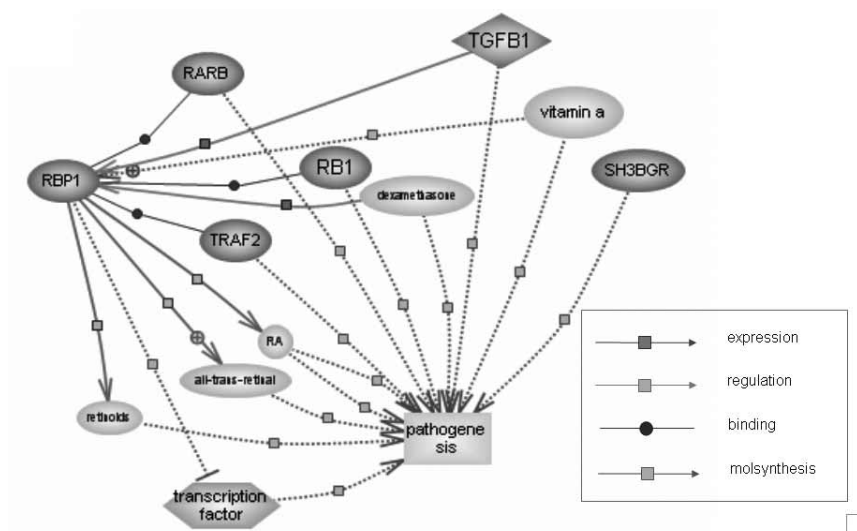
Figure 7. — PathwayAssist™ built pathways between CRBP and SH3BGR proteins. This pathway demonstrates the networks of functional relationships of these two proteins. RBP1 represents CRBP.

ulation of HLA-class I molecules in human cervical carcinoma integrated with high-risk human papillomavirus (HPV) DNA [34]. In that report, the significant mutations of the HLA genotype with reduced HLA-class I molecule expression may possibly be the tactics carried out by HPV to escape the immune attack, thus achieving carcinogenesis. Recently, Kubler *et al*. reported that HLA-class II loci or individual HLA-class II haplotypes might be involved in the pathogenesis of ovarian cancer [35]. In our present study, HLA-DRB expression was significantly higher in cancer tissue compared to normal ovaries (Figure 4). In contrast to the cervical cancer integrated with high-risk HPV DNA, the tumor immune escape mechanism by MHC antigen in ovarian cancer seems to be different. The MHC antigen expression of ovarian cancer is worth further investigation.

The functional interrelationship network of several interested proteins among the 12 protein profiles studied in this report was built by using PathwayAssist™ software. As illustrated in Figure 6, CathB and Gal-1 are functionally related to proliferation, invasion and motility of cancer cells, and both were found to be up-regulated in our study. Figure 7 demonstrates the functional network between CRBP and SH3BGR protein; these two proteins play important role in cancer pathogenesis through many pathways.

In conclusion, we have demonstrated in the present study that 12 protein spots are expressed differentially on ovarian cancers and normal ovaries, and seven identified proteins have not previously been reported in ovarian cancer. Although we could not completely explain all correlations between expressed proteins and their roles in

tumorigenesis, there might be a possibility to find tumor-specific markers among the differentially expressed proteins. Further cloning and functional analysis of these proteins will provide more information on pathophysiologic roles during tumor formation and progression.

Acknowledgements

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Mesothelin gene expression and promoter methylation/hypomethylation in gynecological tumors

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Summary

Purpose: Mesothelin is a cell surface glycoprotein that is present on normal mesothelial cells and overexpressed in several cancers. In this study, we investigated the methylation/hypomethylation status in the promoter region of the mesothelin gene in gynecological tumors. *Methods:* Forty-four ovarian tumor specimens and 16 cases of uterine endometrial carcinoma, and normal tissue specimens were used. Monoclonal antibody (5B2) was employed for the immunohistochemical analysis. The methylation-sensitive single-nucleotide primer extension (Ms-SNuPE) technique was used to quantify the methylation/hypomethylation status at 20 CpG sites in the mesothelin promoter region. *Results:* Mesothelin was expressed in 100% of serous cystadenocarcinoma and 100% of serous borderline tumor of the ovary. None of the germ cell tumors and sexcord-stromal tumors was immunoreactive. Fifty percent of endometrial carcinoma was immunoreactive for mesothelin. The average methylation of CpG sites in ovarian tumors ranged from 6-56% (median: 31%) in mesothelin-positive and 13-79% (median: 43%) in mesothelin-negative samples. In endometrial tumors, the average methylation ranged from 5-52% (median: 28%) in mesothlin-positive and from 15-67% (median: 22%) in mesothlin-negative samples. A correlation was found between mesothelin expression and the average methylation/hypomethylation status as well as methylation/hypomethylation status at four of 20 CpG sites in ovarian samples. No correlation was found in endometrial samples. *Conclusion:* We detected diverse levels of methylation/hypomethylation at CpG sites in the mesothelin promoter region in ovarian and endometrial tumors. We speculate that, although methylation/hypomethylation changes may affect its transcription, other mechanisms may synergically operate in tissue-specific expression and tumor-related mesothelin overexpression.

Key words: Mesothelin; Overexpression; Gynecological tumors; Promoter methylation; Hypomethylation.

Introduction

The human mesothelin gene is a 40-kilodalton carboxy-terminal component of a 69-kilodalton precursor protein whose amino portion is the secreted cytokine known as megakaryocyte-potentiating factor. Mesothelin is a glycosy-phosphatidylinositol-linked cytoplasmic membrane glycoprotein whose function has not been clarified, but it may be involved in cell adhesion [1, 2]. In humans, mesothelin is expressed in normal mesothelial cells lining the body cavities and in some epithelial cells of the kidney, tonsil, trachea, and fallopian tube [3]. In immunohistochemical or gene expression studies of human cancers, mesothelin has been reported in mesothelioma, adenocarcinoma of the ovary, pancreas, lung, stomach, colon, rectum, uterus, and some squamous cell carcinomas [4-15]. Mesothelin has been evaluated as a diagnostic marker of ovarian cancer and mesothelioma. The elevation of serum mesothelin in ovarian cancer and mesothelioma has been identified by several groups [9, 14-16]. However, epigenetic mechanisms of mesothelin gene expression were not well studied. Studies of global gene expression and DNA hypomethylation analysis of pancreatic cancer involving 18 genes showed that, in normal pancreatic tissue, the mesothelin gene is hypermethylated, which means it does not express mRNA or

protein. On the other hand, mesothelin is overexpressed in most pancreatic cancer cases, and the mesothelin promoter was found to be hypomethylated [17]. The methylation and hypomethylation profile of the CpG sites in the promoter region of mesothelin and the correlation with the expression pattern in various human tissues and tumors are still largely unknown.

Mesothelin overexpression has been reported in subtypes of ovarian and endometrial cancer, but the epigenetic alteration of the mesothelin gene is unknown in these tumors. In the present study, we employed immunohistochemical analysis to investigate the expression of the mesothelin gene in gynecological tumors including several histological subtypes of ovarian tumors, endometrial carcinoma, and normal tissue specimens. Using microdissected tissue DNA, we performed quantitative methylation analysis of the 20 CpG sites in the promoter region of the mesothelin gene, and the correlation with its expression was investigated.

Materials and Methods

Cases

Forty-four ovarian tumor specimens, 42 cases of uterine endometrial carcinoma, and normal tissue specimens were obtained from patients surgically treated between 1993 and 2005 at the Department of Obstetrics & Gynecology of Juntendo University Hospital, Tokyo, Japan. This research project

was approved by the local ethical committee, and all samples were obtained with the patients' informed consent. A part of the tissue sample was fixed in formalin and embedded in paraffin for histological diagnosis. Histological diagnosis was made when two pathologists specializing in gynecological disease reached a consensus. The age of the patients ranged from 17 to 72 (mean: 43) years old for ovarian tumors and from 35 to 80 (mean: 55) years old for uterine tumors. Analyzed samples are shown in Table 1.

Immunohistochemistry

The expression of mesothelin was assessed by immunohisto-chemistry using anti-mesothelin monoclonal antibody 5B2 (Novacastra, Newcastle-on-Tyne, UK), an anti-mesothelin anti-body that was generated by immunizing mice with a recombi-nant protein corresponding to 100 amino acids at the NH2 ter-minus of membrane-bound mesothelin [2]. This antibody has been characterized and used for detection in several types of benign and malignant tumor expression studies. For each case, one to three pathology blocks were selected for human mesothelin gene expression analysis. Immunhistochemical staining was performed as follows: 4-μm-thick sections were deparaffinized, treated for 30 min with 3% hydrogen peroxide to block endogenous peroxidase activity, and then with citric acid (pH 6.0) for 10 min at 100°C in a microwave oven for antigen retrieval. Five percent normal goat serum was applied for 30 min to block nonspecific reactions. Sections were incu-bated with the mouse anti-human anti-mesothelin antibody 5B2 (1:50) at 4°C overnight. After rinsing in PBS, slides were treat-ed with Envision+HRP System (Dako Cytomation, Glostrup, Denmark, K4000)-labeled polymer anti-mouse immunoglobu-lin for 60 min. The peroxidase reaction was visualized by incu-bating sections with 0.02%, 3.3-diaminobenzidine tetrahydro-chloride in 0.05M Tris, buffer and then slides were counterstained with hematoxylin. Sections for the negative control were pre-pared using normal mouse serum instead of the primary anti-body. The intensity of the staining was scored from 0-3 (absent, weak, moderate, and strong, respectively). The mesothelin expression was regarded as negative when no cells were stained or only faintly stained over less than 30% of the tumor area. Mesothelin was regarded as positive when more than 30% of the tumor cells were immunostained. Most of the mesothelin staining showed membranous staining, but some cases also showed cytoplasmic staining. No nuclear staining was detected. Most negative cases clearly showed no staining in the majority of tumor cells or normal cells on immunostained slides.

Microdissection and DNA extraction

Serial 8-μm paraffin-embedded tissue sections were cut with a microtome, deparaffinized, and stained with regular hema-toxylin-eosin (HE). The first and last sections were cut to 8-μm thick, stained with HE, and cover-slipped for microscopic examination. Tumor and non-tumor portions were microdis-sected using a 27-gauge needle under an inverted microscope. A laser-assisted microdissection system (Leica laser microdis-section system, Leica Microsystems, Wetzlar, Germany) was also used. Visual inspection revealed that at least 95% of the collected cells were tumor cells. Genomic DNA extract was iso-lated from microdissected tissues using a DNA isolation kit (Qiagen, QIAamp® DNA Micro Kit, Qiagen, Hilden, Germany) following the manufacturer's instructions.

DNA methylation analysis of mesothelin promoter

DNA was modified in 40 μl of water with sodium bisulfite using the EpiTect™ Bisulfite Kit (Qiagen, Hilden, Germany).

Sodium bisulfite converts unmethylated cytosine to uracil, which is replicated as thymine in the subsequent PCR step. Methylated cytosines are resistant to deamination by sodium bisulfite and are therefore replicated as cytosine during PCR. Presumed genomic structures (Sequence position 13,501-14,220 of Gene Bank ID: AL031258) of the mesothelin pro-moter and primers and CpG sites analyzed are shown in Figure 1. The region contains a postulated CpG island, predicted by the CpG island searcher (http://www.uscnorris.com/cpgislands2/cpg.aspx), and an 18-bp upstream enhancer CanScript that con-tains a transcription enhancer factor-dependent MCAT motif [18]. Ten possible transcription start sites described by Hucl *et al*. [18] are also shown. After bisulfite modification, PCR of the mesothelin promoter was performed with the primers as shown in Figure 1 using the JumpTaqTM REDTaq DNA polymerase (Sigma, Saint Louis, MO, USA) under the following PCR con-ditions: 40 cycles of 94°C for 30 sec, 58°C for 60 sec, and 72°C for 50 sec. Approximately 1-2 μl of bisulfite-treated DNA was used as a template for strand-specific PCR amplification. PCR products were electrophoresed on a 1.8% ethidium-bromide stained gel and the DNA fragments were purified from agarose gel slices using the Wizard DNA Clean-up system (Promega, Madison, WI, USA).
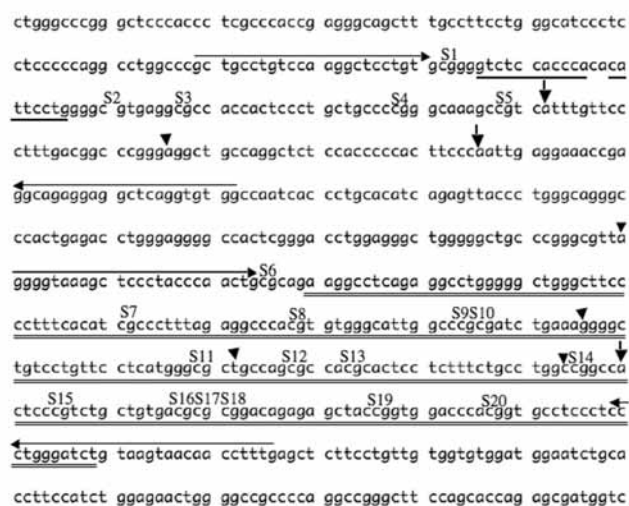
Figure 1. — Genomic region of postulated mesothelin promoter regions (sequence position 13501-14220 of GenBank accession AL031258), postulated CpG islands, and CpG sites analyzed by MsSNuPE. S#, CpG site analyzed for methylation. The position of an 18-bp upstream enhancer CanScript that contains a tran-scription enhancer factor-dependent MCAT motif is underlined. The promoter CpG island, predicted by the CpG island searcher (http://www.uscnorris.com/cpgislands2/cpg.aspx), is double-underlined. PCR primers for the MsSNuPE template are shown by arrows. The consensus initiator sequence is shown by thick overlying arrows, and the nonconsensus start site is shown by arrowheads.

Quantitation of mesothelin promoter methylation/hypomethy-lation status at specific CpG sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE)

DNA is treated with sodium bisulfite, followed by PCR amplification of the target mesothelin promoter CpG island sequence to generate a strand-specific DNA template suitable for Ms-SNuPE analysis. The single nucleotide primer extension assay was first described by Kuppuswamy and others for the

Table 1. — *Summary of ovarian and endometrial tumors analyzed for mesothelin expression and their promotoer methylation staus.*

Sample ID (OVA #)	Histology and/or component	Mesothelin expression	Average methylation (%)	Median methylation (%)	Range of methylation (%)
OVA1	Normal stromal component	-	43	48	1-86
OVA2	Mucinous cystadenoma	-	65	93	2-93
OVA3	Serous cystadenoma	+	37	39	1-65
OVA4	Serous cystadenoma	+	18	18	1-38
OVA5	Mucinous cystadenoma	-	49	47	20-79
OVA6	Clear cell carcinoma	+	34	28	2-64
OVA7	Endometrioid adenocarcinoma	-	17	4	1-96
OVA8	Mucinous cystadenocarcinoma	-	28	10	1-88
OVA9	Serous papillary cystadenocarcinoma	+	56	58	1-100
OVA10	Serous papillary cystadenocarcinoma	+	17	14	6-55
OVA11	Clear cell carcinoma	-	24	22	1-66
OVA12	Serous papillary cystadenocarcinoma	+	18	17	2-42
OVA13	Serous papillary cystadenocarcinoma	+	29	27	8-42
OVA14	Serous papillary cystadenocarcinoma	+	34	31	2-90
OVA15	Endometrioid adenocarcinoma	-	36	31	2-69
OVA16	Serous cystadenofibroma	+	31	37	1-83
OVA17	Mucinous cystadenocarcinoma	-	14	14	8-22
OVA18	Mucinous LMP	-	79	92	4-97
OVA19	Endometrioid adenocarcinoma	-	29	24	7-77
OVA20	Clear cell carcinoma	-	18	15	2-47
OVA21	Clear cell carcinoma	-	70	74	46-94
OVA22	Clear cell carcinoma	-	59	67	3-73
OVA23	Clear cell carcinoma	-	37	36	21-58
OVA24	Mucinous LMP	-	24	20	3-71
OVA25	Mucinous LMP	-	64	74	2-100
OVA26	Mucinous LMP	-	34	30	11-80
OVA27	Serous LMP	+	6	4	1-20
OVA28	Serous LMP	+	62	80	3-96
OVA29	Sertoli cell tumor	-	67	93	2-98
OVA30	Normal ovarian stroma	-	48	54	12-82
OVA31	Arterial wall	-	38	37	12-88
OVA32	Normal ovarian stroma	-	13	8	3-63
OVA33	Normal corpus luteum	-	50	58	6-94
OVA34	Immature teratoma, neural component	-	73	95	1-100
OVA35	Immature teratoma, stromal component	-	47	52	1-97
OVA36	Granulosa cell tumor	-	55	68	1-98
OVA37	Immature teratoma, skin	-	42	41	1-88
OVA38	Immature teratoma, cartirage		43	42	18-69
OVA39	Immature teratoma, neural epithelium		42	41	2-93
OVA40	Fibroma	-	60	63	19-86
OVA41	Brenner tumor, epithelium	-	30	24	7-83
OVA42	Brenner tumor, stroma	-	39	33	7-78
OVA43	Yolk sac tumor	-	60	70	1-94
OVA44	Fibroma	-	40	43	1-75
OVA45	Desmoplastic small round cell tumor	-	57	61	12-84
OVA46	Fibrothecoma	-	43	41	22-92
OVA47	Fibroma	-	42	39	15-93
OVA48	Sertoli-Leydig cell tumor, sertoli cells	-	41	32	1-99
OVA49	Mature teratoma, skin & adnexa	-	53	59	2-88
OVA50	Mature teratoma, thyroid	-	32	38	1-69
OVA51	Strumal carcinoid	-	44	42	23-81
OVA52	Brenner tumor, epithelial component	-	51	55	5-89
OVA53	Brenner tumor, stromal component	-	52	56	18-79
OVA54	Yolk sac tumor	-	58	60	19-90
OVA55	Granulosa cell tumor	-	61	62	25-88
EM1	Endometrioid adenocarcinoma	-	25	26	1-44
EM2	Endometrioid adenocarcinoma	+	18	13	5-69
EM3	Normal endometrium	-	16	13	40
EM4	Endometrioid adenocarcinoma	-	28	30	1-73
EM5	Endometrioid adenocarcinoma	+	5	2	1-24
EM6	Endometrioid adenocarcinoma	+	28	24	0-91
EM7	Endometrioid adenocarcinoma	+	27	26	14-50

Sample ID (EM #)	Hystology and/or component	Mesothelin expression	Average methylation (%)	Median methylation (%)	Range of methylation (%)
EM8	Endometrioid adenocarcinoma	-	15	12	3-65
EM9	Endometrioid adenocarcinoma	-	24	18	3-76
EM10	Endometrioid adenocarcinoma	+	40	40	11-74
EM11	Normal endometrium	-	19	18	7-37
EM12	Endometrioid adenocarcinoma	+	52	56	3-82
EM13	Endometrioid adenocarcinoma	-	67	67	27-97
EM14	Endometrioid adenocarcinoma	+	31	21	0-93
EM15	Endometrioid adenocarcinoma	-	18	8	0-73
EM16	Endometrioid adenocarcinoma	-	23	20	1-56
EM17	Endometrioid adenocarcinoma	+	16	14	6-31
EM18	Endometrioid adenocarcinoma	-	22	24	0-46

The methylation status is expressed as the average % methylation at 20 CpG sites analyzed, median % methylation and the range of % methylation. OVA#, ovarian tumor samples or components; EEM#, endometrial tumors and normal endometrial samples; LMP, tumors of low malignant potential (borderline tumor).

detection of mutations in abnormal alleles [19]. Gonzalgo and Jones [20, 21] modified this method for the quantification of DNA methylation differences at specific CpG sites. Briefly, 2 µl of purified bisulfite PCR product was used in each Ms-SNuPE reaction. One µM of Ms-SNuPE primer (each cg site specific 19-27 mers; sequences of primers available upon request) was labeled with 32P-dCTP or 32P-TTP and 1 U of Jump start polymerase was used for the primer extension reaction. The Ms-SNuPE reaction were performed at 94°C for 3 min, 45°C for 2 min, and 72°C for 2 min. Stop solution (10 µl of 0.1% bromophenol blue, 0.1% xylene cyanol, and 95% formamide) was then added to the reaction mixtures, heated at 95°C for 5 min, and samples were loaded on to 23% denaturing polyacrylamide gels. The radioactivity of gel signals was visualized and quantified using the BAS2500 Image analyzer (Fuji, Tokyo, Japan). Different length primers were designed for the multiplex quantitative analysis of methylation at twenty top stand cytosines in the 5´CpG island of the mesothelin gene. The intensities of bands in the C lane were proportional to the percent of methylation at each CpG site being monitored, and band intensities in the T lanes were proportional to the percent of unmethylated cytosine. The percent of methylation at each CpG site is calculated as the signal intensity of C/ (signal intensity of C+T) x 100 (%). Methylase-treated DNA as well as subcloned and sequence-verified DNA from PCR products was used as methylated and unmethylated CpG controls.

Statistical analysis

The Mann-Whitney U test was used to compare differences in the percent of methylation at CpG sites between mesothelin-negative ovarian tumor or normal ovarian tissue samples and mesothelin-positive ovarian tumors. Similarly, in endometrial samples, mesothelin-negative normal endometrial cells, mesothelin-negative endometrial cancers, and mesothelin-positive endometrial cancers were compared.

Results

Mesothelin gene expression in various ovarian tumors and uterine cancers

Immunohistochemical findings are summarized in Table 1 and representative mesothelin immunostaining is shown in Figure 2. Twenty-five percent of ovarian tumors stained positively for mesothelin. Mesothelin was expressed in five of five serous carcinomas (100%), two of two serous cystadenomas (100%), two of two (100%) serous tumors of low malignant potential (borderline serous tumors), one serous cystadenofibroma, and one of six clear cell carcinomas (17%). The staining was mostly detected on the cell surface membrane. Some tumors also showed cytoplasmic staining. None of the mucinous tumors (2 benign, 4 borderline, and 2 malignant tumors), two Brenner tumors, and three endometrioid carcinomas were all negative for mesothelin. None of the germ cell tumors and sex-cord stromal tumors were immunoreactive.

In endometrial samples, endometrioid uterine adenocarcinoma was frequently positive for mesothelin (8 of 16; 50%). None of the normal endometrial glandular cells, endometrial stromal cells and myometrial smooth muscle cells were immunostained for mesothelin.

Quantification of DNA methylation/hypomethylation status at specific CpG sites in the mesothelin promoter

We used the Ms-SNuPE assay to determine the methylation status of multiple CpG sites in the mesothelin promoter in various gynecological tumor specimens. Representative gels of the SNuPE assay are shown in Figure 3. Table 1 shows the average percent of methylation of all CpG sites analyzed and the range of percent of methylation for each sample. Figure 4 shows a box-and-whisker plot of the average percent of methylation and the percent of methylation at individual CpG sites for mesothelin-negative and positive-samples. The average methylation of these 20 CpG sites in ovarian tumors ranged from 6-56% (median: 31%) in mesothelin-positive samples and 13-79% (median: 43%) in mesothelin-negative samples. The lowest level of methylation (hypomethylated) was detected in serous borderline tumor case #OVA27 and the highest was detected in immature teratoma case #OVA35. In endometrial tumors, the average methylation ranged from 5-52% (median: 28%) in mesothelin-positive samples and from 15-67% (median: 22%) in mesothelin-negative samples.

When each CpG site was analyzed separately in ovarian tumor, the lowest level of methylation was detected at the S8 site (median: 7% in mesothelin-positive ovarian tumor and 15% in mesothelin-negative ovarian tumor). The highest level of methylation was detected at the S3 site (median: 74% in mesothelin-posi-
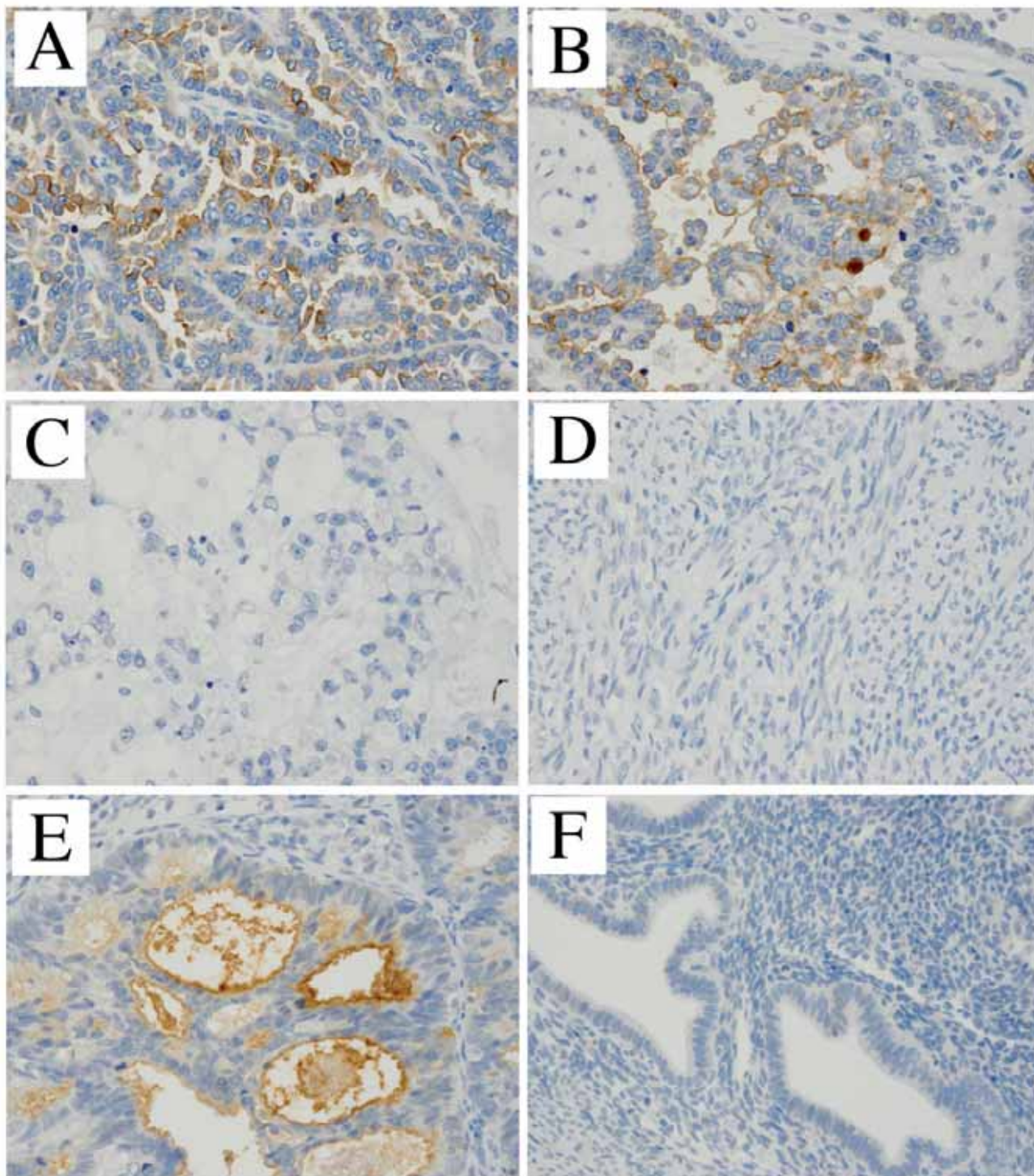
Figure 2. — Immunohistochemical staining of mesothelin in ovarian and uterine endometrial tumors. (A) and (B) Strong mesothelin expression in ovarian serous carcinoma case #OVA9 and OVA10, respectively . Note the predominantly membranous staining. (C) Negative mesothelin staining in ovarian clear cell carcinoma case #OVA21. (D) Negative mesothelin staining in ovarian fibroma case #OVA41. (D) Negative mesothelin expression in normal proliferative phase endometrium. (E) Strong mesothelin expression in endometrial endometrioid adenocarcinoma case #EM6.

Figure 3. — Representative MsSNuPE gels demonstrating various levels of % methylation at each CpG site in ovarian and endometrial tumors. C represents the signal for MsSNuPE reactions incubated in the presence of [32P]dCTP and T represents the signal for MsSNuPE reactions incubated in the presence of [32P]TTP. S# represents the individual CpG sites analyzed.

tive ovarian tumor and 72% in mesothelin-negative ovarian tumor).

One of the mesothelioma samples and respiratory epithelium showed an average of 20% and 25% methylation, respectively (data not shown).

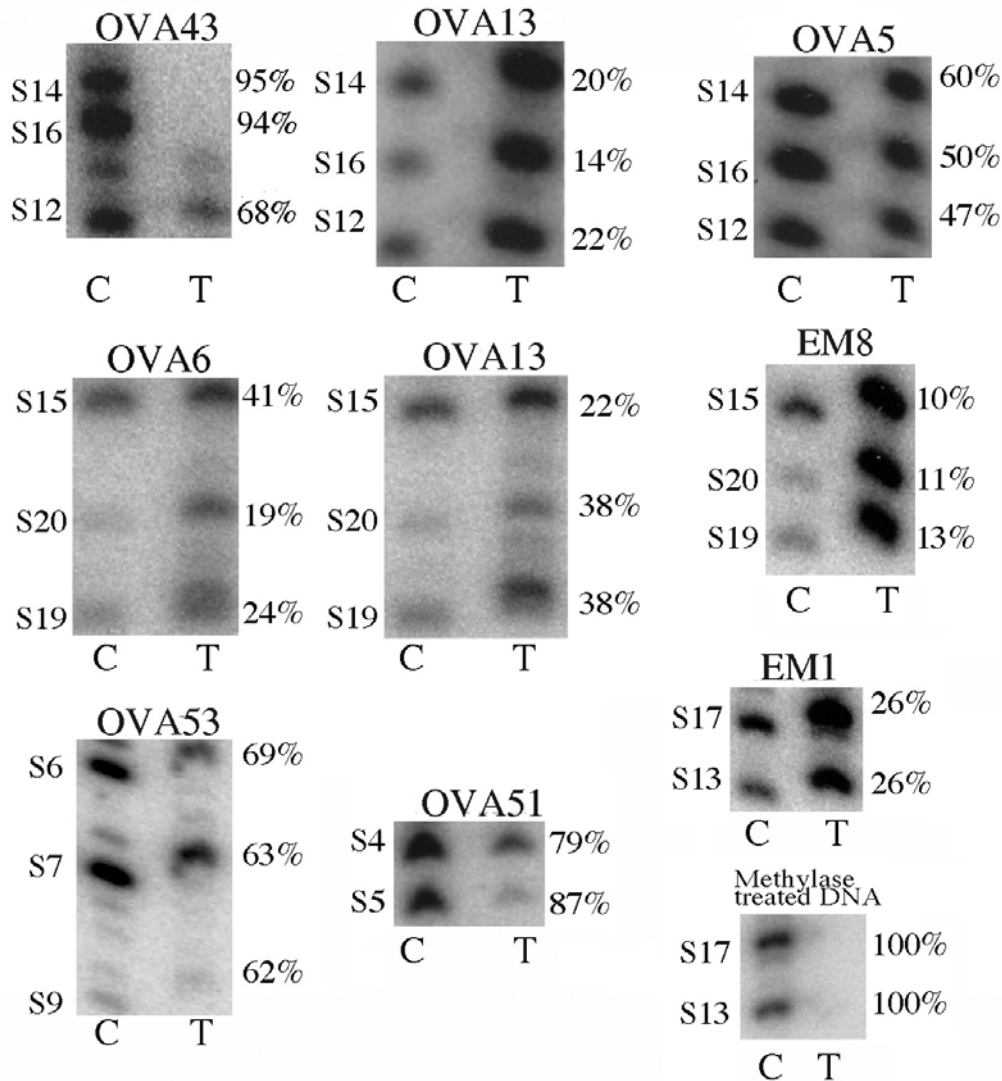*Correlation of mesothelin expression and promoter methylation in gynecological tumors*

By the Mann-Whitney U test, a correlation was found between the mesothelin expression status and the average methylation, as well as, the methylation at the CpG site S9, S14, S15, and S16 in ovarian samples (Figure 4A). No correlation was noted in endometrial tumors (Figure 4B).

