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**Distinguished Expert Series**

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Longitudinal outcomes of high-risk human papillomavirus (HPV) infections as competing-risks events following cervical HPV test at baseline visit in the *NIS-LAMS** cohort


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Summary

Background: The complex natural history of human papillomavirus (HPV) infections following a single HPV test can be modeled as competing-risks events (i.e., no-, transient- or persistent infection) in a longitudinal setting. The covariates associated with these competing events have not been previously assessed using competing-risks regression models. Objectives: To gain further insights in the outcomes of cervical HPV infections, we used univariate- and multivariate competing-risks regression models to assess the covariates associated with these competing events. Study Design and Methods: Covariates associated with three competing outcomes (no-, transient- or persistent HR-HPV infection) were analysed in a sub-cohort of 1,865 women prospectively followed-up in the NIS (n = 3,187) and LAMS Study (n = 12,114). Results: In multivariate competing-risks models (with two other outcomes as competing events), permanently HR-HPV negative outcome was significantly predicted only by the clearance of ASCUS+ Pap during FU, while three independent covariates predicted transient HR-HPV infections: i) number of recent (< 12 months) sexual partners (risk increased), ii) previous Pap screening history (protective), and history of previous CIN (increased risk). The two most powerful predictors of persistent HR-HPV infections were persistent ASCUS+ Pap (risk increased), and previous Pap screening history (protective). In pair-wise comparisons, number of recent sexual partners and previous CIN history increase the probability of transient HR-HPV infection against the HR-HPV negative competing event, while previous Pap screening history is protective. Persistent ASCUS+ Pap during FU and no previous Pap screening history are significantly associated with the persistent HR-HPV outcome (compared both with i) always negative, and ii) transient events), whereas multiparity is protective. Conclusions: Different covariates are associated with the three main outcomes of cervical HPV infections. The most significant covariates of each competing events are probably distinct enough to enable constructing of a risk-profile for each main outcome.

Key words: HPV; Natural history; Outcomes; Competing events; Competing-risks regression model; Transient infection; Persistent infection; Prospective follow-up; NIS Cohort; LAMS Study.

* NIS, New Independent States of the Former Soviet Union; ** LAMS, Latin American Screening Study.
Introduction

The natural history of human papillomavirus (HPV) infections in the uterine cervix is closely linked with the development of cervical intraepithelial neoplasia (CIN), with three outcomes: regression, persistence and progression [1-5]. From a strictly virological standpoint, however, the natural history of cervical HPV infections is much more complex, at least six possible outcomes being distinguished in long-term longitudinal cohort studies: incident infections, early clearance, persistence, fluctuation, late clearance, and recurrence [6-11].

These early observations have been refined by more recent studies on the dynamics of cervical HPV infections at genotype level [12-14]. According to these data, incident high-risk (HR) HPV infections are clearly age-dependent, the 3-year cumulative incidence exceeding 50% after the onset of sexual activity [13-16]. On the other hand, clearance of the virus does not show such strict age-dependence [17], but continues at a rather constant rate from age 30 years onwards when the clearance rates exceed the acquisition rates, resulting in progressively declining age-specific prevalence rates [18-20]. However, not all HPV infections will undergo spontaneous clearance; a substantial proportion of acquired HR-HPV infections remain persistent [19-23]. These persistent infections by the HR-HPV genotypes are considered as prerequisite for developing a progressive CIN and eventually invasive cervical cancer (CC) if left unnoticed [24-29].

To identify the risk factors (covariates) for these optional outcomes of cervical HR-HPV infections is of utmost importance, on one hand, to avoid unnecessary therapies for transient infections that are likely to clear spontaneously, and on the other hand, to detect persistent HR-HPV infections predisposing the patient to an increased risk of developing progressive disease [30, 31]. Until now, most of the studies assessing the covariates associated with these different HPV outcomes have been based on cohort (or cross-sectional) case-control settings, where the outcome of interest (i.e., HPV acquisition, persistence, clearance) has been compared with the controls lacking this event; no acquisition, clearance, no clearance, respectively [12-29]. The most commonly used statistical techniques include conventional survival analysis (Kaplan-Meier or Cox) or Poisson regression [16, 23], while marginal (e.g., GEE, generalized estimating equation) [32] and mixed-effects models have been rarely used, despite their particular suitability for modeling this type of longitudinal data [33]. Yet, another recently introduced statistical technique known as competing-risks regression [34, 35] has been completely neglected in HPV natural history studies so far.

The outcomes of HPV infection can be considered as competing-risks events, being different for HPV-negative and HPV-positive women. Any woman testing HPV-negative is at risk for two competing events when subjected to longitudinal follow-up: i) remain permanently HPV-negative, or ii) develop an incident infection [13-16]. Similarly, baseline HPV-positive women can experience two competing events: i) clearance of the prevalent HPV, or ii) develop a persistent infection [18-29]. Because both incident and prevalent HPV infection can remain persistent or undergo spontaneous clearance, actually only three competing outcomes exist: 1) permanently HPV-negative; 2) transient HPV infection, and 3) persistent HPV infection [8, 11, 31]. These are mutually exclusive, and as such fulfill the criteria of competing-risks events amenable for modeling by the competing-risks regression [34, 35].

The present study is the first where this novel technique is used to model the covariates associated with these three competing-risks events, tested in a sub-cohort of 1,865 women prospectively followed-up in our NIS and LAMS cohort studies [15, 19, 20, 25].

Material and Methods

The NIS and the LAMS Cohort Study

The present analysis is based on the combined cohort of the NIS and the LAMS studies described in recent reports. Both studies are international multi-centre trials testing optional screening tools in three NIS (New Independent States of the Former Soviet Union) countries (Russia, Belarus and Latvia) [36] as well as in two Latin American countries (Brazil and Argentina) [36]. The design and baseline data of both cohorts have been previously detailed [36, 37].

Patients and Study Design

The material of the NIS study cohort comprises 3,187 consecutive women attending six different outpatient clinics in the three NIS countries between 1998 and 2002. These women derived from three different groups: i) cervical cancer screening (= SCR patients); ii) attendants of gynaecologic outpatient clinics (= GYN patients), and iii) patients examined at STD clinics (= STD patients). The mean age of these women at enrollment was 32.6 (± 10.7 SD) years (median 30.6, range 15-85 years) [36]. The study design has been detailed in a series of reports [15, 19, 20, 25]. All eligible women had Pap smear taken and were tested for HR-HPV using HC2 and the first 1,500 women also with PCR and hybridisation. Patients with ASC-US or higher Pap had biopsy confirmation at baseline [15, 19, 20, 25, 36].

The LAMS study is a longitudinal cohort of women enrolled in regions with low, intermediate, and high incidence of CC in Brazil and Argentina [37]. A total of 12,114 women were enrolled by the four clinics. The mean age of these women at enrollment was 37.9 years (median 37.7, range 14-67). In this trial, eight different diagnostic tests were compared as follows: cervical cytology (conventional Pap and LBC) was compared with i) four optional screening tools suggested for low-resource settings: a) visual inspection with acetic acid (VIA), b) visual inspection with Lugol iodine (VILI), c) cervicography, d) screening colposcopy; and ii) with the
new molecular diagnostic tools (HPV testing by Hybrid Capture 2; HC2), performed a) in samples collected by physicians, and b) in those collected by self-sampling devices [37-40]. Women testing positive with any of these techniques were examined by colposcopy.

Prospective Follow-up

Prospective follow-up (FU) is an essential component of both studies. In the NIS cohort, all women who presented with biopsy-confirmed low-grade lesions were assigned for FU, while high-grade lesions were treated. FU data are available for 887 women, of whom 33 patients with baseline CIN3 were excluded from this analysis, leaving 854 women in the final prospective NIS cohort. The mean FU time is 17.2 mo (SD, 11.6 mo; median, 16.6 mo; range 1-43 mo) [15, 19, 20, 25].

In the LAMS study, the same criteria were used to allocate the women into the FU and treatment groups [37-40]. A total of 1,011 women completed at least one FU visit, scheduled at 6-month intervals. The mean FU time is 21.7 mo (SD, 8.09 mo; median, 24.2 mo; range 1-54 mo). All high-grade lesions were promptly treated and followed-up for the same period, using repeated Pap test and colposcopy at 6-month intervals, and HC2 assay at 12-month intervals.

Methods

Because the methods used in these two cohort studies are detailed in several reports [15, 19, 20, 25, 36-40], they are described here only as far as pertinent to elaborating the data used in the present analysis.

Recording the risk factors by questionnaire

In both studies, all women who gave their consent to participate filled in a detailed inquiry concerning the risk factors of HPV, CIN and CC. In combining the two databases, only the variables that were recorded in both cohorts were maintained to make the data consistent. The present analysis is based on the following variables recorded at baseline: age, marital status, years of education, race, age at first sexual intercourse, number of pregnancies, live births, abortions, number of life-time sexual partners, number of sexual partners during the past 12 months, partners’ STD history, mode of contraception, years of hormonal contraception, history of STDs, previous Pap screening history, history of CIN, history of genital warts, and smoking history [36, 37, 41].

Papanicolaou (Pap) smears

In the NIS study, all women were examined using conventional Pap smears [36], whereas in the LAMS study, three methods were used: conventional PAP and two different LBC techniques (DNA-Citoliq; Digene Brazil, Sao Paulo, and SurePath; TriPath, Durham, NC, USA) [38]. In the present analysis, only the results of the conventional Pap test were used (available from all patients).

Directed Punch Biopsy

Directed punch biopsies (and cones) were fixed in formalin, embedded in paraffin, and processed into 5- m-thick hematoxylin-eosin (HE)-stained sections for light microscopy, following the routine procedures. All biopsies were examined among the daily routine in the Pathology Departments of the partner institutions, and diagnosed using the commonly agreed CIN nomenclature.

Detection of HR-HPV DNA by Hybrid Capture 2 assay

In both studies, the principal HPV testing method was Hybrid Capture 2 (HC2) assay, performed using cervical swabs (collected by a physician) or self-sampling devices (tampons, in LAMS study only), as described previously [36,37,40]. HC2 assay (n = 3,084 baseline tests in the NIS and n = 4,694 in the LAMS) was performed using the automated HC2 test system according to the manufacturer’s protocol. The samples were analysed only for the presence of HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Samples were classified as HR-HPV positive with the RLU/CO ≥ 1.0 pg/ml cut-off.

HPV outcomes and competing-risks events

The 854 women from the NIS cohort and 1,011 women from the LAMS study were merged into the same database in the panel format, clustered by woman-ID and FU-visits as the time variable. The combined cohort of 1,865 women was first categorised according to all possible outcomes of their baseline cervical HPV infections during the follow-up, as follows: 1) permanently HR-HPV negative (n = 326), 2) incident HR-HPV infection (n = 59), 3) persistent (prevalent) HR-HPV infection (n = 368), 4) clearance of (prevalent) HR-HPV infection (n = 496), 5) HC2 test not done (n = 153), 6) only one (positive) HC2 test available (n = 270), 7) fluctuating course of HR-HPV infection (n = 63), and 8) only one (negative) HC2 test available (n = 130).

In the next step, these three competing-risks events (always negative, transient, persistent) were defined among those eight outcome categories, following the principles detailed recently [42]. Women testing invariable HC2 negative throughout the follow-up period were included in the first category (permanently HR-HPV negative). The second category (transient HR-HPV infection) comprises all women, among whom either incident (new infection) or prevalent (baseline) HR-HPV infection cleared by the end of FU. All women in whom either prevalent (baseline) or incident HR-HPV infection persisted for at least six months (6M+) [42] were classified as having the third competing event (persistent HR-HPV infection). As described before, inclusion into this category necessitates that the infection persists also in the last FU-visit [42]. Similarly, all women with only one (or no) HC2 assay available, were omitted from the analysis. In the panel data format, all these events were recorded at each FU visit, resulting in a panel with 969 HC2-negative records (for category one women only), 1024 events for 6M+ persistent infections, and 580 (clearance) events for all transient infections.
Table 1. Covariates associated with the three HR-HPV outcomes in an univariate competing-risks regression model (with the other two as competing events).

<table>
<thead>
<tr>
<th>HPV covariate</th>
<th>Permanently HR-HPV negative*</th>
<th>Persistent HR-HPV infection**</th>
<th>Transient HR-HPV infection***</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort:</strong></td>
<td>Shr (95% CI)</td>
<td>Shr (95% CI)</td>
<td>Shr (95% CI)</td>
</tr>
<tr>
<td>NIS</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>LAMS</td>
<td>1.19 (1.02-1.38)</td>
<td>0.46 (0.37-0.56)</td>
<td>0.75 (0.64-0.89)</td>
</tr>
<tr>
<td><strong>CC incidence region:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-incidence region</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Intermediate-incidence region</td>
<td>1.09 (0.94-1.27)</td>
<td>1.05 (0.84-1.31)</td>
<td>0.88 (0.74-1.04)</td>
</tr>
<tr>
<td>High-incidence region</td>
<td>0.27 (0.18-0.39)</td>
<td>1.17 (0.88-1.55)</td>
<td>0.66 (0.50-0.87)</td>
</tr>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above 30 years</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Below 30 years</td>
<td>0.67 (0.57-0.78)</td>
<td>2.06 (1.68-2.53)</td>
<td>1.32 (1.13-1.55)</td>
</tr>
<tr>
<td><strong>Marital status:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living with partner</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Single</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Baseline Pap test ASCUS+:</strong></td>
<td>Shr (95% CI)</td>
<td>Shr (95% CI)</td>
<td>Shr (95% CI)</td>
</tr>
<tr>
<td>PAP negative</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>PAP ASCUS+</td>
<td>1.15 (0.99-1.33)</td>
<td>1.61 (1.32-1.96)</td>
<td>0.97 (0.82-1.13)</td>
</tr>
<tr>
<td><strong>Pap test follow-up:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incident ASCUS+ Pap (yes/no)</td>
<td>0.58 (0.47-0.72)</td>
<td>1.34 (1.06-1.70)</td>
<td>0.99 (0.82-1.21)</td>
</tr>
<tr>
<td>Persistent ASCUS+ Pap (yes/no)</td>
<td>0.81 (0.63-1.04)</td>
<td>2.50 (1.89-3.30)</td>
<td>1.13 (0.88-1.45)</td>
</tr>
<tr>
<td>ASCUS+ Pap cleared (yes/no)</td>
<td>1.23 (1.05-1.44)</td>
<td>0.85 (0.67-1.06)</td>
<td>0.95 (0.79-1.13)</td>
</tr>
<tr>
<td><strong>Years of education:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 11 years</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 8-11 years</td>
<td>0.83 (0.60-1.16)</td>
<td>1.16 (0.68-1.96)</td>
<td>1.03 (0.71-1.51)</td>
</tr>
<tr>
<td>Between 5-8 years</td>
<td>0.97 (0.68-1.36)</td>
<td>0.91 (0.51-1.64)</td>
<td>1.14 (0.77-1.70)</td>
</tr>
<tr>
<td>Less than 5 years</td>
<td>0.99 (0.70-1.39)</td>
<td>0.99 (0.57-1.74)</td>
<td>0.82 (0.54-1.24)</td>
</tr>
<tr>
<td><strong>Race:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Non-white (black, mixed, other)</td>
<td>1.10 (0.91-1.31)</td>
<td>0.56 (0.41-0.77)</td>
<td>1.06 (0.86-1.30)</td>
</tr>
<tr>
<td><strong>Age at onset of sexual activity:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At or above 20 years</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 17 and 20 years</td>
<td>0.88 (0.73-1.04)</td>
<td>1.03 (0.80-1.33)</td>
<td>0.94 (0.77-1.15)</td>
</tr>
<tr>
<td>Between 15 and 17 years</td>
<td>0.85 (0.69-1.04)</td>
<td>1.23 (0.92-1.64)</td>
<td>0.99 (0.79-1.25)</td>
</tr>
<tr>
<td>Below 15 years</td>
<td>0.98 (0.74-1.29)</td>
<td>0.92 (0.58-1.46)</td>
<td>1.33 (0.99-1.79)</td>
</tr>
<tr>
<td><strong>Ever been pregnant:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>1.40 (1.17-1.70)</td>
<td>0.67 (0.54-0.85)</td>
<td>0.76 (0.63-0.90)</td>
</tr>
<tr>
<td><strong>Number of pregnancies:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>1.42 (1.12-1.80)</td>
<td>0.82 (0.62-1.10)</td>
<td>0.89 (0.71-1.12)</td>
</tr>
<tr>
<td>2</td>
<td>1.48 (1.17-1.85)</td>
<td>0.71 (0.53-0.96)</td>
<td>0.76 (0.60-0.97)</td>
</tr>
<tr>
<td>3</td>
<td>1.39 (1.08-1.78)</td>
<td>0.66 (0.47-0.92)</td>
<td>0.74 (0.57-0.96)</td>
</tr>
<tr>
<td>4 or more</td>
<td>1.38 (1.10-1.72)</td>
<td>0.52 (0.37-0.73)</td>
<td>0.66 (0.52-0.84)</td>
</tr>
<tr>
<td><strong>Number of live births:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>1.20 (1.01-1.44)</td>
<td>1.00 (0.79-1.28)</td>
<td>0.83 (0.69-1.01)</td>
</tr>
<tr>
<td>2</td>
<td>1.23 (1.01-1.52)</td>
<td>0.97 (0.72-1.29)</td>
<td>0.70 (0.55-0.90)</td>
</tr>
<tr>
<td>3 or more</td>
<td>1.02 (0.81-1.27)</td>
<td>0.47 (0.31-0.70)</td>
<td>0.63 (0.48-0.84)</td>
</tr>
<tr>
<td><strong>Number of life-time sexual partners:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>2-3</td>
<td>0.78 (0.63-0.97)</td>
<td>1.51 (1.01-2.27)</td>
<td>1.34 (1.01-1.78)</td>
</tr>
<tr>
<td>4-5</td>
<td>0.72 (0.54-0.97)</td>
<td>1.54 (0.94-2.51)</td>
<td>1.17 (0.83-1.66)</td>
</tr>
<tr>
<td>6 or more</td>
<td>0.90 (0.62-1.31)</td>
<td>2.69 (1.63-4.45)</td>
<td>1.59 (1.06-2.39)</td>
</tr>
<tr>
<td><strong>Number of partners during past 12 months:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>0.94 (0.66-1.33)</td>
<td>1.03 (0.63-1.69)</td>
<td>1.45 (0.93-2.25)</td>
</tr>
<tr>
<td>2 or more</td>
<td>0.70 (0.47-1.06)</td>
<td>2.03 (1.20-3.43)</td>
<td>1.96 (1.23-3.13)</td>
</tr>
<tr>
<td><strong>Any sexual partner with diagnosed STD:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>0.90 (0.70-1.15)</td>
<td>1.15 (0.83-1.58)</td>
<td>1.09 (0.83-1.43)</td>
</tr>
</tbody>
</table>
following Table 1. — Covariates associated with the three HR-HPV outcomes in an univariate competing-risks regression model (with the other two as competing events).

<table>
<thead>
<tr>
<th>HPV covariate</th>
<th>Permanently HR-HPV negative*</th>
<th>Persistent HR-HPV infection**</th>
<th>Transient HR-HPV infection***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (95% CI)</td>
<td>SHR (95% CI)</td>
<td>SHR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>p</td>
<td>p</td>
</tr>
<tr>
<td><strong>Mode of contraception:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No contraception</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Oral contraception</td>
<td>1.13 (0.93-1.38)</td>
<td>0.81 (0.63-1.05)</td>
<td>1.05 (0.84-1.30)</td>
</tr>
<tr>
<td>Other contraception</td>
<td>1.07 (0.89-1.29)</td>
<td>0.64 (0.50-0.84)</td>
<td>1.06 (0.87-1.319)</td>
</tr>
<tr>
<td><strong>Oral contraception:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever (current and past)</td>
<td>1.08 (0.93-1.27)</td>
<td>1.04 (0.83-1.29)</td>
<td>1.01 (0.85-1.20)</td>
</tr>
<tr>
<td><strong>History of STD:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>0.93 (0.75-1.17)</td>
<td>1.24 (0.93-1.67)</td>
<td>1.22 (0.97-1.53)</td>
</tr>
<tr>
<td><strong>Previous Pap smear taken:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>1.11 (0.94-1.32)</td>
<td>0.59 (0.48-0.74)</td>
<td>0.69 (0.58-0.82)</td>
</tr>
<tr>
<td><strong>Time since last Pap smear:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 24 months</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 12 and 24 months</td>
<td>1.07 (0.80-1.43)</td>
<td>0.78 (0.48-1.36)</td>
<td>0.82 (0.59-1.15)</td>
</tr>
<tr>
<td>Between 6 and 12 months</td>
<td>0.86 (0.66-1.13)</td>
<td>0.76 (0.49-1.16)</td>
<td>0.74 (0.54-1.01)</td>
</tr>
<tr>
<td>Less than 6 months</td>
<td>0.89 (0.66-1.21)</td>
<td>1.10 (0.71-1.70)</td>
<td>0.94 (0.68-1.30)</td>
</tr>
<tr>
<td><strong>History of previous CIN:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>1.12 (0.83-1.52)</td>
<td>0.73 (0.41-1.27)</td>
<td>1.60 (1.20-2.14)</td>
</tr>
<tr>
<td><strong>Ever been smoker:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever (current and past)</td>
<td>0.93 (0.78-1.10)</td>
<td>0.92 (0.72-1.18)</td>
<td>0.97 (0.80-1.17)</td>
</tr>
<tr>
<td><strong>Duration of smoking:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5 years</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 5 and 10 years</td>
<td>1.31 (0.98-1.76)</td>
<td>0.81 (0.55-1.22)</td>
<td>0.96 (0.70-1.30)</td>
</tr>
<tr>
<td>Longer than 10 years</td>
<td>1.36 (1.02-1.81)</td>
<td>0.66 (0.44-0.97)</td>
<td>0.84 (0.61-1.17)</td>
</tr>
</tbody>
</table>

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; SHR, subhazard ratio; NC, not computable.

Statistical analyses

All statistical analyses were performed using the SPSS 19.0.1. for Windows (IBM, NY, USA) and STATA/SE 12.0 software (STATA Corp., Texas, USA). This longitudinal data file was constructed into a panel format, clustered by woman-ID and using the FU-visits as the time (repeated measures) variable. Competing-risks regression models (STATA strcegg) [34, 35] were first used in univariate mode to estimate the risk, i.e., crude subhazard ratios (SHR and 95% CI) of different covariates to associate with i) permanently HR-HPV negative outcome, ii) transient HR-HPV outcome, and iii) persistent HR-HPV outcome. Multivariate models were constructed to disclose independent covariates, calculating SHRs (95% CI) adjusted for age and all significant univariates. These analyses were repeated for all three competing events, using the other outcomes as competing-risks events [34, 35]. To enable pair-wise comparisons between the outcomes, the competing-risks event in the model was changed appropriately, i.e., transient HPV vs always negative, persistent HPV vs always negative, and persistent HPV vs transient HPV. In all calculations, robust variance estimator (vce) was used, clustered by woman-ID, to account for the repeated sampling of each woman. Woman-ID was not set as an ID-variable, however, because we wanted the STATA strcegg to treat each observation within individual patients as a distinct spell, not as a set of overlapping spells. All tests were 2-sided, and values $p < 0.05$ were regarded as statistically significant.

Results

Table 1 summarizes the results of univariate competing-risks regression analysis of all recorded covariates associated with the three endpoints. Keeping the other two outcomes as competing-risks events in the model, the covariates associated with each of the three outcomes (always negative, transient HPV, persistent HPV) are quite distinct and show different predictive power (SHRs).

The most powerful cofactors (at $p = 0.0001$ level) of permanently HR-HPV negative outcome include i) high-incidence region for CC (negative association), ii) age below 30 years (negative association), iii) incident ASCUS+ Pap smear during FU (negative association), iv) ever pregnant (positive association). Another significant covariates include a) clearance of ASCUS+ Pap during FU, b) parity variables (pregnancies and live births)(positive association), c) number of life-time sexual partners.
Only two very powerful ($p = 0.0001$) covariates were associated with transient HR-HPV infections; i) age below 30 years, and ii) previous Pap screening history (protective). Several others reached $p = 0.001$ significance level, including the cohort itself (LAMS negative), parity variables (multiple protective), and history of previous CIN (increasing the risk). Yet several other covariates showed an association, with lower significance levels.

A long list of covariates are associated with persistent HR-HPV infections at the $p = 0.0001$ significance level. These include: i) LAMS cohort (protective), ii) age below 30 years (risk increased), iii) baseline ASCUS+ Pap smear (increase), iv) persistent ASCUS+ Pap (increase), v) race (non-white protective), vi) multiparity (pregnancies/deliveries)(protecting), vii) number of life-time sexual partners (positive), and viii) previous Pap screening history (protective). Several other significant covariates were disclosed, with lower significance levels (Table 1).

When all these significant covariates of the univariate analysis were entered in multivariate competing-risks regression models, only a few remained significant independent predictors of each HPV outcome (Table 2). Permanently HR-HPV negative outcome was significantly predicted only by the clearance of ASCUS+ Pap during FU ($p = 0.015$). There were three independent covariates of transient HR-HPV infections: i) number of recent (< 12 months) sexual partners (increase the risk), ii) previous Pap screening history (protective), and history of previous CIN (increasing risk). The single most powerful predictor of persistent HR-HPV infections was persistent ASCUS+ Pap during FU, with SHR = 8.85 (95% CI 2.75-28.42) ($p = 0.0001$). Equally powerful but to another direction (protective) was previous Pap screening history, with SHR = 0.06 (95% CI 0.01-0.33) ($p = 0.0001$). Also increased parity was an independent covariate, protective against the persistent outcome ($p = 0.012$).
Table 3.— Significant covariates associated with transient and persistent HR-HPV outcomes in univariate competing-risks regression model (with permanently HR-HPV-negative and transient HR-HPV infections as competing events, respectively).

<table>
<thead>
<tr>
<th>HPV covariate</th>
<th>Transient HR-HPV infection*** (vs permanently HR-HPV negative)</th>
<th>Persistent HR-HPV infection** (vs permanently HR-HPV negative)</th>
<th>Persistent HR-HPV infection** (vs transient HR-HPV infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (95% CI) p</td>
<td>SHR (95% CI) p</td>
<td>SHR (95% CI) p</td>
</tr>
<tr>
<td><strong>Mode of contraception</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No contraception</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Oral contraception</td>
<td>1.05 (0.84-1.30)</td>
<td>0.82 (0.63-1.06)</td>
<td>0.81 (0.69-1.03)</td>
</tr>
<tr>
<td>Other contraception</td>
<td>1.03 (0.83-1.279)</td>
<td>0.80 (0.65-1.00)</td>
<td>0.84 (0.65-1.09)</td>
</tr>
<tr>
<td><strong>Previous Pap smear taken</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>0.66 (0.55-0.79)</td>
<td>0.58 (0.47-0.739)</td>
<td>0.60 (0.48-0.76)</td>
</tr>
<tr>
<td><strong>Time since last Pap smear</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 24 months</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 12 and 24 months</td>
<td>0.82 (0.58-1.14)</td>
<td>0.77 (0.48-1.25)</td>
<td>0.79 (0.49-1.27)</td>
</tr>
<tr>
<td>Between 6 and 12 months</td>
<td>0.71 (0.51-0.979)</td>
<td>0.73 (0.48-1.13)</td>
<td>0.72 (0.47-1.12)</td>
</tr>
<tr>
<td>Less than 6 months</td>
<td>0.94 (0.68-1.319)</td>
<td>1.09 (0.70-1.88)</td>
<td>1.06 (0.68-1.64)</td>
</tr>
<tr>
<td><strong>History of previous CIN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>1.58 (1.16-2.14)</td>
<td>0.75 (0.42-1.32)</td>
<td>0.73 (0.41-1.29)</td>
</tr>
<tr>
<td><strong>Duration of smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5 years</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 5 and 10 years</td>
<td>0.96 (0.70.131)</td>
<td>0.82 (0.55-1.23)</td>
<td>0.85 (0.57-1.27)</td>
</tr>
<tr>
<td>Longer than 10 years</td>
<td>0.82 (0.59.114)</td>
<td>0.85 (0.44-1.96)</td>
<td>0.83 (0.45-1.00)</td>
</tr>
</tbody>
</table>

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; SHR, subhazard ratio; NC, not computable.
The three HPV outcomes are compared pair-wise for their covariates by changing the competing-risks events in the models. Only significant covariates are listed in Table 3. It is obvious that several covariates make distinction between transient HR-HPV outcome and permanently HR-HPV negative outcome, of which six do so at the $p = 0.0001$ level. The covariate profile is even more distinct between persistent infections and permanently HR-HPV negative outcome, with ten covariates predicting the former at the $p = 0.0001$ significance level. These covariates are practically identical with those distinguishing between persistent- and transient HR-HPV infections (9 with $p = 0.0001$) (Table 3).

In the final multivariate model with all significant univariates entered, relatively few covariates are significant in pair-wise comparisons between the three HPV outcomes (Table 4). Number of recent sexual partners and previous CIN history increase the probability of transient HR-HPV infection against permanently HR-HPV negative competing event, while previous Pap screening history is protective. Persistent ASCUS+ Pap during FU and no previous Pap screening history are significantly associated with the persistent outcome (compared with an always negative competing event), whereas multiparity is protective. These three covariates also make the significant distinction between persistent- and transient HR-HPV infections, practically with the same power as obtained when the HR-HPV negative outcome is used as the competing event.

### Table 4. Covariates associated with transient and persistent HR-HPV outcomes in multivariate competing-risks regression model (with permanently HR-HPV-negative and transient HR-HPV infections as competing events, respectively).

<table>
<thead>
<tr>
<th>Competing outcome events</th>
<th>Transient HR-HPV Infection (permanently HR-HPV negative as competing event):</th>
<th>Persistent HR-HPV Infection (permanently HR-HPV negative as competing event):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted SHR</td>
<td>Lower Bound</td>
</tr>
<tr>
<td>Age (30-yrs cut-off) (&gt; 30 yrs ref)</td>
<td>1.18</td>
<td>0.95</td>
</tr>
<tr>
<td>Persistent ASCUS+ Pap smear</td>
<td>1.15</td>
<td>0.81</td>
</tr>
<tr>
<td>Ever been pregnant (never ref)</td>
<td>0.98</td>
<td>0.76</td>
</tr>
<tr>
<td>Number of pregnancies omitted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of live births (0 ref)</td>
<td>0.97</td>
<td>0.86</td>
</tr>
<tr>
<td>Number of life-time sexual partners omitted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of recent (&lt; 12 month) sexual partners (0 ref)</td>
<td>1.28</td>
<td>1.03</td>
</tr>
<tr>
<td>Previous Pap smear taken (never ref)</td>
<td>0.72</td>
<td>0.58</td>
</tr>
<tr>
<td>History of previous CIN (no ref)</td>
<td>1.59</td>
<td>1.14</td>
</tr>
<tr>
<td>Persistent HR-HPV Infection (transient HR-HPV infection as competing event):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (30-yrs cut-off) (&gt; 30 yrs ref)</td>
<td>1.68</td>
<td>0.75</td>
</tr>
<tr>
<td>Baseline ASCUS+ Pap smear omitted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incident ASCUS+ Pap smear omitted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent ASCUS+ Pap smear (no ref)</td>
<td>8.77</td>
<td>2.67</td>
</tr>
<tr>
<td>Race (white ref)</td>
<td>0.93</td>
<td>0.38</td>
</tr>
<tr>
<td>Ever been pregnant (never ref)</td>
<td>0.51</td>
<td>0.12</td>
</tr>
<tr>
<td>Number of pregnancies (0 ref)</td>
<td>0.52</td>
<td>0.34</td>
</tr>
<tr>
<td>Number of live births (0 ref) omitted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of life-time sexual partners (1 ref)</td>
<td>1.13</td>
<td>0.71</td>
</tr>
<tr>
<td>Number of recent (&lt; 12 month) sexual partners</td>
<td>1.03</td>
<td>0.10</td>
</tr>
<tr>
<td>Mode of contraception</td>
<td>1.01</td>
<td>0.52</td>
</tr>
<tr>
<td>Previous Pap smear taken</td>
<td>14.03</td>
<td>1.67</td>
</tr>
</tbody>
</table>

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; @Adjusted for all covariates that were significant in univariate model; Omitted from the model because of collinearity; SHR, subhazard ratio; significant covariates are in bold.
Discussion

As evidenced by several cohort studies, the natural history of HPV has several characteristics that, from a statistical point of view, are infrequently encountered in other fields of infectious disease or cancer research [4-11, 18-31, 33]. Despite the fact that multiple-type infections are common, prevalence, incidence, persistence and clearance of HPV can be measured at genotype level in longitudinal settings with repeated sampling [16, 23, 32, 43]. In settings where repeated measures involve the same subject, the results tend to be correlated [33]. In other words, the probability of detecting any given HPV genotype is greater among women who test positive for another genotype, and similarly, women with biopsy-confirmed CIN are more likely to have the disease in the subsequent visit as well, if repeated within a reasonable time frame. Statistical techniques that i) fail to take these correlations into account would be invalid, and ii) methods that do not exploit all the collected data (in a repeated measures setting) would be inefficient [33]. Marginal (e.g., GEE, generalized estimating equation) [16, 23, 32] and mixed-effects models [33] are both capable of handling these issues, showing a greater efficiency as compared with standard logistic regression and Cox models for studying the natural history of HPV infections.

We recently used GEE [32] and Poisson regression for panel data [16, 23] in modeling the covariates associated with genotype-specific HPV persistence, incident HPV and virus clearance in the prospective Finnish Family HPV Cohort. Both techniques would be technically suitable for analysis of the panel data of the present study as well, but because they only accept a binomial (0/1) dependent variable this would necessitate a separate analysis of the multiple comparisons between the three events of interest, which is not feasible. Thus, we ended up in selecting another method for analysing our data, by taking into account the fact that i) the longitudinal data be utilised in full, ii) dependence of the repeated measurements at FU visits be taken into account, and iii) the multiple-endpoint (no-, transient- and persistent HR-HPV infection) variable be treated in a single model. All these prerequisites are met by the competing-risks regression [34, 35], here used to model the covariates associated with the competing outcomes of cervical HPV infections.

Based on the method of Fine and Gray (1999), competing-risks regression provides a useful alternative to standard Cox regression for survival data in the presence of competing risks [34]. In contrast to the usual survival analysis measuring time-to-failure (e.g., clearance) as a function of observed covariates, the term competing risk refers to the chance that instead of HPV clearance (i.e., transient infection), one will observe a competing event, e.g. virus persistence or no HPV infection at all [34, 35]. During the observation period, detection of any of these competing events impedes the occurrence of the event of interest. This is basically different from the usual censoring that occurs in conventional survival analysis, i.e., loss to follow-up; while censoring obstructs you from observing the event of interest, a competing event prevents the occurrence of the event of interest. In simple terms, competing-risks regression generates hazard for (failure) events of interest, while simultaneously keeping the subjects who experience competing events still “at risk” so that they can be adequately counted as not a chance of failing [34, 35]. Different from the usual Cox regression models producing HR (hazard ratio), this technique reports exponentiated coefficients known as subhazard ratios (SHR). The correlation within multiple records on the same subject is accounted for by using robust variance estimator, clustered by patient-ID, to treat each observation within a patient as an own predictor and not as a set of overlapping predictors [34, 35].

In classical cohort studies [4-11], the natural history of cervical HPV infections has been shown to be extremely complex, with several distinct outcome patterns observed. The natural history of CIN is closely linked with HPV, albeit less complex (regression, persistence, progression) [1-3]. However, given that HPV is the causative agent of CIN, we should separate the natural history of cause (HPV) and effect (CIN), i.e., to distinguish between viral outcomes and clinical outcomes [4-10]. As a virus, HPV in the cervix can cause incident infection, remain persistent, or undergo spontaneous clearance [12-23]. Because both incident and prevalent HPV infections can either persist or undergo clearance, HPV outcomes can be simplified as either i) persistent, or ii) transient HPV infections. Among baseline HPV-negative women, the third possible outcome is permanent absence of HPV, i.e., no incident event during the longitudinal follow-up. Because these outcomes are mutually exclusive, but a woman still remains “at risk” for the other outcomes, these are competing events, making the data suitable for analysis by competing-risks regression [34, 35].

From the clinical point of view, there is a major difference between these three HPV outcomes. While practically no risk for developing CIN and CC is encountered among women who remain constantly HR-HPV-negative, there is a non-negligible risk for incident CIN2+ among women who experience a transient HR-HPV infection [24-29]. On the other hand, several studies have demonstrated very high relative and absolute risks of CIN2+ ascribable to type-specific persistent HR-HPV infections. Clearly, persistent HR-HPV infections represent a sign of increased risk of CC, and as such, 6-month (6M+) or 12-month (12M+) type-specific persistence of HR-HPV have been proposed as powerful surrogates of progressive disease [31, 42, 44]. As recently shown, however, the power of both 6M+ and 12M+ persistent HR-HPV infections as predictors of incident CIN2+ critically depends on the reference category used in the calculations, i.e., whether transient HPV infections or constantly HPV-negative women [31, 42, 44]. This implicates that the risk of incident CIN2+ is also increased among women with transient infections as compared with constantly HPV-negative women [31, 42]. Another way to look at this is to assess the covariates associated with transient infections on one hand and persistent infections on the other hand, as done in the present study treating these outcomes as competing events.
As clearly shown by the present data, the covariates associated with transient HR-HPV and persistent HR-HPV infections are quite different when HPV-negative outcome is used as the competing event, but very similar indeed, when compared to each other (Table 3). These data provide direct confirmation to the recent discussion, why constantly HPV-negative women should always be used as the reference category while calculating the predictive power of 6M+ and 12M+ HR-HPV persistence as surrogate endpoints of progressive disease [31, 42].

In the present analysis, a large number of covariates were associated with each of the three competing events (Tables I-4). However, in multivariate competing-risks regression models, only a few remained significant independent predictors of each event (with the two others as competing events). While permanently HR-HPV negative outcome was significantly predicted only by the clearance of ASCUS+ Pap during FU, there were three independent covariates of transient HR-HPV infections: i) number of recent (<12 months) sexual partners (increase the risk), ii) previous Pap screening history (protective), and history of previous CIN (increased risk). The two most powerful predictors of persistent HR-HPV infections were persistent ASCUS+ Pap during FU (increasing the risk), and previous Pap screening history (protective). Also increased parity was an independent (protective) covariate against persistent HR-HPV infection.

