Vol. XXXIII, no. 1, 2012

European Journal of Gynaecological Oncology

An International Journal

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European Journal of Gynaecological Oncology (ISSN 0392-2936) publishes original peer reviewed works in the fields of female genital cancers and related subjects and also proceedings of gynecologic oncology society meetings all over the world. The Journal is covered by CURRENT CONTENTS, SCISEARCH, RESEARCH ALERT, INDEX MEDICUS, MEDLINE, EMBASE/Excerpta Medica, CURRENT ADVANCES IN CANCER RESEARCH, BIOSIS.
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Covariates of high-risk human papillomavirus (HPV) infections are distinct for incident CIN1, CIN2 and CIN3 as disclosed by competing-risks regression models

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

Summary

Background: In addition to the oncogenic human papillomavirus (HPV), several cofactors are needed in cervical carcinogenesis, but whether the HPV covariates associated with incident i) CIN1 are different from those of incident ii) CIN2 and iii) CIN3 needs further assessment. Objectives: To gain further insights into the true biological differences between CIN1, CIN2 and CIN3, we assessed HPV covariates associated with progression to CIN1, CIN2 and CIN3 were analysed in the combined cohort of the NIS (n = 3,187) and LAMS study (n = 12,114), using competing-risks regression models (in panel data) for baseline HR-HPV-positive women (n = 1,105), who represent a sub-cohort of all 1,865 women prospectively followed-up in these two studies. Results: Altogether, 90 (4.8%), 39 (2.1%) and 14 (1.4%) cases progressed to CIN1, CIN2, and CIN3, respectively. Among these baseline HR-HPV-positive women, the risk profiles of incident CIN1, CIN2 and CIN3 were unique in that completely different HPV covariates were associated with progression to CIN1, CIN2 and CIN3, irrespective which categories (non-progression, CIN1, CIN2, CIN3 or all) were used as competing-risks events in univariate and multivariate models. Conclusions: These data confirm our previous analysis based on multinomial regression models implicating that distinct covariates of HR-HPV are associated with progression to CIN1, CIN2 and CIN3. This emphasises true biological differences between the three grades of CIN, which revisits the concept of combining CIN2 with CIN3 or with CIN1 in histological classification or used as a common endpoint, e.g., in HPV vaccine trials.

Key words: CIN; HPV; Covariates; Progression; Competing-risks regression; Univariate; Multivariate; Prospective follow-up; NIS Cohort; LAMS Study.

Introduction

Since the first evidence on human papillomavirus (HPV) as the causal agent of cervical cancer (CC) and its precursor (CIN) lesions [1-4], a substantial amount of data has accumulated on the potential risk factors of HPV infections [2, 3, 5-7]. Oncogenic HPV types are associated with CC and CIN in nearly 100% of the cases, but it is increasingly clear that several other cofactors are needed to complete the progression to high-grade CIN and invasive CC [2-4, 7-12]. Of these potential covariates of HPV, those associated with reproduction have attracted particular interest, including oral contraception (OC), parity, age at first intercourse, number of sexual partners, age at first full term delivery, age at menarche, and menopause [7, 13-17]. Cigarette smoking is suggested to increase the persistence of oncogenic HPV infections [18-20], and more recently, also drug addiction has been included in the list of potential risk factors [21].

Revised manuscript accepted for publication

Eur. J. Gynaec. Oncol. - ISSN: 0392-2936
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CC precursors have been classified by different approaches, including the 3-grade CIN terminology (CIN1-3) [22]. This concept was challenged by the Bethesda System (TBS), simplifying their classification into two categories (low-grade and high-grade) [23], abandoning the intermediate (CIN2) category. According to the leading European authorities, however, maintaining CIN2 in this classification can be based on solid i) morphological, ii) biological, and iii) clinical arguments [24, 25]. In addition, recent biomarker studies have disclosed some early molecular markers up-regulated in CIN1, and many more late markers being over-expressed only upon transition from CIN2 to CIN3 [26, 27], thus not advocating the clumping together of these two entities [23].

A novel approach to gain further insights in the genuine biological differences between CIN1, CIN2 and CIN3 is to assess whether the HPV covariates needed for progression from normal epithelium to i) CIN1 are different from those required for further progression to ii) CIN2 and iii) CIN3. Until today, four such studies (three for prevalent CIN and one for incident CIN) have been published [7, 28-30], all suggesting that CIN lesions are associated with HPV covariates that are unique for CIN1, CIN2 and CIN3.

All these studies used multinomial regression analysis [7, 28-30], which might not be the optimal technique for modelling particularly the longitudinal data on prospective settings [30]. Prompted by the recent advocates of marginal and mixed-effect models for analysis of HPV natural history data (based on repeated measures of individual women) instead of standard logistic regression models [31], we decided to use competing-risks regression models [32, 33] to analyse the panel data of our combined NIS-LAMS cohort [34] for HR-HPV covariates associated with incident CIN1, CIN2 and CIN3. To control for residual confounding by HPV, only the baseline HR-HPV-positive women (n = 1,105) were included, who represent a sub-cohort of all 1,865 women prospectively followed-up in these two studies [34].

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) [35] as well as in two Latin American countries (Brazil and Argentina) [36]. The design and baseline data of both cohorts have been previously detailed [35, 36].

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 1998-2002. 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 enrolment was 32.6 (± 10.7 SD) years (median 30.6, range 15-85 years) [35]. The study design has been detailed in a series of reports [15-18]. 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, which might not be the optimal technique for modelling particularly the longitudinal data on prospective settings [30].

The NIS-LAMS study comprises 3,187 consecutive women attending six different outpatient clinics in the three NIS countries between 1998-2002. 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 enrolment was 32.6 (± 10.7 SD) years (median 30.6, range 15-85 years) [35]. The study design has been detailed in a series of reports [15-18]. 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, which might not be the optimal technique for modelling particularly the longitudinal data on prospective settings [30].

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 was 17.2 mo (SD, 11.6 mo; median, 16.6 mo; range 1-43 mo) [15-18].

In the LAMS study, the same criteria were used to allocate the women into the FU and treatment groups [36-39]. A total of 1,011 women completed at least one FU visit, scheduled at 6-month intervals. The mean FU time was 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.

Outcomes and endpoints

The data of the 854 women from the NIS cohort and 1,011 women from the LAMS study were merged into the same file, and the combined cohort of 1,865 women was analysed for four outcomes: 1) no progression, 2) progression to CIN1; 3) progression
Table 1. — Disease progression to CIN1, CIN2 and CIN3 endpoints in the NIS and LAMS cohorts.

<table>
<thead>
<tr>
<th>Disease progression</th>
<th>Progressed cases</th>
<th>Progression times to event (mo)</th>
<th>Progression rate (Events/1,000 WMR)</th>
<th>WMR Rate/1000 WMR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CIN1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIS cohort</td>
<td>14</td>
<td>1.6</td>
<td>16.8</td>
<td>11.7-21.8</td>
</tr>
<tr>
<td>LAMS cohort</td>
<td>76</td>
<td>7.5</td>
<td>14.1</td>
<td>12.5-15.6</td>
</tr>
<tr>
<td>Combined</td>
<td>90</td>
<td>4.8</td>
<td>14.5</td>
<td>13.0-16.0</td>
</tr>
<tr>
<td>p = 0.0001</td>
<td></td>
<td>p = 0.35</td>
<td></td>
<td>(95% CI 0.16-0.47)</td>
</tr>
<tr>
<td><strong>CIN2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIS cohort</td>
<td>7</td>
<td>0.8</td>
<td>19.6</td>
<td>8.5-30.6</td>
</tr>
<tr>
<td>LAMS cohort</td>
<td>32</td>
<td>3.2</td>
<td>15.4</td>
<td>12.8-18.0</td>
</tr>
<tr>
<td>Combined</td>
<td>39</td>
<td>2.1</td>
<td>16.2</td>
<td>13.5-18.9</td>
</tr>
<tr>
<td>p = 0.0001</td>
<td></td>
<td>p = 0.53</td>
<td></td>
<td>(95% CI 0.15-0.71)</td>
</tr>
<tr>
<td><strong>CIN3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIS cohort</td>
<td>0</td>
<td>0.0</td>
<td>14668</td>
<td></td>
</tr>
<tr>
<td>LAMS cohort</td>
<td>14</td>
<td>1.4</td>
<td>15.8</td>
<td>12.1-28.1</td>
</tr>
<tr>
<td>Combined</td>
<td>14</td>
<td>1.4</td>
<td>15.8</td>
<td>12.1-28.1</td>
</tr>
<tr>
<td>p = 0.0018</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Papnicolau (Pap) smears

In the NIS study, all women were examined using the conventional Pap smear [35], 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) [37]. 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-µm-thick haematoxylin-eosin (HE)-stained sections for light microscopy, following the routine procedures. All biopsies were examined in the daily routine in the Pathology Departments of the partner institutions, and diagnosed using the commonly agreed CIN nomenclature [22, 24, 25]. Lesions presenting with morphological signs of HPV but not fulfilling the criteria of CIN were called HPV-NCIN (= flat HPV lesions without CIN). In statistical analysis, these lesions were treated as baseline-negative biopsies [35, 36].

Detection of HR-HPV DNA by Hybrid Capture 2 (HC2) assay

In both studies, the principal HPV testing method was HC2 assay, performed using cervical swabs (collected by a physician) or self-sampling devices (tampons, in LAMS study only), as described previously [35, 36, 39]. 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.

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). Frequency tables for categorical variables were analysed using the chi-square test, with likelihood ratio (LR) or Fisher’s exact test for significance. Differences in the means of continuous variables were analysed using non-parametric tests (Mann-Whitney, Kruskal-Wallis) or ANOVA. The incidence rates of CIN1, CIN2, CIN3 were expressed as events/1,000 wmr, and their 95% confidence intervals (95% CI). Incidence rates were compared by RR (rate ratio) statistics (with 95% CI).

This longitudinal data file was constructed into a panel data, clustered by women-ID and using the FU-visits as the time (repeated measures) variable. Competing-risks regression models [32, 33] were first used in univariate mode to estimate the risk, i.e., crude subhazard ratios (SHR and 95% CI) of different HPV covariates to associate with incident i) CIN1, ii) CIN2 and iii) CIN3 (among baseline HR-HPV+ women). Multivariate models were constructed to disclose independent HPV covariates, calculating SHR's (95% confidence intervals) of different HPV covariates to associate with progression to CIN1, CIN2 and CIN3.
### Table 2. Covariates of HR-HPV associated with progression to CIN1, CIN2 and CIN3 in univariate competing-risks regression model (with non-progression and other CIN grades as competing events).

<table>
<thead>
<tr>
<th>HPV Covariate</th>
<th>Progressed to CIN1 (vs non-progression, CIN2, CIN3)</th>
<th>Progressed to CIN2 (vs non-progression, CIN1, CIN3)</th>
<th>Progressed to CIN3 (vs non-progression, CIN1, CIN2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (95% CI)</td>
<td>p</td>
<td>SHR (95% CI)</td>
</tr>
<tr>
<td>Age Above 35 years</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Below 35 years</td>
<td>0.98 (0.52-1.83)</td>
<td>0.951</td>
<td>1.22 (0.48-3.09)</td>
</tr>
<tr>
<td>Marital status: 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>1.12 (0.60-2.09)</td>
<td>0.715</td>
<td>1.66 (0.71-3.87)</td>
</tr>
<tr>
<td>Baseline Pap test ASCUS+: 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>3.00 (1.58-5.71)</td>
<td>0.001</td>
<td>3.93 (1.48-10.45)</td>
</tr>
<tr>
<td>Baseline Pap test LSIL+: PAP LSIL+</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Race 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>0.87 (0.37-2.04)</td>
<td>0.756</td>
<td>0.86 (0.42-3.60)</td>
</tr>
<tr>
<td>Age at onset of sexual activity 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>1.07 (0.65-3.12)</td>
<td>0.368</td>
<td>1.09 (0.35-3.72)</td>
</tr>
<tr>
<td>Between 15 and 17 years</td>
<td>1.49 (0.63-3.55)</td>
<td>0.366</td>
<td>1.70 (0.54-5.32)</td>
</tr>
<tr>
<td>Below 15 years</td>
<td>2.10 (0.72-6.13)</td>
<td>0.172</td>
<td>1.51 (0.36-7.28)</td>
</tr>
<tr>
<td>Ever been pregnant No</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>0.73 (0.40-1.35)</td>
<td>0.326</td>
<td>0.74 (0.31-1.75)</td>
</tr>
<tr>
<td>Number of pregnancies 0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>1.07 (0.51-2.23)</td>
<td>0.851</td>
<td>0.71 (0.21-2.35)</td>
</tr>
<tr>
<td>2</td>
<td>0.63 (0.25-1.59)</td>
<td>0.333</td>
<td>0.84 (0.26-2.78)</td>
</tr>
<tr>
<td>3</td>
<td>0.43 (0.12-1.44)</td>
<td>0.173</td>
<td>0.86 (0.23-3.18)</td>
</tr>
<tr>
<td>4 or more</td>
<td>0.73 (0.30-1.75)</td>
<td>0.489</td>
<td>0.63 (0.17-2.35)</td>
</tr>
<tr>
<td>Number of live births 0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>0.69 (0.32-1.48)</td>
<td>0.350</td>
<td>0.82 (0.31-2.13)</td>
</tr>
<tr>
<td>2</td>
<td>0.91 (0.39-2.09)</td>
<td>0.826</td>
<td>0.22 (0.03-1.75)</td>
</tr>
<tr>
<td>3 or more</td>
<td>1.06 (0.41-2.74)</td>
<td>0.896</td>
<td>0.75 (0.17-3.29)</td>
</tr>
<tr>
<td>Number of life-time sexual partners 1</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>2-3</td>
<td>1.26 (0.57-2.76)</td>
<td>0.559</td>
<td>0.67 (0.13-3.27)</td>
</tr>
<tr>
<td>4-5</td>
<td>1.03 (0.39-2.70)</td>
<td>0.942</td>
<td>2.67 (0.68-10.50)</td>
</tr>
<tr>
<td>6 or more</td>
<td>0.96 (0.30-3.02)</td>
<td>0.950</td>
<td>2.18 (0.45-10.59)</td>
</tr>
<tr>
<td>Number of partners during past 12 months 0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>0.93 (0.22-3.78)</td>
<td>0.922</td>
<td>0.94 (0.12-7.68)</td>
</tr>
<tr>
<td>2 or more</td>
<td>1.03 (0.23-4.59)</td>
<td>0.961</td>
<td>0.83 (0.09-7.28)</td>
</tr>
<tr>
<td>Any sexual partner with diagnosed STD No</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>1.01 (0.40-2.51)</td>
<td>0.986</td>
<td>0.78 (0.18-3.33)</td>
</tr>
<tr>
<td>Mode of contraception 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>2.38 (1.00-5.69)</td>
<td>0.050</td>
<td>2.16 (0.76-6.16)</td>
</tr>
<tr>
<td>Other contraception</td>
<td>1.96 (0.83-4.61)</td>
<td>0.123</td>
<td>0.87 (0.27-2.82)</td>
</tr>
<tr>
<td>Oral contraception 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>1.53 (0.84-2.79)</td>
<td>0.163</td>
<td>2.34 (1.02-5.35)</td>
</tr>
</tbody>
</table>
Covariates of high-risk human papillomavirus (HPV) infections are distinct for incident CIN1, CIN2 and CIN3 as disclosed etc.

Table 2. Covariates of HR-HPV associated with progression to CIN1, CIN2 and CIN3 in univariate competing-risks regression model (with non-progression and other CIN grades as competing events)*.

<table>
<thead>
<tr>
<th>HPV Covariate</th>
<th>Progressed to CIN1 (vs non-progression, CIN2, CIN3)</th>
<th>Progressed to CIN2 (vs non-progression, CIN1, CIN3)</th>
<th>Progressed to CIN3 (vs non-progression, CIN1, CIN2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (95% CI)</td>
<td>p</td>
<td>SHR (95% CI)</td>
</tr>
<tr>
<td>History of STD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td></td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>0.94 (0.40-2.20)</td>
<td>0.895</td>
<td>0.94 (0.28-3.16)</td>
</tr>
<tr>
<td>Previous Pap smear taken</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td></td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever</td>
<td>5.65 (2.03-15.73)</td>
<td>0.001</td>
<td>1.90 (0.70-5.14)</td>
</tr>
<tr>
<td>Time since last Pap smear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 24 months</td>
<td>1.00 (Reference)</td>
<td></td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 12 and 24 months</td>
<td>0.71 (0.28-1.78)</td>
<td>0.466</td>
<td>4.40 (0.55-35.57)</td>
</tr>
<tr>
<td>Between 6 and 12 months</td>
<td>0.81 (0.31-1.83)</td>
<td>0.614</td>
<td>0.40 (0.02-6.44)</td>
</tr>
<tr>
<td>Less than 6 months</td>
<td>0.19 (0.05-0.71)</td>
<td>0.014</td>
<td>2.59 (0.30-21.92)</td>
</tr>
<tr>
<td>History of previous CIN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (Reference)</td>
<td></td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>0.79 (0.19-3.22)</td>
<td>0.746</td>
<td>1.88 (0.44-8.04)</td>
</tr>
<tr>
<td>Ever been a smoker</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1.00 (Reference)</td>
<td></td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Ever (current and past)</td>
<td>1.11 (0.60-2.06)</td>
<td>0.719</td>
<td>5.86 (2.12-16.15)</td>
</tr>
<tr>
<td>Duration of smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 5 years</td>
<td>1.00 (Reference)</td>
<td></td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Between 5 and 10 years</td>
<td>0.55 (0.15-1.99)</td>
<td>0.362</td>
<td>1.64 (0.48-5.59)</td>
</tr>
<tr>
<td>Longer than 10 years</td>
<td>0.88 (0.29-2.57)</td>
<td>0.811</td>
<td>1.58 (0.46-5.37)</td>
</tr>
</tbody>
</table>

*p only HR-HPV positive women included; n=1,105; SHR, subhazard ratio; NC, not computable.

Covariates of high-risk human papillomavirus (HPV) infections are distinct for incident CIN1, CIN2 and CIN3 as disclosed etc. These analyses were repeated for CIN1, CIN2 and CIN3 incident endpoints (1, 2, or 3; recorded at each FU-visit), using the other CIN endpoints and non-progression (endpoint = 0) as competing-risks events [32, 33]. To enable pair-wise comparisons between the CIN grades, the competing-risks event in the model was changed appropriately, i.e., CIN2 vs CIN1, CIN3 vs CIN1, and CIN3 vs CIN2. In all calculations, robust variance estimator (vce) was used, clustered by woman-ID, to account for the repeated sampling of each woman. All tests were 2-sided, and values $p < 0.05$ were regarded as statistically significant.

Results

Table 1 summarizes the key characteristics of progression to CIN1, CIN2 and CIN3 histological outcomes during the follow-up of the NIS (n = 854) and LAMS (n = 1,011) cohorts. These two cohorts are markedly different as to the proportion of the progression events and the rates of incident CIN1, CIN2, and CIN3, reflecting the different baseline status of these women. However, all progression times to the three endpoints are very similar in both cohorts.

Table 2 lists the results of univariate competing-risks regression analysis of all HPV covariates associated with the CIN1, CIN2 and CIN3 incident endpoints among baseline HR-HPV+ women (to control for confounding by HR-HPV, i.e., the common risk factor of all CIN outcomes) [30]. While keeping the other CIN grades and non-progression as competing-risk events in the model, the covariates associated with incident CIN1, CIN2 and CIN3 were quite different. The single most powerful cofactor of incident CIN1 was ever having had a Pap smear (SHR = 5.65, 95% CI 2.03-15.73) ($p = 0.001$), followed by baseline LSIL+ smear (SHR = 3.59), baseline ASCUS+ smear (SHR = 3.0). Other significant covariates were current use of OC (SHR = 2.38, 95% CI 1.0-5.69) ($p = 0.050$) and time since last Pap test < 6 months (protective) (SHR = 0.19, 95% CI 0.05-0.71) ($p = 0.014$). Ever having been a smoker was the most powerful predictor of CIN2 outcome (SHR = 5.86, 95% CI 2.12-16.15) ($p = 0.001$), ever having used OC was another significant cofactor (p = 0.44). Also baseline ASCUS+ smear (but not LSIL+) was significantly associated with incident CIN2 (SHR = 3.93, 95% CI 1.48-10.45) ($p = 0.006$). Baseline ASCUS+, LSIL+ and HSIL Pap were all significantly ($p = 0.001$) associated with incident CIN3. Other significant (or borderline) covariates include number of live births, ever having used OC, and number of recent sexual partners (ambiguous).

All these significant univariate predictors were entered in multivariate competing-risk regression models, and only a few remained significant independent predictors of each CIN endpoint (Table 3). Again, these significant HPV covariates were different for the three CIN outcomes. For incident CIN1: previous Pap history; for CIN2: ever having been a smoker, baseline ASCUS+ Pap test; for CIN3: baseline HSIL Pap test, recent sexual partners.
Progressed to "Adjusted SHR 95% CI Significance

<table>
<thead>
<tr>
<th>CIN 1</th>
<th>*Adjusted SHR</th>
<th>95% CI</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (35 yrs cut-off):</td>
<td>1.91</td>
<td>0.91</td>
<td>4.04</td>
</tr>
<tr>
<td>Baseline Pap test ASCUS+</td>
<td>1.99</td>
<td>0.73</td>
<td>5.35</td>
</tr>
<tr>
<td>Baseline Pap test LSIL+</td>
<td>1.95</td>
<td>0.67</td>
<td>5.63</td>
</tr>
<tr>
<td>Mode of contraception</td>
<td>2.37</td>
<td>0.73</td>
<td>7.46</td>
</tr>
<tr>
<td>Previous Pap smear taken</td>
<td>5.95</td>
<td>1.77</td>
<td>20.06</td>
</tr>
<tr>
<td>CIN2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (35 yrs cut-off):</td>
<td>4.62</td>
<td>0.95</td>
<td>22.68</td>
</tr>
<tr>
<td>Baseline Pap test ASCUS+</td>
<td>4.93</td>
<td>1.71</td>
<td>14.26</td>
</tr>
<tr>
<td>Oral contraception ever</td>
<td>1.34</td>
<td>0.48</td>
<td>3.77</td>
</tr>
<tr>
<td>Ever been smoker</td>
<td>5.79</td>
<td>1.75</td>
<td>19.20</td>
</tr>
<tr>
<td>CIN3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (35 yrs cut-off):</td>
<td>0.36</td>
<td>0.10</td>
<td>1.24</td>
</tr>
<tr>
<td>Baseline Pap test HSIL</td>
<td>175.74</td>
<td>41.56</td>
<td>743.13</td>
</tr>
<tr>
<td>Number of live births</td>
<td>0.99</td>
<td>0.62</td>
<td>1.56</td>
</tr>
<tr>
<td>Number of recent (&lt;12-mo) partners</td>
<td>0.22</td>
<td>0.10</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Only HR-HPV positive women included; n = 1,105; *Adjusted for all covariates that were significant in univariate model; †Mode of contraception dropped from the model because of collinearity; ‡Baseline Pap smear dropped from the model because of collinearity; §LSIL+ and ASCUS+ dropped from the model because of collinearity; ¶SHR, subhazard ratio; significant covariates are in bold.

The three incident CIN endpoints were compared pairwise for their HPV covariates by changing the competing-risks events in the models. Only significant covariates are listed in Table 4. Three covariates made a significant distinction between incident CIN2 and CIN1; baseline ASCUS+ Pap test (SHR = 4.61), ever having used OC (SHR = 2.25) and ever having been a smoker (SHR = 5.99). Not unexpected, there were five HPV covariates that were significantly associated with incident CIN3 (with CIN1 as a competing-risk event): baseline Pap ASCUS+, LSIL+, HSIL, number of live births, and recent partners. The first four also distinguished between incident CIN3 and CIN2 (as competing events).

In the final multivariate model with all significant univariate variables entered (Table 5), ever having been a smoker and baseline ASCUS+ Pap were significant covariates of the CIN2 endpoint as compared with CIN1 as a competing event. HSIL baseline Pap was the single most powerful predictor of incident CIN3, when CIN1 was the competing event. The same was true (with a slightly lower SHR) also when CIN2 was used as the competing event for CIN3.

Discussion

Data on HR-HPV persistence are of little help while assessing the differences between CIN1, CIN2 and CIN3, because HR-HPV seems to be involved in the development of practically all these endpoints [34, 41-44]. A powerful novel approach to gain further insights in the true biological differences between CIN1, CIN2 and CIN3 is to assess, whether the covariates of progression from normal epithelium to CIN1 are different from those required for progression to CIN2 and CIN3. To adequately control for residual confounding by HR-HPV, this assessment needs to be done on baseline HR-HPV positive women [44]. Until now, three such studies have been published [7, 28, 29], all using multinominal regression models to assess the risk profiles for prevalent CIN1, CIN2 and CIN3 endpoints. We recently provided such data in a prospective setting by analysing the HR-HPV covariates for incident CIN1, CIN2 and CIN3 [30].

To make our results [30] comparable with the published three studies [7, 28, 29], we used a similar statistical approach (multinomial logistic regression models) to analyse our longitudinal data. This technique has an advantage to the standard logistic regression or Cox models in that it enables alternating the dependent variable instead of the binomial (0/1) endpoint, which makes possible also the pair-wise comparisons between the three CIN grades [30]. Using this approach in the sub-cohort of 1,105 baseline HR-HPV-positive women, we demonstrated that different HPV covariates are associated with incident CIN1, CIN2 and CIN3, irrespective whether the comparisons are made to cases with no progression or to lower grades of CIN [30]. This suggests that each CIN grade represents a distinct biological entity, with distinct natural history and covariates associated with HPV in disease progression, persistence or regression [42-46].

In a recent review, the appropriate statistical techniques to be applied in the natural history studies on HPV were extensively discussed [31]. It seems obvious that the natural history of HPV has several characteristics that, at least from a statistical perspective, are infrequently encountered in other fields of infectious disease or cancer research [31, 42-44]. The same applies to the natural history of CIN, which also runs a complex natural history, with persistence, progression or spontaneous regression as potential outcomes [45, 46]. 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 [47-49]. In all settings where repeated measures involve the same subject, the results tend to be correlated [31]. 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 at a given visit are more likely to have the disease in the subsequent visit as well, if repeated within a reasonable time frame, e.g., at 6-month intervals. Statistical techniques that fail to take these correlations into account would be invalid, and methods that do not exploit all the collected data (in a repeated measures setting) would be inefficient [31]. Marginal (e.g., GEE, generalized estimating equation) and mixed-effects models 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, which is fully confirmed by our recent experience as well [47-49].
Covariates of high-risk human papillomavirus (HPV) infections are distinct for incident CIN1, CIN2 and CIN3 as disclosed etc.

Table 4. — Significant covariates of HR-HPV associated with progression to CIN2 and CIN3 in univariate competing-risks regression model (with CIN1 and CIN2, respectively, as competing events)@.

<table>
<thead>
<tr>
<th>HPV Covariate</th>
<th>Progressed to CIN2 (CIN1 as competing event)</th>
<th>Progressed to CIN3 (CIN1 as competing event)</th>
<th>Progressed to CIN3 (CIN2 as competing event)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (95% CI)</td>
<td>p</td>
<td>SHR (95% CI)</td>
</tr>
<tr>
<td>Baseline Pap test ASCUS+ 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>4.61 (1.78-11.92)</td>
<td>0.002</td>
<td>40.86 (5.22-319.42)</td>
</tr>
<tr>
<td>Baseline Pap test LSIL+ PAP &lt; LSIL</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>PAP LSIL+</td>
<td>2.20 (0.62-7.73)</td>
<td>0.217</td>
<td>22.24 (5.72-86.50)</td>
</tr>
<tr>
<td>Baseline Pap test HSIL PAP &lt; HSIL</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>PAP HSIL+</td>
<td>5.07 (0.66-38.91)</td>
<td>0.118</td>
<td>166.62 (43.68-635.50)</td>
</tr>
<tr>
<td>Number of live births 0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>0.70 (0.27-1.81)</td>
<td>0.470</td>
<td>0.40 (0.04-3.74)</td>
</tr>
<tr>
<td>2</td>
<td>0.26 (0.03-2.03)</td>
<td>0.202</td>
<td>3.90 (1.04-14.54)</td>
</tr>
<tr>
<td>3 or more</td>
<td>0.56 (0.14-2.28)</td>
<td>0.426</td>
<td>1.86 (0.35-9.88)</td>
</tr>
<tr>
<td>Number of partners during past 12 months 0</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>0.88 (0.09-7.93)</td>
<td>0.911</td>
<td>NC</td>
</tr>
<tr>
<td>2 or more</td>
<td>0.88 (0.11-6.56)</td>
<td>0.903</td>
<td>0.37 (0.20-0.66)</td>
</tr>
<tr>
<td>Oral contraception Never</td>
<td>0.88 (0.11-6.56)</td>
<td>0.903</td>
<td>0.37 (0.20-0.66)</td>
</tr>
<tr>
<td>Ever (current and past)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>0.88 (0.09-7.93)</td>
<td>0.911</td>
<td>NC</td>
</tr>
<tr>
<td>2 or more</td>
<td>0.88 (0.11-6.56)</td>
<td>0.903</td>
<td>0.37 (0.20-0.66)</td>
</tr>
<tr>
<td>Ever been a smoker Never</td>
<td>0.44 (0.03-5.11)</td>
<td>0.052</td>
<td>2.97 (0.92-9.58)</td>
</tr>
<tr>
<td>Ever (current and past)</td>
<td>2.25 (0.99-5.11)</td>
<td>0.052</td>
<td>2.97 (0.92-9.58)</td>
</tr>
<tr>
<td>12 months</td>
<td>0.56 (0.14-2.28)</td>
<td>0.426</td>
<td>1.86 (0.35-9.88)</td>
</tr>
<tr>
<td>0</td>
<td>2.25 (0.99-5.11)</td>
<td>0.052</td>
<td>2.97 (0.92-9.58)</td>
</tr>
<tr>
<td>12 months</td>
<td>0.56 (0.14-2.28)</td>
<td>0.426</td>
<td>1.86 (0.35-9.88)</td>
</tr>
</tbody>
</table>

@only HR-HPV positive women included; n = 1,105; SHR, subhazard ratio; NC, not computable.

As pointed out in our original report [30], the potential weaknesses of the multinomial regression model used in analysis of HPV covariates in incident CIN include the fact that this method i) fails to fully exploit these longitudinal data based on repeated testing of individual women, and ii) more importantly, fails to control for the dependence of these repeated measurements [30]. Incident CIN cases represent count variables (events per person time at risk), and as such would be perfectly suitable for analysis by Poisson regression models. However, useful as Poisson models are in analysing the incident endpoints in panel data [48, 49], this technique only accepts a binomial (0/1) dependent variable and thus would necessitate a separate analysis for each of the multiple comparisons between the three CIN grades, which is not feasible. To overcome the potential caveats of the multinomial regression, 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 be taken into account, and iii) the multiple-endpoint (CIN1, CIN2, CIN3, no progression) variable be appropriately treated in a single model. There prerequisites are met by the competing-risks regression, here used to validate the previous results on HPV covariates in incident CIN1, CIN2 and CIN3, obtained by multinomial regression [30]. 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 [32]. In contrast to the usual survival analysis measuring time-to-failure as a function of observed cofactors, e.g. development of CIN3 in relation to HPV and other covariates, the term competing risk refers to the chance that instead of incident CIN3, one will observe a competing event, i.e., incident CIN1, CIN2 or no progression at all [32, 33]. During the observation period, detection of any of these com-
peting events impedes the occurrence of the event of interest (CIN3). 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. Different from the usual Cox regression models producing HR (hazard ratio), this technique reports exponentiated coefficients known as subhazard ratios (SHR) [32, 33]. Technically, the correlation within multiple records on the same subject is accounted for by using a robust variance estimator, clustered by patient-ID, so as to treat each observation within a patient as an own predictor and not as a set of overlapping predictors [32, 33].

In the present study, all previous calculations based on multinomial regression in the original report [30] were repeated using the competing-risks regression models (Table 2-5). Importantly, as compared with the original data, the key results did not substantially change with this new technique of analysing the data. A detailed discussion of the results concerning the different HPV covariates in CIN1, CIN2 and CIN3 was provided in the original report, and is not repeated here. In the present analysis, the HPV covariates associated with incident CIN1, CIN2 and CIN3 are clearly different. In general, the number of significant covariates declined from CIN1 towards CIN3, as was also the case with the original analysis [30]. Interestingly, practically the same covariates remained significant, with only slight changes in their relative risk (SHR), and 95% CIs (Table 2). However, the most dramatic difference to the original analysis appears among the HPV covariates of incident CIN3, where the role of baseline Pap smear becomes accentuated. Instead of only ASCUS+ in the original data [30], also LSIL+ and HSIL are significant HPV covariates, the latter having the single most powerful predictive value of incident CIN3 (SHR = 146.2). This emphasises the difference between the two techniques of data analysis; while multinomial regression fails to use all recorded data (resulting in several non-computable estimates for CIN3 covariates) [30], competing-risk regression is capable of utilising all the data collected by repeated sampling at all FU visits. This results in more efficient estimates, with only one (a sub-category) of all analysed covariates remaining non-computable (Table 2). Because of a larger number of incident CIN2 and CIN1 cases, the estimates in these two categories are not that dramatically affected by the different techniques of data analysis. Even here, however, the baseline Pap smear will appear among the significant covariates of both CIN1 (ASCUS+, LSIL) and CIN2 (ASCUS+), in contrast to the original analysis, where no significance was established for these covariates.

Like before [30], all significant univariate predictors were entered in multivariate models separately for CIN1, CIN2 and CIN3 (keeping all others as competing-risks events). Again, the significant independent HPV covariates were different for the three CIN outcomes (Table 3). Similar analysis was also completed for each CIN grade, using its immediate precursor as the only competing event (i.e., CIN1 vs non-regression, CIN2 vs CIN1, etc.) (Table 5). Ever having been a smoker and baseline ASCUS+ Pap were significant covariates of CIN2 endpoint (CIN1 as a competing event). HSIL baseline Pap was the single most powerful predictor of incident CIN3, when CIN1 was the competing event, and the same was true also when CIN2 was the competing event. None of these independent significant HPV covariates are exactly identical to those reported in the previous studies on stage-specific risk profiles [30], except for CIN [7, 28, 29]. Direct comparison of these studies is not straightforward, however, the present data is the only information based on a prospective setting (with incident CIN events), while the others used prevalent (baseline) CIN outcomes. However, more important than the individual covariates is the demonstration of the concept that the significant HPV covariates associated with progression from normal epithelium to CIN1 are different from those associated with progression to CIN2 and further to CIN3. All previously published studies are unanimous in demonstrating this [7, 28-30].

Taken together, the present analysis based on competing-risk regression models gives no evidence justifying us to change the original conclusions reached by multinomial logistic regression models, unequivocally demonstrating that different HPV covariates are associated with incident CIN1, CIN2 and CIN3 [30]. These conclusions remain the same, irrespective which competing-risk events are used for the failure event of interest, i.e., all other categories or just the immediate precursor category. Having been confirmed by two fundamentally different techniques of data analysis, these results substantiate the concept that each CIN grade represents a distinct biological entity, as also suggested by the extensive natural history data available for CIN [42, 43, 45, 46]. This should have important implications in at least two fields: 1) lumping together CIN2 and CIN3 in the histological classification of cervical cancer precursors should be revisited, and 2) using the combined CIN2/CIN3 endpoint in any studies assessing the risk factors of cervical cancer should be reconsidered. Of great interest will be to assess whether these different HPV covariate profiles are linked with individual HR-HPV genotypes.

Acknowledgements

The NIS Cohort study has been supported by the INCO-Copernicus Program of the European Commission (Contract No. ERB IC15-CT98-0321), and the LAMS study by the European Commission, INCO-DEV Programme (Contract# ICA4-CT-2001-10013). Special thanks are due to Digene Corp. for providing the Hybrid Capture analyzer, samplers and the test kits at our disposal. We express our thanks to all women who participated in these two cohort studies. Finally, all the members of the NIS and LAMS study research groups are acknowledged for their invaluable contribution to these studies.
References


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Expression of beclin 1, an autophagy-related protein, in human cervical carcinoma and its clinical significance

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Summary

Purpose: To investigate the impact of beclin 1 on prognosis of cervical cancer, we determined the expression of beclin 1 in cervical cancer, cervical intraepithelial neoplasia (CIN) and normal cervical tissues. Methods: A total of 122 cases of cervical cancer, 35 cases with CIN and 31 cases with uterine fibroids were collected at the Cancer Center of Sun Yat University to determine the expression of beclin 1. Results: Beclin 1 positive rate in normal cervical tissues, CIN tissues and cervical cancers was 83.9%, 74.3% and 53.3%, respectively, and it was significantly different between the three groups (p < 0.01). Beclin 1 expression was negatively correlated with cervical cancer differentiation, lymph node metastasis, recurrence and death (p < 0.05). The negative expression is the risk factor affecting overall survival (p < 0.05) and progression-free survival (PFS) (p < 0.05). Multivariate analysis showed that beclin 1 negative expression was an independent risk factor of PFS time. Conclusions: Beclin 1 may play a role in the occurrence and development of cervical cancer. Beclin 1 positive expression in patients indicates a better prognosis.

Key words: Cervical cancer; Autophagy; Beclin 1; Overall survival (OS); Progression-free survival (PFS).

Introduction

Cervical cancer is one of the common malignant tumors in the female reproductive system. According to the statistics, there are approximately 51 million new cases of cervical cancer every year and 80% of the cases are from developing countries with annual deaths of about 28 million [1]. With the extensive cancer screening and continuous improvement of cancer treatment, morbidity and mortality have decreased. However, uncontrolled local tumor, recurrence and metastasis are the leading causes of death. Therefore, it has been an urgent requirement to identify effective treatments and examination indexes in order to reduce relapse and improve survival rate.

It was found that programmed cell death and autophagy inhibition were associated with the occurrence of cancer. Most scholars have divided cell death into apoptosis, autophagy and necrosis [2, 3]. Autophagy is known as type II programmed cell death, which was discovered in recent years as a new type of programmed cell death, and is independent of the non-procaspase [3, 4]. Autophagy is a normal function of cells. It can be induced immediately when the self-steady-state is changed (such as developmental differentiation and tissue reconstruction), or the body is subjected to a variety of stimuli (such as injury, hypoxia, high temperature, infection, hormones, etc.). Through autophagy, the cells can remove excessive or damaged organelles and intracellular components [5, 6].

The occurrence of autophagy is associated with ATG (autophagy-related gene) family genes. Beclin 1 and yeast ATG6, the same series of substances, is a kind of diallelic tumor suppressor gene related to autophagy; it is also a specific gene involved in autophagy in mammals. In 1999, Aita et al. [7] found that the gene encoding beclin 1 is located in the human chromosome 17q21, and successfully cloned the gene beclin 1.

Although the role of beclin 1 has been investigated in tumors such as liver, lung, breast, ovarian, endometrial, esophageal and colon cancers, few studies are available with regard to the role of beclin 1 in cervical cancer. This study was designed to detect the expression of beclin 1 in cervical cancer by immunohistochemical staining to explore the relationship between beclin 1 expression and pathological features, and to elucidate the effect of beclin 1 on prognosis.

Materials and Methods

Clinical information

A total of 188 cases studied were divided into three groups: cervical cancer, cervical intraepithelial neoplasia (CIN) and normal cervix. The experimental group included 122 cervical cancer cases (median age: 39 years) collected from the Cancer Center of Sun Yat-Sen University between January 1998 and December 2003. The criteria for the cases in the experimental group were: 1) patients were diagnosed with cervical squamous cell carcinoma by pathological analysis and initial treatment; 2) IB1~ IIA stages were obtained by FIGO clinical staging; 3) patients received radical hysterectomy and pelvic lymphadenectomy (Wertheim-Meigs operation) in the hospital; 4) radiotherapy, chemotherapy, biological and immune therapy had not been conducted before operation; 5) complicated or secondary cancer did not occur; and, 6) complete clinical data and pathological specimens were available and postoperative follow-up.
information was complete. A second group included 35 cases (median age: 39 years) with CINII-III, and the third group included 31 cases (median age: 40 years) with normal cervix. Clinical staging established by the International Federation of Gynecology and Obstetrics (FIGO) in 1995 was adopted for the patients in this study. Detailed information on the cases is shown in Table 1. There was no significant difference in the age among the three groups.

Pathological analysis

Paraffin-embedded tissue samples from the 188 cases were examined and serial sections (4 µm thick) were cut from the wax block. Monoclonal antibody against beclin 1 (Abcam, USA) was used for immunohistochemical staining. A polymer immunohistochemistry kit containing HRP-conjugated goat anti-rabbit IgG antibody was purchased from Jinqiao Biotechnology Co., Ltd (Beijing, China); 0.01M PBS buffer at pH 7.4 and 3,3-diamine benzidine tetrahydrochloride (DAB) solution were prepared immediately before use. Immunohistochemistry was performed by using a two-step non-biotin labeling method. Briefly, the slides were baked and dewaxed. High-pressure antigen restoration was performed using citric acid solution (pH 6.0), and the slides were rinsed successively with distilled water and PBS buffer (pH 7.4). After endogenous peroxidase activities were inhibited by peroxidase blocker, the slides were washed and then incubated with non-immune goat serum, anti-beclin 1 antibody and washed with PBS. Subsequently, the slides were incubated with HRP-conjugated goat anti-rabbit IgG antibody polymer and washed with PBS. Hematoxylin counterstaining was performed subsequent to DAB colorimetric detection. After treated with 0.1% saline alcohol, the slides were rinsed with tap water, dehydrated with 95% alcohol, dried and sealed with neutral resin. Beclin 1 positive brain sections were used for positive control, and PBS instead of primary antibodies for negative control.