Discussion

Mesothelin shows a strong tissue-specific expression. In normal tissue, only mesothelial cells of the body cavities, respiratory epithelium, and tubal epithelium express

mesothelin[22]. Many tumors including mesothelioma, lung, pancreatic, ovarian, and endometrial cancer are known to over-express mesothelin [4-15]. However, one of the mechanisms of gene transcription, the methylation/hypomethylation status of the mesothelin promoter, is largely unknown. In this study, in order to evaluate the relationships between mesothelin methylational changes and its expression, we analyzed the level of methylation of the 20 promoter CpG sites in gynecological tumors by quantitative Ms-SNuPE methods.

The results of the immunohistochemical staining of various ovarian tumors and endometrial cancers are comparable to those of previous reports [6, 7, 9, 23-26]. Serous ovarian cancer showed the highest rate of mesothelin immunoreactivity. No mucinous tumors of benign, borderline, and malignant categories were stained for mesothelin. Germ cell tumors and sex-cord stromal tumors were all negative for mesothelin.
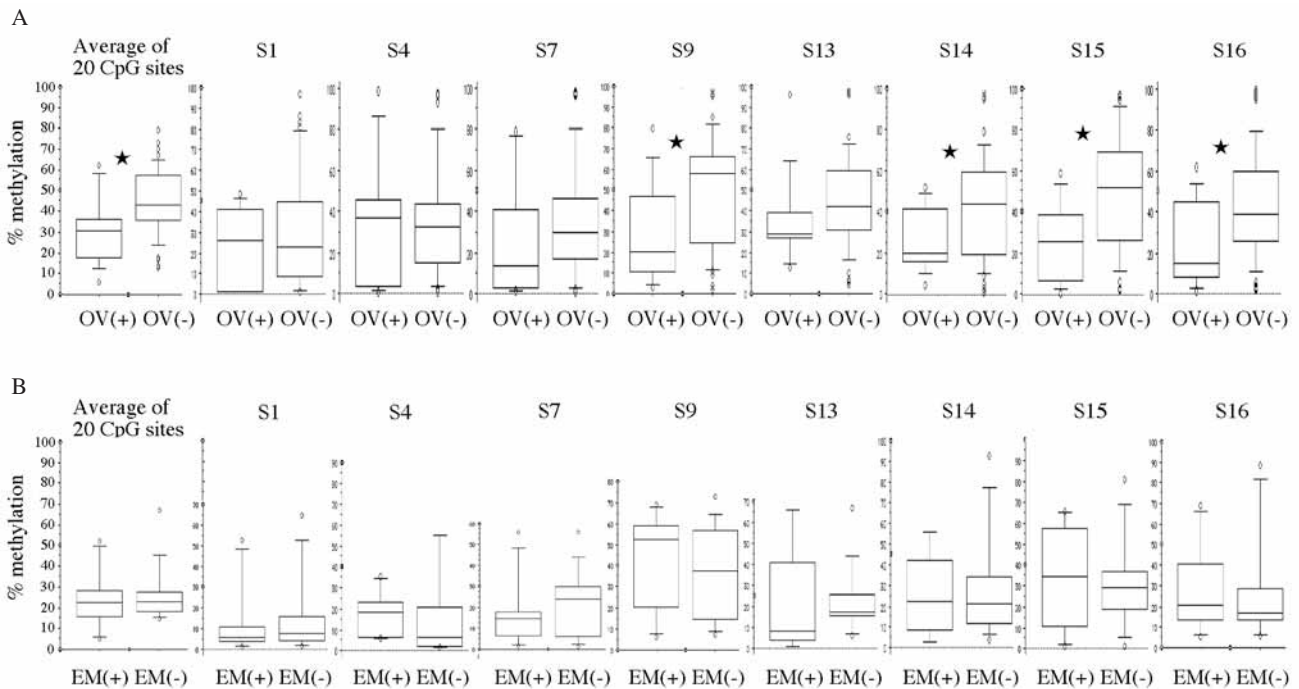
Figure 4. — The extent of mesothelin promoter methylation/hypomethylation. OV(-), mesothelin-negative ovarian tumors or ovarian component; OV(+), mesothelin-positive ovarian tumor; EM(–), mesothelin-negative endometrial cancer and normal endometrial samples; EM(+), mesothelin-positive endometrial cancer. (A) A box-and whisker plot of the average methylation and the methylation at individual CpG sites in ovarian tumors. (B) A box-and whisker plot showing average methylation and the methylation at individual CpG sites in endometrial tumors. The lines within the box indicate the median values, the top and bottom horizontal lines of the box, the 75th and 25th percentiles, respectively, and the top and bottom horizontal lines the 90th and 10th percentiles, respectively. ★, statistically significant differences for percent of methylation in mesothelin-positive and mesothelin-negative samples.

We detected that percentages of methylation/hypomethylation differed from case to case. In serous ovarian cancers showing a strong mesothelin expression (case #OVA10, 12, 13, 14, 16, and 27), we identified predominantly hypomethylated CpG sites. Also, correlation was found between the mesothelin expression status and the average methylation, as well as, the methylation at the four of 20 CpG sites in ovarian samples. We believe that methylation/hypomethylation especially at these 4 CpG sites as well as overall methylation/hypomethylation status of the promoter region may affect its transcriptional machinery in ovarian tumors. A correlation between such a methylational status and mesothelin expression was not evident at the remaining 16 of 20 CpG sites in ovarian tumors.

In pancreatic cancer, hypomethylation of the mesothelin promoter has been described. By systematic analysis of the number of genes for methylation and hypomethylation, Sato *et al*. [17]. found seven genes including mesothelin were overexpressed in pancreatic cancer cell lines and primary pancreatic carcinomas. These genes in pancreatic cancers were strongly hypomethylated in CpG sites in their 5' promoter regions. On the other hand, these genes are normally methylated and not expressed in the non-neoplastic pancreas. Because pancreatic cancers show such a correlation, it is tempting to postulate that

varied levels of methylation/hypomethylation in various ovarian and endometrial tumors may also play a certain role in their mesothelin expression.

Although we only analyzed a single case each, we identified strong hypomethylation in mesothelioma and respiratory epithelium, both of which are mesothelin-immunoreactive. Many serous ovarian and endometrial cancers were predominantly hypomethylated. When several components were separately microdissected and analyzed regarding their methylation from a single tumor (immature teratoma, Brenner tumor, etc.), we occasionally detected different levels of methylation among the components (data not shown). We speculate that mesothelin promoter methylation/hypomethylation may play a certain role in the tissue- specific and tumor type-specific overexpression in certain tumors. We are currently evaluating the methylation levels of various tumors and organs including lung cancer, mesothelioma, and related non-neoplastic lesions.

Promoter analysis of mesothelin demonstrated the 18-bp upstream enhancer CanScript that contains a transcription enhancer factor-dependent MCAT motif [18]. CanScript appears to be a modular element for cancer-specific mesothelin transcription. We noted variable levels of methylation at CpG sites in the region close to CanScript,

which may affect the binding of various factors. Other epigenetic mechanisms such as histone acetylation and histone methylation may also operate.

The function of mesothelin in gynecological tumors and normal tissue remains to be elucidated. Mesothelin may play a role in cell adhesion and the metastatic spread of ovarian cancer [27]. On the other hand, high-grade ovarian serous carcinoma with diffuse mesothelin expression has been correlated with prolonged patient survival [26]. The immunological response to mesothelin-expressing tumor cells may be one of the associated mechanisms. Cohesive cell growth in mesothelin-expressing ovarian tumor cells may prevent tumor dissemination and metastasis [26]. The analysis of the methylational profile of ovarian tumors may facilitate the molecular modulation of mesothelin for therapeutic purposes.

Although less frequently than in ovarian tumors, endometrial cancers have been found to express mesothelin. We found that eight of 16 endometrioid carcinomas of the uterus express mesothelin. On the other hand, absent to very faint mesothelin immunostaining was detected in normal endometrial tissue. Interestingly, we detected predominantly unmethylated alleles in most of the 20 CpG sites studied in mesothelin-positive as well as-negative endometrial cancers and normal endometrial epithelial cells. There may be several possibilities to be considered. For the negative scoring of immunohistochemistry, we regarded completely absent-faint immunoreactivity as staining in less than 30% of the tumor cells. A similar criterion has been previously adopted in many related articles. These tumors may actually show certain levels of gene transcription, and exclusive methylation may not be required. Actually, although this has not been reported, we detected very faint mesothelin staining in normal endometrial glandular cells. Thus, the normal counterpart of these tumors may also transcribe mesothelin, but at very low levels. The transcriptional activity of mesothelin mRNA and expression of mesothelin detected by immunohistochemistry may not always be correlated. Alternatively, tissue-specific methylation and mechanisms for gene up-regulation may be involved.

There are at least three mesothelin variants [2, 28-31]. Mesothelin variant 1 is attached to the cell membrane by a glycosylphosphatidyl inositol (GPI) linkage and appears to be the predominant mRNA in both normal and tumor cells. This variant 1 is also a dominantly expressed protein on the cell surface of ovarian carcinoma cells. Also, the variant is currently considered as a major released form detected in the serum as a diagnostic marker [29, 30]. Variant 2 has a 24-bp insert, and variant 3 has an 82-bp insert, which leads to the premature termination of the protein, resulting in the loss of GPI anchorage and its release from the cell. Variants 2 and 3 are expressed and released much less frequently. Thus, it is reasonable that methylation/hypomethylation changes at promoter CpG sites may predominantly affect the expression of variant 1.

There are several potential transcription start sites [18, 32]. We do not know which sites are involved in ovarian and endometrial tumors. The patterns of usage of these transcription start sites may vary from organ to organ and from tumor to tumor. These possible differences may also affect the methylation levels.

Mesothelin is one of the new promising tumor markers for tumor monitoring. Molecular and vaccine therapies targeting mesothelin are currently being investigated [2, 15, 25, 33]. Further analysis of the regulation of mesothelin expression may also advance the future development of molecular therapeutic approaches.

In conclusion, we have detected variable levels of methylation/hypomethylation at CpG sites in the mesothelin promoter region in ovarian and endometrial tumors, but there was some correlation with its protein expression status. We speculate that although methylation/hypomethylation changes may affect its transcription, other mechanisms may synergically operate in tissue-specific expression and tumor-related mesothelin overexpression.

Acknowledgment

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Prevalence of human papilloma virus infection in pregnant Turkish women compared with non-pregnant women

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Summary

Purpose of Investigation: We aimed to find a prevalence of human papilloma virus (HPV) in order to define the 100 genotypes and subset of 14 oncogenic genotypes in pregnant Turkish women and to compare these with non-pregnant women. *Methods:* Cervical thin-prep specimens were obtained from 164 women in the first trimester pregnancy and 153 non pregnant women. *Results:* 29.2% of pregnant versus 19.6% of non-pregnant Turkish women had at least one of the 100 types of HPV infection - a statistically significant difference. The rate of 14 high-risk HPV genotype infections was significantly higher in pregnant (14.6) compared to non-pregnant Turkish women (9.6%). *Conclusions:* Pregnant Turkish women are at higher risk for all HPV infections including high-risk cervical cancer genotypes.

Key words: Human papillomavirus; Cervical cancer; Turkish women; Prevalence; Genotyping.

Introduction

Statistical analyses released from the World Health Organization (WHO) suggest that cervical cancer is the second most common cancer in women worldwide [1-3]. It is estimated that each year approximately 493,000 new cases are diagnosed and 274,000 women die from cervical cancer worldwide [4]. The presence of HPV DNA in cervical tissues has implicated HPV as a causative agent in genital condylomatas, in lower female genital tract intraepithelial neoplasias, such as cervical intraepithelial neoplasia (CIN), and in invasive cervical carcinomas [5]. It has been demonstrated that HPV DNA can be detected in approximately 99% of all invasive cervical cancers [6]. In addition, HPV DNA is almost always present in condylomatas and high-grade dysplasias, such as CIN III [7]. HPV types 6 and 11 are known to induce exophytic condylomatas affecting the anogenital mucosa and lower vagina [8]. A subset of HPV types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) are regarded as oncogenic, or high-risk, HPV viral types. This subset represents the predominant HPV genotypes detected in high-grade intraepithelial lesions (CIN II and III) and in carcinomas of the lower female genital tract [6-9]. A basic understanding of HPV epidemiology is required to comprehend the role of various HPV types in the development of cervical cancer and to design effective vaccine strategies against the virus. Different populations may harbor varying HPV genotypes in the genital tract [6]. Thus far, the pregnant and non-pregnant Turkish population has not been studied regarding their prevalence of 100 HPV genotypes. Before utilizing HPV vaccines for a particular population, it is imperative to have relevant HPV genotyping data to provide an optimal vaccine to provide the best possible care for that population. For primary prevention, the approach taken was to develop an HPV vaccine and, recently, HPV prophylactic vaccines have become available in many countries including Turkey. These vaccines are type-specific (for HPV 6, 11, 16 and 18) and protection against cancer is expected to be in the 65-75% range, depending on the distribution of HPV genotypes in the population [10]. For secondary prevention, type-specific HPV testing has been proposed as an additional biomarker to stratify women according to risk for precancerous lesions and cancer [11]. This study provides the baseline data that will be accessible to insure that this population can be appropriately included in vaccine trials in the future.

Material and Methods

Over a 1.5-year time period, 317 cervical samples were collected from pregnant and non-pregnant Turkish women attending the Medico-social Unit of Istanbul University and Sisli Etfal Training and Research Hospital outpatient clinic. Ethical Committee approval of the hospitals was obtained prior to sample collection. Patients presenting at the Gynecology and Obstetrics Clinic for a routine physical examination volunteered to participate in the study. The participants included were sexually active with no previous histological diagnosis or treatment and were seeking cervical cancer screening. A history collection and physical examination were performed on patients, and for conventional Pap smears, samples were prepared on a glass slide. The Paps were diagnosed using the Bethesda system (TBS) in which the following terms are used: atypical squamous cells of undetermined significance (ASCUS), low-grade and high-grade squamous intraepithelial lesion (LSIL, HSIL) [12]. ***DNA isolation:*** Genomic DNA was isolated from the thin prep and biopsy samples according to a standard salting-out protocol. The quality of the DNA isolation was tested with the amplification of the beta-globin gene using the following primers: Globin-F: 5'- GAA GAG CCA AGG ACA GGT AC-3' and Globin-R: 5'-

CAA CTT CAT CCA CGT TCA CC-3'. The amplification of 270 bp product showed the success of isolation. In case of failure the isolation was repeated. ***HPV detection and genotyping:*** In the present study we used a PCR-based assay to detect and genotype human papillomaviruses (HPV) in mucosal samples. For the detection of HPV, nested-PCR was applied to amplify the consensus MY09/11 region of HPV with MY09/11 and GP5+/6+ primers [13]. The amplification products were visualized in EtBr stained agarose gel electrophoresis. The presence of 150 bp products indicated HPV infection. ***Multiplex PCR:*** For HPV genotyping, after amplification of the E6/E7 oncogene region of HPV using consensus E6/E7 primers, nested multiplex PCR with type-specific primers was used to genotype each 100 HPVs including 14 high-risk HPVs (16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) [14]. The amplification products were separated in EtBr stained agarose gel electrophoresis. ***Sequencing:*** In patients who tested negative for high-risk HPV, the GP5+/6+ PCR products were sequenced to determine the genotype of HPV. The fragments were sequenced with automated sequencer ABI 3130 PRISM (Applied Biosystems). The resulting sequences were aligned with the Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). ***Statistical analysis:*** We performed statistical calculations using SPSS Version 13.0 for Windows. The chi-square test was performed to assess the statistical significance of differences in the prevalence of HPV infection in pregnant and non-pregnant women and to assess differences in frequency of HPV infection among four seasonal groups; p values of less than 0.05 were considered significant (95% confidence interval).

Results

Mean ages of women in the groups were similar. Parity and gravidity did not differ in either group (Table 1). Thirty non-pregnant Turkish women out of 153 (19.6%) had at least one of the 100 HPV genotypes in their cervical region. Forty-eight women out of 164 in the first trimester gestation were HPV positive (having at least one of the 100 HPV genotypes), which means a high prevalence of HPV (29.2%) in pregnant Turkish women. On the other hand, HPV infection in pregnancy was significantly higher than in non-pregnant women ($p <$ 0.005). The prevalence of high-risk HPV infection (14 genotypes) was 14.6% and 9.8% in pregnant and non-pregnant women of our population, respectively, and the difference was significant ($p < 0.05$). Of the pregnant women, 12.1% were infected by high-risk HPV genotype 18 or 16 which were included in the vaccination against

HPV infection. Of the non-pregnant women 7.7% were infected by genotype 16 or 18 and the difference was significant ($p = 0.01$). Multiple infection rates in both groups were not different and were very low. Only one pregnant women had three HPV types and two women in the non-pregnant group had two different HPV genotypes together. Two patients in the pregnant group had ASCUS in their cervical cytology; one had genotype 18 and the other had genotype 31 HPV infection. There was no abnormal cervical cytology in the non-pregnant group.

Conclusions

The overall prevalence of HPV infection considering non-pregnant Turkish women was 19.6% which is similar to the American-Indian and Asian population; Bell *et al.* found the prevalence of HPV to be 21.25% in American-Indian women and Li *et al.* found a 22% prevalence of HPV infection in the Northern Chinese population with normal cytology [15, 16]. However this overall prevalence of HPV in our population is relatively high compared to other worldwide studies [17]. Stockman *et al.* found the overall prevalence of HPV to be 45.3% in a French population study which is very high compared to ours [18]. Not only the overall prevalence but also the prevalence of high-risk HPV genotypes were so different in these studies which clearly shows the importance of reginal and ethnic variation in HPV.

Previous studies have indicated a seasonal correlation of HPV infection [19, 20]. However, in our study there was no correlation between HPV infection and seasonal variation.

Two prophylactic virus-like particle-based vaccines (one bivalent vaccine against HPV16 and HPV18 and a quadrivalent vaccine against HPV16/18/6/11) have demonstrated efficacy (90-100%) against persistent infection with targeted types when administered in a three-dose schedule to women who are uninfected with those types [21, 22]. The quadrivalent vaccine is also efficacious in preventing related high-grade cervical lesions, with the results from Phase III trials of the bivalent vaccine awaited [21]. However, because vaccine-induced protection is probably relatively specific for targeted types [23], vaccination will not replace the need for Pap screening programs. Therefore, the potential effectiveness of the vaccine in reducing the burden of Pap abnormalities and cancer will be dependent on local epidemiology. This is why we investigated the prevalence of 14 high-risk HPV genotypes in pregnant and non-pregnant women including type 16 and 18 against which vaccines would be effective, and we found a high prevalence of these in both groups. Thus we concluded that HPV vaccine in the Turkish population, especially before pregnancy, would be highly preventive for cervical cancer.

Why are HPV genotypes, including 14 high-risk genotypes, significantly higher in pregnant women? The reason may be due to an attenuated immune system in pregnancy. A woman may be exposed to genital HPV infection many times but most HPV infections could be

Table 1. — *Demographic properties of the groups.*

	Mean age	Parity	Gravidity
Pregnant women	30.56 ± 7.74	2.4 ± 1.2	2.8 ± 1.4
Non-pregnant women	33.25 ± 8:71	2.8 ± 1.3	3.2 ± 1.5

Table 2. — *Distribution of high-risk HPV genotypes in the groups.*

	HPV						
	16	18	31	33	45	56	58
Pregnant women	12	8	1	2	–	1	–
Non-pregnant women	7	5	1	–	1	–	1

eradicated by her immune system in her normal lifetime. However in pregnancy there is hormonal depression of immune reactions. Another reason may be psycho-social factors in that many couples may decrease the frequency of sexual intercourse due to fear of losing their baby, especially in the first three months of gestation, which may increase multipartner behavior in males.

Bell *et al.* found that the incidence of HPV infection was inversely correlated with age. In younger women (< 24 years) HPV infection was significantly higher (41%, $p < 0.005$) compared to all other age groups [15]. We did not investigate the correlation of age with HPV prevalence because our group of pregnant women was already restricted by reproductive age. However a relatively high HPV prevalence may be partially due to the relatively young ages in the pregnant group and also in the control group.

Multiple infection rates by different HPV genotypes were extremely lower than other populations studied worldwide [17].

Acknowledgment

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Analysis of the cytogenetic response in peripheral blood lymphocytes from breast cancer patients following chemotherapy

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Summary

The presence of chromosomal aberrations induced in circulating lymphocytes from breast cancer patients during chemotherapy was analyzed. Ten breast cancer patients undergoing neoadjuvant chemotherapy and ten healthy women (controls) were evaluated. Metaphases were obtained from cultures of peripheral lymphocytes stimulated with phytohemaglutinin and metaphase blockage was achieved with colchicine. One hundred metaphases were analyzed for chromosomal aberrations and 1,000 cells for the mitotic index. No significant differences were observed regarding the frequency of chromosomal aberrations, number of cells with chromosomal aberrations and mitotic index between the controls and patients before chemotherapy. However, after the first chemotherapy cycle, the numbers of chromosomal aberrations and cells with them was greater. After the third cycle, the mitotic index was lower, but the fifth cycle produced an increase in relation to the third and fourth cycles. The results suggest that chemotherapy raises the number of chromosomal aberrations and favors persistence of stable chromosomal abnormalities.

Key words: Breast cancer; Chemotherapy; Chromosomal changes; Mitotic index.

Introduction

Breast cancer is a complex disease that results from the interaction of multiple environmental, hormonal and lifestyle risk factors associated with the individual genome [1]. Its heterogenous clinical course results from different risk factors such as ethnicity, diet, age, environmental factors and cumulative exposure to estrogen. These factors are believed to be responsible for differences in tumor grade, degree of invasion, potential for metastasis and other complex signs of cell growth and survival [2].

Combined treatment started to be provided in 1974 consisting of primary (neoadjuvant) chemotherapy followed by surgery and/or radiotherapy and it has become commonly administered to patients with locally advanced breast cancer or those presenting inoperable margins [3]. This type of treatment may increase survival through eradicating distant micrometastases and diminishing the size of the tumor, thereby enabling surgery that is more conservative [4, 5]. Another important advantage of neoadjuvant chemotherapy is that it makes it possible to observe the response of the primary tumor to treatment [6].

Chemotherapy drugs act on cells to interfere with the growth and division process, mostly in a nonspecific manner. Thus, they are usually toxic to rapidly proliferating tissues with high mitotic activity and short cell cycles [6, 7]. Despite the benefits observed following chemotherapy, there is an increased risk of leukemia among breast cancer patients who undergo this type of treatment [8].

Evaluation of chromosomal aberrations is useful for studying radiosensitivity and risk factors. The micronucleus test on breast cancer patients has demonstrated that patients present greater numbers of micronuclei than controls do [9]. In a study conducted by our group, increased numbers of micronuclei were also demonstrated in patients with risk factors for cancer of the uterine cervix [10]. Several studies have evaluated the radiosensitivity of peripheral lymphocytes by means of culturing [11, 12], through new methodologies with cancer risk factor scores [9, 13, 14] and as treatment assessments [15]. Certain chromosomal abnormalities may characterize cancer with a poor prognosis [16].

Therefore, studying genetic aberrations may be used to analyze genetic damage following cancer treatment, and to determine risk factors. Few studies have analyzed the influence of neoadjuvant chemotherapy on treatments for breast cancer and genetic abnormalities. The aim of the present study was to analyze the presence of chromosomal aberrations induced in peripheral blood lymphocytes, in breast cancer patients. For this, patients were compared with a control group before any treatment and were also evaluated after each sequential chemotherapy cycle.

Patients and Methods

Patients and controls

Ten women with a diagnosis of breast cancer who underwent neoadjuvant chemotherapy without any type of previous antineoplastic treatment or use of immunosuppressor drugs were selected randomly and evaluated prospectively. All of these patients were attended at the Mastology Outpatient Clinic of the Oncological Research Institute (IPON)/Discipline of Gynecol-

ogy and Obstetrics of the Teaching Hospital of the Federal University of the Triângulo Mineiro (UFTM). The samples were collected between 2004 and 2006. The diagnosis was made by means of clinical and mammographic examinations and confirmed by means of puncture for fine-needle aspiration biopsy and/or core biopsy. The anatomopathological staging followed the recommendations of the American Joint Committee on Cancer (AJCC), together with the Committee of the International Union against Cancer (UICC). This staging reflected the extent of the tumor expressed through the TNM system – tumor size (T), presence of axillary node (N) and/or metastasis (M) – and made it possible to then give priority to the most appropriate treatment [17]. Data such as age, ethnicity, side of the breast affected, drugs used in the chemotherapy and type and stage of the tumor were gathered from the patients' medical files.

The controls were healthy female volunteers from the community, i.e., they did not have any diagnosed disease and were not using immunosuppressor drugs. They were approached and invited to participate at the time when blood samples were being collected from the patients. The control and patient groups were paired with regard to age and presence of smoking habit.

The project was approved by the Research Ethics Committee of UFTM and all the patients who agreed to participate signed a free and informed consent statement.

Chemotherapy

The treatment was carried out over six or eight cycles, with 21-day intervals between the cycles and when the total leukocyte count was greater than or equal to 2000/mm^3. The latter was evaluated by means of a leukogram, produced on average two to three days before starting each cycle. The chemotherapy regimen consisted of one of the following combinations: (a) AC: adriamycin (50 mg/m^2) and cyclophosphamide (500 mg/m^2); (b) EC: cyclophosphamide (500 mg/m^2) and epirubicin (50 mg/m^2); or c) CMF: cyclophosphamide (500 mg/m^2), methotrexate (50 mg/m^2) and 5-fluorouracil (600 mg/m^2). Since the treatment was individualized, its maintenance for periods shorter or longer than what was initially prescribed was dependent on the tumor response.

Blood collection

Samples of peripheral venous blood were collected from the patients using disposable sterilized material and following all the principles of asepsis. This was done on two different occasions: (1) before the first chemotherapy cycle; and (2) around 21 days after finishing each cycle, immediately before starting the next cycle. The latter was the amount of time needed for recovery of the medullary aplasia induced by chemotherapy. On each occasion, one sample of 5 ml of blood was collected in a tube containing anticoagulant (heparin, 100 UI/ml), which was used for lymphocyte culturing. After collection, the samples were conserved at 4ºC for a few hours until the cultures were performed.

The same procedure was followed for collecting blood from the healthy volunteers on a single occasion.

Lymphocyte cultures

The presence of chromosomal aberrations was analyzed by means of metaphases obtained from lymphocytes [18]. The blood was collected in RPMI 1640 medium (Gibco®) and/or Dulbecco's medium (Gibco®) and was centrifuged (10 min; 1200 rpm) to separate the leukocytes. The lymphocytes were added to cultures containing 70% RPMI 1640 medium (Gibco®)

and/or Dulbecco's medium (Gibco), 30% fetal bovine serum (Gibco®), 0.3% phytohemaglutinin (Sigma®) and 0.1% of glutamine. The cultures were incubated for 72 hours at 37°C. The metaphases were blocked by adding to each culture 25 μl of colchicine (0.16%), 60 min before cell collection. Two cultures were made from each sample.

After 72 h of incubation, cells were collected and were subjected to hypotonic treatment with 0.075M KCl, for 25 min. Next, they were fixed using a solution of methanol and acetic acid (3:1), three times. After fixing, the cells on their slides were stained using Giemsa solution and Sorënsen buffer for 5 min.

Chromosome analysis

The metaphases were analyzed in a blind test using an optical microscope with an immersion objective lens (magnification of 1000x). To quantify chromosomal aberrations, 100 metaphases were analyzed per individual. Only metaphases with 46 ± 1 chromosomes that were well spread out without overlapping of the chromosomes were used. Structural abnormalities such as gaps, breaks, acentric fragments, rings, dicentric chromosomes, triradial chromosomes, telomeric associations and exchanges were investigated, following the terminology that has been proposed [19, 20]. The mitotic index was determined as the ratio of the number of metaphases per 1,000 cells and was expressed as a percentage.

Statistical analysis

The results were evaluated by means of the Sigmastat 3.1 and Statistica 6.0 software. The Kolmogorov-Smirnov test was used to investigate whether the data presented normal distribution and the Levene test was used to investigate the homogeneity of the variance. Since the distribution was normal, the results were presented as means and standard deviations. ANOVA-F analysis was performed, followed by the unpaired Student's t-test, for comparisons between the controls and patients before chemotherapy, and the paired test between the groups before and after treatment. The significance level was 5%.

Results

Study population

Ten patients with breast cancer and ten healthy female volunteers (forming the control group) were evaluated. The patients' mean age ($\pm$ SD) was 54.10 ± 17.10 years (range 24-86 years) and the mean for the control group was 51.9 ± 17.93 years (range 21-85 years). Nine of the patients (90%) were white and one (10%) was black, while all the women in the control group were white. In six patients (60%), the tumor was in the left breast; three (30%) presented a tumor in the right breast and there was one case of bilateral cancer. In this last patient, 51.7% of the chromosomal aberrations in the metaphases were located in the group E chromosome. None of the members of this patient's family had breast cancer. The histological type most frequently found was ductal carcinoma, in eight cases (80%), while lobular carcinoma was diagnosed in two patients (20%). The chemotherapy regimens used were EC in five cases, AC in four and CMF in one.