When a similar analysis was repeated for pair-wise comparisons between each three outcomes, relatively few covariates proved to be significant independent predictors of each competing event. Number of recent sexual partners and previous CIN history increase the probability of transient HR-HPV infection against a permanently HR-HPV negative competing event, while previous Pap screening history is protective. Persistent ASCUS+ Pap during FU and no previous Pap screening history are significantly associated with the persistent outcome (compared with an always negative competing event), whereas multiparity is protective. These three covariates also make the significant distinction between persistent- and transient HR-HPV infections, practically with the same power as obtained with the HR-HPV negative competing event.

These results might have important practical implications, while providing the potential means to address the burning questions arising after a single HPV test, irrespective of whether HPV-positive or HPV-negative. In the former case, the patient is interested in the eventual outcomes and their predictability. In the latter case, the issues are related to the protective effect of a single negative HPV test, i.e., probability of remaining HPV-negative also in the foreseeable future. If accurately predicted, substantial savings can be achieved while refraining from repeat HPV testing of these HPV-negative women who are likely to remain HPV-negative also in the future. In HPV-positive cases, on the other hand, an accurate distinction of transient infections from HR-HPV persistence would avoid unnecessary treatments of infections that are likely to resolve, but instead help in focusing the efforts of monitoring the women at high risk for incident CIN2+ [24-29, 31, 42].

Taken together, the present analysis of a sub-cohort of 1,865 women in the combined NIS-LAMS cohort used competing-risks regression models to disclose significant covariates associated with three main outcomes (persistent-, transient-, or no HR-HPV infection) of cervical HPV infections, treated as competing events. Covariates associated most significantly with each of the three competing events were distinct enough to enable designing a risk-profile for each outcome. In the next step, the performance of these risk-profiles in predicting the longitudinal outcomes of cervical HPV infections will be tested in this cohort.

Acknowledgements

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Efficacy of neoadjuvant chemotherapy followed by radical hysterectomy in locally advanced non-squamous carcinoma of the uterine cervix: a retrospective multicenter study of Tohoku Gynecologic Cancer Unit

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Summary

Objective: Radical hysterectomy (RH) is a standard treatment for locally advanced non-squamous cell carcinoma (N-SCC) of the uterine cervix, but there have been no reports on whether neoadjuvant chemotherapy (NAC) followed by radical hysterectomy could improve the outcome of patients with this disease.

Materials and Methods: This multicenter retrospective study enrolled 77 patients with Stage IB2 to IIB N-SCC of the uterine cervix. Of these, 27 patients were treated with NAC prior to radical hysterectomy (NAC group) and 50 with RH alone (RH group). The two-year recurrence-free survival (RFS) rate, progression-free survival (PFS), and overall survival (OS) were compared between the two groups. Clinical parameters such as clinical stage, histological type, and postoperative treatment were also examined between the groups.

Results: While the two-year RFS rates were 81.5% and 70.0% in NAC and RH groups, respectively (p = 0.27) and the median PFS was 51 months and 35 months in NAC and RH groups, respectively (p = 0.35), the median OS was 58 months and 48 months in NAC and RH groups, respectively, which was significant (p = 0.0014). The median OS of patients with mucinous adenocarcinoma in NAC group was significantly higher than that in RH group: 58 months versus 37 months (p = 0.03).

Conclusion: NAC prior to RH may offer the prognostic advantage of patients with locally advanced N-SCC of the uterine cervix, especially mucinous adenocarcinoma.

Key words: Uterine cervical carcinoma; Non-squamous cell carcinoma; Neoadjuvant chemotherapy; Radical hysterectomy; Outcome.
Results

Patient characteristics

The median age was 49 and 45 years in NAC and RH groups, respectively. Eleven (40.7%) and 29 (58.0%) patients had Stage IB2 disease in NAC and RH groups, respectively, and 16 (59.3%) and 21 (42.0%) patients had Stage II disease in NAC and RH groups, respectively. In regard to the histological type, 13 patients had mucinous adenocarcinoma, four had endometrioid adenocarcinoma, three had clear cell carcinoma, and seven had adenosquamous carcinoma in the NAC group, while 27 patients had mucinous adenocarcinoma, nine had endometrioid adenocarcinoma, two had clear cell carcinoma, nine had adenosquamous carcinoma, and three had other types in RH group. Of the 27 patients in NAC group and 50 in RH group, 19 (70.4%) and 40 (80.0%) underwent any postoperative treatments, respectively (Table 1).

NAC regimens and number of cycles

Because this was a retrospective and multicenter study, the combination of anti-cancer agents utilized was heterogeneous as shown in Table 2. Of the 27 patients in NAC group, eight received DC: seven patients received two cycles and one patient received three cycles. Five patients received cisplatin alone. Four patients received MEP: one patient received one cycle, two patients received two cycles, and one patient received three cycles. Three patients received TC of two cycles. Three patients received FCAP: one patient received one cycle and two patients received three cycles. Other four patients received cisplatin/CPT-11 of two cycles, cisplatin/Adriamycin of two cycles, cisplatin/mitomycin C of three cycles, and carboplatin/actinomycin D of three cycles, respectively.

Comparison of clinical outcome between NAC and RH groups

The two-year RFS rate was 81.5% in NAC group and 70.0% in RH group (p = 0.27, Table 3). The median PFS was 51 months (range, 14-157 months) in NAC group and 35 months (range, 4-157 months) in RH group (p = 0.35, Table 3). On the other hand, the median OS was 58 months (range, 15-157 months) in NAC group and 48 months (range, 9-157 months) in RH group, which was significant (p = 0.0014, Table 3 and Figure 1A).

Comparison of clinical outcome according to clinical parameters

There were no significant differences in the median PFS and OS between NAC and RH groups according to stage, histological type and adjuvant therapy, except mucinous adenocarcinoma (Table 4). While the median PFS of patients with mucinous adenocarcinoma was 58 months (range, 8-124 months) in NAC group and 33 months (range, 4-125 months) in RH group (p = 0.34), the median OS of those with mucinous adenocarcinoma was 58 months (range, 24-124 months) in NAC group and 37 months (range, 9-125 months) in RH group, which was significant (p = 0.03) (Table 4 and Figure 1B).

Clinical outcome according to therapeutic modality after NAC and radical surgery

The outcome of patients who underwent chemotherapy or chemoradiotherapy or radiotherapy after NAC and RH were compared. As shown in Table 5, chemotherapy after NAC and surgery prolonged PFS and OS, and increased...
Sardi et al. reported a significant improvement of the seven-year survival rate in patients treated by NAC and radical surgery with chemotherapy after NAC and surgery, although they did not reach significance.

Discussion

Numerous phase II studies have reported the favorable effects of NAC in the treatment of locally advanced adenocarcinoma of the uterine cervix. The authors have previously reported the efficacy and safety of NAC with cisplatin plus irinotecan in this disease [10]. However, few randomized clinical trials (RCT) have evaluated the effect of NAC with cisplatin or carboplatin, so platinum agents seem favorable for chemotherapy prior to surgery in N-SCC of the uterine cervix.

The two-year RFS rate and the median OS were better in NAC group than in RH group, which were not significantly different, whereas the median OS in NAC group was significantly longer than in RH group (p = 0.0014). Furthermore, prognostic analysis in clinical parameters showed that the median OS of patients with mucinous adenocarcinoma in NAC group was significantly longer than in RH group (p = 0.003), although other histological types and postoperative treatment did not significantly affect the prognosis of patients between NAC and RH groups. These results suggest that NAC may offer the prognostic advantage of patients with locally advanced N-SCC of the uterine cervix, especially mucinous adenocarcinoma. Because mucinous adenocarcinoma accounts for approximately 70% out of adenocarcinomas of the uterine cervix, NAC may improve prognosis of patients with SCC of the uterine cervix [13, 14], because of the higher incidence of lymph node metastases at a relatively early stage of the disease, and a lower sensitivity to radiotherapy in N-SCC of the uterine cervix [15, 16].

Efficacy of neoadjuvant chemotherapy followed by radical hysterectomy in locally advanced non-squamous carcinoma of the etc.
in uterine cervical carcinoma [18]. Considering these reports together with the present results, chemoradiotherapy or radiotherapy after NAC and surgery may contribute to unfavorable outcome of the patients with uterine cervical adenocarcinoma compared to chemotherapy after NAC and surgery, although further investigation is necessary to confirm the appropriate therapeutic modality following NAC and surgery.

The recent reports demonstrated that taxanes were used effectively in NAC for uterine cervical adenocarcinoma [19, 20]. Most of the institutions joining TGCU had adopted cisplatin-based regimens in the 1990s, and switched to the regimens combining taxanes and platinum derivatives after 2000. Despite the diverse NAC regimens and the small sample size, the authors believe that the present results have provided constructive ideas for the development of new therapeutic strategy for N-SCC of the uterine cervix. An effective chemotherapeutic regimen for N-SCC of the uterine cervix should be urgently adopted in a phase II study and then a RCT that compares a new single NAC and radical surgery with radical surgery alone, is warranted to confirm the present results.

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Expression of p53, p27 and Jab1 protein in epithelial ovarian tumors

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Summary

Objective: The study aimed to investigate expression of p53, p27 and Jun activation domain-binding protein 1 (Jab1) proteins in epithelial ovarian tumors and the values of these factors as discriminating markers for the transformation of borderline tumors to cancers. Methods: Forty-seven cases of paraffin-embedded tissues of epithelial ovarian tumors including 22 cases of benign ovarian tumors, nine cases of borderline tumors, and 16 cases of invasive cancers were used to evaluate expression of p53, p27 and Jab1 proteins by immunohistochemical methods. Results: p53 protein was expressed in 13.6% of the benign tumors, 44.4% of the borderline tumors and 62.5% of the malignant tumors and p27 protein was expressed in 95.5% of the benign tumors, 66.7% of the borderline tumors, and 37.5% of the malignant tumors. Expression of Jab1 protein was observed in 22.7% of the benign tumors, 77.8% of the borderline tumors and 62.5% of the malignant tumors. Expressions of p53, p27 and Jab1 proteins in malignant tumors were all higher than in benign tumors and the expression of p27 protein in malignant tumors was lower than in benign tumors (p < 0.05). Expression of Jab1 protein in borderline tumors was significantly higher than in benign tumors (p < 0.05). Conclusions: Expression of p53, p27 and Jab1 proteins can be used to discriminate between benign and malignant tumors in epithelial ovarian tumors.

Key words: p53; p27; Jab1; Borderline tumor.

Introduction

Epithelial ovarian tumors are classified as benign tumors, borderline tumors, and malignant tumors according to the histological characteristics. Borderline ovarian tumors, which have the characteristics of the intermediate stage between benign and malignancy, were first described by the Taylor in 1929 [1] and named as ‘borderline malignancy’ by the World Health Organization (WHO) and International Federation of Gynecology and Obstetrics in the early 1970s [2].

If there are pushing borders, they are borderline tumors, and if destructive invasion, malignant tumors [3].

The prognoses of borderline tumors are known to be good. However, the prognoses of some borderline ovarian tumors are reported to be poor, and there have been enormous efforts for the last several decades to discriminate histologically diagnosed epithelial borderline ovarian tumors due to the poor prognosis [4].

The recent development of oncogene and tumor suppressor gene examination in molecular biology is being used to find out the cause of the cancers, to estimate the prognosis of the disease and to decide the treatment at a gene level. Among genes, p53 is a tumor suppressor gene which usually regulates cell growth and proliferation but when it is damaged by mutation, it loses its ability to suppress oncogenesis and plays as an oncogene [5, 6].

Another tumor suppressor gene, p27, halts cell divisions by combining with cyclin E/cyclin-dependent kinase (CDK)2 complex and suppressing them, and eventually blocking the progression from the G1 phase to S phase in a cell cycle [7]. The loss of p27 protein means the loss of ability to suppress mitosis of cancerous cells and it is thought to be related to rapid growth of cancer cells. p27 protein is also known to be involved in cellular adhesion and the loss of p27 protein is expected to weaken cellular adhesion and make the metastasis of cancerous cells easier.

In addition to p27 protein, Jun activation domain-binding protein 1 (Jab1) has recently been researched [8]. However, there is a lack of research about Jab1 in regards to epithelial ovarian cancer, especially borderline ovarian cancer.

For this reason, this study was carried out to investigate the outlook of the expressions of p53, p27 and Jab1 proteins in epithelial ovarian tumors through immunohistochemical staining, and the value of these factors as discriminating markers to predict the degeneration of malignancy in borderline ovarian cancers.

Materials and Methods

Forty-seven well preserved tissues from patients who had been diagnosed pathologically as having benign, borderline and malignant ovarian tumors and who underwent surgery in the University Hospital between August 2003 and December 2004.

Clinical records and histopathological investigations

Ages of each patient were recorded and each histological slide was reviewed and then classified as benign, borderline or malignant.
Expression of p53, p27 and Jab1 protein in epithelial ovarian tumors

Immunohistochemical staining

Paraffin-embedded tissues of the selected patients were cut into 4-5 μm sections, dehydrated three times for 5 min, hydrated with 100% alcohol, 90%, 75% and 50% of ethanol for 2 min. and rinsed. Afterwards, the sections were processed with 0.3% of hydrogen peroxide-methanol for 10 min to block endogenous peroxidase within the sections, washed with water, washed again with 50 mM Tris of buffered saline (TBS, pH 7.5), processed with goat serum for 30 min to block non-specific binding sites of the tissue and the remaining solution was removed. Then, the primary antibodies, p53 (DAKO, Carpinteria, CA, USA), p27 (DAKO, Carpinteria, CA, USA) and Jab1 (Spring Bioscience, Fremont, CA, USA) were applied for two hours at room temperature. After the primary antibody application, the tissue was washed with TBS for 5 min three
times and the biotin-binding secondary antibody (1:300; Zymed Co., San Francisco, CA, USA) was applied for 20 min and then stained with the avidin-biotin complex method. Three-amo-no-9- ethylcarbazole (AEC), the color coupler, was used and counter staining was done with Mayer’s hematoxylin.

The results of p27, Jab1 and p53 protein immunohistochemical staining were determined through zoomed microscopy (200 x). If dark brown color stained nuclei were more than 10%, the result was considered to be positive and if less than 10%, it was considered to be negative.

Statistical analysis

SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL., USA) was used for statistical analysis and the chi-square and Fisher’s exact tests were carried out afterwards; a p value less than 0.05, was considered as statistically significant.

Results

Cliniopathological findings

There were 22 cases of benign tumors, nine cases of borderline tumors and 16 cases of malignant tumors among 47 epithelial ovarian tumors. There were eight cases of serous tumors, 13 cases of mucinous tumors and a case of serous fibroma in benign tumors, and there were six cases of serous tumors and three cases of mucinous tumors in borderline tumors. Among malignant tumors, serous tumors were the highest in number with 13 cases and there was also one case of mucinous tumor and one case of clear cell metastatic adenocarcinoma. In terms of age distribution, the mean age of patients with benign tumors was 42.3 ± 5.5 years old, patients with borderline tumor 51.1 ± 6.8 years old, and for patients with malignant tumors it was 56.2 ± 2.1 years old.

Expression of p53 in epithelial ovarian tumors

Among 22 cases, the expression of p53 was shown in 13.6% of the benign tumors (3 cases), in 44.4% of the borderline tumors (4 cases) and in 62.5% of the malignant tumors (10 cases) which shows that the expression rate is significantly higher in malignant tumors (p < 0.05). There was no statistically significant difference between borderline tumors and malignant tumors or between borderline tumors and benign tumors (Table 1).

Expression of p27 in epithelial ovarian tumors

Expression of p27 protein was shown in 95.5% of the benign tumors (21 cases). In comparison, 66.7% and 37.5% were shown in the borderline tumors and malignant tumors, respectively, which demonstrates that the expression rate was significantly higher in benign tumors compared to malignant tumors (p < 0.05) but there was no statistically significant difference between borderline tumors and malignant tumors or between borderline tumors and benign tumors (Table 1).

Expression of Jab1 protein in epithelial ovarian tumors

Among 47 cases, the expression of Jab1 protein was shown in 77.8% of the borderline tumors (7 cases out of 9 cases) which was not significantly different from the expression rate of malignant tumors which was 62.5%. However it was significantly different from the expression rate of benign tumors which was 22.7% (p < 0.05). Also, the expression rate was significantly higher in malignant tumors compared to benign tumors (p < 0.05) (Table 1).

Correlation of the expressions of each protein

Among the 17 cases that expressed p53, 63.6% of them (14 cases) also expressed Jab1 protein and among the 30 cases that did not express p53 protein, 88.0% (22 cases) also did not express Jab1 protein either, which shows that there was a correlation between them (p = 0.05) (Table 2).

On the other hand, among 22 cases with expression of Jab1, 76.9% (10 cases) showed loss of p27 and among 24 cases without expression of Jab1, 63.6% (21 cases) still showed expression of p27, suggesting that there was a significant correlation between the expression of Jab1 protein and loss of p27 (p < 0.05) (Table 3). Also, among 33 cases that expressed p27 protein, 86.2% (25 cases)

<table>
<thead>
<tr>
<th>Benign</th>
<th>Borderline</th>
<th>Malignant</th>
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<tr>
<td>p53</td>
<td>Jab1</td>
<td>p27</td>
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<td>(-) %</td>
<td>(+) %</td>
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<td>86.4</td>
<td>95.5</td>
<td>66.7</td>
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* p53 Jab1 p27
** Jab1 p27
*** p53 Jab1

Table 1. — Expression of p53, p27, Jab1 in epithelial ovarian tumors.

<table>
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<tr>
<th>Jab1</th>
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<th>p27</th>
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<tbody>
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<tr>
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p value 0.000

Table 2. — Comparison of expression of p53 and Jab1 in epithelial ovarian tumors.

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p value 0.021

Table 3. — Comparison of expression of p53 and p27 in epithelial ovarian tumors.

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<tr>
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<td>52.9</td>
<td>86.2</td>
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p value 0.007

Table 4. — Comparison of expression of p53 and p27 in epithelial ovarian tumors.
showed loss of p53 protein and among 13 cases that did not express p27, 52.9% (9 cases) expressed p53, which again suggests that there was a significant correlation between the loss of p27 protein and expression of p53 (p < 0.05) (Table 4).

In other words, as the expression of p53 increased, the expression of Jab1 protein increased, but the expression of Jab1 was significantly related to loss of p27 and the expression of p53 was also significantly related to loss of p27.

Discussion

The histological diagnostic criteria of borderline ovarian cancer are the stroma, without invasion, which is differentiated from malignancy. Borderline ovarian cancer rarely progresses to malignancy. There is a lack of studies on the characteristics of prognostic factors in borderline ovarian tumors. It is important to discriminate between benign and malignancy in the progress of borderline ovarian cancer [4]. We intended to discriminate between borderline tumors and malignant tumors in epithelial ovarian cancers based on the genes p53, p27 and Jab1. The expression of p53 proteins and p27 proteins between benign tumors and borderline tumors was not significantly different but, the expression of Jab1 protein was significantly higher in borderline tumors than benign.

p53 is the most commonly found gene in genetic mutations of several human cancers and the frequency is known to be about 50% [9]. Therefore, it has been extensively researched and the normal form of p53 is known as a tumor suppressor gene. Abnormal mutated p53 protein accumulates in the nucleus, and can be detected on immunohistochemical staining. It is commonly known that the overexpression of p53 protein is related to prognosis of ovarian malignancies but, there are reports saying that expression of p53 is not high in borderline ovarian tumors [10] nor does it have characteristics of benign or early borderline ovarian tumors [11]. There are also findings that the expression of p53 is higher in borderline ovarian tumors than benign ovarian tumors, and it is claimed that the expression of abnormal p53 could be an early phenomenon that contributes to malignant changes in some borderline ovarian tumors, endometriosis and other precancerous lesions [12, 13].

The overexpression rate of abnormal p53 was significantly higher in ovarian malignancies than benign ones in this study, but there were no significant differences between benign tumors and borderline tumors or between malignant tumors and borderline tumors.

p27 protein is located at 12p13 as a powerful tumor suppressor gene and directly suppresses the enzyme of cyclin-CDK complexes and halts cell cycles by suppressing the progression from the G1 phase to S phase [14, 15].

There are reports suggesting that the lower the expression of p27 protein is, the higher the resistance against chemotherapy drugs becomes in epithelial ovarian cancers [16] and that the loss of p27 protein is related to malignant findings and poor prognosis in lung cancers, breast cancers, prostate cancers, oral cancers, brain tumors, lymphomas and colon cancers as well as ovarian cancers [17-24].

On the other hand, Jab1 is a protein that induces cell proliferation by strengthening the activation of c-Jun gene as an activator protein 1 coactivator [25]. There is a negative correlation between the expression of Jab1 protein and expression of p27 protein in epithelial ovarian cancers and it is estimated that it is related to the progress of the cancers and their prognosis [26]. This tendency, the loss of p27 increased significantly statistically as the expression of Jab1 protein increased, is also shown in this study. Moreover, the expression of p53 increased significantly when the expression of Jab1 was high (p < 0.05) and there was a statistically significant correlation between the loss of p27 and expression of p53 (p < 0.05).

Whereas the expression of p27 was shown in 95.5% of benign tumors, it decreased to 66.7% in borderline tumors and 37.5% in malignant tumors, suggesting poorer prognosis as there was more loss of p27 protein expression. While the expression of Jab1 was shown in 22.7% of benign tumors, it was shown in 77.8% of borderline tumors and 62.5% of malignant tumors suggesting poorer prognosis as the expression of Jab1 protein increased. Thus the expression of p53, the loss of p27 expression and the expression of Jab1 protein suggest poor prognosis.

The expression of p53 and Jab1 protein and loss of p27 protein expression were shown significantly more in malignant tumors than benign tumors in this study. The expression of these proteins on immunohistochemical staining can be valuable in the judgment of prognosis as suggested in the studies of Sui et al. [26] or Patah et al. [15]. However, it is known that expression rates of p53 in epithelial ovarian cancers are high but are irrelevant to their prognosis [27, 28]. The expressions of these proteins were not significantly different between borderline tumors and malignant tumors, therefore, studies with bigger samples should be carried out.

p53, p27 and Jab1 are all thought to be valuable factors to discriminate between benign and malignant tumors and the expression of Jab1 protein is assumed to be involved in the malignant degeneration of ovarian tumors. The expression of p53, Jab1 protein and the loss of p27 protein expression were linked together. Borderline tumors are in continuity with malignant tumors, therefore, further research should be considered regarding the oncogenesis of ovarian tumors.

Acknowledgement

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References


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Expression of inflammatory cytokines by adipose tissue from patients with endometrial cancer

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Summary

Obesity results in increased mortality from many forms of cancer. We looked at the levels of gene expression for TNFα, IL-6, IκB kinase (inhibitor of NF-κB), CD68 (glycoprotein expressed on macrophages) and leptin in samples of adipose tissue from individuals with endometrial cancer versus patients with benign conditions. This is a prospective study which included patients of a gynecologic oncology group. A piece of omental tissue was harvested from them during surgery. RNA was purified from all samples. Relative amounts of RNA for IκB, TNFα, IL-6, CD68 and leptin were calculated. Pearson’s correlation method was used to correlate RNA levels with BMI. Logistic regression method was used to compare gene expression for cancer and control groups. The total sample size was 56 (24 endometrial cancer and 32 controls). IκB, TNFα and IL-6 levels increased linearly with increasing BMI in the control group. There was no correlation of IκB, TNFα, IL-6 or CD68 levels with cancer status of the patients. Leptin had a weak protective effect against endometrial cancer (odds ratio = 0.92). Obesity is associated with increased expression of certain inflammatory cytokines in the adipose tissue. However, increased levels of these inflammatory markers in the adipose tissue of the omentum are not associated with presence of endometrial cancer.

Key words: Endometrial cancer; Inflammation; Cytokines; Obesity.

Introduction

Obesity is an epidemic that seriously threatens public health. According to the Centers for Disease Control and Prevention, an estimated 64% of US adults are either overweight or obese (body mass index, BMI, greater than 25, based on the National Health and Nutrition Examination Survey 1999-2000) [1]. Obesity is associated with metabolic dysfunction including resistance to insulin leading to diabetes, and hyperlipidemia associated with an increased risk of cardiovascular disease. Obesity also results in increased mortality from many forms of cancer [2].

In attempting to understand the increased mortality from cancer among obese individuals, a role for inflammation has been suggested. There is increasing evidence that excess adipose tissue induces a chronic inflammatory state. Adipose tissue secretes cytokines such as tumor necrosis factor α (TNFα) and interleukin 6 (IL-6) [3, 4], normally associated with activation of the immune system. Recently published reports go further in suggesting that inflammation associated with obesity is related to infiltration of immune cells, specifically macrophages, into the adipose tissue of obese animals [5, 6]. Also, although cultured adipocytes and pre-adipocytes do not normally express macrophage-specific genes, pre-adipocytes can undergo conversion to macrophages [7]. The relation between cancer and inflammation is complex, but there is evidence that TNF and IL-6 may be linked to cancer development through their downstream target, nuclear factor kappa-B (NF-κB), which promotes cell proliferation, inhibits cell death, or apoptosis and is involved in tumor promotion, angiogenesis and metastasis [8-10]. Excess adipose tissue also secretes increased amounts of leptin, a hormone responsible for appetite regulation [11]. Leptin has also been shown to promote angiogenesis which may contribute to cancer progression [12, 13]. In this study, we measured the expression of inflammatory cytokines and leptin in the omental adipose tissue of patients with endometrial cancer, which has been particularly prevalent in the obese population [14]. We also examined the relationship of these markers to BMI in subjects with benign conditions. Although there are a number of other potential mechanisms for the association of obesity with endometrial cancer, e.g., higher levels of endogenous sex steroids [14, 15], current knowledge suggests that the proinflammatory state associated with obesity may be a potential mechanism for cancer risk.

Methods

This is a prospective study which included 56 patients all of whom were patients of the gynecology-oncology group at Stony Brook University Medical Center between 2006 and 2008 (Table 1). Twenty-four patients in this sample had pathologically confirmed diagnosis of endometrial cancer. For all cases this was a newly diagnosed malignancy and none had had chemotherapy or radiation treatment prior to the surgery. Patients from the control group underwent abdominal operations for benign conditions, such as ovarian cysts, fibroids, etc. All diagnoses were confirmed by pathology reports. Patients with concurrent non-gynecologic malignancy were excluded from the study.

Upon obtaining informed consent from patients, a section of omental adipose tissue was collected during the surgery and stored frozen. All adipose tissue samples were ground under...
Results
The total sample size was 56. There were 24 patients in the cancer group and 32 patients in the control group. Baseline demographics are summarized in Table 1. Patients in the endometrial cancer group were on average ten years older than controls and 83.3% were obese (defined as BMI > 30) versus 50% in the control group (Table 1). The majority of cancer patients (75%) had Stage I disease.

Using Pearson’s correlation method we found that expression of IκB, TNFα, IL-6, CD68 and leptin expression versus BMI and age of the patients in the control group (patients with benign conditions) and linear regression curves were plotted. All five markers as well as age and BMI of the patients were included as independent variables in the multivariable logistic regression models in order to find correlations of their expression in omental tissue with cancer status. Presence versus absence of endometrial cancer was set as a dependent variable for the logistic regression. All calculations were performed with the SPSS 13 software package.

Discussion
The purpose of this study was to examine how the levels of inflammatory markers expressed by adipose tissue vary with increasing BMI and how they relate to the presence of endometrial cancer. A search for the link between obesity and cancer is warranted because of the impact that it has on cancer-related mortality. A prospective study of 900,000 US adults begun in 1982 with a 16-year follow-up found that deaths from cancer were 52% higher for males and 62% higher for females with a BMI > 40 compared to non-obese subjects [2]. Although increased risk was reported for multiple cancers, the relative risk of death from uterine cancer for non-smoking women was especially high at 6.25 in the study of Calle et al. [2].

In this study, we examined whether inflammation associated with excess adipose tissue is associated with endometrial cancer. We used TNFα, IL-6, IκB and CD68 as the markers of inflammatory response in the omental fat harvested from our patients. TNFα and IL-6 are inflammatory cytokines and IκB is a protein responsible for inactivation of NF-κB. CD68 is a glycoprotein expressed on the surface of macrophages. The expression of this gene implies either infiltration of adipose tissue with macrophages of bone marrow origin or differentiation of pre-adipocytes into inflammatory cells. Both phenomena have been hypothesized in the literature [5, 7, 10].

We first looked at whether there was a correlation of the levels of gene expression for IκB, TNFα, IL-6 and CD68 in the omental tissue with BMI of the patients in the control group that underwent surgery for benign conditions. We did observe a statistically significant increase

Table 1. — Basic demographics of the cancer and control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cancer patients</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>61 (44-80)</td>
<td>51 (33-71)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean BMI (range)</td>
<td>36.6 (21.8-51.4)</td>
<td>31.4 (19.1-50.6)</td>
<td>0.013</td>
</tr>
<tr>
<td>% obese (BMI &gt; 30)</td>
<td>83.3</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>% smokers</td>
<td>25</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Cancer stage</td>
<td>Stage 1 n = 18</td>
<td>Stage 0 n = 32</td>
<td></td>
</tr>
<tr>
<td>Stage 2 n = 1</td>
<td>Stage 3 n = 4</td>
<td>Stage 4 n = 1</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Logistic regression models for IκB, TNFα, IL-6 and CD68 (controlled for age and BMI).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio [Exp(B)]</th>
<th>95% Confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IκB</td>
<td>1.21</td>
<td>0.72-2.02</td>
<td>0.465</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.921</td>
<td>0.671-1.26</td>
<td>0.609</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.793</td>
<td>0.596-1.06</td>
<td>0.112</td>
</tr>
<tr>
<td>CD68</td>
<td>0.945</td>
<td>0.677-1.32</td>
<td>0.742</td>
</tr>
</tbody>
</table>

Table 3. — Multivariable logistic regression model for endometrial cancer and leptin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio [Exp(B)]</th>
<th>95% Confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>0.92</td>
<td>0.84-0.97</td>
<td>0.041</td>
</tr>
<tr>
<td>Age</td>
<td>1.12</td>
<td>1.03-1.2</td>
<td>0.005</td>
</tr>
<tr>
<td>BMI</td>
<td>1.08</td>
<td>0.98-1.18</td>
<td>0.109</td>
</tr>
</tbody>
</table>
in the levels of IκB, TNFα and IL-6 associated with increase in BMI (Figure 1). Given the larger mass of adipocytes in the individuals with higher BMI, this upregulation of gene expression is expected to translate into even higher overall levels of these inflammatory cytokines.

TNFα and IL-6 have been shown by numerous studies to be potent activators of NF-κB family of proteins, some of which besides activating normal immune response have been shown to lead to cell growth and proliferation and thus act as oncogenes [8, 9]. There is also evidence that NF-κB can mediate metastasis and angiogenesis [8, 10] and thus play an important role at the later stages of cancer progression. NF-κB is constitutively active in a lot of human tumors including multiple myeloma, leukemias, breast and prostate cancers [8]. NF-κB is a downstream target of not only inflammatory agents, but also some common carcinogens, such as cigarette smoke [8]. Since the levels of inflammatory cytokines increase with increasing BMI, we suspected that endometrial cancer, which is highly associated with obesity, may show association with higher expression of TNFα, IL-6 and IκB (a marker for the amount of NF-κB).

When the data on the expression of inflammatory genes were examined for the relationship to endometrial cancer, we did not see a statistically significant association of IκB, TNFα, IL-6 or CD68 expression by omental adipose tissue with cancer status of the patients (Table 2). Therefore we conclude that expression of inflammatory markers by adipose tissue within the peritoneal cavity is not elevated at least in the early stages of endometrial cancer since the majority of our patients had Stage I disease. However, with the data currently available, it is not possible to determine the significance of inflammatory cytokines for the long-term prognosis of these patients and whether or not there are subpopulations with higher expression of inflammatory genes and poorer clinical outcomes. Also, we only examined the gene expression profile in the omentum of our patients. The benefit of studying the omentum is that it is in a close physical proximity to the sites of gynecological malignancies. However, it is not known whether systemic inflammatory response associated with secretion of cytokines by peripheral fat deposits plays a role in cancer progression.

We also looked at the level of leptin expression in adipose tissue. Some studies have shown that leptin has angiogenic properties and as such may have important implications in cancer progression [13, 17], although these results have not been reproduced in all studies, particularly in vivo models [18]. Other molecular mechanisms by which leptin may promote cell proliferation have also been described. For instance, there is a study from China that looked specifically at proliferation of endometrial cells promoted by leptin-induced activation of COX-2 [19]. There also have been some clinical studies suggesting an association of leptin levels with cancer stage [20]. Ashizawa et al. in 2010 found that elevated serum leptin/adiponectin ratios were associated...
with increased risk of endometrial cancer [21]. Interestingly, there is some evidence that leptin may be synergistic with estrogens as shown in a breast cancer model [22]. Contrary to the data from breast cancer, we found that leptin expression in omental fat appeared to have a mild protective effect against endometrial cancer in our patients (OR = 0.92, p = 0.041).

We conclude that obesity is associated with increased expression of certain inflammatory cytokines in adipose tissue. However, our study did not demonstrate any convincing evidence that increased levels of these inflammatory markers in adipose tissue of the omentum are associated with endometrial cancer.

Our study has important limitations. A relatively small sample size and bias towards early stages of cancer precluded us from stratified analysis according to cancer stage. Also, to really establish the role of cytokines secreted by excess adipose tissue, a study comparing adipose tissue from different depots would be helpful. The relation between obesity, cancer and inflammation may be more complex than could be uncovered in the context of a clinical study and further laboratory studies are needed.

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References


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Wortmannin inhibits proliferation and induces apoptosis of MCF-7 breast cancer cells

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Summary

Objective: The present study aimed to explore the effects of wortmannin in the proliferation and apoptosis of human breast cancer MCF-7 cells. Methods: The authors treated cells with 0, 1, 6.25, 12.5, 25, and 50 nM wortmannin for 24, 48, and 72 hours. Inhibition of proliferation was measured by cell counting kit-8 assay (CCK8). Apoptosis was detected with Annexin V-fluorescein isothiocyanate/propridium iodide double staining by flow cytometry. Additionally, expression of proteins involved in the PI3K pathway, specifically total Akt, phosphorylated Akt (p-Akt), and NF-κB was detected by Western blotting following 24 hours of wortmannin exposure. Results: Higher doses (6.25, 12.5, 25, and 50 nM) of wortmannin significantly inhibited proliferation of MCF-7 cells after 24, 48, and 72 hours of exposure compared with control MCF-7 cells incubated with DMSO alone in DMEM (p < 0.05). This inhibition increased with concentration and duration of treatment. Similarly, wortmannin at 6.25, 12.5, 25, and 50 nM concentrations significantly increased apoptosis of MCF-7 cells following 24 hours of exposure (p < 0.05). Western blotting revealed that increasing concentrations of wortmannin (6.25, 12.5, 25, and 50 nM, 24 hours) increasingly reduced expression of p-Akt and NF-κB; however, expression of total Akt was unaffected at any concentration of wortmannin. Conclusions: Wortmannin inhibits proliferation and induces apoptosis of MCF-7 cells in a dose and time-dependent manner, likely through down-regulation of PI3K/Akt signaling and NF-κB protein expression.

Key words: Wortmannin; MCF-7 cell line; Proliferation; Apoptosis; PI3K/Akt.

Introduction

The phosphoinositide 3-kinase (PI3K)/Akt signal transduction pathway is critical for cell proliferation and differentiation and commonly exhibits changes in tumor cells [1-3]. Indeed, this pathway plays important roles in maintaining the biological characteristics of malignant cells. Further, PI3K/Akt signaling may aid tumor cells in escaping damage produced by anti-cancer drugs [1-3]. Thus, inhibitors of this pathway have been heavily investigated for their potential to block tumorigenesis.

One such PI3K inhibitor is wortmannin. Isolated from penicillium wortmannii in 1957, wortmannin is a steroid metabolite belonging to the viridin family. This compound specifically inhibits catalytic activity of PI3K through the p110 subunit, blocking activation of the PI3K/Akt pathway and thereby inhibiting cell proliferation [4]. Many studies have shown that wortmannin has broad-spectrum anti-fungal activity and anti-inflammatory activities [5], and at least one study has demonstrated its anti-tumor activity [6]. Recent studies have employed wortmannin to treat various advanced tumors, including lung [7] and gastric cancer [8, 9], revealing its potential for cancer therapy.

The PI3K/Akt pathway is crucial in the development and progression of breast cancer, and PI3K inhibitors have been heavily investigated in this setting [10]. However, wortmannin has not been extensively studied for its ability to prevent or treat breast tumors. Therefore, a study was designed to investigate the effects of wortmannin in the proliferation and apoptosis of MCF-7 breast cancer cells positive for estrogen receptor, to determine a potential clinical application for this disease.

Materials and Methods

Cell culture

The human breast cancer MCF-7 cell line was purchased from the Cell Bank of Typical Model Cultivation Preservation Committee, Chinese Academy of Sciences. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 0.01 mg/ml bovine insulin at 37°C with 5% CO2 for ten generations.

Wortmannin administration in MCF-7 cells

MCF-7 cells in the logarithmic growth phase were harvested to 1 × 10^6 cells/ml in single-cell suspension, with 100 µl inoculated into each 96-well culture plate. The medium was extracted and discarded 24 hours later. For each experimental group, six parallel wells were used. To each well 100 µl wortmannin (Alexis Corp., Switzerland) solution, dissolved in DMSO (Sigma) at concentrations of 1, 6.25, 12.5, 25, and 50 nM, were added. Control wells received 0.1% DMSO in DMEM. Cells were then cultured for 24, 48, or 72 h.

Detection of cell proliferation

The colorimetric cell counting kit (CCK8; Dojindo, Japan) was used according to manufacturer’s instructions to determine cell proliferation and viability following wortmannin administration. Absorbance, (A), was measured on a microplate reader at 450 nm optical spectrum. Values were determined three times for each sample, and the average value was used to calculate the inhibition rate. Proliferation inhibition rate = [(1 - (experimental group A value/control group A value)) × 100]. The experiment was performed in triplicate.

Detection of apoptosis

Twenty-four hours after wortmannin or DMSO/DMEM administration, EDTA-free trypsin (Life Technologies, Inc.) was used to detach cells for harvesting. Cell suspensions were centrifuged at 1000 rpm for five minutes and washed twice with PBS. Apoptosis was detected by addition of 5 µl Annexin V-fluorescein isothiocyanate (FITC) and 5 µl propidium iodide (PI)
Inhibition rates were significantly different (interaction rates increased with increasing concentrations or time). Proliferation after 24, 48, and 72 hours (p < 0.05, 1, 25, 50 nM wortmannin inhibited MCF-7 cell proliferation after 24, 48, and 72 hours (p < 0.05). Inhibition rates increased with increasing concentrations or time. Inhibition rates were significantly different (p < 0.05) from proliferation in the control group, which was exposed only to DMSO in DMEM. Pairwise comparisons between groups revealed the statistically significant differences with increasing doses (p < 0.05).