Grading of the pathological observations

All the slides were blindly and independently examined by two pathologists. The cells with brown granules in cytoplasm were recognized as positive cells. Beclin 1 was localized in the cell membrane, cytoplasm and Golgi apparatus. Ten fields with 100 cells in one field were randomly selected and examined with a high magnification lens. The slides were evaluated according to the ratio of positive cells and intensity of the staining. The grading for the ratio of positive cells is based on the following criteria: “0” indicates that the positive cells account for less than 10% of the total cells, “1” indicates that the positive cells account for 10%-25% of the total cells, “2” indicates the positive cells account for 26%-50% of the total cells and “3” indicates that the positive cells account for more than 50% of the total cells. Grading for the staining intensity is based on the following criteria: “0” indicates no staining, “1” indicates light yellow staining, “3” indicates yellow to brown yellow staining, and “2” indicates the staining intensity between “1” and “3”. We used the formula: total grade=grade for positive cell ratio+grade for staining intensity to evaluate each sample. Total grade 0-1 indicates negative, 2-3 weakly positive, 4-5 medium strongly positive, and ≥ 6 strongly positive.
Expression of beclin 1, an autophagy-related protein, in human cervical carcinoma and its clinical significance

Statistical analysis

The SPSS 15.0 package was used for statistical analysis. Chi-square test or Fisher’s exact test were used for categorical data, and rank test for the ranked data. Continuous variables were subjected initially to a normal distribution test. If the continuous variables were in accord with the normal distribution, Student’s t-test was used. Otherwise, the rank test was performed. Spearman rank correlation analysis was used for rank correlation. Life table methodology was used to calculate the survival rate. The Kaplan-Meier method was used to draw survival curves. The log-rank test and Cox proportional hazard model were used for multivariate prognostic analysis; p < 0.05 was considered statistically significant.

Prognostic evaluation

Two indicators, overall survival (OS) and progression-free survival (PFS), were used to evaluate prognosis. OS refers to the time from surgery to the last time of follow-up or death. PFS indicates the time from surgery to clinical or pathological recurrence.

Results

Expression of beclin 1 in normal cervical, CIN and cervical squamous carcinoma tissues

The positive expression rates of beclin 1 in normal cervical, CIN and cervical cancer tissues were 83.9% (26/31), 74.3% (26/35) and 53.3% (65/122), respectively. Chi square analysis showed that the positive rates of beclin 1 were statistically different between the three groups (p < 0.01). There was no significant difference between the positive rate in the normal cervical group and that in the CIN group ($\chi^2$ = 1.099, $p$ = 0.295). The positive rate was significantly different between the normal cervical group and cervical cancer group ($\chi^2$ = 25.270, $p$ < 0.01), and between the CIN group and cervical cancer group ($\chi^2$ = 13.009, $p$ < 0.01) (Figures 1 and 2).

Relationship between beclin 1 expression and the factors associated with cervical squamous cell carcinoma

Statistical analysis showed that beclin 1 expression was negatively correlated with tumor differentiation, lymph node metastasis, recurrence and death (p < 0.05), while was not significantly correlated with age, tumor diameter, FIGO staging, SccAg, depth of cervix invasion, or surgical margin (Table 1).

Effect of beclin 1 and other clinical and pathological factors on the prognosis of cervical cancer: univariate analysis

Statistical analysis showed that tumor differentiation, lymph node metastasis and the negative expression of
beclin 1 were the risk factors for OS ($p < 0.05$), while age, tumor diameter, FIGO staging, SccAg, depth of cervix invasion, surgical margin were not risk factors for OS (Figures 3 and 4). SccAg, tumor differentiation, lymph node metastasis, surgical margin, depth of cervix invasion and the negative expression of beclin 1 were the risk factors for PFS ($p < 0.05$), while age, tumor diameter, FIGO staging were not risk factors for PFS (Figures 3-4).

**Discussion**

At present, tumor research has reached the molecular level. Many studies have confirmed that the occurrence of tumors is related to the activation of oncogenes and inactivation of tumor suppressor genes. Recently, the role of autophagic genes in cancer has become the new research focus. Autophagic genes contain double alleles and are the specific genes involved in autophagy in mammals. The autophagic gene, beclin 1, is frequently absent in a number of malignant tumors, which causes scholars to be attentive. Beclin 1 is the key factor mediating the localization of other autophagic proteins in pre-autophagic processes.
Expression of beclin 1, an autophagy-related protein, in human cervical carcinoma and its clinical significance

Beclin 1 is an important gene involved in the regulation of autophagic bodies in mammals. It can inhibit the growth of tumors by enhancing autophagy. In many tumor cell lines, beclin 1 is expressed at a low level [8, 9, 13]. The key roles of autophagy during the process of tumor development are embodied in the following two aspects. Firstly, autophagy changes the protein metabolism disorder, maintains internal stability and inhibits the occurrence of tumors by adjusting the intracellular peroxide concentration. Secondly, reduction in the function of autophagy increases oxidative stress and accumulation of tumorigenic mutations [14]. In this study, we found that beclin 1 had a positive expression rate of 83.9%, 74.3% and 53.3% in normal cervical, CIN, and cervical cancer tissues, respectively. There was no significant difference between the positive expression rate in normal cervical groups and that in the CIN group. In contrast, the positive expression rate of beclin 1 was significantly different between normal cervical and cervical cancer tissues and between CIN tissues and cervical cancer tissues (p < 0.01). Although the positive expression rate of beclin 1 in the CIN group was not significantly different from that in the normal cervical group, the positive expression of beclin 1 had a declined trend from the normal cervical to CIN group and from the CIN to cervical cancer group. These results indicate that lack of beclin 1 expression may be an early molecular event in cervical precancerous lesions. Decreasing expression of beclin 1 from normal cervix to CIN and from CIN to cervical cancer also suggests that beclin 1 may play a certain role in the occurrence and development of cervical cancer.

The key feature of tumor growth in vivo is its aggressive growth and metastasis, which is the main reason why a tumor is difficult to treat. There are generally the following steps during the process of metastasis of malignant tumors: 1) growth of an early primary tumor; 2) tumor angiogenesis; 3) shedding and invasion of tumor cells into the interstitial tissue; 4) invasion of tumor cells into the vascular system; 5) formation of tumor thrombus; 6) localized growth of secondary tissues and organs; and, 7) continuous spread of metastatic carcinoma [15]. Thus, the invasion of tumor cells into the lymphatic vascular system is the basis for metastasis. Our study showed that lymph node metastasis is not only an independent risk factor for PFS, but also the independent prognostic factor affecting OS. The beclin 1 expression was significantly correlated to tumor differentiation (p < 0.01), lymph node metastasis (p = 0.048), relapse (p = 0.005) and death (p = 0.011). We hypothesized that downregulation of beclin 1, a haplo-insufficient tumor suppressor gene, results in the deficiency of autophagy and apoptosis, genome mutation and increases in the malignant phenotype, thereby increasing the corrosive nature of tumor cells and promoting tumor metastasis. This study also found that 5-year PFS rate and survival rate in beclin 1-negative patients were 68.1% and 81.2%, respectively. In contrast, 5-year PFS rate and survival rate in beclin 1-positive patients were 89.2%, 95.4%, respectively, which was significantly different to those in beclin 1-negative patients.

Multivariate analysis showed that beclin 1 is an independent factor affecting the PFS time. Based on these results, we concluded that beclin 1-positive patients had a better prognosis. Improving autophagic activity in cervical cancer cells may become a new target for biological therapy of cervical cancer.

It has been demonstrated that improving autophagic activity in cervical cancer cells can be used for the treatment of cervical cancer in animal models. Qu et al. [9] and Yue et al. [10] established beclin 1-deficient mice and embryonic stem cells, and showed that Beclin 1 defects significantly increased the incidence of liver cancer, lung cancer and lymphoma. Liang et al., [8] transfected the beclin 1 gene into the human breast cancer cell line MCF-7 (deficient in the expression of beclin 1) and found that the number of autophagic vacuoles was increased, the in vitro proliferation ability of cancer cells and the malignant phenotype was decreased, and the tumor-forming ability of MCF-7 was reduced in nude mice. It has been demonstrated that a variety of anti-tumor drugs exert an anti-tumor effect through increasing the expression of beclin 1. Identification of an anti-tumor drug that can target the molecular mechanism of autophagy is of important significance for the treatment of tumors. Treatment of MCF-7 cells with tamoxifen can cause cell death with typical features of autophagy. Activation of autophagic death in breast cancer cells by tamoxifen is caused by ceramide-mediated upregulation of beclin 1 [16, 17]. With the progress in the molecular biology of cancer, introduction of autophagic death into the treatment of tumors will become a new target for cancer therapy.

In conclusion, beclin 1 may play a role in the occurrence and development of cervical cancer, and patients with beclin 1 positive expression have a better prognosis.

Acknowledgement

The work was supported by Guangdong Provincial Science and Technology Planning Project (No. 2006B35901005).

References


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Comparison of nerve content in removed parametrial tissue after classic radical hysterectomy and nerve-sparing radical hysterectomy - histologic evaluation

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Summary

Purpose of investigation: The aim of this study was to find whether nerve-sparing radical hysterectomy resulted in a lower amount of nerves in the removed parametrial tissue. Methods: Histological specimens from nerve-sparing radical hysterectomy (28 cases) were compared with those obtained after classic radical hysterectomy (26 cases). Width of the parametria and vaginal cuff were measured. Using a point counting technique, nerve areal density was determined in cross sections of resected parametria at 0.5 cm (A), 1 cm (B), 1.5 cm (C) from the cervix. Results: The width of the resected parametria was smaller in the study group (right side \( p < 0.013 \); left side; \( p < 0.011 \)). The nerve areal density in the lateral part of the right parametrium was lower in the study group (\( p < 0.01 \)) (Student’s t-test). Conclusion: Modified radical hysterectomy is less radical and is nerve-sparing.

Key words: Nerve-sparing radical hysterectomy; Parametrial nerve areal density.

Introduction

Nerve-sparing radical hysterectomy in the treatment of cervical cancer is still controversial, especially because of the strongly rooted opinion that only wide (total) resection of the lateral parametria ensures a complete removal of tumor tissue, which may not be the case with nerve-sparing techniques [1, 2]. However, total resection of the parametrium might represent over-treatment, at least in patients with Stage IB disease, especially if tumor diameter is less than 3 cm [3, 4]. Consequently, limiting the radicality of debilitating treatment options seems to be an unavoidable and logical trend, at least for low-stage tumors [5].

Many authors have described the techniques of nerve-sparing surgery [6-12]. However, only Butler-Manuel and co-workers analyzed nerve content in removed parametrial tissue by comparing radical and simple hysterectomy specimens. They found that in radical hysterectomy specimens, uterosacral ligaments contained a greater number of large trunks and free ganglia of sympathetic and parasympathetic nerve systems compared with cardinal ligaments, but free nerve content was higher in the cardinal ligaments [13, 14].

Regarding these findings, Trimbos and co-workers emphasized that the most important act in preserving the utmost of autonomic nerves was lateral dissection of the uterosacral ligament and laterализation of nerve fibers within it [15]. This kind of surgical preparation would not only preserve nerves during uterosacral ligament dissec- tion but would also preserve nerves in the following acts of the modified dissection of the cardinal ligament.

We implemented the Trimbos technique of nerve-sparing radical hysterectomy in 2002. We were curious as to whether this technique in comparison with classic radical hysterectomy really contributes to a lower number of embedded nerves in the removed parametrial tissue. To find the answers to this question we designed a study in which we compared histologic specimens of two groups of patients – those who underwent nerve-sparing radical hysterectomy and control patients who underwent classic radical hysterectomy.

Materials and Methods

Patients

In the years 2005 and 2006, 60 patients were operated on for cervical cancer Stage IB1 to IB2. In 28 patients with IB1 cancer stage we applied the nerve-sparing technique; they represented the study group. A detailed description of the technique can be found elsewhere [15]. The analysis of surgical specimens involved the width of parametrial tissue removed and the length of vaginal cuffs. Nerve areal density of the parametra was determined, and the results were compared with those obtained in the control group patients (\( n = 26 \)) in whom radical hysterectomy was performed for cervical cancer Stage IB1 as well, but before the year 2000 when only classic (non nerve-sparing) radical hysterectomy was performed at our institution [16].

Histologic preparation of the specimens and stereometry

The length of vaginal cuff and the length of parametria were measured before and after the cervix had been divided from the parametria. For histologic examination two consecutive 5 µm thick tissue sections were cut from each paraffin block. The first section was routinely stained with hematoxylin and eosin for tissue morphology assessment. The second section from each block was used for S100 immunohistochemical staining.

Revised manuscript accepted for publication May 2, 2011
Briefly, tissue sections were placed on a poly-L-lysine coated slide, deparaffinized and rehydrated. Antigen unmasking was performed by pressure cooking in citrate buffer (pH 6.0), and primary rabbit polyclonal anti S100 antibodies (DAKO, Glostrup, Denmark) diluted 1:100 were applied, followed by a standard ABC immunohistochemistry protocol using a biotinylated secondary antibody (DAKO, Glostrup, Denmark) and diaminobenzidine as chromogen (Sigma, Munich, Germany). The slides were counterstained with hematoxylin (Figure 1). Nerve areal density was determined by stereometric analysis using a point counting technique by a pathologist who was blinded to the clinical history of the patients [17]. Depending on the availability, this was done at three planes of both the left and the right parametrium, A – representing the medial part of the parametria (originating just at the cervix and extending to almost 0.5 cm laterally from the cervix), B – representing the middle part of the parametria (from 0.5 cm to 1 cm from the cervix), and C – representing the lateral part of the parametria, spreading from 1 cm to a maximum 1.5 cm from the cervix.

Table 1. — Comparison of the parametrial width, vaginal cuff length and nerve areal density in resected parametria.

<table>
<thead>
<tr>
<th></th>
<th>Study group mean (SD)</th>
<th>Control group mean (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right parametrium (mm)</td>
<td>28 15.50 (7.579)</td>
<td>26 22.69 (12.59)</td>
<td>.013</td>
</tr>
<tr>
<td>Left parametrium (mm)</td>
<td>28 15.71 (8.700)</td>
<td>26 22.96 (11.49)</td>
<td>.011</td>
</tr>
<tr>
<td>Vaginal cuff anterior (mm)</td>
<td>25 13.36 (5.830)</td>
<td>24 12.54 (4.191)</td>
<td>.577</td>
</tr>
<tr>
<td>Vaginal cuff posterior (mm)</td>
<td>25 21.64 (7.947)</td>
<td>26 19.31 (5.620)</td>
<td>.231</td>
</tr>
</tbody>
</table>

A, B, C – points of measurements of resected parametrial width.

Statistical analysis
The student’s t-test was used for statistical analysis of differences between the groups. For comparison of adjuvant therapy and survival rates we used the chi-square test. Statistical significance was set at \( p < 0.05 \).

The study was approved by the national medical ethics committee.

Results
The five-year survival rate was 92.85% in the study group and 84.61% in the control group [18]. Of the 28 patients in the study group, 15 (53.57%) received adjuvant radiotherapy because of spread of the tumor more than half the width of the cervix and evident lymphangio-invasion (13 patients; 47.85%), or spread of the tumor more than half the width of the cervix (10 patients; 35.71%). Four of the 15 patients (14.28%) with extensive lymph node infiltration received chemotherapy. None of the study group patients had parametrial metastases. Of the two patients in the study group who died by the end of the observation period, one had positive sentinel lymph nodes and one had lymphangio invasion found on histologic examination. Both patients received postoperative adjuvant radiotherapy.

Of the 26 patients in the control group, 17 (65.38%) received adjuvant radiotherapy because of spread of the tumor more than half the width of the cervix and lymphangio-invasion (13 patients; 50%) or pelvic lymph node metastasis (4 patients; 15.38%), three (11.53%) of them combined with chemotherapy. Also in this group none of the patients had parametral metastases. The four patients with positive lymph node metastases died.

Table 1 shows histological and stereometric data of the enrolled patients. The resection of the cardinal ligament was significantly wider with classic radical hysterectomy, both on the left and the right side. Mean parametrial widths were approximately 7 mm wider in the control group on both sides. The length of resected vaginal cuffs was almost the same in both groups.

Comparison of the stereometric analysis of the nerve areal density revealed no significant differences between the groups in the medial and middle part of the parametria. However, the nerve areal density in the lateral part of the right parametria (C) was significantly lower in the study group \( p < 0.011 \) (Table 1).

Not all stereoscopic data were available for all the patients. This was due to tear of the specimens (medial and middle part: 2 patients in the study and 2 in the control group; lateral part in the control group – 7 patients) and shortness of the parametria (lateral part in the study group).

Discussion
Dysfunctions of the lower urinary tract and distal bowel after radical hysterectomy are well known and have been described by many authors [19-24]. These dysfunctions might be the consequence of disruption of neural struc-
Comparison of nerve content in removed parametrial tissue after classic radical hysterectomy and nerve-sparing radical etc.

tures such as the superior hypogastric plexus, hypogastric nerve, inferior hypogastric plexus, usually occurring during resection of the sacrouterine, cardinal and vesicovaginal ligaments, part of which the aforementioned structures are, or lay in close proximity [25].

Dutch gynecologic surgeons developed a technique which is feasible and effective in patients in terms of nerve-sparing and radicality [15]. As opposed to some other nerve-sparing techniques, this technique provides tangible parametrial tissue, bound to the uterus, which can be histologically assessed.

To analyze the nerve-sparing effect of the implemented surgical technique, we first compared the width of parametrial tissue removed between the group undergoing nerve-sparing (study group) and that undergoing classic radical hysterectomy (control group). We found that it was wider in the control group, indicating that classic radical hysterectomy was more radical, and also implying that for this reason a greater number of nerves had been dissected in the control group. The latter speculation is based on the anatomical knowledge about the nerve fibers running through the parametrium and the surgical landmarks such as the inferior vesical vein and the deep uterine vein [6-12, 25]. The inferior vesical vein is important in preparation of the vesicovaginal ligament and the deep uterine vein is important for the resection of the cardinal ligament. If nerves are to be preserved, parametral dissection should not go beyond these points, otherwise the distal part of the hypogastric plexus (landmark – inferior vesical vein) or the main body of hypogastric plexus (landmark – deep uterine vein) could be embedded in the dissected parametrial tissue [15]. However, in the case with classic radical hysterectomy where the main goal was to dissect the parametrium as widely as possible, the aforementioned landmarks were not respected. Consequently, we believe that the amount of embedded nerves in the dissected parametrial tissue was larger compared with the nerve-sparing technique, although this had not been documented.

Nevertheless, the nerve-sparing effect is achieved not only through a less radical dissection of the parametria, but also through a meticulous preparation of the parametrial tissue as the main characteristic of this surgical technique is gentle lateralization of the hypogastric nerve from the uterosacral ligament and careful dissection of the cardinal ligament avoiding the inferior hypogastric plexus [5, 15]. This is the reason that we subjected the dissected parametrial tissue to stereometric analysis.

Stereometric analysis of the nerve areal density of the parametria involved 1.5 cm of tissue extending from the cervix on their way to the pelvic wall. This tissue represents the common parametrial trunk, where connective fibers of the uterosacral, cardinal and vesicouterine ligaments are still joined, before they disperse on their way to the pelvic wall.

Comparing the removed parametrial tissue of both groups with stereometry, we confirmed a lower nerve areal density only in part C of the right parametrium in the study group. This suggests that the nerve fibers running through the lateral part of the parametria to the lower urinary tract (bladder) were at least partially spared. We presume that the nerve fibers spared were of the distal inferior hypogastric plexus, criss-crossing the parametrial trunk in its lateral side (below the landmark of deep uterine vein [15]), which were in the process of lateralization pushed even further laterally. On the other hand, medially positioned nerve fibers entering the uterus itself could not be spared in this process, and from there on non-significantly different nerve areal density in medial parts of the parametrium (regions A and B) was also expected.

Surprisingly, we confirmed lower nerve areal density only in the right lateral part of the parametria. We presume that with a higher number of specimens this would probably be observed in the left part of parametria as well, although we suspect that the preparation of the parametrial tissue on the left side was not as effective as that on the right side. The reason for this phenomenon is very likely in a less favorable position of the surgeon for the left-side preparation combined with dense interconnection between nerve fibers and supporting connective fibers, which does not permit more effective nerve preservation. However, this might also have been due to insufficient surgical skillfulness in the early period of implementing the new technique.

Considering the available data we should stress that nerve-sparing in the removed parametrial tissue is significant, although only slightly. This implies that lateralization of the sacrouterine ligament with gentle preparation of the hypogastric nerve, careful dissection of the cardinal ligament and not-too-deep dissection of the vesicovaginal ligament in subsequent steps are also important. However, we believe that if the operation should be effective as far as nerve-sparing is concerned, this is achieved by less radical parametral dissection rather than by lateralization of the nerves themselves.

A histologic comparison of two different techniques of radical hysterectomy reveals that the nerve-sparing technique very likely contributes to smaller involvement of nerve fibers in the dissected parametrial trunk. However, this effect is only valid for nerve fibers criss-crossing the parametral trunk (fibers of hypogastric nerve and inferior hypogastric plexus), whereas the effect on nerve fibers entering the uterus is insignificant.

We should emphasize that we are aware of the small number of patients investigated. This is a common characteristic of the studies concerning cervical cancer surgery, and is partly due to declining numbers of patients eligible for this type of treatment. We are also aware that the study was partly retrospective (control group) which might decrease the value of the reported results. However, in the case of early cervical cancer such as IB1, for which the best treatment option is in the opinion of many surgeons less radical parametral dissection [3,4], randomization in two groups of which one (control) would be exposed to wide (classical) parametral dissection, seems ethically controversial. Also, it would be improper to prospectively compare two parametral tissue samples obtained after a different type of operation (nerve-sparing and classical radical hysterectomy), where the former...
would be performed for cervical cancer IB1 and the latter for more advanced cervical cancer. For these reasons we believed that the only way to compare tissue samples after different surgical techniques was the comparison of the samples obtained for cervical cancer IB1, which were in our case obtained in two different time periods, as the surgical techniques for the same stage of disease were different as well. Because the main goal of this study was the analysis of nerve content in the dissected parametrial tissue, this seemed acceptable, and because of dealing with cancer treatment we additionally present the data of adjuvant treatment and survival rates. Regarding the survival rates so far, the nerve-sparing surgical technique seems to be an appropriate treatment for low-stage cervical cancer. Despite all the limitations of the study, this modified radical hysterectomy preserves more tissue, and is consequently nerve-sparing. However, the complete preservation of autonomic nerves cannot be achieved with this type of operation.

References


Comparison of tumor markers and clinicopathological features in serous and mucinous borderline ovarian tumors

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Summary

Objective: The aim of this study was to assess tumor markers and clinicopathological findings of patients with serous and mucinous borderline ovarian tumor (BOT) features. Methods: The study consisted of 50 patients that were diagnosed with and treated for BOT between 2005-2010 in three centers. CA125, CA19-9, and CA125+CA19-9 levels and clinicopathological features were compared in serous and mucinous histotypes. In serous and mucinous BOTs, correlations between tumor markers and demographics such as age, menopausal status, parity, clinical findings (stage, relapse, adjuvant chemotherapy, cytology, lymph node involvement and tumor morphology (cystic-solid content, papilla, septation) were evaluated. Results: There were no significant differences between serous and mucinous tumors in the clinicopathological features such as stage, tumor markers, age, menopausal status, or cytology. In serous BOTs we found a significant relation between elevated CA125+ CA19-9, CA19-9 and recurrence (p < 0.05). Also there was a significant relation between elevated CA125+ CA19-9, CA19-9 and cytology positivity (p < 0.05). We found a significant relation in serous BOTs between elevated CA125+CA19-9, adjuvant chemotherapy and lymph node metastases (p < 0.05). Also In mucinous BOTs with papilla formation we found a significant relation between elevated CA125 and CA125+ CA19-9 (p < 0.05). There was significant relation between cytology positivity and elevated CA19-9 in mucinous BOTs (p < 0.05). Conclusion: Serum tumor markers of serous and mucinous BOTs were different in relation to their clinicopathological features. This may reflect differences of serous and mucinous BOTs.

Key words: Borderline ovarian tumor; Serous; Mucinous borderline ovarian tumor; Tumor markers.

Introduction

Borderline ovarian tumors (BOTs) were first described by Taylor in 1929 [1] and were introduced in 1971 by FIGO as a category of epithelial ovarian tumors [2]. BOT is a different form of both benign epithelial ovarian tumor and invasive epithelial ovarian cancer and accounts for 10-15% of all epithelial ovarian tumors. Clinically BOTs are diagnosed in earlier stage such as Stage I, affect mainly young reproductive women, have low potential for malignancy, including indolent behavior, longer patient survival, and later recurrence as compared with invasive epithelial ovarian tumors [3-6].

The most common histological types of BOTs are serous (65%) and mucinous (35%) tumors [3, 5]. Besides different histological appearances, these subtypes seem to have different etiologies and behavior patterns [6]. There is a clear association between the tumor marker and the histotypes of tumor. Elevated cancer antigen (CA) 125 in serous tumors was significantly more frequent than CA19-9, and elevated CA19-9 levels in mucinous types was more frequent, as shown in several studies [7-9].

Associations between serum tumor markers and clinical and sonographic parameters such as age, premenopausal status, tumor size, stage, and recurrence have been evaluated in many studies, and these have also been compared between serous and mucinous tumors [7-13].

To our knowledge, comparison of serum tumor markers and clinicopathologic features in serous and mucinous subtypes separately, has not been studied yet.

The aim of the present study was to review the clinical characteristics and serum tumor markers CA125 and CA19-9 of patients with BOT with special emphasis on serous and mucinous histology.

Materials and Methods

Fifty patients with BOTs diagnosed and treated in three gynecologic oncology centers between 2005-2010 were studied retrospectively. To be included in the study, a patient had to have complete information about preoperative tumor marker status; CA125, CA19-9. The levels of CA125 were considered positive when ≥ 35 ng/ml and CA19-9 levels were considered positive when ≥ 37 ng/ml [10]. Other tumor markers were not included, because they were not present in the patient records of all BOTs.

Other study characteristics that were analyzed in relation to tumor markers such as demographic characteristics, histotypes, ultrasonographic (US) features, surgery and follow-up were complete in the files. Also, other BOT histotypes such as Brenner, clear cell and endometrioid-type BOTs were excluded. Then, both serous and mucinous groups were divided into three subgroups: elevated CA125, elevated both CA125 and CA19-9, and elevated CA19-9.

Tumor marker groups were compared for other parameters
such as tumor size, age, menopausal status, US features, cytology positivity, etc. Patients were staged according to classification of ovarian carcinomas established by the International Federation of Gynecology and Obstetrics [14]. Pathologically, BOTs are characterized by features of malignant epithelial ovarian tumors, including stratification of epithelial lining of the papillae, formation of microscopic papillary projections, epithelial pleomorphism, atypia, and mitotic activity, without invasion of stroma [15]. Cystic tumors were defined as cysts with clear fluid, and solid contents were defined as dense echogenic fluid. Papillae were defined as small tissues in the cyst wall, septae were defined as walls inside the cyst (but septa and papilla were not further separated into small, large or thin-thick). Peritoneal implants were classified as non-invasive or invasive depending on the absence or presence of stromal invasion of the peritoneum, respectively. Surgery was considered conservative when the uterus and at least a portion of one ovary were preserved. Staging was considered complete when all peritoneal surfaces were carefully inspected and peritoneal washing, multiple random or oriented biopsies, omentectomy and appendectomy in cases of a mucinous tumor were performed.

Statistical analysis
Statistical analysis was performed with the SPSS software (Chicago, USA) 15 version for Windows. Quantitative variables were compared by using Mann-Whitney U test; categorical variables were compared by using the chi-square test.

Results
We studied a total of 50 patients with BOTs: 30 (60%) serous and 20 (40%) mucinous. Demographics and clinicopathological characteristics of the study are shown in Table 1. The mean ages of BOT cases were 42.7 ± 15.7 and 41.1 ± 12.1 years, serous and mucinous, respectively. Ages of patients with serous BOTs were similar with mucinous tumors.

There were no differences between parity, menopausal status, tumor features, tumor contents, tumor bilateralism, rate of positive peritoneal cytology, presence of peritoneal implants, surgical approach, the choice of surgical staging, lymph node metastases, adjuvant chemotherapy, and recurrence in BOTs. Tumor size was 11.0 ± 6.64 for serous and 13.8 ± 9.3 for mucinous BOTs, and tumor size was similar between mucinous and serous BOTs (tumor size 5-10 cm and ≥10 cm). Forty-four (88%) patients had Stage I and six (12%) had Stage II-III in BOTs. According to FIGO stage there were no differences between Stage I and II-III.

Mean CA125 levels were 191.1 ± 165.9 in serous BOTs and 163.9 ± 124.2 in mucinous BOTs. Mean serum CA125 was not significant between serous and mucinous BOTs (191.1 ± 145.9 vs 163.9 ± 94.2, respectively). The elevated CA125 rate was 18 cases (60%) of serous and nine (45%) of mucinous, respectively. Also, mean CA19-9 levels were 38.7 ± 43.5 in serous BOTs and 48.1 ± 34.3 in mucinous BOTs. The elevated CA19-9 rate was 26% (8 cases) and 50% (10 cases) for serous and mucinous tumors, respectively. There were no differences between serous and mucinous BOTs according to high and normal value of CA125, CA 19-9 and CA125+CA19-9.

Correlations between tumor markers and clinical features according to histotypes are shown in Table 2. When the serous tumor group and mucinous group were evaluated by elevated CA125, CA125+CA 19-9 and CA19-9 tumor markers and the marker associations with clinicopathological features, there was no correlation between menopausal status, age, parity, tumor size, cystic features, the presence of septum in the tumor, bilaterality and implant positivity for either serous or mucinous tumors. In serous BOTs there was a significant correlation between elevated CA125+CA19-9 stage, adjuvant chemotherapy and lymph node metastases (p < 0.05). This correlation was also present in mucinous tumors; the presence of papilla was correlated with CA125+CA19-9, and CA19-9 (p < 0.05). In serous BOTs, there was a significant correlation between elevated CA125+CA19-9, and CA19-9 tumor markers and recurrence (p < 0.05). Also there was a significant correlation between CA19-9 tumor markers and cytology positivity (p < 0.05).

Discussion
Serous and mucinous BOTs are the most frequent histotypes of BOTs, accounting for more than 95%. Besides different histological appearances, these subtypes seem to have different etiology and behavior [6], and are assumed to differ from each other in various aspects such as the rate, tumor characteristics and tumor markers. Information on the association of preoperative tumor markers and findings for BOT is very limited and dependent on case series.

The most important issue for serous and mucinous BOTs was the difference in tumor markers. Elevated CA125 was significantly more frequent in serous tumors than CA19-9 and, elevated CA19-9 was more frequent in mucinous types [7-9]. E elevated CA125 rates were present in 36-70% of serous BOTs, and 22-52% of mucinous BOTs [6-11]. On the contrary, elevated CA19-9 rates in 8-27% of serous, and 30.4-65% of mucinous BOTs were reported [10, 16].

In another study, median value of CA125 of 142 mucinous BOT cases was 38.0 while the median value of CA19-9 was 49.5 [17]. We found tumor markers (elevated CA125 and CA19-9 levels) in serous and mucinous BOTs to be similar, but this similarity was not statistically significant. In the literature, serum tumor markers and clinicopathological relation assessment studies are few.

The studies, especially on tumor markers and stage, tumor size, recurrence and this relation were compared by multivariate analyses [16, 18].

In one study, associations between clinical, sonographic and serum tumor marker parameters (CA125, CA19-9, CEA, CA15-3) were analyzed [13]. The study showed that patients who had at least one abnormal serum tumor marker were more likely to have large tumors, bilateral tumors and ascites. Women with normal and abnormal tumor markers did not differ in terms of their mean age, familial history of cancer, parity (including nulliparity), menopausal status, presenting symptoms, or the use of
Comparison of tumor markers and clinicopathological features in serous and mucinous borderline ovarian tumors

Table 1. — Demographics and analyses of clinicopathological characteristics in borderline ovarian tumors.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 50)</th>
<th>Serous (n = 30)</th>
<th>Mucinous (n = 20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (year)</td>
<td>42.08 ± 14</td>
<td>42.73 ± 15.75</td>
<td>41.10 ± 12.169</td>
<td>NS*</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40 years</td>
<td>22 (44%)</td>
<td>15 (50%)</td>
<td>7 (35%)</td>
<td>NS **</td>
</tr>
<tr>
<td>≥ 40 years</td>
<td>28 (56%)</td>
<td>15 (50%)</td>
<td>13 (65%)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>22 (44%)</td>
<td>12 (40%)</td>
<td>10 (50%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Multiparous</td>
<td>28 (56%)</td>
<td>18 (60%)</td>
<td>10 (50%)</td>
<td></td>
</tr>
<tr>
<td>Menopause status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>36 (72%)</td>
<td>22 (73.3%)</td>
<td>14 (70%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>14 (28)</td>
<td>8 (26.7%)</td>
<td>6 (30%)</td>
<td></td>
</tr>
<tr>
<td>Tumor features</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary absent</td>
<td>24 (48%)</td>
<td>16 (53.3%)</td>
<td>8 (40%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Papillary positive</td>
<td>26 (52%)</td>
<td>14 (46.7%)</td>
<td>12 (60%)</td>
<td></td>
</tr>
<tr>
<td>Tumor contents</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic</td>
<td>41 (82%)</td>
<td>26 (86.7%)</td>
<td>15 (75%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Semi-solid</td>
<td>9 (18%)</td>
<td>4 (13.3%)</td>
<td>5 (25%)</td>
<td></td>
</tr>
<tr>
<td>Tumor bilateralism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>33 (66%)</td>
<td>18 (60%)</td>
<td>15 (75%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Bilateral</td>
<td>17 (34%)</td>
<td>12 (40%)</td>
<td>5 (25%)</td>
<td></td>
</tr>
<tr>
<td>Mean tumor size</td>
<td>12.12 ± 7.88</td>
<td>11.0 ± 6.64</td>
<td>13.80 ± 9.37</td>
<td>NS **</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-10 cm</td>
<td>32 (64%)</td>
<td>21 (70%)</td>
<td>11 (55%)</td>
<td>NS **</td>
</tr>
<tr>
<td>&gt; 10 cm</td>
<td>18 (36%)</td>
<td>9 (30%)</td>
<td>9 (45%)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>44 (88%)</td>
<td>25 (83.3%)</td>
<td>19 (95%)</td>
<td>NS **</td>
</tr>
<tr>
<td>II-III</td>
<td>6 (12%)</td>
<td>5 (16.6%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Cytology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (18%)</td>
<td>5 (5%)</td>
<td>4 (20%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Negative</td>
<td>41 (82%)</td>
<td>25 (83.3%)</td>
<td>16 (80%)</td>
<td></td>
</tr>
<tr>
<td>Implant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6 (12%)</td>
<td>5 (16.7%)</td>
<td>1 (5%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Negative</td>
<td>44 (88%)</td>
<td>25 (83.3%)</td>
<td>19 (95%)</td>
<td></td>
</tr>
<tr>
<td>Surgical approach</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparoscopy</td>
<td>7 (14%)</td>
<td>5 (16.7%)</td>
<td>2 (10%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>43 (86%)</td>
<td>25 (83.3%)</td>
<td>18 (90%)</td>
<td></td>
</tr>
<tr>
<td>Choice of surgical staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertility-sparing</td>
<td>11 (22%)</td>
<td>6 (20%)</td>
<td>5 (25%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Comprehensive</td>
<td>39 (78%)</td>
<td>24 (80%)</td>
<td>15 (75%)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (2%)</td>
<td>1 (3.3%)</td>
<td>0</td>
<td>NS **</td>
</tr>
<tr>
<td>Negative</td>
<td>49 (98)</td>
<td>29 (96.7%)</td>
<td>20 (100%)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (8%)</td>
<td>3 (10%)</td>
<td>1 (5%)</td>
<td>NS **</td>
</tr>
<tr>
<td>No</td>
<td>46 (92%)</td>
<td>27 (90%)</td>
<td>19 (95%)</td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (8%)</td>
<td>3 (10%)</td>
<td>1 (5%)</td>
<td>NS **</td>
</tr>
<tr>
<td>No</td>
<td>46 (92%)</td>
<td>27 (90%)</td>
<td>19 (95%)</td>
<td></td>
</tr>
<tr>
<td>Mean CA125 (U/ml)</td>
<td>171.2 ± 115.5</td>
<td>191.1 ± 165.9</td>
<td>163.9 ± 124.2</td>
<td>NS *</td>
</tr>
<tr>
<td>CA125 - High</td>
<td>27 (54%)</td>
<td>18 (60%)</td>
<td>9 (45%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Normal</td>
<td>23 (46%)</td>
<td>12 (40%)</td>
<td>11 (55%)</td>
<td></td>
</tr>
<tr>
<td>Mean CA19-9 (U/ml)</td>
<td>42.4 ± 39.9</td>
<td>38.7 ± 43.3</td>
<td>48.1 ± 34.3</td>
<td>NS *</td>
</tr>
<tr>
<td>CA19-9 - High</td>
<td>18 (36%)</td>
<td>8 (26.7%)</td>
<td>10 (50%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Normal</td>
<td>32 (64%)</td>
<td>22 (73.3%)</td>
<td>10 (50%)</td>
<td></td>
</tr>
<tr>
<td>CA125+CA 19-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>11 (22%)</td>
<td>4 (13.3%)</td>
<td>7 (35%)</td>
<td>NS **</td>
</tr>
<tr>
<td>Normal</td>
<td>39 (78%)</td>
<td>26 (86.7%)</td>
<td>13 (65%)</td>
<td></td>
</tr>
</tbody>
</table>

* Mann-Whitney U test; ** Chi-square test.
Table 2. — Correlations between tumor markers and clinical features according to histotypes.

<table>
<thead>
<tr>
<th>Menopause status</th>
<th>Premenopause (n = 22)</th>
<th>Menopause (n = 8)</th>
<th>p</th>
<th>Premenopause (n = 14)</th>
<th>Menopause (n = 6)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA125</td>
<td>14 (63.6%)</td>
<td>4 (50%)</td>
<td>NS</td>
<td>5 (37%)</td>
<td>4 (66.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>CA125+CA19-9</td>
<td>3 (13.6%)</td>
<td>1 (12.5%)</td>
<td>NS</td>
<td>4 (28.5%)</td>
<td>3 (50%)</td>
<td>NS</td>
</tr>
<tr>
<td>CA19-9</td>
<td>6 (27.2%)</td>
<td>2 (25%)</td>
<td>NS</td>
<td>6 (42.8%)</td>
<td>4 (66.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>&lt; 40 age (n: 15)</td>
<td>&gt; 40 age (n: 15)</td>
<td>p</td>
<td>&lt; 40 age (n: 7)</td>
<td>&gt; 40 age (n: 13)</td>
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<td>9 (60%)</td>
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<tr>
<td>CA125+CA19-9</td>
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<td>&lt; 0.05</td>
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<td>–</td>
<td>NS</td>
<td>5 (33.3%)</td>
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<td>10 (71.4%)</td>
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<td>6 (40%)</td>
<td>3 (60%)</td>
<td>NS</td>
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<tr>
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<td>3 (16.6%)</td>
<td>1 (8.3%)</td>
<td>NS</td>
<td>4 (26.6%)</td>
<td>3 (60%)</td>
<td>NS</td>
</tr>
<tr>
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<td>7 (46.6%)</td>
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<td>1 (100%)</td>
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<td>NS</td>
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<tr>
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<td>1 (100%)</td>
<td>&lt; 0.05</td>
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<tr>
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<td>10 (66.6%)</td>
<td>–</td>
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<td>4 (25%)</td>
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<td>Implant negative (n: 19)</td>
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<td>3 (60%)</td>
<td>NS</td>
<td>9 (47.3%)</td>
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<td>NS</td>
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<td>NS</td>
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<td>3 (60%)</td>
<td>NS</td>
<td>10 (52.4%)</td>
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<td>NS</td>
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<td></td>
<td>Adjuvant chemotherapy negative (n: 19)</td>
<td>Adjuvant chemotherapy positive (n: 1)</td>
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<td>CA125</td>
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<td>3 (100%)</td>
<td>NS</td>
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<td>NS</td>
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<td>7 (36.8%)</td>
<td>–</td>
<td>NS</td>
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<td>CA19-9</td>
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<td>NS</td>
<td>10 (52.2%)</td>
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</tr>
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<td>2 (66.6%)</td>
<td>NS</td>
<td>8 (42.1%)</td>
<td>1 (100%)</td>
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<tr>
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<td>2 (66.6%)</td>
<td>&lt; 0.05</td>
<td>6 (31.5%)</td>
<td>1 (100%)</td>
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<td>1 (100%)</td>
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Variables were compared by using the chi-square test.
Comparison of tumor markers and clinicopathological features in serous and mucinous borderline ovarian tumors

Preoperative computed tomography (CT) scan, first-line laparoscopy and conservative treatment [13]. However abnormal serum tumor markers and other variables were not evaluated according to histotypes in this study. On the contrary in this study, we did not find any significant relation between abnormal markers and tumor size and bilateralism.