Chromosomal aberrations

Chromosomal aberrations were analyzed in the metaphases of lymphocytes from the controls and patients. Out of the total of 4,700 metaphases from the patients analyzed, 213 cells with aberrations were found, with a total of 244 aberrations, thus indicating that some cells had more than one aberration. In the control group, 1,000 metaphases were analyzed and 12 cells with chromosomal aberrations were found. The principal aberrations encountered were simple abnormalities such as chromatid breaks, gaps, chromosomal breaks and fragments. Complex abnormalities such as rings and dicentric, triradial and quadriradial chromosomes were only found in the patients, but with lower frequency (Table 1). Before the chemotherapy, the patients presented a higher frequency of cells with chromosomal aberrations and greater number of cells with chromosomal aberrations than observed in the controls, although without reaching statistical significance (Table 2).

Table 1. — *Description of the types of chromosomal aberrations found in metaphases from breast cancer patients and controls.*

Type of chromosomal aberration	No. of chromosomal aberrations %	
	Controls (%)	Patients (%)
Chromatid gap	3 (25.0)	32 (13.11)
Chromosome gap	2 (16.66)	39 (15.98)
Chromatid break	1 (8.33)	38 (15.57)
Chromosome break	1 (8.33)	67 (27.46)
Ring	0 (0)	3 (1.23)
Dicentric chromosome	0 (0.0)	6 (2.46)
Fragment	5 (41.66)	57 (23.36)
Triradial chromosome	0 (0)	1 (0.41)
Quadriradial chromosome	0 (0)	1 (0.41)
Total number of chromosomal aberrations	12 (12.0)	244 (244.0)
Number of cells with chromosomal aberrations	12 (12.0)	213 (213.0)
Number of metaphases analyzed	1,000	4,700

Table 2. — *Number of chromosomal aberrations (NCA), number of cells with chromosomal aberrations (NCCA) and mitotic index (MI) in the controls and in breast cancer patients who underwent chemotherapy. Values are expressed as means and standard deviations, with minimum and maximum values in between brackets.*

Groups (n)	NCA	NCCA	MI
Controls	1.2 ± 1.2	1.2 ± 1.2	1.9 ± 0.9
(10)	(0-3)	(0-3)	(0.7-4.3)
Before chemotherapy	3.5 ± 3.6*	3.5 ± 3.6*	2 ± 0.8***
(10)	(0-12)	(0-12)	(1-3)
1st cycle	5.2 ± 4.6	4.8 ± 3.8	1.5 ± 0.6***
(10)	(0-16)	(0-13)	(0.2-2.6)
2nd cycle	7.4 ± 5.0**	5.1 ± 3.2	2 ± 2.1
(8)	(0.17)	(1-9)	(0.3-7.1)
3rd cycle	5.5 ± 4.2	4.9 ± 3.0	1.1 ± 0.4#
(8)	(1-14)	(1-9)	(0.7-2)
4th cycle	7.0 ± 4.9	6.2 ± 4.2	1.5 ± 0.6#
(5)	(3-15)	(3-13)	(1.1-2.6)
5th cycle	3.2 ± 1.5	3.2 ± 1.2	2.1 ± 1
(6)	(2-5)	(2-5)	(1-3.5)

n = number of individuals; mean ± standard deviation; V_{MIN} and V_{MAX} = minimum and maximum values, respectively.
ANOVA-F for repeated measurements (F = 3.171; $p < 0.05$); paired t test, * $p < 0.01$ in comparison with the fourth cycle; ** $p < 0.05$ in comparison with the third cycle; *** $p < 0.01$ in comparison with the third cycle; # $p < 0.05$ in comparison with the fifth cycle.

Increases in the total number of chromosomal aberrations and the number of cells with chromosomal aberrations were also observed between the first and fourth treatment cycles, in comparison with the findings before chemotherapy. Statistical significance was reached after the fourth cycle. After the fifth treatment cycle, there was a return to close to baseline values (Table 2). There was no statistically significant difference in mitotic index between the control and patient groups before the chemotherapy, but there was a significant decrease in mitotic index after the third treatment cycle, in relation to before the chemotherapy and after the first cycle, and there was an increase after the fifth cycle (Table 2).

Discussion

The purpose of this study was to analyze the effect of chemotherapy on the frequency of chromosomal aberrations in lymphocytes from women with breast cancer. Cytogenetic studies are a classical means of evaluating mutagenicity and clastogenicity because of their sensitivity of response to agents that induce DNA damage. The frequency of chromosomal aberrations in lymphocytes from human peripheral blood has been used as a marker for the initial effects induced by occupational exposure or chemotherapy in specific types of tissue. Assuming that the mechanisms for chromosomal damage are similar in different types of tissue, the level of damage to lymphocytes may reflect the damage induced in other types of tissue [21]. An accumulation of chromosomal abnormalities may affect critical genes involved in cell proliferation, differentiation and survival and thus direct the processes of the multiple stages in the development and progression of cancer [22].

Among the patients evaluated in this study, 70% were aged between 40 and 69 years, which was in accordance with data showing that the greatest incidence of breast cancer affects women within this age group. The left breast was the one most affected and, since this was more voluminous than the right breast, increased volume of breast tissue might be associated with a greater likelihood of mutations [6]. Only one of the patients was black, and this is in line with other studies that have demonstrated greater incidence among white women [6, 23]. Cytogenetic studies have demonstrated that white women with breast cancer present greater numbers of chromosome abnormalities than women of other ethnicity do [24]. Ductal carcinoma was more frequent than lobular carcinoma, and this was concordant with other studies [25] and with data obtained at our clinic, at which 90.6% of the cases diagnosed were ductal carcinoma [26].

Blood was not collected from all of the patients in relation to all cycles for a variety of reasons, such as cases that received blood transfusions because of leukopenia, difficulty in performing venous puncture and patient debilitation. Despite the small number of patients, the study was shown to be representative, with characteristics similar to those of studies with greater study populations.

The abnormalities in group E chromosomes that were

seen in one patient may have related to inactivation of tumor suppressor genes or activation of proto-oncogenes such as *p53, BRCA, E-cadherin and HER-2,* which are found in the chromosomes of this group [27-30].

To evaluate the residual chromosomal aberrations induced by chemotherapy and obtain the greatest number of metaphases, the blood collection was performed immediately before each cycle. In this way, it was expected to find stable chromosomal aberrations that had not been eliminated by the cells during the repair. The chromosomal aberrations observed most frequently, both in the control group and in the breast cancer patients, were simple abnormalities such as gaps, chromatid breaks, chromosome breaks and fragments. Studies have correlated the presence of chromatid breaks as a response to the action of chemotherapy agents [21]. In tests on G2 radiosensitivity to chemotherapy, greater frequency of breaks and gaps was also observed [31].

The frequency of chromosomal aberrations found in the control group was within the baseline frequency range for healthy individuals, i.e., 1-2% [32]. Both greater frequency of cells with aberrations and greater numbers of aberrations were observed in the patients before the chemotherapy, in relation to the controls, although without any statistically significant difference. This was concordant with other studies [33] and suggests that the cells of cancer patients present a higher frequency of abnormalities. Patients with breast cancer present more DNA damage than is seen in control groups, according to the comet test [34]. They also present greater lymphocyte sensitivity to induction of chromosome damage by means of radiation, as shown by the micronucleus test [35, 36].

Following chemotherapy there was an increased frequency of chromosomal aberrations, reaching significance after the fourth cycle, compared with before the treatment. However, after the fifth cycle, there was a reduction in the frequency of aberrations, such that values close to baseline were reached, thus demonstrating that the cell damage was probably undergoing repair. Among groups of patients with lung cancer and ovarian cancer, increased frequency of micronuclei during the first half of the therapy have been reported, with a peak in the second or third cycle and subsequent decline with continuing treatment, thereby reaching values lower than found before the treatment. Two possible mechanisms may be involved in these results: 1) repopulation with leukocytes may have occurred faster than the formation of cytogenetic damage; or 2) the lymphocytes became resistant to chemotherapy drugs [37]. Other studies have detected accumulations of chromosomal aberrations over the last two cycles of chemotherapy, thus demonstrating the difficulty of recovering the damage induced by chemotherapy [6].

Analysis of cell proliferation and progression in tissues exposed to clastogenic agents may be used to observe these agents' influence on the cell cycle. Any disturbance to the events controlling the progression of cell division may stop the cells from following their normal course.

They might remain halted in one phase, or apoptosis might be induced [38]. No statistically significant difference in mitotic index was found between the controls and breast cancer patients, perhaps because an increase in the number of chromosomal aberrations occurred between these groups. There was a significant reduction in the mitotic index after the third cycle, in relation to before the chemotherapy and after the first cycle, along with an increase in the number of chromosomal aberrations, thus demonstrating that the cells were not undergoing repair yet. However, an increase in the mitotic index was also found in the fifth cycle, compared with the third and fourth cycles, thereby showing that the cells had started to undergo repair of the damage caused by the chemotherapy and taking the number of chromosomal aberrations towards pretreatment values.

The variation between individuals may have been due to differences in how the chemotherapy drugs were metabolized [39] and differences in the degree of reduction of leukocyte numbers [37]. The persistence of high frequencies of cells with rearrangements that seem to be stable for many years after finishing the cyclophosphamide, methotrexate or 5-fluoracil therapy suggests that although these cells present severe aberrations, they become viable progenitors in that they enable survival and cell proliferation. Within this context, there may be increased incidence of hematological diseases secondary [40] or immunological changes [41, 42] after chemotherapy administration for cancer treatment.

In conclusion, the results suggest that evaluation of the cytogenetic damage to lymphocytes from breast cancer patients may be able to estimate the sensitivity to chemotherapy, considering that persistence of stable chromosomal aberrations may lead to increased risk of secondary neoplasia.

Acknowledgements

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Is sentinel node biopsy reliable in large breast tumors?

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Summary

Purpose: The value of sentinel lymph node biopsy (SNB) in patients with larger breast tumors (diameter > 3 cm) has been questioned due to high false-negative rates reported from initial studies. The aim of this study was to analyze the safety and prognostic reliability of SNB in this group of patients. *Methods:* During a 6-year period (2001-2007), 84 women with mean age 51.7 ± 11.6 years diagnosed with a breast tumor larger than 3 cm in diameter on pathological analysis were retrospectively identified from the database of our institution. Sentinel node identification was performed after injection of blue dye subcutaneously at the subareolar area. The sentinel node specimen was sent for frozen section analysis. Regardless of the SNB results, all patients underwent completion axillary clearance. *Results:* Breast surgery consisted of mastectomy in 62 patients (73.8%) and partial mastectomy in 22 patients (26.2%). There were 69 invasive ductal cancers (82.1%), 14 lobular cancers (16.6%) and one case of anaplastic carcinoma (1.3%). Nine tumors (10.7%) were identified to be multifocal after the histopathological report. The mean number of sentinel nodes removed was 1.5 ± 0.7 (range 1-4) while SNB detection was not feasible in three patients (3.6%). Of 56 positive SNBs, seven (12.5%) were not identified by routine hematoxylin and eosin staining during frozen section analysis but were detected by subsequent immunohistochemistry on the final histopathological report. All patients with multifocal tumors presented nodal metastases on pathological analysis (100%), while the rate of nodal metastatic disease in patients with unifocal tumors was 16% (12 patients), although no statistical significance was documented. The overall false-negative rate, defined as the percentage of all node-positive tumors in which the SNB was negative, was 14.3%. The false-negative rate was significantly higher for the group of patients with multifocal tumors (55.5%) compared to the group with unifocal tumors (9.3%) ($p < 0.001$). Conclusions: The present study indicates that sentinel node biopsy is feasible in patients with larger breast tumors (max. diameter > 3 cm), with comparable false-negative and sentinel detection rates (14.3% and 96.4%, respectively). Larger tumor size seems to be associated with increased incidence of nodal metastases while multifocality appears to be related to increased false-negative rates; hence completion axillary clearance should be initially considered for these cases.

Key words: Breast cancer; Sentinel lymph node; Multifocality.

Introduction

Breast cancer is the commonest malignancy among the female population and one of the leading causes of mortality worldwide [1]. For women diagnosed with breast cancer, the assessment of axillary nodal status is a crucial parameter for staging the disease as well as a critical indicator of prognosis. Although axillary lymph node dissection is still the gold standard for evaluating nodal status in breast cancer, the long-term complications of this technique in breast cancer survivors, such as reduced shoulder mobility, shoulder weakness, sensory disturbance, neuralgia and permanent arm lymphoedema [2,3], lead to a wide acceptance of sentinel node biopsy in all cases of early breast cancer. Since Morton and colleagues introduced this technique in 1992 [4], it has been well established and validated in the surgical treatment of early breast cancer. A lot of studies have evaluated the role of the sentinel node in cases of early invasive breast cancer [5, 6] and despite concerns regarding false-negative rates, and variation and long-term implications of failing to identify axillary metastases, this technique has become widely applicable.

In cases of larger breast tumors, the value of SNB has been questioned. Initial studies resulted in high false-negative rates as to 18% for T2-T3 tumors [7] and the reliability of this technique has been doubted, while other studies have reported no statistically significant difference in false-negative rates when SLNB is applied in larger tumors compared to axillary lymph node dissection (8,9). Therefore, further validation trials for added assurance regarding the safety of SLNB in terms of axillary recurrence and survival are necessary.

In this study, the experience with sentinel node dissection for larger breast tumors (> 3 cm) with clinically negative axilla in a tertiary institution is reported, aiming to evaluate the safety of this technique and to define the major factors of false-negative results in this group of patients.

Materials and Methods

A retrospective review of the clinical records of patients diagnosed with breast cancer during a 6-year period was undertaken after approval by the ethics committee of our institution. All cases with a breast tumor larger than 3 cm in diameter on pathological analysis were included in the study. Neoadjuvant chemotherapy or radiotherapy, multifocal tumor diagnosed preoperatively, clinically suspected axillary node metastases and

known allergy to the blue dye were exclusion criteria for the study. Eighty-four women were eligible for inclusion in the study. Sixty-two patients derived from the initial learning and validation phase of SNB when axillary lymph node dissection was routinely added while 22 patients were additionally included afterwards due to large sized tumors > 3 cm where axillary dissection was the optimal surgical approach independently of sentinel node results. All procedures were performed by two surgeons. Sentinel node identification was performed after injection of blue dye (Patent Blue V; Guerbet, Paris, France) subcutaneously at the subareolar area. No radioactive tracer was used in any case. After the excision of the specimen it was sent for frozen section analysis. At least three sections were prepared from the sentinel node or each part of bisected nodes, and examined by hematoxylin and eosin staining (HES). All patients underwent an additional axillary lymph node dissection including levels I and II, even if the frozen section analysis was negative.

The definitive assessment of fixed SLNs included serial sectioning with hematoxylin-eosin staining and anti-cytokeratin immunohistochemical (IHC) staining. The overall false-negative rate, which was the primary end-point, was defined as the percentage of all node-positive tumors in which the SNB was negative.

Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS 13.0, SPSS Inc, Chicago, Illinois, USA; $p < 0.05$ was considered statistically significant.

Results

A total of 84 women with breast cancer met the inclusion criteria and were enrolled in the study. The mean age of the patients was 51.7 ± 11.6 years. Breast surgery consisted of mastectomy in 62 patients (73.8%) and partial mastectomy in 22 patients (26.2%). Primary tumor diameter ranged from 30 to 59 mm (36 ± 0.6) including 60 T2 tumors (71.4 %) and 24 T3 tumors (28.6 %). There were 69 invasive ductal cancers (82.1%), 14 lobular cancers (16.6%) and one case of anaplastic carcinoma (1.3%). The mean number of sentinel nodes removed was 1.5 ± 0.7 (range 1-4) while SNB detection was not feasible in three patients (3.6 %).

Of 56 positive SNBs, seven (12.5%) were not identified by routine hematoxylin and eosin staining during frozen section analysis but were detected by subsequent immunohistochemistry on the final histopathological report. Twelve patients with a negative SNB were found to have positive axillary non-sentinel lymph nodes after the definitive histopathological assessment, resulting to an overall false-negative rate of 14.3%.

Among the 84 patients enrolled in the study, nine (10.7%) had a multifocal tumor at the final histopathological report. All these patients presented nodal metastatic disease on pathological analysis, while the rate of nodal metastases in patients with unifocal tumors was 16% (12 patients), but no statistical significance was documented. The false-negative rate was also significantly higher for the group of patients with multifocal tumors (5 of 9, 55.5%) compared to the group with unifocal tumors (7 of 75, 9.3%) ($p < 0.001$). Nevertheless, due to the small statistical sampling, these differences might not be meaningful.

All patient characteristics are summarized in Table.

Table 1. — *Patient characteristics*.

Patients	84	
Age (mean ± SD), years	51.7 ± 11.6	
Type of surgery		
Total mastectomy	62 (73.8%)	
Partial mastectomy	22 (26.2%)	
Staging/Histopathological analysis		
Primary tumor diameter (mm)	30-59, 36±0.6	
T2	60 (71.4%)	
T3	24 (28.6%)	
Invasive ductal cancer	69 (82.1%)	
Lobular cancer	14 (16.6%)	
Anaplastic cancer	1 (1.3%)	
Number of sentinel nodes removed	1.5 ± 0.7 (range 1-4)	
No *feasible* detection	3 patients (3.6%)	
Focality		
Unifocal tumors	75 (89.3%)	
Multifocal tumors	9 (10.7%)	
Nodal metastatic disease	12 of 75 unifocal tumors (16%)	
	9 of 9 multifocal tumors (100%)	
	Nodal metastatic disease in patients with negative SNB	*False-negative rate*
Overall	12	14.3%
Unifocal tumors	7	9.3%
Multifocal tumors	5	55.5%

Discussion

Sentinel lymph node biopsy is today a well established technique for treating patients with early-stage breast carcinoma. It provides important prognostic information to direct adjuvant treatment and helps avoid the morbidity of unnecessary lymph node dissection. Several studies have demonstrated an increased rate of axillary metastases in patients with larger breast tumors [10,11] suggesting that fewer patients in this group have any benefit from this procedure. Nonetheless, the reliability and feasibility of this technique in multifocal or large unifocal tumors has been poorly investigated and even if some patients with a negative SNB do not require additional axillary lymph node dissection, this would be a major benefit.

Although an association between experience with this technique and the detection rate of the sentinel node has been reported [12], our detection rate of 96.4% is comparable to that of other early validation studies [13,14]. Therefore, although most of the patients enrolled in this study came from the learning curve of this technique, the results indicate that SNB is also feasible in larger breast tumors, in accordance with the results of Bergkvist *et al.* [15] who stated that the surgical experience is not a crucial parameter for higher false-negative results when performing sentinel node biopsy.

The accuracy of SNB in multicentric and multifocal invasive breast cancers has been evaluated by Tousimis *et al.* [16] and it resulted that there were three false-negative SNBs in a total of only four T3 breast tumors. In the Axillary Lymphatic Mapping Against Nodal Axillary Clearance (ALMANAC) trial [17], although patients were not excluded on the basis of large tumor size, only

three of 75 multifocal tumors were T3 and no information was given about the T stage of the three tumors found with a false-negative SNB.

A recent study by Schüle *et al.* [18], evaluated the sentinel node biopsy in patients with breast cancer larger than 3 cm in diameter and it resulted that this technique is reliable in cases of unifocal tumors although there was a statistically higher proportion of false-negative results in multifocal tumors.

In the present study, although multifocality was an exclusion criterion, nine patients were identified to have a multifocal breast tumor after the final histopathological report. The false-negative rate of SNB in this subgroup of patients was statistically higher compared to the subgroup of patients with unifocal tumors, thus proposing that SNB is not reliable in these tumors. In contrast, other studies propose that SNB results are comparable in unifocal and multifocal tumors and state that this technique is reliable even if multifocality is detected preoperatively [16,19]. Therefore, current recommendations for SNB in multifocal tumors might be re-evaluated.

Although larger breast tumor and multifocality have been reported to be independent risk factors for nodal metastatic disease [20], the results of this study support that SNB is also feasible and efficient in cases of T2 and T3 tumors, unless multifocality is detected. Larger multifocal tumors necessitate complete axillary lymph node dissection. A major impediment still remains the lack of an exact definition of a multifocal tumor as it varies considerably among several studies [15,21]. Therefore, an international consensus is essential along with more randomized control trials in order to derive strong recommendations for the optimal treatment of these patients.

Conclusion

Although the reliability of sentinel node dissection in larger breast tumors has been questioned in initial studies, the current study indicates that this technique is feasible in patients with larger breast tumors (max. diameter > 3 cm), with comparable false-negative and sentinel detection rates, unless multifocality is present.

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Laser vaporization in the management of CIN

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Summary

Aims: To evaluate the effectiveness of laser CO_2 vaporization in high-grade cervical intraepithelial neoplasias and to assess the diagnostic reliability of cytology, colposcopy, microbiology and HPV tests in predicting recurrence in a long-term outcome. *Methods:* Forty-four patients affectd by high-grade cervical intraepithelial neoplasia (HG-CIN) were submitted to laser CO_2 vaporization and followed-up a minimum of five years. Vaginal smears for microbiological examination were detected. HPV testing was performed by polymerase chain reaction (PCR). *Results:* The average age of the patients was 19.5 years (range 15-24). The cure rate after a single treatment was 95%. Two cases (5%) revealed HG-CIN persistence after three months. The five year follow-up of all cases submitted to a second laser procedure revealed negative cytologic and colposcopic findings. *Conclusions:* A higher degree of expertise and experience from the colposcopist and long-term follow-up proves the effectiveness of laser vaporization in the management of CIN in young women. It has been suggested that HPV infection alone may not be sufficient to promote carcinogenesis and that other cofactors could be involved. Microbiological tests are important to identify and treat any inflammation which might represent a cofactor of HPV infection in the pathogenesis of cervical dysplasia. Cytocolposcopic long-term follow-up, microbiological and HPV tests can improve regression of disease.

Key words: Cervical intraepithelial neoplasia; HPV; Recurrence; Adolescents.

Introduction

Cancer of the uterine cervix is the second most common cancer among women worldwide, with approximately 493,000 new cases and 274,000 deaths in 2002 [1].

The casual role of HPV in all cancers of the uterine cervix has been firmly established biologically and epidemiologically [2].

High-risk HPV (HR HPV) infections seem to persist longer than low-risk infections [3].

During reproductive age, if a cytological high-grade squamous intraepithelial lesion (HG-SIL) is detected, conservative treatment is mandatory to eradicate the lesion and to preserve reproductive function [4]

Often excisional methods have also been chosen as the treatment for ectocervical high-grade cervical intraepithelial neoplasias because of the significant incidence of microinvasion in excised specimens and reported cases of invasive disease following local destructive procedures [5-8].

The advantages of carbon dioxide laser vaporization in treating CIN have been outlined, but most studies have not provided long enough follow-up periods to acquire sufficient information on recurrence rate and disease progression [9-12].

A skilled colposcopist with cyto-colposcopic follow-up is indicated after conservative treatment of CIN [13-16].

Persistence of HPV is associated with an increased risk of developing cervical dysplasia and cancer. Women who test positive for HPV persistently over time have been shown to be at the highest risk of developing preneoplastic genital disease [17].

It has been suggested that HPV infection alone may not be sufficient to promote cervical carcinogenesis and that other cofactors could be involved [2].

Microbiological tests in abnormal cytology are important to identify and treat any inflammations, even if asymptomatic, which might represent a cofactor of HPV infection in the pathogenesis of cervical intraepithelial lesions [18].

The objective of this study was to evaluate the effectiveness of CO_2 laser vaporization of high-grade cervical intraepithelial neoplasia (HG-CIN) in adolescents and to assess the diagnostic reliability of cytology-colposcopy, microbiological and high-risk HPV testing, improving relapse disease in long-term follow-up.

Materials and Methods

Forty-four patients with abnormal cervical cytology classified as HG-SIL, formulated in agreement with the Bethesda System [19] and histologically confirmed, were recruited.

The patients were interviewed about personal history (age, parity, sexual habits, referred tobacco use, oral contraceptive use, previous cervical treatments and past genital infections) and gave their informed consent.

Microbiological testing was performed by vaginal smears through microbiological cultures and by fresh bacterioscopic examination.

Molecular detection of HR HPV was performed by PCR.

Cytological samples were collected in sterile 1.5 polypropylene tubes and resuspended in 100 μl of digestion buffer with proteinase K, incubated overnight at 37°C, and boiled for 5 min. Aliquots (10 μl) of each were used for PCR amplification. Each cytological sample was analyzed by PCR for HPV open reading frame sequences using the following primers: HPV-16, 5'-ACC gAA ACC ggT Tag TATAAAAgC-3' and 3'-gAT CAT TTg TCT CTg gTT gCA AAT-5'; HPV-18, 5'-CAC ACC ACA ATA

CTA Tgg CgCgCT-3' and 3'-CTg CTg gAT TCA ACg gTT TCT ggC-5'. Every amplification experiment included one negative and one positive control for each viral type. A portion of exon 15 of the human APC gene was routinely amplified as a positive control using the following primers: APC, 5'-gTCCTTCACAgAAtgAAAgATg-3' and 3'-CTg CTT gAA gAA gAC ATA TgTTTCg-5'.

The sizes of the amplified fragments were 576, 360 and 520 bp, respectively. Amplification reactions were carried out in 100 μl of reaction buffer containing 50 mM KCl, 2 mM MgCl2, 10 mM Tris (pH 8.3), 200 μM each deoxynucleotide triphosphate, 2.5 units of Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT), 100 pmol of each primer, and 10 μl of proteinase K-digested sample. Samples were denatured at 95°C for 5 min, followed by 40 cycles of amplification (denaturation at 94°C for 1.5 min, annealing at 55°C for 2 min, except APC, where annealing was at 40°C and 57°C, respectively, and extension was at 72°C for 2 min; the final extension was prolonged to 7 min).

Amplified products (15 μl) were electrophoresed through 1.6% agarose gels. The gels were analyzed by UV after staining with ethidium bromide [20].

The patients underwent colposcopy (Zeiss OM 50 colposcope. Carl Zeiss Inc; Germany) using a 5% acetic acid solution followed by the Lugol test and colposcopic findings were interpreted according to the International nomenclature [21].

Topography and the size of ectocervical lesions were determined by colposcopy and the number of quadrants involved by the abnormal transformation zone (ANTZ) were recorded dividing the cervix into four quadrants (1 quadrant or 25% of the cervix, 2 quadrants or 25-50% of the cervix, 3 quadrants or more than 50% of the cervix, 4 quadrants or more than 75% of the cervix).

The patients whose colposcopic examination detected completely ectocervical lesions and an entirely visible squamo-columnar junction (SCJ) and no more than three quadrants of cervix involved by lesions were considered available for treatment.

Laser vaporization was performed using a laser CO_2 Coherent System 451, connected to a Zeiss OM50 colposcope (Carl Zeiss Inc; Germany); surgical procedures were carried out in day surgery, under colposcopic guidance, without local anesthesia. The mean ablation depth was 7 mm (range 6-8 mm). Laser vaporization was performed in patients whose cytology, colposcopy and histology were in agreement.

The patients were followed-up by cytology and colposcopy every three months for the first year, every six months for the second year and then annually for a minimum of five years.

In any suspicious area of persisting or relapsing lesion, a colposcopic directed biopsy was performed.

In patients with histologically confirmed relapsing of HG-CIN a large electrosurgical excisional procedure (LEEP) was carried out.

The median follow-up was 72 months (range: 60-120 months).

Results

Histological analysis of colposcopy-directed biopsies revealed CIN2 in 11 out of 44 (25%) patients and CIN3 lesions in 33 out 44 (76%) patients.

The average age of patients was 19.5 years (range: 15-24), mean parity 1.7 (0-5), mean age for first sexual intercourse 16 years (14-24), and the mean number of sexual partners four (1-10); 52% referred tobacco use, 32% pre-

Table 1. — *Cytology, histology and HPV test results.*

Initial cytology	Histology	HPV test	
No. of patients (%)		pos 16 / pos 16-18	
High SIL	CIN2 11 (25%)	11	–
	CIN3 33 (75%)	29	4
Total 44		40	4

SIL = squamous intraepithelial lesion; CIN = cervical intraepithelial lesion.

vious cervical treatment for clinical HPV lesions or CIN and 74% for past genital infections.

In every patient pretreatment colposcopy visualized an entirely detectable SCJ and an ectocervical ANTZ.

Six (13%) ANTZ cases showed grade 1 of abnormality and 38 (87%) revealed grade 2.

Cumulative failure rate at first treatment was 5% and where the lesion involved three quadrants the percentage was 4%.

Microbiological examination was negative in 11 (26%) for vaginal secretion and positive in seven (15%) for mycetes, in four (8%) for *Gardnerella vaginalis*, in six (13 %) for *Trichomonas vaginalis*, in eight (16%) for *Chlamydia tracomatis*, in three (6%) for Trichomonas and Gardnerella, in two (5%) for Streptococcus agalactiae, in three (6%) for Enterococci and in two (5%) for Cocchi.

The patients were submitted to proper local and systemic therapy before starting any surgical treatment.

HPV testing resulted positive for HPV type 16 in 40 cases (91%) and for HPV types 16-18 in four (9%). No early or late complications were observed.

The patients fully returned to normal activities within four to six weeks of treatment.

Forty-two (91%) patients had negative cytology and colposcopy at the first year follow-up check.

In two patients (5%) HG-CIN recurrences were observed.

Detectability of SCJ after treatment was considered the parameter defining a satisfactory colposcopic follow-up; in 43 treated cases (97%) the SCJ was entirely visible. In one (3%) it was partially endocervical and visible only after dilation of the cervical canal.

At one-year follow-up seven (16%) patients were positive for HR HVP testing but they had negative cytology.

At two-year follow-up four (9%) patients were positive for HR HVP testing and they also had negative cytology.

The 5-year follow-up of all cases submitted to a second laser procedure revealed negative cytological and colposcopic findings. All had complete regression of cytological abnormalities within a year of treatment.

Discussion

Persistence of HPV is associated with an increased risk of developing SIL and cancer. In younger women, it has been shown that persistent viral detection represents a more accurate measure of risk for development of cervical neoplasia than do tests taken at a single point in time.