**Wortmannin inhibited MCF-7 cell proliferation**

Addition of wortmannin to MCF-7 cell cultures at a concentration of one nM did not affect cell proliferation (Table 1), as detected by colorimetry. However, the addition of 6.25, 12.5, 25, or 50 nM wortmannin inhibited MCF-7 cell proliferation after 24, 48, and 72 hours (p < 0.05). Inhibition rates increased with increasing concentrations or time. Inhibition rates were significantly different (p < 0.05) from proliferation in the control group, which was exposed only to DMSO in DMEM. Pairwise comparisons between groups revealed the statistically significant differences with increasing doses (p < 0.05).

**Wortmannin induced MCF-7 cell apoptosis**

With annexin and PI to label cells undergoing programmed cell death, flow cytometry was used to assess levels of apoptosis of MCF-7 cells 24 hours following treatment with wortmannin. The mean percentage of apoptotic cells was not affected in cells treated with 1 nM wortmannin (Table 2). However, the percentages of early apoptotic MCF-7 cells (defined as annexin-positive/PI-negative) and late apoptotic MCF-7 cells (annexin-positive/PI-positive) were significantly different for cells treated with 6.25, 12.5, 25, and 50 nM doses of wortmannin compared to their respective controls (all p < 0.05). Indeed, apoptosis increased with increasing concentrations of wortmannin. Additionally, percentages of cells undergoing early apoptosis were higher than those undergoing late apoptosis for 6.25, 12.5, 25, and 50 nM wortmannin.

**Wortmannin affected expression of proteins in the PI3K/Akt pathway**

To assess the possibility that wortmannin may alter cell proliferation and induces apoptosis of MCF-7 cells via inhibition of the PI3K/Akt pathway, Western blotting was employed in cells treated with wortmannin using antibodies against three proteins that may be affected: Akt, phosphorylated Akt (p-Akt), and NF-κB p65, which appears to be activated by the PI3K pathway, particularly in cancer biology [11]. Expression of total Akt was not affected 24 hours after any exposure to wortmannin compared with control medium (Figure 1); however, increasing concentrations of wortmannin (6.25, 12.5, 25, and 50 nM) caused larger reductions in expression of both p-Akt and NF-κB p65.
that the drug negatively regulates multiple molecules in the pathway, including PI3K and mTOR, to dephosphorylate Akt and reduce its activity. The present findings indicate that further investigation of the link between NF-κB and Akt signaling is required.

In summary, wortmannin exposure significantly inhibits proliferation and induces apoptosis of MCF-7 breast cancer cells. Such changes may inhibit tumor cell growth and differentiation and reduce the invasiveness of tumor cells via inhibition of the PI3K/Akt signal transduction pathway. These findings suggest that wortmannin offers potential in anti-tumor targeted therapy and drug screening for breast cancer.

References


Discussion

While the pathogenesis of breast cancer is complex and multifactorial, involving multiple genes and environmental interactions, cell signaling plays a vital role in its occurrence and development. The present study demonstrates that breast cancer cells show reduced proliferation after addition of wortmannin, an inhibitor of the PI3K/Akt signaling pathway. These effects in cell proliferation were increasingly evident at higher drug concentrations. Concurrently, wortmannin exposure induced apoptosis of MCF-7 cells. Apoptosis significantly increased with increasing concentration of the drug, consistent with other reports [12].

Because wortmannin is known to specifically inhibit the PI3K/Akt pathway, the authors investigated whether alterations in this pathway might be responsible for the reduced proliferation and increased apoptosis of MCF-7 cells following wortmannin exposure. p-Akt expression decreased with increasing concentrations of wortmannin; however, total Akt expression was not affected at any concentration. This finding indicates that only the active (phosphorylated) form of Akt was affected by wortmannin activity. Therefore, the authors hypothesize that the anti-proliferative activity of wortmannin is correlated with PI3K/Akt signaling pathway activation.

The transcription factor NF-κB is considered a key molecule in regulating oncogene expression, enabling apoptosis inhibition of tumor cells while also promoting metastasis and drug resistance [13]. This protein however, is also an important link in signal transduction pathways, making it an ideal target for cancer prevention and enhancing sensitivity to chemotherapy [14]. This protein is also believed to interact with PI3K/Akt [11]. In this instance, wortmannin exposure down-regulates NF-κB protein levels in MCF-7 cells, demonstrating that inhibition of PI3K by this drug also affects the NF-κB signaling pathway. This finding is supported by recent literature [15, 16]. Poh et al. [16] studied the effects of wortmannin on the PI3K/Akt pathway, demonstrating...
Effective multidisciplinary treatment for ovarian granulosa cell tumor with multiple metastases - a case report


National Hospital Organization Tokyo Medical Center, Division of Obstetrics and Gynecology, Tokyo (Japan)

Summary
Ovarian granulosa cell tumor (GCT) is among the ovarian sex-cord stromal tumors that are classified as borderline malignancies. We report a case of GCT with multiple metastases for which multidisciplinary treatment including surgery, chemotherapy and radiotherapy was effective. A 41-year-old woman underwent left salpingo-oophorectomy because of an ovarian tumor in 2004. Final pathology confirmed a granulosa cell tumor adult type, FIGO Stage IC. In 2008, tumorectomy of the lower abdominal wall metastases was also performed. After three cycles of BEP chemotherapy for metastases of the right lung, liver, paraaortic lymph node and rectus, surgical resection was performed in 2009. In 2010, local radiation was performed for the first lumbar vertebral metastasis. Ovarian GCTs exhibit slow growth but if the surgical stage is IC or higher, there is the possibility of recurrence. It is important to treat recurrent tumors with the combination of surgery, chemotherapy, and radiation therapy.

Key words: Ovarian granulosa cell tumor; Recurrence; BEP.

Introduction
Ovarian granulosa cell tumors (GCT) are among the ovarian sex-cord stromal tumors that constitute nearly 2-3% of all ovarian neoplasms [1, 2]. They are often estrogen-producing tumors and are seen in women of all ages. Although they are classified as borderline malignancies, the prognosis is relatively good. They often have late recurrence after ten years or more. As such, 10-year survival rates of almost 90% have been reported, with 20-year survival rates dropping to 75% [3]. The tumors may spread hematogenously, but most recurrences often remain in the abdominopelvic cavity. However, metastases to the abdominal wall, muscle and bone are rare [3-5].

We report a case of GCT with subcutaneous metastases and metastases to the lung, liver, paraaortic lymph node, rectus, and lumbar spine, in which multidisciplinary treatment including surgery, chemotherapy, and radiotherapy was effective.

Case Report
A 41-year-old (gravid 0, para 0) woman underwent an emergency laparotomy with left salpingo-oophorectomy for a left ovarian tumor with abdominal pain in 2004. Final pathology confirmed a granulosa cell tumor adult type, FIGO Stage IC (pT1cNXM0) (Figure 1). The results of the immunohistochemical study are summarized in Table 1.

Ascitic fluid cytology from the first operation was positive. As the patient wanted to preserve fertility, no additional surgery was performed. Although we explained the need for regular follow-up to her, she suspended follow-up, of her own accord.

The patient underwent a medical examination at our hospital after having had a subcutaneous tumor in the lower abdominal wall in 2008. Since abdominal magnetic resonance (MR) identified subcutaneous tumors in the lower abdominal wall, tumorectomy was performed by a dermatologist in our hospital. The final pathology confirmed metastases of the granulosa cell tumor and surgical margins were negative. No additional treatment was administered, and she was subsequently followed up in our hospital.

In 2009, although she had no complaints, a follow-up thoracoabdominal computed tomography (CT) (Figure 2) scan was performed which indicated metastatic tumors in the right lung, liver, paraaortic lymph node, and rectus. Since multiple lesions were observed at that time it was considered that surgical resection would be difficult. Thereby we determined that chemotherapy was appropriate, with a dose schedule of bleomycin 30 mg/patient (day 2, 9, 16), etoposide 100 mg/m² (day 1-5), cisplatin 20 mg/m² (day 1-5) every three weeks. She received three cycles of BEP therapy.

After three cycles of BEP the thoracoabdominal CT scan (Figure 3) indicated that the liver tumor had disappeared, and that the other metastatic lesions were reduced. After the chemotherapy, the patient went into partial remission. Thereafter, she underwent total abdominal hysterectomy, right salpingo-oophorectomy, pelvic lymphadenectomy, paraaortic lymphadenectomy, partial omentectomy, partial rectus resection, and thoracoscopic partial right lung resection.

Lesions were found in the paraaortic lymph node, rectus, and right lung. Final pathology confirmed metastases of GCT in all sites. Since the microscopic finding of these lesions demonstrated that the there were viable cells, but that they were part of tumors with necrotic changes, the BEP therapy was effective pathologically (Figure 4). The results of the immunohistochemical study are summarized in Table 1.

In 2010, abdominal CT and MR imaging indicated a metastatic tumor in the first lumbar vertebra (L1), and positron emission tomography (PET)-CT indicated accumulation in L1 (Figure 5a, b). The orthopedist and radiologist in our hospital diagnosed the GCT metastases in L1, and local radiation 40 Gy was administered to L1. Seven years have passed since the initial treatment, with no recurrence to date.
Effective multidisciplinary treatment for ovarian granulosa cell tumor with multiple metastases - a case report

Discussion

Ovarian GCTs are borderline malignancies, because they are relatively slow growing tumors and often have a good prognosis. However, due to their slow growth, late recurrence, after ten years or more, has often been reported. After initial treatment in this case, the patient developed multiple metastases to the abdominal wall within four years, to the lung, the liver, the paraaortic lymph node, and the rectus muscle within five years, and to the bone within six years. The initial treatment for ovarian GCTs is surgical resection if the patient does not wish to preserve fertility, and the standard operative procedures are recommended, i.e., hysterectomy, bilateral salpingo-oophorectomy, and partial omentectomy.

Table 1. — Immunohistochemical finding and mitotic index in the primary lesion and metastatic lesion.

<table>
<thead>
<tr>
<th></th>
<th>Primary lesion</th>
<th>Metastatic lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Inhibin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mitotic index</td>
<td>1-2/10 HPF</td>
<td>10-20/HPF</td>
</tr>
<tr>
<td>Ki67 index</td>
<td>a few</td>
<td>10%</td>
</tr>
</tbody>
</table>

Figure 1. — Pathological finding of the initial operation.

a) Left ovary (hematoxylin-eosin stain). Tumor composed of a solid proliferation of small round or oval cells. Some parts of tumor show small clusters and rosette-like patterns. The tumor cells show a high nucleus/cytoplasm ratio and less anisokaryosis. Some cells exhibit coffee bean grooved nuclei. The tumor was diagnosed as a granulosa cell tumor, adult type.

b) Left ovary (immunohistochemical staining for alpha-inhibin). Most of the cells comprising the tumor exhibited alpha-inhibin positivity; otherwise the stromal cells were negative for alpha-inhibin.

c) Ascites cytology. Some clusters consisting of small oval cells appear to be GCT cells.

If the patient hopes to preserve fertility, unilateral salpingo-oophorectomy should be performed after adequate examination of the abdominal cavity [3]. However, if the clinical stage is IC or higher, careful consideration regarding preservation of the adnexa should be made, since some recurrences have been reported [6]. In advanced cases, additional chemotherapy or radiation therapy should be considered after surgery because surgical resection alone is not expected to be able to remove all microlesions. Since this case was FIGO Stage IC, and cytology of the ascites was positive, we had to consider the indication for surgery that would preserve the patient’s fertility. Even though her ovaries were preserved, adjuvant chemotherapy had to be added. However, adjuvant chemotherapy and radiation for ovarian GCTs have not been investigated in a phase III study, but only in small-scale studies. Therefore, sufficient evidence for adjuvant therapy does not exist. While ovarian GCTs often recur in the pelvis, distant metastases are very rare. However, lung metastases, liver metastases, lymph node metastases and bone metastases have been reported, as observed in this case [4, 7-9].

The treatment for recurrent tumor of ovarian GCTs includes surgery, chemotherapy, radiation, and hormone
therapy. Diagnostic imaging, such as CT and MR imaging, is useful for the diagnosis of recurrent GCTs. On the other hand, PET scan has been reported to be less helpful in the diagnosis of recurrence [10]. Since ovarian GCTs are borderline malignancies, their cell growth is slower than cancer cells. Furthermore, due to the lower 18F-fluorodeoxyglucose (18F-FDG) uptake, tumor cells are considered to be false negative in a PET scan. While the lumbar spine metastasis was positive in a PET scan in this case, the standard uptake value (SUV) was slightly high, 3.60, and the diagnostic sensitivity of recurrence and metastases was not considered to be higher with a PET scan compared to CT or MR imaging.

GCTs are estrogen-producing tumors and estradiol is known to be one of the tumor markers of GCTs. Inhibin and anti mullerian hormone (AMH) have been reported to be useful as tumor markers of GCTs but they are off-label examinations in Japan [6, 11]. In this case estradiol did not increase during the recurrence and inhibin and AMH were not examined.

Figure 2. — Lung, liver, paraaortic lymph node (PAN), rectus metastases before the chemotherapy.

a) Lung, b) Liver, c) PAN, d) rectus.
Each arrow indicates a metastatic lesion of the lung, liver, PAN, and rectus.
In chemotherapy, small-scale clinical studies of BEP and PVB for ovarian GCTs have been performed, and the responsiveness for BEP and PVB has been reported to be 37% and 60.5%, respectively [12, 13]. These regimens are performed for ovarian GCTs in Japan. Since these regimens contained bleomycin, which has toxicity for the lungs, they are limited to three or four cycles. As in this case, if multiple metastatic lesions are detected, chemotherapy is recommended alone, but if few recurrent lesions are detected, and they are operable, it is desirable to add the appropriate surgery and radiation therapy.

Radiation therapy is performed when the lesion is localized. In a retrospective study, the lesions disappeared in 43% of cases [14]. It is also useful as a treatment for palliative care [15]. Dubuc-Lissoir et al. reported that radiation therapy was effective for vertebral metastases [4]. Since recurrence of the lesion after chemotherapy was solely within the lumbar spine, which was difficult to remove surgically, radiation therapy was performed.

Hormone therapy, as a gonadotropin releasing hormone analog or aromatase inhibitor, has been reported.
to be effective for GCTs. However, hormone therapy for GCTs has not often been administered in Japan [16, 17].

The clinical risk factors that predict the recurrence of ovarian GCTs have been reported to be age, surgical stage, the presence of residual tumor, and additional treatment. The pathological risk factors that predict the recurrence of ovarian GCTs have been reported to be tumor size, nuclear atypia, mitotic index, Ki-67 index and positivity for alpha-inhibin, but this remains debatable [6, 18-20]. In this case the surgical stage was I, and the mitotic and Ki-67 indexes were not high. However, since the peritoneal cytology was positive, it is suspected that there was a residual tumor in the body at the cellular level.

In conclusion, ovarian GCTs exhibit slow growth, but if surgical stage is IC or higher, there is the possibility of recurrence. We need more careful follow-up after the initial treatment. It is important to treat recurrent tumors with such combination of surgery, chemotherapy, and radiation therapy.

Figure 4. — Pathological findings of the metastatic lesion.
a) Left ovary (hematoxylin-eosin stain). Tumor cells are similar to Figure 1. This tumor was diagnosed as a recurrence of the granulosa cell tumor, adult type.
b) Left ovary (immunohistochemical staining for alpha-inhibin). Most of the cells comprising this tumor exhibited alpha-inhibin positivity.

Figure 5. — First lumbar vertebra (L1) metastasis.
a) MR finding.
b) PET-CT scan finding.
Each arrow indicates a metastatic lesion of the L1; SUV is 3.60.
References

Laparoscopic versus laparotomic approach to endometrial cancer

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Introduction

Endometrial cancer (EC) is the third most common cancer in women worldwide [1, 2]. It is estimated that 70% of patients affected by endometrial tumors had a high body mass index (BMI > 25) and 50% had comorbidity such as diabetes or cardiovascular disease [3].

For patients with early stage EC, FIGO recommends total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH+BSO) with or without lymph node dissection through a laparotomic vertical midline incision [4]. The laparoscopic approach to EC is not a standard surgical procedure. In the last decade several retrospective and prospective studies have demonstrated that the laparoscopic approach is an effective and safe alternative to the open procedure allowing for significant reduction of treatment related morbidities, shorter hospital stay, less pain, and quicker return to daily activities. These advantages are even more pronounced in obese and older patients [5-8].

On this basis the laparoscopic approach has been used in EC. Laparoscopy in early-stage EC represents a minimally invasive technique compared to the laparotomic approach and in many countries this latter approach is being increasingly replaced by laparoscopy [9]. Like laparotomy, operative laparoscopy can accomplish full surgical procedures including complete intraperitoneal surveys, peritoneal washings, removal of adnexae, and performance of pelvic and paraaortic lymphadenectomy and total hysterectomy [10].

In this study we retrospectively reviewed the clinical records of hysterectomy and lymphadenectomy for EC performed by the authors. The aim of our analysis was to compare feasibility, morbidity, long-term safety, and survival rate of the laparoscopic (LPS) approach in early stage EC compared to the traditional laparotomic (LPT) approach.

Materials and Methods

Study design

We retrospectively reviewed the data of all patients who underwent primary surgery from 1997 to 2009. We recorded clinical parameters, surgical stage, histological type, operative and peri-operative complications, time to resumption of normal functions, conversion to laparotomy, overall survival, and disease-free survival. Results: LPS did not increase operative risk and peri-operative complications even in obese and older women. The number of pelvic lymph and aortic nodes removed was similar for the two groups. One hundred and eight patients had a follow-up of 60 months. The two groups were similar for disease-free survival and overall survival.

Conclusions: Laparoscopic approach to EC provides a reduction in postoperative complications and hospital stay compared to the laparotomic approach.

Key words: Endometrial cancer; Laparoscopy; Laparotomy; Hysterectomy.
Inclusion criteria were clinical Stage I to IIA uterine cancer according to FIGO 1988 rules.

Exclusion criteria were previous malignancy, previous hysterectomy, EC histological type I and Stage IA, intra-operative findings of ovarian lesions, metastasis beyond the uterus, and procedures performed by surgeons in training. We also excluded patients undergoing laparotomy because of contraindications to LPS such as increased uterine volume (bulky > 12 week), history of cardiac failure, myocardial infarction, unstable angina or pulmonary obstructive disease, and poorly controlled or contraindicating prolonged Trendelenburg position. Prior abdominal surgery was not considered a contraindication for the LPS approach.

Operative time was calculated from first skin incision to last incision closure. Intraoperative blood loss hemorrhage was calculated as the difference between pre- and postoperative hemoglobin values. [11]. Active bleeding with symptomatic anemia and hemoglobin less than 8 g/dl were considered criteria for blood transfusion. Hospital stay was counted from the first post-operative day until discharge.

Postoperative morbidities recorded were fever (defined as a temperature of 38°C or higher on two occasions over 48 hours), urinary tract infection, respiratory tract infection, wound infection, pelvic lymphocyst with or without abscess, intestinal or ureteric fistula, need to return to operating theatre within 14 days following the primary surgery, deep venous thrombosis, and pulmonary venous embolism. Diagnosis of deep-vein thrombosis and pulmonary embolism was confirmed by venous ultrasound (US) and helical computed tomography (CT) or ventilation-perfusion scan, respectively.

Overall survival period, disease-free survival period, disease recurrence, port-site disease, and any long-term complications were retrieved from hospital records and direct patient reports. We confirmed information and patient status by direct telephone interview and clinical follow-up.

Surgical technique

The typical operative management of a patient is described as follows: routine preoperative investigations such as clinical examination, pelvic US, and hysteroscopy/uterine revision with biopsy were performed in all patients referred to our Institute. Pelviabdominal CT scan and colonoscopy are optional studies required if clinical metastasis is suspected.

Routine bowel preparation, thromboprophylaxis and antibiotic prophylaxis were provided.

Patients were given a general anesthetic and placed in a modified lithotomy position using Allen’s stirrups, and a urinary catheter was inserted.

In all cases (LPT and LPS) surgical staging began with an inspection of the entire abdominopelvic cavity. A sample of peritoneal fluid was obtained for cytologic analysis.

Since 2001 most patients have been screened via the sentinel lymph node procedure [12]. The time required for this procedure was subtracted from the total surgical time.

Laparotomic route

Abdominal access was performed through a vertical midline skin incision and the hysterectomy consisted of an extrafascial total hysterectomy. To minimize the risk of tumor spread we routinely grasped the fallopian tubes bilaterally before starting the laparotomic procedure.

Laparoscopic route

A laparoscopic spoon or a colpotomizer was placed within the uterus for manipulation. Abdominal entry was established via an umbilical 10-mm port for the laparoscope (directly or with open technique [13], two 5-mm ports on either side of the abdominal wall and one 12-mm port supraperitoneally were positioned (additional trocars may be used in accordance with surgical need). The vaginal cuff was sutured by laparoscopy in all total laparoscopic hysterectomies. To minimize the risk of tumor spread during manipulation of the uterus we routinely coagulated the fallopian tubes bilaterally before starting the laparoscopic procedure.

Both in laparotomic and laparoscopic access cytology were obtained on entry into the peritoneal cavity.

Limits of lymphadenectomy were lateral genitofemoral nerve, medial hypogastric artery, posterior obturator nerve, caudal circumflex iliac vein, and inferior mesenteric artery as the cranial limit when performing paraaortic lymphadenectomy.

Statistical analysis

All continuous data is expressed in terms of mean and standard deviation of the mean and range. The unpaired t-test was performed to investigate differences of continuous variables between the groups. Pearson’s chi square test, calculated by the Montecarlo method, was performed to investigate the relationships between group variables. For all tests $p < 0.05$ was considered significant. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) software version 9.0 (SPSS Inc., Chicago, IL, USA).

Results

From 312 patients whose data were reviewed, 210 were considered eligible for the study; 115 in the LPS group and 95 in the LPT group. Table 1 lists the demographic and clinical characteristics of women with EC on the basis of the surgical approach used. The two groups were homogeneous for age, body mass index (BMI), menopausal age, comorbidities, histological type, preoperative stage and surgical risk (previous abdominal surgery, general health).

Conversion from LPS to LPT

Conversion to laparotomy was necessary in six (5%) of the 115 cases managed by laparoscopy. Poor tissue exposure and anesthesia problems (high saturation in CO2) in five patients; one case, for evidence of intraperitoneal tumor dissemination. In no patient was conversion to laparotomy due to obesity or adhesions.

Operative and postoperative results

Surgical time was 280 ± 80 vs 222 ± 82 minutes in the LPS and LTP groups, respectively ($p < 0.0005$).

The median hemoglobin decline was 2.2±1.0 g/dl (range 2.0-2.4) in the LPS group and 2.1±1.4 g/dl (range 1.8-2.4) in the LPT group. Drainage was positioned in 76 patients (80%) and in 63 patients (56%) of the LPS and LPT groups, respectively ($p = 0.001$).

The mean time of postoperative ileus was 2.0 ± 0.9 days in the LPS group and 3.5 ± 2.1 days in the LPT group ($p < 0.0005$). We routinely removed the urinary catheter the day following the surgical procedure in both groups and all patients were voiding spontaneously without any difficulty.
The mean length of hospital stay was 3.1 ± 1.5 in the LPS group and 5.6 ± 1.5 days in the LPT group (p < 0.005).

Table 1.—Population characteristics in the two groups of patients.

<table>
<thead>
<tr>
<th></th>
<th>LPS</th>
<th>LPT</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Study patients (no.)</td>
<td>115</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>65.1 ± 10.8</td>
<td>66.6 ± 10.6</td>
<td>ns</td>
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<tr>
<td>BMI, Kg/m² (mean ± SD)</td>
<td>28.7 ± 6.0</td>
<td>28.6 ± 5.2</td>
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<td>Patients in menopausal status, no. (%)</td>
<td>87 (82)</td>
<td>76 (73)</td>
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</tr>
<tr>
<td>Menopausal age, years (mean ± SD)</td>
<td>50.8 ± 4.7</td>
<td>51.6 ± 3.5</td>
<td>ns</td>
</tr>
<tr>
<td>Histological Type I, no. (%)</td>
<td>83 (81)</td>
<td>82 (86.6)</td>
<td>ns</td>
</tr>
<tr>
<td>Histological Type II, no. (%)</td>
<td>32 (19)</td>
<td>13 (13.4)</td>
<td>ns</td>
</tr>
<tr>
<td>Stage I, no. (%)</td>
<td>83 (88)</td>
<td>86 (90)</td>
<td>ns</td>
</tr>
<tr>
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<td>32 (12)</td>
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<tr>
<td>ASA I/II, no. (%)</td>
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<td>86 (90)</td>
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<tr>
<td>ASA III/IV, no. (%)</td>
<td>11 (9)</td>
<td>9 (10)</td>
<td>ns</td>
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Surgical staging

In Table 2 we report postsurgical staging in both groups. Staging of lymph nodes were histologically documented from the pelvis in both laparoscopic and laparotomic route patients. The number of pelvic lymph nodes removed was similar for the two groups: 18.0 ± 9.6 vs 14.9 ± 7.9 mean ± SD in the LPS and LPT groups, respectively. Paraaortic lymphadenectomy was performed in 25 (22%) cases in the LPS group and in 21 (22%) cases in the LPT group. The mean number of resectioned aortic lymph nodes was 9.8 ± 3.1 in the LPS group compared to 8.9 ± 2.5 in the LPTs group (n.s.). Lymph node metastases were found in 10% of participants and were similar in both groups.

Table 2.—Surgical stage and tumor type after surgical staging in the two groups of patients.

<table>
<thead>
<tr>
<th>Surgical stage (FIGO 1988)</th>
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<th>LPT</th>
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<td>I</td>
<td>88</td>
<td>76</td>
<td>74</td>
</tr>
<tr>
<td>II</td>
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<tr>
<td>Type I</td>
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<td>66</td>
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<tr>
<td>Type II</td>
<td>24</td>
<td>21</td>
<td>29</td>
</tr>
</tbody>
</table>

Intraoperative and postoperative complications in the two groups of patients.

**Intraoperative complications**

<table>
<thead>
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<th>p</th>
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<tbody>
<tr>
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<td>1</td>
</tr>
<tr>
<td>Vein</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Artery</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bladder</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ureter</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nerve</td>
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</table>

**Postoperative complications**

<table>
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</thead>
<tbody>
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<td>4</td>
</tr>
<tr>
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<td>29</td>
<td>60</td>
</tr>
<tr>
<td>Lymphoedema</td>
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<td>13</td>
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<tr>
<td>Venous thrombophlebitis</td>
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<td>2</td>
</tr>
<tr>
<td>Bowel obstruction</td>
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<td>2</td>
</tr>
<tr>
<td>Urinary infection</td>
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<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Wound infection</td>
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<td>4</td>
<td>12</td>
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<td>Subfascial hematoma</td>
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<td>Urinary fistula</td>
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<td>1</td>
</tr>
<tr>
<td>Bowel fistula</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Arhythmia</td>
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<td>2</td>
</tr>
<tr>
<td>Blood transfusion</td>
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<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Antibiotics</td>
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<tr>
<td>Re-admission</td>
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<tr>
<td>Re-operation</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

The mean length of hospital stay was 3.1 ± 1.5 in the LPS group and 5.6 ± 1.5 days in the LPT group (p < 0.005).

**Surgical staging**

In Table 2 we report postsurgical staging in both groups. Staging of lymph nodes were histologically documented from the pelvis in both laparoscopic and laparotomic route patients. The number of pelvic lymph nodes removed was similar for the two groups: 18.0 ± 9.6 vs 14.9 ± 7.9 mean ± SD in the LPS and LPT groups, respectively. Paraaortic lymphadenectomy was performed in 25 (22%) cases in the LPS group and in 21 (22%) cases in the LPT group. The mean number of resectioned aortic lymph nodes was 9.8 ± 3.1 in the LPS group compared to 8.9 ± 2.5 in the LPTs group (n.s.). Lymph node metastases were found in 10% of participants and were similar in both groups.

**Intraoperative complications**

Intraoperative complications were not statistically different between the two groups (Table 3).

In the LPS group we had one case of ureterovesical fold incision that was sutured laparoscopically. There was one case of obturator nerve injury during obturator pelvic node lymphadenectomy. The nerve was quickly sutured by LPS and the patient was submitted to rehabilitation therapy, and after one year no further problem was observed. One case of ureter damage due to accidental bipolar coagulation required anastomosis. No long-term problem was observed. One case of small aortic injury was sutured with a metallic clip.

In the LPT group one patient had a bowel perforation...
due to dense adhesions of the uterus which was diagnosed during the surgical procedure and resolved with a bowel resection and latero-to-end anastomosis, one case of bladder injury was resolved quickly with a suture of the bladder and there was one case of ureter injury.

Postoperative complications

Postoperative complications are shown in Table 3. Fever and wound infections were significantly more common in the LPT group. Other complications were not significantly different between the two groups.

Adjuvant treatment

There was no significant difference between the two groups with respect to adjuvant treatment. In the LPS group, 61 (53%) patients underwent radiotherapy (RT) and seven (7%) underwent chemotherapy (two patients underwent both). In the LPT group, 59 (51%) patients underwent RT and 12 (13%) underwent chemotherapy (one patient underwent both).

BMI and age

The LPS group data was considered on the basis of BMI and age; no significant statistical differences were observed (Tables 4 and 5) in any parameters such as surgical length, hemoglobin decrease, and number of lymph nodes removed. No difference in intraoperative and postoperative complications or adverse events was observed.

Follow-up

One hundred and eight patients had a follow-up of 60 months. The total recurrence rate of the entire sample was 14% (n = 29 patients); eight (13%) of 62 patients in the LPS group had a recurrence versus seven (15%) of 46 patients of the LPT group. In the LPS group, metastases were detected in the vaginal cuff in two patients and distant metastases (brain, bowel, and aortic node) were detected in five patients. There were no port-site recurrences noted in the LPS group. Disease-free survival showed no significant difference between the two groups (Figure 1). No significant difference was found between the two groups when the recurrence rate was compared. At the time of last follow-up, six patients (10%) and four (8.6%) patients died of disease or correlated disease in the LPS and LPT groups, respectively.

Discussion

Our analysis confirms that total laparoscopic access represents a feasible and safe therapeutic procedure for the management of early-stage EC. The LPS approach to EC compared to the LPT approach allows for the reduction of postoperative complications and hospital stay. There were no significant differences in intraoperative complications, surgical performance, postoperative complications, and disease-free survival between the two approaches, even when considering obese and older patients.

In general, one of the principal problems for the surgeon is to choose the most appropriate surgical access approach to perform the operation. The choice must guarantee the patient the lowest possibility of complications and, in the case of oncologic surgery, the greatest oncologic profundity. In a LAP 2 study around a quarter of patients scheduled for laparoscopy only completed the operation after conversion to laparotomy. One of the most frequent reasons for conversion is that obesity has made laparoscopy difficult. This data could lead to the thinking that a large number of patients affected by EC are not candidates for laparoscopy as a high percentage of them are obese. In reality our data, as that of other studies [14], show a low percentage of conversion to laparotomy and in those few cases of conversion the reason was not patient obesity but more than anything, in our opinion, was due to the longer laparoscopic surgical time which encourages the absorption of CO₂ through the tissue and therefore an increase of the gas in the blood with the consequent risk of metabolic acidosis. In our experience this problem can be resolved by working towards maintaining low CO₂ pressure, not superior to 12 mmhg, from the beginning of surgery.

Our data showed that morbidly in obese patients (BMI > 30) treated with LPS was similar to that of non obese patients (BMI < 30) (Table 4). In obese patients, LPS presents some advantages versus LPT, being faster resumption of normal intestinal function, lower risk of wound infection and dehiscence of suture, shorter hospital stay without compromising surgical staging, and no increase in conversion rate. Other authors described the same advantages of LPS versus LPT in obese women [15, 16]. We found, in accordance with other authors, [17] the same results in older patients (Table 5), in that age does not increase perioperative complications and conversion rate. Obesity and age was not a contraindication to LPS.
in EC patients. It is most important, when deciding the surgical approach for these patients, to evaluate the overall health including such things as renal function, respiratory, and cardiovascular conditions [18].

As in previous studies in the literature, we did not record differences between LPS and LPT approaches in terms of intra-operative complications [17-21]. Only data on blood loss was discordant. In a LAP 2 study [7] a slightly higher arterial bleeding was reported and in other studies there was a significant reduction in intraoperative blood loss [22] due to magnification of small blood vessels provided by the current optical systems [23, 24]. In our patients the percentage of hemoglobin decrease was similar in the two groups; no patients required intraoperative transfusion and the number of postoperative transfusions was similar in the two groups.

On the other hand significant advantages of LPS over LPT were obtained in terms of postoperative complications (fever, ileus, and wound infections) and shorter mean length of hospital stay. In contrast, as reported by other authors, laparoscopy was associated with a significantly longer operative time [17-24].

Although data is accumulating which shows that the laparoscopic approach represents a convenient alternative to the laparotomic surgery for EC, various questions remain unanswered, particularly related to oncologic safety. Some authors questioned that the use of a colpotomizer increases the risk of vaginal cuff recurrences [22, 23], positive cytology, and the possibility of port-site metastasis (PSM).

As described in the literature, we found no differences in surgical staging in the two groups. We had one vaginal cuff recurrence in both the LPS and LPT groups. The vaginal cuff recurrence in the LPS group was compatible with histological type (type II) and Stage II in both groups. No increase in positive cytology was observed. After five years post surgical staging we had generated information on 62 patients.

During five years of follow-up, PSM was not found in any patient. Data on the relative risk of parietal metastases in open incisions versus laparoscopy remains controversial [24, 25]. Recently Querleu et al. reported, in a series of 1,216 laparoscopies for uterine cancer, only five PSM (four in cervical cancer and one in an EC). All PSM patients had concomitant metastasis (peritoneal carcinomatosis, vaginal recurrence, and lymph node progression). The authors concluded that PSM represents a rare complication in patients with uterine cancer and cannot be used as an argument against laparoscopic staging in these patients [26].

Several postoperative studies showed that patients treated by LPS had a superior quality of life compared to patients treated by LPT [19, 27-29], however few studies have reported long-term results and oncological findings [21, 22-30]. An important aspect of our study is the length of follow-up (five years). Our data suggest that the surgical approach does not influence disease-free survival (Figure 1) and overall survival. The scientific world advocates new studies on this subject. Awaiting the long-term outcome data from the GOG-LAP2 and the LACE [7-30] trials, which are designed to answer the final questions relating to cancer-free survival following LPS, it is important to collect this information from the experience of the various centres.

The limits of this study were the difficulties in data collection in that all data was retrospective and that four different surgeons performed the operations. However we consider these problems to be insignificant because we carefully examined the standard parameters not subject to personal evaluation and, because the surgeons (experienced in both laparoscopy and laparotomy) performed the operations using the same techniques. We therefore consider our retrospective data to be reliable.

In conclusion, our study describes surgical experience in EC patients confirming and reinforcing previous preliminary reports suggesting feasibility, safety and advantages of the LPS approach to EC treatment. Obesity and age do not compromise LPS performance and these patients can benefit from a minimally invasive technique which leads to superior quality of life without compromising oncological security and survival.

References

Laparoscopic versus laparotomic approach to endometrial cancer


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Human epididymal protein 4 (HE4) is a novel biomarker and a promising prognostic factor in ovarian cancer patients

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Summary
Purpose of investigation: The aim of this work was to compare serum concentrations of HE4 in patients with benign and malignant epithelial tumors and to determine the association of preoperative concentrations of HE4 with some clinicopathologic factors. Methods. We enrolled 94 patients, including 39 females with freshly diagnosed ovarian cancer. HE4 concentrations were measured with ELISA HE4 EIA assay from Fujirebio Diagnostics. Results. Serum concentrations of HE4 differed significantly in patients with ovarian cancer (324.1 pM) compared with benign epithelial tumors (26.1 pM; p < 0.001). There was also a significant difference between HE4 concentrations at diagnosis of ovarian cancer (324.1 pM) and in patients with complete clinical remission (23.3 pM; p < 0.0001). Patients with poorly differentiated tumors had significantly higher concentrations of HE4. Preoperative HE4 levels were higher in patients in whom relapse was noted and who died before the end of the two-year follow-up period. Conclusion. On the basis of these findings and reports in the literature it appears likely that HE4 can complement CA125 in the monitoring of therapy in ovarian cancer and may also serve for prognostication.

Key words: HE4; Ovarian cancer; Prognostic factors; Biomarkers.

Introduction
In spite of great efforts of medical specialists, researchers, and clinicians, ovarian cancer still remains the main cause of death among women with malignancies of the sex organs. Looking at the latest reports on ovarian cancer one may notice a specific “scientific race” for which the prominent goal is to accurately determine risk and prognostic factors, develop methods of early diagnosis, and improve therapeutic efficacy. During the last decade there emerged biomarkers discovered in the serum of patients with ovarian cancer but their sensitivity and specificity was never found to be superior to that of CA125 [1].

The human epididymal protein 4 (HE4) is among the very few biomarkers which have been studied recently and deemed suitable to play a leading role in the diagnostics and screening for ovarian cancer [2-5]. This protein was first discovered in the lining epithelium of the distal part of the epididymis [6] and was later identified exclusively in epithelial cells of various organs [7]. Physiologic expression of human HE4 is highest in the epithelium of the trachea, epididymis, female sex organs (oviducts, endometrium, and endocervix), and salivary glands [7, 8]. Expression of HE4 in the ovary is most noticeable in the metaplastic epithelium of Mullerian inclusion cysts [8]. Drapkin et al. found overexpression of HE4 in serous and endometrioid ovarian carcinomas but not in epithelial tumors of other organs (intestine, breast, lung, kidney or thyroid gland) [8]. It was inferred from these findings that cells of serous and endometrioid carcinomas of the ovary are capable of secreting HE4 to the extracellular space [8].

The first article on the use of HE4 as an oncomarker in ovarian cancer was published by Hellstrom et al. [2] in 2003. During recent years, other researchers followed with a series of reports on the diagnostic importance of HE4 in ovarian cancer [9-12]. Unfortunately, little is known about the prognostic value of this epididymal protein. The present work was undertaken to compare serum concentrations of HE4 in malignant and benign epithelial tumors of the ovary with a focus on correlations of preoperative HE4 levels with other prognostic and clinicopathologic factors.