Another detailed study by Ayhan et al. compared tumor markers and the clinicopathologic relation [10]. In this study the mean values of CA125 and CA19-9 were significantly increased by increasing tumor size and elevated CA125 and CA19-9 relations between age, lymph node metastasis, micropapillary architecture, tumor bilateralism, surgical staging choice, history of smoking and use of oral contraceptive pills were not significant. But elevated CA125 was found to be significant at FIGO stage, parity and implant [10]. This study did not investigate the correlation of tumor markers and variables with histotypes. In our study, we did not find any stage, parity, or peritoneal implant association with tumor markers.

In another study, when the serum CA125 value was 35 IU/ml, the mean tumor size was 7.7 cm, and when CA125 value was ≥ 100 IU/ml, the mean tumor size was 14.2 cm [8]. We did not find a significant relation between tumor size and tumor markers in either group. Tumor marker and stage of the tumor is another issue.

Patients with mucinous borderline tumors tended to have lower tumor stages.

In another study 79.4% of patients with serous BOTs were Stage I-II, and 97.8% of the mucinous BOT patients were Stage I. In this study 20.6% of serous tumors were at Stage III, while only 2.2% mucinous were reported at this stage [6]. Therefore, serous and mucinous tumors have different serum markers at different stages.

Leinhard et al. reported a relation of tumor stage with increased CA125 [19]. Rice et al. reported that in serous BOTs, patients with advanced stage had higher CA125 levels than Stage I patients [20]. In the literature, elevated CA125 rates for serous were 35-66.7% at Stage I, while 71.4-100% of Stage II-IV patients had elevated CA125 [10, 11]. CA125 elevation in mucinous tumors at Stage I was found to be 37%, while this rate was 67% for Stage II-IV tumors in a review of 325 patients [8, 10, 11]. Reports on elevated CA19-9 and stage comparison for both serous and mucinous tumors are few in the literature. In one study, elevated CA19-9 rates for serous Stage I and III BOTs were found to be less than mucinous tumors at the same stage [10]. However, we did not find elevated CA125 and CA19-9 levels according to the stages of serous and mucinous tumors. The other issue is tumor marker and cytology. The rate of high preoperative CA125 level increases in cases of positive peritoneal cytology results was shown. This significant increase was not observed for the positivity of serum CA19-9 [10]. However, we found that cytology was positively associated with elevated CA125 and CA19-9, but was not related to CA125 in the serous group, contrary to this study. Also, we found an elevated CA19-9 association with positive cytology in the mucinous group. But, this and our study were different according to histotypes as the rates of cytology positivity of serous and mucinous tumors were 11 vs 3 and 5 vs 4, respectively, and this study did not compare tumor markers to histotypes as other studies.

The rate of positive peritoneal cytology of serous BOTs is more than mucinous.

In one study, positive peritoneal cytology was found to be 35.7% for serous versus 8.5% for mucinous [6]. However, in our study the rate of positive cytology was similar in both groups, and differences may be due to this finding.

Published reports have shown 38-40% bilaterality for serous tumors and only 8% for mucinous tumors [21]. In another study the bilaterality rate for serous versus mucinous tumors was 27.9% versus 1.1% [6].

Bilateralism and tumor markers were evaluated by Ayhan et al. and found to be non significant [10]. In our study we did not find a significant relation between serum tumor markers and bilaterality for either group. As for relations between serum tumor markers and recurrence there are conflicting results in the literature. In a study consisting of 266 cases with a recurrence rate of 23 (8.6%), progression-free survival (PFS) was related to CA125 [12]. In another study (233 cases, 21 recurrences) in five years PFS analyses showed that CA125 > 144 cases with high recurrence rate and PFS were related to CA125 [19]. In another study Leinhard et al. showed that CA125 elevated preoperatively had a diagnostic value for recurrence, but it was not significant for overall survival [19]. However in another study, recurrence and CA125 were not significantly associated; the elevated CA125 rate was 13.1% for recurrence versus 86.9% for non recurrence [16].

Another study showed abnormal serum tumor marker status was not associated with the risk of recurrence [13], but this study did not evaluate the detailed analyses of the histotypes as we did.

In our study, we found that only in the serous group, elevated CA125 and CA125+CA19-9 were significantly related to recurrence.

An important issue is implants and tumor markers. The rate of implants of serous BOTs was much more than mucinous. In this study the rate of implants was 22.4% for serous versus 3.6% for mucinous [6]. Implant and survival relation has been shown in several studies, but a relation between implant and tumor markers are limited. Ayhan et al. showed peritoneal implants were significantly associated with elevated CA125, but not with CA19-9 [10]. Leinhard et al. showed elevated CA125 was significantly related to implants [19]. However in our study, we did not find any implant association with tumor markers, contrary to these studies.

Another issue is lymph node involvement in BOTs. Combined data from five studies (161 cases) by Fadare indicated that lymph node involvement ranged from 0%-42% (average 27%) in BOTs and estimation of involvement rate was difficult, because most BOTs were not formally staged [22]. Also, lymph node involvement and
tumor marker relation studies are few. In one study, lymph node involvement rate was 8.33%, which was not related to either to CA125 nor to CA19-9, similar to our results.

The rate of intracystic papilla was reported to be between 48-78% [23-25]. Gotlieb et al. showed that mucinous tumors tended to be larger on US than serous tumors; the rate of multilocularity was 50% and contained papillations in 40%. In another study, serous tumors were multilocular in 30% of patients, but presented with solid or papillary patterns in 78% [8].

In another study, 48% of BOTs showed papilla and 24% showed septa, of which 18% were multilocular, and the author indicated that the presence of internal papillae and multiple septa was the most significant sonographic pattern associated with BOT [23]. Another multivariable analysis study indicated that only intracystic papillae were an independent predictor of BOT [24]. However, few studies evaluated tumor markers and papilla. Micropapillary architecture and tumor markers were evaluated by Ayhan et al. and they did not find any significant relation. But, only in the mucinous group, we interestingly found elevated CA125 and CA125+CA19-9 and papilla presence, and we did not find any relation with septa formation [10].

Our study group was small and the role of some features low in both serous and mucinous groups. We compared serum tumor markers with all clinicopathologic features of both serous and mucinous histotypes and all our cases had both elevated CA125 and CA19-9. To the best of our knowledge, there are no similarly designed studies as ours in the literature. There is a need for large series to confirm the knowledge about tumor markers in BOTs, which may reflect differences of serous and mucinous BOTs.

References


Is magnetic resonance imaging useful in early evaluation of women on neoadjuvant chemotherapy for locally advanced cervical cancer?

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Summary

Objective: To evaluate the accuracy of magnetic resonance imaging (MRI) in staging cervical tumors after neoadjuvant chemotherapy (NACT). Methods: 26 women, affected by locally advanced cervical cancer and triaged for surgery after NACT, were submitted to three cycles of neoadjuvant chemotherapy. All patients were submitted to MRI before and after NACT. We evaluated the MRI sensitivity and specificity in staging cervical tumors after chemotherapy, relating MRI findings after NACT with the pathological findings as the gold standard. Results: In our series, MRI sensitivity was 58.8% and specificity was 66.7%. Conclusions: In our study MRI accuracy after NACT was lower than that of MRI used to stage patients with early cervical cancer scheduled for primary surgery, reported by the literature. MRI false negative cases are the major problem because of the delay in application of an effective therapy in non responders to NACT.

Key words: Locally advanced cervical cancer (LACC); Neoadjuvant chemotherapy (NACT); Magnetic resonance imaging (MRI); Early response evaluation.

Introduction

Although the widespread availability of effective screening programs, cervical cancer ranks third worldwide among gynecological malignancies [1]. The optimal treatment strategies are increasingly tailored to the extent of disease, necessitating improvement in pretreatment evaluation [2].

For early cervical cancer (tumor limited to uterine cervix, International Federation of Gynecology and Obstetrics, FIGO, Stage Ia2-Ib1), radical surgery (radical hysterectomy and pelvic lymphadenectomy) or radiotherapy are accepted as the standard treatment. Instead, there is still no agreement on the best approach for bulky (maximum tumor diameter ≥ 4 cm) or locally advanced cervical cancer (LACC, FIGO Stage IIb-IVa) [3].

At present, concomitant chemoradiotherapy (CT-RT) is considered the standard treatment of LACC. A recent meta-analysis showed a highly absolute survival improvement of concomitant CT-RT compared to radiotherapy alone [4].

Recently, neoadjuvant chemotherapy (NACT) prior to surgery has been applied as a new therapeutic option for LACC. This treatment usually uses cisplatin-based agents repeated for three cycles at three-week intervals as the standard regimen. Some studies have supported its effectiveness in shrinking tumor size, controlling micrometastasis and increasing operability rate or improving the outcome of radiotherapy [5].

On the other side, major supposed disadvantages of NACT are the delay of curative treatment in non responders, the development of radio-resistant cellular clones, and the cross-resistance with radiotherapy [6].

How to make use of the advantages that NACT offers and at the same time how to avoid the delay of the effective therapy for non responders is a very important topic in current cervical cancer therapy. Early NACT response predictors are needed to provide a window of opportunity to modify treatment strategy and improve survival in non responders.

In recent years sophisticated radiological examinations, such as magnetic resonance imaging (MRI), have been used to define tumor extension before and/or after treatment so as to better tailor the management of patients affected by cervical cancer. However MRI in patients submitted to NACT seems to be less accurate than MRI used to stage patients with early cervical cancer triaged for primary surgery [7, 8].

The aim of this study was to evaluate the accuracy of MRI in staging cervical tumors after neoadjuvant chemotherapy and to analyze its usefulness in tailoring clinical management of women submitted to NACT.

Material and Methods

Between November 2002 and October 2010, 26 women affected by bulky or locally advanced cervical cancer and triaged for surgery after neoadjuvant treatment were admitted to the Unit of Clinical Obstetrics and Gynecology at the “San Martino” University Hospital in Genoa. These 26 patients were staged according to FIGO criteria and underwent a physical...
examination, tumor biopsy and chest X-ray. Clinical characteristics of the women enrolled in this study are reported in Table 1. In all the cases the tumor staging was completed with an abdominal-pelvic MRI. In the last nine patients of the study the MRI exam was carried out, as now routinely performed in our Institution, with the auxilium of an artificial saline hydrocolpos [9]. MRI examinations were always performed and evaluated by the same radiologist. We used a high field MR scanner (Avanto 1.5 T, Siemens, Erlangen, Germany) with phased-array body coils. High resolution Turbo Spin Echo (TSE) T2-weighted sequences were performed (4 mm slice thickness, 200-220 field of view, matrix 256 x 256, TR 4000 ms, TE 90 ms, ETL 15, number of measurements 3, parallel imaging factor of acceleration 2) in sagittal, para-axial and para-coronal planes oriented towards the uterine cervix long axis. TSE T1-weighted axial images from the pelvis to the upper abdomen were then performed to evaluate the lymph nodes. Contrast medium was not used in any case.

Concerning imaging analysis, a tumor was identified when a signal that was equal to or higher than that of fat replaced low signal intensity of normal cervical stroma in the T2-weighted spin-echo images. Four MRI characteristics of cervical carcinoma were specifically recorded in the pelvic MRI by the radiologist:

- signal intensity in the T2-weighted images (equal to or higher than the surrounding adipose tissue);
- maximal diameter of the lesion;
- infiltration of vaginal fornices;
- infiltration of parametria.

The presence/absence of metastatic lymph nodes (considered positive if the nodal maximal diameter was more than 1 cm) was always reported.

All the 26 study women were submitted to three cycles of neoadjuvant chemotherapy. According to the age and performance status of the patients, they were treated with the TP schedule (paclitaxel 175 mg/m², cisplatin 75 mg/m²) or the TIP sched-

Table 1. — Clinical and pathological characteristics of the patients study (n = 26).

<table>
<thead>
<tr>
<th>Characteristics</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>IB1</td>
<td>1 (3.8%)</td>
</tr>
<tr>
<td>IB2</td>
<td>5 (19.2%)</td>
</tr>
<tr>
<td>IIA</td>
<td>3 (11.5%)</td>
</tr>
<tr>
<td>IIB</td>
<td>16 (61.5%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SCC</td>
<td>22 (84.6%)</td>
</tr>
<tr>
<td>Adc</td>
<td>4 (15.4%)</td>
</tr>
<tr>
<td>Grading of differentiation</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>2 (7.4%)</td>
</tr>
<tr>
<td>G2</td>
<td>13 (51.9%)</td>
</tr>
<tr>
<td>G3</td>
<td>7 (25.9%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (14.8%)</td>
</tr>
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</table>

SCC: squamous cell carcinoma; Adc: adenocarcinoma.
Is magnetic resonance imaging useful in early evaluation of women on neoadjuvant chemotherapy for locally advanced cervical cancer?

Is magnetic resonance imaging useful in early evaluation of women on neoadjuvant chemotherapy for locally advanced cervical cancer? Twenty-three women were submitted to the TP regimen and the other three were submitted to the TIP regimen. After the end of the neoadjuvant treatment every patient was reassessed using the same clinical and imaging procedures described above. All MRI studies after chemotherapy were performed within four weeks (mean 15 days) after the last course of chemotherapy. The MRI response to chemotherapy was recorded relating MRI findings before and after NACT, according to the World Health Organization (WHO) criteria [10]. A complete response (CR) was defined as the disappearance of all known disease, a partial response (PR) as a 50% or more decrease in total tumor size of the lesions and stable disease (SD) was identified when a 50% decrease in total tumor size could not be established nor a 25% increase in the size of one or more measurable lesions was not demonstrated. In conclusion progressive disease (PD) was defined as a 25% or more increase in size of tumor, or appearance of new lesions.

The patients who showed complete or partial MRI response to neoadjuvant treatment underwent surgery, while patients experiencing no change or progression of the disease were treated with salvage chemoradiotherapy. Twenty-three out of the 26 patients were operated: 21 were submitted to Piver III radical hysterectomy, while the other two young patients (mean age 29.5 years old), wishing to retain their childbearing prospects, were treated with vaginal radical trachelectomy. In all these cases a pelvic and paraaortic lymphadenectomy was associated.

Adjuvant postoperative treatment was recommended to patients with pathological stage greater than pT2a, less than 3 mm of uninvolved cervical stroma or lymph node metastasis. Three women were not operated because of SD and parametrial invasion at MRI after NACT. Thus these three patients were submitted to a definitive chemoradiation.

In the 23 patients who were operated, we evaluated the MRI sensitivity, specificity, false-negative rate and false-positive rate in staging cervical cancer after chemotherapy, relating MRI findings after NACT with the pathological findings as the gold standard. The correlation between MRI and pathological findings is about the extension of the tumor. For the pathological specimen, a tumor maximal diameter ≤ 3 mm is considered as microscopic disease and it is valued as a negative finding because its prognosis is equal to that of absent residual disease. A pathological residual disease with a maximal diameter > 3 mm is considered as macroscopic disease.

Results

In our study after NACT a MRI response (CR or PR) [10] was documented in 20 out of 26 patients (76.9%) (Table 2). Six patients demonstrated SD after chemother-
apy with parametrial infiltration and/or lymph node metastasis: three out of these six women were not operated and were treated with salvage chemoradiotherapy (patients n. 6, 17 and 21 in Table 2); the other three patients and the gynecologists were in agreement about the pathological specimen in the 23 patients who were submitted to surgical intervention. 1Correlation between MRI and pathological findings: TP true positive, FP false positive, TN true negative, FN false negative.

Table 2. — Extension of the tumor at MRI pre- and post-NACT and MRI response to chemotherapy in the 26 study patients. \*WHO response to chemotherapy: CR complete response, PR partial response, SD stable disease.

<table>
<thead>
<tr>
<th>Patient</th>
<th>MRI pre-NACT Maximal diameter (mm)</th>
<th>MRI pre-NACT Parametric infiltration (yes/no)</th>
<th>MRI pre-NACT Parametric lymph node metastasis (yes/no)</th>
<th>MRI post-NACT Maximal diameter (mm)</th>
<th>MRI post-NACT Parametric infiltration (yes/no)</th>
<th>MRI post-NACT Parametric lymph node metastasis (yes/no)</th>
<th>MRI response (CR, PR, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>no</td>
<td>no</td>
<td>0</td>
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<td>no</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
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<td>no</td>
<td>23</td>
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<td>yes</td>
<td>PR</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>no</td>
<td>no</td>
<td>50</td>
<td>yes</td>
<td>no</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>no</td>
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</tr>
<tr>
<td>5</td>
<td>60</td>
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<td>20</td>
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<td>no</td>
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</tr>
<tr>
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<td>60</td>
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</tr>
<tr>
<td>16</td>
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<td>45</td>
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</tr>
<tr>
<td>17</td>
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<td>PR</td>
</tr>
<tr>
<td>18</td>
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</tr>
<tr>
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<td>35</td>
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<td>45</td>
<td>yes</td>
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<td>45</td>
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<td>PR</td>
</tr>
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</table>

Table 3. — Correlation between the MRI findings after NACT and the analysis of the pathological specimen in the 23 patients who were submitted to surgical intervention. 1Correlation between MRI and pathological findings: TP true positive, FP false positive, TN true negative, FN false negative.

<table>
<thead>
<tr>
<th>Patient</th>
<th>MRI FIGO stage post-NACT</th>
<th>Maximal lymph node metastasis post-NACT</th>
<th>Pathological FIGO stage post-NACT</th>
<th>Pathological lymph node metastasis post-NACT</th>
<th>MRI pathology correlation (TP,FP, TN, FN)</th>
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<tbody>
<tr>
<td>1</td>
<td>IB1 No IB1 Yes</td>
<td>FN</td>
<td>IB1 No IB1 Yes</td>
<td>IB1 No IB1 Yes</td>
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</tr>
<tr>
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<td>FN</td>
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</tr>
<tr>
<td>3</td>
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<td>FN</td>
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<td>FN</td>
</tr>
<tr>
<td>4</td>
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<td>FN</td>
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<td>0 No 0 No</td>
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<tr>
<td>5</td>
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</table>

Discussion

Today MRI is often used to complete the clinical staging of women affected by invasive cervical cancer. In
primary cervical carcinoma the overall accuracy for staging for MRI is 86% (for determining lesion diameters 93%, vaginal and parametrial invasion 95%, and the presence of nodal involvement 85%). Moreover, MRI is important for demonstrating treatment complications and for detecting of disease recurrence [2].

MRI is also used to evaluate tumor response after neoadjuvant treatment. In fact NACT represents a promising alternative option to standard chemoradiation for LACC, reducing the tumor size and increasing tumor resectability [5].

The rationales for the use of NACT are several. Tumor size reduction permits simplification of surgical procedures and the possible transformation of inoperable tumors in radically resectable ones. Also NACT may increase radiosensitivity decreasing hypoxic cell fraction. Some regimens, especially platinum-based ones, also act directly as radiation potentiators [11, 12].

Finally, response to NACT is an important prognostic factor and this helps in the decision making of the successive therapeutic approach [13]. However the efficiency and the safety of neoadjuvant treatment are currently controversial. Many women submitted to NACT also need adjuvant chemo and/or radiotherapeutic therapy, thus prolonging their treatment and potentially exacerbating associated morbidity.

Above all, the major problem of this new approach seems to be the delay of effective therapy for non-responders, submitted to three or four cycles of NACT during a period longer than nine weeks. In these women the delay may result in missing the optimal time point for surgery [5, 6, 14].

Thus, clinical and radiological evaluations of patients submitted to NACT are very important to plan the appropriate following treatment (radical surgery vs definitive chemoradiotherapy) and not to miss the optimal time point for definitive therapy in non responders.

In a study of Manfredi et al. MRI was performed before and after neoadjuvant therapy in 18 patients with locally invasive cervical carcinoma. MRI was 78% accurate in evaluation of tumor response [7].

In another study carried out by Testa et al. MRI after NACT was less accurate than MRI used to stage patients with early cervical cancer scheduled for primary surgery. Women treated with neoadjuvant therapy are difficult to examine with any imaging method because fibrosis and necrosis change the normal structure of the pelvic organs and disrupt the borders between organs and structures. Moreover the authors underlined that transvaginal ultrasoundography (TVS) and MRI have a similar level of diagnostic performance, in contrast with studies in the past that suggested a very limited role for TVS examination in the evaluation of cervical cancer [15].

In our series of 26 patients we evaluated the performance of MRI in definition of size and parametrial or vaginal extent of invasive cervical cancer after NACT, with pathological findings used as the gold standard.

In our study MRI evaluation after NACT corresponded to the pathological one in 14 out of 23 patients (60.9%), with a sensitivity of 58.8%; in particular the false-negative rate (41.2%) was superior to that of the literature [7, 15].

In our series, we identified seven false-negative cases; most of all in two patients (n. 3 and 6 in Table 3), MRI did not demonstrate the presence of any residual disease, while the pathological analysis documented a macroscopic residual lesion with parametrial infiltration and lymph node involvement. Thus in these two cases the NACT approach delayed the application of an effective treatment and could have negatively influenced the survival chances of the patients. A correct MRI evaluation of the extent of residual disease could avoid surgical intervention.

The last nine patients in the study were evaluated with MRI completed by the auxilium of an artificial saline hydrocolpos, as now routinely performed in our Institution.

In MRI T2-weighted sequences, the elevated contrast intensity difference between the isotonic saline solution, which distends the vagina, and the lesion makes the evaluation of tumoral diameters, morphology (intra or extracervical growth) and of tumor relations with the nearest structures of the pelvis (internal uterine os, vaginal fornix and wall) more immediate and accurate (Figures 1 and 2).

Moreover in case of a large lesion that takes up all the vaginal cavity, fornix involvement evaluation can be very difficult. Hydrocolpos can precisely define the tumor infiltration at this level because the isotonic saline solution that distends the vagina interposes between the vaginal fornix and tumoral mass [9].

In our experience, hydrocolpos is a simple, cheap and safe artifact that makes the radiologist’s interpretation of MRI scans easier and more immediate.

Conclusions

In our study MRI accuracy after NACT was lower than that of MRI used to stage patients with early cervical cancer triaged for primary surgery as reported by the literature [2].

MRI false-negative cases are the major problem in tailoring clinical management of women affected by uterine cervical cancer and submitted to NACT, because the false-negative case delays the application of an effective therapy in patients who do not respond to neoadjuvant therapy and thus salvage options are poor.

It may be useful to associate TVS examination to MRI after NACT and to repeat the staging workup after the first two cycles of chemotherapy, not to miss the optimal time point for definitive therapy in non responders.

With ongoing technological advances, functional imaging techniques, such as dynamic contrast enhanced MRI (DCE-MRI), diffusion weighted MRI, magnetic resonance spectroscopy and F-18-fluorodeoxyglucose positron emission tomography, have significant potential to provide new biomarkers of early tumor response, with an ultimate impact on cancer management [16-18].
References


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3D optical coherence tomography of cervical intraepithelial neoplasia - early experience and some pitfalls

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²Laser Forschungs Labor, Life Science Centre, Ludwig-Maximilians University, Munich (Germany)

Summary

Objectives: To compare two different systems for optical coherence tomography for the diagnosis of cervical dysplasia and to assess potential benefits of three-dimensional imaging. Materials and Methods: OCT images were taken from unsuspicious and suspicious areas of fresh conisation specimens using two different imaging systems, one with the capability to produce three-dimensional images. All OCT images were separately evaluated by two blinded investigators based on a 6-grade classification (normal, inflammation, CIN 1, CIN 2, CIN 3, squamous carcinoma) and later compared to the corresponding histology. Sensitivity and specificity of OCT in detecting cervical dysplasia were determined. Results: OCT images using both OCT systems were taken from 46 sites in ten conisation specimens and later compared to the corresponding histology. CIN lesions were diagnosed correctly by the three-dimensional system with a sensitivity and specificity of 91% and 78% according to the high-resolution OCT system. Conclusions: Both OCT systems used were highly sensitive in identifying cervical intraepithelial neoplasia. Despite technical problems experienced in the present series, we believe that three-dimensional imaging has the potential to further improve the accuracy of optical coherence tomography.

Key words: Optical coherence tomography; OCT; Colposcopy; Intraepithelial cervical dysplasia; CIN.

Introduction

Approximately 500,000 women worldwide are annually diagnosed with invasive cervical carcinoma (ICC) and about 230,000 women die from the disease [1]. Although the incidence of ICC has declined over the last decade, the incidence of cervical intraepithelial neoplasia (CIN) has increased, especially in younger women. If untreated, 15-20% of these women develop severe dysplasia and 5-10% invasive carcinoma [1-3]. In industrialized countries the implementation of screening programs has led to a decline in the number of cervical cancer related deaths. However, this requires a complex diagnostic infrastructure providing cytology, HPV testing, colposcopy and histology [4, 5]. The present screening programs are associated with overall high costs and not feasible for most countries. Therefore, the implementation of new imaging techniques that allow cheap, rapid and non-invasive evaluation of the cervix would be a vast improvement in the prevention of ICC.

Optical coherence tomography (OCT) is a non-invasive high-resolution imaging technique that uses near-infrared light interferometry to visualize the microstructure of tissues. The technique is analogous to B-mode ultrasound imaging with the difference that it uses light as opposed to ultrasound waves. In ultrasound the time delay for an ultrasonic wave to be reflected back to the probe is used to generate an image of the tissue structure. As the speed of light is much greater than the speed of sound, time delay measurements with OCT necessitate a correlation technique known as low coherence interferometry. By providing cross-sectional images in real time with as much as 2 mm penetration depth and high spatial resolution optical coherence tomography fills an important gap between existing imaging modalities [6-8].

In two previous studies we demonstrated that OCT can achieve high-resolution images of cervical epithelium and is highly sensitive in identifying pre-invasive and invasive cancer of the uterine cervix [9, 10]. However, with the current available OCT devices the differentiation between low- and high grade dysplasia is difficult. In two previous studies we demonstrated that OCT can achieve high-resolution images of cervical epithelium and is highly sensitive in identifying pre-invasive and invasive cancer of the uterine cervix [9, 10]. However, with the current available OCT devices the differentiation between low- and high grade dysplasia is difficult. The purpose of this study was to evaluate the feasibility of a new OCT system providing three-dimensional images and to compare the results with the two-dimensional images of our present device.

Materials and Methods

We present a prospective single-institution, institutional review board-approved, ex-vivo study comparing two different OCT systems. Images were taken from 46 sites in ten loop electrosurgical excision procedure (LEEP) specimens and later compared to the corresponding histology. All images were anonymized to preclude identification. Two investigators blinded for the final histological diagnosis evaluated the OCT images using a 6-grade classification (normal, inflammation, CIN 1, CIN 2, CIN 3, squamous carcinoma) as described before [9, 10].

OCT imaging was carried out using two different devices: The Niris imaging system (Imalux Corporation, Cleveland, OH) is an optical fiber-based interferometer with a superluminescence diode (SLD), providing a low-coherent broadband, near infrared (NIR) light. The reusable fiber-optic probe with a diameter of 2.7 mm provides a depth scanning range of ± 1.5 mm and a lateral scanning range of 1.6-2.4 mm. It is used in direct contact with the tissue. The system acquires real-time two-dimensional images of 200 x 200 pixels. The Niris imaging

*Revised manuscript accepted for publication June 8, 2011*
The Vivosight OCT Scanner (Michelson Diagnostics, Orpington, Kent, UK) uses a swept source laser (HSL-2000-12-MDL, Santec Corporation, Ohkusa-Nenjyozaka, Komaki, Japan) with a wave length of 1305 nm. The reusable probe provides a depth scanning range of ± 2.0 mm and a lateral scanning range of 5 mm. Direct contact with the tissue is not necessary. Due to its multislice function the system acquires both two- and three-dimensional images with a lateral resolution better than 7.5 m and an axial resolution better than 10 m. The Vivosight scanner has been approved by the FDA and the EC.

Due to its multislice function the system acquires both two- and three-dimensional images with a lateral resolution better than 7.5 m and an axial resolution better than 10 m. The Vivosight scanner has been approved by the FDA and the EC.

Results
Forty-six OCT images for each system and corresponding histologies were taken from ten LEEP specimens within the first postoperative hour. The patients mean age was 34.1 years (27-44 years) and all women were premenopausal. Indication for conisation was a Pap III in one case, a Pap IIB in four cases and a Pap IVA in five cases according to the Munich nomenclature. All women were HPV high-risk positive.

All images of the Niris system were evaluated by two investigators working independently and being blinded for the final histology. The correlation between OCT images and histology is shown in Table 1. Thirty-six (second investigator: 37) of 46 sites were correctly diagnosed by OCT. All biopsy sites with histologically no dysplasia were correctly interpreted. Two (1) inflammatory changes were misinterpreted as CIN lesions. By comparing the 46 histological results with the corresponding OCT findings and defining a threshold at CIN 2, there were 21 (22) true positive, 18 (18) true negative, 2 (1) false negative and 5 (5) false positive results. The sensitivity calculates to 91% (96%), the specificity to 78% (78%).

Table 1. — By comparing 46 histological results with corresponding OCT findings and defining a threshold at CIN 2, there were 21 (22) true positive, 18 (18) true negative, 2 (1) false negative and 5 (5) false positive results. The sensitivity calculates to 91% (96%), the specificity to 78% (78%).

Investigator agreement was assessed by applying Cohen’s Kappa statistics. The sensitivity calculates to 91% (96%), the specificity to 78% (78%).

Discussion
This study was carried out in order to compare two different OCT systems with the chance to evaluate 3D imaging of cervical epithelium. We had to choose an ex vivo setting as the probe of the Vivosight scanner available at the time of our experiments was not appropriate for in vivo imaging. Meanwhile the company has developed a new soft tissue probe which allows in vivo imaging of cervical tissue and provides the same imaging properties as the topical probe.

The Vivosight scanner provides both 2D and 3D images over a 5 mm x 5 mm area producing up to 2000
cross sections. As in our series image margins frequently showed shadowing effects we reduced the format to 3 mm x 3 mm. Furthermore, we reduced the number of cross sections to 100/mm in order to minimize scan time and file size, to avoid oversampling and to utilize a lateral resolution of better than 7.5 µm.

Due to the higher resolution of the Vivosight scanner we expected more detailed OCT images in comparison to the Niris system. In fact, we obtained excellent results in a number of cases but we were not able to maintain this high standard throughout the study. The probe we were using represented the main cause for difficulties. As even minor movements caused artefacts and superpositions, it became necessary to fix the probe in a support frame. Finding the right distance between probe and epithelium to achieve a satisfactory depth scanning range, to exactly scan the area of interest and to scan the tissue in a right angle represented other problems. We assume that the new soft tissue probe available now will eliminate these difficulties.

The Volume Viewer plugin of the Image J software program (Wayne Rasband 2009) allows slices and volume visualization to be displayed using different interpolation and rendering techniques. The viewing position and the orientation and position of slices or volumes can be arbitrarily chosen. Therefore, 3D images provide not only additional information regarding the epithelial surface but can also visualize layers of interest such as the basement membrane. Figure 4 shows a sagittal (a) and a horizontal (b) plane of a CIN3 lesion. The level of the horizontal plane was chosen close to the basement membrane displaying an uneven column-like appearance as a typical feature of high-grade cervical dysplasia. However, to what extent 3D imaging improves the interpretation of cervical dysplasia has to be evaluated in future studies.

The Niris OCT system which we have routinely used in our out-patient clinic for more than two years produces reliable pictures and the results presented in this study are similar to those reported earlier [9, 10]. The system is

Figure 1. — Niris (left) and Vivosight images (right) of normal epithelium, CIN 3 and inflammation obtained from identical areas. (a) a well recognizable three-layer architecture and a sharp interface between epithelium and stroma optically representing the basement membrane (B). (b) a CIN 3 lesion with the typical irregularity of the epithelial layer. (c) Inflammation. The basement membrane as an optical interface between epithelium and the stroma is existent but less clear. The Vivosight image shows a much brighter epithelium as a correlate for inflammation.
highly sensitive in identifying precancerous lesions, but is not as precise as requested in distinguishing between different CIN grades. A succeeding model with higher resolution and a new light source and optic will be available in 2011 and may improve the specificity as well as the differentiation of cervical dysplasia.

With only 46 specimens included, the calculation of sensitivity and specificity must be judged with caution. Furthermore, there is a bias in our study design as we gained the results from a very selected group of patients. All conisations were carried out to further assess suspicious Pap smears and colposcopy findings.

This study comprises just a small number of patients and one might ask why we present the data at all. Despite our problems with the Vivosight scanner and a large number of inadequate images, the successful images in the series clearly show the great potential of optical coherence tomography. As technical difficulties can be resolved, we are convinced that the future generation of OCT systems represents a substantial progress towards the identification and clinical management of precancerous and cancerous cervical lesions.

Acknowledgements

This study was supported by grants of the Friedrich-Baur-Stiftung and the Muenchener Medizinische Wochenschrift. We gratefully acknowledge the loan of a Niris OCT imaging system by Imalux (Cleveland, OH) and a Vivosight OCT Scanner by Michelson Diagnostics (Orpington, Kent, UK). Dr. H. Stepp is member of the advisory council of Imalux (Cleveland, OH).

References

3D optical coherence tomography of cervical intraepithelial neoplasia - early experience and some pitfalls


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Combination of fertility preservation strategies in young women with recently diagnosed cancer

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Summary

Background/Aims: The study describes clinical management and outcomes of currently available fertility preservation techniques in a set of 154 young female cancer patients. Methods: Patients in reproductive age with newly diagnosed cancer were offered embryo or oocyte cryopreservation, ovarian tissue cryopreservation and the administration of GnRH analogues during chemotherapy. Particular attention was given to the technical aspects and clinical application of these fertility preservation techniques. Results: During the study period (2004-2009), 154 young female cancer patients were offered fertility preservation counseling. Patient's average age was 29.4 years and average parity was 0.7 children. Administration of GnRH analogues (n = 123, 79.9%) and ovarian tissue cryopreservation (n = 15, 9.7%) were the most commonly used fertility preservation strategies. In 20 cases (16.1%), the combination of several fertility preservation techniques was offered to individually selected patients. Conclusions: Combination of fertility preservation techniques gives young cancer patients the best chance for future fertility and should be concentrated in specialized centers.

Key words: Cancer; infertility; Fertility preservation; Oocyte cryopreservation; Ovarian tissue cryopreservation.

Introduction

Incidence of cancer in reproductive age and childhood is on the rise. However, today more and more cancer patients can be completely cured due to earlier diagnosis and the availability of very effective anti-cancer therapies. One of the most common long-term consequences of cancer treatment is infertility due to the destruction of gonadal cells. Of the different modalities of oncology treatment, chemotherapy represents the greatest threat to reproductive function. The most gonadotoxic chemotherapeutics are alkylating agents, platina derivates and taxans. Some of these drugs, especially cyclofosphamide, are also widely used for immunotherapy in rheumatology (systemic lupus erythematodes, rheumatoid arthritis, etc.) [1].

The destruction of ovarian follicles by chemotherapy leads to a disruption of ovarian function. The degree of ovarian function disruption depends on the actual number of primordial follicles (PMF) in the ovary before the onset of chemotherapy. The number of ovarian follicles slowly decreases in life due to follicle maturation in reproductive age and also as a result of their ongoing apoptosis [2]. The actual number of follicles in the ovary is mainly determined by the patient’s age at the time of cancer diagnosis. A complete loss of ovarian follicles leads to acute ovarian failure (AOF). AOF is characterized by amenorrhea and acute menopausal syndrome (hot flushes and night sweating), which occur due to absence of the ovarian steroids (mainly estrogens and progesterone) normally produced by the follicles. The impact of chemotherapy sometimes only causes a significant decrease of PMF (not complete loss). Such cases are asymptomatic with regular menstrual cycle and the normal production of ovarian steroids, but a patient’s ovarian reserve of PMF is considerably reduced and she is at a high risk of the onset of premature ovarian failure (POF) in the years following treatment. That is why all cured cancer patients are strongly advised not to postpone motherhood for too long. POF is defined as the onset of menopause before the age of 40, with amenorrhea and acute menopausal symptoms. In cases where AOF or POF occur, the patient becomes completely infertile due to the absence of PMF containing oocytes, and the only chance of becoming pregnant is to use an oocyte donor.

In the last ten years, a lot of research and clinical effort have been devoted to developing fertility preservation strategies which would preserve the reproductive potential of successfully cured cancer patients. The importance of the future fertility of cancer patients has prompted the creation of a new subspecialty of reproductive medicine recently referred to as oncofertility. This term was suggested by Woodruff in 2004 mainly because this problem involves primarily oncologists and specialists in reproductive medicine. The main goal of this new ‘inter’ discipline is research and development into methods of fertility preservation for patients in connection to cancer diagnosis, treatment and survivorship [3]. Oncofertility amalgamates biomedical and translation research, clinical medicine and the social sciences. Preserving fertility in the face of other medical (non-cancer) conditions is also becoming more prevalent, and so is the postponement of future fertility for social reasons.

Several fertility preservation strategies are currently available for young cancer patients before embarking on their oncology therapy. The optimal approach is chosen on a strictly individual basis and depends on the type of cancer, the type of treatment (e.g., radiation and/or chemotherapy), time available till the onset of treatment,

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Revision manuscript accepted for publication June 10, 2011

XXXIII, n. 1, 2012
the patient’s age, and whether the patient has a partner [4]. To date, the most effective technique, and also the oldest, is embryo cryopreservation (EC). The human embryo is very resistant to damage caused by cryopreservation and delivery rates per embryo transfer using cryopreserved embryos are promising (18-20%) [5]. Oocyte cryopreservation (OC) is an alternative to embryo storage. Freezing mature oocytes has been a technical challenge because mature human oocytes are extremely sensitive to temperature changes. An alternative cryobiology approach exists called oocyte vitrification, which consists of solidifying liquid by ultra-rapid cooling. This renascent and promising technique is becoming an effective alternative to conventional slow freezing protocols [6]. However, both approaches (EC and OC) require in vitro fertilization (IVF) with the need for ovarian stimulation (usually for 2 to 4 weeks), which delays cancer treatment. The EC method additionally requires the participation of the male partner of the cancer patient. When these criteria cannot be met, more experimental preservation methods must be considered. A young female patient with newly diagnosed cancer should be informed about the possibility of ovarian tissue cryopreservation (OTC). After the patient has been cured, thawed ovarian tissue may be later auto-transplanted back to the pelvis to attempt spontaneous conception or carry out ovarian stimulation and subsequent IVF. Other emerging treatment options for fertility preservation include medication to prevent chemotherapy-induced oocyte damage. Clinically the most successful is the administration of gonadotropin-releasing hormone analogues (GnRH-a) during systemic cancer treatment (gonadotoxic chemotherapy or total body irradiation). Several human studies demonstrate that the GnRH-a are well tolerated and may protect long-term ovarian function [7-9]. Although the results of these studies are limited by sample size and lack of any randomized control group, randomized controlled trials are currently underway internationally to evaluate this strategy in young women with cancer. The oocyte donor (OD) program remains an established clinical option with a very high success rate for those who accept the use of non-parental genes or choose not to use experimental techniques or who fail to conceive with the above-mentioned methods.

The aim of this study is to describe the management and outcomes of currently available fertility preservation techniques in a set of 154 female cancer patients in reproductive age. The data presented here show that the most effective clinical results regarding the future fertility of young female cancer patients can be achieved using a combination of currently available fertility preservation techniques.

Materials and Methods

Female patients with newly diagnosed cancer in reproductive age (15-35 years) were referred by their oncologist for counseling at the Brno University Hospital Fertility Preservation Center (FPC) from January 2004 until November 2009. During their appointment, patients were informed about the risk of fertility impairment or fertility loss due to planned cancer treatment and about the possibilities represented by the available fertility preservation techniques. After considering several parameters, such as the type of cancer, proposed treatment protocol (e.g., radiation and/or chemotherapy), time available till onset of treatment, patient’s age and whether the patient had a partner, the individual risk of threat to their fertility was assessed. An individual optimal fertility preservation strategy was advised from one of the following options:

- ovarian stimulation with embryo or oocyte cryopreservation (EC, OC);
- ovarian tissue cryopreservation (OTC);
- administration of GnRH-a during cytotoxic chemotherapy.

In many cases, a combination of GnRH-a co-treatment and one of the cryopreservation techniques was recommended. Every patient signed an informed consent form, containing detailed information about proposed fertility preservation techniques approved by the institutional ethics committee. All patients also had an option to refuse any of the proposed fertility preservation techniques. All administrative and statistical data monitored in the study were processed and statistically evaluated by standard statistical software (SPSS). We shall describe below in greater detail all the techniques mentioned above.