Several prospective studies have shown that women

who are HPV-DNA-positive at baseline have a higher risk of developing CIN3 or invasive cervical cancer during the follow-up than HPV-DNA-negative women [2].

In our series the 5-year failure rate was 18% (percentage of treated patients with absence of recurrent/persistent HG CIN). A single destructive treatment case of invasive carcinoma occurred after a mean follow-up of six years.

Increasing grade of CIN has been identified as an important factor in failure rates but a correlation with grade of CIN has not been found indicating that the size of lesion is more important.

Patients with one quadrant involved had a recurrence rate of 2% (1 case), those with two quadrants involved 5% (2 cases), and those with more than two quadrants involved 9% (4 cases) [22].

Ferenczy [23] reported a 29% failure rate with CIN3, and in those patients whose lesion was greater than 30 mm in size, the failure rate was 38% [23].

Laser vaporization represents a low-morbidity procedure because there are no intra- or postoperative complications [11, 24].

Available cytocolposcopic follow-up is one of the most important reasons to choose an ablative procedure as treatment.

Patients should be examined by an experienced and skilled colposcopist because the most important factor determining the quality of clearance results is the diagnostic accuracy [22].

If colposcopy is satisfactory, the procedure is known to reveal residual disease in the presence of negative cytology. A satisfactory colposcopic follow-up was possible in 99.4% of patients, permitting early diagnosis of persistence.

It is believed that HPV type affects both the absolute risk of viral persistence and progression to dysplasia given viral persistence. HPV 16 appears to be remarkably carcinogenic with an absolute risk of CIN3 approaching 40% at five years of persistence [24].

Persistence of HPV is associated with an increased risk of developing dysplasia and cancer.

Women who test positive for HPV persistently over time have been shown to be at the highest risk of developing preneoplastic genital disease [17].

It has been suggested that HPV infection alone may not be sufficient to promote cervical carcinogenesis and that other cofactors could be involved, such as cigarette smoking, oral contraceptives, immunosuppression, vitamin deficiency, and other sexually transmitted diseases [25-27].

Sexually transmitted diseases have been considered as possible cofactors in the pathogenesis of carcinoma of the uterine cervix, even if no single agent has been identified as particularly significant [28].

The role of *Chlamydia trachomatis* (CT) infection as a risk factor in the development of cervical lesions is controversial. Some authors suggest that combined infection with HPV plays a central role in the etiology of intraepithelial lesions of the uterine cervix and represents a risk for the subsequent development of invasive cervical neo-plasia when associated with other factors, such as cigarette smoking and sexual promiscuity [26, 27, 29-31]. However, other authors suggest that infections due to HPV or CT are independent [32].

Other authors [33, 34] found that women with cervical cytological abnormalities presented higher frequencies of *Mycoplasma hominis* and *Ureaplasma urealyticum* infection.

Patients who present abnormal Pap tests should undergo cervicovaginal microbiologic examinations for potential pathogens, especially before any treatment of SIL, and even more so in persistent or recurrent cases of papillomavirus infections [33].

Therefore abnormal cytology is relevant in identifying and treating any inflammations, even if asymptomatic, which might represent a cofactor of HPV infection in the pathogenesis of cervical intraepithelial lesions.

Available cyto-colposcopic long-term follow-up in selected cases, after destructive procedures, for HG-CIN could be combined with microbiologic and HPV testing which might represent cofactors involved in persistence, recurrence and progressive disease.

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Sonographic value in diagnosis of hemorrhagic ovarian cysts

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Summary

Purpose: To investigate the sonographic characteristics of hemorrhagic ovarian cysts (HOC) and to avoid unnecessary surgery. *Methods:* 113 cases of suspected HOC underwent sonographic and clinical follow-up for three months. *Results:* 104 cases were clinically diagnosed with HOC as the masses disappeared naturally. The mean length of the greatest diameter was 5.12 ± 1.33 cm, and the mean period of disappearance was 3.5 ± 2.4 weeks. There were four patterns of the image: 21 cases (20.2%) showed a diffused dense echo pattern, 25 cases (24.0%) displayed a mixed pattern, 30 cases (28.8%) expressed a sponge-like pattern and 28 cases (27.0%) exhibited a cystic pattern. Ring blood flow with high velocity and low resistance was detected in 41 cases (40%) and there was no internal blood flow. *Conclusion:* HOC showed characteristic features on sonography, which provided useful information to differentiate HOC from ovarian tumors.

Key words: Hemorrhagic ovarian cysts; Sonography; Color Doppler.

Introduction

Most ovarian disorders are benign, with the majority being functional ovarian cysts and benign neoplasms [1]. It is rather difficult for clinical gynecologists to discriminate between ovarian tumors and benign ovarian disorders, such as hemorrhagic ovarian cysts (HOC). The difficulties are caused by the deep location of the ovary in the pelvic cavity, and the insidious clinical symptoms of ovarian diseases. Sonographic or MR imaging can often aid in diagnosis and risk assessment [2, 3]. HOC usually result from bleeding of the ovarian follicle or corpus luteum [4]. To date ultrasound (US) examination is a relatively simple and cheap diagnostic method. Different types of non-neoplastic ovarian cysts vary in sonographic spectrums. HOC may have miscellaneous US features [5], which cause difficulties in differentiation from ovarian neoplasms. Surgery is unnecessary for functional HOC, so it is valuable to distinguish HOC from ovarian tumors correctly.

The aim of this study was to describe the clinical and sonographic characteristics of HOC, and to enhance the diagnostic accuracy of HOC, thus avoiding unnecessary surgery. One hundred and thirteen patients with an adnexal mass were included in the study and the sonographic patterns and clinical outcomes were analyzed.

Materials and Methods

Patients: 113 patients with an adnexal mass detected by clinical examination and sonography were retrospectively evaluated between 6/2002 and 6/2008 at Women's Hospital, School of Medicine, Zhejiang University, China. Pertinent gynecologic histories were obtained on all patients. None of the patients had fever, evidence of infection or a history of ectopic gestation. None of these patients were treated with antibiotics or hormones, and none had clinical evidence or typical ultrasonic characteristic of an ovarian tumor, endometrioma, teratoma, or fallopian hydrops.

The patients included in this study were 13-52 years old (mean, 30 years; median, 28 years). Sixty patients (53%) had acute pelvic and/or lower abdominal pain. There were a variety of other symptoms such as waist soreness, abdominal mass, nausea, vomiting, etc. Some patients displayed no symptoms.

Methods: Patients underwent transvaginal or transrectal US examination by a skilled examiner using Medison 530D and ESAOTE MYLAB50 equipment with a 5-MHz transducer. Patients were examined in the supine position with an empty bladder. Masses were scanned from several angles in an attempt to evaluate all sonographic characteristics. High- and low-gain studies were performed on any anechoic mass for evaluation of internal echoes. Masses were analyzed for size, shape, internal echogenicity, periphery and internal blood flow, peak flow rate (PFR) and resistance index (RI) during systolic period.

Sonographic follow-up was carried out every one to two weeks till three months after the first discovery of an adnexal mass. One hundred and four patients displayed gradual disappearance of the ovarian cyst. Surgery was required in nine patients whose mass remained intact.

Results

One hundred and four HOCs (92%) out of 113 patients had total resolution documented by follow-up sonograms and/or by the clinical disappearance of a palpable mass. The sizes of HOC varied from 2.0 cm to 7.6 cm (mean 5.12 ± 1.33 cm). Time intervals of disappearance of HOC between the first and last examinations ranged from one to ten weeks (mean 3.5 ± 2.4 weeks). The masses in the other nine patients remained intact in the 3-month follow-up. These nine patients underwent surgery, and the pathological results proved to be ovarian serous cystadenoma
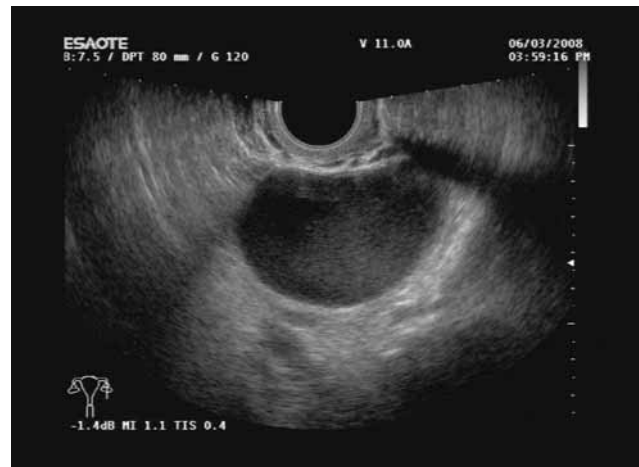
Figure 1. — Homogeneous dense echo pattern (Pattern I).
Figure 3. — Sponge-like echo pattern (Pattern III).

Figure 2. — Mixed echo pattern (Pattern II).
Figure 4. — Low echo pattern (Pattern IV).

(5 cases), ovarian endometriotic cyst (3 cases), and ovarian mucous cystadenoma (1 case), respectively.

According to the internal echogenicity, the sonographic characteristics of HOC were summarized and divided into the following four patterns:

1) Pattern (homogeneous dense echo pattern): 21 cases (20.2%) displayed internal homogeneous dense echo diffused inside the cyst (Figure 1)

2) Pattern (mixed echo pattern): 25 cases (24.0%) showed mixed sonographic features with dense echo and echo-free spaces (Figure 2).

3) Pattern (sponge-like echo): 30 cases (28.8%) displayed a pattern as a micro-network, similar to the sponge image. Buffeting of the internal tremelloid substance was observed when the mass was gently pushed by an ultrasonic transducer (Figure 3).

4) Pattern (low echo pattern): 28 cases (27.0%) showed low-level echo or an echo-free internal space, with sparse fine echogenic dots (Figure 4).

Color Doppler examination showed that the ring blood flow with high velocity and low resistance were detected in 41cases (40%), and there was no internal blood flow in either the dense echo or low echo position .

Discussion

Hemorrhagic ovarian cysts (HOC) are one of the functional ovarian cysts. Some patients with HOC suffer different degrees of pelvic and/or lower abdominal pain to different extents, while others may display no clinical symptoms. It is difficult to discriminate HOC from ovarian tumors under clinical conditions. US examination is a relatively simple and cheap diagnostic method. It is widely accepted as the main method for diagnosis of HOC. Sonographic technology is a valuable tool for gynecologists as it may characterize HOC, improve accuracy of clinical diagnosis and avoid unnecessary surgery.

In this study, 104 patients with adnexal masses displayed a comparable ultrasonic spectrum and clinical characteristics as Reynold [6] and Swire [7] described. Resolution documented by sonograms and clinical evidence of disappearance of a palpable mass confirmed the diagnosis of HOC. HOC differed in the side and internal echogenecity. In the present study, the smallest diameter was 2 cm, and the longest was 7.6 cm (mean 5.12 ± 1.33 cm). It was reported by Jain that the maximum diameter reached 10.0 cm [8]. The size of HOC is related to hemorrhagic volume. The internal echongenecity of HOC

depends on both hemorrhagic volume and time of hemorrhage occurrence. Blood is known to have a variable sonographic appearance, mostly related to the temporal sequence of clot formation and lysis [9]. In a standard case, fresh blood is anechoic, progressing subacutely to a mixed echogenicity, and finally becoming anechoic [10]. In most cases, however, any pattern may exist independently or in combination with one another. Therefore, it is not surprising that HOCs have such variable sonographic features. Coelho *et al.* [11] showed that acute bleeding with higher hemorrhage volume usually exhibited hyperechogenicity, similar with pattern in our study. However, acute bleeding with lower hemorrhage volume might exhibit network-like or sponginess echogenecity similar to Pattern in our study, partly because the blood had not fully coagulated. Pattern and pattern were possible consequences of clot lysis of pattern and pattern, respectively.

HOCs exhibit so much diversity in sonographic features that it is necessary and important to discriminate them from ovarian neoplastic lesions. Clinical observation showed that pattern I and pattern II of HOC were often misdiagnosed as dermoid tumors or ovarian parenchymatous tumors. Pattern III of HOC might be misdiagnosed as ovarian mucous cystadenoma. Pattern IV of HOC might be confused with serous cystadenoma, ovarian endometriosis, and adnexal abscess. Color Doppler US plays an important role in the differential diagnosis of HOC and ovarian tumors. The results of this study showed that approximately 40% of HOC exhibited distinctive ring blood flow with high velocity and low resistance. Since the pathologic basis of most HOC is the bleeding of the corpus luteum, it is not surprising that the distinctive luteal blood flow surrounded HOC. In comparison, serous ovarian cystadenoma and ovarian endometriotic cysts rarely displayed blood flow in the cyst wall. Thus the appearance of the characteristic luteal ring blood flow in sonographic imaging is helpful in discriminating HOC from ovarian neoplastic cysts. In cases where an internal homogeneous dense echo, like echogenecity of parenchymatous tissue is present, examination of blood flow is also very important. The lack of blood flow detected by US indicates a high possibility of HOC. Patients suspected of having HOC are highly recommended to undergo ultrasonic and clinical follow-up due to the beneficial diagnostic accuracy. Our data revealed that the mean time interval for the disappearance of HOC was 3.5 weeks. The longest case lasted ten weeks.

As indicated by this study, the unique sonographic characteristics of HOC provided useful information to discriminate HOC from ovarian tumors. Because HOC can resolve spontaneously, HOC should be included in the differential diagnosis of any adnexal mass that has good sound through-transmission. This differential diagnosis, however, may be extensive, including dermoid, endometrioma, abscess, ectopic pregnancy, cystadenoma, adnexal torsion, and carcinoma. To those patients with suspected ovarian neoplastic lesions smaller than 8 cm, it is especially valuable to conduct cautious sonographic follow-up before the decision to perform surgery. For HOC, a conservative approach monitoring resolution is more acceptable than immediate intervention.

Conclusion

HOCs show characteristic features on sonography, which provide useful information to differentiate HOC from ovarian tumors.

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E-cadherin expression in estrogen receptor-positive and negative breast carcinomas of postmenopausal women

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Summary

Background: Preservation of E-cadherin expression is usually related to non-invasive and well differentiated breast carcinomas. *Purpose:* The aim of this study was to evaluate E-cadherin immunohistochemical expression in estrogen receptor (ER) positive and negative infiltrating ductal breast carcinomas. *Methods:* Twenty-three postmenopausal patients with Stage II, operable, infiltrating ductal breast carcinomas were divided into groups A (ER+; n = 13) and B (ER-; n = 10). E-cadherin immunohistochemical expression was assessed semiquantitatively according to membrane staining intensity and classified as negative (< 10% of cells with stained membranes), positive + (10-50% of cells stained) or positive ++ (> 50% of cells stained). Fisher's exact test was used to compare the distribution of staining intensity in the two groups ($p < 0.05$). *Results:* In group A (ER+), E-cadherin staining was positive in all cases: + (n = 3; 23%) and ++ (n = 10; 77%) compared to three cases (30%) in group B (ER-), + (n = 2; 20%) and ++ (n = 1; 10%). This difference was statistically significant ($p < 0.0005$). *Conclusions:* The present results indicate that E-cadherin expression loss is significantly associated with ER-negative tumors and therefore with a more aggressive phenotype of invasive ductal breast carcinoma.

Key words: Breast; Cancer; E-cadherin; Estrogen receptor; Cell adhesion molecules.

Introduction

The mammary alveoli and ducts that are formed cyclically during pregnancy and lactation consist of bilayered epithelial structures surrounding a central lumen [1]. Luminal cells adhere to each other via E-cadherin, which is also necessary for cell survival, whereas the myoepithelial cells surrounding the luminal layer adhere to each other via P-cadherin [1, 2]. E-cadherin (EC) is a calcium-regulated transmembrane glycoprotein that functions as an epithelium-specific, cell-cell adhesion molecule [3-7]. The physiology of the reproductive tissues, including the breast, is dependent on the preservation of appropriate cell-cell contact, cell adhesion molecules (CAMs) being important for regulating tissue architecture and maintaining tissue integrity [7].

The loss or down-regulation of E-cadherin has been associated with breast cancer progression, and although its practical application as a diagnostic and prognostic marker in breast cancer remains controversial [8], some studies have shown that a reduction in E-cadherin expression constitutes an adverse prognostic marker in breast cancer [8-10]. E-cadherin expression is irreversibly lost in more than 85% of invasive lobular carcinomas (ILC). The loss of EC occurs at onset of the disease, i.e., at the preinvasive stage of lobular carcinoma in situ (LCIS) [1, 8]. However, EC expression in invasive ductal carcinoma, unlike invasive lobular carcinoma, is highly variable [11]. In general, preservation of E-cadherin expression is related to non-invasive and well-differentiated breast carcinomas [12].

Estrogens receptors are expressed in around 60-65% of breast cancer cases, and in these cases, a relatively better

prognosis can be expected compared with tumors that do not express them [13, 14]. Likewise, tissue response to estrogen may be implicated in EC regulation, which, via the estrogen receptor (ER), indirectly represses the Snail transcription factor that down-regulates EC [13]. Thus, in ER-negative breast tumors, the Snail transcription factor would predominate and there would be a corresponding decrease in EC. Nevertheless, despite current controversies, there are few studies comparing E-cadherin expression in ER-positive and ER-negative breast tumors, leading us to design the present study.

Materials and Methods

Patients

Twenty-three patients with operable Stage II infiltrating ductal breast carcinoma, receiving medical care at the Mastology Division, Department of Gynecology, *Getúlio Vargas* Hospital, Federal University of Piauí were included in the present study. The study was approved by the Internal Review Board of the Federal University of Piauí and all the patients signed informed consent forms prior to initiation of the study. All the patients had been menopausal for at least one year and had no history of any previous treatment for breast cancer. Tumor samples were obtained by incisional biopsy at the time of definitive surgery for the purpose of evaluating ER status and to perform immunohistochemistry for E-cadherin. Tumors in which the semiquantitative evaluation of estrogen receptors following immunohistochemical staining was classified as high (≥ 10% immunoreactive cells) were considered positive [14].

Study Design

This was an analytical, cross-sectional study in which patients were divided into two groups: A (ER+; n = 13) and B (ER-; n = 10). All the patients had Her-2 negative tumors. The groups were considered homogenous with respect to age, tumor size, stage, histological grade and axillary status (Table 1).
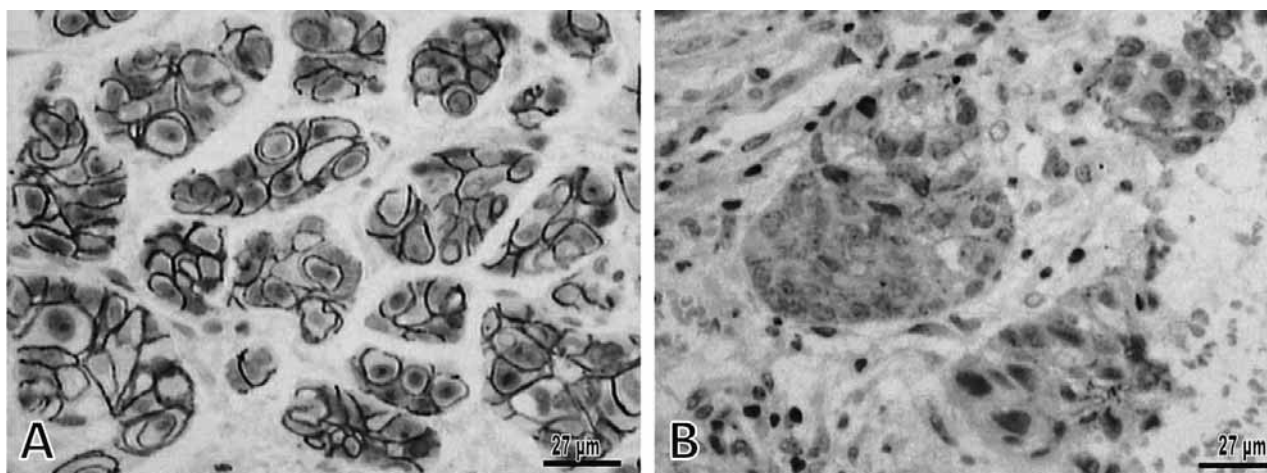
Figure 1. — Photomicrographs of histological sections of invasive ductal breast cancer: (A) In patient # 8, ER-positive, note the high concentration of cell membranes strongly stained brown by the anti-E-cadherin antibody, and (B) in patient # 6, ER-negative, note the sparse cells with plasma membranes weakly stained by the anti-E-cadherin antibody (original magnification 200 x).

Table 1. — *Patient characteristics in the ER-positive and ER-negative groups.*

	A (ER +)	Group B (ER-)	p value
n	13	10	
Age (years)			0.0581
Mean	63.2	58.8	
S.D	6.3	12.1	
Tumor Size (cm)			1.0000
Mean	3.7	3.3	
S.D	0.9	0.6	
Staging (%)			0.3788
IIa	53.8	70.0	
IIb	46.2	30.0	
Histological grade (%)			0.4674
G1	46.1	30.0	
G2	46.1	40.0	
G3	7.8	30.0	
Axillary status (%)			1.0000
N0	46.1	60.0	
N1	53.9	40.0	

Table 2. — *Immunohistochemical staining for E-cadherin in estrogen receptor-positive and negative invasive ductal breast carcinomas.*

Tumor	n	Negative	Staining intensity +	++
ER+	13	0 (0%)	3 (23%)	10 (77%)
ER-	10	7 (70%)	2 (20 %)	1 (10%)

Association between loss of E-cadherin expression and ER-negative breast carcinomas was statistically significant ($p < 0.0002$).

Immunohistochemistry for E-cadherin

All samples were fixed in 10% neutral buffered formalin for 24 hours and then embedded in paraffin. Sections measuring 5 μm were deparaffinized and antigenic recovery was performed using 0.21% citric acid (pH 6) in a pressure cooker for 8 min after pressure was initiated. Next, the slides were incubated with anti-E-cadherin (mouse) antibody, Clone 4A2C7 (Cat. No. 18-0223-Zymed, South San Francisco, CA)* at a dilution of 1:1200 and incubated overnight at 4-8°C. The slides were then washed with PBS containing Tween, excess PBS was aspirated and the secondary reagent (anti-mouse BA 200 – Vector, Burlingame, CA) was instilled, incubation following for 60 min at room temperature. After this, the slides were washed again with PBS-Tween, the excess PBS was aspirated, and the ABC Elite system (PK 6100 – Vector, Burlingame, CA) was instilled. Slides were then incubated for 45 min at room temperature, after which DAB (diaminobenzidine tetra-hydrochloride – REF.: D-5637, Sigma, St. Louis, MO) was instilled. Finally, the slides were washed in abundant distilled water, counterstained with hematoxylin, dehydrated in an absolute ethyl alcohol-xylol series and finally mounted in Permount resin. The cells expressing E-cadherin were identified by dark-brownish staining of the cytoplasmic membrane.

Quantification

E-cadherin expression was evaluated under light microscopy by two observers who were blinded with respect to group identification. These observers semiquantitatively counted the cells in which the membrane was positively stained (400 x magnification) using a system consisting of a light microscope (Nikon Eclipse E-400, optical microscope, Tokyo, Japan) connected to a videocamera (Samsung Digital Camera SCC-131, Seoul, Korea) with capture and transmission to a computer equipped with the Imagelab® software program (Softium Informatica LTDA, São Paulo, Brazil). Only tumor cells with obvious immunohistochemical labeling of the cytoplasmic membrane were considered positive. Immunopositivity was calculated as grade + if 10-50% of cells were positive or grade ++ if more than 50% of cells were positive. Tumors were graded as negative when less than 10% of the cells were stained [15].

Statistical analysis

The Student's t-test was used to test the homogeneity of the two groups with respect to age of the patients and tumor volume. Fisher's exact test was used to evaluate stage, lymph node status and histological grade between the two groups and to calculate the proportion of E-cadherin-positive cells in the estrogen receptor-positive and negative breast carcinomas [16]. Statistical significance was established at $p < 0.05$.

Results

Light microscopy detected a higher concentration of cells in which the membrane was strongly stained by the anti-E-cadherin antibody in the estrogen receptor-positive breast carcinomas compared to the estrogen receptor-negative tumors (Figure 1). Cells with E-cadherin-stained membranes were found in all the patients in group A (ER+), three (23%) being classified as grade + and ten (77%) as grade ++. In comparison, in group B (ER-), seven (70%) were found to be negative for E-cadherin, while only three (30%) were positive, two (20%) of which were classified as grade + and one (10%) as grade ++ (Table 2). This association between a reduction in E-cadherin expression and ER-negative tumors was statistically significant ($p < 0.0005$).

Discussion

Cell adhesion is a significant factor in containing cancer; hence, loss of intercellular adhesion may be associated with unfavorable prognoses [7]. The cadherins comprise a rapidly expanding superfamily of cell adhesion molecules that includes E-, N- and P-cadherin, known as type I cadherins because they were the first to be discovered. They all promote calcium-dependent cell-cell adhesion via homophilic intercellular interactions [5, 7]. The presence of cadherins in the reproductive tract, particularly in the breast, suggests an effect of estrogens in these tissues via cadherin expression [17]. Furthermore, some *in vivo* and *in vitro* studies have suggested a correlation between ER-negative status and the loss of E-cadherin [18, 19].

In the present study, there was a significant reduction in EC expression in the ER-negative, invasive ductal breast carcinomas compared to the ER-positive tumors. Unlike invasive lobular breast cancers in which EC expression is irreversibly lost in the majority of cases, in ductal carcinomas EC expression is highly variable and its relationship with respect to prognosis, histological grade and hormone receptor status is controversial [1, 8].

Some studies have reported preserved EC expression in almost all invasive ductal carcinomas but have found reduced expression to be associated principally with poor differentiation and high tumor grade [8-10, 19, 20]. Other studies have reported a correlation between reduced EC expression, lymph node status and ER status [18, 21]. On the other hand, other studies have failed to find any correlation between EC expression and tumor size, grade, mitotic activity, HER-2 overexpression or ER status [22, 23].

The patients who participated in the present study were homogenous with respect to age, tumor size, histological grade, stage and axillary status. This may have been due to the selection criteria adopted, since only postmenopausal patients with operable, stage II tumors over 3 cm in size were admitted to the study. Irrespective of these morphological prognostic factors, the loss of EC expression was significantly correlated with estrogen receptor-negative status. The association between ER-negative tumors and poorer prognosis may involve, in addition to the loss of EC expression, other molecular markers related to the angiogenesis, proliferation and apoptosis of tumor cells [24-29].

The connection between estrogens and cadherins has long been postulated from *in vivo* studies [7]. Factors that regulate EC expression, particularly the zinc-finger transcription factor Snail, an E-cadherin inhibitor, play an important role in the relationship between EC and prognosis, and may be regulated by steroid hormones [1, 7]. Moreover, several studies have shown that the E-cadherins expressed by the reproductive tissues are responsive to hormonal stimulus by which they control morphological changes in these tissues. The ER indirectly stimulates estrogen-dependent expression of metastatic tumor antigen 3 (MTA3), which in turn transcriptionally represses the cadherin transcription factor Snail [7, 30]. Therefore, it is proposed that estrogen maintains epithelial architecture by constraining Snail repression of E-cadherin [30], suggesting a mechanistic link between ER-negative status, tumor invasion and poor prognosis [1, 7, 30].

Some authors have demonstrated that in non-lobular breast carcinomas, reduced and/or negative EC expression was significantly associated with lack of ER expression and preferentially found in basal-like carcinomas [31, 32]. Therefore, ER-negative status is related to a loss of EC, which was confirmed by the findings of the present study, and this loss of EC may provide an explanation for the unfavorable prognosis of ER-negative breast cancers.

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Granulosa cell tumors of the ovary: a clinicopathologic and immunohistochemical study of 21 cases

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Summary

Purpose: To further study the clinicopathologic and immunohistochemical features of ovarian granulosa cell tumors (GCTs). *Methods:* We retrospectively studied all cases of GCTs diagnosed in our laboratory over the last 10-year period. Immunohistochemistry for inhibin, vimentin, cytokeratin, Ki-67 and p53 was performed on archival paraffin blocks. Pathologic and immunohistochemical findings were correlated with the clinical records of the patients. *Results:* Twenty-one cases (15 of the adult and 6 of the juvenile type) were retrieved. All patients were FIGO Stage I at the time of diagnosis. Recurrent disease was detected in four patients (19 %) during a median follow-up of 36 months (range 2-26 years). Pathology revealed a concomitant theca-cell component in three cases, a Sertoli-Leydig component in one case, and a thecoma in one case. Archival tissue material was available in 12 cases. Immunohistochemistry was positive for: β-inhibin in 12/12 cases (100%), vimentin in 11/12 cases (91.7%), cytokeratin in 3/12 cases (25%), CD34 in 0 cases (0%), and p53 in 2/12 cases (16.7%). The Ki-67 index was < 5% in 12/12 cases (100%). No significant correlations were observed between the pathologic and immunohistochemical parameters examined and the clinical outcome. *Conclusions:* Despite the relatively indolent nature and favorable prognosis of most GCTs, late recurrences are not a rare event even in Stage I patients, necessitating a close and long-term follow-up. The identification of novel prognostic markers, in addition to our traditional staging parameters such as clinical staging, is needed in order to more accurately predict probabilities of recurrence in these patients.

Key words: Adult; Juvenile; Granulosa cell tumor; Ovary.

Introduction

Granulosa cell tumor (GCT) of the ovary is a relatively uncommon sex-cord-stromal neoplasm comprising approximately 2-5% of all ovarian cancers. [1, 2]. GCT is subdivided in two types, known respectively as adult and juvenile, which differ with regard to their clinical and pathologic features [1, 2]. The more frequent adult type of this tumor is usually – but not exclusively – diagnosed in women of reproductive age, while approximately 80% of juvenile GCT cases are diagnosed in the first two decades of life [1]. Both types are often associated with excessive secretion of estrogens and endometrial pathology, leading to symptoms of menstrual irregularities, menorrhagia, or postmenopausal bleeding, although juvenile GCTs may also present with isosexual precocity or abdominal pain due to the presence of a large ovarian mass [1-3].

Despite the tendency of GCTs to recur, often several years after their initial diagnosis, they are characterized as relatively indolent neoplasms. However, the favorable prognosis seems to be largely dependent on the clinical staging at the time of the initial diagnosis [1]. Most previous studies have demonstrated a greater than 90% 5-year survival rate for patients with Stage I disease [2, 4-8]. On the other hand, an aggressive clinical course has been strongly correlated with disease presentation at an advanced stage, and 5-year survival rates for Stage III/IV cases range from 22-50% [5, 7]. Apart from stage, additional prognostic factors, including age, tumor size, tumor rupture and degree of nuclear atypia have also been described in some series [4-6, 9, 10]. However, the accurate identification of novel prognostic indicators which will aid in better evaluating prognosis and designating appropriate treatment for each individual case remains to be established.