Material and Methods
Patients
Serum was obtained from 94 patients with epithelial tumors of the ovary seen at the Department of Gynecologic Surgery and Gynecologic Oncology of Adults and Adolescents and at the Outpatient Clinic of Gynecologic Oncology, Pomeranian Medical University. Some of our patients were referred to us due to the presence of an ovarian tumor. Transvaginal ultrasound was performed in each case to confirm the diagnosis of a tumor prior to surgical intervention. Initially, we enrolled 65 patients but this group was restricted to 56 patients with the histologic diagnosis of an epithelial tumor of the ovary (we excluded patients with endometriosis and other benign non epithelial tumors). These patients were divided into two groups:

A) patients with ovarian cancer (n = 39);
B) patients with benign epithelial tumor (n = 17).

The second part of our patients (n = 38) were admitted to the hospital for second-look laparoscopy in ovarian cancer. Based on histopathological results of clippings we assigned these patients to groups:
C) with histologically confirmed disease free survival (n = 16);
D) with relapse or residual disease (n = 22).
In some patients of group D, relapse was confirmed with computed tomography (CT). All patients provided informed consent to participate in the study.

The groups (A, B, C, D) were compared as to concentrations of HE4 and CA125, and a detailed analysis of data in group A was done. Comparisons were performed in the whole group, in the subgroup with serous cancer, and in patients with FIGO III clinical stage. We analyzed HE4 and CA125 levels depending on the FIGO stage and cellular differentiation. Prognostic usefulness of HE4 was studied by correlating preoperative levels with the fact of disease-free survival (DFS) (no symptoms from the last chemotherapy session to relapse), time of DFS in months, relapse (only patients with complete remission, excluding those resistant to chemotherapy), two-year survival, and death during follow-up. All patients were treated at our Department between 2006 and 2008 and the final verification of clinical data concerning survival was done in January 2011.

**Serum collection**

Blood was collected at admission, one day before surgery, and centrifuged. Aliquots of serum were stored until analysis.

**Marker assays**

CA125. Assays were performed at the Laboratory of Hormones and Oncomarkers of the Department of Gynecologic Surgery and Oncology or at the Central Laboratory of the Independent Public Hospital of the Pomeranian Medical University. CA125 was determined in most patients visiting our Department or Clinic, using commercially available test kits from Abbott. The upper normal value was 35 U/ml.

HE4. Serum HE4 concentrations were measured using the HE4 EIA assay from Fujirebio Diagnostics. This solid-phase, non-competitive immunoassay based on the direct sandwich technique was carried out according to the manufacturer’s instructions. Appropriate controls were within the ranges provided by the manufacturer. The limit of detection (LoD) corresponded to the upper limit of the 95% confidence interval and represented the lowest concentration of the HE4 antigen that can be distinguished from zero. LoD of the HE4 kit was calculated to be < 2.5 pM and the normal upper limit was taken as 150 pM given by the manufacturer.

**Statistical analysis**

Statistics were performed using STATISTICA 9.1 PL software. Means were compared with the nonparametric Mann-Whitney U test or the Kruskal-Wallis test. Qualitative variables were analyzed with contingency tables and the chi-square test. Parametric linear correlations and nonparametric Spearman’s and Kendall’s rank correlations were studied. Receiver operating characteristic (ROC) curves were obtained and the area under the curve (AUC) was calculated with a 95% confidence interval. The sensitivity and specificity values for some clinico-pathologic parameters were determined in group A. AUC = 1 (or 0) corresponded to the ideal ROC curve and the test was deemed useful for AUC ≥ 0.5. The level of significance was taken as p < 0.05.

**Results**

Patient data of group A are presented in Table 1. All the patients from group A were treated with debulking surgery and taxane-platinum-based chemotherapy.

Group B included patients with benign cystadenomas:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Premenopausal</th>
<th>Postmenopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean age 44.2 [32-50]</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Mean age 60.64 [50-79]</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>BMI &gt; 30</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>BMI &lt; 30</td>
<td>12</td>
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<tr>
<td></td>
<td>FIGO I</td>
<td>10</td>
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<tr>
<td></td>
<td>FIGO II</td>
<td>5</td>
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<tr>
<td></td>
<td>FIGO III</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>FIGO IV</td>
<td>1</td>
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<tr>
<td></td>
<td>Grade 1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Grade 3</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Serous</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Mucinous</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Endometrioid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Clear cell</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Undifferentiated</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1. — Percentage of normal and elevated HE4 levels in serum of patients in group A (all, serous tumors, FIGO III) depending on cellular differentiation of the tumor.

*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).*

eight with serous tumors, six with mucinous tumors, and three with cystadenofibroma. The mean age in this group was 48.5 years. There were ten postmenopausal patients with a mean age of 62.3 years and mean HE4 concentration of 40.6 pM (9.39-101.9) and seven patients in reproductive age with a mean age of 27.8 years and mean HE4 concentration of 5.35 pM (2.9-10.6). Five mucinous tumors were diagnosed in postmenopausal patients – their mean HE4 concentration was 58.45 pM (10.41 – 101.9). For serous tumors, the mean HE4 concentration was 22.9 pM (2.9-85.8). While none of the patients in group B had HE4 concentrations above the normal range, 29.4% of them had elevated CA125 levels. There was a linear correlation (R = 0.527161, p < 0.05) and a nonparametric correlation (Spearman’s, Kendall’s, p < 0.005) between concentrations of CA125 and HE4 in group B.

Group C consisted of patients with histologic evidence of clinical remission. There were no tumor cells in histo-
logic specimens, no symptoms of disease, and no signs of any active neoplastic process in CT. All patients had previously undergone optimal surgery and standard chemotherapy based on platinum analogs. The mean age in this group was 53 years (24-75). In 11 patients with serous cancer, the mean HE4 concentration during remission was 32.59 pM (4.54-106.3). In the remaining patients (2 endometrial, 1 mucinous, 1 clear cell, and 1 transitional cell cancer), the mean HE4 concentration was 13.7 pM (4.6-36.9). None of the patients had elevated CA125 or HE4 levels, which was in strict correlation with their clinical status.

Group D comprised patients with previously diagnosed ovarian cancer, in whom laparoscopy and histology disclosed the presence of active disease or who presented with a mass in the pelvis or abdomen appearing during diagnostic imaging to be the sequella of the primary diagnosis. Mean age in this group was 56 years [43-75]. There were 19 cases of serous cancer. Differentiation grade of the cancer was G3 in 13 cases (mean HE4 = 361.6 pM (33.8-1558.9), mean CA125 = 1149 U/ml (23-6000) and G1 or G2 in eight cases (mean HE4 = 171.8 (7.9-700.4), mean CA125 = 158.7 U/ml (7.06-1155.48)). Taking 150 pM as the upper normal limit for HE4, we found that seven patients (33.3%) had elevated HE4 levels at the time of relapse or residual disease was diagnosed. This subgroup increased to 16 patients (76%) when we reduced the upper limit to 70 pM as suggested by Moore et al. [5]. As regards CA125, the upper normal limit of 35 U/ml was exceeded in 11 patients (52%).

Concentrations of HE4 and CA125 in the groups are presented in Tables 2 and 3. Significantly higher concentrations of HE4 and CA125 were found in patients with malignant epithelial tumors at the time of diagnosis.
Human epididymal protein 4 (HE4) is a novel biomarker and a promising prognostic factor in ovarian cancer patients.

At the time of relapse or disclosure of residual disease (group D), compared with patients with benign epithelial tumors (group B) or with ovarian cancer during complete clinical remission (group C). There was close to a significant difference between patients with FIGO II and FIGO III in group A as regards HE4 (p = 0.0578) and CA125 (p = 0.0741) (Table 4). Qualitative analysis evidently disclosed that higher concentrations of HE4 (above 150 pM) were associated with high clinical stage of the tumor. We could clearly see that HE4 levels correlated inversely with cellular differentiation. Poorly differentiated tumors demonstrated higher HE4 values in all groups and subgroups although statistical significance was disclosed only between G1 and G3 (Table 5). Univariate qualitative analysis revealed that normal HE4 concentrations in serum correlated with a highly mature form of cancer in the whole group and in the subgroup of serous cancer. The tendency was similar in high-grade cancer, albeit without statistical significance.

There was no correlation between serum concentrations of HE4 or CA125 and disease-free survival (DFS) (Table 6). Instead, there was a significant correlation between HE4 levels and duration of DFS (Figures 5 and 6). Concentrations of HE4 and CA125 correlated with relapse (Table 6): this was demonstrated by comparing means (Table 6) and carrying out qualitative analysis (Table 5). Table 6 and Figure 2 present the correlation between serum concentrations of HE4 and two-year survival or death during follow-up. Two-year survival was more frequent among patients with lower initial concentrations of HE4. Significance was noted when means were compared (242.72 pM in patients who survived vs 445.78 pM in patients who did not survive two years). The same was shown in qualitative analysis: 69.2% patients who survived two years had normal levels of HE4 whereas 70% patients who did not survive had HE4 levels above 150 pM. Kaplan-Meier curves revealed a statistically significant correlation between survival and

### Table 2. Concentrations in serum of HE4 and CA125 in ovarian cancer patients depending on stage and in patients with benign epithelial tumors.

<table>
<thead>
<tr>
<th>Group</th>
<th>A Mean range</th>
<th>B Mean range</th>
<th>p</th>
<th>A Mean range</th>
<th>C Mean range</th>
<th>p</th>
<th>A Mean range</th>
<th>D Mean range</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE4 [pM]</td>
<td>324.1 [2.5-1574.9]</td>
<td>26.1 [0.0-101.9]</td>
<td>&lt; 0.0001</td>
<td>324.1 [2.5-1574.9]</td>
<td>23.25 [0.0-106.33]</td>
<td>&lt; 0.0001</td>
<td>324.1 [2.5-1574.9]</td>
<td>289.5 [7.91-1558.9]</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Table 3. Concentrations in serum of HE4 and CA125 in ovarian cancer patients during remission and relapse and in patients with benign epithelial tumors.

<table>
<thead>
<tr>
<th>Group</th>
<th>B Mean range</th>
<th>C Mean range</th>
<th>p</th>
<th>B Mean range</th>
<th>D Mean range</th>
<th>p</th>
<th>C Mean range</th>
<th>D Mean range</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE4 [pM]</td>
<td>26.1 [0.0-101.9]</td>
<td>23.25 [0.0-106.33]</td>
<td>NS</td>
<td>26.1 [0.0-101.9]</td>
<td>289.5 [7.91-1558.9]</td>
<td>&lt; 0.000005</td>
<td>23.25 [0.0-106.33]</td>
<td>289.5 [7.91-1558.9]</td>
<td>&lt; 0.00005</td>
</tr>
</tbody>
</table>

### Table 4. Concentration in serum of HE4 and CA125 in ovarian cancer patients depending on FIGO stage.

<table>
<thead>
<tr>
<th>FIGO</th>
<th>WHOLE GROUP A</th>
<th>SEROUS ONLY</th>
<th>p</th>
<th>FIGO II</th>
<th>FIGO III</th>
<th>p</th>
<th>FIGO II</th>
<th>FIGO III</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE4 [pM]</td>
<td>46.07 (0.0-136.31)</td>
<td>46.82 (20.39-102.07)</td>
<td>NS</td>
<td>46.07 (15.64-76.59)</td>
<td>513.48 (0.0-1574.89)</td>
<td>p &lt; 0.0005</td>
<td>46.82 (20.39-102.07)</td>
<td>513.48 (0.0-1574.89)</td>
<td>p = 0.0578</td>
</tr>
<tr>
<td>CA125 [U/ml]</td>
<td>176.8 (15.81-500)</td>
<td>2859.21 (7.38-21442)</td>
<td>NS</td>
<td>176.8 (53.51-300.1)</td>
<td>2018.26 (49.22-22982)</td>
<td>p &lt; 0.005</td>
<td>2859.21 (7.38-21442)</td>
<td>2018.26 (49.22-22982)</td>
<td>p = 0.0741</td>
</tr>
</tbody>
</table>

There was no correlation between serum concentrations of HE4 or CA125 and disease-free survival (DFS) (Table 6). Instead, there was a significant correlation between HE4 levels and duration of DFS (Figures 5 and 6). Concentrations of HE4 and CA125 correlated with relapse (Table 6): this was demonstrated by comparing means (Table 6) and carrying out qualitative analysis (Table 5). Table 6 and Figure 2 present the correlation between serum concentrations of HE4 and two-year survival or death during follow-up. Two-year survival was more frequent among patients with lower initial concentrations of HE4 whereas 70% patients who did not survive had HE4 levels above 150 pM. Kaplan-Meier curves revealed a statistically significant correlation between survival and
HE4 levels (Figures 3 and 4). Using ROC curves to determine sensitivity and specificity of the method in group A, we found the following probabilities of occurring relapse as 0.753, DFS 0.483, two-year survival 0.754, and death 0.163 for HE 4. For CA125, the probabilities were: 0.778, 0.557, 0.675, and 0.667, respectively. We repeated this analysis for patients with serous tumors in group A and found the following areas under ROC curves for HE4: relapse 0.828, DFS 0.5, two-year survival 0.5, and death 0.862. The respective values in the case of CA125 as far as preoperative diagnosis of ovarian tumors is concerned [14, 15].

HE4 is one of the most interesting and promising novel biomarkers which may be useful for routine management of patients with ovarian cancer and in the differential diagnosis of pathologies of the adnexes [16, 17]. Huhti et al. [16] demonstrated that combined measurements of HE4 and CA125 in serum are much more valuable than determinations of each of these markers alone. For example, elevated levels of CA125 and HE4 in patients with a pathology of the ovary found at ultrasonography will suggest an ovarian malignancy whereas elevated serum level of CA125 and normal level of HE4 points more to endometriosis or to another benign lesion. Vice versa, elevated HE4 and normal CA125 may rather be associated with a tumor of a female sex organ other than the ovary (e.g. endometrial cancer) [16].

Most studies on HE4 in ovarian cancer carried out in recent years have focused on the diagnostic importance of this oncomarker and on prediction of ovarian cancer [3, 5, 9]. It was demonstrated that 32% of ovarian cancer cases reveal expression of HE4 and no expression of CA125. Logically, determination of both markers should markedly improve prediction of ovarian cancer [1].

Discussion

There are reports suggesting that various histologic subtypes of ovarian cancer are in fact different nosologic units revealing a different natural history of onset, clinical course, and prognosis. Biomarkers should be helpful in the determination of the precise biology of ovarian epithelial tumors and in consequence should have an impact on individualized therapies [13]. During recent decades, the sensitivity and specificity of some onco-markers in serum have been measured in patients with ovarian cancer. None of them performed any better than CA125 [1]. Unfortunately, elevated levels of this antigen are observed in malignancies of organs other than the ovary and in non-oncologic diseases, limiting the usefulness of CA125 as far as preoperative diagnosis of ovarian tumors is concerned [14, 15].

HE4 is one of the most interesting and promising novel biomarkers which may be useful for routine management of patients with ovarian cancer and in the differential diagnosis of pathologies of the adnexes [16, 17]. Huhti et al. [16] demonstrated that combined measurements of HE4 and CA125 in serum are much more valuable than determinations of each of these markers alone. For example, elevated levels of CA125 and HE4 in patients with a pathology of the ovary found at ultrasonography will suggest an ovarian malignancy whereas elevated serum level of CA125 and normal level of HE4 points more to endometriosis or to another benign lesion. Vice versa, elevated HE4 and normal CA125 may rather be associated with a tumor of a female sex organ other than the ovary (e.g. endometrial cancer) [16].

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Human epididymal protein 4 (HE4) is a novel biomarker and a promising prognostic factor in ovarian cancer patients.

June 2010, the American FDA (Food and Drug Administration) approved the HE4 test from Abbott for monitoring treatment of ovarian cancer patients. Shah et al. [12] reported that the sensitivity of differentiating ovarian cancer from benign lesions of the adnexes in patients at an average population attributable risk is 58.8% for CA125 and 61.8% for HE4. These figures rose to 79.4% and 80.4%, respectively, when ovarian cancer patients were compared with healthy individuals. A minor increase in sensitivity was observed for women at an increased population attributable risk. Hevrilesky et al. [4] demonstrated that the sensitivity of HE4 and CA125 for early diagnosis of ovarian cancer is 82.7% and 45.9%, respectively, and 92.5% and 58.5% respectively, for advanced cancer. The specificity of CA125 and HE4 was 98.2% and 86.4%, respectively. It was suggested that high sensitivity and specificity warrants the use of the test for screening of the general population [4]. Moore et al. [5] found that the sensitivity in ovarian cancer was 72.9% for HE4, 43.3% for CA125, and 76.4% CA125 and HE4 combined. Taking the upper normal limit for HE4 as 150 pM, we found above-normal levels of this marker in 43.6% of patients with freshly diagnosed ovarian cancer and in 38.1% with relapse. Normal levels of HE4 were confirmed in all patients with benign lesions and in patients during remission. However, when we set the upper normal limit at 70 pM after Moore et al. [5], HE4 was elevated in 63.2% of patients at diagnosis of ovarian cancer and in 76% of patients at relapse. One may rightly ask whether the upper normal limit given by the manu-
facturer conforms with the pattern of HE4 in healthy women and as a consequence, whether the sensitivity of the test for diagnosing ovarian malignancies is reduced at this limit. Based on our results, we believe that the normal range for HE4 awaits verification. Anastasi et al. [18] demonstrated that mean values of HE4 do not change in a statistically significant manner during the menstrual cycle, ranging from 37.5 pM to 46.6 pM in women before the age of 35 years and from 39.7 pM to 45 pM in older women, not exceeding 70 pM even when two standard deviations were added. Moore et al. [5] reported that the mean HE4 concentration was 58.6 pM in women with benign ovarian pathologies, 70.8 pM during postmenopause, and 51.2 pM during premenopause. Wang et al. [19] found 34.1 pM as the mean value for HE4 in healthy women and 39.1 pM in benign tumors. In the present study, the mean value for HE4 in patients with benign ovarian pathologies (group B) was 26.1 pM (95% CI = 8.5-43.8), again markedly below the assumed upper normal limit of 150 pM. In no patient of this group did the concentration of HE4 exceed 70 pM.

HE4 and CA125 are often used in specific algorithms aimed at early detection of ovarian cancer [3, 10, 20, 21] which take into account high or average population

Figure 7. — ROC curves for CA125 and HE4 in patients with malignant epithelial tumors of the ovary. A) relapse, B) death, C) disease-free survival, D) two-year survival.
attributable risk, hormone status (pre- or postmenopause), and diagnostic imaging findings. Risk of Ovarian Malignancy Algorithm (ROMA) developed by Moore et al. [5] in 2010 is one of these algorithms. According to these authors, its sensitivity is 94.3%, clearly above 84.6% for the previously used Risk of Malignancy Index (RMI) algorithm. An interesting study was done by Anderson et al. [21] who found that HE4 and CA125 levels in serum are able to predict ovarian cancer several years prior to the final diagnosis. Based on these results it appears that the greatest benefit could be expected for BRCA1 mutation carriers who are candidates for preventive salpingo-oophorectomy, in whom the timing of the operation could be optimized. We were able to demonstrate that HE4 is markedly increased in patients with high FIGO clinical stage. Normal levels of HE4 were observed in all patients with FIGO I and FIGO II, whereas above-normal levels were found in 73.9% of patients with FIGO III of group A and in 76.5% of patients with serous cancer. These differences were statistically significant. We also found that all patients with highly mature forms of cancer had normal levels of HE4 as opposed to the finding of above-normal levels in most patients with poorly differentiated cancer (Figure 1). In this case, the difference was also statistically significant. In line with our results, Van Gorp et al. [9] observed differences in HE4 concentrations depending on the stage of the tumor but failed to find statistically significant differences between grades 1, 2, and 3.

Anastasi et al. [22] reported in 2010 that HE4 may serve as an early biomarker of relapse but their study was done in eight ovarian cancer patients only, in whom complete remission was achieved after the first chemotherapy cycle. During follow-up, HE4 and CA125 levels were increased in five patients. The increase in HE4 preceded the increase in CA125 by five to six months. In the present study, the upper normal limit of 70 pM for HE4 was exceeded in 76% of patients with relapse of ovarian cancer (group D) while only 52% of them had above-normal levels of CA125. The basic objective of the present study was to determine whether one of the most promising novel biomarkers for ovarian cancer may also serve as a prognosticator. So far, there are only two studies discussing prognostic significance of human epididymis protein 4 in epithelial ovarian cancer [23, 24]. Results of Paek and colleagues [23] demonstrated that elevated serum HE4 level was related to the advanced stage of ovarian cancer and was a poor prognostic factor for PFS. Steffensen et al. [24] found HE4 to be a strong independent indicator of worse prognosis in epithelial ovarian cancer unlike CA 125 and HER2. Yamashita et al. [25] discussed the prognostic importance of HE4 expression in 137 female patients with pulmonary adenocarcinoma and found that expression heralded a poor prognosis and indicated a shortening of DFS and overall survival times. We observed that HE4 levels at diagnosis did not correlate with the prognosis of complete remission but were implicated in the duration of remission as seen from the Kaplan-Meier curves presented in Figures 3 and 4. The duration of complete remission was significantly longer in patients with normal HE4 concentrations in serum. In other words, relapse was less frequent in patients with normal HE4 levels. Elevated HE4 concentrations in group A were found in 57.1% of relapse cases and in 72.7% of patients with serous cancer (Figure 2). Similar figures were observed for two-year survival and moreover, there was a clear tendency to elevated HE4 levels in ovarian cancer patients who did not survive for at least two years from diagnosis. Normal HE4 concentrations in two-year survivors of group A were disclosed in 69.2% of the whole group and in 71.43% of serous cancer cases ($p = 0.0348$ and $p = 0.0575$, respectively). Using ROC curves we were able to confirm that HE4 performs as well or better than CA125 as a biomarker for prognostication in ovarian cancer.

It can be inferred from the literature on HE4 patterns and from our present results that HE4 may be a valuable supplement to CA125 for the monitoring of therapy in ovarian cancer and may also serve as an important prognostic factor. It is too early now for final conclusions until the results can be corroborated in a large group of patients. In spite of the growing knowledge on the potential applications of HE4 in clinical practice, there remain “gaps” which need to be filled in quickly.

References

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Summary

Purpose: Cervical cancer is a significant health burden in many countries. Long-term cost of care is still not well understood. We aimed to evaluate the long-term burden of illness and healthcare resource utilization associated with cervical cancer, cervical intraepithelial neoplasia (CIN) and genital warts from the care provider perspective. Method: We developed a health state-transition Markov model to portray the algorithm of treatment of stages of cervical cancer, CIN and genital warts by tracking a hypothetical lifetime cohort of 12-year-old girls. Costs in this study were unit cost; capital costs and labor costs were included in the unit cost for inpatients and out-patients. Results: The highest incidence of CIN and genital warts was observed in women aged 20-30 years old. For cervical cancer, the highest incidence was 45-55 years. Death rate was estimated at 2%, 8%, 84% and 94% in cervical cancer Stage IA1, IA2-IIA, IIB-IV A and IVB, respectively. The estimated mean direct cost per patient with cervical cancer Stage IA1, IA2-IIA, IIB-IVA and IVB, respectively. Cost for survival or death case was indifferent. The overall lifetime costs from the provider perspective were evaluated at 859.1 million Baht ($26.7 million US) per a cohort of 100,000 women which corresponds to approximately 4,244 million Baht ($131.8 million US) for the current number of Thai 12-year-old girls. Conclusions: HPV-related diseases impose health and cost burdens in Thailand. The national immunization programme to reduce this burden as well as further research to evaluate the impact is keenly expected.

Key words: Cost; Cervical cancer; HPV-related diseases.

Introduction

Cervical cancer is a significant health burden in many countries. Approximately 80% of all cases occur in developing countries and predominantly in people of low socio-economic status [1-4]. The death rate is approximately 50% worldwide [5].

In Thailand cervical cancer is identified as a key public health problem [6]. About 8,000 Thai women develop cervical cancer each year [7-9]. Age standardized incidence rate is 24.7 per 100,000 women [10].

A number of clinical studies have shown that the cause of cervical cancer is attributable to the infection with human papillomavirus (HPV) [11-15]. On basis of this evidence, effort is undergoing to develop vaccines that prevent HPV infection. Both of the licenced HPV vaccines can protect women from HPV type 16 and 18 that cause majority of cervical cancer. While quadrivalent vaccine can also protect against HPV type 6 and 11 which mostly cause genital warts [16]. Several mathematical models have been published to evaluate the cost effectiveness of new vaccines [17-27]. One of the important factors for the evaluation of cost-effectiveness is reliable long-term treatment cost. Many studies on cost estimation were carried out in Western countries [28-32]. A study on cost of care in Thailand was evaluated five years after completion of treatment, but did not represent lifetime costs of treatment [33].

Given less understanding in the long-term situation of HPV-related disease, we, therefore, aimed at evaluating the lifetime burden of illness and healthcare resource utilization associated with cervical cancer, cervical intraepithelial neoplasia (CIN) and genital warts from a care provider perspective.

Method

We developed a health state-transition Markov model [34, 35] to portray the algorithm of treatment of stages of cervical cancer, CIN and genital warts using TreeAge software (TreeAge Inc., Williamstown, MA, USA). In the model, we simulated a hypothetical lifetime cohort of 12-year-old girls until age 100 years (Figure 1). Costs in this study were unit cost (capital costs and labor costs were included in the unit cost) for in-patients and out-patients at King Chulalongkorn Memorial Hospital (Table 1). Costs were expressed in Thai Baht with the conversion rate of 35 Baht per US dollar. Probabilities at each chance node were systematically reviewed from the Thai healthcare context if available, otherwise data from other regions were used under substantial consensus by three authors with expertise in oncology gynecology (Table 2) [36-42].

Sensitivity analysis

Beta and Gamma distribution were applied to any probabilities and to unit costs in probabilistic sensitivity analysis, respectively. Monte Carlo simulation was performed to obtain the
Discussion

Treatment costs are essential for healthcare policy authorities in assessing benefits such as HPV testing [23] or the national immunization program [43-45]. To our knowledge this was the first attempt to portray lifetime information concerning cervical cancer, CIN and genital wart patients in terms of burden of illness and healthcare costs.

Results

Model validation

The incidence rate according to the model was 24.3/100,000 which is comparable to the incidence rate given by the National Cancer Institute of Thailand of 24.7/100,000 [10]. The cervical cancer mortality rate from the model was 7.99/100,000 which is slightly more than the crude death rate given by the Ministry of Public Health of 5.2/100,000 for the year 2007.

Results from model

Estimated number of HPV related cases and death

Our calculation suggested that in a lifetime cohort of 100,000 women, there would be 56,621 women with HPV-related disease including genital warts, CIN1, CIN2/3 and cervical cancer, of which 632 would die from cervical cancer (Table 3).

Estimated individual cost of treatment

An individual lifetime cost of treatment is shown in Table 4. Minimum and maximum boundaries are also displayed, using the Monte Carlo simulation technique. Costs per cervical cancer death seem to be indifferent from cost per case.

Estimated lifetime cost of treatment

Overall treatment costs of HPV-related diseases were approximately 859.1 million Thai Baht per a lifetime cohort of 100,000 women. The trend was to increase rapidly from the age of 12 years and peaked at the age of 30 years. After age 30 years, this burden was dominated by cervical cancer treatment costs (Figure 2).

Table 1. — Unit cost for treatment of genital warts, CIN and cervical cancer.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Unit cost (Baht)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical treatment of warts</td>
<td>3348.24</td>
</tr>
<tr>
<td>Surgical treatment of warts</td>
<td>5941.56</td>
</tr>
<tr>
<td>Cryotherapy</td>
<td>638.10</td>
</tr>
<tr>
<td>Conization</td>
<td>33,805.70</td>
</tr>
<tr>
<td>TAH</td>
<td>39,842.29</td>
</tr>
<tr>
<td>Radical Hysterectomy</td>
<td>101,830.80</td>
</tr>
<tr>
<td>Vaginectomy</td>
<td>39,156.97</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>270,857.28</td>
</tr>
<tr>
<td>Radiation</td>
<td>52,575.45</td>
</tr>
<tr>
<td>Palliative care</td>
<td>66,142.96</td>
</tr>
</tbody>
</table>

Table 2. — Selected baseline values used in the model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base case</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual probability of death (all-causes)</td>
<td>0.001</td>
<td>[36]</td>
</tr>
<tr>
<td>10-14*</td>
<td>0.0134</td>
<td></td>
</tr>
<tr>
<td>95-99</td>
<td>0.8103</td>
<td></td>
</tr>
<tr>
<td>Annual incidence of cervical cancer</td>
<td>24.7 per 100,000</td>
<td>[10]</td>
</tr>
<tr>
<td>Annual incidence of CIN1</td>
<td>120 per 100,000</td>
<td>[37]</td>
</tr>
<tr>
<td>Annual incidence of CIN 2/3</td>
<td>80 per 100,000</td>
<td>[37]</td>
</tr>
<tr>
<td>Annual incidence of genital warts</td>
<td>231 per 100,000</td>
<td>[31]</td>
</tr>
<tr>
<td>5-year cancer survival</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IA1 (survival of recurrence)</td>
<td>94.3%</td>
<td>[38]</td>
</tr>
<tr>
<td>Stage IA2, IB, IIA (survival of recurrence)</td>
<td>83.3%</td>
<td>[39]</td>
</tr>
<tr>
<td>Stage IIB-IVA (survival of recurrence)</td>
<td>67.6%</td>
<td>[41]</td>
</tr>
<tr>
<td>Stage IVB</td>
<td>22%</td>
<td>[42]</td>
</tr>
</tbody>
</table>

* Calculated in 5-year age categories, only lowest, middle and highest age groups are shown.
A study in Italy also reported similar findings, although CIN1 (5,381 Thai Baht) to CIN2/3 (49,933 Thai Baht).

We imposed nearly one-third of cervical cancer costs. In 12-year-old girls (Figure 2), and, the lifetime monetary burden of 129.6 million Thai Baht per cohort of 100,000 12-year-old girls (Table 4), an estimation of 3,585 Thai Baht per wart case (Table 4), and in younger women with the peak at age 22 years. The cost of treatment per individual case death (Thai Baht).

Table 3 shows more than a 9-fold increase in cost from our model. Cost of treatment for individual cases and death (Thai Baht).

Our study estimated 36,150 wart cases per a cohort of 100,000 12-year-old girls (Table 3). The incidence started in younger women with the peak at age 22 years. The cost estimation of 3,585 Thai Baht per wart case (Table 4), and, the lifetime monetary burden of 129.6 million Thai Baht per cohort of 100,000 12-year-old girls (Figure 2), imposed nearly one-third of cervical cancer costs.

Table 4 shows more than a 9-fold increase in cost from our model. Cost of treatment for individual cases and death (Thai Baht).

The difference was not as large as in our study [46, 47] (Table 5).

As expected, this study also showed increasing cost of care with increasing severity of cervical cancer (Table 4). These estimates were consistent with figures reported by Ricciardi et al. [32] in 2009 and Arveux et al. [28] in 2007 (Table 5). In those studies, there was a 75% increase in treatment costs for Stage II compared to Stage I disease, whereas our study showed more than a twofold increase in such stages (Table 4).

Our estimated costs of treatment were substantially less expensive when compared to other countries (Table 5). These might not be due to differences in physician practices but may be dominated by the difference in cost of living. However, this expenditure appeared to be inadequate in relation to our average family income of 226,320 Thai Baht per year [48]. According to the National Statistic Office in Thailand, there were 494,053 individual 12-year-old girls, which accounted for our estimated cases of 279,738 HPV-related diseases as well as corresponding to 4,244 million Thai Baht for direct medical care costs.

It should be noted that there are some limitations of this study. First, cost information was achieved from the unit cost of King Chulalongkorn Memorial Hospital, a tertiary government teaching university hospital. This may be an under-estimation when compared to private hospitals or an over-estimate when compared to secondary or primary hospitals. However, we used the Monte Carlo simulation to display the spread of expected values in terms of minimum and maximum.

Second, due to the lack of country information on age-specific incidence of genital warts and CIN, an alternative approach was to adopt previous studies from other countries. Over- or under-estimation of the true incidence of diseases may have occurred.

Lastly, diagnosis costs, follow-up costs, indirect costs and psychological costs were not taken into account. Therefore, from a provider perspective, all costs in this study should be considered as the lower boundary of the overall costs. Estimates from this study may lay a baseline for further study of cost-effectiveness analysis.

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References


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Laparoscopic surgery compared to traditional abdominal surgery in the management of early stage cervical cancer

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2Pathology, Akdeniz University School of Medicine, Antalya (Turkey)

Summary
The purpose of the study was to compare laparoscopic total radical hysterectomy with classic radical hysterectomy regarding parametrial, and vaginal resection, and lymphadenectomy. Methods: Laparoscopic or laparotomic total radical hysterectomy with advantages and disadvantages was offered to the patients diagnosed as having operable cervical cancer between 2007 and 2010. Lymph node status, resection of the parametra and vagina, and margin positivity were recorded for both groups. Data were collected prospectively. Statistical analysis was performed with the SPSS statistical software program. Results: Totally, 53 cases had classical abdominal radical hysterectomy and 35 laparoscopic radical hysterectomy, respectively. Parametrial involvement was detected in four (11.4%) cases in laparoscopic radical surgery versus nine (16.9%) in laparatomic surgery. All the cases with parametrial involvement had free surgical margins of tumor. Also there were no significant statistical differences in lymph node number and metastasis between the two groups. Conclusion: There is no difference in anatomical considerations between laparoscopic and lapararadical surgery in the surgical management of cervical cancer.

Key words: Cervical cancer; Laparoscopy; Radical hysterectomy.

Introduction
Early stage of cervical cancer except IB2 is treated by radical hysterectomy plus pelvic and/or paraaortic lymphadenectomy. Classic abdominal radical surgery is the standard of care. Laparoscopic total radical hysterectomy has been applied to cervical cancer management for less than 20 years and fewer than 2,000 cases have been reported. Up to now, gynecologic oncology centers have reported their experience on laparoscopic radical abdominal hysterectomy from different countries [1, 2]. Also this is the first large study about laparoscopic radical hysterecomy compared to open abdominal radical hysterectomy from Turkey.

Adequate radical resection of parametrial and paravaginal tissue, and lymph node status are the most important aspects of radical cervical cancer surgery. Also it is an important question to know whether it is possible to remove parametrical-vaginal tissue as in classic surgery. In the literature there are a few studies comparing the two types of surgery and most of these studies are retrospective analyses [3-6]. On the other hand, parametrial resection is only defined theoretically. Thus we compared total radical laparoscopic hysterectomy with classic radical hysterectomy prospectively and especially resection of the lateral and deep parametrium.

Materials and Methods
This study was prospectively designed to compare two types of surgical techniques according to patient preferences. The cases diagnosed as having cervical cancer by cervical biopsy and who underwent laparoscopic or classic radical hysterectomy between 2007 to 2010 were enrolled in the study. The research project was approved by a suitably constituted Ethics Committee of the institution. FIGO Stages I-IIA, except IB2, cases in good health status were submitted to surgical treatment. Staging of the patients was performed with gynecologic examination under anesthesia. All the cases were informed about the advantages and disadvantages of the two types of surgeries. All cases were operated according to their preference for laparoscopy or laparotomy. Intraoperative and postoperative complications, surgical margins, and lymph node status were recorded. All the cases game written informed consent. The women accepted as inoperable during gynecological examination under anesthesia (Stage IB or higher) or submitted to surgery but discovered as inoperable during the operation (metastatic nodes discovered by frozen section or macroscopic parametrial invasion or intraabdominal disease) were excluded from the study. Data were collected prospectively.

Technique
All patients had bowel preparation, started prophylactic antibiotic (cephoxitin) medication one hour before surgery, and were on low-molecular weight heparin for two weeks beginning from the day before surgery. Two types of surgery were performed using the same principle and technique in the intraabdominal part. First of all, the patients were evaluated looking for parametrial invasion, extracervical or intraabdominal disease for operability. Abdominal radical hysterectomy was performed as described by the classic technique.

In the laparoscopy group, patients were placed in the lithotomy position. After dilatation of the cervix with a Hegar dilator (no. 8), an uterine manipulator (Clermont-Ferrand, Karl Storz, Tuttingen, Germany) was inserted. An infraumbilical 1 cm vertical or transverse incision was performed and a Verres needle was inserted for the created pneumoperitoneum. After the pneumoperitoneum was created a 10 mm trocar and 0 degree laparoscope were introduced. Under direct vision of the laparoscope, two 5 mm and one 10 mm trocars were inserted as seen.
in Figure 1. Intraabdominal pressure was adjusted as 12-13 mmHg, and then patients were placed in a head-down position at 25-30 degrees. The abdominal cavity including the pelvis was evaluated carefully for extracervical metastatic disease. Parapectal and paravesical spaces were developed by incision of the lateral peritoneum and round ligament. Pelvic lymphadenectomy including the bilateral common iliac, external iliac, internal iliac and obturator lymph nodes was performed. In cases with tumor diameter 3-4 cm, paraaortic lymphadenectomy until the left the renal vein was added. Lymph nodes were removed with an endobag. After completion of lymphadenectomy, the posterior peritoneum was cut. Bilateral ureters were dissected and lateralized until the pelvis. The anterior peritoneum was incised and the bladder was separated from the cervix. The uterine artery and superior vein were cut and ureteral dissection was completed without scrapings the ureter and cutting the vesicouterine ligament until the bladder. Sacrouterine/recuteouterine ligaments were coagulated and cut with ligasure or bipolar cutting forceps. After that, deep uterine veins were coagulated or clipped and cut at the lateral pelvic wall (Figure 1). The last step was coagulating and cutting the paravaginal tissue and vagina. Specimens were removed vaginally and the vaginal cuff was closed by the abdominal or vaginal route.

Statistical analysis were performed using the SPSS 18 statistical software program. Independent-samples t-test was used for statistical analysis and p < 0.05 was accepted as statistically significant.

Results

Totally 88 patients were submitted to radical surgery. Fifty-three (60.2%) of these patients had classical radical hysterectomy and 35 (39.7%) cases laparoscopy, respectively. Mean age was 51.4 in the laparatomy group and 49.2 in the laparoscopy group. There were no statistical differences between the two groups for other demographic characteristics (Table 1). Mean lymph node number did not differ between the groups, although metastases to lymph nodes were common in the laparatomy group but not differ between the groups, although metastases to lymph nodes were common in the laparatomy group but without statistical significance (Table 2). Parametrial involvement was also the same (16.9% vs 11.4% in the laparoscopy group, p = 0.478). All cases had free surgical margins of tumor. Adjuvant treatment (chemoradiation-CT/RT) was given to 17 (32.0%) patients in the laparotomy group and seven (20.0%) in the laparoscopy group (p = 0.159). Totally 13 (24.5%) units of redblood cells (RBCs) were transfused in the laparotomy group and eight (23.5%) in the laparoscopy group with no statistically difference. Mean operative time was longer in the laparoscopy group (190 minutes vs 250) (p = 0.001). There was no difference in total complication rates (Table 1). Postoperative intraabdominal bleeding developed in one case of the laparoscopy group which required laparotomy. This case had hypertension, diabetes mellitus and chronic hepatitis B infection. There were no bleeding sites during laparatomy. The patient was discharged post-operatively without any problems and is well without any complications now. There were three recurrences in the laparatomy group and none in the laparoscopy group. There was no difference between the two groups for mean follow-up period (Table 2).