Embryo cryopreservation

Before EC all patients were stimulated with recombinant FSH follitropin alfa (Gonal-F, Merck Serono) together with the gonadotrophin releasing hormone antagonist cetrorelix (Cetrotide 3 mg, Merck Serono) according to a standardized protocol. Ovarian stimulation started at day 2 of the patient’s menstrual cycle with a daily dose of 75-225 IU of FSH according to basal FSH levels and vaginal ultrasound (US) results. On day 7, cetrorelix in a dose of 3 mg was added to FSH to prevent any premature LH surge and oocyte retrieval was planned for day 10-14 of the patient’s menstrual cycle. Oocytes were collected by US-guided vaginal needle aspiration under short general anesthesia. Collected oocytes were assessed by an embryologist and mature oocytes were fertilized with spermatozoa from the patient’s partner using the intracytoplasmatic sperm injection (ICSI) technique. The partner of the patient gave written informed consent for the use of his spermatozoa which was collected before fertilization. All embryos created by fertilization were grown in vitro in an embryo culture (Vitrolife) for 2-4 days according to their growth potential, which was assessed daily by an embryologist. Usually at day 3 of culture, embryos were cyropreserved using the slow freezing technique by using a controlled rate freezer (Planer Kryo 10). The commercial freezing set Freeze-Kit 1TM (Vitrolife) was used for embryo preparation before the cryopreservation procedure. After successful cancer treatment, cryopreserved embryos can be used to achieve pregnancy by frozen-thawed embryo transfer (FET). To produce good pregnancy rates, the endometrium of recipient woman has to be prepared by administering estrogen and progesterone. We used estradiol valerate and micronized progesterone oral tablets for endometrium preparation according to standardized protocol [10]. FET was performed if endometrial thickness was at least 7 mm (measured by vaginal US). Embryos were always thawed one day prior to embryo transfer by using the commercial embryo thawing kit Thaw-Kit 1TM (Vitrolife). After the thawing procedure, embryos were cultivated for 24 hours in a standard IVF medium and only those embryos that showed further development (50% or more of surviving blastomeres) were used for embryo transfer.
Oocyte cryopreservation

This second possible technique is suitable mainly for women without a regular male partner or adolescents. Ovarian stimulation and the oocyte collection process have been described in the previous paragraph. Collected mature oocytes containing both polar bodies (MII oocytes, metaphase of second meiotic division) can be cryopreserved by the conventional slow freezing technique or by vitrification (ultra-rapid cooling). The conventional slow freezing technique used in our study is described in detail by Fabri et al. [11]. Oocytes were prepared for freezing with the ready-to-use commercial media system Oocyte Freeze (Medicult) containing 1,2-propanediol and sucrose as cryoprotectants and later cryopreserved in straws using a controlled rate freezer (Planer Kryo 10). The vitrification technique used by our lab is described by Kuwayama et al. [12]. For vitrification we also used the commercial media system Vitrification Cooling (Medicult) containing ethylene glycol, propandiol and sucrose as cryoprotectants and eight vitrification straws (High Security Vitrification Straw) for oocyte storage. According to strict European regulations (European Tissue Directive, 2004/23/EC), we used the so-called “closed” vitrification system, which avoids direct contact between the cryopreserved material and liquid nitrogen. The oocyte thawing process is analogous to the embryo thawing technique. Oocytes cryopreserved using the slow freezing technique were processed with the OocyteThaw (Medicult) thawing kit according to the manufacturer’s instructions. Previously vitrified oocytes were thawed using a Vitrification Warming set (Medicult). Thawed oocytes were fertilized using standard ICSI and cultivated in vitro for 2–4 days according to their growth potential, which was assessed daily by an embryologist. Written informed consent of a patient’s male partner allowing embryo transfer was required. Embryo transfer was performed by the standardized technique described in the previous paragraph.

Ovarian tissue cryopreservation

Ovarian tissue cryopreservation, the third technique available, was developed according to the previously published studies of several authors [13–16]. Ovarian tissue was obtained using the laparoscopic approach under general anesthesia and zero CO₂ pneumoperitoneum. In a majority of cases, a sample of ovarian cortex sized 10 x 20 x 1-2 mm was obtained from both ovaries. In some patients with a severe risk of premature ovarian failure due to planned highly gonadotoxic chemotherapy, one entire ovary was removed. In all cases, a further 2–3 small (1 x 1 x 1 mm) samples of ovarian cortex were removed from both ovaries at random from the ovarian surface. Theses samples were assessed by a pathologist to exclude any metastatic involvement of the ovaries by primary diagnosis: malignancy. Ovarian tissue intended for cryopreservation was transported to the embryology lab in a cryopreservation medium (G-Fert, Vitrolife) at human body temperature. The tissue was inspected under the microscope in a laminar box of the embryology lab. If 2-5 mm in diameter ovarian follicles were found to be growing in the sample, they were aspirated and oocytes were sought in the follicular fluid. Providing the patient had previously agreed, retrieved oocytes found in the follicular fluid could be matured in vitro and later cryopreserved as described in the previous paragraph. The acquired ovarian cortex tissue was cut lengthwise into smaller pieces, approximately 5 x 1 x 1 mm, with the help of scissors. In cases of whole ovary removal, the ovarian cortex was separated from the ovarian medulla by digital dissection or with the help of scalpel. Ovarian medulla is not suitable for cryopreservation because it does not contain PMF. Small strips of ovarian cortex were incubated in the cryopreservation medium EF52 Freezing Kit (Vitrolife) for 90 min and loaded into cryotubes (4-6 strips to each cryotube). After careful identification, cryotubes with ovarian tissue were inserted into a controlled rate freezer (Planer Cryo 10) and cryopreserved according to the protocol used for routine embryo freezing. Whenever the vitrification technique was applied, the following commercially made vitrification kits were used: RapidVit Cleave (Vitrolife) or Vitrification Cooling (MediCult). Cryoprotectant exposure was performed according to the manufacturer’s instructions and cryotubes loaded with processed ovarian strips were plunged directly into liquid nitrogen.

Administration of GnRH-a during chemotherapy.

We advise the administration of GnRH-a to our young female cancer patients during chemotherapy in order to prevent or decrease the rate of ovarian damage. The administration of GnRH-a during chemotherapy to prevent ovarian damage is sufficiently effective, especially in cases where less gonadotoxic chemotherapy regimens are being administered. This was confirmed by our previous observational study on female Hodgkin lymphoma (HL) patients [8]. This technique is used separately or in combination with the other fertility preservation methods described above. If less gonadotoxic chemotherapy is planned to cure a patient’s malignancy, then the administration of GnRH-a is advised as a stand-alone technique to prevent ovarian damage. If highly gonadotoxic chemotherapy protocols must be used to achieve a patient’s complete cure (e.g., alkylating agents, high cumulative doses, myeloablative chemotherapy before bone marrow transplantation), the patient is advised to combine GnRH-a administration during chemotherapy with one of the cryopreservation fertility saving techniques. The GnRH-a triptorelin (Diphereline SR 3 mg, Ibsen) is administered to female cancer patients during the whole time of chemotherapy in the form of intramuscular injection once a month (usually together with the pulse of chemotherapy).

Results

Over a period of five years (2004–2009), a cohort of 154 patients scheduled for gonadotoxic chemotherapy or immunotherapy was referred for consultation to prevent fertility impairment. The basic characteristics of the patient set are described in Table 1. The average age of patients included in the study was 29.4 ± 6.3 years. All women were Caucasian. The average body mass index (BMI) in the set of patients was 23.8 ± 3.1 kg/m². The majority of women were nuliparas or primiparas. The mean parity in this set of patients was 0.7 ± 0.3 children per patient. The length of systemic chemotherapy or immunotherapy was 5.2 ± 1.8 months on average. The mean time from cancer diagnosis to the start of systemic cancer treatment (needed to perform the planned methods of ovarian protection) did not exceed 14 days.

The spectrum of oncology or rheumatology diagnoses of patients referred for fertility preservation counseling is described in Table 2. The majority of treated patients suffered from newly diagnosed malignancy of the blood or lymphatic system. The most common diagnosis was Hodgkin lymphoma. This malignancy has very good...
Combination of fertility preservation strategies in young women with recently diagnosed cancer

The commonly used technique of fertility preservation was offered to patients and their counseling physician. The most common diagnoses referred to fertility preservation were systemic lupus erythematoses with visceral involvement, breast malignancies, gynecological malignancies, head or neck malignancies, and leukemia. All referred patients were individually consulted regarding their risk of fertility impairment or loss due to planned gonadotoxic chemotherapy. All patients were offered one or more fertility preservation techniques chosen by the patients and their counseling physician. The most commonly used technique of fertility preservation was GnRH-a administration during chemotherapy (79.9%). Ovarian tissue cryopreservation was the second most preferred fertility preservation technique (10.4%). In 20 cases (16.1%), a combination of more than one fertility preservation technique was offered to individually selected patients. In these cases, administration of GnRH-a during chemotherapy or immunotherapy was combined with one of the cryopreservation strategies (EC, OC or OTC). Where a combination of fertility preservation techniques was applied, the case was categorized for statistical evidence into one of the cryopreservation sub-groups. A small number (5.2%) of referred patients decided not to use any of the offered fertility preservation supportive strategies before or during their oncology therapy. Detailed results of the fertility preservation techniques are summarized in Table 3 and analyzed in more detail in the following paragraphs.

**Embryo cryopreservation**

Embryo cryopreservation was the first technique implemented into our Fertility Preservation Program. This routinely available method in reproductive medicine was utilized by our first oncology patient in May 2004. The results of embryo cryopreservation and its usage by our women cancer patients are summarized in Table 4. Since 2004, we have used this technique in four patients and gained 18 oocytes suitable for fertilization. We have successfully frozen 12 embryos in total, with a mean number of 3.3 embryos per patient. The embryos of three patients, who successfully came through their oncology treatment, are still cryopreserved and ready for FET. In one case, cryopreserved embryos have been used for treating infertility caused by the oncologic therapy. This 21-year-old young woman was diagnosed with advanced stage Hodgkin lymphoma. Her chemotherapy was postponed for 16 days while her oocytes were acquired. Four embryos were cryopreserved for later use. After almost four years following successful cancer treatment the FET of three embryos was accomplished. These embryos were successfully thawed and transferred, but unfortunately the patient did not become pregnant. The FET was possible to carry out because the woman still had the same male partner, who agreed with the procedure. This patient is now dependent on hormonal replacement therapy and her infertility is being treated by oocyte donation.

**Oocyte cryopreservation**

During the study period, oocyte cryopreservation techniques were used by three cancer patients. Details of these cases are presented in Table 5. A total number of 15 oocytes were cryopreserved (5.0 oocytes per patient). Four of the 19 retrieved oocytes (21.0%) were not suitable for cryopreservation due to oocyte immaturity (diploid germinal vesicle oocytes) or their degeneration during the preparation procedure. None of these three cancer patients have yet asked to use their oocytes to become pregnant. The disadvantage of both this tech-

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### Table 1. — Basic characteristics of the set of patients referred for consultation to prevent fertility impairment (Assisted Reproduction Center, Department of Obstetrics and Gynecology of Brno University Hospital, years 2004-2009).

<table>
<thead>
<tr>
<th>Follow-up parameter</th>
<th>Mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.4 ± 6.3</td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
<td>23.8 ± 3.1 kg/m²</td>
</tr>
<tr>
<td>Parity</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian (100%)</td>
</tr>
<tr>
<td>Length of systemic chemotherapy</td>
<td>5.2 ± 1.8 months</td>
</tr>
<tr>
<td>Time to treatment start</td>
<td>13.5 ± 2.6 days</td>
</tr>
</tbody>
</table>

### Table 2. — The spectrum of oncology or rheumatology diagnoses of patients referred for fertility preservation counseling (Assisted Reproduction Center, Department of Obstetrics and Gynecology of Brno University Hospital, years 2004-2009).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>ICD 10 code</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin lymphoma (HL)</td>
<td>C81</td>
<td>101</td>
<td>65.6</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma (NHL)</td>
<td>C82-85</td>
<td>15</td>
<td>9.7</td>
</tr>
<tr>
<td>Leukemia</td>
<td>C91-97</td>
<td>12</td>
<td>7.8</td>
</tr>
<tr>
<td>Systemic lupus erythematoses (SLE)</td>
<td>M32</td>
<td>11</td>
<td>7.1</td>
</tr>
<tr>
<td>Breast malignancy</td>
<td>C50</td>
<td>5</td>
<td>3.2</td>
</tr>
<tr>
<td>Gynecology malignancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(vagina, uterus, ovary)</td>
<td>C51-56</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>Gastrointestinal malignancy</td>
<td>C18-20</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>Bone malignancy</td>
<td>C40-41</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Malignancy of head and neck</td>
<td>C00-13</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>154</td>
<td>100</td>
</tr>
</tbody>
</table>

ICD - International Classification of Diseases.

### Table 3. — Representation of fertility preservation techniques chosen by the patient and their counseling physician (Assisted Reproduction Center, Department of Obstetrics and Gynecology of Brno University Hospital, years 2004-2009).

<table>
<thead>
<tr>
<th>Fertility preservation technique</th>
<th>No. of patients</th>
<th>% from total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo cryopreservation (EC)</td>
<td>4</td>
<td>2.6</td>
</tr>
<tr>
<td>Oocyte cryopreservation (OC)</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Ovarian tissue cryopreservation (OTC)</td>
<td>16</td>
<td>10.4</td>
</tr>
<tr>
<td>GnRH analogues (GnRH-a)</td>
<td>123</td>
<td>79.9</td>
</tr>
<tr>
<td>No fertility protection</td>
<td>8</td>
<td>5.2</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combination of fertility preservation techniques</th>
<th>No. from total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(cryopreservation+ GnRH analogues)</td>
<td>20</td>
</tr>
</tbody>
</table>

.prognosis quod vitam – five-year survival is 85.98% [17]. The other commonly occurring diagnoses referred to FPC were systemic lupus erythematoses with visceral involvement, breast malignancies, gynecological malignancies and head or neck malignancies.

All referred patients were individually consulted regarding their risk of fertility impairment or loss due to planned gonadotoxic chemotherapy. All patients were offered one or more fertility preservation techniques described in the methodology section. Table 3 describes the fertility preservation techniques chosen by the patients and their counseling physician. The most commonly used technique of fertility preservation was
Table 4. — Results of embryo cryopreservation and their usage by women cancer patients. (Assisted Reproduction Center, Department of Obstetrics and Gynecology of Brno University Hospital, years 2004-2009).

<table>
<thead>
<tr>
<th>Monitored parameters - embryo cryopreservation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (stimulated IVF cycles)</td>
<td>4 cycles</td>
</tr>
<tr>
<td>Total number of retrieved oocytes</td>
<td>18 oocytes</td>
</tr>
<tr>
<td>Mean number of oocytes per cycle</td>
<td>4.5 oocytes</td>
</tr>
<tr>
<td>Total number of created embryos</td>
<td>15 embryos</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>83%</td>
</tr>
<tr>
<td>Total number of cryopreserved embryos</td>
<td>12 embryos</td>
</tr>
<tr>
<td>Percentage of embryos not suitable for cryopreservation</td>
<td>20%</td>
</tr>
<tr>
<td>Mean number of cryopreserved embryos per cycle</td>
<td>3.3 embryos</td>
</tr>
</tbody>
</table>

Table 5. — Results of oocyte cryopreservation in women cancer patients. (Assisted Reproduction Center, Department of Obstetrics and Gynecology of Brno University Hospital, years 2004-2009).

<table>
<thead>
<tr>
<th>Oocyte cryopreservation - monitored parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (stimulated IVF cycles)</td>
<td>3 cycles</td>
</tr>
<tr>
<td>Oncology diagnoses</td>
<td>2x HL, 1x NHL*</td>
</tr>
<tr>
<td>Total number of retrieved oocytes</td>
<td>19 oocytes</td>
</tr>
<tr>
<td>Mean number of oocytes per cycle</td>
<td>6.3 oocytes</td>
</tr>
<tr>
<td>Total number of cryopreserved oocytes</td>
<td>15 oocytes</td>
</tr>
<tr>
<td>Mean number of cryopreserved oocytes per cycle</td>
<td>5.0 oocytes</td>
</tr>
<tr>
<td>Percentage of oocytes not suitable for cryopreservation</td>
<td>21%</td>
</tr>
<tr>
<td>(diploid GV oocytes, fragmentized oocytes)</td>
<td></td>
</tr>
<tr>
<td>Oocyte cryopreservation technique</td>
<td>slow freeze (Planer) 2x vitrification (MediCult) 1x</td>
</tr>
<tr>
<td>Mean time of chemotherapy postponement</td>
<td>18.6 days</td>
</tr>
</tbody>
</table>


The administration of GnRH analogues during chemotherapy to protect ovarian function was introduced into clinical practice in our center in 2003. During the follow-up period (2004-2009) of this study, we administered this medication to 123 patients during chemotherapy. This has been the most widely used method (79.9%) from the portfolio of fertility preservation techniques offered. Quite a large proportion of the women (n = 20, 16.1%) receiving GnRH analogues during chemotherapy have chosen to combine this approach with one of the cryopreservation techniques (EC, OC or OTC) (Table 3). The protective effect on ovarian function of administering GnRH analogue during chemotherapy has been described in detail in our previously published prospective case-control study on patients (n = 72) with newly diagnosed Hodgkin lymphoma. We documented a statistically smaller number of premature ovarian failure cases in the group of patients receiving GnRH analogues compared to the control patient group with follow-up periods of six and 12 months. After analyzing the study group according to the gonadotoxicity of chemotherapeutical regimens used, it was clear that the protective effects of GnRH analogue co-treatment were statistically significant only in the patient subgroups receiving less gonadotoxic chemotherapeutics [8]. These observations thus show the unsatisfactory protective effect of administering GnRH analogues to protect ovarian function in advanced cancer cases, where high dosage combined chemotherapy has to be administered.

GnRH analogues

The technique of ovarian tissue cryopreservation has been developed in our laboratory since 2005. Initially, we used a Planer controlled rate freezer; in 2007 we started to freeze ovarian tissue by the vitrification technique. During the period of this study the technique was applied in 16 cases of young female patients prior to highly gonadotoxic chemotherapy. In the majority of cases, patients suffered from blood or lymph node systemic malignancy (81%) - Hodgkin lymphoma (9x), non-Hodgkin lymphoma (3x) and acute myeloid leukemia (1x). A summary of ovarian tissue cryopreservation cases is presented in Table 6. The average age of women taking advantage of this technique was 26 years. The youngest patient was 13 years old and she decided for the procedure after taking advice from her parents. The patient set consisted of mostly nulliparous women (88%). All women were Caucasian. Average BMI in the sample was 23.7 kg/m². The length of systemic chemotherapy or immunotherapy averaged 7.1 months. Average time from fertility preservation counseling to the beginning of chemotherapy was very short, not exceeding one week. Ovarian tissue harvesting was conducted for all cases in this subgroup by laparoscopic surgery. The length of surgery did not exceed 60 min and no surgical complications were observed. The patient length of stay in hospital was two days at most. In the majority of cases (88%), only an ovarian cortex sample (approximately sized 10 x 20 x 1-2 mm) was removed from both ovaries. In the last two patients, where there was a very high risk of permanent ovarian failure, one entire ovary was removed. All of the ovarian tissue cryopreserved samples have yet to be thawed for the auto-transplantation and restoration of ovarian function. The digested characteristics of the patient set and the results of ovarian tissue cryopreservation are reported in Table 7.

Ovarian tissue cryopreservation

The technique of ovarian tissue cryopreservation has been developed in our laboratory since 2005. Initially, we used a Planer controlled rate freezer; in 2007 we started to freeze ovarian tissue by the vitrification technique. During the period of this study the technique was applied in 16 cases of young female patients prior to highly gonadotoxic chemotherapy. In the majority of cases, patients suffered from blood or lymph node systemic malignancy (81%) - Hodgkin lymphoma (9x), non-Hodgkin lymphoma (3x) and acute myeloid leukemia (1x). A summary of ovarian tissue cryopreservation cases is presented in Table 6. The average age of women taking advantage of this technique was 26 years. The youngest patient was 13 years old and she decided for the procedure after taking advice from her parents. The patient set consisted of mostly nulliparous women (88%). All women were Caucasian. Average BMI in the sample was 23.7 kg/m². The length of systemic chemotherapy or immunotherapy averaged 7.1 months. Average time from fertility preservation counseling to the beginning of chemotherapy was very short, not exceeding one week. Ovarian tissue harvesting was conducted for all cases in this subgroup by laparoscopic surgery. The length of surgery did not exceed 60 min and no surgical complications were observed. The patient length of stay in hospital was two days at most. In the majority of cases (88%), only an ovarian cortex sample (approximately sized 10 x 20 x 1-2 mm) was removed from both ovaries. In the last two patients, where there was a very high risk of permanent ovarian failure, one entire ovary was removed. All of the ovarian tissue cryopreserved samples have yet to be thawed for the auto-transplantation and restoration of ovarian function. The digested characteristics of the patient set and the results of ovarian tissue cryopreservation are reported in Table 7.
Rapid advances in the field of oncofertility have been made in last decade [18]. Our study presents the clinical outcomes of quite a large cohort of female patients requiring fertility preservation counseling. The complete portfolio of fertility preservation techniques offered to patients is another important strength of our study. One of the limitations of results presented here is the difficulty in following up the future fertility of the patients in the long term. The introduction of an international registry of patients who have used certain fertility preservation techniques and the later follow-up of their children could improve our knowledge about clinical efficacy.

Embryo cryopreservation has historically been the first available technique for preserving fertility in young female cancer patients. This technique became the gold standard of care with the best overall results for conception after successful cancer treatment. The pregnancy rate per embryo transfer varies between 10-30 % [19]. In our cohort, we reported embryo transfer of frozen-thawed embryos after successful cancer treatment, but the patient, unfortunately, did not conceive. The main disadvantage of this technique is the need for ovarian hyperstimulation with FSH and postponing the planned cancer treatment by an average of 2-4 weeks. Nevertheless, usage of “soft” hyperstimulation protocols (clomifene, tamoxifene, letrozole) is possible in cases of estrogen sensitive tumors [20]. A further drawback for this technique is its unavailability to young single women patients without any regular male partner, who would provide sperm for oocyte fertilization (analogically it is also unavailable for adolescents and children). This method is less preferred at our center because of the aforementioned inconveniences of embryo cryopreservation and the availability of new fertility preservation techniques. This technique has been especially relegated in favor of OTC, which does not require ovarian stimulation or postponing the cancer treatment.

The cryopreservation of oocytes could be the favored option for women without a male partner. On the other hand, ovarian stimulation and postponing the start of chemotherapy is still necessary. Conventional slow freezing with ice crystal formation inside the cell could seriously damage the oocyte (cytoskeleton breaks, zona pellicuda hardening) and reduce its future fertility compe-
IVF/ICSI cycle is usually required to achieve pregnancy. Ovarian hyperstimulation with a conventional and the oncology disease is in long-term complete remission when childbearing is desired by the treated couple, Auto-transplantation should be carefully scheduled to the period during which hormones of the transplanted ovarian tissue continue to function is limited. Moreover, the period during which hormones of the transplantation of ovarian cortex strips. The patient's written consent is required [25]. The risk of cancer is high due to the suboptimal laboratory procedures used. The technique was later significantly improved on animal models [23]. The main goal of this strategy is to save intact primordial follicles for later use, i.e., after the patient has been cured of cancer [13, 16, 24]. In comparison with EC or OC techniques, our patients who decided for OTC did not have to lose valuable time before starting chemotherapy to save ovarian reserve [7, 9]. Our previous published results indicate the insufficient protective effect of GnRH analogues in preventing primordial follicle destruction by highly gonadotoxic myeloablative chemotherapeutic regimens before bone marrow transplantation [8]. Hopefully, we will be able to evaluate exactly the protective effect of GnRH analogues by double blind, multicenter, prospective, randomized trials, which are currently ongoing. The main advantage of this fertility preservation technique is its simplicity and non-invasiveness. In addition, the amenorrhea induced by administering GnRH analogues helps to protect pancytopenic and immuno-compromised patients during chemotherapy against heavy menstrual bleeding and any need for the extensive usage of expensive hematopoietic factors and blood derivatives. According to our clinical experience with fertility preservation counseling, we recommend offering GnRH-analogue co-treatment during chemotherapy to the majority of our young female cancer patients. This approach is strongly recommended in can-
References


Acknowledgements

This work was supported by Internal Grant Agency (IGA) of the Ministry of Health of the Czech Republic-No. NS/9661.


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Mammographic features in infertile women as a potential risk for breast cancer: a preliminary study

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Summary

The purpose of the present study was to evaluate breast mammographic features, particularly mammographic density in a selected population of infertile women and to assess if these women should be considered at higher risk for breast cancer. The prevalence of female infertility in Western countries is approximately 10-15% and since causes affecting the female are involved in 35-40%, concerns have developed about the future health of these women, specifically whether infertility could represent a risk factor for future cancer development. Moreover, infertility is now often treated with medication and procedures that could modify the hormonal environment and be cofactors in the cellular changes towards cancer development. Mammographic breast density is a useful marker for breast cancer risk and breast density is considered one of the strongest risk factors for breast cancer. Breast density is associated with known breast cancer risk factors such as reproductive and menstrual factors including serum estrogen and progesterone concentrations. In Italy the National Federation for Breast Cancer (FONCAM) guidelines suggest the usefulness of mammography from 35 years of age for women who undergo infertility hormone therapy (FONCAM Guidelines, 2005). According to this recommendation 294 women aged ≥35, with primary infertility, sent to our breast service before joining an IVF program were recruited and then underwent clinical examination and X-ray mammography. Women were divided into two groups: dense breast (DB) and non-dense breast (NDB). Univariate analysis was employed to evaluate if there was an association between mammographic density and other risk factors. Evaluation of mammographic features showed the presence of BI-RADs C and D in the sample of 200 (68%) patients with DB and in 94 (32%) patients with NDB BI-RADS A and B. Univariate analysis showed that there were no statistically significant differences between the groups BD and NDB as regards age at mammography, age at menarche, BMI and family history for breast cancer, while ovulatory etiology of infertility was found to be associated with high mammographic density (p < 0.05). In conclusion, bearing in mind that 68% of our study sample had high breast density, we can assume that patients with primary infertility might represent a group at high risk for breast cancer, particularly if infertility is due to an ovulatory factor. We suggest breast screening from the age of 35 in infertile patients who undergo treatment with fertility drugs in accordance with FONCAM recommendations. This might allow the identification of higher risk patients who need more closely monitored breast examinations.

Key words: Infertility; Fertility drugs; Breast density; Breast cancer risk.

Introduction

Infertility is defined as the failure to conceive after one year of regular unprotected intercourse. Its prevalence in Western countries is approximately 10-15% [1] and since causes affecting the female are involved in 35-40%, concerns have developed about the future health of these women - specifically whether infertility could represent a risk factor for future cancer development. Moreover, infertility is now often treated with medication and procedures that could modify the hormonal environment and be cofactors in the cellular changes towards cancer development [2].

In recent years in Western countries the demand for infertility services has increased; in the USA prescription of fertility drugs increased almost 2-fold between 1975 and 1991 [3]. In Europe the number of reported assisted reproductive therapy (ART) cycles reached 418,111 in 2005 compared with 367,966 in 2004, equivalent to an increase of 13.6% [4]. Hormonal therapy results in the proliferation of ovarian cells and up to 5-fold increases in serum estrogen and progesterone concentrations [5-7]. These effects have raised concerns about the potential role of fertility drugs as a breast cancer risk. A number of studies have examined fertility drug use in relation to breast cancer risk [8-21]. A few studies have suggested possible risk increase [9, 10] or decrease [14, 15], whereas other studies have reported no association with risk of breast cancer [8, 11-13, 16-22].

Mammographic breast density is a useful marker for breast cancer risk and breast density is considered one of the strongest risk factors for breast cancer [23]. Breast density is associated with known breast cancer risk factors such as reproductive and menstrual factors [24] including serum estrogen and progesterone concentrations.

In Italy the National Federation for Breast Cancer guidelines suggest the usefulness of mammography starting at age 35 years for women who undergo hormone therapy (FONCAM Guidelines, 2005) [25].

According to this recommendation all women aged over 35 that undergo fertility treatment at our department have a breast examination performed.

Purpose of the present study is to evaluate breast mam-
mographic feature, particularly mammographic density in a selected population of infertile women and to assess if these women can be considered as at higher risk for breast cancer.

Materials and Methods

Ethical approval for this single-center observational study was granted by the Medical Research Ethics Committee of our institution, and written informed consent was obtained from all patients. The study was carried out from January 2007 to November 2009 at the Department of Gynecology and Obstetrics, University of Rome “Sapienza” among women with primary infertility sent to our centre for breast advice prior to entering an assisted fertilization program.

According to the protocol we selected only women aged ≥ 35 with primary infertility who had never undergone fertility drug treatments. After recruitment the women were interviewed by a physician (trained in medical research) and collected information included: age, etiology of infertility (if known), family history of breast cancer (two or more cases), previous administration of hormonal contraceptive therapy (yes/no), and age at menarche (years). Height without shoes (m) and weight in light clothes (kg) were registered by a trained nurse for the calculation of body mass index (BMI).

All recruited women, according to FONCAM recommendations, underwent clinical examination and X-ray mammography (XRM). In all cases conventional XRM was performed at our Department of Radiological Sciences using digital image formation and computed radiography. At least two views per breast were obtained. Mammograms were interpreted in accordance with the guidelines of the American College of Radiology (ACR) Breast Imaging Reporting and Data system (BI-RADS) by three physicians (two radiologists and a breast specialist) blinded to the clinical data.

The diagnostic quality of mammograms was assessed according to the British criteria “PGMI” [25]. Based on the BI-RADS lexicon, patients were then assigned to one of the four categories of breast parenchymal density distribution [26]: type A, the breast is almost entirely fat (glandular parenchyma < 25% of the total area of both breasts); type B, scattered fibroglandular densities (25-50%); type C, heterogeneously dense breast tissue (51-75%); type D extremely dense (> 75% glandular). It is a well known fact that sensitivity of mammography is reduced in type 3 and 4 [39-41], and the patients participating in our study were therefore divided into two groups: dense breast (DB) which included BI-RADS type C and D and nondense breast (NDB) which included BI-RADS type A and B. In case of contradictory judgments, the classification assigned by at least two readers out of three was considered correct.

The presence of focal disease at mammography or request for further diagnostic tests (i.e., presence of focal disease at mammography or request for further diagnostic tests). This selection produced a final sample of 294 women. All mammographic examinations were considered as class P (perfect) or G (good) according to the British “PGMI” criteria.

Table 1 lists the data collected at the anamnestic interview: demographic information, reproductive history, family medical history and anthropometric measurements.

Evaluation of mammographic features showed the presence of BI-RADS C and D in the sample of 200 (68%) patients with DB and in 94 (32%) patients with NDB BI-RADS A and B (Table 2). Assessment of inter-operator variability did not show any statistically significant differences; Cohen’s kappa values ranged from 0.85 to 0.89 (p = 0.001) thus indicating a high level of agreement.

Univariate analysis to assess the association between qualitative and quantitative variables and mammographic breast density showed that there were no statistical significant differences between the two groups of BD and NDB (Table 3) regarding age at mammography, age at menarche, BMI and family history of breast cancer while ovulatory etiology of infertility was found to be associated with high mammographic density (p < 0.05).

Discussion

We hypothesized that women with primary infertility might have denser breasts than the general population. In the literature there is no information about the characteristics of these women because mammography screening programs for breast cancer are offered after age 50 years and mammographic examinations are not routinely recommended for women under the age of 40 or for those undergoing fertility treatments [27, 28]. In Italy, FONCAM guidelines suggest the usefulness of mammography starting at 35 years of age for women undergoing fertility drug treatment.

Mammographic breast density (MD) has consistently been one of the strongest risk factors for breast cancer, with risk estimates that are three-to five-fold greater for women in the highest quartile of density than for women of similar age in the lowest quartile [29]; 16% to 32% of breast cancers may be attributed to this trait [30, 31] with an even larger estimated proportion among pre-menopausal women [32]. The relationship between MD and breast cancer is thought to be multifactorial, and in
Mammographic features in infertile women as a potential risk for breast cancer: a preliminary study

Table 1. — Main characteristics of the study population: percentage of qualitative variables, mean value and standard deviation for the quantitative variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at mammography (years)</td>
<td>38.9 ± 3.0</td>
<td>21.6</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.4 ± 1.4</td>
<td>10.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.7 ± 2.5</td>
<td>43.3</td>
</tr>
<tr>
<td>Family history for breast cancer</td>
<td>275 (93.5%)</td>
<td>24.7</td>
</tr>
<tr>
<td>No</td>
<td>19 (6.5%)</td>
<td>15.3</td>
</tr>
<tr>
<td>Previous administration of hormonal contraceptive therapy</td>
<td>117 (58.5%)</td>
<td>9 (3.1%)</td>
</tr>
<tr>
<td>Infertility etiology</td>
<td>140 (47.6%)</td>
<td>32 (%)</td>
</tr>
<tr>
<td>Ovulatory factor</td>
<td>100 (34%)</td>
<td>9 (3.1%)</td>
</tr>
<tr>
<td>Tubal disease</td>
<td>127 (43.3)</td>
<td>94 (32)</td>
</tr>
<tr>
<td>Male infertility</td>
<td>73 (24.7)</td>
<td>9 (3.1%)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>73 (24.7)</td>
<td>9 (3.1%)</td>
</tr>
</tbody>
</table>

Table 2. — Mammogram classification according to the BI-RADS system and categorization into two groups: dense breast (DB) which included BI-RADS type C and D, and non breast dense (NDB) which included BI-RADS type A and B.

<table>
<thead>
<tr>
<th>BI-RADS category</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>63</td>
<td>21.6</td>
</tr>
<tr>
<td>B</td>
<td>31</td>
<td>10.4</td>
</tr>
<tr>
<td>C</td>
<td>127</td>
<td>43.3</td>
</tr>
<tr>
<td>D</td>
<td>73</td>
<td>24.7</td>
</tr>
<tr>
<td>A-B (non breast dense; NDB)</td>
<td>94 (32)</td>
<td>9 (3.1%)</td>
</tr>
<tr>
<td>C-D (dense breast; DB)</td>
<td>200</td>
<td>68.0</td>
</tr>
</tbody>
</table>

Table 3. — Main characteristics of the patients versus mammographic features: percentage of qualitative variables, mean value and standard deviation for the quantitative variables in the two groups of non-dense breast (NDB) and dense breast (DB). To assess the association between mammographic density and qualitative variables Pearson’s chi-square was employed, whereas for quantitative variables Student’s t-test was used. Significant level was α = 0.05.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-dense breast (NDB) (n = 94; 32%)</th>
<th>Dense breast (DB) (n = 200; 68%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at mammography (years)</td>
<td>39.1 ± 2.8</td>
<td>38.9 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>Age at menarche (years)</td>
<td>12.3 ± 1.4</td>
<td>12.4 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4 ± 2.5</td>
<td>22.9 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Ovulatory etiology of infertility</td>
<td>32 (10.8%)</td>
<td>108 (36.8%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Family history of breast cancer (yes)</td>
<td>5 (1.8%)</td>
<td>14 (4.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous administration of hormonal contraceptive therapy (yes)</td>
<td>36 (12.3%)</td>
<td>86 (29.2%)</td>
<td></td>
</tr>
</tbody>
</table>

early studies the main explanation was thought to be due to ‘masking bias’ [33] but Boyd and colleagues [32] found that compared with women with density in less than 10% of the mammogram, women with more than 75% density had an increased risk of breast cancer (odds ratio [OR] = 4.7; 95% confidence interval [CI]: 3.0, 7.4), whether detected by screening (OR = 3.5; 95% CI: 2.0, 6.2) or detected within 12 months of a negative screening examination (OR = 17.8; 95% CI: 4.8, 65.9).

A recent meta-analysis [29] illustrates a high prevalence of increased density in the general population (31% to 43% had a BI-RADS of 3 or 4). Importantly, a larger proportion of premenopausal women have dense breasts, with estimates of 37% among premenopausal women [34] compared with 12% among postmenopausal women. Even without significant differences in association by menopausal status, the attributable risk is much higher in younger women (26%) than in older women (7%) [23, 24, 32, 33]. This underscores the importance of MD for potential risk prediction in younger women.

In our study population 68% of women were classified as DB according to BI-RADS score. This value is significantly higher than the 37% reported by Celine et al. [34].

This difference could be attributed to the fact that we have considered a select sample of women with primary infertility and therefore nulliparous.

The role of nulliparity as risk factor for breast density has been discussed in several studies.

De Waard et al. [35] postulated that breast density could be the biological relationship between parity and breast cancer risk because women who have had several pregnancies show lower MD than nulliparous women. Similarly Boyd et al. [23, 24] found that DB is less extensive in women who are parous and less extensive in those with a larger number of live births.

The breasts of nulliparous women often show a large quality of undifferentiated epithelial breast tissue more susceptible to carcinogenic stimuli such as endogenous and exogenous female hormones [36]. Other studies provided evidence of independent effects of breast density and parity [37]. Finally, Van Gils et al. [38] in their case control study found that breast density was not simply an explanatory factor in the relationship between parity and breast cancer. They postulated that parity and MD may interact and nulliparous women with high breast density could possibly represent a high-risk group for breast cancer.

In addition, infertility itself may be a risk factor for breast density as 35-40% of cases have pathologies of the female reproductive organs including ovulatory dysfunction, the most common cause of female infertility [39].

In our series ovulatory etiology of infertility was found to be associated with high mammographic density. This result underlines the role of sexual hormones in the pathogenesis of MD.

In addition, our sample consisted of women who wanted to undergo treatment with fertility drugs.

The role of these drugs in the pathogenesis of breast cancer has not been demonstrated, but is still widely debated.

In a recent meta-analysis [8] the relationship between fertility drugs used in ART procedures and the risk of breast cancer were examined: combining the result of several studies [8-21], the authors found that the risk of...
breast cancer was not significantly associated with fertility drug treatment (RR 0.99; CI 0.89-1.11).

Analysis of the relationship between number of fertility treatment cycles and cancer risk has shown that there was no statistically significant trend in risk of breast cancer across the number of cycles of therapy (RR 1.04; 95% CI 0.88-1.22).

Regarding age, the distribution of MD changes with increasing age reflected a reduction in glandular tissue and increase in fat. The decline in density with age may seem paradoxical, as breast cancer incidence increases with age, but this apparent paradox may be resolved by reference to a model of breast cancer incidence that is based on the concept that breast tissue age, or breast tissue exposure rather than chronological age, is the relevant measure for describing the incidence of breast cancer. Breast tissue “age” is closely associated with exposure of breast tissue to hormones and growth factors, and to the effects that menarche, pregnancy and menopause have on these exposures and on susceptibility to carcinogens [23, 24, 34].

Breast tissue exposure is greatest at the time of menarche, falls with pregnancy, is further reduced in the perimenopausal period and is least after menopause.

Thus, women with DB would have an increased risk of breast cancer that persists over time even when, due to age-related involution of fat, the breast does not appear dense on mammography.

This study has some limitations. One concerns the reliability of mammographic classification which was performed qualitatively and not by a computer-assisted method. Furthermore, the BI-RADS system was developed to alert the referring clinician that the ability to perform mammographic classification which was performed qualitatively and not by a computer-assisted method. Furthermore, the BI-RADS system was developed to alert the referring clinician that the ability to classify MD, i.e. Boyd’s rating system, showed a large gradient for breast cancer risk after adjustment for the effect of all other generally recognized risk factors for breast cancer [40].

The lack of a control group, randomization in the selection of patients and lack of follow-up makes it impossible to evaluate if women with infertility really represent a group at higher risk for breast cancer. In fact the higher prevalence of MD observed in our population study compared to data reported in the literature might be due to age. Finally, in this study MD was not adjusted for any potential confounding factors [41-43].

In conclusion, bearing in mind that 68% of our study sample had a high breast density, we can assume that patients with primary infertility might represent a group at high risk for breast cancer, particularly if infertility is due to ovulatory factor.

We suggest breast screening from the age of 35 in infertile patients who undergo or want to undergo treatment with fertility drugs in accordance with FONCAM recommendations.

This might allow the identification of higher risk patient who need more closely monitored breast examinations.

On the other hand this recommendation could increase the number of tests required and therefore costs benefits. Careful prospective randomized trials are required to determine whether there is an association between infertility, mammographic density and breast cancer risk together with cost benefits of mammography screening from age 35 in a subgroup of potential higher risk women.

References


Mammographic features in infertile women as a potential risk for breast cancer: a preliminary study


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Prognosis of primary peritoneal carcinoma: effect of cytoreductive surgery combined with neoadjuvant chemotherapy after laparoscopic diagnosis and evaluation: a multi-center trial

F. Yang, J. Wang, H. Li, X. Tong
Shanghai First Maternity and Infant Healthy Hospital, Tongji University School of Medicine, Shanghai (China)

Summary

Objective: To evaluate the clinical outcome and prognosis of patients with primary peritoneal carcinoma (PPC) treated with cytoreductive surgery and neoadjuvant chemotherapy after laparoscopic diagnosis. Material and Methods: We retrospectively reviewed 29 patients with PPC, treated between March 2001 and June 2009 at three hospitals. All patients underwent laparoscopy to diagnose and evaluate whether they were good candidates for optimal cytoreductive surgery. After confirmed to be PPC histologically, the patients who were not suitable to undergo optimal cytoreductive surgery received chemotherapy for three to six cycles before operation, and then underwent cytoreductive surgery, followed with chemotherapy again for six cycles. The study included patient demographics, surgery procedures, surgery stage, pathologic findings, chemotherapy programs, and outcomes. Results: The mean age of the 29 patients was 58.5 years. One patient was at Stage IIIB, 23 at Stage IIIC, and five at Stage IV. The rate of optimal cytoreductive surgery was 79.3%. At the time of this review, three patients had stable disease - two with progressive disease, eight were partial responders; 16 patients were alive without evidence of disease, seven were alive with disease, and six had died from disease. The mean and median overall survival time was 46 and 48 months. Conclusion: Combination of neoadjuvant chemotherapy and cytoreductive surgery after laparoscopic diagnosis and evaluation is effective in the treatment of patients with PPC.