The aim of the present study was to further investigate the clinical, pathologic and immunohistochemical features of GCTs and briefly review the existing literature.

Materials and Method

A retrospective study of patients with adult and juvenile GCT of the ovary was carried out. All cases diagnosed at the pathology laboratory of our hospital over a 10-year period (from 1996 to 2005) were included. Histological diagnosis was determined during routine pathologic assessment. The relative clinical and pathology reports as well as representative slides for each case were retrieved.

The clinical data, including patient age, presenting symptoms, menopausal status, treatment received, and the final clinical outcome were correlated with the pathologic data and immunohistochemistry results. The pathologic data included the following parameters: tumor size and location, histological type, degree of nuclear atypia and mitotic rate of tumor, associated endometrial abnormalities and disease stage at the time of diagnosis. For the purposes of staging, all patients had been submitted to peritoneal washings and pelvic lymphadenectomy.

Immunohistochemistry, formalin-fixed paraffin-embedded tissue blocks were retrieved from the archives of our laboratory.

Additional sections were obtained for the application of a streptavidin-biotin immunohistochemical method. The specimens were stained using the Ventana automated immunostainer, with the following primary antibodies: vimentin (mabVg, Euro-Diagnostica), pan-cytokeratin (mono clone 80, Monosan), β-inhibin (mono E4, Serotec), Ki-67 (mono MIB-1, Dako), p53 (mono D07, Dako), CD34 (mono QBend/10, Novocastra).

Results

After reviewing the archival files of our laboratory, we were able to retrieve 21 cases of GCTs, 15 (71.4%) of the adult and six (28.6%) of the juvenile type, among 560 cases of malignant ovarian tumors, indicating an incidence of 3.75%. Follow-up data were available for all patients. The median follow-up period was 36 months (range 2-26 years). Archival tissue material was available for the performance of immunohistochemistry in 12/20 cases (60%).

Clinical data: Table 1 shows the clinical features of all patients included in the study. Patient age ranged from 17 to 73 years (mean: 47.8 years). The mean age of adult patients was 58.6 years (range 49 to 73 years) while in the juvenile group the mean age was 20.8 years (range 17-31 year). Eleven patients (52.4%) were postmenopausal, nine (42.9%) premenopausal, while one patient (4.8%) was pregnant. Among the adult group, most patients were postmenopausal (11/15 cases, 73.3%) and presented with abnormal postmenopausal bleeding. In the remaining four adult premenopausal cases the presenting sign was menstrual irregularities in three cases and abdominal pain in one. In the group of juvenile GCT, five of our patients presented with abdominal pain while a pregnant patient was asymptomatic at the time of diagnosis. Fourteen patients (14 cases, 66.7%) were submitted to total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAHBSO), and all remaining patients (7 cases, 33.3%) to unilateral salpingo-oophorectomy (USO). With regard to staging, all of our patients (21 cases, 100%) were Stage I at the time of diagnosis. Recurrences were noted in four cases (three of the adult and one of the juvenile type). Time elapsed between the initial operation and recurrence was two, six, 20 and 25 years (mean interval: 13.2 years). Two adult cases relapsed in the pelvis after a disease-free interval of two and 25 years, respectively, while one adult case presented with a liver metastasis 20 years postoperatively. The case of the juvenile GCT relapsed with distant metastasis in the lung after a disease-free period of six years. Local recurrences were treated with secondary surgical debulking, while chemotherapy was administered in patients with metastatic disease. All our patients were alive and disease-free at the time of the last follow-up.

Pathologic and immunohistochemical data: Table 2 shows the pathologic features of our studied material. Various histological patterns of growth were observed including mainly microfollicular, macrofollicular, trabecular, solid and diffuse patterns (Figures 1 and 2). In the majority of our cases the tumor was located in the

Table 1. — *Distribution of clinical characteristics in 21 patients with GCT.*

Characteristics (IGF-II concentration, application time)	Frequency/range (mean)*	Percent (%)
Age (years)		
– All	17-73 (47.8)*	
– Adult group	49-73 (58.6)*	
– Juvenile group	17-31 (20.8)*	
Menopausal status		
– Premenopausal	9/21	42.9
– Postmenopausal	11/21	52.4
– Pregnant	1/21	4.8
Presenting symptoms		
– Postmenopausal bleeding	11/21	52.4
– Menstrual irregularities	4/21	19.0
– Abdominal pain	5/21	23.8
– Asymptomatic	1/21	4.8
Stage		
I	21/21	100
II	0/21	0
III	0/21	0
IV	0/21	0
Recurrences		
– Local	2/21	9.5
– Distant metastases	2/21	9.5
Treatment		
1) Primary		
– TAHBSO	14/21	66.7
– USO	7/21	33.3
2) Adjuvant		
– Secondary debulking	2/21	9.5
– Chemotherapy	2/21	9.5

TAHBSO: total abdominal hysterectomy with bilateral salpingo-oophorectomy; USO: unilateral salpingo-oophorectomy.

Table 2. — *Distribution of pathological findings in 21 patients with GCT.*

Characteristics (IGF-II concentration, application time)	Frequency/range (mean)*	Percent (%)
Histological type		
– Adult type	15/21	71.4
– Juvenile group	6/21	28.6
Tumor location		
– Right	7/21	33.3
– Left	13/21	61.9
– Bilateral	1/21	4.8
Tumor size (cm)		
– All	3-30 (9.1)*	
– Adult type	3-19 (7.0)*	
– Juvenile	5-30 (14.4)*	23.8
Mitotic rate		
– Low	21/21	100.0
– Moderate	0/21	0
– High	0/21	0
Nuclear atypia		
– Low	20/21	95.2
– Moderate	0/21	0.0
– Severe	1/21	4.8
*Endometrial abnormalities**		
– Hyperplasia	6/14	42.9
– Polyps	3/14	21.4
– Adenocarcinoma	2/14	14.3
– None	3/14	21.4

** Only among women submitted to TAHBSO.

Fig. 1

Fig. 2

Fig. 3
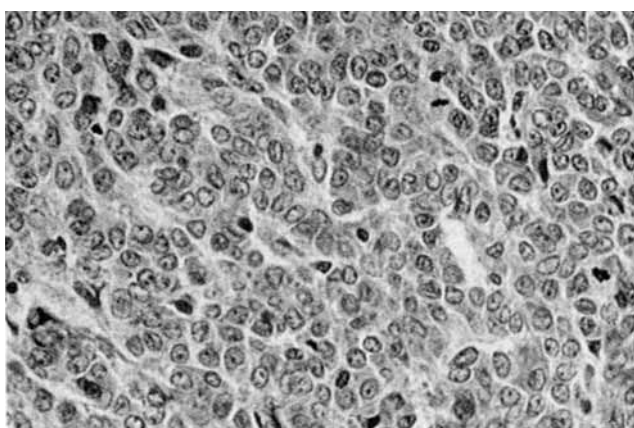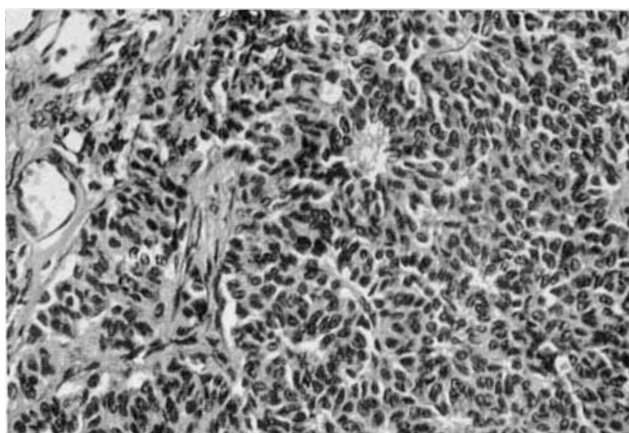
Figure 1. — Histological section of a juvenile granulosa ovarian tumor showing a cystic pattern (hematoxylin-eosin x 25).

Figure 2. — Histological section of a juvenile granulosa ovarian tumor showing a solid pattern with Call-Exner bodies (hematoxylin-eosin x 120).

Figure 3. — Histological section of the recurrent juvenile granulosa tumor with prominent mitotic activity (hematoxylin-eosin x 250).

left ovary (13 cases, 61.9%). In seven cases (33.3%) the right ovary was involved, while bilateral involvement was noted in one case (4.8%). Tumor size in all cases ranged from 3-30 cm (mean: 9.1 cm), with a mean size of 14.4 cm in the juvenile types and a mean size of 7 cm in the adult cases. With the exception of one case associated with distant metastasis, which displayed prominent mitotic activity (Figure 3), the mitotic rate was low (< 5 mitoses/10 HPF) and the degree of nuclear atypia was also low in all remaining cases. A theca-cell component was found in three (adult) cases while a Sertoli-Leydig component was found in the case of the juvenile type which recurred with metastatic disease. In 11 out of 14 patients (78.6%) who were submitted to TAHBSO, concomitant endometrial abnormalities were found as follows: endometrial adenocarcinoma was diagnosed in two cases, endometrial hyperplasia in six cases and endometrial polyps in three cases. An ovarian thecoma was also found in one (adult) case.

Immunohistochemistry was positive for β-inhibin in 12/12 cases (100%), vimentin in 11/12 cases (91.7%), cytokeratin in 3/12 cases (25%), p53 in 2/12 cases (16.7%) and CD34 in 0 cases. The Ki-67 index was < 5% in 12/12 cases (100%).

No significant correlations were observed between the pathologic and immunohistochemical parameters examined and the clinical outcome.

Discussion

Adult and juvenile GCTs are described as two histologically and clinically distinct neoplasms and are usually studied as separate clinicopathologic entities although they represent different subtypes of the same tumor [11]. In the present study we examined both subtypes to compare their features and investigate the differences with regard to their particular biological behavior.

GCTs may be detected in almost any age group, from the first to the tenth decade of life, with a mean age of 52 years at the time of diagnosis [11]. Nevertheless, juvenile GCTs in particular are more age-specific in comparison to their adult counterpart, occurring mostly in prepubertal girls and only rarely in older patients [11]. In this study the mean age of all patients was 48 years. The youngest patient with an adult type of tumor was 45 years old, while the oldest patient with a juvenile GCT was a pregnant 31-year-old woman. As regards the clinical presentation, major differences were noted not only between premonopausal and postmenopausal patients but also among adult and juvenile cases. More specifically, all postmenopausal women in our series presented with abnormal postmenopausal bleeding, while premopausal patients presented with menstrual irregularities or with abdominal pain. The latter symptom was far more common in cases with the juvenile type of tumor and was

directly related to the larger tumor size found in this group (mean size of 14.4 cm, versus a mean size of 7 cm in the adult cases). As previously described [2, 12] and as normally expected, larger tumors are much more likely to cause persistent , abdominal or pelvic pain or to be complicated with hemorrhagic rupture into the abdominal cavity, occasionally mimicking a ruptured ectopic pregnancy [4, 13]. Concomitant endometrial abnormalities may also be found, and are consistent with increased estrogen production by the tumor [2, 11]. In our series the most common endometrial pathology found in association with GCTs was endometrial hyperplasia, followed by endometrial polyps and adenocarcinoma.

The primary treatment for patients with suspected GCTs is surgery [2, 11]. The surgical procedure should be conservative unless there are obvious signs of advanced cancer, and should aim at establishing a definite histological diagnosis, completing the process of staging and achieving optimal tumor debulking [2, 11]. For patients presenting with Stage I disease, a total abdominal hysterectomy with bilateral salpingo-oophorectomy is typically performed in postmenopausal women or when childbearing is not an issue, while the more conservative approach of a unilateral salpingo-oophorectomy may represent a more appropriate option for younger premenopausal women who wish to retain their fertility [11]. Treatment options for recurrent or advanced-stage disease remain a controversial issue, and mainly include the performance of secondary surgical debulking and the administration of chemotherapy agents , either alone or in combination [2].

Although GCTs are considered to be of low-grade malignant potential, in a significant percentage (10-50%) of patients, late and/or several relapses tend to occur, often many years after the initial diagnosis [14]. The median time of relapse is approximately four to six years postoperatively, although in several previous reports relapses appearing more than 20 years later have been described [2, 6-8, 15, 16]. This fact necessitates a close, long-term follow-up of patients in order to detect recurrent disease in time and to intervene accordingly. For patient monitoring the measurement of serum levels of inhibin has been recommended [11, 17]. Although inhibin production is restricted neither to the ovary nor to GCTs (both the placenta and some epithelial ovarian cancers, especially of the mucinous type, also secrete this molecule), it seems that a consistently rising level of inhibin in a postmenopausal woman with a history of GCT is a strong predictor of recurrence and should warrant further investigation [2, 11, 17, 18]. A common site of recurrence is the pelvis, followed by disease spread to the upper abdomen [4, 6, 12]. Distant metastases involving the lung and bones may also be found but seem to be a rare event [2]. In our series, recurrences were noted in four patients: two cases relapsed in the pelvis, one case presented with liver metastasis and the remaining case relapsed with distant metastasis in the lung. Interestingly, the disease-free interval in two out of these four cases was 20 and 25 years, respectively, which

further supports the described propensity of GCTs for late recurrences.

According to previous studies and literature reviews, mainly FIGO stage, and to a much lesser degree other parameters such as tumor size, tumor rupture and degree of nuclear atypia, seem to influence the clinical outcome of women with GCTs [4-6, 9, 10]. Some authors have further suggested that age, Ki-67 index, p53 and DNA ploidy may also significantly affect prognosis, but the clinical value of these markers remains to be validated [4, 19-22]. In the present study we also investigated the prognostic significance of some previously described yet still debatable factors, including but not limited to tumor size, mitotic index, nuclear atypia, Ki-67 index and p53 immunostaining in women with both adult and juvenile GCTs. Our failure to reach any significant correlations between the studied parameters and patient outcome could be due to the small number of cases included in our study. This is a common problem which has been reported by many authors and is normally attributed to the relative infrequent occurrence of this neoplasm [14, 23].

In conclusion, additional large scale prospective and retrospective studies are needed to further define the exact clinical significance of all previously described parameters in GCTs and provide further insight into the molecular pathways that regulate the development of these indolent neoplasms as well as their transformation to a metastatic and potentially life-threatening disease. Furthermore, as suggested by Auranen et al., recent advances in molecular genetics carry great promise for radical improvements in our understanding of the biological pathways that regulate granulosa cell proliferation, with the potential to significantly contribute as well in the development of novel prognostic and therapeutic markers for GCT patients [23].

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Uterine involvement in advanced epithelial ovarian cancer

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Summary

Background: With an increasing trend for sparing fertility in gynecologic malignancies, we tried to assess uterine involvement in all stages of epithelial ovarian cancer (EOC) in an evidence-based study. *Method and Material:* From September 1999 to September 2005, 177 patients with epithelial ovarian cancer underwent staging laparatomy in the Gynecologic Oncology Department, Vali Asr University Hospital, Tehran, Iran. Staging data from patient files and pathologic reports were analyzed. *Result:* Of the 177 cases with EOC, 26% of patients were in Stage I, 13.6% Stage II, 53.1% Stage III and 7.3% Stage IV. Uterine Involvement was 17.9% with serosal involvement in 25 cases (78.1%) and myometrial involvement in seven cases (21.9%). Of these cases 84.4% were in Stages III or more and all had omental involvement (Stage IIIa 7.4%, Stage IIIb 14.8%, Stage IIIc 63% and Stage IV 14.8%). Only 15.6% cases of normal appearing omentum had uterine tumoral involvement. *Conclusion:* Only eight cases had myometrial involvement out of 177 cases of EOC (all in Stage III). All the eight patients had omental or gross pelvic tumoral involvement. In this study we found that in the absence of gross pelvic or omental involvement in EOC, there is really none or minimal chance of myometrial involvement. Future multicenter studies with more cases will show whether standard hysterectomy by multiple serosal biopsies could be replaced.

Key words: Epithelial ovarian cancer; Conservative surgery; Uterine involvement; Omental cake.

Introduction

Ovarian cancer is the second most common gynecological cancer and is the leading cause of death from gynecological malignancies [1]. Invasive epithelial ovarian cancer (EOC) accounts for approximately 70% of all ovarian malignancies. The most common type of EOC is serous, which is bilateral in 50% of these patients [2]. At diagnosis about 70% have already spread to the upper abdomen or beyond; however, only 25% of epithelial ovarian cancers are limited to the ovaries at the time of diagnosis [3, 4]. For patients with Stage I to IV disease, conventional therapy consists of total abdominal hypterectomy with bilateral salpingo-oophorectomy and debulking surgery followed by systemic platinum/taxane-based chemotherapy [5, 6].

Ovarian cancer generally affects older women with less than 17% of invasive cancers occurring in women under 40 years of age for whom preservation of reproductive function is an important clinical goal [1]. Ovarian cancer in young women generally compromises definitively reproductive performance with the exception of cases of unilateral salpingo-oophorectomy performed for Stage 1A1 [7].

During the past two decades there has been a trend toward less radical surgery in patients with early-stage breast cancer, cervical cancer, vulvar cancer, and ovarian germ cell malignancies [8]. Benefits of this therapeutic approach include reduced operative morbidity and mortality, enhanced patient self-image, and in the case of ovarian cancer, retention of reproductive function.

The term conservative surgery indicates a surgical procedure that allows removal of the ovarian tumor together with adequate staging. Potential benefits of conservative surgery will include not only the preservation of fertility but also the maintenance of endocrine function. The potential risks are an increase in the probability of recurrence and death, and an increase in further benign surgery. Due to the fear of leaving microscopic contralateral tumor and thereby compromising curability, most authors are reluctant to perform conservative surgery in all other Stage I invasive ovarian cancers. Morice *et al*. showed most recurrent lesions were on the remaining ovary and consequently, the spared ovary was the first recurrence in the majority of patients [6].

In our 12-year experience of EOC surgery, we observed that gross uterine involvement in all stages is relatively uncommon even in the presence of diffuse omental or peritoneal disease. This study is an evaluation of uterine pathologic data in EOC patients during the last six years in our department.

Method and Materials

Subjects for this investigation included all patients with invasive EOC who were treated with radical surgery at Gynecologic Oncology Department of the Vali-Asr University Hospital, Tehran, Iran, between 1999 and 2005. Hospital records and available histological material for each patient were used as sources for patient data. Pathology slides were reviewed by one pathologist to confirm cell type, histologic differentiation, and stage of disease. Tumors were classified histologically according to the World Health Organization (WHO) system and were staged according to the International Federation of Gynecology and Obstetrics (FIGO) system. Statistical analysis of the data

was performed using univariate and multivariate analyses. Proportions were compared using the chi-square statistic from the corresponding contingency tables. Statistical significance was determined at the 0.05 level.

Results

Patient demographics and tumor characteristics in cases studied are shown in Table 1. The mean age of the patients was 47.7 years (range 18-82 years). Of the patients 28.8% were young (≤ 40 years) and 45% of these cases were in Stage I-II.

As shown in Table 2 there was uterine involvement in 17.9%, serosal involvement in 25 cases (78.1%) and myometrial involvement in seven cases (21.9%). Of these cases 84.4% were in Stage III or more and all had omental involvement (Stage IIIa 7.4%, Stage IIIb 14.8%, Stage IIIc 63% and Stage IV 14.8%). Only15.6% cases of normal appearing omentum had uterine tumoral involvement.

Discussion

Although the incidence of epithelial ovarian cancer increases with age, reaching a maximum in the seventh decade of life, it does occur in women of childbearing age as well. Approximately 17% of all epithelial ovarian cancer occurs in women ≤ 40 years of age [9].

The standard management of epithelial ovarian cancer involves primary surgery including total abdominal hysterectomy and bilateral salpingo-oophorectomy, tumor debulking, omentectomy, pelvic/paraaortic lymph node biopsies, and multiple peritoneal biopsies and washings of the pelvis and abdomen.This is followed by adjuvant chemotherapy, and second-look surgery in selected cases. Many young women with early-stage ovarian cancer wish to maintain reproductive capability [9].

Fertility-sparing surgery in the face of a frankly malignant epithelial cancer requires formal staging to determine whether conservative surgery is contraindicated (e.g., by occult peritoneal or lymph-node spread). In the absence of such spread following thorough staging, conservative surgical management can be an option for those women who are highly motivated and desire to preserve their childbearing potential. Chemotherapy has been efficient in treating the microscopic lesions. When these Stage IA epithelial cancers are high grade, adjuvant chemotherapy is typically advised [10].

Women with frankly malignant epithelial cancers can be managed with fertility-sparing surgery when disease is confined to one ovary. In such instances the opposite ovary should be inspected carefully for occult metastasis or a synchronous tumor. When the histology is other than mucinous, for which bilaterality is uncommon, bivalving the opposite ovary might be prudent [10]. Colombo *et al*. [11] analyzed the outcomes of 99 women under the age of 40 with Stage I ovarian carcinoma, 56 of whom (including 36 Stage IA, 1 Stage IB, and 19 Stage IC patients) were treated by conservative surgery. Relapse occurred in three Stage IA (grades 1-3) patients, but only one occur-

Table 1. — *Patient demographics and tumor characteristics in studied cases (N = 177).*

Stage	Percent of patients	Total
I	26	
II	13.6	
III	53.1	
IV	7.3	100%
Cell type		
Serous	74.7	
Mucinous	7.2	
Endometrioid	7.7	
Clear cell	2.1	
Mixed serous mucinus	2.1	
Poorly differentiated adenocarcinoma	5.2	
Other	1	100%

Table 2. — *Characteristics of patients with uterine involvement.*

Stage	No. of patients	No. of patients with uterine involvement
I	42	0
II A	8	1 (12.5%)
II B	13	4 (30.8%)
II C	3	0 (0%)
III A	13	2 (15.4%)
III B	123	4 (25%)
III C	66	17 (25.8%)
IV	13	4 (30.8%)

rence was in the residual ovary, and that case was remedied by a second operation [10].

In our cases we decided to conserve the uterus. Classical surgical treatment of Stage 1C ovarian cancer includes hysterectomy. The oncological efficacy of removing the uterus in such early cases can reasonably be doubted. Our decision to keep the uterus is to preserve the reproductive performance of these patients. The advantages of removing the uterus are minimal. Serous metastatic microscopic implants can be present but the uterine peritoneum is a small part of all pelvic peritoneum. Endometrial metastasis is infrequent in early stages and may be detected eventually by curettage. Chemotherapy could also be considered efficient in this type of metastasis. Conservation of the uterus for reproductive performance can be proposed in young women without impairing their vital prognosis, thus showing that bilateral salpingo-oophorectomy does not affect uterine function or vascularization.

In conclusion, in early-stage ovarian cancer Stage 1C, conservation of reproductive performance should include uterine conservation, thus permitting subsequent oocyte donation.

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Case Reports

Primary non-Hodgkins lymphoma of the ovary in the background of human immunodeficiency virus (HIV): A bold and curative approach to treatment

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Summary

Non-Hodgkins lymphoma of the ovary is a rare disease and there is only one previously documented case arising in a patient with human immunodeficiency virus (HIV). In this report, the authors discuss the management of a case of non-Hodgkins Lymphoma of the ovary occurring in a patient with HIV and demonstrate that treatment regimens may be successfully implemented in this immuno-compromised population without an increase in adverse effects.

Key words: Non-Hodgkins Lymphoma; Ovary; Human Immunodeficiency Virus.

Introduction

There were an estimated 39.5 million (34.1 million-47.1 million) people worldwide living with HIV at the end of 2006 according to the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organisation (WHO). Based on UNAIDS estimates, approximately 63% of people living with HIV in the world are from the sub-Saharan Africa region [1]. Just over 5,000,000 South Africans were HIV-positive in 2004, which constitutes 11% of the population [2].

There has been considerable improvement in access to antiretroviral therapy for adults in sub-Saharan Africa with an estimated 100,000 people receiving antiretroviral therapy in 2003, increasing to more than 1.3 million in 2006 [3]. In South Africa antiretroviral treatment is initiated once the CD4 count is below 200 cells/μl, in the absence of other AIDS-defining illnesses.

Inkosi Albert Luthuli Hospital based in Durban, South Africa, is a state-run facility privileged to have access to the latest chemotherapeutic and biologic therapies as well as a fluorodeoxyglucose (FDG)-positron emission tomography/computerised tomography (FDG-PET/CT) scan facility. This institution also has to contend with the raging HIV pandemic and HIV-associated malignancies. The lack of a cancer registry in South Africa is a major limiting factor towards performing an accurate assessment of the impact of HIV-associated malignancies in our health system [2].

We discuss the presentation and management of a rare case of HIV-associated primary non-Hodgkins lymphoma of the ovary.

Case Report

A 25-year-old para 1 woman presented with an eight-month history of right-sided iliac fossa pain. There was no associated weight loss or night sweats reported.

On enquiry into previous medical illnesses, we learned that she had been diagnosed with extrapulmonary tuberculosis, diagnosed on a positive acid fast bacilli (AFB) culture of the ascitic fluid in 2000. She was treated with a course of anti-TB treatment for 12 months. She was also diagnosed with HIV in 2005 and has been on antiretroviral treatment since diagnosis.

She was initially evaluated at her regional hospital. An ultrasound pelvis done showed a mixed echogenic mass with solid and cystic components noted superior to the uterus and extending into both adnexae measuring approximately 7.7 cm in diameter. A laparotomy was performed at the referral centre which revealed a large cystic right ovarian tumour measuring 16 cm x 17 cm x 10 cm. In view of her age and parity, only a right oopherectomy was performed. Histology revealed high-grade non-Hodgkins B cell lymphoma with CD20 immunopositivity. Positive staining for Ebstein-Barr virus was also demonstrated.

She was subsequently referred to our clinic. We requested a FDG-PET/CT scan (Figure 1) as a baseline staging investigation which revealed an FDG avid mass in the right adnexal region. Sub-centimetre paraaortic lymph nodes were noted on computed tomography (CT) scan with normal FDG uptake.

The patients' serum beta-2 microglobulin (beta 2M) level was 2.7 mg/l and serum lactate dehydrogenase (LDH) level was 305 U/l. The patient refused a bone marrow aspirate, and trephine biopsy. Her baseline CD4 count was 468 cells/μl.

She was subsequently started on a course of R-CHOP (Rituximab 375 mg/m^2 IV d1, Cyclophosphamide 750 mg/m^2 IV d1, Doxorubicin 50 mg/m^2 IV d1, Oncovin 1.4 mg/m^2 IV d1, Prednisone 100mg/day p.o D1-5). She received six cycles of this regimen. Her CD4 count was repeated thereafter and was found to be 369 cells/μl. The use of systemic chemotherapy and rituximab was well tolerated by the patient without haematological toxicities.

Response to treatment was evaluated with a repeat FDG-PET/CT scan (Figure 2).

She subsequently received involved field radiotherapy (IFRT) to a dose of 30 Gy in 2 Gy fractions to the right adnexal region. She did not experience any adverse effects to the radiotherapy. The patient remains alive and well to date.
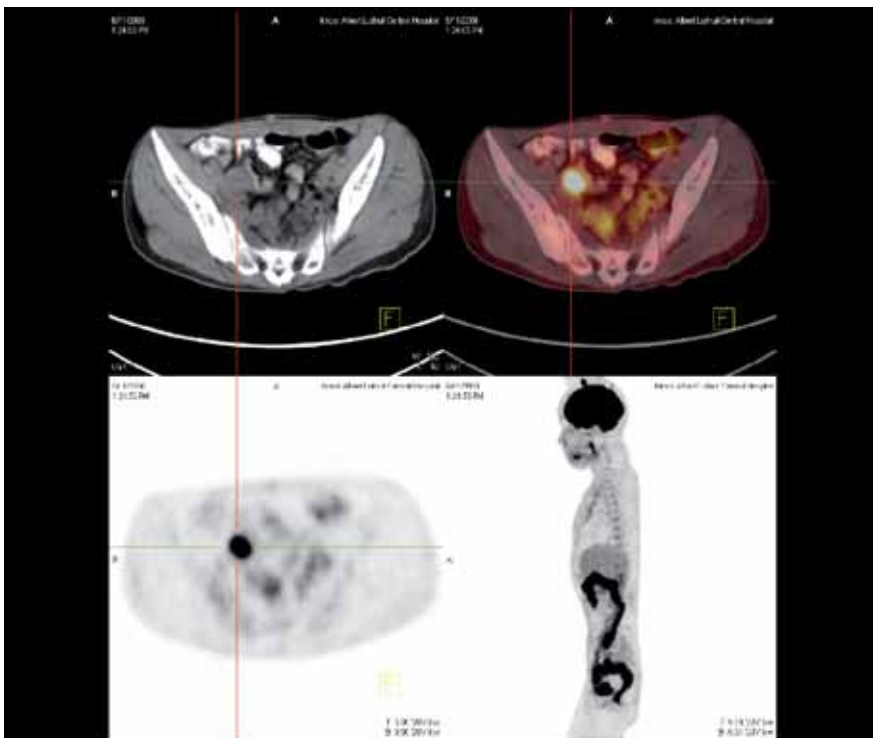
Figure 1. — FDG- PET/CT scan image on presentation with FDG uptake noted in the right adnexal region.
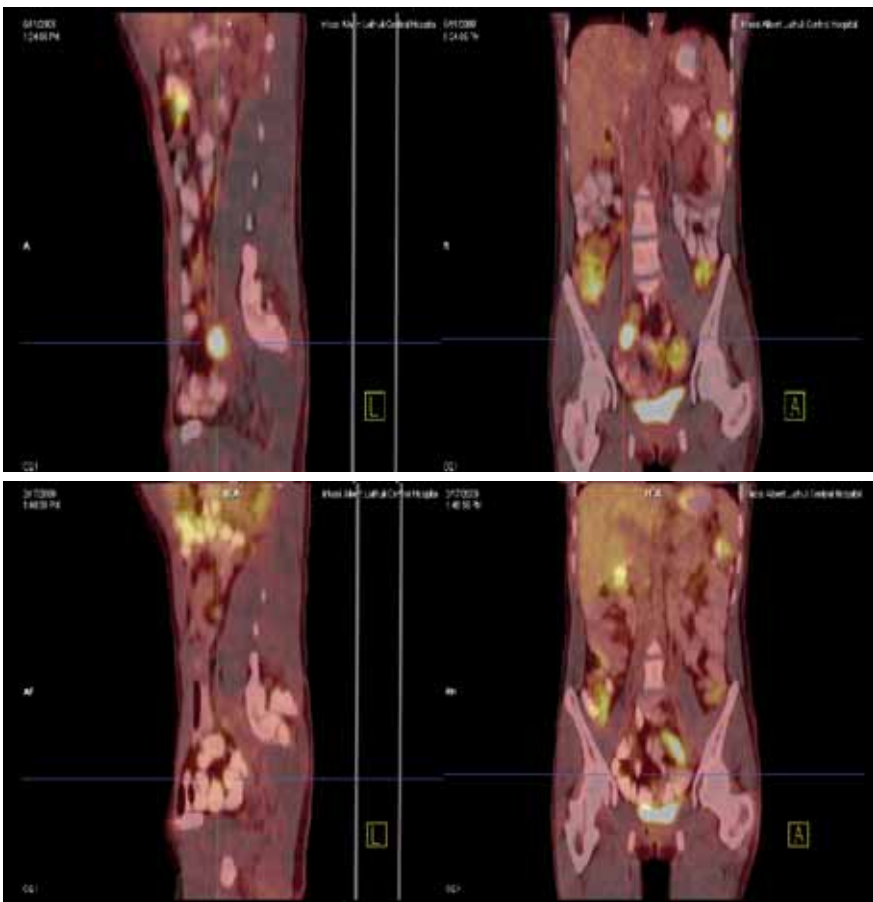
A. Pre-treatment

B. Post-treatment

Figure 2. — Comparison of FDG-PET scan images before and after treatment.