### Table 1. — Characteristics of the cases.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Laparatomy (n = 53)</th>
<th>Laparoscopy (n = 35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>51.4 (31-79)</td>
<td>49.2 (28-60)</td>
<td>0.487</td>
</tr>
<tr>
<td>Mean gravida</td>
<td>3.4 (1-8)</td>
<td>3.8 (1-9)</td>
<td>0.738</td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>35 (66.0%)</td>
<td>26 (74.2%)</td>
<td>0.417</td>
</tr>
<tr>
<td>Non-squamous</td>
<td>18 (33.9%)</td>
<td>9 (25.7%)</td>
<td></td>
</tr>
<tr>
<td>Mean operative time</td>
<td>190 (90-310)</td>
<td>250 (180-500)</td>
<td>0.000</td>
</tr>
<tr>
<td>Transfusion</td>
<td>13 (24.5%)</td>
<td>8 (22.8%)</td>
<td>0.892</td>
</tr>
<tr>
<td>Parametrial invasion</td>
<td>9 (16.9%)</td>
<td>4 (11.4%)</td>
<td>0.478</td>
</tr>
<tr>
<td>Postoperative complications</td>
<td>3 (5.6%)</td>
<td>2 (5.7%)</td>
<td>0.992</td>
</tr>
<tr>
<td>Infection</td>
<td>1 (1.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self catheterization</td>
<td>2 (3.7%)</td>
<td>1 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. — Lymph node status, adjuvant treatment and recurrence.

<table>
<thead>
<tr>
<th>Region</th>
<th>Laparatomy (n = 53)</th>
<th>Laparoscopy (n = 35)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pelvic nodes</td>
<td>28.2 (10-46)</td>
<td>26.7 (12-72)</td>
<td>0.146</td>
</tr>
<tr>
<td>Metastasis</td>
<td>9 (16.9%)</td>
<td>3 (8.5%)</td>
<td>0.266</td>
</tr>
<tr>
<td>Mean paraaortic nodes (n = 37)</td>
<td>17.9 (4-37)</td>
<td>14.1 (10-18)</td>
<td>0.09</td>
</tr>
<tr>
<td>Adjuvant treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT/CT</td>
<td>17 (32.0%)</td>
<td>7 (20.0%)</td>
<td>0.159</td>
</tr>
<tr>
<td>Recurrence</td>
<td>3 (5.6%)</td>
<td>–</td>
<td>0.156</td>
</tr>
<tr>
<td>Mean follow-up (months)</td>
<td>30.3 (5-51)</td>
<td>29.0 (5-50)</td>
<td>0.382</td>
</tr>
</tbody>
</table>

### Discussion

Although classical abdominal radical hysterectomy is still considered the standard treatment of early-stage cervical cancer, laparoscopic radical abdominal and assisted vaginal radical hysterectomy have been widely accepted with increasing experience and technology. The major advantages of minimally invasive surgery are less postoperative pain and bleeding, a shorter recovery time and hospitalization or lower rate of postoperative infection [7]. In addition, it is possible to detect details of pelvic structures as magnified features of laparoscopy. Parametrial, paravaginal tissues, vascular, lymphatic structures of the pelvis can be identified easily. Thus dissection of parauterine tissue and lymph nodes may be easier than laparotomy.

Surgical management of cervical cancer in early stages requires radical resection of parametral and paravaginal tissues, and upper part of the vagina. In type III radical hysterectomy according to the Piver classification [8], the parametrium should be removed as much as possible laterally. Also in type C radical hysterectomy according to the Querleu and Morrow classification [9], the vascular area (C1) or nerves (C2) of the parametrium should be removed according to stage of disease. There are some questions about removing all tissue as defined original figure. Deep uterine veins are an important land mark for radical surgery as seen in the study figure. Up to now, there has been no showing resection of the parametrium...
Laparoscopic surgery compared to traditional abdominal surgery in the management of early stage cervical cancer

In this respect to our knowledge. In the many studies reported in the literature, this phase of the operation is defined just theoretically. In the current study, we performed lateral and deep parametrial resection. Also adequate vaginal resection is important for radicality. Vaginal resection was adequate for all the cases in the study.

Lymph node dissection is an important crucial step in radical surgery of cervical cancer. It may be therapeutic and prognostic. There are many studies in the literature reporting that laparoscopic pelvic and paraaortic lymphadenectomy can be performed as laparotomy [3-5] with more nodes in the laparoscopy group [10]. Also the number of lymph nodes is important for adequacy of lymphadenectomy. Twenty lymph nodes are accepted as the cutoff limit for pelvic or paraaortic lymphadenectomy [11, 12]. There are a few comparative reports having 20 or more lymph nodes yielded during laparoscopic total radical hysterectomy [3, 5, 10, 13]. In our study mean lymph node number for pelvic lymphadenectomy was 26.7. Metastatic lymph node number was more frequent in the laparotomy group but without statistical significance. This may due to more patients having larger tumors (3-4 cm) in the laparotomy group or may be an incidental finding. Level of paraaortic lymph node dissection is hardly a debated issue. Some centers do paraaortic lymph node dissection under the inferior mesenteric artery. In our practice for laparoscopy and laparotomy, we do paraaortic node dissection until the left renal vein. We believe if there is any indication for paraaortic lymphadenectomy (larger tumor, pelvic nodal metastasis and parametrial invasion), it should be performed until the left renal vein.

Recurrence rate indicates success of the treatment modalities. It affects the survival of patients directly. In our study, recurrence rate was 5.6% in the laparotomy group and none in the laparoscopy group. In the literature recurrence rate for laparoscopy varies between 0 and 16.3% [3, 14, 15]. Also in our study there were no patients with positive surgical margins in either group. Ghezzi et al. [16] reported 6.0% positive parametrial margins in the laparoscopic radical hysterectomy group and 6.2% in the laparotomy group with no statistical differences between the two groups.

Major intraoperative complications of laparoscopic radical hysterectomy are reported in different ranges. Rates of these complications are no different from open abdominal surgery [5, 6]. The major intraoperative complications are vessel, bladder, rectal and ureteral injuries. Vessel injuries are more important and need emergency intervention by laparoscopy or laparotomy. A few cases need management with laparotomy. Bladder and rectal injuries are the other frequent organ complications of laparoscopic radical surgery. Intraoperative detection of these complications are mandatory. If they are not diagnosed intraoperatively, catastrophic results including septic shock may be unavoidable. Bladder and rectal injuries are usually managed by laparoscopy during surgery. Rarely is laparotomy performed. Campos et al. [17] reported that four (13.7%) cases had major intraoperative complications including one bladder, one ureteral and two rectal injuries. They managed ureteral and bladder injuries by laparoscopy. One rectal injury was corrected via the vaginal route. The last case with rectal injury was managed by open loop colostomy. Li et al. [5] reported eight (8.8%) cases with intraoperative complication as four iliac vain and four bladder injuries. They performed laparotomy for one iliac vain injury and one cystotomy case. In the study, there were no intraoperative complications in either group.

The most frequent postoperative complication of radical hysterectomy – either laparotomy or laparoscopy – is bladder dysfunction. There was no difference in the two types of surgery in our study. Bladder dysfunction usually relieves with time. Also, Li et al. [5] reported that the urinary retention occurrence rate was 32.2% with no difference using open radical hysterectomy. Chen et al. [15] found that 15.9% of patients had voiding dysfunction after one year of laparoscopic radical abdominal hysterectomy. Intraabdominal bleeding is a life threatening postoperative complication of radical surgery. Laparoscopic surgery reduces blood loss compared to open surgery and postoperative intraabdominal bleeding is encountered rarely. However, there was no difference in
blood transfusion rate in our study. This result arises from one case having postoperative intraabdominal bleeding which required transfusion of four units of RBCs in the laparoscopic group. This case had hypertension, diabetes and chronic hepatitis B infection. Laparotomy was performed and no detected bleeding area was diagnosed. Intraabdominal hematomas were evacuated and a drain was put in. No postoperative infection or thromboembolic process occurred in any case in the study.

The major disadvantage of laparoscopic surgery is operative time. Mean operative time for radical laparoscopic hysterectomy is reported as between 92 and 420 minutes [18, 19]. Also it is longer in radical laparoscopic surgery than laparotomy in the comparative studies with 196 to 371 minutes [3-6, 10]. Our result is comparable with the literature but longer than laparotomy in the laparoscopic group (250 vs 190 minutes). Operating time may be reduced by increasing experience, a standardized technique and using new technologies such as pulsed cautery.

Postoperative adjuvant treatment is given to cases having major risk factors as parametrial involvement, metastatic lymph nodes, positive surgical margins and deep stromal or serosal invasion. Totally 32% of laparotomy and 20% of laparoscopy groups had adjuvant therapy.

Conclusion

Laparoscopic total radical hysterectomy with advantages of minimally invasive surgery allows resection of the lateral and deep parametrium, vagina and lymph nodes as classic radical hysterectomy. Although this study was a prospective case control study, it is was not a randomized study. Prospective randomized studies are needed.

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Labeling of microvessel density, lymphatic vessel density and potential role of proangiogenic and lymphangiogenic factors as a predictive/prognostic factors after radiotherapy in patients with cervical cancer

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Summary

Introduction: Angiogenesis, formation of a new blood vessel from the existing vascular network, is essential for tumor growth, progression and metastasis. Vascular endothelial growth factor (VEGF) has been identified to be one of the most important factors of angiogenesis. VEGF-C, a novel member of the family, is a relatively specific lymphangiogenic growth factor. It is tempting to suggest that cervical cancer is one of the most common malignancies in a woman’s life. Its prognostic factors are tumor stage, lymph node status, histologic type, level of hemoglobin. However, little is known about the predictive/prognostic significance of angiogenesis in cervical cancer. Objective: This prospective study is an attempt to evaluate serum VEGF-A, VEGF-C, microvessel density (MVD), and lymphatic vessel density (LMVD) in cervical cancer and the correlations with clinicopathologic features. Material and Methods: Blood samples were collected from 58 patients affected by FIGO I-IV stage cervical cancer, who were admitted to the Department of Oncology and Brachytherapy Collegium Medicum in Bydgoszcz of Nicolaus Copernicus University. Serum VEGF-A/VEGF-C concentrate was determined by means of a quantitative sandwich enzyme immunoassay (ELISA). All tumor samples were taken from cross section during the first brachytherapy. Then they were examined by immunohistochemical studies with podoplanin antibody and anti-CD31 antibody. The present analysis was used to evaluate MVD and LMVD. Results: The median serum VEGF-A was 734.76 pg/ml (range 86.39 pg/ml - 2200.00 pg/ml), and VEGF-A was only correlated with after treatment hemoglobin concentration (p = 0.046, R = –0.3450). The median serum VEGF-C was 145.72 pg/ml (range 131.08 - 233.60 pg/ml). Serum VEGF-C levels measured in patients were associated with primary tumor size. We observed significantly higher serum VEGF-C in localized disease (FIGO I, II) in comparison to advanced tumors (232.44 pg/ml vs 152.45 pg/ml; p = 0.034). The median LMVD was 6.25 (range 3.5-10.0) and median blood vessel density was 12.5 (range 9.5-23.0). We found significantly higher lymphatic vessel density in patients with G1/G2 grade of differentiation than in those with G3 (9.93 vs 6.25; p = 0.0398). We observed a statistically significant correlation between MVD and LMVD; (p = 0.032). Conclusion: In conclusion, our study suggests that serum VEGF-A, VEGF-C, LMVD and MVD play an important role in tumor growth and progression in cervical cancer. Nonetheless, further studies are essential to explore the underlying mechanism.

Key words: Cervical cancer; Angiogenesis; VEGF; Microvessel density,

Introduction

Poland is among the European countries with one of the highest levels of cervical cancer, with as many as 3,345 new incidents and 1,819 deaths only in 2006 [1]. It affects young women with the incidence rate increasing with age; the highest risk age group is 45-55 years. The most frequent histopathologic type is plano-epithelial carcinoma in keratinizing or non-keratinizing forms, accounting for around 95% of malignant cervical cancers [2]. The risk of involving lymph nodes accounts for 1-5% in FIGO Stage IA, 20% in IB and up to 45% in advanced tumors (FIGO III-IV). The choice of treatment is dependent on prognostic factors such as tumor size, patient’s age and general condition, type and grade of tumor differentiation, invasion of lymph vessels or blood vessels, lymph node status and level of hemoglobin before treatment [3-5]. These results inspired a search for a new predictive factor which would help determine patients with high risk of metastasis to lymph nodes and/or relapse.

Angiogenesis is the process of the formation of a new blood vessel from the existing vascular network. It is essential for tumor growth, progression and metastasis. It plays a crucial role in many phenomena, both physiological and pathological, and it consists of many stages [6, 7]. Vascular endothelial growth factor (VEGF) participates in the increase in vascular permeability, edema, extravascular fibrin deposits, the formation of vascular malformations, angiogenesis, arteriogenesis, fibrogenesis and lymphangiogenesis [7-9]. One of its isoforms is VEGF-C, which was first described in 1996 [9], and
increased VEGF-C expression is observed in hematological diseases and malignancies.

Lymphangiogenesis coexists with the processes of angiogenesis, for example as a result of VEGF-C stimulation, which in turn stimulates the processes of angiogenesis by the receptor VEGFR-2 and/or stimulates the processes of lymphangiogenesis by the receptor VEGFR-3. Factors inducing and inhibiting angiogenesis are known but still little is known about the processes of lymphangiogenesis [10].

This prospective study was an attempt to evaluate the influence of serum VEGF-A, VEGF-C, microvessel density (MVD) and lymphatic vessel density (LMVD) on the result of tumor treatment in women with cervical cancer, and the correlation of these factors with clinicopathologic features.

Material and Methods

Patients

The research was carried out in a group of 58 patients qualified for brachytherapy for cervical cancer. All women were patients of the Department and University Hospital of Oncology and Brachytherapy Collegium Medicum in Bydgoszcz of Nicolaus Copernicus University in Torun between May 2005 and November 2006. HDR was applied in 33 women and LDR in 25 women. Combined treatment consisting of brachytherapy with surgical removal of reproductive organs was administered in 33 patients in Stage I-IIA. In 17 women in Stage IIB, III and IV basic treatment was radiotherapy (brachytherapy + external beam radiotherapy). Six women were additionally treated systemically. Eight patients underwent only brachytherapy due to coexisting diseases or distant metastasis. In two out of these patients palliative chemotherapy was applied due to the coexistence of another tumor. The average age of the patients was 56, the youngest was 36 and the oldest 87. The first follow-up visit took place six weeks after completing the treatment, and the next were scheduled for every three months. Seven patients were lost to treatment as they never turned up for a follow-up visit. Their case histories are unknown and as such they were excluded from the analysis of their response to treatment. The period of follow-up observation ranged between two and 31 months.

Serum assay

Blood samples were collected from the basic vein on an empty stomach, once, at seven o’clock in the morning before the start of the treatment. VEGF-C and VEGF-A were determined by means of a sandwich enzyme immunoassay for quantitative detection of human antibodies (ELISA, Bender MedSystem detection kit). The blood was delivered to the Diagnosis Laboratory of the Centre of Oncology in Bydgoszcz. After a 10-minute centrifugation at 3000 RPM, the blood serum was frozen and stored at –70°C until examination. All serum VEGF-A/VEGF-C analyses were performed at the same time and in the same batch. Prior to the assay, the serum was thawed at room temperature and all reagents were prepared. The tests were performed with human VEGF-A/VEGF-C (ELISA). According to the test protocol, 58 sera of cervical cancer patients were assayed, two for each patient. The intensity of staining in the wells was measured immediately after the reaction had stopped in the spectrophotometer at wavelength lambda = 450 nm and at 600 nm as a referential length.

Intratumoral angiogenesis and lymphangiogenesis

Tumor samples from the uterine cervix affected by cancer were taken during the first brachytherapy under brief general anesthesia. Tumor samples were put into a brine-filled container and delivered to the Tumor Pathology Department in the Centre of Oncology in Bydgoszcz. Tissue fragments were prepared by means of cross-section into 4 µm strips, transferred into buffered formalin (citrate buffer) and stored in paraffin, where they remained until assaying. Xylene and 3% perhydrol solution were applied in the process of deparaffining. Next, the samples were incubated for 10 min in 0.5% pepsin solution at 37°C to expose antigen determinants. The next stage was incubation of rabbit serum with an antibody directed against podoplanin (anti-human podoplanin. Mouse monoclonal antibody; clone 4D5aE5E6BMS 1105 Bender MedSystem) coating on antigens of antibody. The samples were then washed in water and dehydrated and preserved by means of xylene and Canadian balm.

All slides were evaluated by two independent researchers by means of an optical microscope Olympus BX51 (Olympus Optical Co. Ltd., Japan). At a magnification 10x and 20x the so-called ‘hot spots’, areas of the highest density of the vessels, were subjectively chosen. The number of such areas was different in different slides, therefore from 1-3 hot spots were chosen and the mean was calculated. The photos were taken with a Color View 3u digital camera combined with the microscope.

Microvessel density in tissue sample

Test results included 52 slides because the samples of six patients were too small. The analyzed samples were reported as follows: in 38 there was no reaction with the antibody or a massive inflammatory component, which made the evaluation of blood vessels impossible; in three samples, a weak reaction with the antibody, and in four a reaction only in the big vessels. This precluded the identification of blood vessels in the samples and excluded them from further analysis. In each case the areas of the highest density of blood vessels were searched for. The rule to evaluate three hot spots was followed. Since in some samples there were fewer areas, the number of blood vessels in one, two or three areas was evaluated. Next, the mean of microvessel density in the tissue sample was calculated.

Lymphatic vessel density in tissue sample

Test results for lymphatic vessels also included 52 slides. In eight of the samples no stroma was observed; six samples were non-diagnostic, and in three there was no reaction, which excluded them from further analysis. In each case the areas of the highest density of blood vessels were searched for. The rule to evaluate three hot spots was followed as aforementioned.

Statistical analysis

Statistical analysis was carried out by means of computer software Statistica 6.0 (StatSoft, Inc.2001). The equality of continuous distributions with a normal distribution was calculated by means of the Kolmogorov-Smirnov test corrected by the Liliefors test, and the equality of variances was assessed with Levene’s test. Inter-group comparison of the variables whose distribution did not differ from the normal distribution was assessed by Student’s t-test for independent trials. For these variables which did not satisfy the condition of the equality of variances, Student’s t-test with a separate assessment of variances was carried out. Comparison of variables whose distribution did not satisfy the conditions of normality was made by means of Mann-Whitney U test for assessing independent trials.
The degree of correlation between continuous variables was measured with the Pearson linear correlation coefficient. To assess the dependence between variables in range scale and continuous variables, the analysis of regression was carried out by means of the General Regression Model (GRM) from Statistica 6.0 software package. The influence of selected parameters on progression-free survival was assessed by Cox’s proportional hazards model. Variables which in Cox-regression analysis gained statistical significance were included in the initial model. The risk of affecting lymph nodes and no response were assessed by means of the logical regression model. In order to do that, the quotients of chances and the correlating 95% confidence intervals were calculated.

In all statistical analyses $p < 0.05$ was taken as the border value for probability coefficient.

Results

The research was carried out on a group of 58 cervical cancer patients. A detailed characterization is given in Table 1. There were patients in all FIGO stages: 23 women in Stage I (39.7%), 18 women in Stage II (31%), and 13 women in Stage III (22.4%). Four patients were in Stage IV cancer, extending to the neighboring organs and/or with distant metastasis. The total follow-up observation period of the 58 patients ranged from 2-31 months (the mean follow-up observation period was 9.7 months). Out of 51 women 20 (39.2%) had a relapse of the cancer, 16 (31.3%) had local relapse, four (20%) had metastasis, including two with another cancer diagnosed. The mean time of the relapse was 8.2 months (range 1-23 months).

Detailed data concerning the age, VEGF-A, VEGF-C concentration in blood serum, MVD, LMVD and the level of hemoglobin (Hb) in blood before treatment are presented in Table 2. The median serum VEGF-C was 145.72 pg/ml (range 131.08 pg/ml to 232.44 pg/ml). Median serum VEGF-A was 734.76 pg/ml (range 86.39 pg/ml to 2200.00 pg/ml), and VEGF-A was only correlated with after treatment hemoglobin concentration ($p = 0.046$, $R = -0.3450$). Serum VEGF-A was not correlated with other parameters like tumor stage, histological grade, age, before treatment Hb concentration, or platelet count.

The dependencies between variables in the whole group were assessed by means of Pearson’s analysis. Due to small specification of the antibody to staining blood vessels (MVD) and no reaction in the majority of the analyzed samples, the assessment was possible in only 13 slides. In the analysis a statistically significant correlation was only observed between MVD and LMVD in the tumor ($p = 0.032$).

On applying the analysis of regression no differences were found between VEGF-C concentration in blood serum and FIGO stages ($p = 0.30801$, $R = 0.2850$). Similarly, no correlation was observed between LMVD and tumor stage ($p = 0.60585$, $R = 0.2421$).

**Relationship between VEGF-C concentration in blood serum, LMVD and tumor stage according to FIGO staging system**

In the present study, VEGF-C concentration in blood serum and LMVD were compared in the two groups with regard to stage of the disease: in the FIGO Stage I-II group and in the FIGO Stage III-IV group. A higher level of VEGF-C in blood serum was observed in early stages of the disease in comparison with advanced stages, where the level was lower ($p = 0.0354$). With regard to LMVD in the tumor no statistically significant differences were found between the two groups (Table 3).

**VEGF-C concentration in blood serum, LMVD and the grade of tumor differentiation**

The level of VEGF-C in blood serum and LMVD was compared depending on the grade of tumor differentiation in the two groups, G1-G2 vs G3 (Table 4). Higher LMVD was observed in patients with grade 1 and 2 compared to grade 3, where the number of vessels was smaller, and the result was statistically significant ($p = 0.0398$) (Table 4).

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**Table 1. Patient characteristics.**

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>Before treatment</th>
<th>6 weeks after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 58</td>
<td>N = 37</td>
</tr>
<tr>
<td>Stage I</td>
<td>33 (50%)</td>
<td>21 (60%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>18 (28%)</td>
<td>13 (38%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>13 (22%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>4 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Histological grade</td>
<td>n = 31</td>
<td>n = 19</td>
</tr>
<tr>
<td>G1</td>
<td>2 (6%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>G2</td>
<td>21 (68%)</td>
<td>13 (61.9%)</td>
</tr>
<tr>
<td>G3</td>
<td>8 (26%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>Treatment:</td>
<td>n = 58</td>
<td>n = 36</td>
</tr>
<tr>
<td>Brachytherapy + Operation</td>
<td>33 (56.8%)</td>
<td>24 (72.7%)</td>
</tr>
<tr>
<td>Brachytherapy + External Beam Therapy</td>
<td>17 (29.3%)</td>
<td>9 (52.9%)</td>
</tr>
<tr>
<td>Brachytherapy alone</td>
<td>8 (13.7%)</td>
<td>3 (37.5%)</td>
</tr>
</tbody>
</table>

**Table 2. Differences in levels of circulating VEGF-C, MVD, LMVD and clinicopathological parameters in patients with cervical cancer.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (36-87 years)</td>
<td>58</td>
<td>56.12 ± 13.43</td>
</tr>
<tr>
<td>Hemoglobin levels before treatment (g/dl)</td>
<td>58</td>
<td>12.14 ± 1.84</td>
</tr>
<tr>
<td>Serum VEGF-C (pg/ml)</td>
<td>55</td>
<td>209.7 ± 165.82</td>
</tr>
<tr>
<td>Serum VEGF-A (pg/ml)</td>
<td>37</td>
<td>848.46 ± 568.06</td>
</tr>
<tr>
<td>Lymphatic vessel density (LMVD)</td>
<td>34</td>
<td>20.00 ± 6.30</td>
</tr>
<tr>
<td>Microvessel density (MVD)</td>
<td>13</td>
<td>16.68 ± 8.86</td>
</tr>
</tbody>
</table>

**Table 3. Correlation of VEGF-C and LMVD with FIGO stage in patients with cervical cancer.**

<table>
<thead>
<tr>
<th>FIGO stage</th>
<th>VEGF-C concentration (pg/ml)</th>
<th>Lympathic vessel density (LMVD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, II</td>
<td>33 232.44 ± 188.21 0.0354</td>
<td>21 8.95 ± 6.70 0.3280</td>
</tr>
<tr>
<td>III, IV</td>
<td>13 152.45 ± 60.63</td>
<td>12 6.70 ± 7.94</td>
</tr>
</tbody>
</table>

Black a value of $p < 0.05$.

---
In terms of VEGF-C in different grades of tumor differentiation, the differences were not statistically significant (p = 0.2458).

Analysis of selected factors influencing the response to cervical cancer treatment

Higher LMVD was observed in patients with grade 1 and 2 compared to grade 3, where the numbers of vessels were smaller, and in 51 patients who reported for follow-up examination, the influence of the factors of angiogenesis, lymphangiogenesis, patients' age and the level of Hb on the result of treatment was assessed. It was measured by the grade of tumor regression, which was assessed based on clinical and diagnostic examinations. Six weeks after the termination of the treatment complete response (CR) was confirmed in 37 patients, partial response (PR) was observed in four patients, and ten patients had progression of disease (PD). During the first follow-up visit no differences were found in the aforementioned parameters in groups CR vs PR+PD.

The following examinations of the patients brought similar observations, after three, six and nine months, respectively. The only exception was the level of Hb, which in the sixth month after treatment was statistically lower in the group with PD than in the group with CR. (p = 0.0336). Due to the small sample size or no patients with a PR, the assessment of the response to treatment was impossible for the next observations.

In the next part of the investigation the risk of no response to treatment was compared between groups with: low (≤ 6.25) and high (> 6.25) lymph vessel density, low (≤ 145.7) and high (> 145.7) serum VEGF levels, and between early (FIGO Stage I-II) and advanced stages of disease (FIGO III-IV). No statistically significant influence of any of the analyzed parameters on the increased risk of lymph node invasion was found. No correlation was found between LMVD and patients’ age.

Discussion

In the etiopathogenesis of cancer the processes of angiogenesis and lymphangiogenesis are considered to be of crucial importance. Recently there has been an increased interest in this subject, as it is considered to be an area which raises great expectations and is of great significance. The main research trends are targeting a search for new predictive and prognostic factors. The potential of using some of these issues in developing targeted therapies are also worth noting.

Researching factors of angiogenesis and lymphangiogenesis involve a multitude of methodological and interpretative problems which have not yet been solved.

ELISA is the most commonly used method due to the ease of obtaining the pathologic material and the possibility of repeated determination of these factors.

Like any other method, ELISA is in some ways limited; for instance, secretion of VEGF in healthy cells and in tumor cells in organisms (in vivo) is unknown. Thus it is difficult to set a norm above which VEGF-C concentration is associated with the neoplastic process [10]. Observations indicate that secretion varies between different localizations of the disease and histopathologic type of tumor [11]. Determining is done with different commercial sets whose sensitivity and specificity varies, which poses difficulties in comparing results obtained by different laboratories. Moreover, we lack information as to which material is the best for determination. VEGF concentration is higher in blood serum than in plasma, which is due to the presence of blood platelets. The concentration in plasma depends on the type of the anticoagulant for blood platelet stabilization [11-13]. The obtained results frequently happen to be so diverging that they make it impossible to draw conclusions enabling researchers to work out uniform standards of determining VEGF concentration [12]. Similarly, the interpretation of VEGF concentration and other proangiogenic factors produced by the cancer, but also released by blood platelets and leucocytes in the coagulation process, is a problem which has not yet been solved [12, 14]. These interpretative difficulties are related to the fact that VEGF-C con-
centration constitutes a balance in the plasma between a free fraction of VEGF-C and a blood platelet-related VEGF-C fraction.

In scientific research it is pointed out that there is a large dispersion of the obtained results. This, according to some authors, discriminates VEGF-C as a useful marker in laboratory diagnostics [4, 15]. It is assumed that this divergence results from inflammation. From early stages of the disease, cancer is accompanied by inflammation processes with different intensity, and the activated leukocytes secrete many cytokines, including VEGF-C [16-18]. In the present study, like in most other studies, the obtained results were fairly divergent: the mean concentration of VEGF-C in blood serum was 209.7 pg/ml+/–165.82 pg/ml [17, 19-22].

A crucial and yet unsolved problem in determining VEGF-C is determining the cutoff point between low concentration connected with physiological processes ongoing in the organism and high concentration indicating cancer; especially in healthy people quite a huge difference in proangiogenic factor concentration can be observed [11, 23].

Based on multiple studies, Gasparini and other experts [12, 24] have presented international recommendations for determining the processes of angiogenesis and lymphangiogenesis. They include the guidelines for applying the antibody directed against endothelial molecule CD31, which is described to be useful for staining small and large vessels in normal tissue and in tumors. On the other hand, the study reports that this antibody has low specificity and that it does not stain lymphatic vessels [12, 24]. Despite these limitations CD31 was chosen for the study mostly due to the fact that it does not stain lymphatic vessels, which allows the determination of the real number of blood vessels in the tumor. In case of other antibodies the reaction takes place with both blood and lymphatic vessels, making the independent assessment of either vessel group impossible. Another factor which limits the usefulness of an antibody directed against CD31 is its presence in cancerous and inflamed cells. These processes are frequently connected with each other. Our observations confirm the problem. An extensive inflammatory component, expressed in the intensity of the reaction with the antibody anti-CD31 in the entire field of view, masked the presence of blood vessels and in many situations made the interpretation of the results impossible. The antibody directed against molecule CD31 is characterized by the fact that during identification of the antigen and triggering the immunoreactivity of the antigen, due to microwave activity, it may lead to lost activity, making it impossible to read the results. This happens mainly if cancer is accompanied by an extensive inflammatory component. In the analyzed material there were slides in which inflammation processes prevented the researchers from determining microvessel density (MVD). These slides were excluded from the analysis, as the objective was to search for small vessels which formed as a result of intensified angiogenic processes.

In the present study VEGF-C concentration in blood serum, LMVD and MVD were analyzed in relation to the progression of the disease according to FIGO stages. In the scientific literature there is a big divergence in obtained results. The studies by Mathur et al. and others indicated that the higher the levels of VEGF-C concentrations and other proangiogenic factors are the more advanced stage of the disease, which is opposite to what was observed in the present study [15, 25, 26]. Mathur et al. [15] studied VEGF-C content in cervical dysplasia and cervical cancer patients. They observed that VEGF-C concentration in blood serum increased with cervical dysplasia stage CIN 1,2,3, and that it was higher in cervical cancer than in cervical dysplasia patients. The concentration was highest in advanced disease and in patients with distant metastasis. However, there are multiple studies which do not confirm such relationship [4, 10, 11, 17, 27, 28].

In scientific research it is pointed out that there is a big divergence in obtained results. The studies by Mathur et al. and others indicated that the higher the levels of VEGF-C concentrations and other proangiogenic factors are the more advanced stage of the disease, which is opposite to what was observed in the present study [15, 25, 26]. Mathur et al. [15] studied VEGF-C content in cervical dysplasia and cervical cancer patients. They observed that VEGF-C concentration in blood serum increased with cervical dysplasia stage CIN 1,2,3, and that it was higher in cervical cancer than in cervical dysplasia patients. The concentration was highest in advanced disease and in patients with distant metastasis. However, there are multiple studies which do not confirm such relationship [4, 10, 11, 17, 27, 28].

In the present study we also dealt with MVD in tumors. Apart from the correlation between MVD and LMVD, no association with other clinical or pathological parameters was observed. The obtained results should be treated with a healthy degree of scepticism. The fact that there was a restricted number of women with stained MVD was a considerable limitation of the study and had a vital impact on its results. In studies by other authors concerning MVD the results are quite diverse. The conclusions reached by different authors are contradictory. In some studies no association with clinical and pathological parameters was found, while in others MVD was associated with the stage of disease [31-36], with metastasis to lymph nodes [37] or with overall survival [33, 38].
In the next stage of the present study VEGF-C concentration in blood serum and LMVD was investigated in relation to the grade of tumor differentiation. It was demonstrated that higher levels of LMVD were associated with G1 and G2 grade, compared to G3, respectively, 9.9 vs 5.25 (p = 0.0398). The differences in VEGF-C content in particular groups of patients did not reach levels of statistical significance. Gao et al. [39] analyzed slides of 147 cervical cancer patients in FIGO Stage IA and IB. The authors confirmed the correlation between LMVD and the grade of tumor differentiation.

The next stage of the present study was to compare the level of Hb with VEGF-C concentration in blood serum, LMVD and MVD in tumors. Decrease in the level of Hb was accompanied by increase in LMVD, but the observed trend did not reach levels of statistical significance. In the published sources there have been few authors investigating the relationship between the level of Hb and the processes of angiogenesis and lymphoangiogenesis. Ferrero et al. [40] analyzed 72 ovarian cancer patients. They demonstrated an inverse correlation between the level of Hb and MVD in tumors. Such relationship was not confirmed by other authors dealing with this subject, like Kayaa et al. or Gasinska et al. [28, 38].

In the present study the predictive value of proangiogenic and lymphoangiogenic factors for prognosis was not confirmed. The differences in concentration of the analyzed parameters were not statistically significant between groups with CR, PR and PD. Higher LMVD and over-expression of VEGF-C in tumors correlates with metastasis to the lymph nodes and poor prognosis [10, 17, 41]. Such relationship is confirmed in esophageal, stomach, thyroid and pancreatic cancer. It makes it possible to assume that the concentration of VEGF-C circulating in the organism should be higher, although the results obtained by different authors are different [4, 10, 15, 27, 33, 38, 41]. Many data from the published literature reports indicate that LMVD in tumors may correlate with overall survival and disease-free survival rate. It is assumed that intensification of lymphoangiogenic processes may lead to tumor progression and worse prognosis. The correlation between LMVD and worse prognosis was confirmed in breast cancer, melanoma and in head and neck cancers, but it was not confirmed in oral and ovarian cancer [29, 37, 39, 41]. In the published literature the explanation of this situation is sought. It is emphasized that a key role is played by the ignorance of different mechanisms at a molecular level, small number of patients taking part in studies, and, mostly, no established methodological standards influencing the results of the conducted tests [11, 12, 14, 24]. The risk of no response to treatment in uterine cervical cancer patients is an important issue, and as such it was compared between early (Stage I-II) and advanced (Stage III-IV) of the disease according to FIGO. The risk of no response to treatment increased with the more advanced stages of the cancer (p = 0.0030). The results are similar to those obtained by other authors, in which tumor stage was the strongest prognostic factor in Cox’s regression and multivariate Cox regression analysis [3, 34, 39, 42].

The present study did not demonstrate any influence of VEGF-C concentration in blood serum, LMVD or MVD in tumors, FIGO stage, patients’ age and Hb concentration on the risk of lymph node invasion. In the published literature there have been discrepancies in the results obtained in particular laboratories. The authors emphasize that the mechanisms of this process are not fully known [11, 24, 28, 29, 39, 42]. Such correlation seems to be obvious and expected. Its existence was first confirmed by Tamura et al., where they demonstrated that the risk of invasion of mediastinum lymph nodes in case of non-small cell lung carcinoma increases with VEGF concentration in blood serum [43]. However, subsequent publications did not confirm this relationship [4, 11, 44]. Increased risk of metastasis to lymph nodes with high activity of proangiogenic and lymphoangiogenic factors is explained in several ways. One of the hypotheses assumes, that by means of VEGF-C activation the proliferation of lymphatic cells of endothelium is stimulated and new vessels are formed. They might induce lymph node metastasis. This hypothesis seems to be supported by the fact that newly formed lymphatic vessels are characterized by a dysfunction, which makes metastasis to lymph nodes easier [45].

Progress in the knowledge of molecular biology has initiated a multitude of studies. Yet, a practical application of the presently available information to patients’ advantage in clinical data remains to be a challenge. The above-mentioned research tools, new technologies and strategies may facilitate progress in the area. Nevertheless, from the perspective of personal experience this goal still seems to be far off.

References

Labeling microvessel density, lymphatic vessel density and potential role of proangiogenic and lymphangiogenic factors as a etc. 405


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A single nucleotide polymorphism in the 5’ untranslated region of RAD51 and ovarian cancer risk in Polish women

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Summary
Background. DNA repair gene polymorphisms are known to influence cancer risk. The RAD51 gene encodes proteins essential for maintaining genomic stability by playing a central role in homology-dependent recombinational repair of the DNA double-strand breaks. Aims. We investigated the association of polymorphisms in the DNA repair genes RAD51-135G>C and 172G>T with ovarian cancer risk. Methods. 120 Polish ovarian cancer patients and 120 healthy controls were genotyped for RAD51 (135G>C and 172G>T) by PCR-RFLP. Results. In the present work no association was detected between ovarian cancer risk and 172G>T polymorphism of the RAD51 gene. The 135G>C polymorphism was associated with ovarian cancer risk. We found evidence of an increased ovarian cancer risk in CC homozygotes (OR 12.97 [95% confidence interval {CI} (5.73 - 29.36)]) but not in heterozygotes (OR 0.55 [95% CI 0.23 - 1.29]). We demonstrated a significant positive association between the RAD51 variant 135C allele and ovarian carcinoma, with an adjusted odds ratio (OR) of 6.24 ($ p < .0001$). Conclusion. The results indicated that the polymorphism 135G>C of RAD51 may be positively associated with ovarian carcinoma in the Polish population. Further studies on the role of the RAD51 gene on ovarian cancer are warranted.

Key words: RAD51; Ovarian cancer; Gene polymorphism.

Introduction
Ovarian cancer is detected in more than 3,000 women a year and two-thirds of these die within the five subsequent years [1]. There are no valuable diagnostic methods used worldwide for early recognition of ovarian cancer, and the first unspecific signs of the disease are frequently ignored by patients. Hence, tumours are detected mostly in advanced clinical stages. The risk of ovarian cancer is increased by several factors such as age, childbearing status, infertility, dietary factors, gynaecological diseases (endometriosis, ovarian cysts, pelvic inflammatory disease), and gene mutations [2, 3].

So far the most significant and recognized risk factor for ovarian cancer has been the presence of breast cancer-1 (BRCA1) or breast cancer-2 (BRCA2) gene mutations. They are responsible for about 5-10% of ovarian cancers [4].