Key words: Laparoscopic diagnosis; Neoadjuvant chemotherapy; Cytoreductive surgery; Primary peritoneal carcinoma; Multi-center trial.

Introduction

Primary peritoneal carcinoma (PPC) is a malignancy that spreads widely inside the peritoneal cavity, involving mostly the omentum with minimal or no ovarian involvement [1, 2]. However histopathologic, immunohistochemical, and clinical similarities have been observed between PPC and serous epithelial ovarian cancer (EOC) [3-6]. Most of these patients were diagnosed with malignant ovarian carcinoma; optimal cytoreductive surgery is difficult to perform for patients with advanced stage of PPC, which is the main reason why those patients had a worse prognosis.

Therefore, we considered if tumors could be reduced before surgery, if there would be more chances to perform optimal cytoreductive surgery. All of the patients of our study were confirmed to be PPC first by laparoscopic biopsy. If they were not good candidates for optimal cytoreductive surgery, evaluated by laparoscopy, they received chemotherapy for three to six cycles first, and then underwent cytoreductive surgery, followed by chemotherapy for six cycles postoperatively. The aim of this study was to evaluate the clinical outcome and prognosis of patients with PPC treated with cytoreductive surgery combined with neoadjuvant chemotherapy after laparoscopic diagnosis and evaluation.

Revised manuscript accepted for publication September 30, 2010
All patients in our study were confirmed to have PPC by laparoscopic biopsy. After evaluation of laparoscopy, they received chemotherapy: 11 with paclitaxel (175 mg/m²) and carboplatin (area under the curve 5); 18 patients received paclitaxel (175 mg/m²) and cisplatin (75 mg/m²) for three to six cycles, and then underwent cytoreductive surgery. Standard procedures for cytoreductive surgery consist of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and maximal debulking of metastatic tumors. Systematic pelvic and/or aortic lymphadenectomies are allowed. Then all patients receive treatment with the same chemotherapy program again as before for six cycles.

Each patient’s clinical status was determined up to the last available date. Survival time was measured in months from the time of diagnosis to the date of last follow-up or death. Survival curves were generated using Kaplan-Meier survival analysis. Data were analyzed by SPSS 16.0 (SPSS Inc., Chicago, IL).

Results

Table 1 outlines the clinicopathologic characteristics of the patients. Cytoreductive surgeries followed by chemotherapy were performed by laparotomy in eight patients and in 21 by laparoscopy. At the end of the primary debulking operation, eight patients (20.7%) had documented macroscopic residual disease (≥ 1 cm), making the overall rate of optimal cytoreduction 79.3%.

The mean and median survival was 46 and 48 months. The survival curve for overall survival of all patients is shown in Figure 1. Patients who had optimal cytoreduction had a longer mean survival (46 months) than those who had suboptimal cytoreduction (41 months; Figure 2, \( p = 0.042 \)). Also there was a difference in survival among stages of groups (Figure 3, \( p = 0.032 \)).

At the time of this review, two patients had progressive disease, three patients had stable disease, eight were partial responders, and 16 were complete responders (Table 2); 16 patients were alive without evidence of disease, seven were alive with disease, and six had died from disease.

Discussion

PPC is a rare primary peritoneal tumor [7]. It is believed to arise from the secondary Müllerian system, which comprises the pelvic and lower abdominal mesothelial lining. The mesothelium of the peritoneum and the germinal epithelium of the ovary arise from the same embryologic origin, therefore the peritoneum may retain the multipotentiality of the Müllerian system and allow a primary carcinoma to develop. Thus, PPC shares many of the clinical and histologic features [7] of papil-
Histologic type

Table 1. — Clinicopathologic characteristics of PPC patients.

<table>
<thead>
<tr>
<th>PPC (n = 29)</th>
<th>mean ± SD or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>58.5 ± 9.4</td>
</tr>
<tr>
<td>Mean CA125 (U/ml)</td>
<td>2374.4 ± 1137.4</td>
</tr>
<tr>
<td>Mean volume of ascites (ml)</td>
<td>629.4 ± 315.6</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>43.0 ± 6.5</td>
</tr>
<tr>
<td>Mean survival (months)</td>
<td>46.3 ± 4.2</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>19 (65.5%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>11 (37.9%)</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>14 (48.3%)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>5 (17.4%)</td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td></td>
</tr>
<tr>
<td>IIIa</td>
<td>1 (3.5%)</td>
</tr>
<tr>
<td>IIIb</td>
<td>23 (79.3%)</td>
</tr>
<tr>
<td>IV</td>
<td>5 (17.2%)</td>
</tr>
<tr>
<td>Surgery procedure</td>
<td></td>
</tr>
<tr>
<td>Laparotomy</td>
<td>8 (27.6%)</td>
</tr>
<tr>
<td>Laparoscopy</td>
<td>21 (72.4%)</td>
</tr>
<tr>
<td>Optimal debulking</td>
<td>23 (79.3%)</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>26 (89.7%)</td>
</tr>
<tr>
<td>Mucoid</td>
<td>2 (6.98%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1 (3.5%)</td>
</tr>
</tbody>
</table>

Table 2. — Response rate to adjuvant chemotherapy.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>16</td>
</tr>
<tr>
<td>PR</td>
<td>8</td>
</tr>
<tr>
<td>SD</td>
<td>3</td>
</tr>
<tr>
<td>PD</td>
<td>2</td>
</tr>
<tr>
<td>NE</td>
<td>0</td>
</tr>
</tbody>
</table>

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

Patients with PPC have been reported to have significantly worse [10-12] or similar [3, 13-15] survival rates compared to those with EOC. The outcome of PPC depends on age, surgery stage, size of focus, residual tumor, and physical state. Nam et al. [15] reported that the outcome of PPC is mainly decided by the degree of cytoreductive surgery. Most people go to doctors when there are significant symptoms which means at an advanced stage. The rate of optimal cytoreduction in PPC has been reported to vary from 33% to 70% [16, 17], which resulted in less than 1.0 cm residual tumor.

We use laparoscopy to explore PPC and evaluate the criteria for resectability, which is minimally invasive surgery. Because of the minimally invasive character of laparoscopy, patients can recover in a shorter time than with laparotomy [17], and thus chemotherapy is not postponed given their poor physical state. Moreover, the possibility of spread of malignant cells is much lower than with laparotomy. In our group of patients, we did not find metastasis from the puncture location in laparoscopy. Therefore, laparoscopy is a reliable method of exploring PPC in advanced-stage ovarian cancer and for selecting candidates for neoadjuvant chemotherapy in order to conduct complete cytoreduction surgery. It also contributes to a better quality of life for patients found to have unresectable disease [18].

The presence of residual disease after surgery is one of the most important adverse prognostic factors for survival [19]. Neoadjuvant chemotherapy has been proposed as an alternative approach to conventional surgery as the initial management of bulky ovarian cancer, with the goal of improving surgical quality. Primary surgical cytoreduction followed by chemotherapy usually is the preferred management of advanced (Stage III or IV) PPC and ovarian cancer. It helps in selecting patients for feasible and relative cytoreductive surgery. As the tumors can be reduced through chemotherapy before surgery, it is more likely that optimal surgery can be performed. In our group, 79.3% patients had optimal cytoreductive surgery after neoadjuvant chemotherapy, which is a higher rate of optimal cytoreductive surgery than previous reports [16, 17]. Also, in our study, the mean survival time in the optimal group was higher than non-optimal group.

Since 1979, cisplatin-based multi-agent chemotherapy has been regarded as the standard treatment for patients with epithelial ovarian cancer and, consequently, for patients with PPC [20]. In 1996, a randomized Gynecologic Oncology Group trial demonstrated a significant survival advantage for patients with advanced EOC whose residual disease was > 1.0 cm treated with paclitaxel plus cisplatin compared to similar patients who were treated with cisplatin plus cyclophosphamide [23]. As a result of this study, the combination of paclitaxel and cisplatin is considered the first-line chemotherapy for patients with serous epithelial ovarian cancer. Of 17 patients in our study, two patients had progressive disease, three patients had stable disease, eight were partial responders, and 16 were complete responders; 16 patients were alive without evidence of disease, seven were alive with disease, and six had died from disease. We had a higher rate of complete response than Nam et al. [15] reported.

The median survival time after cytoreductive surgery with combined neoadjuvant chemotherapy reported in our study (48 months) was comparable to that of an earlier study [16] (41 months) and far longer than the 11.3-17.8 months reported previously [13, 14, 20, 22]. This may be related to our combination treatment with neoadjuvant chemotherapy and cytoreductive surgery after laparoscopic diagnosis and evaluation. Although a larger sample is needed, we believe that the combination of neoadjuvant chemotherapy and cytoreductive surgery after laparoscopic diagnosis is effective in the treatment of patients with PPC, which prolongs patient survival time.
References


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Could endometrial cytology be helpful in detecting endometrial malignancies?

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Introduction

Hysteroscopy with endometrial biopsy is the best tool to undergo endometrial pathologies [1]. To date, many studies have highlighted the goodness of hysteroscopy in diagnosing endometrial pathologies, as compared with dilatation and curettage [2], ultrasonographic imaging [3] and blinded endometrial biopsies [4]. Fewer reports have assessed the goodness of endometrial cytologic sampling in predicting endometrial pathologies [5-9]. We believe that the wide diffusion of hysteroscopic techniques led gynecologists to avoid endometrial cytologic sampling, even if it is overall useful in diagnosing endometrial malignancies [5, 6].

The aim of this short report is to check if endometrial cytology performed with a cervical brush agrees with histological findings obtained from hysteroscopic biopsies.

Patients and Methods

This study was conducted on 37 women who agreed to undergo cytologic endometrial sampling before hysteroscopy. Five women were non menopausal and 32 were menopausal. Indications for hysteroscopic examination were irregular menstrual bleeding, abnormal postmenopausal bleeding, and sonographic abnormal patterns.

Cytologic endometrial samples were performed with a sterile endocervical brush. A Saint Martin’s forceps was applied on the cervix, gently tractioning the uterus while the sterile brush was introduced within the uterine cavity. A convex 2.5 mHz ultrasonographic probe placed over the pubis, with the bladder moderately repleted, was used to check the brush position within the uterine cavity. Then, the brush was rotated 360° again, first close to the uterine fundus and following, close to the tubal angles.

Samples were fixed with 2% formalin on slides, and stained with Papanicolaou color for cytological examination (100x). No liquid-based methods were used to prepare microscope slides. Sometimes, some endometrial fragments were placed on slides, allowing histological assessment.

After the sampling, an ultrasonographic probe evidenced a hyperecogenic pattern within the uterine cavity due to air introduced with the brush. Such marker confirms the goodness of the sample.

Some days after endometrial cytology sampling, patients underwent office hysteroscopic biopsies.

Results

Table 1 shows the rates of normal patterns, hyperplasias (not atypical), endometrial polyps, and endometrial cancer for both cytological and histological findings. Kappa values are reported for each pattern. Additionally, overall kappa with significance is shown in the last column on the right. The concordance is poor for hyperplastic patterns, slight for normal patterns, fair for endometrial polyps, and almost perfect for endometrial cancer. Thus, overall concordance is fair for cytological and histological findings (p = 0.006).

The cytological sampling was easy in all patients who complained of pelvic discomfort or mild painful sensations like menstrual pain.

Key words: Endometrial cytology; Endometrial biopsy; Diagnosis.
Could endometrial cytology be helpful in detecting endometrial malignancies?

Table 1. — Histologic and cytologic patterns.

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Histology</th>
<th>k</th>
<th>Overall k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pattern</td>
<td>26</td>
<td>22</td>
<td>0.180</td>
</tr>
<tr>
<td>70.3%</td>
<td>59.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia (not atypical)</td>
<td>6</td>
<td>5</td>
<td>0.041</td>
</tr>
<tr>
<td>16.2%</td>
<td>13.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancers</td>
<td>1</td>
<td>6</td>
<td>0.251</td>
</tr>
<tr>
<td>2.7%</td>
<td>16.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Rates and k values for each pattern. Overall k provide an estimation of the concordance for all patterns.

Discussion

These results do not suggest the use of endometrial cytology as a screening test for detecting endometrial pathologies. However, endometrial cytology seems very able to detect endometrial cancer. This has been reported by other authors with tools able to provide endometrial fragments and/or with a liquid-based preparation of the sample [6, 7, 9]. However, in light of the wide use of hysteroscopy for detecting endometrial cancer and other endometrial diseases in Italy, it seems that endometrial cytology does not have any clinical use. However, when office hysteroscopy or other endometrial sampling tools are not wanted, clinicians should counsel patients about endometrial cytology as a practical and inexpensive tool for detecting endometrial malignancies. The goal of endometrial cytologic samples should be to remove some tissue fragments in order to improve pathological examination. Every endometrial sampler device and tool that allows this kind of tissue sampling improves diagnostic accuracy of endometrial cytology [11, 12]. However, ultrasound guidance and Martin’s forceps on the cervix allow the removal of some endometrial fragments with a common endocervical brush, without a cost-effectiveness disadvantage.

Conclusions

Endometrial cytology with the usual endocervical brush and sonographic guide may be helpful in detecting endometrial malignancies, if hysteroscopic biopsies, dilatation and curettage, or other blinded endometrial biopsies are unwanted or impossible.

References


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Evaluation of treatment results and prognostic factors in early-stage cervical carcinoma patients treated with postoperative radiotherapy or radiochemotherapy

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Introduction

Patients with early-stage cervical cancer (FIGO Stage IB and IIA) can be effectively treated with either radical surgery or radiotherapy (RT). Five-year overall and progression-free survival rates are comparable in both treatment modalities but treatment associated morbidity may differ [1]. Genitourinary symptoms are more commonly seen in the surgery group, whereas gastrointestinal symptoms are more common in patients selected to undergo RT or radiochemotherapy (RCT). The choice of treatment modality depends on institution experience, the gynecologic oncologist or radiation oncologist preference, patient general health, age and characteristics of the lesion [2].

With external beam RT and brachytherapy, the 5-year survival rate is 86-92% for Stage IB, 75% for Stage IIA and 60% for Stage IIIB disease. The overall pelvic failure rate in Stage IB is approximately 5% to 8%, and 15-20% in Stage IIA disease [2].

Several clinical and histopathologic unfavorable features (lymph node metastases, parametrial invasion, positive surgical margin) have been shown to increase the pelvic recurrence and distant metastasis rates for patients treated surgically. In this group of patients the addition of postoperative adjuvant RT/RCT has demonstrated improved outcomes [3-5]. Combined treatment modality (radical surgery followed by RT/RCT) comes with a price for the patient – an increased incidence of side-effects.

With treatment break > 14 days showed significance for DFS and DSS. MLNR was found as a valuable prognostic factor for all endpoints (LRC, DFS, DSS and OS). The rate of grade 3-4 late toxicity was 3.6% and 2%, respectively. Conclusion: Postoperative RT/RCT is an effective treatment modality for early-stage cervical cancer patients with unfavorable features and provides satisfactory local control and survival rates with low morbidity.

Key words: Cervical cancer; Radiotherapy; Radiochemotherapy; Prognostic factors; Metastatic lymph node ratio; Side-effects.

Materials and Methods

Patients

From January 1992 to December 2007, 256 cervical cancer patients treated with postoperative RT/RCT at Ege University Faculty of Medicine, Department of Radiation Oncology were retrospectively reviewed. Patients with at least six months of follow-up were included in this analysis. Median age of the patients was 47 years (range: 25-78).

The histology was squamous cell carcinoma in 201 (78.6%), adenocarcinoma in 29 (11.3%), adenosquamous cell carcinoma...
in ten (3.3%), and other in 16 (6.3%). All patients were staged according to FIGO staging system. Preoperative examination consisted of a medical history, complete blood count and biochemical tests, clinical examination under general anesthesia, chest X-ray, and abdominopelvic ultrasound (US). In case of suspicious findings in X-ray or US, computerized tomography (CT) of the thorax, abdomen and/or pelvis was performed.

One hundred and thirty-five (52.7%) patients had FIGO Stage IB1, 52 (20.3%) had Stage IB2, 35 (13.7%) had Stage IIA, and 34 (13.3%) had Stage IIB disease. The type of surgical procedure was as follows: Wertheim operation in 175 (68.4%) patients, total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BSO) +lymphadenectomy in 45 (17.6%), and suboptimal surgery (TAH+BSO)/total abdominal hysterectomy with a unilateral salpingo-oophorectomy (TAH+USO)/TAH in 36 (14%) patients. Median and mean number of removed lymph nodes were 20 and 23, respectively (range: 1-95). Median and mean number of metastatic lymph nodes was two (range: 1-15) for both. Metastatic lymph node ratio (MLNR) was defined as number of metastatic lymph nodes divided by the number of dissected lymph nodes. MLNR was evaluated in three categories (0; 1-10%; >10%) and respectively, 142 (55.5%), 27 (10.5%) and 31 (12.1%) patients were in each group. In 56 (21.9%) patients, we were unable to determine MLNR due to the lack of lymphadenectomy and/or the lack of information of number of retrieved lymph nodes in the pathology reports.

Surgical margin was positive in 24 (9.4%), parametrial invasion was present in 34 (13.3%) and endometrial extension was present in 26 (10.2%) patients. Lymphovascular space invasion (LVSI) was detected in 82 (32%) patients. The median pretreatment hemoglobin (Hb) level was 12.5 g/dl (range: 8.5-15.1). Patient and treatment-related characteristics are shown in Table 1.

### Treatment

Postoperative RCT was applied in patients with lymph node metastases, positive/close surgical margin, bulky tumor (> 4 cm), and parametral involvement, whereas postoperative RT was applied to patients with suboptimal surgery and/or with LVSI. Weekly concomitant cisplatin with the dose of 40 mg/m² was administered to patients after 1999 and 47 (18.4%) patients received concomitant chemotheraphy with RT. RT consisted of external RT and intracavitary brachytherapy in 236 (92.2%), and only external RT in 20 (7.8%) patients. External RT was applied with Co 60 teletherapy unit until the end of 1993 to 38 (14.8%) patients and with 6-18 MV linear accelerators to the rest of the patients thereafter. External RT was applied with 1.8 Gy daily fractions with a median total dose of 54 Gy (45-59.4 Gy). Radiotherapy portals were designed above the L5 vertebral and below the obturator foramina for the initial phase up to 45 Gy or 50.4 Gy. For patients with positive surgical margins and metastatic pelvic lymph nodes external RT dose was escalated to 54 Gy. Brachytherapy was applied with high-dose rate afterloader (HDR) microelectron device to 0.5 cm depth from mucosal surface. Brachytherapy dose and fractionation changed during years (1 x 925 cGy from 1992 to 2000 and 3 x 600 cGy from 2000 to present). LVSI was detected in 82 (32%) patients. The median pretreatment hemoglobin (Hb) level was 12.5 g/dl (range: 8.5-15.1). Patient and treatment-related characteristics are shown in Table 1.

### Follow-up

Patients were followed with physical and gynecological examination, pap smear, and laboratory tests at 3-month intervals for the first two years, at 6-month intervals for three years, and annually thereafter. Acute and late toxicity was graded according to the Radiation Therapy Oncology Group/European Organisation for Research and Treatment of Cancer (RTOG/EORTC) toxicity criteria [8]. For survival endpoints — locoregional control (LRC), disease-free survival (DFS), disease-specific survival (DSS), and overall survival (OS) — time interval was defined as the time from surgery to the date of first relapse, disease-specific death or any death.

#### Statistical analyses

Statistical analyses were performed by Statistical Package for Social Sciences (SPSS) for Windows version 13.0 (SPSS Inc, Chicago, IL). Failure free and overall survival rates were estimated using the Kaplan Meier method, and patient and treatment groups were compared using the log-rank test. Multivariate analyses were performed with Cox proportional hazard models to test the differences between groups. All statistical tests were two-tailed with a significance level of 0.05.

### Results

Median follow-up time was 60.5 months (range: 6-202 months). Five-year LRC, DFS, DSS and OS rates were 90.8%, 83.4%, 91.2%, and 85%, respectively. There were 30 (15.2%) patients with failures, of whom 17 had isolated locoregional failure, six had locoregional and distant failure, and 16 had distant failure. The location of isolated locoregional failures was as follows: cervical.
stump in six, pelvic nodes in five, cervical stump+pelvic nodes in one, pelvic side wall in two and other in three. The median time for locoregional failure was 24 months (range: 4-90 months) and 35 months (range: 7-168 months) for distant metastases. The distribution of treatment related acute and late toxicity is indicated in Table 2.

Table 2. — Distribution of acute and late term RT-induced toxicities.

<table>
<thead>
<tr>
<th>Acute</th>
<th>Late</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>17 (14.4)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>76 (29.7)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>–</td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
</tr>
<tr>
<td>Genitourinary</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>22 (8.6)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>75 (29.3)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>4 (1.6)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>24 (9.4)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>22 (8.6)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
</tr>
</tbody>
</table>

GI: gastrointestinal system; GU: genitourinary system.

Univariate analysis

Significant prognostic factors for LRC were the presence of LVSI (p = 0.03), tumor size > 4 cm (p = 0.01), the presence of endometrial involvement (p = 0.008), and higher MLNR (p < 0.001). There was a trend for poorer LRC for the presence of parametrial invasion (p = 0.07), positive surgical margin (p = 0.09), and treatment break > 14 days (p = 0.09).

Similarly significant prognostic factors for DFS were tumor size > 4 cm (p = 0.001), parametrical invasion (p = 0.04), endometrial involvement (p = 0.02), treatment break > 14 days (p = 0.006) and higher MLNR (p < 0.001). There was a trend for poorer DFS, for pre-treatment hemoglobin level ≤ 10 g/dl (p = 0.06), and positive surgical margin (p = 0.08).

For DSS; tumor size > 4 cm (p = 0.01), parametrical invasion (p=0.02), endometrial involvement (p = 0.03), positive surgical margin (p = 0.02), treatment break > 14 days (p = 0.01), and higher MLNR (p=0.001) were found as significant factors. For OS; older age (p = 0.01), advanced stage (p = 0.03), tumor size > 4 cm (p = 0.005), pretreatment Hb level ≤ 10 g/dl (p = 0.02), parametrical invasion (p = 0.03), and higher MLNR (p = 0.001) were found as significant factors. There was a trend for poorer OS in patients with positive surgical margin (p = 0.06) and treatment break > 14 days (p = 0.09).

The impact of median number of dissected LNs on local control and survival has been evaluated for patients ≤ 20 vs > 20 LNs removed and 5-year LRC (90.3% vs 93.7%, p = 0.4), for DFS (78.4% vs 89.8%, p = 0.2), for DSS (88.6% vs 92.8%, p = 0.3) and for OS (84% vs 83.9%, p = 0.99) respectively.

The influence of surgery type, optimal (Wertheim /TAH+BSO+lymphadenectomy) vs nonoptimal (TAH+BSO/TAH+USO/TAH) on the rates of local control and survival was evaluated. No significant difference was detected among patients treated with optimal vs non optimal surgery for LRC (91% vs 86%; p = 0.6), for DFS (90% vs 92%; p = 0.8), for DSS (83% vs 82%; p = 0.9) and for OS (84% vs 86%; p = 0.3), respectively. Five-year survival rates for these prognostic factors are listed in Table 3.

Multivariate analysis

Tumor size was shown as an important prognostic factor for LRC, DFS and DSS. Pretreatment Hb level (Hb ≤ 10 g/dl) was found to be a poor prognostic factor for OS. Endometrial involvement showed significance for LRC, and DFS. Treatment break (> 14 days) showed significance for DFS and DSS. MLNR was found as a valuable prognostic factor for all endpoints (LRC, DFS, DSS and OS). Univariate and multivariate analysis of prognostic factors is shown in Table 3.

Discussion

The current study evaluated the treatment results of FIGO Stage IB and II cervical cancer patients treated with postoperative RT/RCT at a single center within a period of 16 years. Five-year LRC, DFS, DSS and OS rates were 90.8%, 83.4%, 91.2%, and 85%, respectively which are consistent with several other series in the literature [9-13]. The only randomized trial assessing the benefit of postoperative RT in cervical cancer was the Gynecologic Oncology Group (GOG) 92 trial which evaluated early-stage cervical cancer patients without lymph node metastases, but was at a high risk of recurrence (bulky tumor, deep stromal invasion and LVSI). This study demonstrated that the addition of postoperative RT showed a 46% reduction in the risk of recurrence and a statistically significant reduction in the risk of progression or death [3].

For Stage I-IIA patients with lymph node metastases or positive margin or parametrial invasion, the addition of weekly cisplatin to postoperative RT was shown to improve progression free survival (80% vs 63%) and OS (81% vs 71%) [14]. Since the current study is a retrospective evaluation we can not assess the absolute benefit of RT or RCT on local control or survival. However the survival and toxicity rates of the patients treated with surgery followed by RT/RCT were satisfactory and similar to the other combined treatment modality study results in the published literature [9, 11].

Lymph node metastases have been shown to be an independent predictor and the most important prognostic factor for OS in cervical cancer patients [15, 16]. Five-year DFS rate was 57% in lymph node positive patients compared to 88% in lymph node negative patients [17]. Liu et al. analyzed 140 early-stage cervical cancer patients treated with surgery and postoperative RT and demonstrated that pelvic lymph node metastases was a
significant prognostic factor for DFS (77% vs 57%) and OS (91% vs 52%) [18]. In our analysis patients with lymph node metastases had worse five-year survival rates for all endpoints and respectively for LRC (94.5% vs 77.1%, \( p < 0.001 \)), for DFS (65.6% vs 88.5%, \( p < 0.001 \)), for DSS (78.1% vs 94.9%, \( p < 0.001 \)); and for OS (71.2% vs 89.1%, \( p < 0.001 \)).

Recently metastatic lymph node ratio has been introduced in surgical oncology as a prognostic factor for several cancer types such as colorectal cancer and pancreatic cancer [19, 20]. Researchers from Vienna evaluated the impact of lymph node density (LND) (the ratio of positive lymph nodes to the total number of lymph nodes removed) on survival in 88 cervical cancer patients treated with postoperative radiochemotherapy. They stratified patients into two groups according to LND; patients with LND \( \leq 10\% \) versus patients with LND > 10% and demonstrated that patients with LND > 10% had impaired DFS and OS rates compared with patients with LND \( \leq 10\% \) [21]. We demonstrated that patients with MLNR > 10% had poorer survival rates for all endpoints of the study (LRC, DSS, DFS and OS). Five-year overall survival rate was 63% for the patients with MLNR > 10% and 80% for the patients with MLNR \( \leq 10\% \).

During the pelvic lymph node dissection for cervical cancer it is suggested to dissect as many of the nodes as possible. The average number of lymph nodes need to be removed is 25-35 [22]. It is well documented that the percentage of nodal involvement increases from 10.5% positive nodes when less than 20 nodes are removed; to 26.5% when more than 50 nodes are removed [23]. Pieterse et al. conducted a prospective trial and demonstrated that the extent of lymphadenectomy significantly affected DFS in patients with pelvic LNs metastases. In

### Table 3. — Evaluation of prognostic factors affecting 5-year locoregional control, disease-free, disease-specific and overall survival rates.

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>LRC</th>
<th>DFS</th>
<th>DSS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>univ.</td>
<td>multiv.</td>
<td>univ.</td>
<td>multiv.</td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.27</td>
<td>0.16</td>
<td>0.16</td>
<td>0.01</td>
</tr>
<tr>
<td>≤ 47</td>
<td>93.1</td>
<td>86.1</td>
<td>92.7</td>
<td>89.5</td>
</tr>
<tr>
<td>&gt; 47</td>
<td>88.3</td>
<td>80</td>
<td>89.2</td>
<td>79.7</td>
</tr>
<tr>
<td>Stage</td>
<td>0.11</td>
<td>0.14</td>
<td>0.1</td>
<td>0.03</td>
</tr>
<tr>
<td>IB1</td>
<td>94.9</td>
<td>88.5</td>
<td>94.7</td>
<td>90</td>
</tr>
<tr>
<td>IB2</td>
<td>89.3</td>
<td>74.1</td>
<td>87.7</td>
<td>80</td>
</tr>
<tr>
<td>IIA</td>
<td>83.2</td>
<td>83</td>
<td>91.9</td>
<td>82.6</td>
</tr>
<tr>
<td>IIB</td>
<td>82.7</td>
<td>74.2</td>
<td>80.6</td>
<td>75.7</td>
</tr>
<tr>
<td>LVSI</td>
<td>0.03</td>
<td>0.49</td>
<td>0.29</td>
<td>0.79</td>
</tr>
<tr>
<td>Absent</td>
<td>94.3</td>
<td>85.4</td>
<td>91.5</td>
<td>86.2</td>
</tr>
<tr>
<td>Present</td>
<td>84.9</td>
<td>79.7</td>
<td>90.5</td>
<td>82.3</td>
</tr>
<tr>
<td>Tumor size</td>
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<td>0.04</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>≤ 4 cm</td>
<td>95</td>
<td>90.2</td>
<td>92</td>
<td>88.7</td>
</tr>
<tr>
<td>&gt; 4 cm</td>
<td>84.4</td>
<td>61.6</td>
<td>77</td>
<td>70</td>
</tr>
<tr>
<td>Hemoglobin g/dl</td>
<td>0.84</td>
<td>0.06</td>
<td>0.26</td>
<td>0.02</td>
</tr>
<tr>
<td>≤ 10</td>
<td>66.7</td>
<td>50</td>
<td>75</td>
<td>66.7</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>90.8</td>
<td>82.9</td>
<td>91</td>
<td>97</td>
</tr>
<tr>
<td>Parametral invasion</td>
<td>0.07</td>
<td>0.04</td>
<td>0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>No</td>
<td>91.9</td>
<td>84.8</td>
<td>92.8</td>
<td>86.5</td>
</tr>
<tr>
<td>Yes</td>
<td>82.7</td>
<td>74.2</td>
<td>80.6</td>
<td>75.7</td>
</tr>
<tr>
<td>Vaginal invasion</td>
<td>0.95</td>
<td>0.7</td>
<td>0.81</td>
<td>0.53</td>
</tr>
<tr>
<td>No</td>
<td>90.8</td>
<td>83.5</td>
<td>91.6</td>
<td>85.1</td>
</tr>
<tr>
<td>Yes</td>
<td>90.4</td>
<td>81.0</td>
<td>88</td>
<td>84.4</td>
</tr>
<tr>
<td>Endometrial involvement</td>
<td>0.008</td>
<td>0.003</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>85.6</td>
<td>92</td>
<td>86.2</td>
</tr>
<tr>
<td>Yes</td>
<td>76.6</td>
<td>64.2</td>
<td>78.7</td>
<td>74.8</td>
</tr>
<tr>
<td>Surgical margin</td>
<td>0.09</td>
<td>0.08</td>
<td>0.02</td>
<td>0.21</td>
</tr>
<tr>
<td>Negative</td>
<td>91.8</td>
<td>84.5</td>
<td>92.8</td>
<td>87</td>
</tr>
<tr>
<td>Positive</td>
<td>81.4</td>
<td>74</td>
<td>77.3</td>
<td>68</td>
</tr>
<tr>
<td>Treatment break (days)</td>
<td>0.09</td>
<td>0.006</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>≤ 14</td>
<td>91.2</td>
<td>84.7</td>
<td>91.9</td>
<td>86.5</td>
</tr>
<tr>
<td>&gt; 14</td>
<td>86.8</td>
<td>70.1</td>
<td>74.7</td>
<td>71.1</td>
</tr>
<tr>
<td>MLNR</td>
<td>0.000</td>
<td>0.01</td>
<td>0.000</td>
<td>0.001</td>
</tr>
<tr>
<td>0</td>
<td>96.4</td>
<td>90.1</td>
<td>95.7</td>
<td>89.1</td>
</tr>
<tr>
<td>1-10%</td>
<td>86.1</td>
<td>75.1</td>
<td>84.1</td>
<td>80.8</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>76.1</td>
<td>62.8</td>
<td>73</td>
<td>63.5</td>
</tr>
<tr>
<td>Histopathology</td>
<td>0.39</td>
<td>0.5</td>
<td>0.6</td>
<td>0.77</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>90.5</td>
<td>84.5</td>
<td>91.7</td>
<td>87</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>92.9</td>
<td>87.1</td>
<td>90.8</td>
<td>83.5</td>
</tr>
</tbody>
</table>

AJCC: American Joint Comittee on Cancer; LVSI: Lymphovascular space invasion; MLNR: Metastatic lymph node ratio; RT: radiotherapy; LRC: Locoregional control; DFS: Disease free survival; DSS: disease specific survival; OS: Overall survival.
patients with less than ten LN dissected in that particular study, 5-year DFS was less than 20% [24]. In our series the median and the mean number of removed lymph nodes were 20 and 23, respectively (range: 1-95). We observed slightly lower LRC, DFS, DSS and OS rates for patients with ≤ 20 LNs dissected but the differences were not statistically significant. It is possibly due to the administration of postoperative pelvic RT which controls possible subclinical disease in the non dissected pelvic lymph nodes.

There are several unfavorable prognostic factors that researchers reached consensus; these are advanced stage, large tumor volume (> 4 cm), deep stromal invasion, lymphovascular invasion, as well as parametrial involvement and close/positive surgical margins in patients with early-stage cervical cancer [18]. In these cases, most studies indicate that adjuvant radiation therapy could improve prognosis even if lymph nodes are negative [3, 24]. Atahan et al. reported the treatment results of 141 patients treated with surgery and postoperative RT/RCT and multivariate analysis revealed that the level and the number of metastatic lymph nodes and administration of concomitant chemotherapy were prognostic factors for OS, DFS and LDFS. Moreover endometrial involvement was found significant for DFS and distant metastases free survival (DMFS) [9]. In the current analysis we demonstrated poorer OS rate for advanced stage, poorer LRC rate for the presence of LVSI, and poorer LRC, DFS, DSS and OS rates for bulky tumor (> 4 cm), poorer DFS, DSS and OS rates for parametrial invasion, poorer LRC, DFS and DSS rates for endometrial involvement and poorer DSS for positive surgical margin.

The influence of age and histopathologic type on LRC and survival is not clearly determined and the results of previous studies have been inconclusive [2]. In our analysis patients younger than 47 years of age had better OS rates. We did not observe any LRC control or survival difference among histopathological types.

The impact of treatment break on local control and survival has already been shown in RT/RCT trials for curatively treated head and neck and cervix cancer patients. For cervical cancer, prolongation of the overall treatment time is associated with an estimated loss of local control of 0.3-1.6% daily [2]. In our analysis treatment break > 14 days is associated with decreased DFS and DSS rates.

Tumor hypoxia has been linked to tumor progression, the development of treatment resistance, and thus poor prognosis [2]. It has been known that Hb level has an important role in tumor radioresponsiveness. Mayr et al. analyzed 88 cervical cancer patients treated with RCT and demonstrated that low Hb (<11.2 g/dl) level is associated with lower LRC and DDS rates [25]. In the current analysis patients with Hb level ≤ 10 g/dl had statistically significant poorer OS rates (66.7% vs 97%) compared to patients with > 10 g/dl.

The locoregional and survival rates of patients with parametrial invasion and positive surgical margin were found to be poorer even with the administration of RT/RCT. Therefore during the preoperative gynecologic examination the gynecologist should discriminate patients who may need postoperative RT and refer these patients to a radiation oncologist. Moreover selecting single modality treatment will protect the patient from increased side-effects of multimodality treatment [1].

In the literature, the rate of late toxicities range from 3% to 30%. This wide range is due to the differences in reporting (all grades vs grade 3-4) and utilization of various toxicity systems (RTOG vs LENT-SOMA). Among these studies gastrointestinal toxicity appears to be the most common toxicity followed by genitourinary toxicity. The GOG 92 trial reported 6.6% grade 3-4 toxicity in the RT arm and 2.1% toxicity in the observation arm [3]. Recent studies reported grade 3-4 late toxicities between 3%-4.7% [2]. Atahan et al. reported the rate of all grade 3-4 late toxicity in eight (6%) patients including both gastro-intestinal (GI), and genito-urinary (GU) toxicity [9]. During a median of 60.5 months (range: 6-202 months) of follow-up, grade 3-4 late GI toxicity in eight (3.2%) and GU in four (1.6%) and skin toxicity in two (0.8%) were observed in our series. The incidence of grade 3 and 4 late toxicities seen in our study was similar to the other mentioned series [3, 9].

In conclusion, our analysis yields similar results with the published literature on the treatment outcome of early-stage cervical cancer. External RT combined with brachytherapy provides satisfactory local control and survival rates. Lymph node metastases, higher MLNR, tumor size (> 4 cm), endometrial involvement, and low Hb status seem to be the most important prognostic factors for early-stage cervical cancer.

References


Evaluation of treatment results and prognostic factors in early-stage cervical carcinoma patients treated with postoperative etc.


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Small cell neuroendocrine carcinoma of the cervix: analysis of the prognosis and role of radiation therapy for 43 cases

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Department of Gynecology Oncology, Zhejiang Cancer Hospital, Hangzhou (China)

Summary

Objective: To explore the outcomes and pattern of recurrence in patients with small cell neuroendocrine carcinoma of the cervix (SCNEC), and to determine the effects of adjuvant radiation therapy on survival in patients with early-stage disease. Methods: A retrospective analysis of 43 patients with SCNEC was carried out at Zhejiang Provincial Tumor Hospital between January 1985 and August 2007. All pathological specimens were examined and definitively diagnosed by two independent pathologists. The radiotherapeutic efficacy and prognosis for SCNEC were explored. Patient survival status was analyzed with the Kaplan-Meier method and survival rate was compared with the log-rank test; p < 0.05 was considered statistically significant. Results: Of 43 patients, 32 were early-stage and 11 were advance-stage. The median age was 45 years (range 25-85 years). There were 21 cases of metastasis or progression occurring in the lungs, retroperitoneal lymph node and brain within two years. In early-stage patients, distant metastasis or progression occurred in 13 cases within two years. The estimated 5-year survival rate for the entire group was 29%. Median overall survival for patients with early-stage disease was 89.6 months and 34.4 months for patients with advance-stage disease (p = 0.001). The 3-year survival for early-stage patients who received postoperative adjuvant chemotherapy was 57.1% compared with 56.4% for those who underwent adjuvant chemoradiotherapy, and their median survival periods were 84.7 and 89.1 months, respectively (p = 0.671). Conclusion: We confirmed the unfavorable prognosis related to early nodal and hematogenous metastasis in SCNEC, resulting in a relatively poor prognosis; clinical staging was an important prognostic factor. Chemoradiotherapy may be provided for advance-stage patients. For early-stage patients, the efficacy and site of postoperative adjuvant radiotherapy need further evaluation.

Key words: Small cell carcinoma; Neuroendocrine; Uterine cervix; Prognosis treatment outcome radiation.

Introduction

Small cell neuroendocrine carcinoma arising from the uterine cervix is an uncommon malignancy comprising less than 3% of all cervical malignancies [1, 2]. It is known to be highly malignant and is associated with the lowest rate of survival of the cervical cancers due to the tumor’s propensity for distant spread. The time interval of a definitive diagnosis to recurrence is less than 35 months and median survival period is around 14.19 months. Five-year survival rates vary from 14%-30% [3, 4]. Long-term survival can be achieved only in patients with limited stage disease. Due to the rarity of this disease, it has been difficult to conduct prospective trials. Clear treatment recommendations for SCNEC have not been defined. How to treat SCNEC patients more effectively and improve their survival rates have become a great challenge for gynecological oncologists. Currently there is a heated debate on the effect and mode of radiation for SCNEC. For SCNEC patients beyond clinical Sage IIA, the survival period never surpassed 30 months in the literature reports [5-7]. Among the SCNEC patients admitted and treated at our hospital, two Stage IIB patients who underwent radiotherapy survived for 112 and 114 months, respectively.

We performed a retrospective review to explore the outcomes and pattern of recurrence in 43 patients with SCNEC. The objectives were to compare the effects of adjuvant chemoradiotherapy versus adjuvant chemotherapy on the survival rate in patients with early-stage disease and to explore the role of radiation of SCNEC so as to provide rationale for the therapy of SCNEC.

Materials

Clinical data

During the period January 1985 to August 2007, a total of 43 cases of SCNEC at first diagnosis were admitted and treated at our hospital. All were definitively diagnosed by two pathologists after a second examination of specimen slides. All cases received gynecological examinations, chest films and such imaging studies of the abdomen and pelvis as computed tomography (CT) and magnetic resonance imaging (MRI). Staging was in strict accordance with the FIGO International Federation of Gynecology and Obstetrics criteria for cervical carcinoma. The early stages were IB-IIA and advanced stages were IIB-IV. The radical surgical approach was extensive hysterectomy plus pelvic lymph node dissection with or without paraaortic lymph node dissection. The radical radiation was extra-pelvic plus intracavitary radiotherapy; the cumulative dose of Point A was 73-77 GY, postoperative adjuvant radiotherapy external whole pelvic radiotherapy at a dose of 45 Gy/25 sessions (1.8 Gy per session, 5 sessions per weeks). In case of common iliac lymph node or abdominal aortic lymphatic metastases, abdominal paraaortic radiotherapy was provided at a dose of 40 Gy. Most patients received the chemotherapeutic protocol of EP (etoposide & cisplatin). The adjuvant chemotherapeutic protocol was cisplatin 60 mg/m² + VP16 100 mg (di-5), and concurrent chemotherapy was one course of chemotherapy within 24 hours of the initiation of radiotherapy.
Small cell neuroendocrine carcinoma of the cervix: analysis of the prognosis and role of radiation therapy for 43 cases

Cisplatin 30 mg/m² + VP16 50 mg (dl-5), repeated for 3-4 weeks. Since 2007, two patients received the TP protocol of chemotherapy, i.e. taxol 175 mg/m² + cisplatin 60 mg/m². All patients received an average of four courses (range: 2-6).