Discussion

Non-Hodgkins lymphoma (NHL) occurs 60-200 times more frequently in the HIV-infected population than in the general population [2]. Approximately 1-6% of HIV-positive patients develop lymphoma each year [4].

The incidence of NHL appears to be on the wane with the increasing use of highly active antiretroviral therapy (HAART) [4]. It is thought that immune reconstitution accompanying the use of HAART offers a protective effect on the development of AIDS-related lymphoma and may account for the declining incidence of NHL. A decline in CD4 counts has also been documented to increase the likelihood of developing lymphoma [5].

The vast majority of lymphomas observed in the HIV population are of B cell origin. The classification of HIV-associated lymphomas can be broadly divided into five main groups: polymorphic lymphoid proliferations (5% of all HIV-associated lymphomas), systemic NHL of various histological subtypes that are more commonly seen in the immunocompetent (80%), plasmablastic lymphoma of the oral cavity (3%), primary central nervous system lymphoma (15%), primary effusion lymphoma (4%), and Hodgkin's lymphoma. The latter is not an AIDS-defining illness [4].

Systemic NHL occurs more commonly in advanced HIV infection, with CD4 counts below 100 cells/μl. Most of these represent high-grade lymphomas with patients often presenting with advanced stage. The most frequent sites of involvement are the gastrointestinal tract, lung, liver, bone marrow and central nervous system [4].

Extranodal NHL accounts for 20-24% of all NHL. They most commonly arise from the gastrointestinal tract, lung, central nervous system and skin as well as the thyroid and salivary glands. While the female genital tract and particularly the ovary may be sites of metastatic involvement, primary genital tract lymphomas are rare neoplasms, accounting for less than 0.5% of all genital cancers and representing 1.5% of all non-Hodgkin's disease. The most common genital sites are the ovary, cervix and corpus of the uterus. Primary lymphomas of the vagina or vulva are uncommon. These tumours usually occur in the 5th decade of life, although the age at presentation is variable. The common presenting symptoms include intermittent vaginal bleeding, dyspareunia, vaginal discharge and pelvic pain [6].

The commonest histological subtype of primary ovarian NHL is diffuse large B-cell lymphoma. Genital lymphomas tend to have a less aggressive course than nodal NHL. It also has a low incidence of recurrence and is associated with a good prognosis. The 5-year survival rate is between 80-90% [6].

On account of the rarity of the disease, there is no established consensus on management. Based on the literature, the mainstay of treatment for primary genital lymphomas is radiotherapy alone or in combination with surgery and/or chemotherapy [6].

In the pre-HAART era, prognosis for patients with AIDS-related lymphomas was dismal with a median survival of five to eight months. Reduced-dose chemotherapy regimens were often used in these patients. Currently patients are treated with HAART and standard dose chemotherapy, which has resulted in response rates comparable to those seen in immunocompetent patients [2]. The introduction of HAART has resulted in an improvement in overall survival when compared to historical controls based on recent reports from a number of groups. The complete remission rate and overall survival with CHOP chemotherapy has improved with the addition of HAART to chemotherapy resulting in a shift in the treatment goal towards complete remission [5].

Adverse effects of chemotherapy include myelosuppression resulting in opportunistic infections as well as drug interactions between chemo-agents and antiretrovirals [2]. Prophylaxis against opportunistic infections remains a major consideration due to the anticipated decline in CD4 cell counts while on chemotherapy in both immunocompetent and immunocompromised patients. Established guidelines in the management of HIV infection suggest that prophylaxis against *Pneumocystis carinii* pneumonia should commence when the CD4 count falls below 200 cells/mm³ and against mycobacterium avium complex when it falls below 50 cells/mm³ [5].

HIV-associated systemic NHL has frequent expression of the CD20 receptor which has led to extensive investigations into the role of rituximab in combination with chemotherapy [4]. Rituximab in addition to chemotherapy has yielded improved response rates (70% complete response with a 59% 2-year survival) [5].

There is only one other reported case on HIV-associated primary NHL of ovary, although in that case the patient was unable to receive treatment due to financial constraints [7]. In countries such as South Africa, we are constantly challenged by the need to offer standard-of-care treatment protocols in the backdrop of the HIV/AIDS epidemic and its attendant morbidities. This case emphasises the need for an appropriate patient selection to ensure that HIV-positive patients may still be in a position to be treated with regimens utilised in immunocompetent patients.

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High levels of xenoestrogens in patients with low-grade endometrial stromal sarcoma - report of two cases

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Summary

Background: Endometrial stromal sarcomas (ESS) are rare uterine tumors with unknown etiological risk factors, but estrogen-dependent growth promotion. *Cases:* We present two patients with advanced ESS, who had increased levels of p,p-DDE; hexachlorobenzene; PCB 28; PCB 52; PCB 101; PCB 138; PCB 153 and PCB 180 in abdominal adipose tissue. Other xenoestrogens were within expected limits for the non-exposed European population. *Conclusion:* Increased levels of xenoestrogens in patients with ESS may be involved in the pathogenesis of ESS. Chronic exposure to xenoestrogens may be a risk factor for tumor progression.

Key words: Endometrial stromal sarcoma; Estrogen; Xenoestrogen; Pathogenesis.

Introduction

Endometrial stromal sarcomas (ESS) are rare uterine tumors, representing less than 1% of all gynecological malignancies. They have no known etiological risk factors such as exogeneous carcinogenic agents. Only one cell culture study with normal endometrial stromal cells reports a sarcomatous transformation after treatment with the carcinogen N-methyl-nitro-N-nitrosoguanidine [1]. The individual steps involved in malignant transformation of endometrial stromal cells are largely unknown, but progression of disease is estrogen-dependent [2].

Hyperestrogenism may occur due to endogeneous and exogeneous factors. Endogenous hyperestrogenism can occur either locally within endometriotic tissues or systemically due to pregnancy, adiposity and polycystic ovarian syndrome. Exogenous hyperestrogenism can be induced by ovulation-stimulating drugs in protocols of assisted reproduction and estrogen-containing hormone replacement therapy [2]. Some environmental substances are also known to induce exogenous hyperestrogenism. Such xenoestrogens are a heterogeneous group of chemicals that differ from naturally occurring estrogens. Of particular concern are hormonally active environmental agents such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD= dioxin) and other persistent compounds such as polychlorinated biphenyls (PCBs) and organochlorine pesticides that bioaccumulate and magnify within the food chain [3].

We analyzed the following xenoestrogens in two patients with advanced EES: p,p-DDE, o,p-DDD; p,p-DDD; o,p-DDT; p,p-DDT; alpha endosulfan; beta endosulfan; dieldrin; endrin; isodrin; cis-chlordane; trans-chlordane, alachlor; o,p-methoxychlor; p,p-methoxychlor; 1,2,3-tricholorbenzol; 1,2,4-trichlorbenzol; 1,3,5-trichlorbenzol; 1,2,3,5-tetrachlorbenzol; 1,2,4,5-tetrachlorbenzol; 1,2,3,4-tetrachlorbenzol; pentachlorobenzene, hexa-chlorobenzene; alpha-HCH; beta-HCH; gamma HCH; delta HCH; hexachlorobutadiene; trifluralin; PCB 28; PCB 52; PCB 101; PCB 138; PCB 153 and PCB 180.

After freeze drying of the homogenized and freeze dried formalin (3%) fixed tissue the samples were soxhlet extracted by toluene/ethanol. An aliquot of the extract was used for lipid determination. Further sample treatment comprised a multi-step cleanup by column liquid chromatography. Measurement of the analytes was done by GC/HRMS (dioxins and dioxin-like PCBs) and GC/LRMS (indicator-PCBs, organochlorine pesticides) respectively. The quantification was done by isotope dilution using ^{13}C-labeled standards, which were added prior to extraction.

Case Reports

Case 1: A patient had presented with FIGO Stage I ESS at age 38. She had two recurrences at age 46 and 50 years resulting in a colectomy. Formalin-fixed adipose tissue from the second recurrence revealed increased levels of the following xenoestrogen: p,p-DDE: 370 mg/g; hexachlorobenzene: 300 ng/g; PCB 28: 84 ng/g; PCB 52: 86 ng/g; PCB 101: 420 ng/g; PCB 138: 150 ng/g; PCB 153: 320 ng/g; PCB 180: 120 ng/g.

Case 2: A 62-year-old woman presented with FIGO Stage III ESS. Formalin-fixed tissue of the omentum was analyzed. The following xenoestrogens were increased: p,p-DDE: 3100 ng/g; p,p-DDT 250 ng/g, hexachlorobenzene: 410 ng/g; gamma-HCH 86 ng/g; PCB 28: 34ng/g; PCB 52: 44 ng/g; PCB 101: 190 ng/g; PCB 138: 840 ng/g; PCB 153: 2000 ng/g; PCB 180: 880 ng/g.

Both patients had levels of dioxin which are considered within normal limits for non-exposed Europeans.

Discussion

The ubiquitous occuring xenostrogene can be either ingested via the food chain or inhaled when bound to dust. Some xenoestrogens can cross the placenta and are present in breast milk. The lipophilic characteristics of these compounds allow bioaccumulation in animals and particularly in humans, who represent the end of the food

chain. Xenostrogens have been linked to increased cancer risk in exposed people [4] and their toxicity has led to a ban in many countries world wide (e.g. PCBs). This is the first time that hormonally active environmental agents have been demonstrated in the abdominal adipose tissue of patients with advanced ESS. In particular p,p-DDE, p,p-DDT, hexachlorobenzene and Lindan (γ-HCH) were demonstrated in significantly higher concentrations than those considered "normal" in European countries [5]. The observed concentrations compared to those of acutely exposed people living close to production plants and storage facilities of organochlorine pesticides [6].

Progression of most ESS is influenced by steroid hormones after binding to their receptors, in particular estrogen receptor isoform alpha, which has been demonstrated in 80% of ESS [7]. Exogenous and endogenous estrogens as well as xenoestrogens may lead to a growth stimulation of tumor cells. At present it is unclear if and how xenoestrogens are involved in tumor progression of ESS. Sequence variations of enzymes can account for differences in effects of xenoestrogen. Martuci and Fishman [8] suggest that genetic polymorphisms in cytochrome P450 (CYP) 1A1 and CYP1B1 are associated with interindividual susceptibility to organochlorines. CYP1A1 and CYP1B1 are phase I drug-metabolizing enzymes that are critical to both metabolism of xenobiotic and naturally occurring estrogens. Furthermore, the C1558-T polymorphism of the aromatase gene CYP19A1 has been associated with increased susceptibility to estrone and estradiol. The prevalence of the mutated T/T phenotype is similar in the general Caucasian population and 20 analyzed European ESS patients (personal unpublished data). It has also been suggested that exposure to DDE, a metabolite of DDT, and other pesticides may cause conformational changes in the estrogen receptor alpha [9].

In conclusion, xenoestrogens were demonstrated in increased concentrations in both analyzed patients with ESS. Chronic exposure to xenoestrogens may be involved in the pathogenesis of ESS and/or associated with an increased risk of tumor progression.

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Cisplatin-induced syndrome of inappropriate antidiuretic hormone (SIADH) in a patient with neuroendocrine tumor of the cervix: a case report and review of the literature

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Summary

We present a case of the syndrome of inappropriate antidiuretic hormone (SIADH) secondary to cisplatin therapy in a patient with advanced-stage large cell neuroendocrine carcinoma of the cervix. This occurred after the first cycle of cisplatin and then again after the second cycle. Carboplatin was substituted for cisplatin, and there were no further episodes of SIADH.

Key words: Cisplatin; SIADH; Cervix; Syndrome of inappropriate antidiuretic hormone; Neuroendocrine tumor.

Introducton

Cisplatin is the most commonly used agent in patients diagnosed with cervical carcinoma requiring systemic therapy. Cisplatin is associated with known toxicities, primarily neuropathy and nephropathy. The nephropathy associated with cisplatin usually results in acute elevations in serum blood urea nitrogen (BUN) and creatinine as well as loss of potassium and magnesium. Severe hyponatremia secondary to cisplatin is rare. The syndrome of inappropriate antidiuretic hormone (SIADH) leads to severe hyponatremia and water intoxication with central nervous system changes and has been reported in assocation with cisplatin use [1-4]. We present a case of cisplatin-induced SIADH.

Case Report

A 40-year-old G2P2002 woman with no significant past medical history presented to the emergency room with complaints of vaginal bleeding and was found to have a large, 10 cm necrotic cervical mass on pelvic examination. The cervical mass extended to the upper half of the vagina, bilateral fornices and parametria. Cervical biopsies showed poorly differentiated carcinoma with squamous features and extensive necrosis. Immunostains chromogranin and synptophysin were consistent with large cell neuroendocrine carcinoma. Computed tomography (CT) scans of the chest, abdomen, and pelvis revealed liver lesions and pelvic and aortic adenopathy. The treatment plan for the International Federation of Gynecology and Obstetrics (FIGO) Stage IVB large cell neuroendocrine carcinoma of the cervix consisted of six cycles of cisplatin/etopside, each cycle administered over three days.

The patient underwent her first cycle of cisplatin/etoposide without incident. Three days after chemotherapy, she presented to the emergency room (ER) with complaints of lethargy, nausea, vomiting, and dizziness. Her physical examination was unremarkable, with no neurological deficits. Laboratory values revealed a severely decreased sodium level compared to her prechemotherapy level. A head CT revealed no significant abnormalities, and random cortisol level was within normal limits. She was admitted and treated with fluid restriction to slowly correct her sodium level, and was discharged home with a sodium level of 127 meq/l and serum osmolarity of 260 mOsm/kg. Outpatient laboratory values later revealed a sodium level of 140 meq/l. The patient underwent a second course of cisplatin/etoposide and again presented to the ER with weakness, dizziness, and lethargy. Laboratory values revealed a serum sodium level of 117 meq/l. She was again admitted to the hospital for five days for correction of hyponatremia and was discharged home with a serum sodium level of 133 meq/l and no additional electrolyte derangements. The chemotherapy regimen was subsequently changed from cisplatin/etoposide to carboplatin/etoposide. The patient was given carboplatin/etoposide for the remainder of her treatments and had no additional adverse reactions requiring hospitalization.

Discussion

Antidiuretic hormone (ADH) is synthesized in the posterior pituitary and primarily exerts its effects on the collecting tubule of the nephron. It is controlled by a complex system including receptors in the kidney, hypothalamus, pulmonary vein, and left atrium. Excessive secretion of ADH results in impaired water excretion, which can result in water intoxication and hyponatremia. Severe hyponatremia can lead to central nervous system alternations, including confusion, fatigue, seizures, coma, and even death.

The exact mechanism by which cisplatin causes the syndrome of inappropriate antidiuretic hormone (SIADH) is unknown; however, there are known renal and neurotoxin effects of cisplatin. Cisplatin is associated with both proximal and distal tubule damage due to activation of cisplatin in the renal tubules. Impaired sodium reabsorption in the proximal tubule leads to increased fluid in the

descending loop of Henle. Reabsorption of fluid in the distal nephron is insufficient to overcome the fluid overload, and sodium and water excretion increases. Increased sodium in the distal tubule causes a reduction in renal blood flow and glomerular filtration rate (GFR) due to increased vascular resistance. The decrease in GFR further decreases sodium and water resorption. Neurotoxicity due to cisplatin may be due to cisplatin-induced damage to Schwann cells, which comprise the myelin sheath surrounding nerves. DNA damage and segmental cell loss is a possible explanation of demyelination seen in cisplatin-induced neuropathies.

SIADH as a result of chemotherapeutic agents has been previously reported in the literature, usually in association with vincristine/vinblastin and other alkylating agents. Cases of cisplatin-induced SIADH have been reported in the literature as early as the 1980s. In 1982, Levin reported a case of SIADH in a patient with a malignant thymoma following cis-dichlorodiammineplatinum (CDDP) administration [1]. Porter reported a case of SIADH following cisplatin therapy for ovarian cancer in 1985 [2]. Numerous cases have been reported in Japan, including a case of SIADH associated with intrathoracic cisplatin infusion in a patient with a malignant thymoma reported in 1996 [3].

Another potential cause of hyponatremia following chemotherapy administration is Renal Salt Wasting Syndrome (RSWS). Cao *et al*. reported a case of cisplatin-induced hyponatremia in a patient with squamous cell carcinoma of the esophagus [4]. The patient was subsequently diagnosed with RSWS, which is characterized by hyponatremia, excessive sodium excretion, and abnormal renal function. To distinguish between SIADH and RSWS, the urinary excretion of sodium should be checked as the urinary excretion in RSWS is excessive and the urinary excretion in SIADH is normal or decreased. This is an important distinction as the treatment of RSWS is different from that of SIADH. The treatment of RSWS is sodium supplementation.

We believe the cisplatin was the cause of this patient's SIADH as she had no adverse reactions to the carboplatin regimen. Recognizing SIADH as a cause of post-chemotherapy hyponatremia could expedite proper treatment and prevent life-threatening seizures, coma, and death. RSWS should also be considered as a differential diagnosis of post-chemotherapy hyponatremia.

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Choroidal melanoma metastasized to the ovary: case report and review of the literature

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Summary

Background: Malignant melanoma metastases to the female genital tract in only 2.5% of cases. Melanoma is characterized by clinical variability and unpredictable biological behavior with long remissions and relapses that develop rapidly. *Case and review:* A 57-year-old woman was admitted for hypogastric pain and weight loss. She had presented enucleation of the right eye six years before for malignant choroid melanoma. Gynaecological examination revealed enlarged ovaries. Bilateral salpingo-oophorectomy, hysterectomy, and omentectomy were performed. Final pathology diagnosed a choroidal metastatic melanoma (CMM). The patient died seven months later. Only seven cases of CMM have been reported in the literature. Patients affected by CMM ranged in age from 38 to 83 years (median 51.2 years), the time to relapse ranged from 3-25 years (median 51.2 years), the size of the cysts ranged from 4-17 cm (median 9.7 cm) and the survival period ranged from 2-14 months (median 8.1 months). *Conclusion:* Malignant melanoma is misdiagnosed because of lack of discriminatory symptoms, increased tumor markers, characteristic imaging findings and the capacity to mimic other tumors. Today CMM still represents a challenge for gynecologic oncologists.

Key words: Choroidal melanoma; Ovary; Metastasis; Diagnosis; Staging; Treatment.

Introduction

Melanoma is a malignant neoplasm of neuroectodermal origin characterized by melanin production that usually affects the skin, adrenal glands and ocular choroid. It is characterized by clinical variability and unpredictable biological behavior with long remissions and relapses that develop rapidly. Metastases to the lungs and liver are the most common cause of death.

Malignant melanoma (MM) accounts for only 3% of cancers that affect female patients and results in less than 1% of cancer deaths [1]. However, in recent years its incidence has been increasing. Primary MM of the reproductive tract usually arises from the vulva (3-7% of MM) [2, 3], secondary or primary melanomas of the upper genital tract are very rare [4].

To the best of our knowledge, only seven cases of ovarian metastasis from previous choroidal melanoma have been reported in the literature. Patients affected by CMM ranged in age from 38 to 83 years (median 51.2 years), the time to relapse ranged from 3-25 years (median 51.2 years), the size of the cysts ranged from 4-17 cm (median 9.7 cm) and the survival period ranged from 2-14 months (median 8.1 months) [5-11].

In this report we present the eighth case of choroidal metastatic melanoma (CMM) of the ovary treated at our department describing the clinical aspects and histopathological features, while discussing the differential diagnosis of MM affecting the ovary.

Case Report

Clinical history

A 57-year-old postmenopausal woman was admitted for hypogastric pain and weight loss (5 kg in 1 month). Her history was characterized by enucleation of the right eye six years before for malignant choroid melanoma. Gynecological examination revealed enlarged ovaries that had a polycystic appearance on ultrasound scan. Free fluid was in the Douglas pouch. Tumor markers (CA 125, CA 15-3, CA 19-9, CEA, AFP, TPA, NSE and PSA) were within the normal range. The patient underwent midline laparotomy for ovarian cancer. Cytologic evaluation of the free fluid aspirated from the Douglas pouch did not reveal malignant cells. At laparotomy two blackish enlarged ovaries were found. The left (11 x 8 x 5 cm) and the right ovary (4.5 x 2 x 5 cm) were resected. The frozen section revealed metastases of CMM. Macroscopically the omentum showed tiny (1-2 mm), dark-brownish petechial lesions, and omentectomy was therefore performed. Further systematic intraabdominal exploration revealed a pancreatic mass and an enlarged metastatic paraaortic lymphnodes, considered unresectable by the surgeon. An extrafascial hysterectomy was also performed.

Because of the advanced metastatic disease and poor prognosis the patient received only a symptomatic and palliative therapy. She died seven months later.

Clinical methodology and findings

The resected lesion was fixed in 10% buffered formalin and multiple extensively sampled sections were then routinely embedded in paraffin and stained with hematoxylin and eosin (Figures 1-2). Cytoreduction procedures were total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy.

Fig. 1
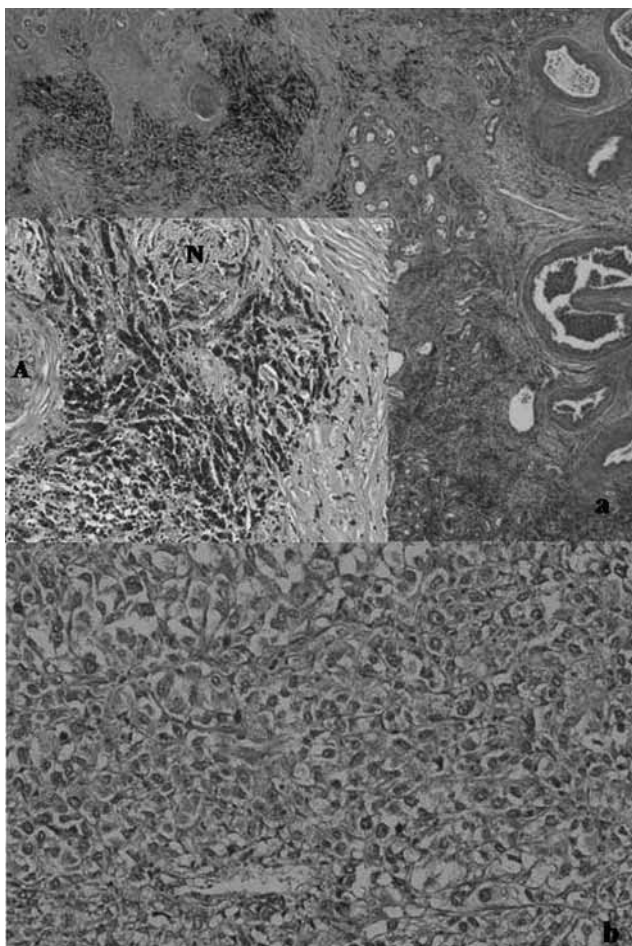
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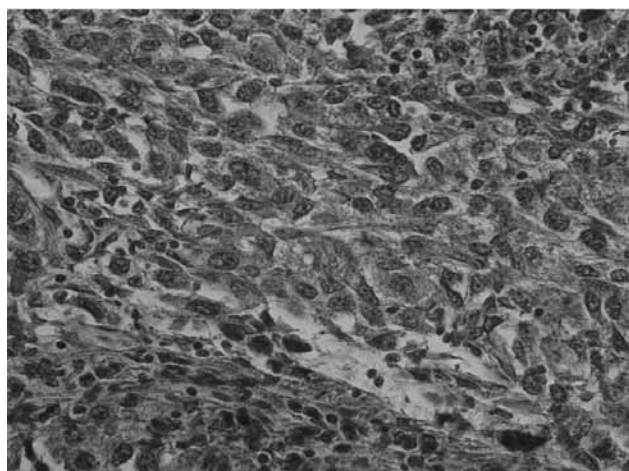
Fig. 3

Figure 1. — a: Malignant melanotic cells with melanin pigment in the ovarian medulla. Perineurium is infiltrated by MM cells [artery (A), nerve (N)]. b: Tumor cells with signet-ring appearance and clear cytoplasm.

Figure 2. — Nested pattern is also present: melanoma cells with epitheloid-like appearance and spindle shaped cells tending to form groups and surrounded by fibrous septa.

Figure 3. — Macroscopic appearance of the dark brownish ovary.

Pathological findings

Final pathology diagnosed metastases of CMM in both ovaries and the omentum. Diagnostic esophagogastroscopy and a transabdominal US examination confirmed the metastatic involvement of the stomach and pancreas.

Histological findings

The left ovary, measuring 4 cm, had a smooth surface and showed cystic lesions filled with a clear fluid and showing a smooth surface with dark spots (Figure 3). The right ovary showed small multilocular cystic lesions, 2 mm in diameter, filled with clear fluid and with a smooth internal and external surface. Microscopic examination revealed bilateral serous cystadenofibroma and metastases of CMM.

The omental sample showed an enlargement of 1 cm and two smaller dark blue areas of 0.5 mm; the cut surface revealed a slightly nodulated, black tumor. Microscopic investigation diagnosed metastases of CMM.

Discussion

Involvement of the female genital tract from extragenital cancers is uncommon. The most common extragenital cancers metastasizing to the female genital tract are breast and gastrointestinal carcinomas [12]. Malignant cutaneous melanoma accounts for only 2.5% of cases metastasizing to the female genital tract and the ovaries are most often affected (75-80% of the cases) [13].

The rarest melanoma metastasizing to the ovary is choroid melanoma. To our knowledge only seven cases have been completely described in the literature [5-11] (Table 1).

Ocular melanoma presents an incidence in the general population ranging from 0.5-1 per 100,000 [10, 14, 15], 50% of patients die in 15 years [12, 10], and 20% metastases occur within five years [16]. It has a latent progression with metachronous metastasis up to 42 years from the first diagnosis [17].

Table 1. — *Characteristics of the patients with ovarian metastasis from previous choroidal melanoma.*

Author	Age	Primary	Symptoms	Ovarian	Other	Imaging	Tumor	IHC	Treatment	Follow-up
Dawson et al. [5]	38	Right eye (choroid spindle cells, pigmented)	Pelvic mass size of an orange	Left ovary (4 y 5 mo, spindle cell)	Right arm Sixth dorsal spine (14 y 5 mo)	–	–	–	BSO	NED (9 mo)
Ben David et al. [6]	62	Left eye (choroid)	Vaginal bleeding left lower abdominal pain nausea	Left ovary (25 y, epitheliod cells, pigmented)	Omentum small and large bowel (25 y)	Semisolid pelvic tumor (USG)	–	–	TAH+ BSO+ OM	CX
Thiery et al. [7]	35	Right eye	Vomiting, diarrhea, weight loss, abdominal swelling	Both ovaries (15 y 3 mo)	Abdomino pelvic (15 y 3 mo)	–	–	–	BSO	CX DOD (8 mo)
Santeusanio et al. [8]	47	Right eye (choroid spindle cells, pigmented)	Pelvic pain	Right ovary (14 y, 17 x 10 x 6.5 cm spindle cells)	No other site	Right adnexal mass (USG) NR	NR	S-100 HMB-45 MART1 vimentin Ki 67 (< 10%)	TAH+BSO+ appendectomy + random peritoneal and omental sampling	(14 mo)
Rey-Caballero et al. [9]	38	Left eye (choroid)	Hypo gastric pain vaginal spotting	Left ovary (9 y, 7 cm)	No other site	Round solid mass in the Douglas	–	–	TAH+BSO+ LFN+OM	Interferon NED (7 mo)
Coutts et al. [10]	83	Left eye (choroid, epithelioid and spindle cells)	Vaginal bleeding	Right ovary (3 y, nodules)	Systemic widespread (3 y)	Autopsy	–	–	Palliative care	DOD (2 mo)
Bloch-Marcotte et al. [11]	50	Left eye (choroids)	Abdominal pain	Right ovary (20 y, 4 cm)	Liver	Heterogeneous Ovarian soft tissue (CT)	–	Melanin A HMB-45	Laparoscopic ovariectomy	CX (2-5 mo)
Index case	57	Right eye	Hypo gastric pain body weight loss (5 kg in 1 mo)	Both ovaries [6 y, left ovary (11 x 8 x 5 cm), right ovary (4.5 x 2 x 5 cm) epithelioid and spindle cells]	Abdomino pelvic (6 y)	Polycystic mass, free fluid in the Douglas pouch (USG)	NR	–	TAH+BSO+ omental sampling	DOD (7 mo)

TAH: total abdominal hysterectomy; BSO: bilateral salpingo-oophorectomy; OM: omentectomy; LFN: lynphadenectomy; Y:years; Mo:months. NR: normal range; USG: ultrasonography; CT: computer tomography; NR: normal range; IHC: immunochemistry ; CX: chemotherapy; NED: no evidence of disease, DOD: died of disease.

Although occult metastasis to the ovary from a primary MM has been reported in up to 18% of women in autopsy studies [18], symptomatic MM is very rare.

It is predominantly diagnosed in women in reproductive age (80%) (average age 35 years), usually unilateral and associated with a poor prognosis [19]. Women of reproductive age may be more prone to metastatic ovarian involvement because the higher blood flow to the premenopausal ovary [20]. The recurrences often occur after a long period of remission (10 years after the initial diagnosis), probably because female hormones might influence the natural history of melanoma [18]. Also, clomiphene has been suspected of increasing the risk of ovarian metastatic melanoma but data have not been able to support this hypothesis [21].