Despite the growing knowledge about ovarian cancer, no effective screening program has been discovered so far. Therefore, the identification of new risk factors for ovarian cancer in the population of women is urgently needed, and an analysis of some gene polymorphisms could be an interesting option.

It is known that defects in the DNA double-strand breaks (DSB) repair pathway may play a role in development and progression of various cancers. DSB in DNA may be rectified by either homologous recombination (HR) and nonhomologous end joining (NHEJ) [5, 6]. RAD51 is involved in homologous recombination and repair of double-strand breaks in DNA and DNA cross-links and for the maintenance of chromosome stability [7].

Two common RAD51 SNP (single nucleotide polymorphism), 135G>C and 172G>T in the 5 UTR have been reported to be associated with altered gene transcription [7].

This SNP is located in the regulatory element of the RAD51 promoter and is suggested to be associated with messenger RNA expression.

RAD51 gene 135G>C and 172G>T polymorphism have been studied as risk factors for various cancers such as breast, colorectal, head and neck and ovarian cancer [8-12]. It is known that variants in the RAD51 gene that interact biologically with BRCA1 and/or BRCA2 may be associated with modified ovarian cancer risk in women who carry BRCA1/2 mutations [13-15].

However, little is known about the interconnections between RAD51 polymorphisms and ovarian carcinoma occurrence in patients without BRCA1/2 mutations [12]. Therefore, the purpose of this study was to determine the frequency of RAD51 135G>C and 172G>T polymorphism in ovarian tissue of Polish women treated for ovarian cancer and the possible influence on the risk of development of this neoplasm.
A single nucleotide polymorphism in the 5′ untranslated region of RAD51 and ovarian cancer risk in Polish women

Materials and Methods

Ovarian cancer patients

The study group consisted of Polish women (n = 120) belonging to the Caucasian population, with recognized ovarian cancer, who qualified for tumor debulking surgery at the Department of Gynaecological Surgery of the Institute of Polish Mother’s Memorial Hospital between 2000 and 2007. All tumours were staged according to the criteria of the International Federation of Gynaecology and Obstetrics (FIGO). The full characteristics of the examined group are presented in Table 1.

One hundred and twenty age- and ethnically-matched women with normal ovaries served as controls. The healthy ovaries were removed during the hysterectomy procedure and bilateral salpingo-oophorectomy was performed due to the presence of uterine fibroids. The Local Ethics Committee approved the study and each patient gave written informed consent.

The ovarian tissue samples (cancerous and non-cancerous) were fixed routinely in formaldehyde, embedded in paraffin, cut into thin slices and stained with hematoxylin/eosin for pathological examination. DNA for analysis was obtained from an archival pathological paraffin-embedded tumour and healthy ovarian samples which were deparaffinised in xylene and distilled water. To ensure that the chosen histological material was representative of cancerous and non-cancerous tissue, every tissue sample which qualified for DNA extraction was initially checked by a pathologist. The DNA samples were extracted using QIAmp Kit (Qiagen GmbH, Hilden, Germany). DNA purification was achieved according to the manufacturer’s instructions.

Genotype determination

Single nucleotide polymorphisms 135G>C and 172G>T of RAD51 gene were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), using primers 5′-TGG GAA CTG CAA CTC ATC TGG-3′ (forward) and 5′-GCT CCG ACT TCA CCC CGC CGG-3′ (reverse).

RAD51 135G>C genotyping was analysed by PCR amplification of a 175-bp region around nucleotide 135. This region contained a single MvaI site that was abolished in the 135C allele. Wild type alleles were digested by MvaI resulting in 86- and 71-bp products. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product.

PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. PCR amplification was performed in a final volume of 25 μl. The reaction mixture contained 5 ng genomic DNA, 0.2 μmol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstadt, Germany), 2.5 mM MgCl₂, 1 mM dNTPs and 1 unit of Taq polymerase (Qiagen GmbH, Hilden, Germany). The PCR cycle conditions were 94°C for 30 sec, 65°C for 45 sec, and 72°C for 50 sec, and a final extension step of 72°C for 10 min. The product after PCR was digested with NgoMIV (New England BioLabs) overnight. The products were separated in 7% polyacrylamide gel. The 172G/G genotype produced two bands (110 and 21 bp), whereas the 172T/T genotype produced only one band (131 bp) and the 172G/T heterozygote displayed all three bands (131, 110 and 21 bp).

Statistical analysis

For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy–Weinberg equilibrium was assessed using the standard χ²-test. Genotype frequencies in cases and controls were compared by χ²-tests. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression; p values < 0.05 were considered to be significant. STATISTICA software (Statsoft, Tulsa, OK, USA) was used to perform analyses.

Table 1. — Characteristics of the 120 ovarian cancer patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>54</td>
</tr>
<tr>
<td>Range</td>
<td>37-79</td>
</tr>
<tr>
<td>Histology of tumor</td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>46 (38.3)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>6 (5.0)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>33 (27.5)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>7 (5.8)</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>23 (19.2)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (4.2)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>35 (29.2)</td>
</tr>
<tr>
<td>II</td>
<td>0 (0)</td>
</tr>
<tr>
<td>III</td>
<td>77 (64.2)</td>
</tr>
<tr>
<td>IV</td>
<td>6 (5.0)</td>
</tr>
<tr>
<td>no data</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>2 (1.7)</td>
</tr>
<tr>
<td>G2</td>
<td>34 (28.3)</td>
</tr>
<tr>
<td>G3</td>
<td>70 (58.3)</td>
</tr>
<tr>
<td>No data</td>
<td>14 (11.7)</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>50 (41.7)</td>
</tr>
<tr>
<td>absent</td>
<td>70 (58.3)</td>
</tr>
<tr>
<td>Tumor wall infiltration/injury</td>
<td></td>
</tr>
<tr>
<td>present</td>
<td>70 (58.3)</td>
</tr>
<tr>
<td>absent</td>
<td>50 (41.7)</td>
</tr>
<tr>
<td>Size of tumor</td>
<td></td>
</tr>
<tr>
<td>&lt; 5 cm</td>
<td>32 (26.7)</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>88 (73.3)</td>
</tr>
<tr>
<td>Menarche</td>
<td></td>
</tr>
<tr>
<td>&lt; 12 years old</td>
<td>26 (21.7)</td>
</tr>
<tr>
<td>&gt; 12 years old</td>
<td>94 (78.3)</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10 (8.3)</td>
</tr>
<tr>
<td>1</td>
<td>22 (18.4)</td>
</tr>
<tr>
<td>2 and more</td>
<td>88 (73.3)</td>
</tr>
<tr>
<td>Number of deliveries</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16 (13.3)</td>
</tr>
<tr>
<td>1</td>
<td>34 (28.3)</td>
</tr>
<tr>
<td>2 and more</td>
<td>70 (58.4)</td>
</tr>
</tbody>
</table>
Table 2. — Distribution of RAD51 135G>C genotype frequencies in patients with ovarian cancer and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Ovarian cancer n = 120</th>
<th>Controls n = 120</th>
<th>OR (95% CI) a</th>
<th>p b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>135G&gt;C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>13 (10.8)</td>
<td>33 (27.5)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>15 (12.5)</td>
<td>69 (57.5)</td>
<td>0.55 (0.23 - 1.29)</td>
<td>0.247</td>
</tr>
<tr>
<td>C/C</td>
<td>92 (76.7)</td>
<td>18 (15.0)</td>
<td>12.97 (5.73 - 29.36) &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>41 (17.1)</td>
<td>135 (56.3)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>199 (82.9)</td>
<td>105 (43.7)</td>
<td>6.24 (4.09 - 9.51) &lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Data in boldface are statistically significant.

Table 3. — Distribution of 172G>T RAD51 genotype frequencies in patients with ovarian cancer and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Ovarian cancer n = 120</th>
<th>Controls n = 120</th>
<th>OR (95% CI) a</th>
<th>p b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>172G&gt;T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>23 (19.2)</td>
<td>29 (24.2)</td>
<td>1.00 Reference</td>
<td></td>
</tr>
<tr>
<td>G/T</td>
<td>62 (51.7)</td>
<td>58 (48.3)</td>
<td>1.34 [0.70-2.59]</td>
<td>0.466</td>
</tr>
<tr>
<td>T/T</td>
<td>35 (29.1)</td>
<td>33 (27.5)</td>
<td>1.33 [0.64-2.76]</td>
<td>0.548</td>
</tr>
<tr>
<td>G</td>
<td>108 (45.0)</td>
<td>116 (48.3)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>132 (55.0)</td>
<td>124 (51.7)</td>
<td>1.14 [0.79-1.63]</td>
<td>0.521</td>
</tr>
</tbody>
</table>

Data in boldface are statistically significant.

Table 4. — Haplotype distribution and frequencies of RAD51 gene polymorphisms in the ovarian cancer patients and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Patients (N = 120)</th>
<th>Controls (N = 120)</th>
<th>OR (95% CI) a</th>
<th>p b</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G-G/G</td>
<td>6 (5.0%)</td>
<td>15 (12.5%)</td>
<td>1.00 Reference</td>
<td></td>
</tr>
<tr>
<td>G/G-G/T</td>
<td>7 (5.8%)</td>
<td>7 (5.8%)</td>
<td>2.5 [0.60-10.26]</td>
<td>0.353</td>
</tr>
<tr>
<td>G/G-T/T</td>
<td>6 (5.0%)</td>
<td>10 (8.3%)</td>
<td>1.5 [0.37-5.99]</td>
<td>0.823</td>
</tr>
<tr>
<td>G/C-G/G</td>
<td>8 (6.6%)</td>
<td>14 (11.7%)</td>
<td>1.42 [0.39-5.16]</td>
<td>0.823</td>
</tr>
<tr>
<td>G/C-G/T</td>
<td>6 (5.0%)</td>
<td>28 (23.3%)</td>
<td>0.53 [0.14-1.95]</td>
<td>0.266</td>
</tr>
<tr>
<td>G/C-T/T</td>
<td>6 (5.0%)</td>
<td>15 (12.5%)</td>
<td>1.00 [0.26-3.81]</td>
<td>0.729</td>
</tr>
<tr>
<td>C/C-G/G</td>
<td>18 (15.0%)</td>
<td>9 (7.5%)</td>
<td>5.00 [1.44-17.27]</td>
<td>0.019</td>
</tr>
<tr>
<td>C/C-G/T</td>
<td>43 (35.8%)</td>
<td>13 (10.8%)</td>
<td>8.26 [2.66-25.64]</td>
<td>0.0002</td>
</tr>
<tr>
<td>C/C-T/T</td>
<td>20 (16.7%)</td>
<td>9 (7.5%)</td>
<td>5.55 [1.62-19.02]</td>
<td>0.0112</td>
</tr>
</tbody>
</table>

Data in boldface are statistically significant.

Table 5. — Dependency of genotypes and frequencies of the alleles of RAD51 gene polymorphism on tumour grade in patients with ovarian cancer.

<table>
<thead>
<tr>
<th>Stage</th>
<th>I (n = 35)</th>
<th>II + III + IV (n = 85)</th>
<th>OR (95% CI) a</th>
<th>p b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RAD51 135G&gt;C</strong></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>10 (28.6%)</td>
<td>16 (18.8%)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>4 (11.4%)</td>
<td>10 (12.9%)</td>
<td>0.64 (0.15-2.60)</td>
<td>0.394</td>
</tr>
<tr>
<td>G/C</td>
<td>21 (60.0%)</td>
<td>59 (69.3%)</td>
<td>0.56 (0.22-1.44)</td>
<td>0.345</td>
</tr>
<tr>
<td>G/C</td>
<td>24 (34.3%)</td>
<td>42 (24.7%)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>4 (65.7%)</td>
<td>128 (75.3%)</td>
<td>0.62 (0.34-1.15)</td>
<td>0.176</td>
</tr>
<tr>
<td><strong>RAD51 172G&gt;T</strong></td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>10 (28.6%)</td>
<td>20 (23.5%)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>G/T</td>
<td>5 (14.3%)</td>
<td>10 (11.8%)</td>
<td>1.00 [0.26-3.72]</td>
<td>0.740</td>
</tr>
<tr>
<td>T/T</td>
<td>20 (57.1%)</td>
<td>55 (64.7%)</td>
<td>0.72 [0.29-1.81]</td>
<td>0.654</td>
</tr>
<tr>
<td>G/T</td>
<td>25 (35.7%)</td>
<td>50 (29.4%)</td>
<td>1.00 Ref</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>45 (63.4%)</td>
<td>120 (70.6%)</td>
<td>0.75 [0.41-1.35]</td>
<td>0.420</td>
</tr>
</tbody>
</table>

*p = 120, aaccording to FIGO criteria; bCrude odds ratio (OR), 95% CI = confidence interval at 95%, *Chi square.

**Results**

Table 2 shows genotype distribution RAD51 135G>C polymorphism between ovarian cancer patients and controls. It can be seen from the table that there were significant differences (p < 0.05) between the two investigated groups. The distribution of genotypes for 135G>C SNP in ovarian cancer patients vs controls was: 10.8% vs 27.5% for GG, 12.5% vs 75.5% for GC and 76.7% vs 15.0% for CC genotype, respectively. A strong association of the C/C genotype [OR = 12.97 (5.73-29.36)] and the C allele [OR = 6.24 (4.09-9.51)] with ovarian cancer was observed. In patients the observed frequencies of the G/G, G/C and C/C genotypes differed significantly (p < 0.05) from the distribution expected from the Hardy-Weinberg equilibrium.

The distribution of genotypes of 172G>T polymorphism in ovarian cancer patients did not differ significantly compared to that predicted by the Hardy-Weinberg distribution. No significant deviation from Hardy-Weinberg distribution of genotypes characterising controls was revealed either. The distribution of genotypes for ovarian cancer patients was estimated as: 19.2% for GG, 51.7% for GT and 29.1% for TT genotype. The controls distribution was: for CC- 24.2%, CT- 48.3%, TT- 27.5%, respectively. We did not find any significant difference in genotype and allele frequencies in patients with cancer and controls. The frequency of genotype and allele distribution in ovarian cancer patients in comparison to controls are summarised in Table 3.

The association between the haplotype analysis of RAD51 and ovarian cancer was also investigated. Haplotype analysis according to wild-type of G135G-G172G showed a high association with ovarian cancer (Table 4). The findings indicated that a statistically significantly increased risk of ovarian cancer was associated with the combined C/C-G/G genotype (OR, 5.00; 95% CI, 1.44–17.27) and C/C-T/T genotype 5.55 [1.62–19.02]. The higher risk of ovarian cancer occurrence was associated with the combined C135C-G172T genotype (OR, 8.26; 95% CI, 1.62–19.02) but no altered risk was associated with other haplotypes.

FIGO staging were related to the RAD51 135G>C and 172G>T polymorphism. The histological stage was evaluated in all cases (n = 120). Stage II, III and IV were grouped together for the purposes of statistical analysis (Table 5). No differences between RAD51-135G>C and 172G>T genotype distributions in these groups were observed. There was a lack of correlation between genotypes of the polymorphisms and ovarian cancer invasiveness.

**Discussion**

As mentioned before RAD51 is an important component of double-stranded DNA-repair mechanisms that interacts with both BRCA1 and BRCA2.

The contribution of polymorphisms of DNA repair
genes in developing ovarian cancer is still being investigated. Therefore we analysed the role of 135G>C and 172G>T genetic variation in homologous recombination repair gene RAD51 and risk of this cancer. A single nucleotide polymorphism was identified in the 5’ untranslated region of the RAD51 gene and was shown to influence gene transcription activity. RAD51 expression is often increased in various malignancies.

A single-nucleotide polymorphism (SNP) in the 5’ untranslated region (UTR) of RAD51, 135G>C has been suggested as a possible modifier of ovarian cancer risk in BRCA1 and BRCA2 mutation carriers [13-16].

In the Polish population RAD51 135C allele showed a protective tendency against ovarian cancer in women harboring BRCA1 mutations. The results reveal that women who harbor the C allele have almost twice the reduction in breast and ovarian cancer risk compared with women who harbor only the G allele [14].

Unfortunately, it is difficult to find reports directly binding RAD51 172G>T with ovarian cancer in the literature.

Few studies have investigated the association between RAD51 172G>T SNP and risk of breast cancer. In a large European and Korean case-control study of patients with breast cancer, the 172T variant genotypes of RAD51 were found to be associated with a non-significantly reduced risk of breast cancer [17, 18].

Conversely, in a recent case-control study of epithelial ovarian cancer (EOC), none of the 135G>C and 172G>T variants of RAD51 were associated with a reduction in risk [12]. Only some polymorphisms in XRCC2 and XRCC3 genes were associated with EOC risk.

A study performed in Australian women did not shown any association between variants in RAD51 and XRCC2 and ovarian cancer etiology [19].

Finally, in a study examining ovarian cancer cases, an association was observed with the RAD51 135G>C allele, suggesting that this polymorphism is associated with disease risk in the context of BRCA1 and BRCA2 [14, 13, 20]. RAD51 is the first gene to be reliably identified as a modifier of risk among BRCA1/2 mutation carriers.

There is a lack of significant data for RAD51 135G>C and 172G>T polymorphisms in ovarian cancer without BRCA1/2 mutations. Therefore in our study we investigated these polymorphisms in women with this cancer without any BRCA1/2 mutation context.

In our earlier studies we analysed RAD51 polymorphism in sporadic breast cancer [8]. Our results suggest that the 135G>C polymorphism of the RAD51 gene may not be linked to breast cancer nor be considered as an additional marker of this disease.

In the present work our results have shown the important role of RAD51 135G>C polymorphism for ovarian carcinoma occurrence in Poland. In this study RAD51 C/C genotype increased the risk of ovarian cancer in a Polish population. There was a 13-fold increased risk of ovarian carcinoma for individuals carrying RAD51-C/C genotype compared with subjects carrying RAD51-G/G, G/C genotype, respectively. The combined genotype of C135G-G172G, C135G-G172T and C135C-T172T was associated with ovarian cancer risk and may have an impact on identification of a high-risk population. In the present study the frequency of 135C allele among ovarian cancer patients was higher than the 135G allele (82.9% vs 17.1%). We realise that this may be due to the small population enrolled in the study or de novo mutations in ovarian cancer. It is possible that the presence of the C allele is in linkage disequilibrium with another, so far unknown, mutation located outside the coding region in the RAD51 gene, which may be of importance for RAD51 concentration in plasma. RAD51 G135C polymorphism was not related to cancer grade. The reason for this can be a relatively small group of A, B and C grade subjects enrolled in our study.

To our knowledge this is the first study linking the 172G>T polymorphism of the RAD51 gene with ovarian cancer.

Finally we suggest that RAD51 135G>C might be used as a predictive factor of precancerous lesion for ovarian cancer in a Polish population. Further studies on the role of these genes on ovarian cancer are warranted.

References


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Metastatic bone involvement in vulvar cancer: report of a rare case and review of the literature

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¹Radiation Therapy Unit, Second Department of Radiology, University Hospital of Athens "Attikon", Athens
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Summary

Purpose: Bone metastasis secondary to vulvar carcinoma is an infrequent clinical entity. Only ten cases have been published in the literature. We describe a case of squamous vulvar carcinoma, that presented with cervical vertebral involvement, as a part of distant spread. MRI of the cervical spine was performed, showing an osteolytic lesion with spinal cord compression. Conclusion: This case was unique in presenting vertebral metastasis eight months after chemotherapy and radiotherapy.

Key words: Vulvar cancer; Bone metastasis; Radiotherapy.

Introduction

The incidence of vulvar cancer in women is increasing, especially in younger women [1-4]. The incidence of invasive vulvar cancer is approximately 2.5 per 100,000 women per year in Germany [2]. For the UK similar age-adjusted incidence rates have been reported [5]. The main route of dissemination is by transcocelomic spread and through the lymphatics. Hematogenous spread is uncommon. Metastasis to bones from these tumors is rare and is reported in a few series [6, 7]. The prevalence of bony metastases secondary to vulvar carcinoma is reported to be far minor, 10% [6, 7]. In other gynecological squamous cell carcinomas such as cervical cancer, the prevalence is 15-29% [6, 8, 9]. Bone metastases from epithelial ovarian carcinoma are rare, usually discovered postmortem. The survival of these patients is poor. Since 1966 only three cases of endometrioid ovarian carcinoma with metastasis to the skeletal structures have been described in the literature [10-16]. Metastasis to bone from endometrioid adenocarcinoma is rare but when metastasis occurs it usually locates in the axial skeleton [11]. Skeletal metastasis from carcinoma of the cervix occurs in 0.8-23% of cases. The majority of bone metastases are either in the long bones or in the vertebral [12]. Spinal cord compression is an infrequent event in the natural course of metastatic vulvar carcinoma. A review of the literature showed only a few patients who developed radiculopathy or spinal cord compression secondary to bone metastases (Table 1). Malignant epidural spinal cord compression (MESCC) is a medical emergency that needs rapid diagnosis and treatment to prevent paraplegia. Patients with malignancy who present with a new onset of neurological signs and symptoms should undergo emergent evaluation including magnetic resonance imaging (MRI) of the entire spine. Simultaneously, spine surgery and oncology teams should be consulted immediately. The initiation of early treatment can improve the quality of life and survival of the patients.

Case Report

A 69-year-old woman presented to a local gynecologist with a vulvar mass, distention of the abdomen, loss of appetite and vague abdominal discomfort of three months duration. The patient had a medical history of hypertension and a negative family history for malignancies. Initial physical examination revealed a vulvar mass with associated bilateral inguinal lymphadenopathy. Radiograph of the chest was normal. A diagnosis of vulvar malignancy was made based on multiple biopsies. The histopathology report of the specimen showed a squamous cell carcinoma, if cN+ is fixed or ulcerated, pre-op chemo-RT (45-50 Gy with cisplatin, 5-FU, and/or mitomycin C) provides about 50% complete response. Surgical salvage for persistent or recurrent disease includes bilateral lymph node dissection, while if there is extra nodal capsular extension, then a boost to
has significant potential for distant metastasis. Bony metastasis from vulvar malignancies is a rare pathologic entity, whose behavior is not completely understood. Reviewing the literature, only ten cases have been reported. In 1976 Seltzer et al. [15] presented one case of multiple bone metastases. In 1978 Brufman et al. [16] reported a case of tibial involvement. As mentioned by Brufman et al., different gynecological tumors tend to spread through the lymphatic system rather than by the hematogenous system. This might be a possible explanation for bone metastases being infrequent in gynecological malignancies [16]. Sharma et al. [17] in 1985 found three cases of clinically Stage III (FIGO classification). Postoperatively, these patients were found to have metastatic carcinoma of the bones. These findings suggest that patients undergoing surgery with advanced disease should have a bone scan or a bone survey as part of the preoperative workup [18]. Abdul-Karim et al. [18] in 1990 reported four cases of asymptomatic skeletal metastases during autopsies. It was supposed that osseous metastases might not be rare but significantly more common than clinically expected.

Table 1. — Cases of metastatic bone lesions related to vulvar carcinoma.

<table>
<thead>
<tr>
<th>Author/Group</th>
<th>No. of patient</th>
<th>Age</th>
<th>Dose to primary</th>
<th>Dose to metastatic</th>
<th>Localisation</th>
<th>Time to metastasis</th>
<th>Survival after primary diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brufman et al. [16] (1978)</td>
<td>1</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>Tibia</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Abdul-Karim et al. [18] (1980)</td>
<td>4</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>Vertebrae</td>
<td>NA</td>
<td>9 months</td>
</tr>
<tr>
<td>Fischer et al. [6] (2005)</td>
<td>1</td>
<td>68</td>
<td>59.4 Gy</td>
<td>-</td>
<td>Humeral</td>
<td>4 months</td>
<td></td>
</tr>
<tr>
<td>Seltzer et al. [15] (1978)</td>
<td>1</td>
<td>59</td>
<td>NA</td>
<td>3990 cGy</td>
<td>Vertebrae</td>
<td>12 months</td>
<td>-</td>
</tr>
<tr>
<td>Sharma et al. [12] (1985)</td>
<td>3</td>
<td>68</td>
<td>5000 cGy</td>
<td>3000 cGy</td>
<td>Humerus</td>
<td>4 months</td>
<td>9 months</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>5200 cGy</td>
<td>Femur</td>
<td>2 months</td>
<td>9 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>NA</td>
<td>Tibia</td>
<td>9 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fibula</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA: not applicable.

Discussion

Vulvar cancer should not be considered a disease that remains confined to the pelvis and abdomen. This disease has significant potential for distant metastasis. Bony metastasis from vulvar malignancies is a rare pathologic entity, whose behavior is not completely understood. Reviewing the literature, only ten cases have been reported.

In 1976 Seltzer et al. [15] presented one case of multiple bone metastases. In 1978 Brufman et al. [16] reported a case of tibial involvement. As mentioned by Brufman et al., different gynecological tumors tend to spread through the lymphatic system rather than by the hematogenous system. This might be a possible explanation for bone metastases being infrequent in gynecological malignancies [16]. Sharma et al. [17] in 1985 found three cases of clinically Stage III (FIGO classification). Postoperatively, these patients were found to have metastatic carcinoma of the bones. These findings suggest that patients undergoing surgery with advanced disease should have a bone scan or a bone survey as part of the preoperative workup [18]. Abdul-Karim et al. [18] in 1990 reported four cases of asymptomatic skeletal metastases during autopsies. It was supposed that osseous metastases might not be rare but significantly more common than clinically expected.
In 2005 Fischer et al., [6] described a case of multiple osseous involvement in a woman with a history of vulvar carcinoma who presented with pain confined to the bones. The epithelial origin of the lesion was confirmed by immunohistochemical examinations (overexpression of pan-cytokeratin MNF116) [5]. The atypical location should alert the physician to suspect distant metastases, rather than locoregional disease. The treatment modalities available for bone lesions are individualized with a definite role of corticosteroids, RT, chemotherapy, surgery and biphosphonates. The pretreatment degree of neurologic dysfunction and the radiosensitivity of the tumor are the strongest predictors of therapeutic outcome. Radiotherapy is an important part of the management of metastatic involvement and it helps in pain relief, cytoreduction of tumor, prevention of progressive neurologic dysfunction and structural damage to the cord.

Malignant epidural spinal cord compression is a common neurologic complication of cancer. It represents a medical emergency that needs rapid diagnosis and treatment to prevent ongoing emergent evaluation including magnetic resonance imaging of the entire spine. The most common treatment offered is radiotherapy [19]. Available evidence suggests that the radiotherapy dose should be tailored to the individual patient, depending on the subtype of the tumor, the extent of metastatic disease and expected survival. Risk stratification for the optimum dose prescription for patients with spinal cord compression is recommended. If epidural spinal cord compression is diagnosed, corticosteroids should be administered. If indicated, patients should undergo maximal tumor resection and stabilization, followed by postoperative radiotherapy. Emerging treatment options such as stereotactic radiosurgery and vertebroplasty may be able to provide some symptomatic relief for patients who are not surgical candidates [15].

Conclusion

Bone staging should be performed in advanced cancer stage in order not to overlook metastasis. The treatment of choice is always radiotherapy and should be administered as soon as possible. In case of a metastatic lesion in the spinal cord, an MRI study is the exam of choice.

References


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Large cell neuroendocrine carcinoma arising in mature cystic teratoma: a case report and review of the literature

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Department of Obstetrics and Gynecology, National Defense Medical College, Tokorozawa (Japan)

Summary

Background: Malignant transformation of ovarian mature cystic teratoma is rare, and occurs in approximately 1% of all cases. The most common histology arising in mature cystic teratoma is squamous cell carcinoma. Less frequently, malignant transformation is represented by an endocrine tumor. To date, only five cases of large cell neuroendocrine carcinoma (LCNC) arising in a mature cystic teratoma of the ovary have been reported. Clinical case: A 69-year-old woman presented with a 15-cm left ovarian mass, and was diagnosed with Stage IV large cell carcinoma neuroendocrine carcinoma (LCNC) arising in mature cystic teratoma (MCT) of the left ovary. The patient received adjuvant chemotherapy with paclitaxel and carboplatin, however, residual tumors increased in size. Six months after the debulking surgery she succumbed to the disease. A literature review revealed LCNC of the ovary showed excessively aggressive phenotype in malignant transformation from ovarian mature cystic teratoma. Conclusion: The present case of LCNC arising in MCT had an exceedingly poor prognosis, which was suggested in the previous five cases reported.

Key words: Ovarian tumor; Mature cystic teratoma; Large cell neuroendocrine carcinoma; Paclitaxel; Carboplatin.

Introduction

Malignant transformation of mature cystic teratoma (MCT) has been reported to occur in about 1% of patients with MCT. More than 80% of malignant transformations were squamous cell carcinomas [1]. Other histological subtypes arising from a MCT include adenocarcinoma, and adenosquamous cell carcinoma [2], however, the frequencies of these tumors are extremely rare.

To date, only five cases of large cell neuroendocrine carcinoma arising in a MCT of the ovary have been reported [3-5]. In this study, we report a case with large cell carcinoma neuroendocrine carcinoma arising from a MCT together with review of the literature.

Case Report

The patient was a 69-year-old woman, gravida 2, para 2. She was diagnosed as having clinical Stage IIIE diffuse large cell lymphoma three years before, and received combination chemotherapy of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone. She had been in complete remission for 26 months; however, her left ovarian tumor was detected by a routine screening with positron emission tomography (PET-CT). She complained of slight tenderness in the lower abdomen, and magnetic resonance (MR) images showed a 15-cm left ovarian tumor which had enhanced solid parts with fatty tissue (Figure 1a). CT suggested multiple metastases including left subclavian lymph nodes, paraaortic lymph nodes, and both lungs. Several tumor markers of her serum were elevated: CA125 37.5 U/ml, CA19-9 139 U/ml, CEA 5.7 ng/ml, SCC 1.3 ng/ml, IL-2R 900 U/ml, and NSE 170 ng/ml. Subsequently, she underwent left salpingo-oophorectomy and biopsy of peritoneal dissemination and subclavian lymph nodes. Approximately 500 ml of yellowish oily ascites was observed at laparotomy. A 15-cm pelvic tumor was derived from the left ovary the adhering to the pelvic wall tightly. The tumor had preoperatively ruptured with intraperitoneal dissemination. The left ovarian tumor was completely removed; however, several residual tumors including pelvic and paraaortic lymph nodes with a diameter more than 4 cm were left.

Macroscopically, the solid tumor showed a yellow and white appearance, and cystic lesion containing hair balls, sebaceous materials, bone, and fatty tissue (Figure 1b). Pathologically, large tumor cells showed solid growth in the benign teratoma (Figure 2a). Partially, tumor cells with solid growth had unclear nucleoli and a high nuclear/cytoplasmic ratio (Figure 2b), resembling pulmonary small cell carcinoma. However, in most solid growths, tumor cells showed strong atypia with large eosinophilic cytoplasm and clear nucleoli (Figure 2c). These cells were immunohistochemically positive for synaptophysin (Figure 2d) and CD56, and partially positive for chromogranin A (Figure 2e) and cytokeratin. Detailed microscopic examination revealed there were no other ovarian epithelial neoplasms such as mucinous tumor. The tumor was diagnosed as a large cell carcinoma neuroendocrine carcinoma arising in mature cystic teratoma. Also, these cells were observed in the subclavian lymph nodes; her disease was diagnosed as Stage IV.

She received two cycles of combination therapy with paclitaxel (175 mg/m²) and carboplatin (AUC = 6) postoperatively. However, peritoneal disseminations and metastatic lymph nodes progressed in size. She and her family did not want additional chemotherapy, and she died six months after the first surgery.

Discussion

Primary large cell carcinoma neuroendocrine carcinoma (LCNC) of the ovary is an extremely rare disease. A search with MEDLINE over two decades showed that 27 cases with ovarian LCNC have been reported (Table 1). [3-12]. Including the present case, 27 cases were available for clinical staging: 11 cases (41%) with Stage I, two cases (7%) with Stage II, six cases (22%) with Stage III,
and eight cases (30%) with Stage IV. Seventeen cases died of disease within 36 months, and seven cases had no evidence of disease during the follow-up period ranging from 8-120 months. Among these seven cases with no evidence of disease, four cases had Stage I disease and three cases had Stage III disease. Three Stage III cases underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by platinum-based chemotherapy; however, the follow-up period of two cases was within a year. Seven (64%) of 11 cases with Stage I disease died of disease within three years, suggesting that prognoses of LCNC cases seem to be quite poor in comparison with epithelial ovarian cancers. Additionally, stage distribution was compared with the patients with squamous cell carcinoma (SCC) arising from MCT. According to the review of 277 cases that had a SCC component, clinical stage was available in 241 cases: 120 cases (50%) in Stage I, 45 cases (19%) in Stage II, 66 cases (27%) in Stage III, and ten cases (4%) in Stage IV [1]. The cases with a LCNC component had significantly advanced stage in comparison with patients with a SCC component (p < 0.001).

The origin or precursor of LCNC of the ovary remains unclear. Most LCNC cases have been associated with ovarian surface neoplasms, such as mucinous cystic tumors. The hypothesis is that LCNC arises from the neuroendocrine cells that are located in surface epithelial-stromal tumors or teratoma [3]. To our knowledge, five cases with LCNC arising in MCT have been reported in the literature. Including the present case, only six cases have been described in the English literature [3-5]. Four were associated mucinous adenocarcinoma, and two cases had a LCNC component only in mature cystic teratoma. Three cases presented with abdominal pain, and another three cases presented with abdominal distension. Stage distribution was as follows: one case (17%) with Stage II, one case (17%) with Stage III, and four cases (67%) with Stage IV. Five cases received systemic chemotherapy after surgery, and one case underwent surgery only. Four of these cases died of disease within 12 months, and another case died of disease within 36 months. Only one case showed no evidence of disease, although the follow-up period was 11 months. The prognosis of LCNC arising from teratoma is presumed to be extremely poor among LCNC tumors of the ovary, which might be explained by advanced stages at diagnoses. Our case was not associated with epithelial tumors by detailed pathologic examination, and we believe that she was the second case of LCNC arising from mature cystic teratoma that had no association with mucinous neoplasia. Considering a precursor lesion in the case, direct transformation existed from MCT to LCNC.

The present case revealed that a combination with paclitaxel and carboplatin was not effective against LCNC arising from MCT. Most ovarian LCNC cases received platinum-based chemotherapy; it can be concluded that there is no standard and effective regimen for these tumors.

**Conclusion**

We have reported a case with LCNC arising from teratoma with a review of the literature. The tumor seemed to be extremely aggressive, showing a high frequency of distant metastasis and chemo-resistant phenotype. An extremely rare case report as described here would be helpful for decision making, when a case with ovarian LCNC presents.
Figure 2. — Pathological images and immunohistochemical stainings of the ovarian tumor. (a) Large tumor cells showing solid growth in benign teratoma (hematoxylin and eosin, x4). (b) Tumor cells in small foci of solid growth had unclear nucleoli and a high nuclear/cytoplasmic ratio, resembling pulmonary small cell carcinoma (Hematoxylin and eosin, x10). (c) Tumor cells showing strong atypia with large eosinophilic cytoplasm and clear nucleoli in most of the solid growth (hematoxylin and eosin, x10). (d) Tumor cells positive for synaptophysin (x10). (e) Tumor cells partially positive for chromogranin A (x10).
Table 1. — A literature review of non-small cell endocrine tumors of the ovary.