Follow-up
The follow-up period began from the first day of definite diagnosis until August 10, 2010. Follow-up rate was 100%, and the median follow-up period was 36 months (5-141.2 months).

Statistical methods
The SPSS 15.0 statistical package was used. Clinical data are described with values of percentages or medians. Total survival time was counted from the definitive diagnosis until initial recurrence or progression. Survival status was analyzed by the Kaplan-Meier method, and the log-rank test was used to compare the survival rate; \( p < 0.05 \) was considered as having statistical significance.

Results
General data: There were a total of 43 patients with a (median age of 45 years old - range: 25-85). Fifteen had Stage IB1, six Stage IB2, 11 Stage IIA, two Stage IIB, one Stage IIIA, three Stage IIIB and five Stage IV. Thirty-two early-stage patients received comprehensive therapy: postoperative adjuvant chemotherapy (n = 8) and postoperative adjuvant chemoradiotherapy (n = 20). Demographic characteristics of the patients and therapeutic protocols are shown in Table 1.

Time and location of metastasis or recurrence: At the final follow-up, 23 patients had either metastasis or progression. Among them, the metastatic locations for two cases remained undefined. One case had a local pelvic recurrence without receiving adjuvant radiotherapy. The remaining 20 cases had distant metastasis. The metastatic sites were in descending order lungs, retroperitoneal lymph node, breast and brain (in Table 2). There were four cases of brain metastasis; among them, three cases had concurrent lung metastasis and one case bony and systemic metastasis. Thirteen (41%) early-stage patients had distant metastasis or progression within two years, and one Stage IIA patient had metastasis to the lung at 31.2 months after diagnosis (Table 3).

Survival rate: The follow-up period was up to August 2010. Twenty-two patients had already died. The median survival time was 37 months. The 3-year OS (overall survival) rates for 32 early-stage patients and 11 advanced-stage patients were 56.7% and 27.3%, respectively. The median survival periods were 89.6 and 34 months, respectively (\( p = 0.001 \)) (Figure 1). Among 11 advanced-stage patients, the median survival period was 16 months. Nine cases died within two years. After radiotherapy, two Stage IIB patients survived for 112 and 114 months, respectively, and one had still survived at the
time of last follow-up. The early-stage patients received either postoperative adjuvant chemotherapy (n = 8) or postoperative adjuvant chemoradiotherapy (n = 20). The 3-year OS rates of the two patient groups were 57.1% and 56.4%, respectively. The median survival periods were 84.7 and 89.1 months, respectively (p = 0.671) (Figure 2). The median progression-free survival (PFS) period was 93.3 and 81.9 months, respectively (p = 0.569).

Table 1. — Characteristics of patients with SCNEC.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean age (range)</th>
<th>FIGO stage (N)</th>
<th>Tumor size (%)</th>
<th>Tumor homology (%)</th>
<th>Primary treatment modality (n)</th>
<th>Adjuvant therapy for early-stage patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IB1 15</td>
<td>≤ 4 cm 34</td>
<td>Pure 34</td>
<td>Surgery only 1</td>
<td>Chemoradiation 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IB2 6</td>
<td>&gt; 4 cm 9</td>
<td>Mixed 9</td>
<td>Radiation only 1</td>
<td>Radiation 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIA 11</td>
<td></td>
<td></td>
<td>Chemotherapy only 5</td>
<td>Chemotherapy 8</td>
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<tr>
<td></td>
<td></td>
<td>IIB 3</td>
<td></td>
<td></td>
<td>Multimodality therapy 36</td>
<td>Neoadjuvant chemotherapy 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIB 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV 5</td>
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<td></td>
<td></td>
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Table 2. — Outcome and patterns of recurrence or metastasis in a 2-year period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
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<td>Recurrence rate</td>
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<td></td>
</tr>
<tr>
<td>Early stage</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Advanced stage</td>
<td>8</td>
<td>63</td>
</tr>
<tr>
<td>Sites of recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Chest</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pelvis</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Unknown sites</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — Rate and site of first recurrence in patients with early-stage disease.

<table>
<thead>
<tr>
<th>N</th>
<th>Local, only</th>
<th>Distant, only</th>
<th>Local and distant</th>
<th>Unknown sites</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>11 (34%)</td>
<td>1 (3%)</td>
<td>2 (6%)</td>
<td>14 (43%)</td>
</tr>
</tbody>
</table>

Discussion

**Prognosis of SCNEC**

Small cell carcinoma of the cervix is a rare gynecological malignancy. The natural history of this disease differs from the more commonly seen squamous cell or adenocarcinoma. It is noted for its very aggressive behavior and has the poorest prognosis of the various cervical carcinomas, even after multimodal therapy. In our cohort of 43 patients, the 3- and 5-year OS rates were 45% and 29%, respectively. The median survival period was 45 months. The 3-year OS rates of early- and late-stage patients were 56.7% and 27.3%, respectively. The difference had statistical significance. In some previous retrospective reports, the 3-year survival rate of SCNEC was around 30% and the 5-year OS rate around 20-25 [8, 9]. The 5-year OS rates of early to middle, and late patients were 36.7% and 0-8.9%, respectively [10]. It was reported in the literature that patients with diseases beyond Stage IB1 had a survival period of over 30 months [5]. Clinical and imaging staging are important prognostic factors [8-11]. The prognosis of this disease is limited due to tumor propensity for distant hematogenous metastases. Even in early stage, 40% to 60% of patients experience lymph node metastasis and hematogenous metastasis within one year of diagnosis [8, 12, 13]. In our cohort, 23 patients had metastasis or progression at the last follow-up, and the rate of definitive distant metastasis was 87%. The metastatic sites were in descending order lungs, retroperitoneal lymph nodes, and breast. Four brain metastasis patients had concurrently lung and other distant metastases. No single patient had brain metastasis alone. Even for the early-stage patients, the rate of distant metastasis or progression within two years was as high as 41%. Only one Stage IIA case had lung metastasis at 31 months after diagnosis. As recently reported by Lee et al. [13] the median PFS of SCNEC was merely 16.9 months. The rate of recurrence or distant metastasis for early patients was up to 67%, and it was obviously higher than 6% for cervical squamous cell carcinoma at the same stage [12]. As demonstrated by the analytic results of Kasamatsu et al., the recurrence rate of early SCNEC patients was up to 70%, and 80% occurred outside the pelvic cavity [14]. As reported by Zinvanovic et al., the 3-year PFS of SCNEC was only 22% and the median progression period nine months [8].

**Therapeutic approach for SCNEC**

Because of the rarity of the disease the Gynecologic Oncology Group attempted to study small cell cervical carcinoma (protocol 66) between 1982 and 1986, but failed to recruit sufficient numbers of patients. To date, most studies on neuroendocrine cervical carcinoma are comprised of only small series and case reports; no large-scale multicenter study has been conducted on the disease, and the optimal initial therapeutic approach has not been clarified. It is generally established that the etoposide/platinum (cisplatin) (EP) chemotherapeutic protocol and comprehensive therapy could reduce distant metastasi-
sis and improve the prognosis [6, 8, 15]. As reported by Huang et al. [16] the 3-year survival rate of SCNEC on comprehensive therapy reached 45% [16]. Although some authors had doubts of the surgical efficacy for SCNEC patients [14], surgeons and patients at most medical institutions have chosen operations as one of the important therapeutic options for early-stage patients [6, 17, 18].

In the present study, there were 32 early-stage patients. Among them, 29 opted for surgery, as did another five Stage IIB patients; small cell lung cancer (SCLC) is sensitive to radiotherapy thus it is one of the important therapeutic options for SCLC. Although the biological behaviors of SCNEC and SCLC are quite similar, the radiotherapeutic efficacy and mode of SCNEC have remained a major focus of debate and clinicians are actively exploring answers.

Role of radical radiation therapy in the treatment of early SCNEC

The possible role of radical radiation therapy in the treatment of early SCNEC is unknown. Chemoradiotherapy has been widely applied for localized and diffuse stages of SCLC. Surgery is reserved only for Stage IA patients with a tumor size less than 2 cm, containing drug-resistant non-small cell components or the resection of residual foci insensitive to radiotherapy [19-21]. Currently there has been no literature report of any efficacy study on radical radiation therapy in patients with early-stage SCNEC. In our cohort, one Stage IB1 patient who underwent simple radiotherapy who was in advanced age survived for 92 months. It is expected that more treatment centers of gynecological tumors will cooperate and conduct comparative studies of early SCNEC operations versus radiotherapy so as to search for more optimized and effective therapy for early SCNEC.

There is a heated debate on the postoperative adjuvant radiotherapy. Although SCNEC has a frequent occurrence of early lymphatic and distant metastasis, many scholars hold the opinion that most SCNEC patients have a local pelvic recurrence prior to the onset of distant metastasis. Thus it has been recommended that early-stage SCNEC patients receive pelvic adjuvant radiotherapy to boost the local control rate [5, 10]. The team of Hoskins [12] reported that 31 SCNEC patients received adjuvant radiotherapy and chemotherapy and achieved a 3-year tumor-free survival rate of 57%. There was a pelvic recurrence in four cases (13%), and two recurrent cases occurred beyond the pelvic radiotherapy field although they underwent routine abdominal paraaortic radiotherapy. In the authors’ opinion, it was impossible for the operation to achieve such a high local control rate. Adjuvant radiotherapy could markedly reduce local and retroperitoneal lymph node metastasis [12]. However, some reports have stated that postoperative radiotherapy was harmful. In 2007, Lee et al. [9] reported that the 5-year survival rates of early SCNEC patients accepting and declining adjuvant radiotherapy were 40.2% and 53.9%, respectively. We surmised that postoperative adjuvant radiotherapy boosted the toxic and unpleasant effect, delayed the initiation of chemotherapy and lowered the survival rate [9].

A comparison of efficacy was conducted between the regimens of postoperative adjuvant chemoradiotherapy and adjuvant chemotherapy in early-stage patients. In our research it was found that the 3-year OS rates were 57.1% and 56.4%, respectively, in two patient groups, and the median survival periods were 84.70 and 89.126 months, respectively. Apparently the result showed that the overall survival period of postoperative adjuvant chemoradiotherapy was shortened. At the same time, we considered such a fact that most patients opting for adjuvant chemoradiotherapy had one or more high postoperative pathological risk factors while those on adjuvant chemotherapy often had no high-risk factors. Among eight patients, undergoing postoperative adjuvant chemotherapy except for one infiltrative case of the outer membrane, infiltrations involved superficial interstitial and even mucous layers in the other seven cases. Among 20 cases undergoing postoperative adjuvant chemoradiotherapy, infiltration of the superficial interstitium occurred in only two cases after neoadjuvant chemotherapy while the other 18 cases had one or more high-risk factors. As reported by Kasamatsu et al. [14] the recurrence rate was as high as 70% in the postoperative SCNEC patients with such high-risk pathological factors as infiltration of deep interstitium, tumor embolus within the lymphovascular space and lymphatic metastasis. Yet for the patients with a depth of interstitial infiltration less than 6 mm, there was no tumor recurrence [14]. The definitive efficacy of adjuvant chemoradiotherapy in early-stage SCNEC patients needs to be analyzed by studies of a larger sample size. In the present study, among 20 patients on adjuvant chemoradiotherapy, no single case had pelvic metastasis and eight patients underwent adjuvant chemotherapy. One case had pelvic metastasis. Considering the fact that small cell carcinoma was sensitive to radiotherapy, we recommended the SCNEC patients with high postoperative risk factors receive adjuvant chemoradiotherapy to boost the local control rate.

Effect of radiation for advanced stage

The chemoradiotherapeutic efficacies of advance-stage SCNEC patients are currently well established. It was reported that post-chemoradiotherapeutic OS rates showed no marked differences between medium and advance-stage SCNEC and cervical squamous cell carcinoma. The median survival period was around seven months for late-stage SCNEC. For SCNEC patients beyond the clinical stage of IIA, a survival period of over 30 months has never been reported in the literature [5-7]. In our cohort, two Stage IIB patients received chemoradiotherapy and survived for 112 and 114 months, respectively, and one of them had an onset of abdominal paraaortic lymph node metastasis at 20 months after pelvic chemoradiotherapy. Then there was a long-term survival after abdominal aortic radiotherapy. The pathological tissues of these two patients were definitively diagnosed after numerous re-examinations and eliminations of mis-
diagnoses with the combination of electron microscope and immunohistochemistry by two pathologists. Thus an aggressive regimen of chemoradiotherapy could achieve a relatively long-term survival in medium and late-stage SCNEC patients with localized disease foci.

**Prophylactic brain and paraaortic radiotherapy for SCNEC**

SCNEC and SCLC share many similar pathological and biological behaviors. Often there is an early onset of lymphatic and distant metastasis resulting in the failure of therapy. Currently all SCLC patients of localized and diffuse stages undergo prophylactic hilar and mediastinal lymph node radiotherapy in spite of the status of lymphatic metastasis [20]. It has remained unclear whether or not SCNEC should receive prophylactic abdominal paraaortic radiotherapy similarly as SCLC, and the number of the relevant literature reports is still quite limited. Hoskins et al. once reported the routine use of abdominal paraaortic radiotherapy for SCNEC, but the case numbers were too few to analyze the efficacy [12]. Considering both the unpleasant effects and indefinite efficacy of radiotherapy, we only selected abdominal paraaortic radiotherapy for the patients with common iliac lymph node or abdominal aortic lymphatic metastasis. The brain metastatic rate of localized SCLC was around 20%-30% while that of diffuse SCLC was around 40%. It was because prophylactic whole brain radiotherapy boosted the 3-year survival rate by 5.4%. Thus prophylactic whole brain radiotherapy became a routine procedure [22-24]. However, its efficacy for SCNEC has been debated. According to the literature reports, the brain metastatic rate of SCNEC was markedly lower than that of SCLC, and the patients with established brain metastasis often had concurrent lung metastasis [5, 25]. None of the patients had solely brain metastasis, and the SCNEC patients required no prophylactic whole brain radiotherapy [5, 25]. It was also reported in the literature that the rate of brain metastasis of early SCNEC patients was up to 25%. Thus prophylactic whole brain radiotherapy was recommended [26]. In our cohort, all four patients with brain metastasis had onset of lung and other distant metastases. Thus none of them received prophylactic whole brain radiotherapy. 

**Conclusion and limitation of this study**

As compared with cervical squamous and adenocarcinomas at the same stages, SCNEC frequently has the clinical feature of an early occurrence of lymphatic and distant metastasis, and the recurrence and survival periods of the patients become markedly shortened with a poor prognosis. All patients in our study came from the same hospital. Their clinical data and follow-up information were complete. The treatment principles and methods of early and late-stage patients were basically the same; the former group was dominated by surgery and complimented with chemoradiotherapy or chemotherapy while chemoradiotherapy remained the major therapy for the latter group. The surgical approaches, radiotherapeutic regimens and doses were roughly the same. The predominant chemotherapeutic protocol was EP. The limitation was that there were too few cases to evaluate the efficacy of adjuvant radiotherapy in early-stage SCNEC patients so as to reach any definitive conclusion on whether or not there is a need for prophylactic abdominal paraaortic and whole brain radiotherapy. Furthermore there is still no efficacious comparison of surgery versus radical chemotherapy in early-stage patients. With the constantly improved proficiency of pathological diagnosis at our hospital, around eight to ten cases of SCNEC are newly diagnosed each year. In recent years a rising trend has been demonstrated. With the cooperation of other large tumor treatment centers, more prospective studies with a larger sample size need to be conducted to explore the optimal therapy for SCNEC so as to improve the prognosis of SCNEC patients.

**References**

Small cell neuroendocrine carcinoma of the cervix: analysis of the prognosis and role of radiation therapy for 43 cases


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Possible effects of insulin-like growth factor-I, IGF-binding protein-3 and IGF-1/IGFBP-3 molar ratio on mammographic density: a cross-sectional study

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Summary

The purpose of this study was to examine the possible effects of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio on mammographic density and assess whether this relationship was similar in subgroups of pre- and postmenopausal women. A group of 341 Italian women of childbearing age or naturally postmenopausal who had performed mammographic examination at the section of radiology of our department a maximum three months prior to recruitment were enrolled. A blood sample was drawn for determination of IGF-1, IGFBP-3 levels and IGF-1/IGFBP-3 molar ratio was calculated. On the basis of recent mammograms the women were divided into two groups: dense breast (DB) and non-dense breast (NDB). To assess the association between mammographic density and IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio showed that IGF-1 levels and molar ratio varied in the two groups resulting in higher mean values in the DB group whereas IGFBP-3 showed similar values in both groups (DB and NDB). After stratification of the study population by menopausal status, no association was found. Our study provides strong evidence of a crude association between breast density, and plasma levels of IGF-1 and molar ratio. IGF-1 and molar ratio might increase mammographic density and thus the risk of developing breast cancer.

Key words: IGF-1; IGFBP-3; IGF-1/IGFBP-3; Molar ratio; Mammographic density; Breast cancer risk.

Introduction

Insulin-like growth factor-1 (IGF-1) is an essential growth factor for the regulation of proliferation and apoptosis in normal mammary cells [1, 2]. There is increasing evidence of the role of IGF-1 in the growth of tumors in a number of different cancers such as prostate, colon and lung cancer [3, 4] including breast cancer [2, 5, 6]. The IGF-1 mechanism of action in carcinogenesis and development of breast cancer is complex and multifactorial [1, 2, 5-11]. IGF-1 circulates in the blood and it has a very short lifetime in free form, approximately 12 minutes; its action is strongly influenced by the association with one of six existing different insulin-like growth factor binding proteins (IGFBPs) which increases its average lifetime to about 12 hours [1, 2]. Over 90% of IGF-1 in circulation is bound to form-3 of IGFBP (IGFBP-3). This complex remains stable in the blood due to the presence of a binding protein, specific protease inhibitor [1, 7].

In the extravascular space, the lack of this inhibitor allows specific metalloproteases to break the link between IGF and IGFBP-3 thus favoring the association between IGF and its specific cellular receptor (IGFR) expression in the breast tissue where IGF-1 fulfills its regulatory role [8].

In addition to the regulatory effect on the action of IGF-1 the IGFBP-3 present in the tissue also seems to directly promote cell apoptosis independently of IGF-1 [7-10]. Several studies have demonstrated an association between the IGF system and breast cancer risk in premenopausal women [6, 12-14], suggesting that IGF-1 might interact with the estrogen signal to increase cell proliferation [15, 16]. Other more recent studies have reported a possible association between the IGF system and carcinogenesis also in postmenopausal women [17].

The debate concerning the association between menopausal status, IGFBP, IGF-1 and breast cancer risk is therefore still open [18, 19]. On the other hand, breast density is currently considered as the strongest breast cancer risk factor [20]. Women with mammographic density ≥ 75% have a five-fold increased risk of developing breast cancer compared to women with fatty breast tissue and density < 5% [21-24].

Given the regulatory function of IGF-1 on the proliferation of normal breast tissue, the question has been raised whether there is a possible association between IGF and breast density. Some authors have shown a significant association between IGF and breast density in premenopausal women [13, 15, 25-28]. The results reported in the literature related to postmenopausal women are still discordant, as most authors found no correlation between IGF and breast density [25-29], whereas some authors reported a weak relationship also in postmenopausal women [30] whether they were receiving hormonal therapy [31] or not [30].

The main objective of the present study was to analyze whether there is a relationship between plasma levels of IGF-1, IGFBP-3, IGF-1/IGFBP-3 molar ratio and mam-
The objective of the study was explained to all the selected subjects. The first phase of the study included signing of an informed consent form, collection of recent mammograms as well as drawing of blood samples for the evaluation of serum IGF-1 and IGFBP-3 and calculation of IGF-1/IGFBP-3 molar ratio.

Patients were divided into two groups: dense breast (DB) or non-dense breast (NDB) according to the mammographic parenchymal category assigned at the evaluation of the presented mammograms. Subsequently patients were stratified by menopausal status.

**Materials and Methods**

Ethical approval for this single-center, prospective study was granted by the Medical Research Ethics Committee of our institution and written informed consent was obtained from all patients.

The sample was built up in the order of presentation and 7,000 women were selected among those who spontaneously turned to the Breast Care section of the Department of Obstetric and Gynaecological Sciences and Urological Sciences of the University of Rome “Sapienza” for a breast examination between March 2005 and March 2007.

According to the protocol we selected only Italian women of childbearing age (regular menstrual cycles during the past year) or naturally postmenopausal women (absence of menstrual cycles for at least 12 months) who had performed mammographic examination, negative for breast cancer pathology, at the section of radiology of our department maximum three months prior to recruitment. Premenopausal women were enrolled in the study only if they had undergone mammographic examination within the first ten days of the menstrual cycle.

After recruitment the women were interviewed by a medical doctor.

Women with a clinical history positive for breast cancer and/or for colon and lung cancer, administration of hormone therapy for up to 12 months before recruitment such as menopause hormone replacement therapy (HRT) and hormonal contraceptives, participation in assisted fertilization programs and previous breast reduction or augmentation surgery were excluded from the study.

The objective of the study was explained to all the selected subjects. The first phase of the study included signing of an informed consent form, collection of recent mammograms as well as drawing of blood samples for the evaluation of serum IGF-1 and IGFBP-3 and calculation of IGF-1/IGFBP-3 molar ratio.

Patients were divided into two groups: dense breast (DB) or non-dense breast (NDB) according to the mammographic parenchymal category assigned at the evaluation of the presented mammograms. Subsequently patients were stratified by menopausal status.

**Mammographic classification**

To determine the mammographic parenchymal category all mammograms were examined by three physicians (two radiologists and a gynaecologist and breast specialist) all blinded to the clinical data and to the classification already assigned. Particular attention was paid to the craniocaudal projections of both breasts and the distribution of glandular parenchyma was qualitatively evaluated in percentage of the total area of the breast. The patients were then assigned to one of the four categories of breast parenchymal density distribution established by the Breast Imaging Reporting and Data System (BI-RADS): type 1, the breast is almost entirely fat (glandular parenchyma < 25% of the total area of both breasts); type 2, scattered fibroglandular densities (25%-50%); type 3, heterogeneously dense breast tissue (51%-75%); type 4 extremely dense (> 75% glandular).

It is well known that the sensitivity of mammography is decreased in type 3 and 4 [32, 33] and the patients participating in our study were therefore divided into two groups: dense breast (DB) which included BI-RADS type 3 and 4, and non breast dense (NDB) which included BI-RADS type 1 and 2.
A total of 7,000 women were assessed for eligibility; 3,099 were excluded because they did not meet the inclusion criteria and 3,560 were excluded according to exclusion criteria. This selection produced a final sample of 341 women. Sampling strategy is illustrated in Figure 1.

Evaluation of mammographic features showed the presence in the sample of 196 (57.5%) patients with DB (BI-RADS 3 and 4) and 145 (42.5%) patients with NDB (BI-RADS 1 and 2). Assessment of inter-operator variability did not show statistically significant differences; Cohen’s Kappa values ranged from 0.85 to 0.89 (p = 0.001) thus indicating a high level of agreement.

To assess the relationship between mammographic density and plasma levels of IGF-1, IGFBP-3 and molar ratio variably in the two groups, correlation analysis either in premenopausal or in postmenopausal patients (Table 1).

As regards menopausal status, 150 (43.9%) women were premenopausal and 191 (56.1%) were postmenopausal at the time of mammography. Mean age was 43.9 years for premenopausal women and 61 years for postmenopausal women.

Comparing the association between plasma level of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio and breast density after stratification of the study population by menopausal status (premenopausal and postmenopausal), it was observed that there was no association either in premenopausal or in postmenopausal patients (Table 2).

Discussion and Conclusion

There is an increasing interest in early detection of risk factors for developing breast cancer. Mammographic density is one factor [20-24] but the IGF system has recently been shown to have a role in the development of breast cancer [2, 5-7, 12-14]. However, it is not yet clear whether these factors are interrelated and if and how they are influenced by menopausal status [24-31].

The purpose of this cross-sectional study was to examine the possible effects of IGF-1, IGFBP-3 and IGF-1/IGFBP-3 molar ratio on mammographic density and assess whether this relationship was similar in subgroups of pre-and postmenopausal women.

The study sample was fairly homogeneous as only Italian Caucasian women were enrolled, while women of different ethnic origins were excluded due to the possibility that plasma levels of IGF-1 and IGFBP-3 and parenchymal density might vary among different ethnicities. This choice was dictated by the need to build a homogeneous study sample, as previous studies of IGF-1 and IGFBP-3 reported in the literature seem not to have paid attention to ethnic differences but only to geographic location thereby suggesting an environmental rather than genetic influence, whereas parenchymal density is thought to differ according to ethnicity rather than geographical location [27, 34, 35]. Also women who had received HRT for up to 12 months before recruitment

<table>
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<tr>
<th>Variables</th>
<th>Non-dense breast (n = 145; 42.5%)</th>
<th>Dense breast (n = 196; 57.5%)</th>
<th>p value</th>
<th>Molar ratio (mean)</th>
<th>DB (n = 124; 62.7%)</th>
<th>NDB (n = 26; 12.7%)</th>
<th>p value</th>
<th>IGFBP-3 (ng/ml; mean)</th>
<th>DB (n = 119; 63.4%)</th>
<th>NDB (n = 72; 36.6%)</th>
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<td>Serum peptide assays</td>
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<td>IGF-1 (ng/ml; mean)</td>
<td>96.6 ± 35.0</td>
<td>109.6 ± 36.1</td>
<td>0.001</td>
<td>25.5 ± 7.6</td>
<td>29.4 ± 8.6</td>
<td>0.001</td>
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<td>28.7 ± 8.7</td>
<td>31.0 ± 9</td>
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<tr>
<td>IGFBP-3 (ng/ml; mean)</td>
<td>3.8 ± 1.0</td>
<td>3.8 ± 0.8</td>
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<td>3.8 ± 0.9</td>
<td>3.7 ± 0.9</td>
<td>NS</td>
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<td>Molar ratio</td>
<td>25.5 ± 7.6</td>
<td>29.4 ± 8.6</td>
<td>0.001</td>
<td>28.7 ± 8.7</td>
<td>31.0 ± 9</td>
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NS = not significant.
were excluded from this study because the use of post-menopausal hormones has been reported to lower circulating IGF-1 levels and increase breast density [31, 36]. Particular attention was paid to the uniformity of blood sampling for determining IGF-1 and IGFBP-3 levels. Analysis of a single sample was considered sufficient, as most authors claim that one evaluation can predict long-term levels of these peptides [37-39]. In premenopausal women, the blood sample was drawn between 8 am and 11 am after an overnight fast between the 6th and 10th day of the menstrual cycle as these values may vary according to the menstrual cycle [40]. Blood analysis was carried out in a single block by one single laboratory technician who was blinded to the parenchymal classification. Using this strategy, peptide levels measured in our group of Italian women were generally lower than those reported by other authors [41].

This study has some limitations. One concerns the reliability of mammographic classification which was performed qualitatively and not by a computer-assisted method. However, BI-RADS mammographic classification is the most common technique used in the USA for the assessment of mammographic density [32]. In order to further reduce the risk of measurement error, women were enrolled only if they had undergone mammographic examination at our center not more than three months before recruitment and blood sampling. Rigid criteria were furthermore used for assessing breast density [42]. Using this method, inter-operator variances were not statistically significant as there was a high level of concordance in the evaluation carried out by the three blinded readers. Furthermore, the BI-RADS system was developed to alert the referring clinician that the ability to detect small cancers in the dense breast is reduced and it not related to the risk per se [33].

A second limitation is that the temporality of the relation between growth factors and breast density cannot be determined due to the cross-sectional design. Finally an analysis of the potential confounders of the relationship between mammographic density and plasma level of IGF-1, IGFBP-3 and molar ratio was not carried out.

Our results showed that IGF-1 values and molar ratio were higher in the DB group compared to the NDB group. IGFBP-3 values were similar in the two groups. When the levels of growth factors were compared to breast density stratifying by menopausal status, no association was found.

Previous studies showed that breast cancer risk rose steadily with increased percentage of the breast area with a dense appearance on a prediagnostic mammogram and this association was not explained by other breast cancer risk factors [21-24, 43]. It is still not known through what mechanism breast density is related to cancer risk [23, 44].

On the other hand current breast density reflecting the proportion of stromal and epithelial proliferation may simply indicate the area of susceptible tissue (number of epithelial cells) or may represent the interaction between stromal and epithelial proliferation influenced by local growth factors, including IGF-1 [45]. Growing evidence indicates that breast development and involution are influenced by IGFs [which increase proliferation] and IGFBPs (which reduce proliferation) [46]. Thus, greater breast density may be a consequence of higher IGF and molar ratio levels and an associated increase in proliferation and/or of decreased IGFBP levels with a resulting reduction in the involution process.

Our study provides strong evidence of a crude association between breast density and plasma levels of IGF-1 and molar ratio, but unlike previous studies by other authors, they do not confirm that IGF-1 can be considered determinant in breast density either in premenopausal [13, 15, 24-28] or in postmenopausal women [29-31].

In conclusion, on the basis of our results it is reasonable to assume that the role of IGF-1 and molar ratio in the pathogenesis of breast cancer is mediated through mammographic density. Thus IGF-1 and molar ratio might increase the risk of cancer by increasing the mammographic density.

Further studies are required to clarify these issues, particularly the mechanisms regulating the IGF bioavailability in the biological systems which may explain the development of not only breast cancer, but also prostate, colon and lung cancer in which growth factors have been implicated.

References


Alterations in the mortality and growth cycle of cervical cancer cells treated with electroporation at different electric strengths

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Summary

Objective: To explore the biological effects of electric fields of various strengths on Hela cells. Methods: Electroporation experiments were performed using Hela cells. Changes in cell mortality, cell vitality, cell cycle, and apoptosis status were examined. In addition, temperature changes in the surrounding tissue were measured. Results: Cell proliferation was markedly inhibited after treatment with field strengths of 2-2.5 kV/cm. The expression of caspase-3 increased significantly in cells treated with field strengths of 1.5-2.5 kV/cm. Field strengths of 1.75-2.5 kV/cm produced complete cancer cell ablation. G2 phase frequency increased significantly after treatment with field strengths of 2-2.5 kV/cm. During this process, the maximum temperature increase in the pulsed electric field was 4.9 ± 1.17°C under free air convection. Conclusions: IRE can be used alone for the treatment of cancer, and its thermal effect is negligible. Cell death was caused by the effects of IRE and apoptosis. The tumor cells must be destroyed completely, or the altered cell cycle may lead to tumor recurrence and accelerated growth.

Key words: Pulsed electric field; Cervical cancer cells; Cell cycle; Apoptosis.

Introduction

The development of techniques based on pulsed electric fields, such as electrochemotherapy, electrogenetherapy, and the newly developed irreversible electroporation (IRE) has been rapid. These techniques are all based on the phenomenon of membrane electroporation. The term electroporation describes an electromagnetic biological phenomenon of increased cell membrane permeability after a pulsed electric field is applied to the cells. The increased membrane permeability is caused by altered transmembrane potentials and the formation of nanochannels in the bilayer lipid membranes [1-3]. Depending on whether the permeability of the cell membranes can return to the normal physiological state, electroporation is divided into reversible electroporation (RE) and irreversible electroporation (IRE), which is also known as irreversible electrical breakdown (IREB) [4-6].

RE allows chemotherapeutic drugs (such as bleomycin and cisplatinum) and macromolecules, which do not normally cross the lipid bilayer membrane, to enter the cells freely. Using RE, chemotherapeutic drugs are injected into tumor cells in a pulsed electric field; this injection is typically performed with the following classical parameters: field strength, 1000 V/cm; pulse width, 100 s; and number of pulses (p), 8. These drugs then enter the tumor cells and exert a strong local anti-cancer effect. The use of RE can reduce the systemic drug dose required, selectively increase local tumor cell toxicity, and avoid the adverse effects caused by systemic chemotherapy [7-11]. Gene therapy is an alternative method for cancer treatment, and a wide variety of vectors, such as adenovirus and liposomes, are used in gene transfer. However, these vectors are not satisfactory because of their biologically unsafe nature, high immunogenicity, and lack of stability. In contrast, when used as a vector for gene transfer, the pulsed electric field is free of these disadvantages.

IRE has been studied extensively in vitro. IRE can effectively kill microorganisms and has been used for water sterilization [12]. Recently, IRE was applied to rat liver and was found to completely ablate the target regions without causing thermal damage to the surrounding normal tissue. The ablation area had a clear border and the large blood vessels and bile ducts of the target area were intact [13]. Rubinsky used IRE as a new and minimally invasive surgical technique to ablate a volume of tissue in large animals and then observed the long-term effects. Because of the presence of intact and functional large blood vessels in the ablation area, the recovery of the target region was extremely rapid. The integrity of the blood vessels and the connective tissue indicated that IRE affected only the cell membranes. The area for ablation can be predetermined by the Laplace equation [14]. These results suggest that the use of IRE as a minimally invasive technique in cancer treatment is promising, especially for tumors that are located near large blood vessels, where surgery is difficult.

The karyoplasmic ratio is large in tumor cells, and the specific inductive capacity of cancer cells is larger than that of normal cells [15]. These characteristics make it possible for pulsed electric fields to selectively kill cancer cells rather than normal cells. This selectivity provides an advantage over other thermal ablation methods for the treatment of cancer. However, the cell death pathway that is activated by pulsed electric fields is still unclear. Both necrosis and apoptosis may occur in the cells in the target area were intact [13]. Rubinsky used IRE as a new and minimally invasive surgical technique to ablate a volume of tissue in large animals and then observed the long-term effects. Because of the presence of intact and functional large blood vessels in the ablation area, the recovery of the target region was extremely rapid. The integrity of the blood vessels and the connective tissue indicated that IRE affected only the cell membranes. The area for ablation can be predetermined by the Laplace equation [14]. These results suggest that the use of IRE as a minimally invasive technique in cancer treatment is promising, especially for tumors that are located near large blood vessels, where surgery is difficult.

The karyoplasmic ratio is large in tumor cells, and the specific inductive capacity of cancer cells is larger than that of normal cells [15]. These characteristics make it possible for pulsed electric fields to selectively kill cancer cells rather than normal cells. This selectivity provides an advantage over other thermal ablation methods for the treatment of cancer. However, the cell death pathway that is activated by pulsed electric fields is still unclear. Both necrosis and apoptosis may occur in the cells in the target...
area, and a combination of the morphological features of the two forms of cell death has been observed. The strong electric field around the electrode may lead to coagulative necrosis, while apoptosis may dominate where the electric field is weaker.

There are many unanswered questions regarding pulsed electric fields. Can it activate apoptosis or will it cause a mild injury that will be repairable and will allow cell survival? If the cells do survive, do they display changes in their cell cycle and growth state? What is the field intensity that will cause ablation of the tumor cells with a minimal thermal effect? To answer these questions, we used pulses with a fixed pulse length and frequency, made gradual changes to the electric field strength, and simulated the attenuation of the electric field within the tissue. We also recorded the responses of Hela cells to different field strengths.

Materials and Methods

Cell culture

Hela cell lines were provided by the Ultrasonic Research Institute of Chongqing Medical University. The cells were cultured in RPMI 1640 medium (HyClone, Logan, UT, USA) with 10% neonatal calf serum (Sijiqing, Hangzhou, China) at 37°C under 5% CO₂. Cells in the logarithmic phase of growth were digested with 0.25% trypsin (HyClone), and the digestion was terminated by addition of serum. The cells were then placed into 10-ml centrifuge tubes and centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The cells were washed three times with phosphate-buffered saline (PBS, HyClone) and resuspended at a concentration of 1 × 10⁶ cells/ml.

Electroporation

The pulse width was 100 s and the frequency was 1 Hz, with eight pulses in a set. On the basis of the field-strength gradient, the cell suspension was divided into a control group that did not receive any electrical field treatment and 0.5-, 0.75-, 1-, 1.25-, 1.5-, 1.75-, 2-, 2.25-, and 2.5-kV/cm groups. Each group was treated with one set of pulses (8 pulses in total). The 1.5–2.25-kV/cm groups were treated with three more sets of pulses, and the gap between each pulse was maintained at 1 min in order to eliminate thermal effects. A total of 760 µl of cell suspension from each group was added to the electrode cuvette (Bio-Rad Inc., Hercules, CA, USA; gap distance of the electrode cuvette, 4 mm), and the pulse generator (Department of Education of High Voltage and Electrical New Technology, Chongqing University, China) was connected to the experimental apparatus. Real-time wave formation was recorded by an oscilloscope (Tektronix TDS3032B; Beaverton, OR, USA). The differences between the set voltage, the pulse width, and the recordings on the oscilloscope were less than 10%. The temperature was measured immediately after the pulse by using a thermocouple device (Model: JK-8; Changzhou Jin Ai Lian Electronic Technology, China; test accuracy: 0.1°C). The theoretical temperature rise of the differential electric field was calculated using the formula

\[
\Delta T = \frac{E^2 \times t}{r \times \rho \times c_p}
\]
where \( r \) indicates the impedance of the solution, \( p \) indicates the density of the PBS solution, and \( p \) indicates the specific heat of the PBS solution (Table 1).

<table>
<thead>
<tr>
<th>Field strength (kV/cm)</th>
<th>Theoretical temperature increase (°C)</th>
<th>Actual temperature increase (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.68</td>
<td>0.16 (0.15)</td>
</tr>
<tr>
<td>0.75</td>
<td>1.53</td>
<td>0.30 (0.26)</td>
</tr>
<tr>
<td>1</td>
<td>2.72</td>
<td>0.56 (0.37)</td>
</tr>
<tr>
<td>1.25</td>
<td>4.25</td>
<td>0.90 (0.43)</td>
</tr>
<tr>
<td>1.5</td>
<td>6.12</td>
<td>1.50 (0.87)</td>
</tr>
<tr>
<td>1.75</td>
<td>8.33</td>
<td>2.13 (0.68)</td>
</tr>
<tr>
<td>2</td>
<td>10.88</td>
<td>3.10 (1.65)</td>
</tr>
<tr>
<td>2.25</td>
<td>13.77</td>
<td>4.43 (2.0)</td>
</tr>
<tr>
<td>2.5</td>
<td>17</td>
<td>4.90 (1.17)</td>
</tr>
</tbody>
</table>

* Values are presented as means (SD).

Trypan blue staining

At 0 h after treatment with the pulsed electric field, 100 µl of the cell suspension was added to 0.4% trypan blue solution and allowed to stain for 5 min. The blue cells and the total number of cells were counted with a hemacytometer (Shanghai Precision Instrument Co., Ltd., Shanghai, China). The mortality rate was calculated as follows: (the total number of cells in the control group – the number of viable cells in the experimental group)/the total number of cells in the control group \( \times 100\% \).

Giemsa staining

Cells treated with different electric field parameters were suspended in 10 µl of the medium, transferred to a 6-well plate (10 × 10 mm), and then cultured with coverslips at 37°C under 5% CO2. The plate was gently rotated to uniformly disperse the cells. After the surviving cells adhered to the coverslips, the coverslips were washed three times with PBS, and the cells were fixed with 4% paraformaldehyde for 10 min. The coverslips were allowed to air dry. Staining with 100 µl of Giemsa (1:50) was performed for 20 min. The coverslips were rinsed three times with water and allowed to air dry.

MTT assay

A total of 100 µl of cells from each group were treated with pulsed electric fields, and then, 300 µl of the culture medium was added to 96-well plates for 24 h for culture. Next, 20 µl of MTT solution was added, and the cells were allowed to grow for an additional 4 h. The supernatant was then removed, 150 µl of dimethyl sulfoxide was added to each well, and the plates were incubated for 10 min. An enzyme-linked immunosorbent assay (ELISA) was used to measure the absorption value at a wavelength of 490 nm.

Immunohistochemistry

For immunohistochemical analysis, 10 µl of the treated cell suspension from each group was cultured on a coverslip (10 × 10 mm) in 30 µl of medium in 6-well plates, which were then rotated gently to uniformly disperse the cells. The cells were cultured at 37°C under 5% CO2 for 6 h. The presence of caspase-3 was tested by SP immunohistochemistry, and the working dilution of both the primary (rabbit anti-human polyclonal antibody; Bio-Rad Inc.) and secondary antibodies (species anti-rabbit; Bio-Rad, Inc.) was 1:200.

Flow cytometry

After treatment with pulsed electric fields for 6 h, 500 µl (1 × 10^4 cells) of cells from each group were stained with annexin V-FITC and propidium iodide. After complete mixing, the solution was allowed to react for 15 min at room temperature in the dark. The cells were tested by flow cytometry, and FACScan CellQuest was used for analysis.

Statistical analysis

The SPSS 10.0 statistical software (SPSS Inc., Chicago, IL, USA) was used to perform single-factor analysis of variance. The results were expressed as mean (SD). If there were differences, the LSD \( t \) test for homogeneous variances or the Games-Howell test for non-homogeneous variances was used to compare differences between the groups. The level of significance was \( p < 0.05 \).