Preoperative diagnosis of ovarian MM presents some difficulties, and usually the diagnosis is made retrospectively after laparotomy.

Similarly to ovarian MM, also CMM can present as a solitary ovarian metastasis [8, 9, 22], or as in the present case as widespread disease [5-7, 10, 11].

Most metastatic tumors involve both ovaries, conversely, ovarian metastases from melanoma are mostly unilateral [19]. On the contrary, Thiery et al. [7] reported a CMM involving both ovaries, as in the present case (Table 1).

Our patient showed diffuse intraabdominal metastatic disease six years after the initial surgery of the primary choroid melanoma even though serum levels of tumor markers were within the normal range.

US examination did not discriminate the ovarian mass; in fact in our case, as reported in the literature, ovarian metastases at US examination presented with images similar to those of primary tumors, multilocular masses and without typical findings that can differentiate them [23]. Magnetic resonance imaging could characterize the lesion only in the presence of a conspicious amount of melanin which causes a peripheral high signal change on T1-weighted images (in contrast to central increases of activity in dermoids and endometriomas), which happens only in one-third of patients [14, 24].

However, when a relapsed melanoma is suspected a positron emission tomography (PET) scan should be performed to detect subclinical metastases and to stage the disease [25].

Melanoma of the ovary represents diagnostic difficulties because the tumors do not have a consistent appearance, and on histology they can be mistaken for germ cell and sex cord stromal tumors [19, 26, 27]. Steroid cell tumors in particular show as abundant eosinophilic cytoplasm as melanomas but they are usually not as mitotically active as melanomas. Although steroid cell tumors may contain lipofuscin pigment, teratomatous elements (primary melanoma), spindle cells and melanin pigment are supportive of melanoma [26]. Moreover immunohistochemistry using melanocytic and sex cord-stromal

markers, such as inhibin and calretinin [28], is likely to be useful, but ovarian sex cord stromal neoplasms and steroid cell tumors are not uncommonly positive with some melanocytic markers, including S-100 and melan-A [29-30]. MM of the ovary rarely shows positivity for inhibin or calretinin [31]. On the contrary, the most specific melanocytic marker HMB 45 may occasionally be positive in ovarian steroid cell tumors [29]. In our cases the diagnosis was made only after laparotomy because of a typical picture of metastatic melanoma of macroscopic and microscopic findings (Figures 1-3).

The diagnosis of primary ovarian MM requires the absence of a primary extraovarian tumor, the detection of an unilateral ovarian tumor with teratoid elements, histological evidence of melanocyte junctional activity, and a good correlation of patient age and symptoms with the cases reported in the literature [26].

The principal treatment is surgery ranging from an ovarian cystectomy to extensive debulking. Medical therapy consists of chemotherapy and immunotherapy. Cisplatin and dacarbazine are the most effective drugs used in patients with persistent or recurrent disease, whereas alfa-interferon and interleukin-1 are promising in metastatic melanoma [15, 32-34].

Even if surgery and use of cisplatin-based chemotherapy appear to improve the outcome, the total response rates of melanoma to adjuvant chemotherapy remain within range of only 20%, with a complete response rate of less than 10% [35].

Hence, several combined therapeutic strategies are commonly adopted because CMM mimics primary ovarian cancer and leads to unnecessary aggressive cytoreductive surgery (Table 1). Secondary ovarian involvement should be diagnosed preoperatively to avoid over-treatment and to provide adequate palliative therapy.

In conclusion, melanomas of the ovary are very rare and very ominous. It is important for the gynecologist to suspect melanoma of the ovary when a patient with a history of a previous malignant melanoma (cutaneous, mucosal) has symptoms such as bleeding, pain, swelling, and abdominal mass.

Malignant melanoma of the ovary represents several diagnostic problems. It is often misdiagnosed because of non discriminatory symptoms, normal levels of tumor markers, non characteristic imaging findings and the capacity to mimic other tumors. Furthermore, in cases of CMM, because of its latency, the diagnosis of metastasis from a melanoma is difficult because the patient may have been in remission for many years or because the antecedent is unknown. Although the first case of choroidal metastatic melanoma of the ovary was described in 1922 [5], CMM still today represents a challenge for gynecologic oncologists.

Acknowledgments

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Muscle metastasis of low-grade endometrial carcinoma seven years after diagnosis: A case report

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Summary

Background: Early-stage low-grade endometrial carcinoma has an excellent prognosis. In few cases local relapse and/or distant metastases can occur. We report the muscle as an unusual site of metastasis. *Case:* A 69-year-old woman underwent surgery for FIGO Stage IA, grade 1 endometrioid adenocarcinoma of the endometrium. After four years she had local relapse without response to chemoradiation, requiring pelvic exanteration. Three years later she was diagnosed with a deltoid muscle metastasis confirmed histologically and bone metastases. After failing hormone therapy, chemotherapy was administered. She died eight months after diagnosis of the bone and muscle metastases. *Conclusion:* Low-risk endometrial carcinoma can behave like a high-risk group. Furthermore, this report describes, to our knowledge, the first case of endometrial carcinoma muscle metastasis.

Key words: Endometrial cancer; Early stage; Muscle metastasis; Bone metastases.

Introduction

Endometrial carcinoma is the most common gynaecological cancer. The majority of cases are diagnosed at an early stage, FIGO Stage I, with a 5-year overall survival of 80%-90% [1]. Pelvic recurrence, although relatively uncommon, is usually associated with distant metastases involving the liver and lung. Bone metastases are infrequent but there are a few cases reported in the literature [2].

We report an unusual evolution of Stage IAGI endometrial cancer. The main unexpected feature was the diagnosis of deltoid muscle metastasis. As far as we know, no previous case of muscle metastasis of endometrial cancer has been reported.

Case Report

A 69-year-old woman underwent surgery for endometrial carcinoma with total abdominal hysterectomy and bilateral salpingo-oophorectomy. The final diagnosis was FIGO Stage IA, grade 1 endometrioid adenocarcinoma.

Four years after surgery, the patient presented with vaginal bleeding. A computed tomography (CT) scan showed a large mass infiltrating the floor of the vagina and sigma. Following diagnosis of pelvic recurrence, she was moved to another institution where she received treatment with concurrent chemoradiotherapy. External pelvic radiation was delivered to the pelvis with a total dose of 45 Gy in 25 fractions. Two courses of chemotherapy based on cisplatin (75 mg/m²) plus paclitaxel (135 mg/m²) were administered on the first and last day of radiation therapy. A CT scan performed after treatment showed a minor response, so total pelvic exenteration was performed. The vagina, the sigma, the bladder and the surrounding soft tissues were infiltrated by moderate, grade 2, endometrial adenocarcinoma.

Three years later, the patient came to our office complaining of swelling of her left upper arm. On physical examination, there was a fixed mass measuring 4 x 4 cm in diameter. Magnetic resonance imaging (MRI) showed a large 4 cm mass in the deltoid muscle (Figure 1). A bone scan revealed multiple metastases in the spine and a body CT scan was unremarkable. A muscle biopsy was performed. The pathology showed adenocarcinoma that resembled endometrioid adenocarcinoma positive for estrogen and progesterone receptors (ER/PR), CK7 positive and CK20 negative (Figure 2). Since the tumour had positive hormonal receptors, we decided to start treatment with progestins. After three months of hormonal therapy, we documented disease progression with spine pain getting worse and new bone lesions in her bone scan. A course of palliative radiation was given to the painful bone metastatic sites. Hormonal therapy was stopped and the patient was put on chemotherapy with paclitaxel plus carboplatin. After two courses of treatment, the patient was hospitalized due to dyspnea. A large pleural effusion and liver metastases were documented. Unfortunately, the patient died two months later.

Discussion

The large majority of Stage I endometrial cancer cases without poor prognostic factors have a 5-year overall survival ≥ 90% [1]. However, approximately 11% of endometrial cancer patients develop a recurrence [3]. The distribution of recurrent disease is varied, with some studies demonstrating local recurrence in 50% of patients and others reporting that the majority of recurrences are distant or multifocal [4, 5]. A small number of women will develop an isolated central pelvic recurrence. The role of surgery in this group of women is undefined. Nevertheless, in those women who have previously received radiation therapy or who have failed to respond to radiation, pelvic exenteration may represent the only potentially curative option with overall 5-year survival rates of approximately 20%-40% [6, 7].

Although haematogenous metastases are infrequent in endometrial cancer, the incidence may be higher in patients with pelvic failure. In these cases, metastases to the lung and liver may be observed. Bone metastases are
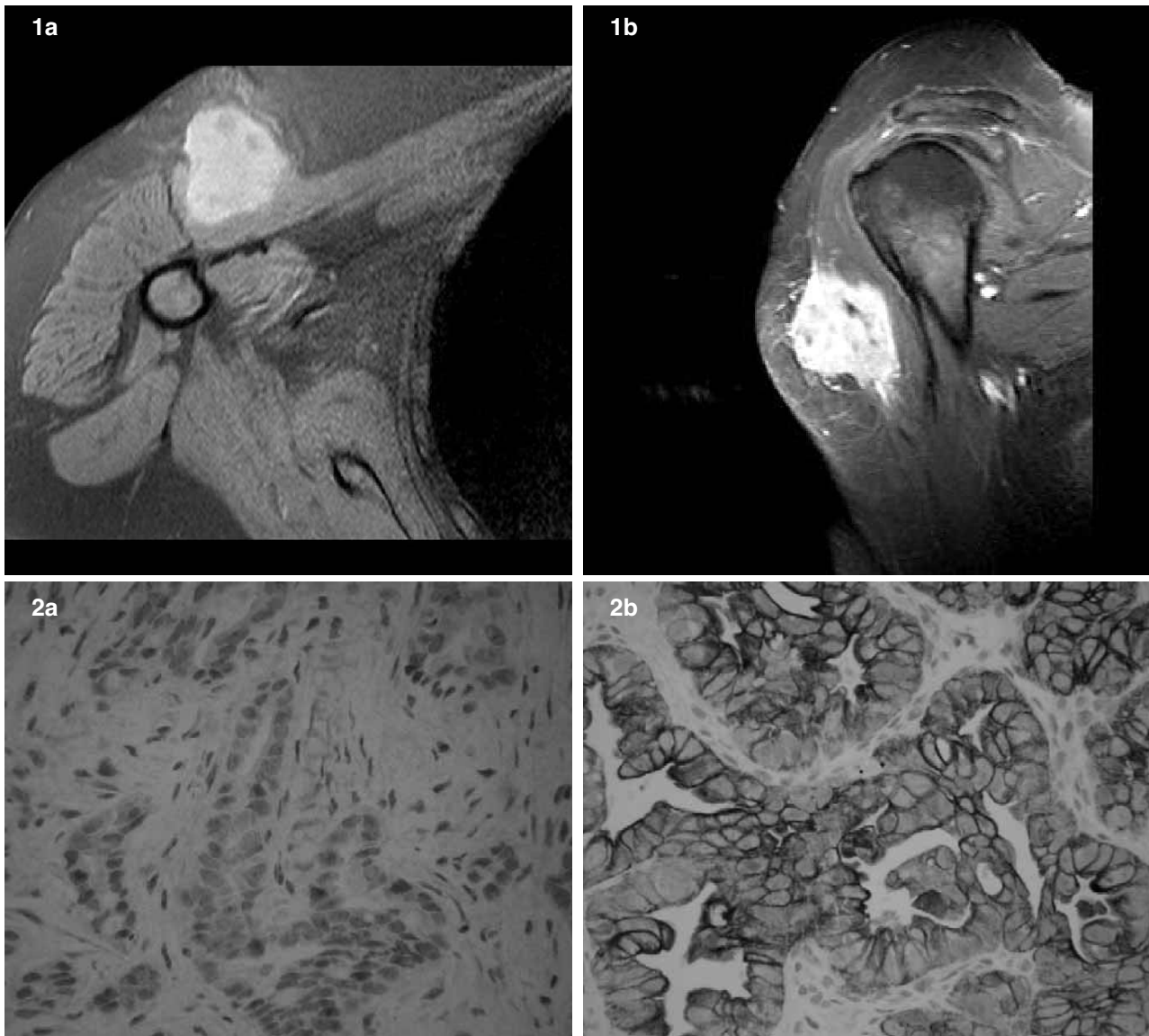
Figure 1. — MRI of the deltoid muscle mass: saggital section (1a) and cross section (1b).
Figure 2. — Immunohistochemistry of the deltoid muscle biopsy confirming an adenocarcinoma of gynaecologic origin: CK20 negative (2a) and CK7 positive (2b).

relatively uncommon with a reported incidence from 2% to 6% [2].

Our case is interesting for different reasons. Firstly, spine metastases were diagnosed three years after the pelvic exenteration and seven years after initial diagnosis. The average length reported in the literature is three years from initial diagnoses. Secondly, an unexpected lesion, deltoid muscle metastasis, was discovered at the same time.

Endometrial cancer usually disseminates by direct invasion or via the lymphatic route. Dissemination to the vertebrae occurs via Batson's plexus and the vertebral venous plexus. In our case, taking into account the simultaneous metastases to multiples sites in the spine and in the deltoid muscle must be the result of bloodstream dissemination [8].

Once endometrial cancer has recurred, the treatment options are quite limited, either hormonal therapy or chemotherapy. The majority of studies of hormonotherapy employing oral progestins, including medroxyprogesterone acetate (MPA) and megestrol acetate, have shown response rates of 11%-56%. Although the majority of responses are relatively short, some patients remain free from disease progression for more than 12 months. The relative lack of side-effects of progestins compared to chemotherapy initially led to their widespread use in unselected patient populations. In recent years, their use has often been confined to the better differentiated cases, which usually correspond with those demonstrating positive hormone receptors [9].

In our case, the metastatic disease was confined to bone

and muscle, but the tumour was moderately differentiated and ER/PR positive so we started MPA. Unfortunately, it did not work and we had to switch to carboplatin (area under of the curve of 6) and paclitaxel (175 mg/m^2) (CP). Results of second-line chemotherapy are generally poor, and only taxanes have response rates greater than 20% in this setting. The most active regimen tested in randomised trials to date is the triplet of cisplatin (50 mg/m^2), doxorubicin (45 mg/m^2), and paclitaxel (160 mg/m^2; TAP). This regimen produces a high percentage of grade 3-4 haematology toxicity and requires granulocyte growth factor support [10]. CP is widely used because of its relative ease of administration and the results from phase II studies [11]. The TAP regimen is being compared with CP in a large Gynecologic Oncology Group trial (GOG #209) which includes women with Stage III or IV disease or recurrent endometrial carcinoma.

This case illustrates that Stage IA well differentiated endometrial cancer can relapse as high grade tumours even in unusual sites. The prognosis of metastatic endometrial cancer remains poor with our current therapy. The emerging results with drugs targeting the PI3K-PTEN-AKT pathway are promising [12, 13].

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Pure Sertoli cell tumor.
A case report and review of the literature

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Summary

Pure Sertoli cell tumor (SCT) is a rare sex cord tumor and a subtype of Sertoli-Leydig cell tumors according to the WHO Classification. They lack a Leydig cell component and do not contain the immature neoplastic stroma found in the neoplasms of the Sertoli-Leydig cell category. The age of the patients ranges between two and 79 years. Sertoli cell tumors occur in women of reproductive age but a few can also occur in children. The most common clinical presentation when occurring in children is isosexual pseudoprecocity. Women of reproductive age and postmenopausal women frequently present with abdominal pain, swelling and menstrual abnormalities. Occasionally SCTs occur in patients who have Peutz-Jeghers syndrome. The tumors are hormone functional in 40-60% of cases. They are often estrogenic, occasionally also androgenic or rarely both. Grossly they are usually yellow to brownish, solid or with several cystic areas. Microscopically they show always almost a tubular growth pattern, but they may also have other growth patterns which can be extensive, making the correct diagnosis difficult. These histologic patterns may result in SCTs mimicking other ovarian tumors. The immunohistochemical panel which usually includes EMA, inhibin, chromogranine, CD99 and calretinin is often helpful in establishing the diagnosis. Most SCTs are Stage I, unilateral, cytologically bland, and clinically benign, but occasional examples are high stage. About 11% of Stage I tumors have worrisome histologic features that may portend an adverse outcome.

Key words: Ovary; Sertoli cell tumor; Pure Sertoli cell tumor; Immunohistochemistry.

Introduction

Pure Sertoli cell tumors are rare sex-cord tumors as designated by the World Health Organization (WHO) [1]. They may be found in patients of any age, with an average age of 30 years. These tumors commonly produce hyperestrinism [2], but they may also be associated with virilization or rarely with a progestational decidual reaction [1, 2]. When these tumors occur in children, isosexual pseudoprecosity is the main clinical symptom. When occurring in reproductive age the main clinical manifestations are menometrorrhagia, amenorrhea, hirsutism, breast atrophy, clitoral hypertrophy and hoarsenes [1-4]. They are all unilateral, usually solid and yellow in color. The diameter of these tumors ranges from 0.8 to 3 cm with the majority being in the range of 4-12 cm. The microscopic patterns of SCT can be extremely variable. Several major categories have been described which may coexist in the same tumor [3, 5]: 1) Well differentiated, which is composed of tubules lined by Sertoli-like cells; 2) Intermediate in which the Sertoli-like cells are arranged in outlines of immature tubules, cords and aggregates; 3) Poorly differentiated which is composed of sheets of spindle shaped cells arranged in a sarcomatoid pattern; and 4) Retiform in which the typical elements of SCT coexist with formations resembling the rete of the ovary or testis. The prognosis of SCTs is usually good and correlates with the stage and degree of differentiation of the tumor. We present a case of rare sex-cord tumor of the ovary with the typical macroscopic and microscopic features of a pure Sertoli-cell tumor and a relevant review of the published literature.

Case Report

A 47-year-old, para 3, gravida 2 woman with a history of hypertension and hypothyroidism under medication, presented to our hospital with a 2-week history of intermittent pain localized in the right lower quadrant of the abdomen and secondary amenorrhea for the last five months. She had also noted hypertrichosis, localized mainly in the androgen-effected areas of the skin, such as upper lips, cheeks, abdomen and lumbus, enlargement of the clitoris and central type of obesity. Her menarche was at the age of 12 years and she normally had menses for five days in a 30-35 day cycle. Laboratory tests by repeated counting did not show increased levels of hormones such as free testosterone, delta$_4$-androstenedione and DHEAS. CA125 level was normal but the level of 17(OH)-progesterone was high. Ultrasound sonography of the ovaries revealed a mass in the right ovary. Subsequently the patient underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. The tumor had a tan to yellow cut-surface and a lobular appearance. The tumor measured 8 x 7 x 5 cm. At microscopic examination the tumor was characterized by tubules with lumens lined by well differentiated columnar cells (Figure 1). The tubules were separated by hyalinized collagenous tissue (Figure 2). In addition to the predominantly round tubules, cells were also arranged in cords with oval to round bland nuclei. No Leydig cells or other types of neoplastic cells were seen in this tumor in spite of extensive sampling and cutting of the specimen. The endometrium was weekly proliferative and the glands were
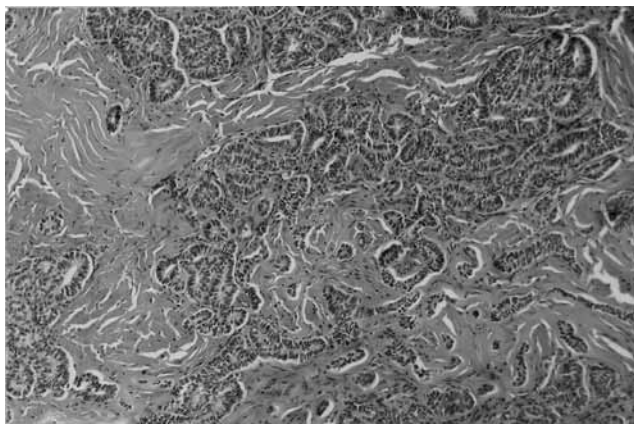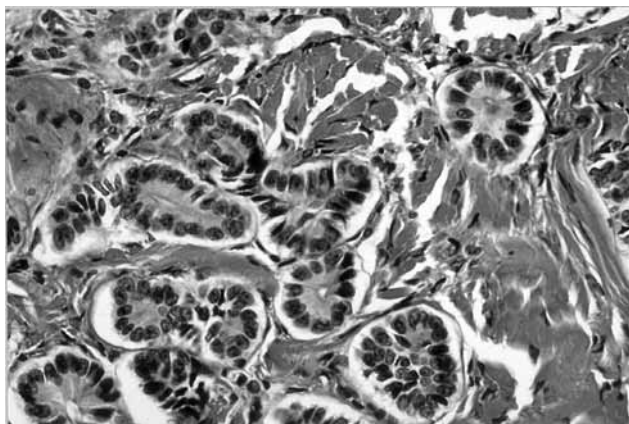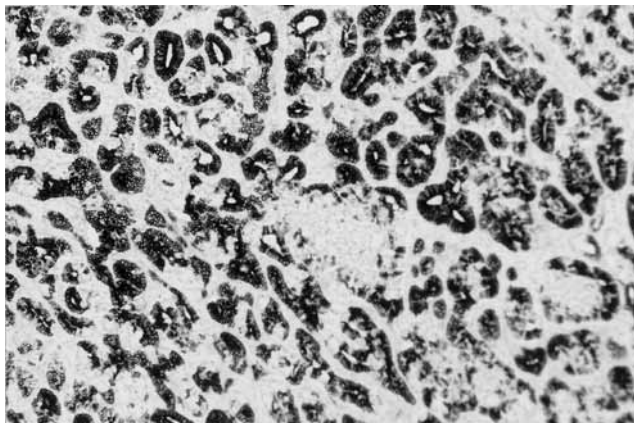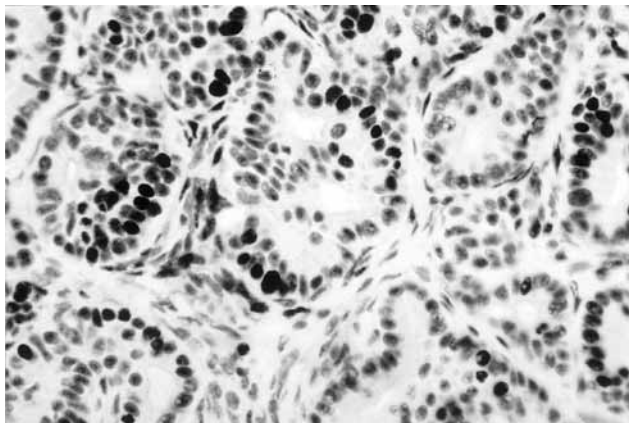
Figure 1. — Sertoli cell tumor H&E x100. Tubules with lumens lined by well differentiated columnar cells.
Figure 2. — Sertoli cell tumor H&E x400. The tubules of the tumor are separated by hyalinized collagenous tissue.
Figure 3. — The neoplastic cells show marked positivity for a-inhibin.
Figure 4. — The nuclei of the neoplastic cells are positive for progesterone.

lined by weekly proliferative endometrium. There was no mitotic activity. A few Nabothi cysts were present in the cervix. At immunohistochemical examination the neoplastic cells showed marked reactivity for low and high molecular weight cytokeratins, a-inhibin (Figure 3), CD99 and calretinin. Progesterone was expressed in 90% of the nuclei of the neoplastic cells (Figure 4). There was negativity for vimentin, CEA, CA125, CA19-9, TTF1, synaptophysine and chromogranine and estrogen. The diagnosis of a "pure Sertoli-cell tumor" was finally established. Our patient was free of disease 20 months after surgery.

Discussion

Sertoli cell tumors make up about 4% of Sertoli-Leydig cell tumors according to the WHO Classification [1]. The term Sertoli cell tumor was used by Morris and Scully [6]. Pure Sertoli cell tumors of the ovary are quite rare neoplasms. They typically occur in women of reproductive age but a few can also occur in children [7, 8]. When occurring in women in reproductive age or in postmenopausal women the patients usually present with abdominal pain or swelling and menstrual abnormalities [8, 9]. Sometimes the tumor can also be an incidental finding [10, 11] in a routine gynecologic examination.

When occurring in children isosexual pseudoprecosity is the main clinical symptom [7, 8]. Patients with SCT may have elevated levels of serum estrogen, progesterone and luteinizing hormone [12, 13]. Estrogen production may result in menstrual abnormalities or postmenopausal bleeding and endometrial hyperplasia, depending on the menopausal status of the patient [14]. Progesterone production results in decidualization of the endometrium or peritoneum [15]. Testosterone production results in amenorrhea or virilization [11]. The secretion of androgen suggests the possible presence of unsampled Leydig cells. Occasionally SCTs present in patients with Peutz-Jeghers syndrome [7, 8], which is characterized by mucocutaneous pigmentation, hamartomatous polyps and occasionally carcinomas of the gastrointestinal tract, adenoma malignum of the uterine cervix and sex-cord tumors of the ovaries. SCT may rarely cause hypertension due to renin production [10, 11]. Grossly SCTs are unilateral and the two ovaries are involved with equal frequency. They are well circumscribed, solid neoplasms with a smooth and lobulated external surface and a yellowish sectioned surface. Areas of hemorrhage and or cystic degeneration may be seen in larger tumors. The most common microscopic pattern shows a tubular

growth pattern. The tubules can be round or elongated, hollow or solid or a combination of these features. The tubules are lined by columnar to cuboidal cells with moderate to abundant amounts of pale to eosinophilic cytoplasm. The nuclei are typically oval or spherical with a small nucleolus. Mitotic figures are usually scanty (< 1 per 10HPF) [11, 12].

The microscopic pattern of SCT can be variable: 1) Well differentiated which is composed of tubules lined by Sertoli-like cells; 2) Intermediate in which the Sertoli like cells are arranged in outlines of immature tubules, cords and aggregates; 3) Poorly differentiated which is composed of sheets of spindle shaped cells arranged in sarcomatoid pattern; and 4) Retiform in which the typical elements of SCT coexist with formations resembling the rete of the ovary or testis [13]. Heterologus elements such as mucinous glands, bone, skeletal muscle and cartilage may be present in some tumors [3, 11, 14]. Immunohistochemical staining of SCT shows reactivity for low molecular weight keratins, a-inhibin [16], vimentin, S100 and SMA. Recently the markers calretinin CD99, NSE and MART-1 have also been added to the immunohistochemical panel [17]. The tumor is typically negative for the epithelial membrane antigen, placental alkaline phosphatase. The differential diagnosis of SCT includes mucinous tumors, low-grade endometrioid carcinoma [19], carcinosarcoma, tubular Krukenberg tumor, tubular carcinoid [20] and ovarian tumors of probable Wolffian [20] origin and dysgerminoma [11, 22]. The most important differential diagnosis is endometrioid carcinoma of the ovary. It is well known that endometrioid carcinoma of the ovary may have a Sertoli-like appearance which even allows the use of the term "sertoliform endometrioid carcinoma" [11]. The oxyphilic variant of SCT can rarely cause any problems in the differential diagnosis with other oxyphilic [23] tumors. In these cases clinical information may be helpful because this variant of SCT may be associated with Peutz-Jeghers syndrome. The most important predictors of biological behavior are the stage and degree of differentiation of the tumor. Patients with Stage I tumor have been reported to have a 95% five-year survival, while some series report a 100% mortality rate in patients with tumors in Stage III or higher. The complete cure consists of hysterectomy and bilateral salpingo-oophorectomy in women who have children. In women who want to bear a child the surgeons apply usually unilateral salpingo-ophorectomy [24]. In conclusion we have presented a rare sex cord tumor of the ovary with the typical microscopic features of a pure SCT. Postoperatively the signs of hyperandrogenemia subsided and the patient improved clinically.

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Immunohistochemical findings in primary fallopian tube cancer. Case report

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Summary

Primary fallopian tube carcinoma is a rare malignancy, representing about 1% of female genital tract malignancies. We present a case report and compare the medical performance with accessible data from the literature as well as present immunohistochemical analysis of estrogen, progesterone, and proliferative together with basic cytokeratin reactions. We found that immunohistochemical expression of ER-β was dominant over ER-α which encourages further evaluations to be performed on a larger number of samples, especially taking into account the very scant progesterone receptor expression we noted. On the basis of the course of disease under study, etiological problems and the possibility of clinical misdiagnosis have been discussed. The low prevalence rate and lack of clear symptoms of this type of carcinoma makes the final clinical diagnosis almost impossible without an intraoperative histopathological study. Multicenter studies are needed to improve the understanding of possible risk factors.

Key words: Primary fallopian tube cancer; Oncogenes; ERα; ERβ; CK7; CK20.

Introduction

Primary fallopian tube cancer is a rare disease, representing about 1% of all female genital tract malignancies [1]. Its etiology is poorly known. Moreover, little is known about its risk factors, prophylaxis or prognostic factors. Formerly it was thought that risk factors were similar to the ones defined in fallopian tube cancer, nonetheless more and more reports proving their distinction appear [2]. The incidence of primary fallopian tube cancer has risen over the last decades, predominantly among women of higher economic status [3]. Difficult preoperative diagnostics is a clinical problem. Only 4% of cases are correctly diagnosed before an operation. Treatment is based on radical or as comprehensive as possible cytoreductive surgery followed by chemotherapy (cisplatin, paclitaxel). A five-year survival rate ranges from 22 to 57% [3].

As a matter of fact immunohistochemical markers used to distinguish ovarian carcinoma, such as cytokeratin 7 (CK7) or cytokeratin 20 (CK20), are virtually the same for tubal cancer.

Until now, it has also been controversial whether ovarian epithelium carcinomas possess steroidogenic enzymes, but several studies have previously shown that ovarian epithelial normal and tumor cells expressed progesterone and estrogen receptors α and β (ERα and ERβ) and are thus potential targets of ER-mediated effects on proliferation [4, 5].

The aim of our study was to describe medical performance in a case of fallopian tube cancer as well as perform immunohistochemical detection of estrogen receptors (ERα and Erβ), progesterone receptor (PgR), and proliferation marker Ki67 as well as basic cytokeratin CK7 and CK20 markers.

Case Report

Clinical Summary

A 72-year-old woman with a BMI of 24.6 presented with pain in the hypogastrium of two months duration. Her history included two natural deliveries and she had her last menses at the age of 49 years. She had been treated due to arterial hypertension for 20 years and there was no family history of oncological diseases.