<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>Chief complaint</th>
<th>Associated component</th>
<th>Stage</th>
<th>Surgery</th>
<th>Adjuvant therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirasawa [3]</td>
<td>56</td>
<td>Abdominal pain</td>
<td>Mucinous adenocarcinoma and teratoma</td>
<td>II</td>
<td>TAH+BSO+PN</td>
<td>not done</td>
<td>DOD 10 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>47</td>
<td>Abdominal pain</td>
<td>Adenocarcinoma, NOS</td>
<td>III</td>
<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>NED 11 months</td>
</tr>
<tr>
<td>Chènevert, et al.[5]</td>
<td>53</td>
<td>Abdominal pain</td>
<td>Mucinous adenocarcinoma and teratoma</td>
<td>IV</td>
<td>TAH+BSO+Omx+PN sampling</td>
<td>Paclitaxel and Carboplatin</td>
<td>DOD 5 months</td>
</tr>
<tr>
<td>Chènevert, et al.[5]</td>
<td>53</td>
<td>Abdominal pain</td>
<td>Mucinous adenocarcinoma and teratoma</td>
<td>IV</td>
<td>TAH+BSO+Omx</td>
<td>Cisplatin and Etoposide</td>
<td>DOD 7 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>25</td>
<td>Abdominal pain</td>
<td>Mature cystic teratoma</td>
<td>IV</td>
<td>BSO+Omx+App</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 36 months</td>
</tr>
<tr>
<td>This study</td>
<td>69</td>
<td>Abdominal pain</td>
<td>Mature cystic teratoma</td>
<td>IV</td>
<td>LSO</td>
<td>Paclitaxel and Carboplatin</td>
<td>DOD 6 months</td>
</tr>
</tbody>
</table>

**With teratoma component**

<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>Chief complaint</th>
<th>Associated component</th>
<th>Stage</th>
<th>Surgery</th>
<th>Adjuvant therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirasawa [3]</td>
<td>56</td>
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<td>II</td>
<td>TAH+BSO+PN</td>
<td>not done</td>
<td>DOD 10 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>47</td>
<td>Abdominal pain</td>
<td>Adenocarcinoma, NOS</td>
<td>III</td>
<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>NED 11 months</td>
</tr>
<tr>
<td>Chènevert, et al.[5]</td>
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<td>Abdominal pain</td>
<td>Mucinous adenocarcinoma and teratoma</td>
<td>IV</td>
<td>TAH+BSO+Omx+PN sampling</td>
<td>Paclitaxel and Carboplatin</td>
<td>DOD 5 months</td>
</tr>
<tr>
<td>Chènevert, et al.[5]</td>
<td>53</td>
<td>Abdominal pain</td>
<td>Mucinous adenocarcinoma and teratoma</td>
<td>IV</td>
<td>TAH+BSO+Omx</td>
<td>Cisplatin and Etoposide</td>
<td>DOD 7 months</td>
</tr>
<tr>
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<td>25</td>
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<td>Mature cystic teratoma</td>
<td>IV</td>
<td>BSO+Omx+App</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 36 months</td>
</tr>
<tr>
<td>This study</td>
<td>69</td>
<td>Abdominal pain</td>
<td>Mature cystic teratoma</td>
<td>IV</td>
<td>LSO</td>
<td>Paclitaxel and Carboplatin</td>
<td>DOD 6 months</td>
</tr>
</tbody>
</table>

**Without teratoma component**

<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>Chief complaint</th>
<th>Associated component</th>
<th>Stage</th>
<th>Surgery</th>
<th>Adjuvant therapy</th>
<th>Outcome</th>
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</thead>
<tbody>
<tr>
<td>Chen, et al.</td>
<td>44</td>
<td>Dyspnea, abdominal distension</td>
<td>Mucinous intraepithelial adenocarcinoma</td>
<td>I</td>
<td>TAH+BSO+Omx</td>
<td>Paclitaxel and Carboplatin</td>
<td>DOD 4 months</td>
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<tr>
<td>Ohira, et al.</td>
<td>33</td>
<td>Lower abdominal pain</td>
<td>Endometrioid adenocarcinoma</td>
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<td>Irinotecan and Nedaplatin</td>
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<tr>
<td>Collins, et al.</td>
<td>34</td>
<td>Weight loss</td>
<td>Mucinous BT with small foci of mucinous adenocarcinoma</td>
<td>I</td>
<td>TAH+BSO+Omx</td>
<td>Cisplatin and Cyclophosphamide</td>
<td>DOD 8 months</td>
</tr>
<tr>
<td>Jones, et al.</td>
<td>22</td>
<td>Abdominal distension</td>
<td>Mucinous cystadenoma</td>
<td>I</td>
<td>TAH+BSO+Omx</td>
<td>not done</td>
<td>DOD 10 months</td>
</tr>
<tr>
<td>Eichhorn, et al.[10]</td>
<td>77</td>
<td>Vaginal bleeding and pelvic mass</td>
<td>Endometrioid adenocarcinoma</td>
<td>I</td>
<td>TAH+BSO</td>
<td>Radiation</td>
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<tr>
<td>Eichhorn, et al.[10]</td>
<td>45</td>
<td>Abdominal distension and pain</td>
<td>Mucinous BT with small foci of mucinous adenocarcinoma</td>
<td>I</td>
<td>TAH+BSO+Omx</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 36 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>59</td>
<td>Abdominal pain</td>
<td>High grade adenocarcinoma, NOS</td>
<td>I</td>
<td>BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>NED 28 months</td>
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<td>Veras, et al.</td>
<td>54</td>
<td>Pelvic mass</td>
<td>Mucinous adenocarcinoma and endometrioid adenocarcinoma</td>
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<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>NED 66 months</td>
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<td>55</td>
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<td>Mucinous BT with intraepithelial adenocarcinoma</td>
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<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>NED 68 months</td>
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<td>Chemotherapy</td>
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<td>Eichhorn, et al.[10]</td>
<td>36</td>
<td>Right lower quadrant pain</td>
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<td>Mucinous BT</td>
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<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 2 months</td>
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<tr>
<td>Chen, et al.</td>
<td>73</td>
<td>Abdominal mass</td>
<td>Microinvasive mucinous adenocarcinoma</td>
<td>III</td>
<td>TAH+BSO+Omx</td>
<td>Paclitaxel, Cisplatin and Adriamycin Cisplatin-based chemotherapy</td>
<td>DOD 4 months</td>
</tr>
<tr>
<td>Eichhorn, et al.[10]</td>
<td>58</td>
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<td>Mucinous BT with small foci of mucinous adenocarcinoma</td>
<td>III</td>
<td>TAH+RSO+Omx</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 8 months</td>
</tr>
<tr>
<td>Choi, et al.</td>
<td>71</td>
<td>Pelvic mass</td>
<td>Serous carcinoma</td>
<td>III</td>
<td>TAH+BSO</td>
<td>Paclitaxel and Carboplatin</td>
<td>NED 8 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>53</td>
<td>Ascites</td>
<td>Endometrioid adenocarcinoma</td>
<td>III</td>
<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>NED 37 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>22</td>
<td>Abdominal pain</td>
<td>Mucinous BT with small foci of mucinous adenocarcinoma</td>
<td>IV</td>
<td>RSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 3 months</td>
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<tr>
<td>Veras, et al.</td>
<td>63</td>
<td>Ascites</td>
<td>Endometrioid adenocarcinoma</td>
<td>IV</td>
<td>TAH+RSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 9 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>42</td>
<td>Pelvic pain</td>
<td>None</td>
<td>IV</td>
<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>DOD 20 months</td>
</tr>
<tr>
<td>Veras, et al.</td>
<td>39</td>
<td>Abdominal pain</td>
<td>Mucinous adenocarcinoma</td>
<td>IV</td>
<td>TAH+BSO</td>
<td>Cisplatin-based chemotherapy</td>
<td>AWD 8 months</td>
</tr>
<tr>
<td>Ahmed, et al.</td>
<td>30</td>
<td>Asymptomatic</td>
<td>Mucinous cystadenoma</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy; RSO, right salpingo-oophorectomy; LSO, left salpingo-oophorectomy; PN, lymphadenectomy; Omx, omentectomy; App, appendectomy; BT, borderline tumor; DOD, dead of disease; NED, no evidence disease; AWD, alive with disease; N/A not available.
References


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Successful response to docetaxel treatment in recurrent ovarian granulosa cell tumor: a case report

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Summary

Background: Ovarian granulosa cell tumor (GCT) is primarily treated surgically. Treatment for advanced or recurrent disease includes primary or adjuvant chemotherapy. Data about the efficacy of treatment with paclitaxel are limited, without data about the role of docetaxel in treating recurrent GCT. Case: A 68-year-old patient with Stage IA ovarian GCT diagnosed ten years earlier, presented with a third episode of recurrent disease. Following the first event of recurrent disease, she underwent a second laparotomy followed by BEP chemotherapy. Because of new liver masses, she was treated with paclitaxel, with complete response. Following diagnosis of new liver lesions, third-line chemotherapy with docetaxel was initiated, resulting in stable disease and a PFI of 24 months. Conclusion: Docetaxel might be a good alternative for treating recurrent GCT.

Key words: Granulosa cell tumor; Docetaxel.

Introduction

Granulosa cell tumor (GCT) represents 5% of all ovarian cancers and accounts for 70% of the ovarian sex cord-stromal tumors, with an incidence of 0.4 to 1.7 per 100,000. Adult GCT may occur at any age, but usually presents during the perimenopausal or early post-menopausal period. Most patients with GCT present with Stage I disease (78-91%), have a good prognosis, and require no further postoperative adjuvant treatment. However, they require long-term follow-up, owing to the potential for recurrent disease, with a median time-to-relapse of approximately four to six years [1]. Treatment of advanced or recurrent GCT consists of surgical debulking, followed by platinum-based chemotherapy, with the BEP (bleomycin, etoposide, cisplatin) regimen producing the best response rates [2, 3]. During the last several years, the limited data available indicated the possible activity of taxanes in GCT, as a single agent or in combination with other drugs [4-6].

Docetaxel is a semi-synthetic taxane agent that acts by disrupting the essential cellular microtubular network; thus, it interferes with the mitotic and interphase cellular functions. Several studies demonstrated that the use of docetaxel instead of paclitaxel in the standard platinum-based regimen used for treating epithelial ovarian cancer might result in less neuropathy and equivalent efficacy [7, 8]. Although docetaxel has exhibited significant activity in epithelial ovarian cancer, to the best of our knowledge, only one study, including three patients with recurrent GCT treated with docetaxel has been reported. We herein report a patient with recurrent ovarian GCT with a marked response to docetaxel treatment.

Case Report

A 68-year-old patient with ovarian GCT, diagnosed ten years earlier, presented to our department with a new episode of recurrent disease. At the time of her initial examination (1996), she was diagnosed with Stage IA following TAH-BSO and surgical staging. Six years later, she was diagnosed with the first relapse, manifested by intraperitoneal lesions. Consequently, she underwent secondary cytoreduction followed by BEP administration, with a complete response. During the following four years, she experienced two more episodes of recurrent disease including the current one that preceded our report. The second-line chemotherapy chosen for the second episode of recurrent disease consisted of weekly paclitaxel. This regimen achieved clinical complete response after nine cycles. However, it was discontinued after a total of 15 cycles, when the patient complained of neurotoxicity-related symptoms. After progression-free-interval of four months, a computed tomography (CT) scan revealed new liver lesions (the largest was 2 cm). Based on the excellent results that were achieved with the previous taxane treatment, we chose docetaxel as the third-line chemotherapy. Docetaxel (25 mg/m²), given on days 1, 8, and 15, in a 28-day cycle, was started in February 2006. The patient completed 14 cycles of docetaxel, after which the disease was stable, without any evidence of new metastatic lesions on physical examinations and repeated CT scans (every 3 to 5 months). She tolerated the treatment well, with only two episodes of significant side-effects (CTCAE v3.0): grade 2-3 diarrhea managed by supportive care and grade 2-3 stomatitis, which resolved spontaneously.

Discussion

Because GCT is a rare malignancy, clinical trials aimed at determining which treatment regimens have the highest efficacy in advanced or recurrent disease are limited; therefore, optimal chemotherapy for this disease has not been defined. In the last decade, the BEP combination was reported as the preferred regimen for recurrent ovarian GCT, with 33% complete response and 50% partial response rates [9]. Other regimens with reported
anti-tumor activity include cisplatin, vinblastine plus bleomycin (PVB or VBP), cyclophosphamide, doxorubicin plus cisplatin (CAP), carboplatin plus etoposide, and doxorubicin alone. Several in vitro studies demonstrated the effect of paclitaxel on ovarian granulosa cells. These studies showed that paclitaxel causes a significant but reversible inhibition of granulosa cell steroidogenesis. We believe that docetaxel, which has the same inhibitory effect, might be as effective as paclitaxel for treating GCT.

Here, we report on a patient with refractory GCT who experienced successful treatment with docetaxel, resulting in long-term, stable disease. Even though docetaxel was given as a third line, there was a significant response, with a relatively long progression-free-interval with only minor side-effects. In the last few years, only a few investigators have reported the role of paclitaxel in treating ovarian GCT, with only one report of docetaxel treatment for these patients. Tresukosol et al. reported the first case of a patient with recurrent ovarian GCT who had a dramatic response to paclitaxel treatment. In their case, partial response was noted after the second cycle of paclitaxel, and it was maintained for 12 months [5]. Powell et al. reported on recurrent juvenile GCT treated with six cycles of paclitaxel and bleomycin as salvage chemotherapy, which resulted in 44 months of disease-free survival [6]. Brown et al. evaluated the efficacy of taxanes in treating 37 patients with ovarian sex cord tumor (SCT). The total response rate in the 34 patients who were treated with paclitaxel as a single agent was 18%, and 60%, when combined with platinum. Unlike the good results with paclitaxel in this report, the three patients who were treated with docetaxel exhibited progressive disease [4]. In a later publication, Brown et al. compared the results of two chemotherapy regimens in patients with ovarian SCT (21 patients with BEP vs 44 patients with paclitaxel) [10]. There was no significant difference in response rate (82%) for newly diagnosed patients, with a median overall survival of 97.2 months and 52 months, respectively. Among patients treated for recurrent measurable disease, the response rate was higher in the BEP group but was not statistically significant, 71% and 37%, respectively. The median progression-free survival rate was similar in both groups (11.2 and 7.2 months, respectively). However, the toxicity profile was better with taxane regimens. They concluded that taxanes demonstrated activity against SCT of the ovary and might be less toxic than BEP [10].

Our patient experienced significant clinical and symptomatic improvement upon docetaxel treatment. To the best of our knowledge, this is the first case in the literature involving response to docetaxel in patients with recurrent GCT. Further experience with this drug is needed to establish its efficacy in this group of patients.

References


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Cisplatin-gemcitabine as palliative chemotherapy in advanced squamous vulvar carcinoma: report of two cases

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Summary

Vulvar cancer (VC) is a rare disease, usually diagnosed in a stage still amenable to potentially curative treatments, including surgery and/or radiation therapy with or without chemotherapy. Several patients however present at diagnosis with metastatic disease and another 30-50% will relapse. Prognosis of metastatic or recurrent disease not amenable to salvage surgery or radiotherapy is very poor. Evidence about the efficacy of chemotherapy in this setting is limited and its role still remains unclear. At present there is no standard treatment for advanced VC and patients are usually treated with schedules adopted for chemoradiation or extrapolated from cervical cancer. We report our experience using a cisplatin-gemcitabine regimen in two cases of metastatic squamous cell VC. No response was obtained with this schedule. No other data are available in the literature about the choice of a cisplatin-gemcitabine regimen in this patient subset. The paucity of evidence about the role of palliative chemotherapy in metastatic VC justifies any effort to implement knowledge. For this reason we think it is notable to also report a negative experience. It is not possible for us to conclude that this chemotherapy would be unable to provide any benefit in a larger sample of patients; nonetheless we think that new agents, rather than combinations of older drugs, could hopefully provide more benefit.

Key words: Vulvar cancer; Chemotherapy; Gemcitabine.

Introduction

Vulvar cancer (VC) is a rare disease which mostly affects elderly women; it represents about 4% of all gynecological malignancies with an incidence of about two cases for every 100,000 women per year [1].

Usually VC has a squamous cell carcinoma histology and is diagnosed in a stage still amenable to potentially curative treatments, including surgery and/or radiation therapy with or without chemotherapy [2]. Several patients however present with metastatic disease at diagnosis and another 30-50% will develop a relapse within two years; recurrences are usually located in the groin [3].

Prognosis of metastatic or recurrent disease that is not amenable to salvage surgery or radiotherapy is very poor. Evidence about the role of chemotherapy in this setting is limited and its role still remains unclear, also because results remain disappointing [4].

While chemotherapy has shown activity on chemo-naive patients in the neo-adjuvant setting [5-9], many single agents (cisplatin, mitoxantrone, bleomycin, piperazinedione) have shown no response or short duration response in pretreated women with recurrent VC [2, 9]. Furthermore, only a modest activity with a response rate of 14% was achieved by the administration of a 3-weekly schedule of paclitaxel in recurrent or metastatic VC women in a phase II trial conducted by EORTC-GCC [10] while Olawaiye et al. [2] and recently Bacha et al. [11] reported an interesting activity of erlotinib in two and one cases, respectively.

Cormio et al. [4] found that combination chemotherapy with the doublet cisplatin-vinorelbine was an active and well tolerated regimen, also in previously irradiated women reporting a 40% overall response rate. Nonetheless, at present there is no standard treatment for advanced VC. This may be in part explained by the rarity of the disease and in part by the fact that patients are usually elderly and frequently present with important comorbidities, such as impairment of renal and cardiac function in particular [9], thus they are not ideal candidates for clinical studies.

The difficulties in enrolling patients and the lack of a standard treatment represent an obstacle in planning and conducting randomized controlled trials. Thus patients are usually treated with schedules adopted for chemoradiation or extrapolated from cervical cancer therapy.

In a phase III GOG trial on advanced cervical carcinoma Monk et al. [12] randomized 513 patients to receive four cisplatin containing doublets (with gemcitabine or vinorelbine or topotecan or paclitaxel, respectively) to assess the toxicity and efficacy. Though cisplatin combined with paclitaxel showed a favorable trend over other treatment arms, no significant difference in OS was found in this study [12], while a lower hematological toxicity was observed in the gemcitabine containing arm. Therefore the authors suggested that differences in chemotherapy scheduling and pre-existing morbidity are important in individualizing therapy [12].

We think it is noteworthy to report our experience about the use of a cisplatin-gemcitabine regimen in two cases of advanced squamous cell VC. To the best of our knowledge this is the first report in this patient subset.
Case Reports

Case 1

A 65-year-old woman with various comorbidities (congestive heart failure, hypertension, atrial fibrillation, diabetes mellitus and Graves’ disease) who experienced a left groin recurrence of squamous VC was treated with a palliative chemotherapy consisting of 75 mg/m2 cisplatin on day 1 and 1000 mg/m2 gemcitabine on days 1 and 8 every three weeks. She was previously submitted to a left hemivulvectomy with inguinal-femoral lymph node dissection and, because of her comorbidities, to adjuvant concurrent chemotherapy with cisplatin and radiation therapy (CT + RT) for a poorly differentiated carcinoma which was staged as pathological T1b N2c M0 (FIGO Stage IIIc). The treatment was well tolerated and only G2 neutropenia was observed. Treatment was stopped after two cycles because of local disease progression.

Case 2

A 73-year-old woman with a poor performance status due to cancer symptoms, also affected by hypertension and atrial chronic fibrillation, was treated with the same chemotherapy schedule (75 mg/m2 cisplatin on day 1 and 1000 mg/m2 gemcitabine on days 1 and 8 every three weeks). She presented with metastases to the left supra-clavicle lymph nodes and with left groin disease progression. She experienced disease progression during concurrent adjuvant cisplatin and capetecinate CT + RT. She had previously undergone radical vulvectomy with bilateral inguinal-femoral lymph node dissection for a moderately differentiated squamous VC, staged as pathological T1b N2c M0 (FIGO Stage IIIc). Treatment was relatively well tolerated (G2 anemia and G3 neutropenia were respectively observed) but despite a sizable reduction of the palpable left supraclavicular nodal metastases treatment was stopped after three cycles because of pulmonary progression of the disease.

Discussion

Commonly a patient with metastatic vulvar cancer presents with one or more comorbidities and a relatively unfavorable performance status, resulting in a major vulnerability to chemotherapy side effects. Therefore, decision making about the preferred treatment is difficult because chemotherapy in the palliative setting should mostly provide improvement in quality of life and disease control.

We chose a cisplatin-gemcitabine combination therapy which is effective against various cancer types (i.e., squamous non-small cell lung cancer, bladder, biliary tract), considering the favorable hematological toxicity profile reported [12] compared with other regimens and initial data of promising activity against cervical cancer [13].

Unfortunately, despite a manageable toxicity profile this regimen has shown no positive activity in our experience. The disheartening lack of response and paucity of evidence about the role of palliative chemotherapy in metastatic VC justify any effort to implement knowledge. For this reason we think it noteworthy to also report a negative experience.

It is not possible for us to conclude that this chemotherapy would be unable to provide any benefit in a larger sample of patients, nonetheless we think that new agents, rather than combinations of older drugs, could hopefully provide more benefit.

An interesting option may probably be offered in the near future by the use of agents such as anti-EGFR targeted therapy, i.e., erlotinib [12, 11] or erbitux [14], which seem to be active and with a different toxicity profile than chemotherapy (mostly cutaneous side effects).

References


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A case of extramedullary solitary plasmacytoma arising at the uterine cervix

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Summary
Extramedullary plasmacytomas are localized plasma cell neoplasms that arise in tissues other than bone and bone marrow. Primary plasmacytomas of the female genital tract are extremely rare and present a substantial diagnostic challenge. We report a case of a 38-year-old woman who presented with an endocervical polypoid. Surgical removal of the polyp was carried out. The final pathological report revealed primary plasmacytoma of the uterine cervix. The diagnosis was further facilitated by the use of immunohistochemistry and clonal immunoglobulin heavy-chain gene rearrangement. We performed a simple hysterectomy by laparoscopy on the patient and kept a close follow-up. She has remained well for more than eight years. The clinical characteristics and histopathologic findings of plasmacytoma of the uterine cervix are discussed.

Key words: Cervical plasmacytoma; Primary plasmacytoma; Uterine cervix.

Introduction
Plasmacytomas are localized, usually focal plasma cell neoplasms that occur in visceral structures, soft tissue, or bone [1]. Primary plasmacytomas of the uterine cervix are extremely rare and present a differential diagnostic challenge. To our knowledge, only nine cases have been reported in the published literature to date [1-9]. We report a case of a 38-year-old woman who presented with an endocervical polypoid. The diagnosis of plasmacytoma was facilitated by the use of immunohistochemistry and molecular clonality studies. After performing a simple hysterectomy by laparoscopy, the patient has remained well for more than eight years of follow-up.

Case Report
A 38-year-old woman presented for a routine health examination in September 2002 and a uterine cervical polypoid was found. She was referred to the Department of Gynecology in our hospital in October 2002. Physical examination revealed a 1 cm, pink, polypoid lesion extruding from her cervix showing no parametrium or vagina abnormality. Surgical removal of the polyp was done in the outpatient clinic. Unfortunately the final pathological report revealed primary plasmacytoma of the uterine cervix. To make the differential diagnosis of multiple myeloma, a series of laboratory tests were carried out. Complete blood count and urinalysis were normal. Abdominal ultrasound (US) and chest radiograph were unremarkable. Serum immunoglobulin levels were normal: IgG 11.9 g/l (normal range 8-15 g/l), IgA 1.35 g/l (normal range 0.85-3 g/l), IgM 0.56 g/l (normal range 0.5-2.5 g/l), k 2.71 g/l (normal range 1.72-3.83 g/l), k 1.46 g/l (normal range 0.81-1.92g/l), k/λ 1.86 (normal range 1.47-2.95 g/l). Serum and urine immunoaffixative electrophoresis and bone marrow biopsy showed no abnormality. After reviewing the case with hematologists, laparoscopic-assisted vaginal hysterectomy was carried out in November 2002. No residual lesions were found in the hysterectomy specimen. The patient recovered soon after the surgery. There has been no clinical evidence of recurrence for more than eight years of postoperative follow-up.

Pathologic findings
Microscopically, the polypectomy specimen was filled with a dense aggregate of mature and immature plasma cells. Plasma cells were present with a morphologic spectrum ranging from mature forms to highly atypical cells with large nuclei, hyperchromatic clumped chromatin, and prominent nucleoli (Figure 1). In poorly differentiated areas, the tumor showed obvious plasmablast differentiation characteristics. Tumor giant cells could be found with brisk mitotic activity and atypical mitotic figures. Immunohistochemical stain revealed CD138 (+), CD38 (+), CD19 (−) (Figure 2), CD56 (−), CD20 (−), λ-light chain (+), κ-light chain (−), CD3 (−), ALK (−), cytokeratin (−), CD10 (−), estrogen receptor (−) and progesterone receptor (−). The monoclonal population with an aberrant phenotype, (CD38+/CD19−/CD56−) distinguished the plasmacytoma from the plasma cells with a normal immunophenotype (CD38+, CD19+, CD56−) [10]. Genetically, IgH gene rearrangement was found by using FR3A primer determination. The subsequently obtained hysterectomy specimen weighed 75 g. It revealed chronic cervicitis in the cervix, and there was also chronic endometritis and multiple myomata in the uterus. We did not find plasma cells in the endocervix nor in the endometrium.

Discussion
Extramedullary plasmacytoma (EMP) is a rare category of plasma cell tumors. It may originate at any site, but occurs predominantly in the upper respiratory tract. The female genital tract is rarely the primary site for hematologic malignancies. Plasmacytomas of the uterine cervix...
cervix are extremely rare [11]. Our review of the literature revealed only nine cases localized to the uterine cervix since 1949 [1-9]. Based on this literature review, the median age at diagnosis was 39.4 years. Clinical symptoms, which are often nonspecific and mimic other disorders, are usually vaginal bleeding or vaginal discharge related to intercourse. One patient presented with pelvic pain. Physical examination findings are frequently inflamed-appearing cervicitis or mass lesions. The diagnosis of primary plasmacytoma of the uterine cervix usually requires biopsy confirmation. Due to the paucity of published data, this rare tumor poses a diagnostic challenge both for clinicians and pathologists. For pathologists, the possibility of a neoplastic process should be considered when highly atypical plasma cells are seen on a Pap test or cervical biopsy. The lesion is histopathologically characterized by infiltrates of plasma cells of diverse maturity and by their monoclonal immunoglobulin products [12]. Immunohistochemical stain and also gene rearrangement analysis should be suggested. The differential diagnosis of multiple myeloma should be excluded by a series laboratory tests such as normal serum immunofixative electrophoresis and bone marrow biopsy. Pathologically, differentiation from plasma cell-rich cervicitis can be excluded by the utility of immunophenotypic and molecular techniques [1].

For the treatment, our review of the literature revealed that one patient received only conization of the uterine cervix, three had a hysterectomy, two received local radiotherapy, two received a surgical excision of the tumor followed with irradiation and chemotherapy, and one patient was treated with three-dimensional conformal radiotherapy after conization.

In most reports on EMP, nearly all patients successfully achieve local control of 80% to 100%. The ten-year disease-free status and overall survival ranges from 50% to 80% [13]. As for plasmacytoma of the uterine cervix, our review of the literature revealed that the clinical follow-up of these patients ranged from three months to three or more years, and local recurrence or persistent disease was documented in three patients. Because of the rarity of this disease, there is no known guideline for the treatment and prognosis. Our patient underwent a simple hysterectomy by laparoscopy. As there was no residual lesion left in the hysterectomy specimen, we did not perform any other medical therapy. The patient recovered very soon and had no complications. However we are continuing a close follow-up every year. The patient has remained well for more than eight years. This is the only reported patient who has had such a long clinical follow-up to now. Thus we think simple hysterectomy may be a good and safe treatment for primary plasmacytoma of the uterine cervix. Nonetheless, more cases and experience are required to evaluate the optimal treatment and prognosis of this rare disease. Since about 30%-50% of patients will develop disease progression to myeloma [14], we should pay attention to the long term follow-up.

References

Primary endometrial natural killer (NK)/T cell lymphoma: case report and review of literature

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Summary
Primary natural killer (NK)/T cell lymphoma of the uterus is extremely rare. A case of primary NK/T cell lymphoma arising from the endometrium of the uterus was diagnosed by curettage which demonstrated the typical pathological characteristics of NK/T cell lymphoma. The patient received induction chemotherapy but refused hysterectomy. Later she developed bone marrow infiltration and eventually died on day 76 after diagnosis. The natural course of primary endometrial (NK)/T cell lymphoma was very aggressive. Conventional cytotoxic chemotherapy may be ineffective for this aggressive disease.

Key words: Endometrium; NK/T cell lymphoma; Extranasal type.

Introduction
Primary lymphoma of the female genital tract is uncommon with a frequency of 0.002% in all patients with extranodal lymphoma [1]. NK/T cell lymphomas are rare and often occur in the nasal or nasopharyngeal region. They are occasionally present in the upper digestive tract, skin, soft tissue and spleen. Involvement of the gynecological tract by NK/T cell lymphomas is extremely rare. A few cases of NK/T cell lymphoma arising in the uterus have been reported [2-6] and only one case with primary NK/T cell lymphoma involving the endometrium of the uterus has been reported in the English literature [2]. In this report, we describe a rare case of primary NK/T cell lymphoma arising from the endometrium which later progressed to bone marrow infiltration.

Case Report
A 36-year-old female was admitted with signs of intermittent vaginal bleeding and mild fever for two weeks. Her medical history indicated no suspicious signs (including Epstein-Barr virus-related disease history and past gynecological history). She had normal menstrual cycles. Physical examination revealed a mildly elevated temperature of 38.0°C. No visible skin lesions were found. Gynecological examination revealed hardness and swelling of the cervix. A 6 cm × 5 cm mass was palpable at the posterior aspect of the vagina fornix. The white blood cell count was 3.16 × 10⁹/l with 53.8% neutrophils and 36.4% lymphocytes. Her serum lactase dehydrogenase (LDH) was 345 U/l (normal value: 114~240 U/l). Chest computerized tomography (CT) and nasal sinus CT scan were normal. Enhanced CT of the abdomen and pelvis showed enlargement of the uterus without significant density change. Transvaginal ultrasonography demonstrated a mass occupying the middle and lower uterine segment and upper cervix (Figure 1: c). However, her fever had not receded but was aggravated. She did not respond well to broad-spectrum antibiotics. No evidence of any infection was found in her body. Follow-up bone marrow aspirate revealed progression of lymphoma with 24% lymphoblasts (POX staining was negative) (Figure 1: c). Flow cytometry of bone marrow cells indicated that 65.8% NK cells (CD3-CD56+) and 44% T cells (CD3+) took up the lymphocytes. The CD3-CD56+ NK cells expressed CD2, CD7, CD8, HLA-DR, and did not express CD5, CD56, and CD16. Among CD3+T cells, the percentage of CD2-CDS+ T cells was 46.78%. Monoclonal T-cell receptor (TCR)-γ rearrangement gene and immunoglobulin heavy chain-H (IgH) was detected on the bone marrow sample. The disease was restaged as Ann Arbor Stage IVB, IPI 3 or FIGO Stage IV. The patient eventually passed away due to multiple organ failure 76 days after diagnosis.

Discussion
Primary extranodal lymphoma involving the female genital tract is rare [7]; the prevalence was reported to be as high as 1.1% in a study of 1,467 cases [8] and as low...
phoma. General symptoms of uterine lymphoma include vaginal bleeding, fever of unknown origin and/or abdominal or pelvic pain [13]. EBV may play a causative role in some cases of lymphoma-like lesions of the cervix [14]. Its infection is closely related to nasal-type NK/T cell lymphoma as well as extranasal NK/T cell lymphoma as described in this case [15, 16].

Treatment of female genital tract lymphoma includes surgery, radiation, chemotherapy alone or a combination of these therapies. The optimal therapy is still unknown because of the rarity of primary uterine lymphomas. Many patients with early-stage cervical lymphoma received only local therapy in the form of surgery and

Figure 1. — Transvaginal three-dimensional ultrasonography: a, b 4.6 × 3.0 × 3.8 cm and 3.8 × 2.9 × 3.3 cm low echoes (arrows) can be detected on the posterior wall of middle and lower segment of uterus and posterior wall of cervix; disturbance blood flow signal was detected in the mass; resistance index (RI): 0.47-0.51. c the mass (2.2 × 1.6 cm, arrow) reduced significantly after chemotherapy.

Figure 2. — NK/T cell lymphoma of the endometrium: a residual endometrial gland (arrow, a, × 200) can be found under the background of neoplastic cells. Higher magnification shows medium to large-sized lymphoid cells with irregular nuclear contours; piece-meal necrosis and lymphoid cells infiltration in endometrial tissues can be observed (b × 400). Immunohistochemical staining showed strong reactivity of lymphoid cells by antibodies against the T cell-associated antigen CD3 (c × 400) and the NK cell associated antigen CD56 (d × 400) and granzyme B (e × 200). In situ hybridization for EBER shows positive nuclear signals in many tumor cells (f × 400).
radiation but remained disease free for two or more years [11] so total hysterectomy may be one of the optimal treatments for a solitary uterine lymphoma at early stage. It was reported that both children and adolescents with early-stage extranodal nasal-type NK/T-cell lymphoma treated with primary radiotherapy had a favorable prognosis [17], but the role of radiotherapy in extranasal type NK/T-cell lymphoma remains to be defined. In this case, the patient does not respond to chemotherapy alone. Failure of chemotherapy may be due to the fact that this type of lymphoma was likely insensitive to solitary chemotherapy. A potential reason for the resistance in NK/T cell lymphomas is the high expression of p-glycoprotein which can lead to resistance of chemotherapy, particularly, the anthracyclines [18]. The L-asparaginase-containing regimens have been proposed to bypass the presence of p-glycoprotein and have shown excellent activity in refractory extranodal NK/T-cell lymphoma. Therefore, this type of agent can be considered as a second-line treatment option [19, 20]. More aggressive treatment or a combination of treatment options can be used in the future for patients with primary endometrial NK/T cell lymphoma.

References

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Müllerian papilloma in a patient with Proteus syndrome: case report and review of the literature

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Summary

Background: Müllerian papilloma is a rare benign tumor of the female genital tract. It mostly affects girls less than five years old. After the treatment long-term follow-up is needed as there is a chance of recurrence, but even then the prognosis is excellent. Case: A 19-year-old girl with Proteus syndrome presented with vaginal bleeding. The histological examination revealed Müllerian papilloma of the uterine cervix and large bilateral ovarian cystadenomas. The patient was treated with a radical operation, because there were signs of more aggressive behavior in the tumor. The patient is alive and free of disease five years after the operation. Conclusion: The medical care of patients affected by rare disorders depends heavily on experiences gained from cases published in the medical literature. Since there is not much experience with tumors in Proteus syndrome we believe that this case can aid in shedding light on this subject.

Key words: Müllerian papilloma; Proteus syndrome.

Introduction

Müllerian papilloma is a rare benign tumor of the female genital tract. Its precise incidence is not known as there are only case reports in the literature [1-5]. It was first described in 1951 by James who observed a benign polypoid tumor of the uterine cervix in a three-year-old girl [6]. For a long time there has been disagreement about the histogenesis of this tumor. The first descriptions named it benign mesonephric papilloma as it was thought to arise from remnants of an involuted mesonephric duct [7-9]. Ulbright in 1981 was the first to propose that the tumor was of Müllerian origin [8]. Later on there were several reports on immunohistochemical and ultrastructural findings that confirmed this hypothesis and thus the term benign Müllerian papilloma was applied [1, 2, 4].

It mainly occurs in prepubertal girls less than five years of age, and the major symptom is intermittent and non-cyclical vaginal bleeding. It is less common in postmenarchal girls where the main symptom is intermenstrual or contact bleeding [1, 2, 10]. It is important that in prepubertal groups, the tumor can clinically and histologically mimic malignant embryonal rhabdomyosarcoma (sarcoma botryoides), a more common and aggressive tumor in this age group. Moreover, the differential diagnosis includes papilloma, condyloma, papillary carcinoma (villoglandular and pure papillary), verrucous carcinoma, clear cell adenocarcinoma and endodermal sinus tumor [11]. Treatment of Müllerian papilloma is usually by local excision, avulsion of the tumor with forceps, and ligation, and also laser surgery or fulguration is an option. Nevertheless long-term follow-up is needed as there is a chance of recurrence but even then the prognosis is excellent [2].

Proteus syndrome is a rare disorder of progressive, asymmetric and disproportionate overgrowth of tissue [12]. Commonly involved tissues include connective tissue, bone, fat and skin but it appears that it can affect any tissue. It is assumed that progressive tissue overgrowth usually plateaus after adolescence [12]. As a discrete clinical entity it was first described in the literature in 1979 by Cohen and Hayden [13] and assigned its name in 1983 by Wiedemann et al. to denote its variability in clinical expression [14]. There are less than 100 cases published in the literature [15]. The cause is still not known but it is thought to arise from a postzygotic somatic mutation as the condition would be lethal in the nonmosaic state [16]. There is a great variability in clinical presentation of Proteus syndrome thus diagnosis can sometimes be difficult to make as there are no diagnostic tests available at this time. Table 1 lists the revised diagnostic criteria for Proteus syndrome currently used [17]. It appears that this syndrome has for a long time had a suspected but not proven predisposition to various tumors [17]. Most apparent is the association with bilateral ovarian cystadenomas and monomorphic adenomas of the parotid gland [18]. These two types of tumors are so specific for Proteus syndrome that they are included in the diagnostic criteria [17].

We present a case of Müllerian papilloma in a patient with Proteus syndrome. The case is unique in that it was a rare benign tumor occurring in a rare clinical syndrome. Herein, the clinical findings of the patient are discussed and the current clinical diagnostic criteria for Müllerian papilloma reviewed.

Case Report

A 19-year-old girl was referred to our gynecological department because of irregular menstrual and contact bleeding for the previous three months. In her past medical history it was notice-
able that at the age of one she had undergone a vaginoscopy because of vaginal bleeding, the cause of which was vaginitis. She was born with a congenital anomaly of her left hand. For the correction of macrodactily, she had had ten reconstructive operations of her left hand and at the age of seven she was diagnosed with Proteus syndrome. As a child she had asthma, but at the beginning of puberty the condition ceased. Her sexual development was normal, periods started at the age of 13 and until three months before they had been regular. She reported stronger vaginal discharge over the last two years but it was never bloody.

A gynecological examination under general anesthesia revealed the presence of a 2 x 2 cm papillary mass on the anterior portion of the uterine cervix. There were numerous submucosal papillary formations up to 5 mm on the anterior vaginal wall that spread to the left vaginal fornix. There was a normal sized uterus and two intraabdominal tumors palpated and no signs of parametrial infiltration. On magnetic resonance imaging a tumor in the uterine cervix and large ovarian masses on both sides were seen. The mass on the left side was 8 x 7 cm and on the right side 10 x 12 cm. The biopsy of the cervical tumor at first revealed a well differentiated villoglandular mucinous adenocarcinoma, but in the differential diagnosis there was also a Müllerian papilloma. After the revision of histology by three different pathologists, including one abroad, the diagnosis was confirmed as Müllerian papilloma with intense squamous metaplasia that at the age of one she had undergone a vaginoscopy because of vaginal bleeding, the cause of which was vaginitis. She was born with a congenital anomaly of her left hand. For the correction of macrodactily, she had had ten reconstructive operations of her left hand and at the age of seven she was diagnosed with Proteus syndrome. As a child she had asthma, but at the beginning of puberty the condition ceased. Her sexual development was normal, periods started at the age of 13 and until three months before they had been regular. She reported stronger vaginal discharge over the last two years but it was never bloody.

A gynecological examination under general anesthesia revealed the presence of a 2 x 2 cm papillary mass on the anterior portion of the uterine cervix. There were numerous submucosal papillary formations up to 5 mm on the anterior vaginal wall that spread to the left vaginal fornix. There was a normal sized uterus and two intraabdominal tumors palpated and no signs of parametrial infiltration. On magnetic resonance imaging a tumor in the uterine cervix and large ovarian masses on both sides were seen. The mass on the left side was 8 x 7 cm and on the right side 10 x 12 cm. The biopsy of the cervical tumor at first revealed a well differentiated villoglandular mucinous adenocarcinoma, but in the differential diagnosis there was also a Müllerian papilloma. After the revision of histology by three different pathologists, including one abroad, the diagnosis was confirmed as Müllerian papilloma with intense squamous metaplasia.

Table 1. — Revised Proteus syndrome diagnostic criteria [17].

<table>
<thead>
<tr>
<th>General criteria</th>
<th>Specific criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>All of the following:</td>
<td>Either</td>
</tr>
<tr>
<td>Mosaic distribution of lesions</td>
<td>Category A or,</td>
</tr>
<tr>
<td>Sporadic occurrence</td>
<td>Two from category B or,</td>
</tr>
<tr>
<td>Progressive course</td>
<td>Three from category C</td>
</tr>
</tbody>
</table>

Specific criteria categories

A. 1. Cerebriform connective tissue nevus
c

B. 1. Linear epidermal nevus

2. Asymmetric disproportionate overgrowth
   
   One or more:
   
   a. Limbs:
      
      Arms/legs
      
      Hands/feet/digits
      
      Extremities
   
   b. Hyperostoses of the skull
d
   c. External auditory meatus
e
   d. Megaspondylodyplasia
   
   e. Viscera:
      
      Spleen/thymus
   
   3. Specific tumors before 2nd decade
      
      One of the following:
      
      a. Ovarian cystadenoma
      
      b. Parotid monomorphic adenoma

C.1. Dysregulated adipose tissue

   Either one:
   
   a. Lipomas
   
   b. Regional absence of fat

2. Vascular malformations

   One or more:
   
   a. Capillary malformation
   
   b. Venous malformation
   
   c. Lymphatic malformation

3. Lung cyst

4. Facial phenotype

   All:
   
   a. Dolichocephaly
   
   b. Long face
   
   c. Down slanting palpebral fissures and/or minor ptosis
   
   d. Low nasal bridge
e
   
   e. Wide or anteverted nares
   
   f. Open mouth at rest

To make a diagnosis of PS all the general criteria need to be available and also various specific criteria.