Results

Trypan blue cell count 0 h after treatment with pulsed electric field

After 0 h, the trypan blue cell count showed no significant differences in the cell mortality between the control group and the 0.5-1.5-kV/cm groups, while there were significant differences between the cell mortality in the control group and the 1.75-2.5-kV/cm groups \((p < 0.01)\). The result for the 1.75-kV/cm group was different from those for the 0-1-kV/cm groups, but not from those for the 1.25-2.25-kV/cm groups. The result for the 2.5-kV/cm group was also different from that for the 2.5-kV/cm group. The result for the 2.5-kV/cm group showed differences when compared with those for the 0.175-kV/cm groups \((p < 0.01)\) and the 2.25-2.5-kV/cm groups \((p < 0.05)\; \text{Figure 1})\).

Trypan blue cell count 6 h after treatment with pulsed electric field

Six hours after treatment with the previously mentioned electrical parameters, the trypan blue cell count showed significant differences between the cell mortality in the 2-kV/cm group and the 0.175-kV/cm groups \((p < 0.05)\). In addition, significant differences were found among the 2.25-kV/cm group, the 2.5-kV/cm group, and the 0.175-kV/cm groups \((p < 0.01)\). No differences were found among the 0.175-kV/cm groups.
Twenty-four hours after treatment with the previously mentioned electrical parameters, the trypan blue cell count showed no significant differences among the control group and the 0.5-0.75-kV/cm groups ($p > 0.05$). Significant differences were found among the control group and the 1-1.25-kV/cm groups ($p < 0.05$) and among the control group and the 1.5-2.5-kV/cm groups ($p < 0.01$). These results suggest that as the exposure time increased, the cells treated with a field strength greater than 1 kV/cm and less than 1.75 kV/cm showed a relatively slow process of death. Cells treated with a field strength of 2-2.5 kV/cm showed a rapid process of death.

Giemsa staining

After 6 h of treatment with an electric field, the surviving cells (Hela cells attached at 6 h) were fixed in situ by Giemsa staining. At this time, pyknosis, which is characteristic of apoptotic cells, could be observed, and completely dead and non-adherent cells were lost in the process of staining.

Cell viability testing with MTT

**MTT value under different field strengths (8 $p \times 1$ set)**

There were significant differences among the control group and the other groups (Figure 10, $p < 0.05$ and $p < 0.01$). Significant differences were also found among the values for the 0.5-1.75-kV/cm groups. A significant difference was found between the values for the 2-kV/cm group and the 2.25-kV/cm group ($p < 0.05$).

**MTT value under the action of field strength (1.5-2.25 kV/cm, 8 $p \times 3$ sets)**

Significant differences were found among the MTT values for the 1.5-kV/cm 8 $p \times 2$ group, the 1.75-, 2-, and 2.25-kV/cm 8 $p \times 2$ groups, and the 1.5-kV/cm 8 $p \times 3$ group ($p < 0.01$). Cells were completely ablated under the 1.75-2.25-kV/cm 8 $p \times 3$ conditions, and there were no viable cells. Cells were not completely ablated under the 1.5-kV/cm 8 $p \times 3$ conditions, and no differences were found compared to the 1.75-2.25-kV/cm 8 $p \times 2$ conditions ($p > 0.05$).

Detection of caspase-3 expression by immunohistochemical analysis of Hela cells after electric field treatment

SP immunohistochemistry showed significantly increased caspase-3 protein expression with the 1.5-2.25-kV/cm 8 $p \times 1$ pulsed electric fields when compared with the expression in the control group. Brown-yellow immunohistochemical particles were located in the cytoplasm and the nucleus (Figure 8).
Alterations in the mortality and growth cycle of cervical cancer cells treated with electroporation at different electric strengths

Real-time temperature increase in cells under different experimental parameters

The theoretical temperature increase of the differential field strength was calculated according to the previously mentioned formula. The real-time temperature increase was 5°C when the field strength was 2.5 kV/cm. The cell temperature increase was negligible after treatment with 0.5-2.5 kV/cm because the temperature was not high enough to damage the cells.

Discussion

With increasing electric field strength, RE shifted to IRE, and the increasing energy was able to ablate the tumor cells completely. However, the ultimate goal of this study was to determine the field strength that was able to ablate the diseased tissues with the minimum amount of energy so that the patients were able to tolerate the adverse effects. Our research shows that the 1.5-2.5 kV/cm levels of field strength were close to the median lethal dosage, and that an increased number of pulses was able to ablate the tumor cells completely. Thus, field strength plays a relatively important role, but the role of the pulse number is also important. It has been demonstrated that an increased number of pulses with optimal intervals can kill tumor cells without heat superposition.

Under the conditions of air convection and proper temperature used in this study, the heat generated by energy dissipation had a negligible impact on the cells, and there was no thermal effect. This is consistent with the findings of a previous study [16].

In this study, we stimulated the cells with gradually increasing electric field strengths in order to observe the changes in the mortality rates of the tumor cells at 0, 6, and 24 h after treatment with the various electric fields. The results showed that 0-6 h after treatment with electric fields of 0.5-1.75 kV/cm, no significant differences in cell mortality were found. However, after the cells were treated for 24 h with an electric field strength of 1 kV/cm, the mortality rate increased significantly. When the field strength reached 2 kV/cm for 24 h, immediate cell death occurred in more than 60% of the population and cell debris was seen, which indicated that the cells showed IRE and disintegrated when the field strength reached a certain level. Thus, there was an obvious dose-effect relationship between cell death and the electric field strength. The process of cell death after treatment with a field strength of 1-1.75 kV/cm was slow and may have involved apoptosis. Although the flow cytometry test showed significantly increased apoptosis at 6 h after treatment with field strengths above 2 kV/cm, slow death continued to occur at 24 h after treatment in some cells. At the same time, the immunohistochemical assay showed
that the expression of the apoptotic protein caspase-3 in cells treated with a field strength greater than 1.5 kV/cm was significantly higher than that in the control cells, which was consistent with the results of the flow cytometry test. Therefore, the treatment of cells with a pulsed electric field of a certain intensity can cause cell death and cell apoptosis.

Notably, the 24-h MTT cell viability test showed that at 6 h after treatment with the 1-1.75-kV/cm electric fields, the cell viability detected was still close to that of the untreated control group, although the trypan blue cell count showed a 40% mortality rate. These data show that the complete ablation of tumor cells by the pulsed electric field was particularly important, and these results are in agreement with the results of Miller et al. [17]. In order to explore the mechanisms underlying these results, we studied the changes in the cell cycle by flow cytometry after treatment with an electric field and found that the numbers of cells in the G2 phase in the treatment groups were higher than that in the control group. This difference was especially prominent between the control group and the 2.25-kV/cm and the 2.5-kV/cm groups. The unablated cells had a changed cell cycle phase, and the proliferation of these cells may have been accelerated. This observation suggests that in the clinical application of pulsed electric fields for the treatment of cancer, the complete killing of the tumor cells is very important to ensure that any recurrence or accelerated growth of the tumor can be avoided.
How can we optimize the combination of electrical parameters for ablating the tumor? On the basis of the original parameters, we chose field strengths above 1.5 kV/cm, which is close to the median lethal dosage, and the number of pulses was increased to three sets. We found that cervical cancer Hela cells were completely ablated without thermal effects by a field strength of 1.75-2.5 kV/cm, 1 Hz, 8 p × 3. We did not try pulses with the lower field strengths. We anticipate that the results of these experiments can also be used for in vivo tumor tissue as long as the geometric properties of the tumor cells do not change. In addition, because the electric field affects the membrane, successful tumor tissue ablation is also closely related to the electrode shape [18, 19].

Conclusion
In summary, IRE may be used alone as a novel and minimally invasive technique for the complete ablation of cancer tissues without a thermal effect. Both the electric field intensity and the number of pulses are important. Coagulation necrosis and apoptosis are involved in the cell death induced by IRE. It is worth noting that the complete destruction of tumor cells is critical when a pulsed electric field is used for tumor ablation, or the change in the cell cycle from the electric field treatment may lead to tumor recurrence or accelerated growth of the tumor cells.

Acknowledgment
This work was supported by grants-in-aid for scientific research from the Chongqing Natural Science Foundation. We thank Yao Cheng Guo for his technical assistance in electroporation.

References

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Retrospective study comparing irinotecan and pegylated liposomal doxorubicin in treatment of recurrent platinum-refractory/resistant epithelial ovarian cancer

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Summary
Purpose: The standard regimen for platinum-resistant/refractory recurrent epithelial ovarian cancer (EOC) remains to be determined. In this study, we retrospectively compared the effect of irinotecan (CPT-11) and pegylated liposomal doxorubicin (PLD) in the treatment of platinum-resistant recurrent EOC. Methods: Thirty patients who received salvage chemotherapy with CPT-11 or PLD were included in the study. CPT-11 (100 mg/m²) was administered intravenously on days 1, 8 and 15 every four weeks. PLD (50 mg/m²) was administered on day 1 every four weeks. Treatment was repeated, provided that no disease progression or intolerable toxicity occurred. Results: Response rate in the CPT-11 group and PLD group showed no difference at 26.7% (p = 0.66) in both, while non-PD rate was 73.3% vs 33.3% (p = 0.05), respectively. Progression-free survival after CPT-11 treatment and PLD treatment was 28.4 weeks and 16.8 weeks (p = 0.07), respectively. Hand-foot syndrome and mucositis were more common in the PLD group than in the CPT-11 group (p = 0.05). Conclusions: The results indicate that CPT-11 is a promising drug for the treatment of platinum-resistant recurrent EOC.

Key words: Ovarian cancer; Recurrence; Platinum-resistant; Irinotecan; Liposomal doxorubicin.

Introduction
Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy, accounting for approximately 8,000 new diagnoses and 4,000 deaths annually in Japan [1]. Patients are usually treated with cytoreductive surgery, followed by platinum/taxane chemotherapy, and the initial response rate for these treatments exceeds 70% [2]. Despite such a good initial response, however, the majority of patients experience a relapse, with a median disease-free interval of 18 to 24 months. Recurrent cases are classified into three categories: platinum-sensitive (relapse after ≥ 6 months of initial platinum therapy); platinum-resistant (relapse within 6 months of initial platinum therapy); or platinum-refractory (stable disease or progressive disease during initial platinum therapy). According to guidelines issued by the National Comprehensive Cancer Network (NCCN), while platinum-based combination therapy should be considered in recurrent cases classified as platinum-sensitive, non-platinum monotherapy is recommended in recurrent cases classified as platinum-resistant/refractory [3]. The standard regimen, however, remains to be determined. Pegylated liposomal doxorubicin (PLD) is approved by the US Food and Drug Administration for use in patients with EOC whose disease has progressed or recurred after platinum-based chemotherapy, and PLD has become a commonly used treatment option for patients with recurrent platinum-resistant/refractory EOC. Irinotecan (CPT-11), a semi-synthetic derivative of camptothecin and topoisomerase I inhibitor, is widely used for platinum-resistant EOC in Japan [4]. In this retrospective study, we compared the effect of CPT-11 and PLD in the treatment of platinum-resistant recurrent EOC.

Materials and Methods
Patients
We retrospectively reviewed the medical records of women with platinum-refractory/resistant recurrent EOC who received CPT-11 or PLD. Thirty patients in whom salvage chemotherapy was commenced between May 2006 and May 2010 were included in the study. All patients underwent initial surgery and primary chemotherapy consisting of a platinum/taxane regimen, and were followed-up at the Department of Obstetrics and Gynecology, Keio University Hospital, Tokyo. All treatments were performed by staff of the same gynecologic oncology group. Decisions with regard to the salvage chemotherapy were usually made by the attending clinician. Any regimen that contained a platinum or taxane drug was counted as one regimen. For example, if a patient received a platinum/taxane regimen as first-line chemotherapy and then, after recurrence, received another platinum/taxane regimen as second-line chemotherapy, the number of regimens was counted as two. Except one patient, none of the patients in the CPT-11 group had received prior treatment with CPT-11, topotecan (TOP) or some other topoisomerase I inhibitor, and none of the patients in the PLD group had been treated with anthracyclines, including PLD. Data were collected on age, International Federation of Gynecology and Obstetrics (FIGO) staging, histologic type, histologic grade, prior chemotherapeutic treatment, site of recurrence, interval between prior chemotherapy and date of recurrence and progression-free survival (PFS) after recurrence.
Definition of chemotherapy sensitivity of prior chemotherapy

“Refractory,” “resistant,” and “sensitive” at first recurrence were defined as follows: refractory: progression, partial remission, or stable disease during primary chemotherapy; resistant: complete remission and relapse < 6 months of termination of primary chemotherapy; sensitive: complete remission and relapse ≥ 6 months after termination of primary chemotherapy.

Treatment schedule, response evaluation and toxicity assessment.

The treatment cycle consisted of four weeks. Irinotecan (100 mg/m²) was administered intravenously over 90 min on days 1, 8 and 15 every four weeks. Pegylated liposomal doxorubicin (50 mg/m²) was administered on day 1 every four weeks. Treatment was repeated for up to eight cycles, provided that no disease progression or intolerable toxicity occurred.

Response was based on 2-dimensional measurement of lesions based on computed tomography (CT) or magnetic resonance imaging (MRI). Complete response (CR) was defined as no evidence of disease on images obtained, with normalization of serum CA125 level. Partial response (PR) was defined as a > 50% decrease in tumor size. Progressive disease (PD) was defined as a > 25% increase in tumor size or the appearance of a new lesion. Stable disease (SD) was defined as neither sufficient shrinkage to qualify as PR, nor sufficient increase to qualify as PD. The CA125 response criteria were not used; however, the patients were considered as showing no PR or change if there was an increase in CA125. CT or MRI were performed every two to three cycles during chemotherapy and every three to six months after chemotherapy. Progression-free survival (PFS) was defined as the interval from the first day of administration of salvage chemotherapy to the day of disease progression.

All adverse effects were classified according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTC), version 3.

Statistical analysis

The Fisher exact test or chi-square test was used to compare clinical background and toxicity between the CPT-11 and PLD groups. The relationships between response rate or non-PD rate and age, histology, number of prior regimens and treatment-free interval (TFI) were analyzed with the Fisher exact test. Patients were categorized by age (< median vs ≥ median), histology (serous vs non-serous), regimen (CPT-11 vs PLD) and TFI (0-3 months vs 4-6 months). Factors influencing PFS were estimated by the Kaplan-Meier method and analyzed with the log-rank test. Statistical calculations were performed using the SPSS Statistics software package, version 17.0 for Windows (SPSS, Chicago, IL).

Results

Patients

Median age at time of salvage chemotherapy was 63 years (range: 37-77 years). Clinical stage and histology were as follows: clinical stage (IIIA, 1; IIIB, 1; IIIc, 14; IV, 14); histology (serous, 18; clear cell, 4; endometrioid, 2; mucinous, 2; other, 4). Median TFI after prior chemotherapy was 3.3 months. Recurrent disease was solitary in five cases and multiple in 25. No patient underwent interval debulking surgery or secondary debulking surgery. Fifteen patients received monotherapy with CPT-11 and 15 with PLD. The clinical background in each regimen is shown in Table 1. No significant differences were observed between the CPT-11 group and the PLD group. The median number of prior chemotherapy regimens was 2.1 (range: 1-3) for CPT-11 and 2.1 (range: 1-5) for PLD. The median number of salvage chemotherapy cycles was 3.9 (range: 2-7) for CPT-11 and 4.1 (range: 2-8) for PLD.

Clinical effect of CPT-11 and PLD

The relationships between clinical factors and response rate or non-PD rate with salvage chemotherapy are shown in Table 2. In total, response rate and non-PD rate in all cases were 26.7% (95% CI: 10.9%-42.5%) and 53.3% (95% CI: 35.4%-71.2%), respectively. PFS after salvage chemotherapy was 22.6 weeks (range: 6.0-107.4 weeks). Age, histology, disease site or TFI showed no association with response rate or non-PD rate in any case.

Response rate in the CPT-11 group and PLD group showed no difference at 26.7% (95% CI: 4.3%-49.1%) (p = 0.66) in both, while the non-PD rate was 73.3% (95% CI: 50.9%-95.7%) vs 33.3% (95% CI: 9.5%-57.1%) (p < 0.05), respectively. Non-PD rate was significantly better in the CPT-11 group than in the PLD group. PFS after CPT-11 treatment and PLD treatment was 28.4 weeks (range: 6.4-107.4 weeks) and 16.8 weeks (range: 6.0-34.7 weeks) (p = 0.07), respectively (Figure 1).

Toxicity

A total of 59 cycles of CPT-11 was administered, and the CPT-11 dose on days 8 and 15 was skipped in 19% and 19% of patients, respectively. Forty-four cycles of CPT-11 were administered after the first cycle. In the
CPT-11 group, 12 of 44 (27%) cycles were delayed, and median delay per one cycle was 3.8 days. Forty-six cycles of PLD were administered after the first cycle. In the PLD group, 17 of 46 (37%) cycles were delayed, and median delay per one cycle was 4.4 days.

The adverse events in the CPT-11 and PLD groups are shown in Table 3. No significant differences were observed in hematologic toxicities between the two groups. Hand-foot syndrome (HFS) and mucositis were significantly more common in the PLD group ($p < 0.05$). Although diarrhea and nausea were more common in the CPT-11 group than in the PLD group, the differences were not significant.

### Discussion

Despite a high clinical complete remission rate, EOC patients still exhibit a high rate of recurrence and require chemotherapy. According to guidelines issued by the NCCN, while platinum-based combination therapy should be considered in recurrent cases classified as platinum-sensitive, non-platinum monotherapy is recommended in recurrent cases classified as platinum-resistant/refractory [3]. A number of randomized phase III studies on PLD, TOP, gemcitabine (GEM) and paclitaxel (PTX) for recurrent EOC have been reported [5-8]. Ten Bokkel et al. compared TOP and PTX in 235 recurrent EOC cases and reported that TOP showed a level of efficacy at least equivalent to that of PTX, as manifested by an increased response rate and significantly longer time to progression [8]. Gordon et al. performed a randomized phase III study to compare the effect of TOP and PLD in 474 recurrent EOC cases and concluded that PLD yielded comparable efficacy, a favorable safety profile and convenient dosing, thus supporting its candidacy as a valuable treatment option in recurrent EOC [6]. However, the subset analysis of platinum-refractory/resistant EOC showed a trend in favor of TOP over PLD in terms of PFS ($p = 0.733$), with a median of 13.6 vs 9.1 weeks, respectively, and overall survival (OS) ($p = 0.455$), with a median of 41.3 vs 35.6 weeks, respectively [6]. Mutch et al. performed a randomized phase III trial comparing GEM with PLD in 195 platinum-refractory/resistant recurrent EOC cases and reported that median PFS was 3.6 vs 3.1 months, median OS was 12.7 vs 13.5 months and overall response rate was 6.1% vs 8.3% in the GEM and PLD groups, respectively [7]. From these results, it remains difficult to determine the standard monotherapy regimen for platinum-refractory/resistant recurrent EOC.

Irinotecan is a topoisomerase I inhibitor, and is widely used in platinum-refractory/resistant recurrent EOC in Japan. Matsumoto et al. retrospectively analyzed the effect of CPT-11 in 28 platinum-refractory/resistant recurrent EOC patients and reported that response rate (CR+PR) was 29% and that median time to progression was 17 weeks [4]. These results are believed to be promising.

In this study, we compared the effect of CPT-11 and PLD in platinum-refractory/resistant recurrent EOC, retrospectively. Although the response rate was comparable, both non-PD rate and PFS were better in the CPT-11 group than in the PLD group at 73.3% vs 33.3% ($p < 0.05$) and 28.4 weeks vs 16.8 weeks ($p = 0.07$), respectively. Both CPT-11 and TOP are topoisomerase I inhibitors, and this type of drug may be effective for platinum-refractory/resistant recurrent EOC.
One of the important purposes of salvage chemotherapy is palliation of symptoms and maintenance of quality of life (QOL). Therefore, toxicity is an important consideration in choice of chemotherapy regimen. In this study, no significant difference was observed in hematologic toxicities or most non-hematologic toxicities between the CPT-11 and PLD groups. However, HFS and mucositis were more common in the PLD group. Finally, tolerability was equivalent between the two groups. Assessment of QOL will be an essential factor in choice of drug.

Conclusion

In conclusion, the results indicate that CPT-11 is a promising drug for the treatment of platinum-resistant recurrent EOC. Further randomized phase III studies are required to elucidate the efficacy of CPT-11 in the treatment of platinum-refractory/resistant recurrent EOC in comparison with PLD.

References


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Summary

Objective: To assess the immunohistochemical expression of BerEP4, a new epithelial antigen in ovarian cancer. Methods: We studied 62 cases of ovarian cancer in which BerEP4, CEA and CA-125 were investigated by an immunohistochemical method. We evaluated the correlations among immunohistochemical positivity and the grading, histotype and stage of disease. Results: BerEP4 was positive in 45 out of 62 cases (72.58%), CA-125 in 36 out of 62 cases (58.06%) and CEA in ten out of 62 cases (16.13%). BerEP4 was present both in serous and in mucinous tumors (80.96% vs 80.77%). CA-125 was mainly expressed in serous vs mucinous tumors (66.67% vs 57.69%). CEA was more prevalent in mucinous vs serous tumors. Ber-EP4 was mainly expressed in G1 (75%) and G2 (77.27%), CA-125 was more present in G1 and G3 (both 62.50%) than G2 (50%), whereas CEA showed positivity in G1: 12.50%, G2: 22.73% and G3: 12.50%. There were no differences among the three antigens studied with regard to clinical stage. Conclusions: In our study Ber-EP4 was positive in 45 out of 62 cases (72.58%) of primary epithelial ovarian cancers. The presence of this antigen seemed to be related to the histotype and grading but not to clinical stage.

Key words: Epithelial antigen; BerEP4; Ovarian cancer.

Introduction

Several studies have been performed on the impact of CA-125 both for cancer screening [1-3] and follow-up [4-7] of patients treated for ovarian cancer. In Western and Northern Europe, as well as in the USA, ovarian cancer is the third most frequent cancer of the genital tract with an estimated 191,000 newly diagnosed cases per year worldwide. Because of its insidious onset, the disease is diagnosed in 70% of cases in an advanced stage. Thus ovarian cancer is the fifth leading cause of cancer-related deaths in women [8]. Furthermore the development of an effective technique for detection of early-stage ovarian cancer is an unrealized goal [9].

The clinical application of tumor markers in ovarian cancer is used also for the follow-up of women at risk of familial ovarian cancer [10, 11] as well as the diagnosis on serous peritoneal spilling of malignant potential (immunohistochemistry) [12] or for the differential diagnosis between the secondary gastrointestinal tumors (Krukenberg) and primary ovarian tumors which is done by immunohistochemistry [13].

Immunohistochemical evaluation of some tumor-associated antigens has pointed out some sensitivity than in serologic studies of this tumor, probably because in the first stages of disease, in which immunohistochemistry is often positive, the amount of tumoral cells producing antigens is not enough to determine their intake in peripheral blood in measurable quantities. Many of the antigens are tested with immunohistochemical methods by monoclonal antibodies in ovarian cancer, in order to make a correct histopathologic diagnosis and to discriminate, in dubious cases, a primary or secondary ovarian origin.

BerEP4, an epithelial antigen recently introduced into clinical practice, which consists of two glycoproteins of 34 and 39 KD, respectively, is located both on the surface and in cytoplasm of the epithelia, with the exception of those of squamous and mesothelial origin [14].

To our knowledge, in the literature there are few data on BerEP4 antigens in ovarian cancer and in other gynecological tumors.

The aim of our study was to evaluate immunohistochemical positivity of BerEP4, CEA and CA-125 in ovarian cancer and the correlation of these antigens with grading, histotype and stage of disease.

Material and Methods

We studied 62 cases of malignant ovarian tumors and investigated the presence of BerEP4 and simultaneously of CEA and CA-125 antigens by immunohistochemistry. The study was approved by our local ethical Committee.

The mean age of the women was 57.5 years (range 19-90). The 62 cases were represented by 55 ovarian cancers: 21 serous, 26 mucinous, four clear cell and four endometrioid tumors. Then three mixed mesodermal, two yolk sac tumor, one granul- and one metastatic. Tumor grades were: 1) 24 patients (38.71%); 2) 22 patients (35.48%); and 3) 16 patients (25.81%). With regard to FIGO stage, 15 had IA, four IB, seven IC, 32 III and four IV.

The immunohistochemical study was performed at the Institute of Pathology of the University of Sassari.

The surgical specimens were fixed in 10% neutral buffered formalin and paraffin-embedded to carry out 4 µm sections; they then were stained by hematoxylin-eosin. Some sections were expelled onto glass slides before being treated with 0.1% poly-L-Lysine in order to increase their adhesiveness.

Revised manuscript accepted for publication July 28, 2011

Eur. J. Gynaec. Oncol. - ISSN: 0392-2936
Antigens were studied in neoplastic tissue by immunohistochemistry, using polyclonal antibodies for CEA and monoclonal antibody (Mab) for CA-125 and BerEP4. The immunodetermination was performed using the immunoperoxidase avidin-biotin complex (ABC method). Endogenous peroxidase was inhibited by the Heyderman and Neville procedure. Diaminobenzidine was the chromogen. No immune rabbit serum was used as a negative control.

With regard to the score used to quantify the positivity of the method we marked 1+ in case of weak intensity of staining, 2+ in case of stronger intensity, and 3+ in case of high intensity related to over 50% of the tumoral cells. The 2+ positivity was assumed as the cut-off of the method.

Results

The positivity for the three tested antigens with indication of the score is shown in Table 1.

CEA was positive (at least 2+) in ten out of 62 cases (16.13%) with 34 cases completely negative. CA-125 resulted positive (at least 2+) in 36 out of 62 cases (58.06%) with 14 cases completely negative. Immunohistochemical positivity was observed only in glandular cells, especially on the top, in some cases with a thin granular staining. BerEP4 was positive (at least 2+) in 45 out of 62 cases (72.58%), with six cases completely negative where-as it was positive in 31 out of 45 cases of endometrial carcinoma (68.9%) in our previous study [15].

As with the other two antigens, Ber-EP4 is mainly located on the top of cells and did not have any particular location in the neoplastic tissue. In some cases there was a spread of membranous staining that showed a marked limit between a glandular cell and the one beside.

With regard to the correlation between positivity for the three antigens and histotype and grading (Table 2), Ber-EP4 was slightly present in mucinous tumors in comparison to the serous (21/26 80.77% vs 17/21 80.96%). We observed no positivity for BerEP4 in either metastatic ovarian tumors or yolk sac tumors.

Ber-EP4 was directly proportional to tumor differentiation (75% of positivity in G1 vs 62.50% in G3), whereas CA-125 and CEA showed no revelant difference regarding grading. Also there were no differences among the three antigens studied with regard to clinical stage.

Discussion and Conclusions

Early diagnosis of malignant ovarian tumors represents an important issue for social medicine, especially towards future projection (progress in imaging techniques, availability of specific markers) so that the patient may benefit from primary therapy with adequate staging and optimal debulking.

The risk of malignancy index, which is a simple scoring system based on menopausal status, ultrasound and serum concentration of CA-125, was able to differentiate malignant and benign pelvic masses efficiently to optimize therapy [16].

With regard to new markers studied in the literature, the multitude of antigens and several biological factors tested do not seem to be useful in early biochemical diagnosis, especially when serum levels are determined.

Ber-EP4, a recently introduced epithelial antigen in clinical practice, is not present on mesothelial cells. The data can suggest its use in the immunocytochemical study of cells recovered from the peritoneal cavity.

There are few data regarding the study of BerEP4 in ovarian cancer. Davidson et al. [17] evaluated BerEP4 in association with four antigens (CA-125, CEA, BG8 and B72.3) in 94 samples of fresh pleural, peritoneal and pericardial effusions from patients diagnosed with gynecological malignancies. These authors reported that BerEP4 had a sensitivity in detecting malignant cells (immunocytochemical positivity) in 78% of cases which is only a little lower than that of CA-125 (88%). Furthermore BerEP4 and B72.3 appeared to be the best markers when both sensitivity and specificity were considered, followed by BG8, while CEA and CA-125 had limited roles in the detection of metastases from gynecological tumors owing to the low sensitivity of the former and the low specificity of the latter [17].

Comin et al. [18] suggested Ber-EP4 and estrogen receptor (ER) as the markers with the greatest discriminatory power in differentiating epithelioid mesothelioma of the peritoneum from serous papillary carcinoma of the ovary. Okamoto et al. [19] concluded that combined immunostaining for Ber-EP4 and the anti-calretinin antibody was helpful for the differential diagnosis between mesothelial cells and not only serous type, but also mucinous, endometrioid and clear cell types of ovarian cancer cells in cytologic specimens.

<table>
<thead>
<tr>
<th>Positive (%)</th>
<th>Negative</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ber-EP4</td>
<td>CA-125</td>
<td>CEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/62</td>
<td>11</td>
<td>25</td>
<td>20</td>
<td>45/62 (72.58)</td>
</tr>
<tr>
<td>14/62</td>
<td>12</td>
<td>24</td>
<td>12</td>
<td>36/62 (58.06)</td>
</tr>
<tr>
<td>34/62</td>
<td>18</td>
<td>7</td>
<td>3</td>
<td>10/62 (16.13)</td>
</tr>
</tbody>
</table>

Table 2. — Correlation between positivity of the three tested antigens (Ber-EP4, CA-125 and CEA) and histotype and grading of ovarian cancer.

<table>
<thead>
<tr>
<th>No cases</th>
<th>BerEP4</th>
<th>CA 125</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>24</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>G2</td>
<td>22</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>G3</td>
<td>10</td>
<td>62/50</td>
<td>58</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>45</td>
<td>36</td>
</tr>
</tbody>
</table>

Discussion and Conclusions

Early diagnosis of malignant ovarian tumors represents an important issue for social medicine, especially towards future projection (progress in imaging techniques, availability of specific markers) so that the patient may benefit from primary therapy with adequate staging and optimal debulking.

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In our study Ber-EP4 was positive in 72.58% of primary epithelial ovarian cancers studied, with a prevalent membranous staining but with no characteristic topographic distribution. The presence of the antigen seemed related to the histotype and grading but not to clinical stage.

Further studies on larger series are necessary to have definitive conclusions on the expression of this antigen by more differentiated tumoral tissue.

References

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Outcomes of conservative surgery in early epithelial ovarian carcinoma

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Department of Gynecologic Oncology, Women’s Hospital, School of Medicine, Zhejiang University, Hangzhou (China)

Introduction
Epithelial ovarian carcinoma is the most common histological type in ovarian malignancies. Although 80% of epithelial ovarian carcinomas are found in post-menopausal women, they do occur in women in reproductive age as well. The 25th FIGO Annual Report showed that about 14% of epithelial ovarian carcinomas would occur in women younger than 40 years, and 62% of ovarian cancers diagnosed before 40 years would be at Stage I-IIA [1].

Surgery is the cornerstone of treatment for epithelial ovarian carcinoma. The standard surgical treatment for early-stage epithelial ovarian carcinoma includes hysterectomy with bilateral salpingo-oophorectomy, omentectomy, peritoneal sampling, lymph node sampling, and peritoneal washing for cytology, with or without appendectomy. However radical surgery means loss of fertility in those young patients who wish childbearing. In order to preserve fertility in young patients, an operation to preserve the uterus and contralateral ovary has been proposed for selected epithelial ovarian carcinoma patients. As early as 1969, Munnell [2] reported 190 cases of unilateral epithelial or nonepithelial ovarian cancer treated at his institution. The age range of women was 20 to 77. The 5-year survival was 79% for the patients operated radically and 74% for those conservatively. The reports about conservative surgery for epithelial ovarian carcinoma have accumulated in the past decade, but most were from European and American populations [3-14]. Indications of conservative surgery for epithelial ovarian carcinoma are not still uniform, and clinical outcomes and fertility status following surgery were discrepant as well.

The aim of this study was to investigate clinical outcomes and fertility results of conservative surgery for early epithelial ovarian carcinoma cases to provide evidence of an available management strategy for young patients.

Materials and Methods
Between 1999 and 2007, a total of 17 patients with primary epithelial ovarian carcinoma underwent conservative surgery in the Department of Gynecologic Oncology, Women’s Hospital, School of Medicine, Zhejiang University, China. Histology diagnoses were reconfirmed by an experienced pathologist in the Department of Pathology. Histology was classified by the World Health Organization (WHO, 1999) criteria and tumor grade was defined by the classification of Shimizu et al. (2000) [15]. Tumors were staged according to the Federation of Gynecology and Obstetrics staging system (FIGO, 1988).

Clinicopathological characteristics, including the age of patients, tumor histology and differentiation, clinical stage, surgery, adjuvant chemotherapy, survival status, menstruation and pregnancy after surgery of 17 patients treated with conservative surgery were reviewed and collected. The information was obtained from medical records and telephone interviews. Follow-up for the patients after surgery included physical examination, serum CA125 testing and an ultrasonographic (US) scan every two months during the first year, then every three months for two years, then every six months for two years, and yearly afterward.

Results

Patient characteristics
The mean age of 17 patients at the time of surgical procedure was 23.2 years (range 16-30 years). All of the patients were nulliparous. Seventeen tumor histologic types included 13 (76%) mucinous carcinomas: one (6%) serous, one (6%) endometrioid, and two (12%) mixed...
occurred in young women for whom preservation of fertility postmenopausal women, but has been documented to

Discussion

regimens were paclitaxel (175 mg/m²) and carboplatin
for one patient with Stage IA and grade 1. Chemotherapy
adjuvant platinum-based combined chemotherapy, except
pathological diagnoses were normal.

as borderline tumors. Three patients with a normal
were pathologically confirmed as benign tumors and two
as borderline tumors. Three patients with a normal
underwent tumor resection or cystectomy; of those, three
were pathologically confirmed as benign tumors and two
as borderline tumors. Three patients with a normal
anterior ovarian surgery biopsy and all pathological diagnoses were normal.

Totally 16 patients received 3.8 (range 2-6) courses of
adjuvant platinum-based combined chemotherapy, except
for one patient with Stage IA and grade 1. Chemotherapy
regimens were paclitaxel (175 mg/m²) and carboplatin
(AUC=5) or carboplatin (AUC=5) and cyclophosphamide
(600 mg/m²) (Table 2).

Recurrence and survival

The patients were followed-up for 61 months (17~115).
All 17 patients were alive until the end of follow-up.
Sixteen patients were disease-free during the follow-up
period. One patient with Stage IC, grade 1, and mixed
serous and mucinous carcinoma recurred in the rectouter-
inal pouch and left iliac lymph node 36 months after the
primary surgery. She underwent hysterectomy, appendec-
tomy, and tumor resection. The second debulking surgery
was optimal with no gross residual tumor. The histologi-
cal type was the same as for the first surgery, but tumor
grade was grade 2. She received a further six courses of
paclitaxel and carboplatin chemotherapy and was alive
without any evidence of disease six months after the end
of chemotherapy.

Ovarian endocrine function and fertility results

Of the 16 patients treated by chemotherapy, 15 had
regular menses as before. Only one patient who had
undergone unilateral salpingo-oophorectomy and con-
tralateral ovarian cystectomy complained of temporary
menopause during three courses of chemotherapy. Her
menstruation restarted two months later but the cycle was
longer and flow decreased.

Among 16 disease-free patients, eight attempted preg-
nancy. Of those, five conceived naturally and totally there
were six term babies and one abortion. Another three pa-
tients were infertile, but the reasons were unclear. No con-
genital malformations were found in any of the offspring.

Discussion

In general, epithelial ovarian carcinoma is a disease of
postmenopausal women, but has been documented to
occur in young women for whom preservation of fertility

Table 1. — Clinicopathological characteristics of 17 patients with epithelial ovarian carcinoma who underwent conservative surgery.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age</td>
<td>23.2 (16–30 years)</td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>13 (76%)</td>
</tr>
<tr>
<td>Serous</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>10 (59%)</td>
</tr>
<tr>
<td>IC</td>
<td>6 (35%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>15 (88%)</td>
</tr>
<tr>
<td>G2</td>
<td>2 (12%)</td>
</tr>
</tbody>
</table>

Table 2. — Adjuvant chemotherapy for 16 patients.

<table>
<thead>
<tr>
<th>FIGO stage and tumor grade</th>
<th>No. of patients</th>
<th>Paclitaxel</th>
<th>Carboplatin</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage IA</td>
<td>Grade 1</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Stage IC</td>
<td>Grade 1</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Grade 2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Stage IIIA</td>
<td>Grade 1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

needs to be clinically considered. It is widely accepted
that fertility-sparing surgery can be applied in FIGO
Stage IA and grade 1 tumors. However, some studies
revealed that conservative management was probably
available for those beyond this phase of disease in the
past more than ten years. Zanetta et al. [3] reported 99
young patients with Stage I ovarian carcinomas. Of those,
56 including 24 higher than Stage IA and 22 higher than
grade 1 underwent fertility-sparing surgery and 43
patients with similar stage and grade underwent radical
surgery. With a median follow-up of seven years, five
(9%) women treated conservatively had recurrence and
five (12%) of those treated radically. Similarly, Schilder
et al. [4] reported a total of 52 patients treated by ferti-
ility-sparing surgery, who were composed of 42 Stage IA
and ten Stage IC diseases that included 38 grade 1, nine-
grade 2, and five grade 3 tumors. The estimated survival
was 98% at five years and 93% at ten years. Furthermore,
Raspagliesi et al. [5] reported ten high-risk epithelial
ovarian carcinoma patients who underwent conservative
surgery, including two Stage IA; grade 3; two Stage IC;
and four Stage IIIA; and four Stage IIIC diseases. All patients
were alive and disease-free at a median of 70 months of
follow-up period, suggesting fertility-sparing surgery
may be also available for some women with higher than
Stage 1, grade 2 epithelial ovarian cancer. However, this
opinion is still debated. Morice et al. [6, 7] reported 25
patients treated by conservative surgery, including 19
Stage IA (9 grade 1 and 10 grade 2), one Stage IC, two
mucinous and serous. Ten (59%) patients were FIGO
Stage IA, six (35%) IC, and one (6%) IIIA. Tumor grade
was grade 1 in 15 (88%) and two (12%) were grade 2.
Clinicopathological characteristics of all patients are
summarized in Table 1.

All the patients underwent conservative primary oper-
ations with complete surgical staging, including multiple
washing for cytology, diaphragmatic smearing, unilateral
salpingo-oophorectomy, omentectomy, peritoneal sam-
ping, retroperitoneal lymph node sampling, and append-
dectomy for mucinous or mixed histologic subtype. Five
patients who had contralateral ovarian tumors or cysts
underwent tumor resection or cystectomy; of those, three
were pathologically confirmed as benign tumors and two
as borderline tumors. Three patients with a normal
anterior ovarian surgery biopsy and all pathological diagnoses were normal.

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longer and flow decreased.

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nancy. Of those, five conceived naturally and totally there
were six term babies and one abortion. Another three pa-
tients were infertile, but the reasons were unclear. No con-
genital malformations were found in any of the offspring.
Stage 2, and three unknown stage diseases. At the medium 47 months of follow-up, three Stage IA and all Stage IC or higher patients recurred, and three of them died. Four years later, they conducted a multicenter study on 34 patients with epithelial ovarian carcinoma treated conservatively. Seven patients with Stage IA disease (2 grade 1, 4 grade 2, and 1 grade 3) and four with Stage IC or higher disease experienced recurrence, and four died at a medium of 47 months of follow-up. Thus, their results indicated that conservative surgery should not be considered for patients staged higher than FIGO Stage IA.

In our study, 17 patients with early epithelial ovarian carcinoma, including ten FIGO Stage IA, six Stage IC, and one Stage IIIA, as well as 15 grade 1 and 2 grade 2, underwent conservative surgery. All of 17 patients were alive and 16 were disease-free during a medium of 61 months of follow-up. Only one Stage IC patient recurred at 36 months after primary surgery but still survived till the follow-up end. Ours and other investigations indicate that conservative surgery can be considered for young patients with FIGO Stage I including grade 1 and grade 2 epithelial ovarian cancer who desire further childbearing, but caution is necessary if this surgical procedure is planned for those with higher than Stage I or grade 3 disease.

Complete surgical staging should be performed during conservative surgery to exclude the presence of occult extratubal disease. The incidence of microscopic implants in a gross normal appearing ovary was not consistent among different series. It was estimated there was a 12% risk for microscopic carcinoma on the contralateral normal appearing ovary [2], but only 2.5% (3/118) of occult contralateral ovarian involvement was found in Stage I epithelial ovarian carcinoma in a recent report [16]. We did not find microscopic metastasis by biopsy in three normal appearing ovaries, like a previous study [3]. In addition, since ovary biopsy may induce adhesion and subsequent infertility, this procedure is not advisable for a normal appearing ovary.

It is generally believed that fertility potential is not obviously decreased in women who undergo conservative surgery for ovarian cancer. Zanetta et al. [3] reported 27 pregnancies in 20 patients treated conservatively. Schilder et al. [4] reported that 17 patients conceived and had 26 term deliveries and five spontaneous abortions in 24 patients who attempted pregnancy, and Park et al. [8] reported 22 term pregnancies in 19 women attempting to conceive. However, Morice et al. [6] showed that only four patients had four pregnancies among 18 disease-free patients. In our data, five women conceived spontaneously in eight attempting pregnancy, and no congenital malformations of the babies were found. Our data suggest that conservative surgery is an effective procedure for women with ovarian cancer who desire childbearing.