The following were found on examination: mobile resistance of 4-5 cm diameter in the right uterine adnexa. An outgrowth of heterogeneously risen echogenicity and irregular contour, measuring 42 x 45 mm, was found in the right adnexa by ultrasonography (US). The remaining organs of the small pelvis and abdominal cavity were unchanged. Free fluid in the peritoneal cavity was not found. On magnetic resonance imaging (MRI) with intravenous contrast medium, a heterogeneous signal was detected connected to the uterus from the posterior and right side (a well-restricted oval area - 42 x 27 x 40 mm), which amplified after the administration of contrast medium, possibly originating from the right ovary. No spread of the lesion to neighbouring organs was visualized. CA-125 marker level was: 86.70 U/ml and chest X-ray showed no focal lesions. A diagnosis of an adnexal tumor was made – probable ovarian cancer. The patient underwent laparotomy.

The suspicion of malignant infiltration, approximately 5 cm in diameter, was intraoperatively found in the right fallopian tube, while fallopian serosa was found to be smooth. The ovaries were bilaterally visually unchanged. A single adhesion of the urinary bladder peritoneum to the sigmoid colon was seen on the surface of about 8 mm. There was a the suspicion of a neoplastic lesion of metastatic potential in the adhesion. The remaining organs of the pelvis and abdominal cavity were visually intact.

The uterine corpus together with the adnexa was removed in a typical way followed by omentectomy and appendectomy. The common iliac, inner and obturator lymph nodes were collected bilaterally. Final postoperative outcome was serous pap-

illary carcinoma of the right oviduct G2 (the cancer did not infiltrate the serosa). There was metastatic focus in the sigmoid-bladder adhesion but the iliac and obturator lymph nodes (12 pieces in total) were intact. Neoplastic cells were present in the cytological smear from the peritoneal cavity.

The postoperative course was uncomplicated. The patient underwent supplementary treatment (chemotherapy). At the 26-month follow-up period after the oncological therapy there was no neoplastic relapse.

Method

The resected specimen was fixed in 10% buffered formaldehyde solution for 24 hours and embedded in paraffin blocks at 56°C. For routine histology, hematoxylin-eosin staining was performed. For immunohistochemical studies, a representative section from the resected specimen was selected. The following biological markers were investigated: cytokeratin (CK), CK 7, CK 20, estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), progesterone receptor (PGR) and proliferation marker Ki-67. CK was assessed using monoclonal mouse antibody, Clone AE1/AE3 (Dako, Denmark) at 1:100 dilution; CK 7 was assessed using monoclonal mouse antibody, Clone OV-TL 12/30 (Dako, Denmark) at 1:100 dilution; CK 20 was assessed using monoclonal mouse antibody, Clone K_S20.8 (Dako, Denmark) at 1:100 dilution; ERα was detected with a mouse monoclonal antibody, Clone 1D5 (Dako, Denmark) at dilution 1:200; ERβ was detected with a mouse monoclonal antibody, Clone EMR02 (Novocastra) at dilution 1:150; PGR was detected with a mouse monoclonal antibody, Clone PgR 636 (Dako, Denmark) at dilution 1:100; Ki-67 was detected with a mouse monoclonal antibody, Clone MIB-1 (Dako, Denmark) at dilution 1:150. The sections were deparaffinized in xylene and hydrated through graded alcohol. Antigen unmasking was performed using heat treatment in a microwave oven at 750 W for 7 min. Sections were allowed to cool in the buffer at room temperature for 30 min and were rinsed in deionized H_2O three times for 2 min each. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 20 min. After rinsing in PBS, the sections were incubated with studied primary antibodies for one hour at room temperature. Antibody-antigen reaction was revealed with EnVision system (Dako, Denmark). Staining was routinely developed using 3,3'-diaminobenzidine as a chromogen (Dako, Denmark). Sections were counterstained with hematoxylin. The expression of studied proteins was evaluated with the use of light microscopy. Appropriate positive and negative immunohistochemical controls were done.

Final pathological findings

Histopathological examination of the studied tumor revealed serosal papillary carcinoma of the right side of the oviduct, grade 2 according to the WHO classification.

Immunostaining for ERα, ERβ, PGR and Ki-67 was restricted to the nucleus of the tumor cells, whereas CK and CK 7 staining was membrane and cytoplasmic. ERα was observed in 30% (focally to 50%) of the cancer cells, ERβ in > 90% of the cancer cells, PGR < 5% of the cancer cells (only focally), and Ki-67 in 60% of the cancer cells; CK and CK 7 were positive in the cancer cells and CK20 was negative.
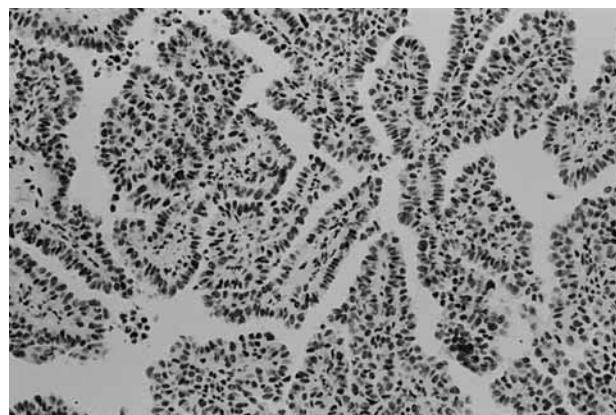
Figure 1. — Positive strong nuclear immunostaining for ERβ assessed in the majority of cancer cells (original magnification - 200x).

Discussion

Primary fallopian tube cancer is a rare female sex organ tumor, similar to ovarian carcinoma but characterized by a worse prognosis [1]. Therefore, it should be treated as a separate disease entity that is independent of other pathological processes of the minor pelvis in women. Primary serous neoplasms in women are found within the borders of the ovaries, fallopian tubes and peritoneum. The above-mentioned three neoplasms could be considered to constitute merely a variety of the same carcinogenesis process, but in fact little is known about primary fallopian tube cancer [2]. It is assumed that hormonal contraception, similarly to parity and breast-feeding, reduces the risk of primary fallopian tube cancer as well as ovarian carcinoma, in contrast to serous peritoneal carcinoma [2, 3]. This may indicate a different route of development for peritoneal carcinoma. The findings of a multicenter clinical case control study published by Moore *et al*. [4] did not prove any differences in survival rates between patients treated for one of the two above-mentioned neoplasms. A problematic issue in case of primary fallopian tube cancer is the difficult differential diagnosis. It can often be clinically misinterpreted as a fallopian tube abscess [5]. According to Riska and Leminen [6], only 4% of fallopian tube cancers are correctly diagnosed before surgery. A similar problem occurred in the case presented here. Based on the interview, physical examination, laboratory diagnostics, US and MRI with contrast, the following diagnosis was made: adnexal tumor – probable ovarian cancer. This diagnosis, however, was confirmed by the intraoperative procedure. Genetic mutations associated with the presence of the BRCA gene may play a key role in this cancer pathogenesis [7]. According to Callahan *et al*. [8], the presence of the BRCA gene enables carcinogenesis in the fimbriae of the oviduct to a greater extent than in the ovary. Moreover, in women with primary fallopian tube cancer a drop in the expression of genes coding for p53

and p27 (kip1) proteins responsible for apoptosis, has been found. On the other hand, in 57% of female patients with advanced stage of the disease, an elevated expression of HER-2/neu oncogene (human epidermal growth factor receptor 2), encoding a receptor protein for cellular growth factor has been found [9]. In ovarian tumor samples the level of ER-α mRNA were similar or slightly higher than those in normal ovaries, while ER-β mRNA was decreased [10]. In another study both ER-α and ER-β mRNAs were expressed in 80% of ovarian cancer samples, however, the ER-α to ER-β ratio was higher suggesting that overexpression of ER-α relative to ER-β mRNA could be a marker of ovarian carcinogenesis [11]. So far no study has shown either gene or protein estrogen and progesterone receptor expression in tubal carcinoma. Although this was only a case study it has been clearly shown that the immunohistochemical expression of ER-β was dominant over ER-α which encourages us to perform further confirmatory evaluations on a larger number of samples, especially taking into account very scant progesterone receptor expression.

Among different cytokeratin combinations, different patterns of CK7 and CK20 staining have so far been mostly used to distinguish between different histological subtypes of ovarian carcinoma [11]. We also attempted to used them to see whether any similarities exist in tubal and ovarian cancers as to the expression of characteristic histological markers. Indeed, absence of CK20 expression with concomitant positive CK7 immunostaining can be characteristic of serous ovarian carcinomas [12], which would not indicate the effectiveness in distinguishing between tubal and ovarian cancer. It is also not surprising that the majority (60%) of cancer cells exhibited high Ki-67 staining since the proliferative potential of these types of tumors is widely accepted.

A deeper understanding of the potential risk factors for primary fallopian tube cancer requires multicenter studies. Furthermore, a need exists for the identification of prognostic factors regarding the treatment at various stages of disease progression.

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Good prognosis for primary ovarian pure nongestational choriocarcinoma using the EMA/CO regime

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Summary

Nongestational choriocarcinoma of the ovary is a rare germ cell tumor with a worse prognosis than gestational choriocarcinoma. In this report we present a case of nongestational chrriocarcinoma where the EMA/CO regime was applied. The clinical features, management, and outcome are discussed.

Key words: Primary ovarian pure nongestational choriocarcinoma; EMA/CO regime.

Introduction

Nongestational choriocarcinoma of the ovary is a rare germ cell tumor and the pure type is less frequent than mixed type among other germ cell tumors [1]. Pure the nongestational choriocarcinoma is extremely rare and accounts for less than one percent of primitive germ cell tumors of the ovary and aggressive tumors [2].

Although the surgical approaches are well identified, there is no concensus on the medical treatment of nongestational ovarian choriocarcinoma.

In this report we present a patient with nongestational choriocarcinoma treated with optimal debulking surgery and chemotherapy with the EMA/CO regimen (etoposide, methrotrexate, actinomycin-D, cyclophosphamide, vincristine) which resulted in the cure of the patient.

Case Report

A 21-year-old woman was referred to our hospital because of abdominal pain. She had a history of a vaginal delivery six months earlier as her only confirmed pregnancy. Pelvic examination revealed bilateral adnexal masses. Pelvic ultrasound (US) scan showed evidence of large complex masses measuring 13 x 11 cm in the right adnexal region and 14 x 12 cm in the left adnexal region, containing both solid and cystic components. A computed tomography (CT) scan showed large bilateral masses which were mostly solid and displaced the uterus. Physical examination documented normal findings. Laboratory findings showed high levels of βhCG (1869 mIU/ml) and CA-125 (86 IU/ml).

Explorative laparatomy including bilateral cyctectomy and appendectomy was performed. As the intraoperative frozen section evaluation reported benign findings, the operation was ended. However, the final pathology report with paraffin blocks showed a diagnosis of choriocarcinoma. The patient consulted with the Oncology Department and decided to be treated with the EMA/CO regimen. Three courses of the EMA/CO regimen were performed with 21-day intervals. Before the first courses

βhCG was ≥ 100,000 mIU/ml and the prognostic score of FIGO was seven. After the third course βhCG level was 99.2 mIU/ml. Thus one more EMA/CO regimen was performed. After the fourth cure, βhCG level was 3.09 mIU/ml. However, the 7 x 5 cm semisolid cystic lesion which was documented by pelvic CT, showed optimal debulking surgery was needed. The second laparotomy demonstrated a 6 x 6 multilocular cystic mass in the right ovary, and a normal left ovary. Peritoneal washing material was collected for cytological examination. Total hysterectomy, bilateral salpingo-oophorectomy, total omentectomy and pelvic lymph node dissection were performed. Pathological examination revealed no tumor invasion in the lymph nodes of the omentum.

After the second operation, the patient was treated with an extra six courses of the EMA/CO regimen. After the sixth cure, βhCG was below 1 mIU/ml. In the first six months after therapy the patient was followed monthly with hCG levels and US. At the end of the six months, controls were performed at 3-month intervals. The patient was well and free of disease at the end of 12 months with normal laboratory and imaging findings and no recurrence was documented. She continues to undergo checkups every six months.

Results

Pathology report: On gross examination a hemorrhagic circumscribed mass was observed. Microscopically, mononuclear cells, hemorrhagia and necrosis were found. Nuclear pleomorphism, hyperchromasia and nucleoli were predominant (Figure 1). Immunohistochemically, βhCG was observed in the syncytiotrophoblasts (Figure 2).

Genetic analysis: We performed genetic analyses from the patient's blood and tumoral tissue to distiguish nongestational choriocarcinoma from gestational choriocarcinoma. DNA was isolated from paraffin blocks and peripheral blood sampling was carried out using the Invisorb Spin Tissue Mini Kit (Invitek, Germany) according to the manufacturer's instructions. For QF-PCR aneuploidy screening, the ChromoQuant TM version 2 kit was used in DNA samples. Samples were analyzed using a 1-
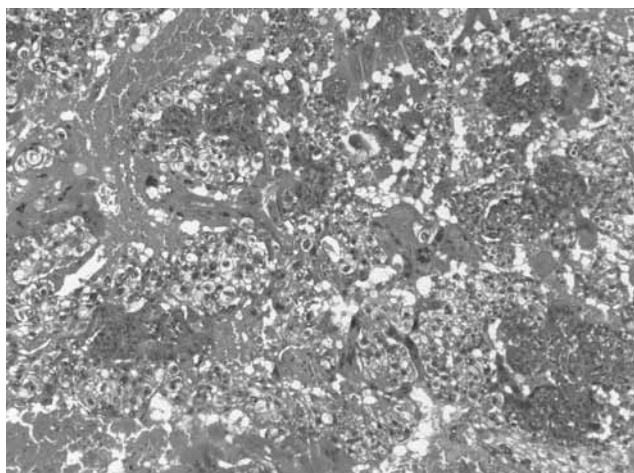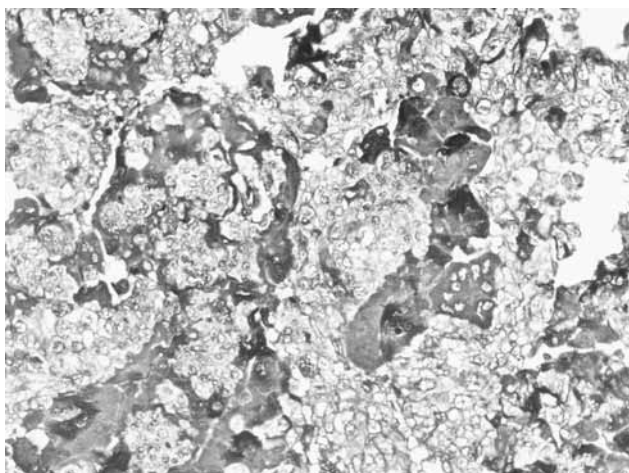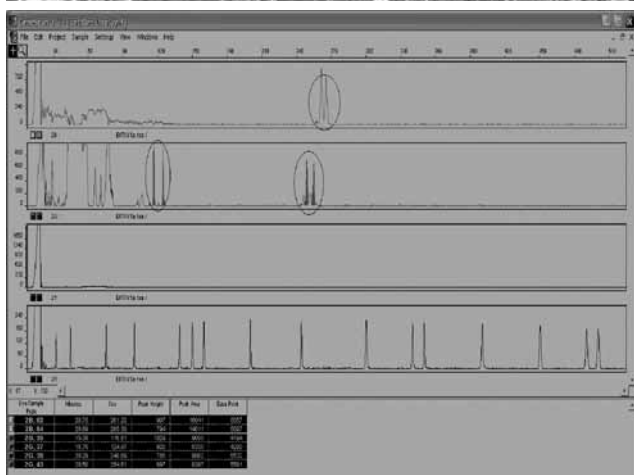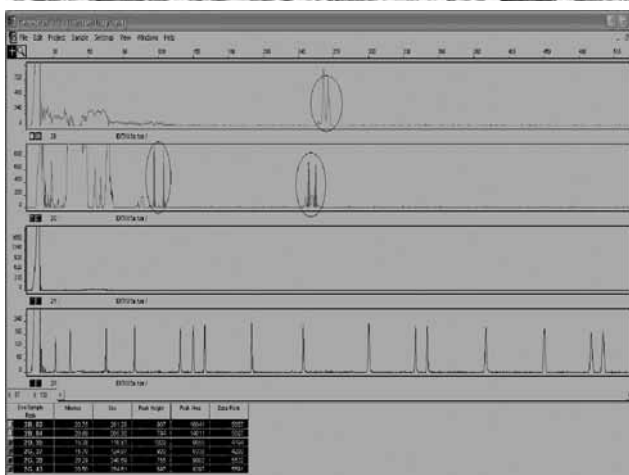
Figure 1. — A mixture of cytotrophoblastic and syncytoiotrophoblastic cells can be seen (H&E x 100).
Figure 2. — Immunohistochemically βhCG positive cells were observed (streptavidin biotin HRP, x 100).
Figure 3. — QF-PCR analysis of extra XY markers from the patient's blood.
Figure 4. — QF-PCR analysis of extra XY markers from tumor paraffin blocks.

tube multiplex QF-PCR in which four STR markers were included. The four markers for chromosomes were SR, DXYS218, DXS6803, and DXS6809 [3] (Table 1). As a result, the same STR regions in both tumor tissue and patient blood were established (Figures 3/4). This result supported the diagnosis of primary ovarian pure nongestational choriocarcinoma.

Table 1. — *List of SRY markers analyzed with the ChromoQuant version 2 kit [3].*

Marker	Label	Het.	Choromosomal location of known alleles	bp
SRY	6-FAM		Yp11.2	Y:463
DXYS218	PET	0.63	Xp22.32 Yp11.3	(PAR1)266,270,274,278,282, 286,290,294
DXS6803	VIC	0.68	Xq12-Xq21.33	106,110,114,118,120,124,128
DXS6809	VIC	0.75	Xq	238,242-6,250,252-4-8,260-2- 6-8,270-4

Discussion

Nongestatiaonal pure ovarian choriocarcinoma is an extremely rare and very malignant tumor with only 30 cases described to date [4]. Ovarian choriocarcinomas typically occur in children and young adults [2] presenting with pain and an adnexal mass [2]. Since nongestational choriocarcinoma appears like its gestational counterpart, serum βhCG levels are useful in both diagnosing and monitoring the response to therapy for nongestational choriocarcinoma. It should be noted that normal levels of βhCG do not eliminate the presence of metastases or recurrences [1]. In our case after the first operation and EMA/CO therapy the βhCG level was normal but we established a metastatic pelvic mass and performed a second operation.

Choriocarcinoma of the ovary may originate from an ovarian pregnancy or by metastasis from another part of the genital tract (mainly the uterus) or as a germ cell tumor differentiating in the direction of trophoblastic structures commonly with other neoplastic germ cell ele-

ments [1]. Gestational choriocarcinoma includes the first two groups mentioned. The latter origin encompasses nongestational choriocarcinomas. In our patient the pathologist did not determine any ovarian or uterine gestational substance during examination of the surgical material. To distinguish nongestational choriocarcinoma from gestational choriocarcinoma we performed genetic analyses from the patient's blood and tumor tissue in paraffin blocks. We observed XX chromosomes and the same alleles in both examinations. This result supported the diagnosis of primary ovarian pure nongestational choriocarcinoma. Thus we planned a chemotherapy regime for nongestational choriocarcinoma.

Nongestational choriocarcinoma of the ovary is a highly malignant germ cell neoplasm which has fulminant progression [5]. Although gestational choriocarcinoma tends to spread primarily via the blood stream, nongestational choriocarcinoma shows lymphatic and intraabdominal spread as well as hematogenous spread and ovarian choriocarcinomas of germ cell origin may be less responsive to chemotherapy than gestational choriocarcinomas [1]. Goswami *et al*. reviewed 30 cases of nongestational choriocarcinoma in the English literature and their report demonstrated that there was no concensus on the optimal chemotherapy following surgery [4]. Due to its rarity, long-term results with chemotherapy have not been specifically described. Therefore both the MAC (methotrexate, actinomycin, cyclophosphamide) and BEP (bleomycin, etoposide, cisplatin) regimens have been used in patients with nongestational choriocarci-

noma. For our patient we used the EMA/CO regimen after surgery. At present our patient's general health is good with no recurrence.

In conclusion, in nongestational choriocarcinoma of the ovary we recommend surgery followed by the EMA/CO regimen as soon as possible to prevent fulminant progression.

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Primary malignant mixed müllerian tumour of the fallopian tube. Report of a case

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Summary

Malignant mixed müllerian tumour of the fallopian tube is an extremely rare lesion and to date only approximately 50 cases have been reported. The tumour is seldom distinguished preoperatively from other more common lesions or ovarian cancer. We report a case of a 60-year-old woman who presented to our hospital with pelvic pain. There was no clinical evidence of ascites or adenopathy. Ultrasound and abdominal and pelvic computed tomography showed a left adnexal mass. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were carried out. Grossly the left side of the fallopian tube was dilated and the cut surface revealed a solid mass filling the entire lumen. Histological examinations showed a malignant mixed müllerian tumour. The tumor was an admixture of both carcinomatous and sarcomatous elements. The carcinomatous element was composed of well to moderately differentiated squamous cell carcinoma and the sarcomatous component was made up of anaplastic spindle shaped cells with hyperchromatic nuclei. An immunohistochemical study was performed. The patient was admitted to the anticancer hospital for further treatment. The prognosis of a primary malignancy of the fallopian tube is poor and depends more on staging than on histologic type and grade.

Key words: Fallopian tube; Mixed müllerian tumour; Immunohistochemistry.

Introduction

Primary malignancies of the fallopian tube are rare accounting for about 0.3-1.1% of all gynaecological malignancies [1]. Malignant mixed müllerian tumour of the fallopian tube is an extremely rare lesion and to date only approximately 50 cases have been reported [2, 3]. The tumour is seldom distinguished preoperatively from other more common lesions or ovarian cancer.

Case Report

A 60-year-old woman presented to our hospital with pelvic pain. At physical examination there was no evidence of ascites or adenopathy. Ultrasound and abdominal and pelvic computed tomography (CT) showed a left adnexal mass. Chest radiographic findings were normal. Bone and liver scan findings were negative. CA125 tumour marker levels were checked and found to be mildly elevated. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were carried out. Grossly the left side of the fallopian tube was 8 cm in length and 6 cm in its widest luminal dilatation. The entire tubal lumen was obliterated by a solid mass. Histological examination showed a malignant mixed müllerian tumour. The tumour was an admixture of both carcinomatous and sarcomatous elements. The carcinomatous element was composed of well to moderately differentiated squamous cell carcinoma (Figure 1) and the sarcomatous component was made up of anaplastic spindle shaped cells with hyperchromatic nuclei (Figure 2). The tumour elicited a high mitotic rate and areas of necrosis. Microscopically transition from benign columnar epithelium of the tubal lumen to the neoplastic epithelium was found (Figure 3). The tumour infiltrated the entire thickness of the fallopian wall and

the mesosalpinx. Sections of both ovaries, uterine cavity and cervix were unremarkable. The right fallopian tube showed features of chronic non specific salpingitis. An immunohistochemical study showed that vimentin was positive in the sarcomatous component. A considerable number of spindle shaped cells were immunoreactive with smooth muscle actin (Figure 4), CK AE1 (Figures 5, 6) and CK AE3. Desmin and CA125 were reactive in a few cells. The patient was admitted to the anticancer hospital for further treatment.

Discussion

Fallopian tube malignancy was first described by Renaud in 1847 [1]. It was proposed that the diagnosis of tubal cancer be based on three conditions: 1) the main tumour should be in the tube, 2) microscopically the mucosa should be involved principally, and 3) microscopically transition from benign columnar epithelium must be found [4]. The etiology remains unknown. Infertility and chronic salpingitis were believed to increase the incidence but have not yet been proven. There are at least 1,500 cases of malignant tubal lesions reported in the literature but only approximately 50 were identified as mixed müllerian tumours (MMT) [1-5]. Malignant mixed müllerian tumours are uncommon neoplasms of the female genital tract that histologically are defined by the presence of malignant epithelial and stromal elements. MMT can arise from the cervix, fallopian tube and pelvic peritoneum but the endometrium and the ovary are the most common primary sites [6]. Patients with fallopian tube malignancy usually present with pelvic pain, a pelvic mass, postmenopausal bleeding and serosanguinous vaginal discharge [1, 4, 6]. Imaging studies like hysterosalpingography helps in detecting intraluminal growth. A CT scan help in localizing spread to other

Fig. 1
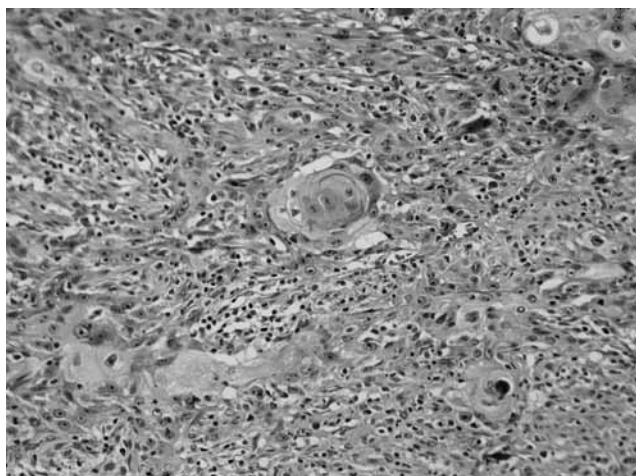
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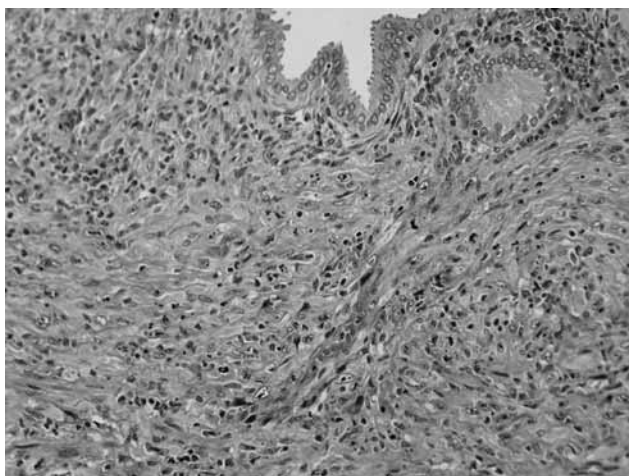
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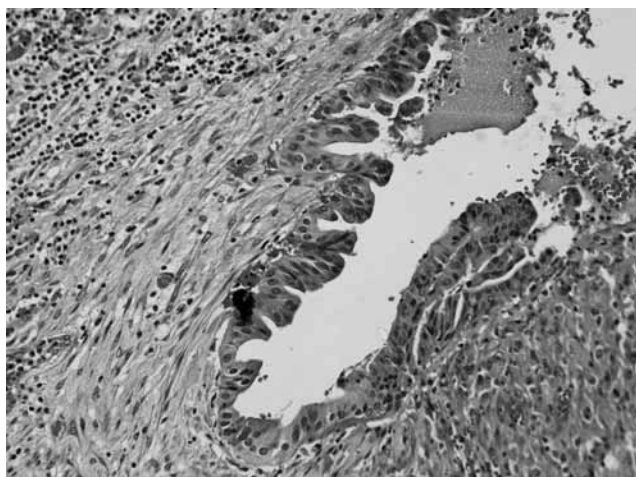
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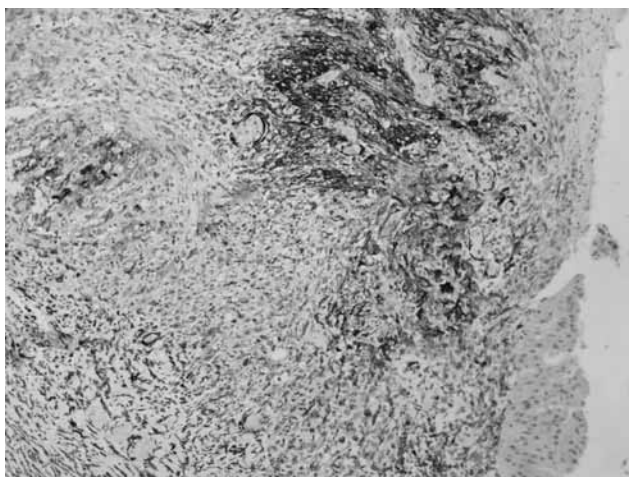
Fig. 5

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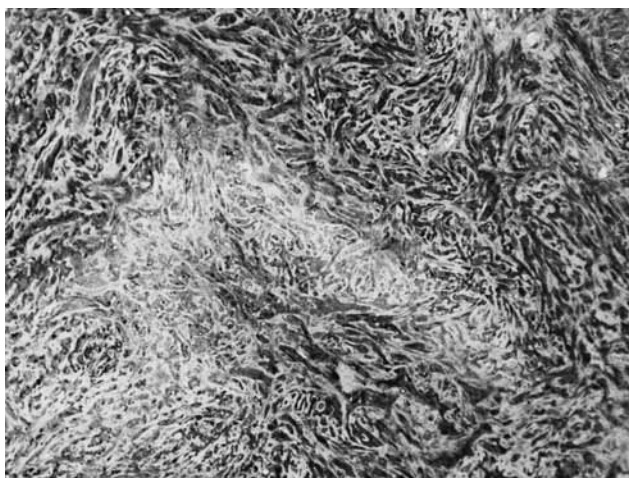
Figure 1. — Well differentiated squamous cell carcinoma (H&E x 200).
Figure 2. — Spindle shaped cells of the sarcomatous component (H&E x 200).
Figure 3. — Dysplastic epithelium of the fallopian tube (H&E x 200).
Figure 4. — The sarcomatous component was immunoreactive with smooth muscle actin (SMA x 100).
Figure 5. — The squamous and sarcomatous components were strongly immunoreactive with CK AE1 (x 20).
Figure 6. — The sarcomatous component was immunoreactive with CK AE1 (x 100).

intraabdominal or retroperitoneal sites. Increased levels of CA125 have been described in some patients [1-7]. Mixed müllerian tumours are initially chemosensitive but have an aggressive clinical course, typically with early relapse after treatment and a poor long-term prognosis. The median survival is 18 months. Radiotherapy is of no help [8-12]. The prognosis depends more on staging than on grade. No morphological factor has been found to correlate with survival, but a tendency was observed for MMTs with a high epithelial nuclear grade, a predominance of the mesenchymal component, or a rhabdomyoblastic mesenchymal component to be associated with more aggressive behaviour. Moreover, patients with a predominating carcinomatous component had a higher response rate to chemotherapy than patients with a predominating sarcomatous component [13-16].

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