1 Cerebriform connective tissue nevi are skin lesions characterized by deep grooves and gyrations as seen on the surface of the brain.

2 Asymmetric, disproportionate overgrowth should be carefully distinguished from asymmetric, proportionate overgrowth.

3 The facial phenotype has been found, to date, only in PS patients who have mental deficiency and, in some cases, seizures and/or brain malformations.
Table 2. — Müllerian papilloma (cases reported in the English literature).

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient age</th>
<th>Symptom(s)</th>
<th>Location</th>
<th>Macroscopic appearance</th>
<th>Treatment</th>
<th>Outcome</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>James [6]</td>
<td>3 years</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>Small papilloma like tumor, friable, bleed easily</td>
<td>Local excision</td>
<td>NSR, 6 months/10 years*(selzer)</td>
<td></td>
</tr>
<tr>
<td>Novak et al. [9]</td>
<td>14 months</td>
<td>Bleeding, vaginal mass</td>
<td>Vagina</td>
<td>Liverlike protrusion from the vagina</td>
<td>Local excision</td>
<td>NSR, 6 months</td>
<td></td>
</tr>
<tr>
<td>Selzer and Nelson [10]</td>
<td>3 years</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>Papillomatous growth</td>
<td>Local excision</td>
<td>NSR, 8,5 years</td>
<td></td>
</tr>
<tr>
<td>Selzer and Nelson [10]</td>
<td>3 years</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>Polypoidal structure, friable, bleed on manipulation</td>
<td>Local excision</td>
<td>NSR 1.5 year after second excision/2 years</td>
<td>6 months from first treatment small polyoid mass on the cervix seen, the base of the polyp was excised</td>
</tr>
<tr>
<td>Janovski and Kasdon [7]</td>
<td>5 years</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>1 cm polyoid mass on the posterior cervical lip and smaller on the anterior cervical lip growing into the anterior vaginal fornix</td>
<td>Local excision</td>
<td>NSR, 4 months</td>
<td></td>
</tr>
<tr>
<td>Norris and Taylor [25]</td>
<td>1 day</td>
<td>Vaginal mass</td>
<td>Vagina</td>
<td>Grapelike mass protruding from introitus</td>
<td>Biopsy</td>
<td>Mass regressed, NSR, 8 years</td>
<td>Norris and Taylor[25]</td>
</tr>
<tr>
<td>Norris and Taylor[25]</td>
<td>1 day</td>
<td>Vaginal mass</td>
<td>Vagina</td>
<td>0.5 cm soft nodule</td>
<td>Local excision</td>
<td>NSR, 8 months</td>
<td></td>
</tr>
<tr>
<td>Ulbright et al. [8]</td>
<td>5 years</td>
<td>Vaginal mass</td>
<td>Vagina</td>
<td>5 x 3 cm mass located in the vaginal wall, vaginal mucosa intact and uninvolved</td>
<td>Resection of the tumor through abdominal approach</td>
<td>NSR, 1 year</td>
<td></td>
</tr>
<tr>
<td>Andrews et al. [21]</td>
<td>3 years</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>2 x 1.5 x 0.5 cm red, soft polyp</td>
<td>Local excision</td>
<td>NSR, 10 months</td>
<td></td>
</tr>
<tr>
<td>Lüttges and Lübbe [4]</td>
<td>5 years</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>0.8 cm polyoid tumor, bleeding on contact</td>
<td>Pevulsion with forceps</td>
<td>Not stated</td>
<td>2 years later recurrent 1.8 cm papillary tumor at the site of former excision</td>
</tr>
<tr>
<td>Schmedding et al. [1]</td>
<td>2 years</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>Soft, red polyp, bleed easily</td>
<td>Local excision</td>
<td>NSR, 6 months</td>
<td></td>
</tr>
<tr>
<td>Smith et al. [22]</td>
<td>4 years</td>
<td>Bleeding, pain</td>
<td>Cervix</td>
<td>Papillary lesion</td>
<td>Biopsy, 1 year later rebiopsy and fulguration</td>
<td>No bleeding or pain, 11 months</td>
<td>1 year after treatment recurrence, biopsy without complete excision (concern of significant cervical destruction)</td>
</tr>
<tr>
<td>Dobbs et al. [23], Abu et al.</td>
<td>52 years</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>Polypoid mass</td>
<td>Ligation, local excision, biopsy</td>
<td>Over the period of 40 years more than 10 recurrences</td>
<td>At the age of 49 borderline malignant changes in the papilloma noted, due to severe cerebral palsy radical treatment not possible, at biopsy 3 years later areas of clear cell carcinoma</td>
</tr>
<tr>
<td>McCluggage et al. [19]</td>
<td>24 years</td>
<td>Pain</td>
<td>Vaginal mass</td>
<td>4 cm intramural nodule with overlying mucosa</td>
<td>Local excision</td>
<td>Not stated</td>
<td>Primigravida in 16th week of pregnancy</td>
</tr>
<tr>
<td>Cohen et al. [20]</td>
<td>13 years</td>
<td>Vaginal mass</td>
<td>Vagina</td>
<td>13 x 10 mm polyoid lesion with a cauliflower-like dark red surface</td>
<td>Not stated</td>
<td>Not stated</td>
<td>Acetaminophen-related Lyell’s syndrome a year before with numerous pigmented scars all over the body, melanin in epithelial cells and melanocytes present in the tumor</td>
</tr>
<tr>
<td>Arbo et al. [26]</td>
<td>2 years</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>Polypoid glandular epithelium</td>
<td>Local excision</td>
<td>Not stated</td>
<td></td>
</tr>
<tr>
<td>Mierau et al. [2]</td>
<td>4 years</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>4.8 x 0.9 x 0.9 cm grape-like mass 'minute' erosion on the vaginal wall</td>
<td>Local excision</td>
<td>NSR, 4 years</td>
<td></td>
</tr>
<tr>
<td>Mierau et al. [2]</td>
<td>9 days</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>1 cm frond-like papillomatous mass</td>
<td>Fulguration</td>
<td>NSR, 8 months</td>
<td></td>
</tr>
<tr>
<td>Lane et al. [5]</td>
<td>18 months</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>1 cm frond-like papillomatous lesion</td>
<td>Local excision</td>
<td>NSR, 6 weeks</td>
<td></td>
</tr>
<tr>
<td>Hollowell et al. [11]</td>
<td>15 months</td>
<td>Bleeding</td>
<td>Cervix</td>
<td>Villous lesion</td>
<td>Local excision</td>
<td>NSR, 6 weeks</td>
<td></td>
</tr>
<tr>
<td>Reck-Burneo et al. [27]</td>
<td>2 years</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>4 cm mildly lobulated, pedunculated mass</td>
<td>Local excision</td>
<td>NSR, 3 years</td>
<td></td>
</tr>
<tr>
<td>Tumini et al. [28]</td>
<td>9 years</td>
<td>Bleeding</td>
<td>Vagina</td>
<td>Papillar mass</td>
<td>Local excision</td>
<td>Not stated</td>
<td></td>
</tr>
</tbody>
</table>

Note: NSR no signs of recurrence
plasia, high proliferative index and focally atypical cells, which could have indicated severe inflammation or the beginning of malignant progression. The tumor was strongly positive for CA 125 and negative for CD 10, while estrogen and progesterone receptors were uniform and strongly positive.

Due to large bilateral ovarian tumors and Müllerian papilloma with high mitotic activity and focus of atypical cells, we performed abdominal hysterectomy with bilateral salpingo-oophorectomy. Both ovarian tumors were sent for frozen section biopsy. The definite histology was Müllerian papilloma of the uterine cervix and bilateral cystadenomas of the ovaries with a focus of high epithelial cell atypia in the right one. Figures 1-3 show the Müllerian papilloma of the uterine cervix and the removed bilateral cystadenomas of the ovaries in our patient. The operation and postoperative period were without complications. Six months later we performed another procedure to eliminate the small papillomas on the anterior vaginal wall. We biopsied one and performed laser destruction of the others. Histology of the biopsied tissue was only squamous epithelium. A similar procedure was also performed 4.5 years after the first operation, and histology of the biopsied tissue was vaginal adenosis.

The patient is alive and well with no signs of recurrence of the disease five years after the surgery.

Discussion

Müllerian papilloma is a rare lesion of the uterine cervix and vagina [1, 2]. To date only 22 cases have been published in the English literature (Table 2). It occurs almost exclusively in children usually between two and five years of age; occasionally in adults. The main symptom is vaginal bleeding [3], sometimes pain [19] or a painless vaginal mass [20] could be the leading symptom. Macroscopically tumor usually appears as a grape-like cluster or papillary mass present on the cervix or vagina but it could also represent as a small erosion [2] or intramural nodule [8, 19]. Tumor could be located on the cervix or vagina and of all published cases 13 were vaginal tumors and nine were cervical. Histologically it is most often described as papillary structures composed of branching fronds with fibrovascular cores lined by cuboidal to columnar epithelium [1, 2, 4]. There have only been three published articles [2, 8, 21] on ultrastructural findings in Müllerian papilloma, the most recent and detailed being one from Mierau et al. They were the first who demonstrated the presence of mucin and glycogen granules in neoplastic cells, which further supported the Müllerian origin of this tumor. The second important thing they stated – that the histological description of this tumor was composed of a fibrovascular core is misleading, since the spindle cells within the supporting stalk did not display the features of fibroblasts and virtually no collagen was found within the extracellular matrix. Immunohistochemical studies showed the surface epithelial cells to be reactive to cytokeratin, epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA). The subepithelial spindle cells were reactive for vimentin, but not for muscle specific actin, desmin or myoglobin [2].

There are few cases of recurrences of Müllerian papilloma published in the literature [22-24] and in all cases simple excision was a suitable mode of treatment. Even incomplete excision has been reported to be curative for tumors presenting during the newborn period [2]. It is thought that maternal hormones induce the intrauterine development of tumors seen in infants and since only those who represent with bleeding come to our attention it is possible that some of these tumors spontaneously regress [2]. Only one case of malignant transformation of Müllerian papilloma was observed following a series of recurrences over a period of 40 years [2].

It has been strongly suspected, but not a proven predisposition to tumors in Proteus syndrome [18]. Knudson’s two-hit hypothesis for neoplasms developing in Proteus syndrome was proposed [18]. Regarding this hypothesis one allele for the postulated ‘proteus gene’ might mutate in a somatic cell, resulting in the somatic mosaicism of Proteus syndrome. The first hit would be cellular overgrowth, producing generally known manifestations of Proteus syndrome-like hamartomas. In some patients, a second hit on the other ‘Proteus allele’ might result in the uninhibited growth of neoplasms [18]. To our knowledge Proteus syndrome has never been associated with Müllerian papilloma.

In our case the proliferative index was high and there were focally atypical cells in the tumor, which could have indicated severe inflammation or the beginning of malignant progression so the future behavior of the tumor was difficult to predict; there were also large bilateral ovarian masses present and we had to carry out a more radical procedure. Also the patient was diagnosed with Proteus syndrome and since we do not know the behavior of tumors in this syndrome this added to our decision to perform a more radical operation.

Conclusions

The medical care of patients affected by rare disorders depends heavily on experiences gained from cases published in the medical literature. We believe that this is the first case of a Müllerian papilloma described in a patient with Proteus syndrome. The case is unique as there was the occurrence of a rare benign tumor in a rare syndrome. Since there is little information on tumors in Proteus syndrome we believe that this case can aid in shedding light on the subject.

References


“Intestinal-type” mucinous adenocarcinoma of the vulva: a report of two cases

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Summary

Background: “Intestinal-type” mucinous carcinoma of the vulva is extremely rare with very few cases reported in the literature. Case report: The authors report two cases. In both cases, restaging was performed with total body computed tomography (CT) scan, gastroscopy, and colonoscopy that showed no other site of disease. A radical vulvectomy with bilateral systematic inguinal lymphadenectomy was performed, and in both cases no residual disease was found. Conclusion: Intestinal-type mucinous carcinoma of the vulva has a poor prognosis. Strict endoscopic follow-up of the colon is mandatory in such cases, considering the high propensity of gastrointestinal (GI) tumors.

Key words: Adenocarcinoma of the vulva; Intestinal-type.

Introduction

Primary adenocarcinomas of the vulva are rare and mostly originate from Bartholin’s glands [1-5]. Other possible origins are sweat glands [1-8], Skene’s glands [1-7], minor vestibular glands [1, 2, 4, 5, 8], aberrant mammary tissue [1-8] or endometriotic implants [1, 5]. Villoglandular adenocarcinoma of colonic-type is a rare variant of vulvar adenocarcinoma of unknown origin, with only few cases reported to date on the vulva [1-5, 7] and in the vagina. It is characterized by villoglandular architecture, mucinous-type epithelium with intestinal differentiation (goblet cells), and direct apposition of the tumor with the surface epithelium. The possibility that these tumors may originate from cloacal remnants has been raised [1-6, 8]. In fact, Novak and Woodruff [9] were the first to propose that misplaced cloacal remnants could be found in the vulva. The hypothesis that such misplaced remnants may undergo malignant transformation into a villoglandular adenocarcinoma of colonic-type was raised later by Tiltman and Knutzen [4].

The authors report two additional cases and review the literature discussing this rare disease.

Materials and Methods

Case 1

A 59-year-old, gravida 5, para 3, obese woman was referred to the hospital for a nodular vulvar lesion with mild discomfort and burning sensation. During vulvoscopy, a papillary epithelium with atypical vessels was seen on the left and posterior vestibular mucosa (Figure 1) and the lesion extended up to the hymenal ring.

Case 2

A 42-year-old white woman, gravida 1, para 1, was referred for a small vulvar lesion of one cm. An incisional biopsy disclosed cloacogenic adenocarcinoma of the vulva. Complete restaging was performed with total body CT scan, gastroscopy, and colonoscopy that showed no other site of disease. The patient underwent complete surgery consisting in radical vulvectomy with colpectomy and inguino-femoral lymphadenectomy. Pathologic examination revealed adenocarcinoma of intestinal-type with vulvar-vagina, extension without nodal involvement. During follow-up with yearly colonoscopy, she was found to have dysplastic polyp in the sigmoid colon, and is alive without disease at 39 months after primary diagnosis.

An excisional biopsy disclosed a well-differentiated adenocarcinoma of intestinal-type with mucin secreting cells. Hysteroscopy showed normal endometrium for patient’s age, apart from a benign polyp. Transvaginal ultrasonography (TVUS) did not reveal ovarian abnormalities. GI endoscopy and colonoscopy did not detect any lesion examining the esophagus, stomach, duodenum, and colon. CT scan was also negative. Tumor markers (AFP, CEA, CA125, CA15.3, CA19.9) were all within normal limits. Following patient’s informed consent, a radical left emivulvectomy with colpectomy of the lower one-third, inguino-femoral lymphadenectomy, and panniculectomy were performed. Post-operative healing was good and the patient was discharged from the hospital six days after surgery. Follow-up was negative for 36 months, while a CT scan demonstrated two metastatic lesions in the liver and also pelvic and aortic nodes involvement. Colonoscopy showed a two by two cm lesion in the upper part of the rectum, and pathologic examination disclosed grade 2 mucinous adenocarcinoma. Fine-needle biopsy of the bone marrow was consistent with secondary involvement. First line chemotherapy resulted in complete remission of all lesions, but six months later the patient developed diffuse recurrent disease, and the patient died of disease 18 months after diagnosis.

References


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had been considered, but no endometriosis was found, and the tumor was significantly different from a breast and endometrioid carcinoma [11].

The histopathologic aspect of this case is in many aspects identical to that of the other seven similar cases reported elsewhere [1-5, 7, 11]. It consisted of a villoglandular adenocarcinoma in direct continuity with the epidermis. The immunophenotypic pattern [12] of this case, namely a strong cytokeratin 7 and weak cytokeratin 20 staining, is comparable to that in the case reported by Rodriguez et al. [7]. Such profile raises questions about the origin of this rare disease. Origin from an area of cloacogenic metaplasia has been suggested but remains speculative [1-6, 8]. In all the cases described, the clinical behavior of this rare malignant neoplasm seems to be rather indolent, and patients are generally doing well, either after radical vulvectomy or wide local excision. Bilateral inguinal lymph node dissection has been performed in four of the reported cases [1-4] and only one disclosed an ipsilateral metastasis [4]. Despite this apparent low-risk of metastasis, the authors' knowledge of this tumor is limited, and either ipsilateral or bilateral inguinal lymph node dissection appears to be indicated [11].

It is interesting to note that the first patient had recurrent disease in multiple sites after primary excision. The authors do not know whether this was a real recurrence from the primary vulvar tumor or if it was a secondary metacronous intestinal cancer. However, also the second patient developed atypical colonic hyperplasia, suggesting that the colonic tract should be carefully evaluated, both at diagnosis and during follow-up, in these patients.

Conclusions

Both pathologists and clinicians should be aware of the existence of this rare tumor. However, more cases are needed to fully understand its origin and to better establish its long-term prognosis.

Discussion

To the knowledge of the authors, these are two other cases of mucinous carcinoma “intestinal-type” of the vulva, also known as adenocarcinomas of cloacogenic origin [1-5, 7]. The possibility that the lesion in the two patients might have been metastatic and of colonic origin was ruled out by a complete negative clinical workup. Furthermore, the immunohistochemical profile showed cytokeratin 7 expression, which is not consistent with an intestinal origin [10, 11]. Primary adenocarcinomas of the vulva are rare, but mostly arise from Bartholin’s glands. Contrary to this case, Bartholin’s glands adenocarcinomas are deeply infiltrative, do not involve the overlying epidermal surface, and an in situ component is usually present in the adjacent benign glands [2, 8]. A sweat gland origin is also unlikely because these adenocarcinomas lack this typical villoglandular pattern and do not contain diastase-resistant periodic acid-Schiff material [2, 3]. A paraurethral Skene’s or minor vestibular gland origin has also been excluded because in this case, the tumor was located away from the introitus and the urethra. An origin from aberrant mammary tissue or endometriosis

References


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A rare case of umbilical and vaginal metastasis from endometrial cancer - review of the literature

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Summary

Introduction: Metastases from primary endometrial cancer to the umbilicus are extremely rare. This unusual site of metastases has been described as Sister Joseph’s nodule. Material and Method: We present a case of a 73-year-old Caucasian woman with a BMI of 30, type II diabetes mellitus, hypertension, and umbilical and vaginal metastasis of endometrial adenocarcinoma (FIGO Stage IIIa, G2). Total abdominal hysterectomy and bilateral salpingo-oophorectomy by Pfannenstiel dissection, had been performed eight months before. The size of the umbilical mass was 2 x 2 cm. A second laparotomy including full recession of the umbilical ring, omentectomy, bilateral inguinal lymph nodes and excision of the upper one-third of the vagina was performed. Histological diagnosis revealed metastases of the same origin with her primary disease. Conclusion: The exact mechanism of implantation of cancer cells at the site of the umbilical ring is still unclear. Perhaps malignant cells penetrated the thickness of the uterine wall and spread intraperitoneally to reach the umbilical ring. The exfoliation of cells from the primary tumor via the fallopian tubes could be another possible explanation. Unfortunately, the presence of umbilical metastasis is a poor prognostic feature and sign of advanced neoplastic disease. The survival rate of these patients is influenced by the type of treatment and time of the diagnosis.

Key words: Endometrial carcinoma; Sister Mary Joseph nodule; Umbilical metastases.

Introduction

Endometrial carcinoma is the most common malignancy of the female tract. About 2% to 3% of women will develop endometrial cancer during their lifetime [1]. The median age at diagnosis is the sixth decade, and only 20-25% will be diagnosed premenopausally [1]. Endometrial adenocarcinoma invades and spreads locally, but can also have lymphatic or hematogenous spread. Metastases from malignancies of the female tract and especially from primary endometrial cancer to the umbilicus are extremely rare. This unusual site of metastases has been described in the past only in a few cases in the English literature and it has been described as Sister Joseph’s nodule [2]. Only 2% of these rare metastases are of endometrial origin. In case of an umbilical malignant tumor, 75% correspond to a Sister Joseph’s nodule. This secondary localization of the malignancy could appear before, during or after the initial diagnosis of the primary tumor. We present a rare case and to the best of our knowledge this is the first one in our country.

Case Report

We present a case of a 73-year-old Caucasian woman with a body mass index (BMI) of 30, type II diabetes and hypertension, who was referred to our department with umbilical and vaginal metastasis of endometrial adenocarcinoma (FIGO Stage IIIa, G2). The initial diagnosis, staging and surgical treatment with total abdominal hysterectomy and bilateral salpingo-oophorectomy by Pfannenstiel dissection had been performed eight months before. Pelvic lymphadenectomy was not performed at that time. Peritoneal washings and vaginal and parametrial resection margins were free of disease.

On admission, the size of the umbilical mass was 2 x 2 cm and occupied the whole umbilical ring (Figures 1 and 2). There was also an exophytic vaginal recurrence of 1.5 x 2 cm. Magnetic resonance imaging abdominal scan revealed bilateral enlarged inguinal lymph nodes. CA 19-9 serum level was 198 IU/ml and CA 125 was 32 IU/ml. A second laparotomy including full recession of the umbilical ring, omentectomy, bilateral inguinal lymph nodes and upper one-third of the vagina was performed. Histological diagnosis revealed metastases of the same origin with her primary disease. She underwent external beam radiotherapy of 20 Gy in four daily fractions of 5 Gy over one week. The post treatment period was uneventful.

Discussion

It is already known that endometrial cancer spreads most commonly by direct extension to adjacent structures and also by transstitial passage of exfoliated cells, lymphatic and hematogenous dissemination [1]. The exact mechanism of implantation of cancer cells at the site of the umbilical ring is still unclear. Perhaps malignant cells penetrate the thickness of the uterine wall and spread intraperitoneally to reach the umbilical ring. The exfoliation of cells from the primary tumor via the fallopian tubes could be another possible mechanism [3]. Although these seem to be the most probable mechanisms of spread, other mechanisms must also play some role, because positive peritoneal washings have been reported in patients who have had a prior tubal ligation [3, 4]. Unfortunately, the presence of umbilical metastasis is a poor prognostic feature and sign of advanced neoplastic disease. The survival rate of these patients is influenced by the type of treatment and time of the diagnosis. Delay...
A rare case of umbilical and vaginal metastasis from endometrial cancer - review of the literature

Figure 1. — Umbilical metastasis from endometrial cancer.
Figure 2. — Umbilical ulceration due to metastatic disease.

of the diagnosis or treatment could lead to poor prognosis ranging between two and 11 months [3, 5]. Palliative treatment is not an option for these cases. The combination of adjuvant therapy and surgical reduction of the tumor seems to offer a better survival rate (up to 21 months) compared to surgery alone (7.4 months) [2, 5, 6]. Multidisciplinary teams and further studies are needed in order to offer the best choice to women.

References

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Hydatidiform mole in a perimenopausal and primary infertility patient: case report

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Summary

We present a case of a 56-year-old woman with primary infertility who complained of amenorrhea for five months and vaginal bleeding for two months. Initially she was misdiagnosed with endometrial cancer due to her disease history and older age, but eventually a diagnosis of complete hydatidiform mole was confirmed and then laparoscopic total hysterectomy was performed. The patient has been followed-up as an outpatient for more than one year, and has an excellent prognosis. To our knowledge, this is the first case of hydatidiform mole in a woman with primary infertility during the perimenopausal stage. Even though hydatidiform mole is rare in primary infertility patients during perimenopause, it should always be considered in case of misdiagnosis.

Key words: Gestational trophoblastic disease; Hydatidiform mole; Perimenopausal women; Primary infertility.

Introduction

Gestational trophoblastic diseases (GTD) encompass a diverse group of lesions that include hydatidiform mole (HM), invasive mole, choriocarcinoma and placental site trophoblastic tumor [1]. Grossly HM is composed of various sizes of grape-like vesicles. Microscopic examination demonstrates hydropic degenerated villi with circumferential trophoblastic cell proliferation, without structure normal embryonic. It is divided into a complete and partial mole. HM is the most frequently occurring GTD [2]. GTD generally occurs in women of reproductive age and rarely presents in patients beyond that group age, and is especially rarer in those women who suffer from primary infertility at the same time. In China, the incidence of GTD is on average 0.78 per 1,000 pregnancies [3]. It generally occurs in women of reproductive age with an age range of 20 to 34 years [4]. When it occurs in women older than 50 years, it frequently represents a malignant disease. Until now there have been few series reported in the world literature of gestational diseases occurring in women older than 50 years. Even rarer are reports of benign trophoblastic disease in perimenopausal women combined with primary infertility. We present a rare case of complete HM in a perimenopausal woman with primary infertility.

HM is a difficult condition to diagnose in women past reproductive age due to its very low incidence. The most frequent symptom in patients with molar pregnancy is abnormal vaginal bleeding, which is usually related to endometrial tumor.

Numerous studies support the fact that benign HM is extremely rare in perimenopausal and postmenopausal women. Patients in the sixth decade are not expected to be pregnant, and physicians may not even think of checking β-hCG levels. Therefore, the diagnosis of HM may be missed. On the other hand, in women of perimenopausal age, the possibility of GTD may be considered for those patients who have perimenopausal syndrome or endometrial tumor. These patients usually present with abnormal vaginal bleeding and excessive uterine size. Transvaginal ultrasound usually shows an enlarged uterus containing mixed material with minor blood flow within the uterine cavity.

In the world literature [5-7], it is well established that the occurrence of GTD in women older than 50 years is rare, and symptoms of the disease become more and more atypical. GTD is extremely rare in perimenopausal women with primary infertility, with no publications being reported either at home and abroad.

In 1997, Davidson et al. [8] described a case of a perimenopausal 60-year-old woman diagnosed with a molar pregnancy after only three months of amenorrhea. Rabczynski et al. [9] in 2000 reported a case of complete HM in a 59-year-old woman; however, the history of amenorrhea was not addressed. In the largest series, Ramondetta et al. [10] presented a case of a 60-year-old woman with evidence of a pelvic mass and pulmonary metastasis. Jequier and Winterton [11] reviewed 109 cases of GTD in women older than 50 years. They found malignant disease in 28.4% (31 patients), benign moles in 47.7% (52 patients), and an unclear pathologic diagnosis in the remaining 23.9%. The age of the patients with benign moles and malignant neoplasms ranged from 50 to 59 years (mean, 52.2 years) and 51 to 64 years (mean, 54.2 years).

A few studies showed that ovarian cells of perimenopausal women had different levels of degeneration, and hazard of HM in women over 50 years of age was much higher in 20-35 year old patients [12]. The research by Feng et al. [13] pointed out that the diagnosis of pregnancy and pregnancy-related disease should be considered in older women presenting with abnormal vaginal bleeding. Once GTD in women aged 50 years or more is diagnosed, chemotherapy should be given as soon as possible.

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tumor markers negative (AFP = 1.79 ng/ml, CEA = 1.32 ng/ml, CA125 = 31.45 u/ml, and CA199 = 3.10 u/ml). A pelvic ultrasound showed an enlarged uterus with complex echoes in the uterine cavity. CDFI: no blood signals (Figure 1). Computed tomography of the pelvis indicated malignant uterine disease (Figure 2).

Due to the high level of sexual hormone determination, urine human chorionic gonadotropin (hCG) level was checked and showed mild positivity, so we suspected that the mass might be related with pregnancy. Accordingly, serum hCG level was determined, which was greater than 200,000 mIU/ml; finally GTD was diagnosed.

The patient underwent suction curettage which grossly revealed a large amount of vesicular tissue, mixed with blood clots; a total of 3000 ml. Considering the huge size of the uterus, suction curettage was incomplete and had to be repeated after one week. We extirpated a total amount of 30 g product; histology showed blood clots and decidual tissue with hydropic trophoblastic cell proliferation (Figure 3). Serum \( \beta \)-hCG was reduced to 4309.5 mIU/ml four days after curettage. The pathology confirmed the diagnosis of benign molar pregnancy.

The patient was classified as high risk according to the WHO Prognostic Scoring System for GTD. She had no desire for further pregnancies, so laparoscopic hysterectomy was subsequently performed five days after curettage. During the operation, we found that uterine size corresponded to three gestational months, was soft in consistency, and there were two fibroid nodules 4 x 3 cm in size extruding from the surface of the anterior wall and fundus of the uterus. No abnormalities were found in bilateral adnexal tissue and the operation was successful. Serum \( \beta \)-hCG was reduced to 1330.4 mIU/ml seven days after operation. Histological pathology showed fibrinous exudate of the uterine cavity, a few pieces of endometrial tissue and uterine leiomyoma. Since the patient had high risk factors for hydatidi-
form mole, prophylactic chemotherapy was proposed, but the patient refused due to personal reasons. She was discharged on the 10th day after the operation, and ultrasound showed no abnormality.

The patient received careful follow-up of hCG as an outpatient, and it took seven months after hysterectomy for serum hCG to regress to normal levels. Up to today symptoms like vaginal bleeding, cough, hemoptysis and metastasis signs have not appeared, and gynecological examinations have shown no abnormalities.

Conclusions

The rarity of a molar pregnancy in a 56-year-old amenorrheic woman brings this patient’s menopausal status into question. Serum luteinizing hormone and follicle-stimulating hormone levels are increased and the estradiol levels are decreased. One may not think of checking hCG in women older than 50 years with a 5-month history of amenorrhea. Even though this patient had amenorrhea for five months, she most likely was considered to be in a transitional period of progressive loss of ovarian function and sporadic ovulatory cycles. At first we almost misdiagnosed this case as endometrial carcinoma, which was attributed to the older age and history of primary infertility. Hydatidiform mole should be suspected in any woman with vaginal bleeding. Thus estimations of serum and urine human chorionic gonadotropin are necessary in perimenopausal patients with vaginal bleeding and history of primary infertility. Diagnosis in this age group depends on a high level of suspicion, and hysterectomy should be considered due to the high risk of postmolar gestational trophoblastic tumor after uterine suction evacuation. Effective procedures to prevent misdiagnosis lie in improving consideration of GTD in perimenopausal women and improving the level of diagnostic techniques.

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An apparently benign vulvar mass: possibly a rare malignancy

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Summary

Background: Vulvar dermatofibrosarcoma is a rare fibrous tumor of intermediate grade malignancy, with a tendency for local recurrence, and rarely metastasizes. Management should be multidisciplinary. This is a report of an apparently benign vulvar mass delayed diagnosis of vulvar dermatofibrosarcoma. Case report: A 42-year-old woman was referred to our hospital because of a vulvar tumor lasting 16 years, although several gynecological procedures and a total laparoscopic hysterectomy had been performed two years before. During this long period the lesion did not change morphological features and remained asymptomatic. Only a benign vulvar mass was diagnosed. Then, the swelling became evident showing erythematous skin with an aspect of “peau d’orange”, leading the patient to consult a specialist. A firm vulvar swelling was observed in the anterior third of right labia majora continuing with about 3 cm of cord on top, quite movable above the underlying tissue but not on the overlying tissue. A wide excision was performed. The pathological examination showed positive margins. One month later an extensive deeper excision was performed. Histology confirmed a diagnosis of dermatofibrosarcoma. Immunohistochemistry was strongly positive for CD34. Conclusion: Vulvar lesions always require complete pathologic examination even in case of features of benign tumor to exclude a dermatofibrosarcoma. The role of the pathologist is essential to ensure negative microscopic margins and to avoid local recurrence.

Key words: Vulvar neoplasms; Dermatofibrosarcoma; Vulvar mass.

Introduction

Dermatofibrosarcoma protuberans (DFSP) is a rare cutaneous soft tissue sarcoma, accounting for approximately < 1% of all these tumours with an incidence of 0.8-5 cases per million per year, occurring most commonly during the fourth and fifth decades of life [1]. DFSP is considered locally aggressive with a high rate of local recurrence but its metastatic potential is low, with rare local and distant metastasis, usually via hematogenous spread to the lungs. Of all DFSP 85-90% are low-grade lesions; the others contain a high-grade fibrosarcomatous component and are considered to be intermediate-grade lesions [2]. DFSP exhibits an indolent growth pattern and in many cases symptoms are long-lasting [2]. The lesion mainly affects the trunk and lower extremity but it has also been found in the upper extremity, head and neck, and groin area. Some lesions develop in sites of previous trauma, particularly scars. The vulva is an uncommon site of involvement, and only approximately 28 cases are reported in the literature [3]. We report a case of a vulvar DFSP to discuss clinical and histopathological features, and the appropriate management.

Case Report

A 42-year-old woman, gravida 2, caesarean section 2, was referred to our hospital because of a vulvar tumor lasting 16 years. Total laparoscopic hysterectomy was performed two years before because of myomatosis. The anamnesis revealed a firm vulvar swelling in the anterior third of the right labia majora continuing with about 3 cm of cord on top (Figure 1). The mass was quite movable above the underlying tissue but not on the overlying tissue. It had been quiescent until six months before, when the swelling became evident with skin erythema. Ultrasound scan showed a complex capsulated mass of 21 x 9 mm continuing with hyperechogenic cord-like echos. A wide excision was performed, and the pathological examination showed positive margins. One month later an extensive deeper excision was performed.

Figure 1. — A firm, erithematous, subcutaneous nodule over the anterior third of the right labia majora.
period the lesion did not change morphological features and remained asymptomatic. A benign vulvar mass was diagnosed, therefore allowing the progression of the lesion. Then, the swelling became evident showing erithematous skin with an aspect as “peau d’orange”, leading the patient to consult the specialist.

Clinical presentation of vulvar DFSP is characterised by ulceration, pain, bleeding and the appearance of a nodule, but most patients are asymptomatic for a long period as in our report. Indeed, due to its indolent nature, the tumour is often undetected in the early stages and usually misdiagnosed at presentation [4]. Therefore, in cases of vulvar lesions it is mandatory for a complete examination even if the feature is like a benign tumour because DFSP can be excluded only with a pathological examination.

The treatment of choice is surgical excision with wide margins of 3-5 mm up to 5 cm of normal skin [5]. High recurrence rates despite “negative” margins can occur because large portions of the true margins are not evaluated on standard histological specimens and because it is difficult to identify “finger-like” projections, responsible for tumor recurrence. Mohs’ micrographic surgery has been advocated to ensure precise margin control with microscopic examinations of deep and lateral margins [6]. This procedure is associated with reduced recurrences [7] and offers preservation of normal tissue in the vulva, which is crucial for cosmetic and functional reasons. Mohs’ micrographic surgery was not performed in our patient because the first histological specimen showed “positive” margins only in one specific direction, at the supero-lateral right margin deep in the pubic adipose tissue.

Grossly, the tumour appeared as a gray-white nodule with focal erosion of the overlying skin. Lesion size was approximately 4 cm and it was not pigmented. The tissue sample was fixed in formalin, then routinely processed and embedded in paraffin. The sections were stained with haematoxylin-eosin. Additional sections were cut and subjected to immunohistochemical studies using antibodies to the following antigens: CD34; CD31, S100; desmin, actin, CD68, vimentin, HMB45, Mart-1, Pan-CK, Ki67. Histological examination showed dense, uniform and monomorphic spindle-shaped tumour cells (Figure 2) arranged in a storiform pattern with little pleomorphism (Figure 3). It was poorly circumscribed with diffuse and intricate infiltration of both the dermis and the subcutaneous adipose tissue in a typical honeycomb pattern. Honeycomb was characterised by tumour cells extending between and surrounding groups of preexisting fat cells, which remained viable, a pathognomonic feature of DFSP. The overlying epidermis was thinned, atrophic with focal erosion. Nerve and muscle invasion was not observed, nor was necrosis or angiolymphatic invasion present. Focal infiltration of lymphocytes and plasma cells involved the periphery of the tumour. No areas of fibrosarcomatous differentiation were found. Mitotic activity was moderate (<5/10 high-power fields). Immunohistochemically DFSP cells were strongly positive for CD34 (Figure 3) and negative for S100, desmin. In view of these morphological and immunohistochemical findings, the diagnosis of DFSP of the vulva was made.

Discussion and Conclusion

Vulvar DFSP is rare: a total of 28 cases have been reported in the literature as reviewed by Ghorbani and colleagues [3]. This is the first report of vulvar DFSP with delayed diagnosis, although the lesion was discovered 16 years before and the patient had undergone several gynaecological procedures. During this long period the lesion did not change morphological features and remained asymptomatic. A benign vulvar mass was diagnosed, therefore allowing the progression of the lesion. Then, the swelling became evident showing erithematous skin with an aspect as “peau d’orange”, leading the patient to consult the specialist.

Clinical presentation of vulvar DFSP is characterised by ulceration, pain, bleeding and the appearance of a nodule, but most patients are asymptomatic for a long period as in our report. Indeed, due to its indolent nature, the tumour is often undetected in the early stages and usually misdiagnosed at presentation [4]. Therefore, in cases of vulvar lesions it is mandatory for a complete examination even if the feature is like a benign tumour because DFSP can be excluded only with a pathological examination.

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An apparently benign vulvar mass: possibly a rare malignancy

The presence of a spindle cell proliferation within the skin allows a differential diagnosis with invasive spindle cell squamous cell carcinoma, spindle cell melanoma, neural tumours, smooth muscle tumours (leiomyosarcoma) and angiosarcoma [8]. Strong positivity for CD34 staining in this histologic context is diagnostic of DFSP, while the lack of staining with cytokeratins argues against a carcinoma [9, 10]. Likewise, negativity for S100 and HMB45 argues against neural and melanocytic origin, respectively. The lack of staining with smooth muscle actin and desmin excludes leiomyosarcoma and the lack of staining with CD31 in this context excludes angiosarcoma [8]. The overlap of histological features makes the differential diagnosis difficult between DFSP and dermal fibrous histiocytoma [10]: foam cells, giant cells and inflammatory cells were better represented in fibrous histiocytomas, as well as more ovoid cells and increased mitotic activity. The typical infiltration of fat is usually observed in DFSP. Most fibrous histiocytomas are only focally positive for CD34 and histiocytes express stronger immunoreactivity for CD163 than DFSP [11].

Areas of fibrosarcomatous differentiation have been associated with unfavourable cause, higher tendency to recurrence and increased risk of metastasis [12].

Because DFSP tends to spread in microscopic projections away from the visible lesion, very wide local excision is required for tumour control. Adjuvant radiotherapy administered either before or after surgery, significantly reduces the risk of local recurrence in patients who have close or positive surgical margins [13].

In conclusion, vulvar DFSP is a subtle pathology with asymptomatic clinical presentation. Any vulvar lesion always requires a complete pathologic examination even in case of features of benign tumour to exclude a DFSP. The role of the pathologist is essential to ensure negative microscopic margins and to avoid local recurrence.

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