It remains controversial whether hysterectomy and contralateral salpingo-oophorectomy should be performed after completion of childbearing. Since the remaining ovary contributes to maintenance of endocrine function, radical surgery is no longer performed for young women in many institutions. Based on our and others follow-up data [8], the remaining ovaries after conservative surgery on the whole have long-term survival. It may be acceptable, just as suggested by Colombo et al. [16], that in any case the final choice should be individualized and risk factors of relapse should be taken into consideration.

In conclusion, conservative surgery can be considered for young patients with FIGO Stage I, including grade 1 and grade 2 epithelial ovarian cancers, who desire further childbearing.

References


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Case Reports

Desmoplastic small round cell tumor (DSRCT) arising in the ovary: report of a case diagnosed at an early stage and review of the literature

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Summary

Background: Desmoplastic small round cell tumor (DSRCT) is a rare sarcoma tumor affecting mainly young adult males. It rarely has an ovarian involvement. Case: A 29-year-old woman presented to her gynecologist for amenorrhea. The laboratory results demonstrated a menopausal status and the ultrasound revealed a large mass of the right ovary. The right ovary was completely removed by laparoscopy. Pathology, cytology and immunochemistry revealed a DSRCT. In January 2009 a left salpingo-oophorectomy was performed. After 35 months from diagnosis there was no clinical evidence of disease recurrence. Conclusion: DSRCT is a rare ovarian tumor in adolescence with a general poor outcome. Every ovarian mass regardless of age should be approached with caution.

Key words: Desmoplastic small round cell tumor; Ovarian neoplasm; Early stage tumor; Treatment; Good response.

Introduction

Desmoplastic small round cell tumor (DSRCT) is a rare and aggressive malignant soft tissue sarcoma that mainly occurs in young males, associated with an extremely poor prognosis even after demolitive surgery and aggressive chemotherapy. The male to female ratio is 4:1. We present a case of a young woman that initially presented an ovarian mass and was thought to have ovarian cancer before pathology, cytology and immunochemistry confirmed the diagnosis of DSRCT.

Case Report

A 29-year-old woman, who had used contraceptive pills for 14 years because of irregular cycles, menstruated spontaneously after she stopped taking them. After a short period of resumption of oral contraceptives, she interrupted the assumption of the pill because of an expressed desire for a pregnancy and presented amenorrhea. At her gynecologist visit the ultrasonographies revealed a large mass on the right ovary and the laboratory results showed menopausal status. The patient underwent a laparoscopic right ovariectomy. Also several biopsies were taken intraoperatively from the posterior wall of the uterus, bowel wall, peritoneum and omentum that were not found to be involved with the tumor. Anatomical pathology confirmed a right ovarian mass 8.5 cm in size and weighing 132 g. Microscopic examination demonstrated a tumor rich in necrotic cells composed by small cells with poorly defined borders which were arranged in large fields and nests with the tendency to form trabecule and surrounded by a few blood vessel stroma.

The tumor cells had small and medium-sized round nuclei with dotted chromatin. Nucleoli were small or unclear. The cytoplasm was poorly eosinophilic. In one of the pieces examined there was connective tissue, probably of the ovary, with a dense chronic inflammatory infiltrate. Histologically, PAS-D staining revealed absence of mucus and glycogen. Immunohistochemical findings showed that the tumor cells were negative for PLAP, beta-hCG and AFP, which excluded a germ cell tumor like an embryonal carcinoma or a dysgerminoma. The lymphocytes were CD45+. A part of the tumor cells were strongly positive for epithelial membrane antigen (EMA) and immunoreactive for pancytokeratin. The neuro-endocrine differentiation was revealed by immunostaining for synaptophysin and chromogranin. CD99 was highly expressed in most of the tumor cells, but there was no immunoreactivity for vimentin, inhibin, GFAP or CD117. The possible options then were a small cell carcinoma of the ovary (with a preference for the hypercalcemic type), a primitive neuroectodermal tumor (PNET) and a DSRCT. The CD99 immunoreactivity excluded, but not completely, a diagnosis of PNET which became even more definitive after the immunostaining against CD56 and desmin showed reactivity; S100 and TTF1 were negative. The final diagnosis confirmed a multiphenotypic small cell tumor with differentiated epithelial, neuroendocrine and muscular cells, morphologically and immunohistochemically comparable with DSRCT (Table 1). However, the tumor did not have a full typical desmoplastic aspect. Indeed, while on one side, the WT1 staining (Dako, with antibodies against the N-terminal of the WT1 protein) showed a strong dot-like cytoplasmatic positivity in around 50-60% of the tumor cells (which offered an additional argument for the diagnosis of DSRCT [1]), on the other side, the diagnosis of DSRCT was not cytogenetically supported by the FISH, because a few days later it did not show any EWS gene rearrangement.

After the operation performed in July 2008, the first of four
cycles of adjuvant chemotherapy with VIDE (vincristine, ifosfamide, doxorubicin, etoposide) was given. The first abdominal/pelvic CT did not reveal any disease activity. The patient underwent chemotherapy, but the somministration of the last cycle was delayed for two weeks through neutropenia. The patient recovered in the hospital and the doses was reduced of 25%. A month later another abdominal/pelvic CT was performed and also this time it did not show any disease activity. The patient was still alive 35 months from diagnosis of DSRCT and pelvis debulking + P6 chemotherapy.

Discussion

The first DSRCT case was reported in 1987 as an undifferentiated malignant epithelial tumor involving serosal surfaces of the scrotum and abdomen in a young male [2]. This tumor, in fact, mostly affects adolescents and young males in nearly 80% of cases and the male to female ratio is 4:1. There is no known organ or area of origin but it mostly occurs in the abdomen, although thoracic and paratesticular primary sites have been reported in the literature [3, 4]. Since then, not many other cases have been described and ovarian involvement by DSRCT has been even more rarely described [5-12]. Indeed, before our case, only 13 DSRCT cases with ovarian involvement have been reported in the literature. In the world literature, bilateral involvement of the ovaries is described in 50% (7 of 14) of cases (Table 2). In our case, there was only involvement of the right ovary. Unilateral primary involvement of the left ovary has been described only by Fang et al. [10]. Because the disease can be misdiagnosed or remain undetected, tumors frequently grow large within the abdomen and metastasize to other parts of the body – also through the lymph nodes or blood stream. Sites of metastasis include the diaphragm, spleen, liver, large and small intestine, abdominal cavity, lungs, bones, uterus, bladder, genitals, central nervous system and the brain. DSRCT with primary ovarian involvement is seen in young women with age ranging from 11 to 29 years old. Herein we have reported a case of DSRCT involving primarily the right ovary in a 29-year-old woman.

Tumor markers

Based only on cytological and histological examination, it is very difficult to diagnose DSRCT; a large numer-

Table 1. — Immunohistochemistry of the tumor in our case.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAP</td>
<td>Negative</td>
</tr>
<tr>
<td>β-HCG</td>
<td>Negative</td>
</tr>
<tr>
<td>AFP</td>
<td>Negative</td>
</tr>
<tr>
<td>CD45</td>
<td>Positive</td>
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<tr>
<td>EMA</td>
<td>Focal positive</td>
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<tr>
<td>Pancytokeratin</td>
<td>Focal positive</td>
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<tr>
<td>Synaptophysin</td>
<td>Positive</td>
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<tr>
<td>Chromogranin</td>
<td>Positive</td>
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<tr>
<td>CD99</td>
<td>Diffuse positive</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Negative</td>
</tr>
<tr>
<td>GFAP</td>
<td>Negative</td>
</tr>
<tr>
<td>Inhibin</td>
<td>Negative</td>
</tr>
<tr>
<td>CD117</td>
<td>Negative</td>
</tr>
<tr>
<td>Desmin</td>
<td>Positive</td>
</tr>
<tr>
<td>CD56</td>
<td>Positive</td>
</tr>
<tr>
<td>S100</td>
<td>Negative</td>
</tr>
<tr>
<td>TTF1</td>
<td>Negative</td>
</tr>
<tr>
<td>WT1-1</td>
<td>Positive 50-60%</td>
</tr>
<tr>
<td>EWS</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table 2. — DSRCTs with ovarian involvement.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age Side</th>
<th>Tumor location</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young et al.</td>
<td>15 both</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>DOD at 18 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and pelvic</td>
<td>multiagent chemotherapy</td>
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</tr>
<tr>
<td>Young et al.</td>
<td>15 both</td>
<td>Intraabdominal</td>
<td>Hysterectomy</td>
<td>No follow-up</td>
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<tr>
<td></td>
<td></td>
<td>and pelvic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young et al.</td>
<td>14 right</td>
<td>Pelvic and</td>
<td>Surgical debulking</td>
<td>No follow-up</td>
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<td></td>
<td></td>
<td>omentum</td>
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</tr>
<tr>
<td>Zaloudek et al.</td>
<td>22 both</td>
<td>Pelvic</td>
<td>Surgical debulking +</td>
<td>DOD at 18 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>multiagent chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Slomovitz et al.</td>
<td>11 right</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>DOD at 11 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and pelvic</td>
<td>multiagent chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Parker et al.</td>
<td>23 right</td>
<td>Pelvis</td>
<td>Surgical debulking +</td>
<td>PD. No further</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pacl/Cis x 4 cy</td>
<td>treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Surgical debulking +</td>
<td>SD, PD after</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Etop/Cis + Emtx</td>
<td>3 mo,</td>
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<td>unresponsive</td>
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<td>to further</td>
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<td></td>
<td></td>
<td></td>
<td>chemotherapy.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DOD at 20 mo</td>
</tr>
<tr>
<td>Fang et al.</td>
<td>13 left</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>Alive NED at</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and Pelvis</td>
<td>multiagent chemotherapy</td>
<td>7 mo after</td>
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<td></td>
<td></td>
<td>multiagent chemotherapy</td>
<td>therapy</td>
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<td></td>
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<td>radiotherapy</td>
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</tr>
<tr>
<td>Fang et al.</td>
<td>23 both</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>PR. Follow-up</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and pelvic</td>
<td>multiagent chemotherapy</td>
<td>ongoing</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>multiagent chemotherapy</td>
<td></td>
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</tr>
<tr>
<td>Engohan-Aloghe et al.</td>
<td>21 right</td>
<td>Pelvis</td>
<td>Surgical debulking +</td>
<td>PR. Follow-up</td>
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<td></td>
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<td></td>
<td>multiagent chemotherapy</td>
<td>ongoing</td>
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<tr>
<td>Sang H. Lee et al.</td>
<td>16 both</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>PR, then PD.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and pelvic</td>
<td>VACIE</td>
<td>AWD at 28 mo</td>
</tr>
<tr>
<td>Ota et al.</td>
<td>26 both</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>DOD at 23 mo</td>
</tr>
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<td></td>
<td></td>
<td>and pelvic</td>
<td>P6 protocol</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>chemotherapy + radiotherapy</td>
<td></td>
</tr>
<tr>
<td>Ota et al.</td>
<td>19 both</td>
<td>Intraabdominal</td>
<td>Surgical debulking +</td>
<td>DOD at 11 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and pelvic</td>
<td>BDP</td>
<td></td>
</tr>
<tr>
<td>Our case</td>
<td>29 right</td>
<td>Pelvis</td>
<td>Surgical debulking +</td>
<td>NED at 22 mo</td>
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<td>VIDE x 4 cy</td>
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</tbody>
</table>

NED: no evidence of disease; DOD: dead of disease; AWD: alive with disease; PD: progressive disease; PR: partial response; SD: stable disease.
Embr. & alv. Rhabd. – + – Neuroblastoma +
Germ cell tumor +
Wilm's tumor + –

Lymphoma – – – – + ±

Small cell carcinoma + + – + +

Ewing sarcoma – + – – + +

Equilibrative nucleoside transporter 4 (ENT4) is a protein that in humans is encoded by the SLC29A4 gene and which catalyzes the reuptake of monoamines into presynaptic neurons, thus determining the intensity and duration of monoamine neural signaling [14]. CA-125 levels have not been well investigated in patients with DSRCT. Increased levels of CA-125 have been reported in a few cases of DSRCT [7, 9, 10, 12, 15, 16]. Ordóñez and Sahin [16] studied the trend of CA-125 serum level in a 34-year-old patient with DSRCT and found a decreased level after she had received chemotherapy after which her tumor was removed, but it became elevated again when the disease recurred. Hence, CA-125 is more a tumor marker for epithelial ovarian cancer, but it could be used as a marker of persistent and recurrent disease. Moreover an elevation of its levels should not mislead the clinician into diagnosing a primary ovarian cancer, but rather should lead the clinician to consider DSRCT, at least, in the differential diagnosis.

Clinical diagnosis

As with the majority of women with ovarian cancer, even the patients with DSRCT show symptoms much later and they are vague and non-specific. The patients present symptoms like abdominal bloating, abdominal pain, symptoms related to obstruction of the viscus and ascites, and also unspecific symptoms like nausea, vomiting and weight loss. In a case described by Fang et al. [10], a patient presented with symptoms of acute appendicitis with high fever, which had never been described before. The most important clinical signs are the presence of an ovarian mass, especially if it is irregular, fixed and bilateral, the presence of multiple nodules in the Douglas pouch, abdominal distension due to ascites or caused by partial occlusion of intestines or pelvic spread of the disease. Usually patients with an ovarian mass also present amenorrhea. In young patients, an ovarian mass is usually a functional cyst due to anovulation that will resolve spontaneously within several days to two weeks. Ovarian masses due to hemorrhagic corpus luteum can be up to 10 cm and the rupture can cause acute abdomen. However also ovulating patients can present, although rarely, with a functional ovarian cyst which can cause abdominal or pelvic pain. An accurate examination of the abdomen can be helpful, especially in case of peritoneal spread of the cancer, because of an occasionally painful lump known as a “Sister Mary Joseph nodule”, a peculiar sign which can be detected in the umbilicus secondary to...
Desmoplastic small round cell tumor (DSRCT) arising in the ovary: report of a case diagnosed at an early stage and review etc.

metastatic cancer in this location [17, 18]. After clinical examination, it is important to perform US and tumor marker investigations. In addition to the standard markers like CA-125, CA19-9 and SCC (squamous cell carcinoma antigen), it is very important to determine also markers like AFP, hCG and LDH, especially in patients under 20 years of age who present with an abdominal or pelvic mass in order to exclude a germ cell tumor. Whether in case of positivity or in case of negativity of those markers, surgery remains the initial approach to DSRCT treatment, also because a definitive diagnosis is only made through it. A useful tool to lead the clinician to suspect a malignancy could be the risk of malignancy index (RMI). The formula of the index is U x M x CA-125 and comprises the ultrasound score (U) (from 1 to 5 points depending on the presence of multilocular cysts, presence of a solid mass, evidence of metastases, presence of ascites and bilateral involvement), the level of a menopausal status (M) (3 is the maximum score and it is given to women in menopause), CA-125 is considered in its absolute value. Depending on the RMI value we can have:

<table>
<thead>
<tr>
<th>RMI</th>
<th>Tumor risk</th>
</tr>
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<tbody>
<tr>
<td>&lt; 25%</td>
<td>&lt; 3%</td>
</tr>
<tr>
<td>25-250</td>
<td>20%</td>
</tr>
<tr>
<td>&gt; 250</td>
<td>75%</td>
</tr>
</tbody>
</table>

Preoperative investigations can include non invasive examinations like CT, MRI, Intravenous pyelogram (IVP), chest X-ray, double contrast barium enema (DCBE) and invasive examinations like lymphography, peritoneal cytology and laparoscopy. However, in the end, the diagnosis can only be made by tissue examination (tissue is the issue).

Treatment

The tumor – in both men and women – is associated with an extremely poor prognosis, even after aggressive surgery and chemotherapeutical intervention. Schwarz et al. [19], after a retrospective survey of 32 patients who underwent aggressive surgical debulking, chemotherapy, and radiotherapy, reported an overall progression-free five-year survival rate of 18%. Median survival is reported to be less than 30 months [20]. In 2002, Elhajj et al. described the first case of a patient with DSRCT with ovarian involvement treated with aggressive surgical debulking and multiagent chemotherapy (etoposide, cisplatinum and cyclophosphamide) who survived for 42 months after diagnosis [8]. In 2006, Church et al. reported two cases of women with intrabdominal and pelvic tumor localization, both treated with chemotherapy: in the first case the patient died at the 27th month after she had resolution of symptoms for a year and progressive disease unresponsive to further chemotherapy; the other patient showed a complete response after four cycles and recurrence six weeks after treatment. She died within 12 months from the diagnosis [19]. There is only one case described by De Lena et al. in 1998 in which the patient survived with no evidence of disease 15 months after surgical debulking and three alternating chemotherapy regimens [21]. Because of the rarity of this disease, a gold standard therapy is not yet established. Many authors have reported some patients respond to high-dose (P6 protocol) chemotherapy, maintenance chemotherapy, debulking surgery, cytoreductive surgery, and radiation therapy. Kushner et al. treated 12 patients with P6 protocol chemotherapy, including four courses of cyclophosphamide, doxorubicin, and vincristine alternating with three cycles of ifosfamide and etoposide, but also in this case, the overall progression-free five-year survival was only 18%. Most patients relapsed and died soon after diagnosis [22]. Other treatment options include: hematopoietic stem cell transplantation, intensity-modulated radiation therapy, radiofrequency ablation, stereotactic body radiation therapy, intraperitoneal hyperthermic chemotherapy, and clinical trials. Prognosis depends on stage of the cancer, but nonetheless it remains really poor. Despite aggressive therapy, three-year overall survival has been estimated at 44% and the five-year survival rate remains around 15% [3, 23, 24].

Conclusion

DSRCT is a very rare tumor and the occurrence in women is even more rare, but a good gynecologic oncologist should be familiar with it. The recent world literature showed an increase in diagnosis of this tumor which means that in reality it occurs more than it seems. It should be considered in the differential diagnosis of ovarian cancer. Our experience indeed proves that a patient affected by this neoplasm, if initially diagnosed as having it and if treated with aggressive cytoreductive surgery in combination with aggressive adjuvant chemotherapy, could have a favorable outcome like our patient who did not show any clinical evidence of disease recurrence at the last follow-up.

References


Peritoneal mesotheliomas mimicking adnexal tumors. Clinicopathological characteristics of four cases and a short literature review

Pathology Laboratory, 2nd Department of Surgery, Aretaieion University Hospital, Athens (Greece)

Summary

Three cases of peritoneal benign cystic mesotheliomas in women 32-34 years of age and one case of peritoneal malignant mesothelioma in a 47-year-old woman are reported. All cases presented with abdominal discomfort and/or pain and the physical and radiological diagnostic methods showed adnexal tumors. The cystic mesotheliomas developed in the cul-de-sac and the right pelvic sidewall, presented as multiple small cysts or large multicellular cystic mass. The malignant mesothelioma showed extensive infiltration of the omentum the intestinal loops and the surface of the uterus and adnexa, with bilateral hydrosalpinx and ascites. All cases presented histological and immunohistochemical characteristics consistent with tumors of mesothelial origin. No history of asbestos exposure was reported. The correct diagnostic and therapeutic approaches to these neoplasms are discussed.

Key words: Mesothelioma; Peritoneum; Immunohistochemistry.

Introduction

Peritoneal mesothelioma is a rare disease, with an incidence approximately one per million, and accounts for about 20% to 30% of all cases of mesothelioma [1]. Benign cystic mesotheliomas generally affect women of reproductive age and have a benign biological course, but local recurrence is possible [2]. On the other hand malignant mesothelioma is a diffuse tumor arising most commonly in the pleura (> 90%), secondly in the peritoneum (6-10%), but also in other serosal surfaces. Asbestos is believed to be the main causative agent, but also other factors such as radiation, peritonitis and SV40 have all been implicated [3, 4]. We present our institution experience over the last 15 years, where three cases of benign cystic mesotheliomas and one case of malignant mesothelioma in female patients, all of which affected the peritoneal cavity and mimicked ovarian tumors, were documented.

The aim of this study is to highlight the special features and immunohistochemical characteristics that lead to the correct diagnosis and treatment.

Case Reports

Case 1

A 36-year-old female was operated laparoscopically due to acute abdomen related to a multicystic lesion of the right adnexa. During surgery a small amount of ascitic fluid was found and multiple cysts measuring 0.5-2 cm were observed, loosely attached to the cul-de-sac wall. The cysts were filled with serous fluid. Conversion to an open approach was decided and the cystic lesions were resected en bloc. The ovaries, uterus and appendix were normal and the patient had no asbestos exposure history. She was discharged on the fourth postoperative day. She was followed for five years and was free of disease clinically and by transabdominal sonography (TAS) yearly.

Case 2

A 33-year-old female was admitted to our hospital suffering abdominal discomfort for the last three months. Physical examination revealed a palpable mass on her left lower abdominal quadrant which was smooth and soft. Computed tomography scanning (CT scan) of the abdomen and transvaginal ultrasound (TVS) revealed a large cystic mass probably arising from the left adnexa. During exploratory laparotomy a 19 cm cyst with a thin, semi-translucent wall filled with serous fluid was revealed, loosely attached to the peritoneal surface of the pelvic sidewall. Frozen section biopsy of the tumor was negative for malignancy. The uterus and adnexa were normal. The patient had no history of asbestos exposure. She underwent an uneventful postoperative period and she was discharged on the fifth postoperative day. Seven years later she remains disease free.

Case 3

A 32-year-old woman with a cystic mass probably arising from the right adnexa, found incidentally by TAS and TVS, and no other findings from the abdominal CT scan underwent exploratory laparotomy. Many small cysts 0.5-3 cm with thin translucent walls attached to the right pelvic sidewall were revealed and were carefully resected en bloc. Once again the patient had no history of asbestos exposure. She had a urinary tract infection postoperatively and received antibiotics so she was discharged on the seventh postoperative day. In her follow-up visits every year for three years she had no complaints and TAS showed no findings consistent with disease recurrence.
Case 4

A 47-year-old woman was admitted to our hospital due to severe hypogastric pain and fluctuating fever and she was already under per os antibiotics for four days without any improvement of the symptoms. Physical examination showed diffuse abdominal tenderness and gynecological examination revealed pain on palpation of both adnexa. Laboratory exams showed mild anemia (Hb 10.5 g/dl). TAS and TVS revealed bilateral adnexal masses and the diagnosis of pelvic inflammatory disease was made. She was treated with intravenous antibiotics and she was discharged five days later after great clinical improvement, continuing orally administrated antibiotic therapy.

Three months later the patient was re-admitted suffering from severe hypogastric pain once again. An abdominal CT scan was performed, which demonstrated findings consistent with bilateral hydrosalpinx and a mild ascitic fluid collection with no further findings. Tumor markers were within normal range. Cytological examination of the ascitic fluid proved the existence of malignant cells. Exploratory laparotomy was decided and numerous neoplasmatic foci were found among the intestinal loops, which were adherent, while the omentum was infiltrated by a large number of brownish-white nodules. The pelvic cavity was full of neoplasmatic masses of brownish color, soft and friable in consistency, covering the outer surface of the uterus and adnexa. Total hysterectomy and bilateral oophorectomy as well as epiploectomy were performed. She underwent an uneventful postoperative period and she was discharged on the fifth postoperative day.

Histopathological examination

All specimens were fixed in buffered formalin. A special histochecmical study was undertaken to exam the presence of proteoglycans and epithelial mucous (PAS and mucicarmine stains). Immunostains were performed to investigate the presence of high and low molecular weight keratins (MoAbAE), CA-125, CEA (Monosan), EMA, (Monosan) factor VIII, vimentin, secretory component (Dako monoclonal antibodies) and calretinin (Invitrogen), with appropriate positive and negative controls.

Histological examination showed that the walls of the cysts in the first three cases were formed of loose connective tissue. The interior surface was smooth and covered by cuboidal or flattened mesothelial cells (negative to mucin stain and negative to immunostains for factor VIII, CA-125, vimentin, CEA and secretory component. There was a positive reaction only to keratin, both high and low molecular weight (Figure 1). The diagnosis was benign cystic mesothelioma.

Fourth case: Histopathological examination revealed a malignant mesothelioma. Microscopy showed the development of a malignant neoplasm with a papillary and partially solid arrangement of the neoplastic cells. The cells were relatively small, uniform, cuboid and showed moderate nuclear atypia with moderate mitotic activity. Focal necrosis and hemorrhagic infiltration of the tumor but no psammomma bodies were noted in the multiple histological sections examined. Small papillary neoplasmatic nodules were growing on the serosal surface of the tubes and the uterine corpus and minute papillary nodules were observed on the ovarian surface. The resected omentum was also extensively invaded from the neoplasm.

The differential diagnosis considered an extra-ovarian peritoneal serous carcinoma and malignant mesotheliomas. Immunostains for high and low molecular weight cytokeratins (moAb-Immunon) were highly positive in all cells and also to normal mesothelial cells (Figure 2). Staining for vimentin (V-9), moAb (Immunon, CEA moAb (Monosan), CA-125 (moAb-CIS Diagnostics), factor VIII, secretory component (poly-DAKO) and WF1 (Immunon) were negative in neoplastic cells.

The total morphology of this neoplasm in combination with the immunohistochemical study was consistent with a malignant mesothelioma. Postoperatively the last patient received two cycles of chemotherapy but she refused the rest of the treatment protocol and was lost to follow-up after a 7-month period.

Discussion

Mesotheliomas are mesenchymal neoplasms originating from the serous lining of the pleural, pericardial or peritoneal space. Benign cystic mesotheliomas (BCMs) are well described tumors of unknown etiology. These lesions tend to occur in young women of reproductive age, as in our cases, and the most common predisposing factors are pre-
vious surgery, pelvic inflammatory disease, or endometriosis. These conditions interfere with peritoneal re-absorption, so origin via reactive and inflammatory response is proposed rather than neoplastic. The ovary and uterus are the most common sites affected, and rarely do BCMs arise in the pleural cavity [5]. The presence of epithelial hyperplasia and/or atypia may create problems in the differential diagnosis from malignant mesothelioma (MM).

MM is a rare malignancy with increasing incidence worldwide [6]. It mostly affects adults over 40 years of age. In contrast to BCM, these lesions mainly affect the pleura and only 20% are noted in the peritoneum. MM has been proven to be related to chronic exposure to asbestos, as well as has been reported following radiation therapy, mica exposure, recurrent peritonitis and administration of thorium dioxide [7, 8]. The mechanism through which asbestos offends the peritoneum is unknown and usually the time interval between exposure and diagnosis of the disease is quite long, estimated approximately in 25-40 years [9]. Three major histological subtypes of MM are recognized: epithelial, fibrous (sarcomatous) and mixed. The first type predominates among series and is characterized by papillary and tubular patterns of growth. The tumor cells are usually cuboidal or polygonal, with moderate cytoplasm and central nucleus. Fibrous mesotheliomas have a sarcomatous appearance and in most cases are made up of malignant appearing spindle cells that grow in fascicles within a relatively scant amount of fibrous stroma. The mixed type, which is easily recognized, is identified under the term biphasic MM and consists of a mixture of epithelial and sarcomatous components.

The clinical symptoms and signs of mesotheliomas are not specific to the disease, and the level of clinical suspicion is relatively low due to the rarity of the condition. Diffuse abdominal or pelvic pain, an incidental clinically or radiologically demonstrated mass lesion and/or increased abdominal girth due to ascites, are the most common initial findings [10]. Also MM can produce symptoms of partial or complete bowel obstruction, like nausea, vomiting, malaise and abdominal distention.

Diagnostic modalities include US and CT, but preoperative diagnosis is often not conclusive and there are no protocols for diagnostic imaging. Prompt diagnosis is often established postoperatively, after extensive histopathological examination. Routine laboratory tests have not proven useful and cytology after needle aspiration of the ascitic fluid might be positive for malignant cells in MM, but with no further information and high rate of false-positive and false-negative results [11]. Among tumor markers CA-125 could be elevated and new tumor markers are being investigated like mesothelin, soluble mesothelin related proteins (SMRP) and osteopontin markers are being investigated like mesothelin, soluble tumor markers CA-125 could be elevated and new tumor markers are being investigated like mesothelin, soluble mesothelin related proteins (SMRP) and osteopontin.

The invasive techniques available for final diagnosis are laparoscopy and exploratory laparotomy with an adequate tumor biopsy.

BCMs are managed with complete surgical resection either laparoscopically or in an open fashion. The laparoscopic approach has been described, but due to the danger of rupture and seeding of a thin walled cyst without confirmed preoperative diagnosis, open surgery seems the safer approach. Aggressive surgical approaches including cytoreductive surgery with peritonectomy are recommended. Anti-estrogens and gonadotrophin-releasing analogues, sclerosing therapy with tetracycline and lately hyperthermic peritoneal perfusion with intra-peritoneal chemotherapy have also been attempted in individual cases with varied degrees of success [13]. Because these tumors tend to recur, follow-up management of these patients is difficult but necessary and includes physical examination and abdominal US or CT scan, without any established guidelines.

On the other hand, treatment of MM requires a multidisciplinary approach combining surgery, chemotherapy and irradiation. Diffuse MM of the peritoneum remains in the peritoneal cavity throughout its clinical course and morbidity and mortality arises from complications of disease progression inside the abdomen and pelvis. Combined locoregional treatment consisting of aggressive cytoreductive surgery and intraperitoneal chemotherapy has strong rationale. Hyperthermic intraperitoneal chemotherapy (HIPEC) has shown promising results and immunotherapy using the target mesothelin and other agents is under investigation [14]. Radiotherapy is added to the management plan after HIPEC and systemic chemotherapy in the form of whole abdominal irradiation with limited success [15]. Prognosis of MM is severe, with a median reported survival between six to nine months [16].

**Conclusion**

Peritoneal mesotheliomas are rare clinical entities with no specific symptoms or laboratory and imaging characteristics. A high index of clinical suspicion and lastly surgical exploration and histopathological examination set the correct diagnosis in order to provide the most appropriate management. A multidisciplinary approach is promising when dealing with MM, which makes many physicians have a nihilistic attitude towards the disease.

**References**


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Perivascular epithelioid cell tumor (PEComa) of gynecologic origin: a clinicopathological study of three cases

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Summary

Perivascular epithelioid cell tumors (PEComas), occasionally associated with the tuberous sclerosis complex, are characterized by varying amounts of spindle and epithelioid cells with clear to eosinophilic cytoplasm that display immunoreactivity for melanocytic markers, most frequently HMB-45. Perivascular epithelioid cell tumor of gynecologic origin is very rare, and there have been only a few reported cases. This study describes the clinical, histological, and immunohistochemical features and prognoses of three cases of gynecologic origin. Two of the three tumors were confined to the uterus and one to the vagina. None of the patients had tuberous sclerosis complex. Immunohistochemistry indicated that all three cases expressed at least one melanocytic marker, and HMB45 was a positive marker for all of them. These markers can be found in both epithelial cells and spindle cells. Except for MiTF, which was located in the nucleus, all the other antibodies were located in the cytoplasm. The three cases have been followed up for 26, 22, and three months, respectively, with disease-free survival in all cases. We conclude that PEComas of gynecologic origin have morphological and immunohistochemical features of the PEComa family, which are rare and should be included in the differential diagnosis with other tumors. Until more cases of this rare tumor are evaluated with longer follow-up, firm criteria for malignancy remain uncertain.

Key words: PEComa; Perivascular epithelioid cell tumors; Gynecologic origin.

Introduction

PEComas were defined by the World Health Organization (WHO) in 2002 as “mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells.” The concept of a family of neoplasms derived from these distinctive cells was first advanced by Bonetti et al. They noted the presence in both angiomylolipoma (AML) and clear cell “sugar” tumor of the lung (CCST) of “an unusual cell type … immunoreactive with melanocytic markers, and exhibiting an epithelioid appearance, a clear-acidophilic cytoplasm, and a perivascular distribution” [1].

The PEComa family of tumors has subsequently grown to include AML, CCST, lymphangioleiomyomatosis (LAM), and a number of unusual visceral, intraabdominal, soft tissue, and bone tumors, which have been described under a variety of names, including clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres, abdominopelvic sarcoma of perivascular epithelioid cells, and primary extrapulmonary sugar tumor, among others [2].

In the 2003 WHO classification of gynecologic neoplasms, only PEComas of the uterine corpus were recognized. However, PEComas have also been described in the cervix [3], vagina [4], pelvis [5], broad ligament [6] and ovary [7]. They are also known to show an overwhelming female preponderance [8, 9]. The uterus seems to be one of the two most frequently reported anatomic sites of origin for PEComas [10]. To better understand the pathological features, immunophenotype, clinical behavior, and prognosis of these rare tumors, we studied three PEComas of gynecologic origin that had been defined as PEComas by immunohistochemistry in the Department of Gynecology and Obstetrics of our hospital since 1998.

Methods

Cases and inclusion criteria

Cases that had been previously coded as “perivascular epithelioid cell neoplasm” since 1998 in the Department of Gynecology and Obstetrics of our hospital were retrieved from our consultation archives. Three cases of gynecologic origin were retrieved for this study. Two of the three tumors were confined to the uterus and one to the vagina. The patients were 50, 44, and 33 years old, and none had tuberous sclerosis complex.

Clinical data collection and follow-up

All cases were studied with respect to general state of health with special attention to features predictive of clinical behavior and whether they had tuberous sclerosis complex. All were followed to better understand the prognosis of these rare tumors.

Pathological features

All cases were studied with respect to tumor site and size, growth pattern (circumscribed or infiltrative), cellularity and nuclear grade, mitotic figures/50 high power fields (HPF), atypical mitotic figures, coagulative tumor cell necrosis, vascular invasion, and epithelioid or spindled morphology.

Immunohistochemical studies

Immunohistochemical studies for pan-cytokeratin (AE1/AE3, 1:100, Dako), S-100 protein (polyclonal, 1:2000, Dako), [alpha]-smooth muscle actin (1A4, 1:100, Dako), desmin (DE-R-11, 1:50, Dako), vimentin (Vim3B4, 1:200, Dako), melanoma

microphthalmia transcription factor (D5, 1:50, Dako), c-kit (CD117, 1:100, Dako), and CD34 (QBEND10, 1:200, Dako) were performed. Cases were scored as negative, 1+ (5-25% positive cells), 2+ (26-50% positive cells), and 3+ (> 51% positive cells). Appropriate negative controls were used.

Results

Pathomorphology features

In the present study, it was shown that the cells of perivascular epithelioid cell tumor of the uterus were epithelioid or spindle cells with light eosinophilic or translucent cytoplasm. Mitotic figures were rare and necrosis was not observed. Thin- or thick-walled blood vessels were readily visible around the smooth muscle with unclear boundaries. The remaining small nodules were formed by smooth muscle cells and were proliferating without atypia or invasive growth. The cells of the perivascular epithelioid cell tumor in the vagina were clear cells with transparent cytoplasm and small round nuclei. Mitotic figures were rare, and tumor cells had an adenoid or acinar-like structure. These pathological features were similar to those of vascular epithelial cells described in the past.

Immunohistochemical findings

In this study, the tumors were positive for HMB45(3/3), SMA(2/3), HHF35(2/3), Vim(+) (1/3), and MITF (2/3). They were uniformly negative for Melan-A, CD10 (-), and S100 (-). All of the three cases expressed at least one kind of melanocytic marker, and HMB45 was a positive marker for all. These markers can be found in both epithelial cells and spindle cells. Except for MITF, which was positively positioned in the nucleus, all other antibodies were located in the cytoplasm.

Prognosis

In the present study, the three cases were followed for 26, 22, and three months, respectively, and disease-free survival was observed in all cases. None had tuberous sclerosis complex.

Discussion

Bonetti et al. first proposed a cellular link among these unusual mesenchymal lesions and lymphangiochomatosis (LAM) [11], following reports of HMB-45 immunoreactivity and the presence of premelanosomes in both clear cell “sugar” tumor (CCST) of the lung and the epithelioid clear cell component of angiomylipoma (AML) of the kidney and liver [12-15]. They subsequently suggested the descriptive term “perivascular epithelioid cell” (PEC) for the distinctive cell type found in these three lesions, and hypothesized that the so-called PEC may originate from the walls of blood vessels, based on the observation that these cells are frequently intimately related to such structures [1]. Folpe et al. presented their experience with 26 PEComas of soft tissue and the gynecologic tract, the largest reported series to date [2]. Their study indicated that there was a marked female predominance. They concluded that PEComas of soft tissue and gynecologic origin may be classified as benign, of uncertain malignant potential, or malignant. Small PEComas without any worrisome histologic features are most likely benign. PEComas with nuclear pleomorphism alone (“symplastic”) and large PEComas without other worrisome features have uncertain malignant potential. PEComas with two or more worrisome histological features should be considered malignant. Almost all uterine PEComas were located in the body of the uterus; however, an unusual instance of this rare tumor presenting as a polypoidal cervical mass in a young female was also reported [3].

From a molecular genetic perspective, the recurrent chromosomal alterations in both renal and extrarenal tumors further support the concept of PEComa as a distinctive tumor entity regardless of anatomical location [16]. PEComas are related to genetic alterations of the tuberous sclerosis complex (TSC), an autosomal dominant genetic disease due to loss of TSC1 (9q34) or TSC2 (16p13.3) genes, which seem to have a role in the regulation of the Rheb/mTOR/p70S6K pathway [17].

PEComas show a marked female predominance and are usually composed of epithelioid, but occasionally spindle cells with clear to granular eosinophilic cytoplasm and focal perivascular accentuation [18]. Our study
showed that the cells of perivascular epithelioid cell tumors of the uterus were epithelioid or spindle cells with light eosinophilic or translucent cytoplasm. Mitotic figures were rare and necrosis could not be observed. Thin- or thick-walled blood vessels were readily visible around the smooth muscle with unclear boundaries. The remaining small nodules were formed by smooth muscle cells that were proliferating without atypia or invasive growth. The cells of perivascular epithelioid cell tumor occurring in the vagina were clear cells with transparent cytoplasm and small round nuclei. Mitotic figures were rare, and the tumor cells had an adenoid or acinar-like structure. These pathological features were similar to those of vascular epithelial cells described in the past.

Nearly all PEComas show immunoreactivity for both melanocytic (HMB-45 and/or Melan A) and smooth muscle (actin and/or desmin) markers [8]. Fukunaga [7] described four cases of uterine PEComa in 2005. Immunohistochemically, the tumors were positive for vimentin (4/4), HMB45 (4/4), h-caldesmon (4/4), alpha-smooth muscle actin (3/4), muscle actin (2/4), and desmin (3/4). They were uniformly negative for Melan A, CD10, and S-100 protein. In this study, the tumors were positive for HMB45 (3/3), SMA (2/3), HHF35 (2/3), vim(+) (1/3), and MITF (2/3). As in Fukunaga’s study, they were uniformly negative for melan A, CD10(-), and S100(-).

The prognosis of PEComas has received close attention. A 79-year-old woman with a large uterine mass that recurred two years following resection was reported. The patient died within months after resection of the recurrent tumor. It was suggested that uterine PEComas should be regarded as tumors with uncertain malignant potential [19]. Among the four cases of uterine PEComa reported by Fukunaga, one patient died of intestinal metastasis 17 months after surgery. The other patients were well with no evidence of disease 8, 12, and 36 months after surgery [7]. Jeon reported a 9-year-old girl who was diagnosed with PEComa of the uterus with metastasis. After multimodal treatment with chemotherapy as well as radiotherapy after surgery, there was no evidence of recurrence or further metastasis. She remained disease-free 1.5 years after her initial diagnosis [20, 21]. In our study, the three cases were followed for 26, 22, and three months, respectively, and disease-free survival was observed in all cases. Because of the limited data about the prognosis of PEComas, it is still difficult to establish firm criteria for malignancy.

In conclusion, PEComas of gynecologic origin have the morphological and immunohistochemical features of the PEComa family. These tumors are rare, and differential diagnosis is needed. Until more cases of this rare tumor are evaluated, with longer follow-up, firm criteria for malignancy remain uncertain.

Acknowledgment

Supported by the Science and Technology Planning Project of Guangdong Province, China, 2008B060600037; Supported by Medical Scientific Research Foundation of Guangdong Province, China, A2008027.

References


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Primary gynaecological tumours mistaken for metastases: report of two cases with review of literature

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Summary

We describe two neoplasms of rare occurrence, one of ovarian and the other of uterine origin that were sent for consultation. Both lesions were diagnosed as metastatic carcinomas by pathologists with special interest in gynaecological pathology. The cases were referred for a second opinion because of subsequent failure to identify the primary source. We discuss the differential diagnoses, the need for generous sampling particularly in ovarian mucinous neoplasms and the value of including particular antibodies in the panel to aid the diagnostic process. Metastatic tumours mimicking primary tumours are always challenging. These two cases illustrate the need to be vigilant against the reverse scenario as well.

Key words: Ovary; Mucinous neoplasms; Uterus; Neoplasms; Sex cord-like differentiation.

Introduction

The distinction between primary and metastatic carcinomas is not always straightforward even with the application of an exhaustive panel of immunohistochemical markers. The existence of metastatic tumours that mimic native tumours and vice versa, adds to the diagnostic challenge. This is an all too familiar dilemma for a gynaecological pathologist who is confronted with an ovarian mucinous tumour. We describe two cases to highlight this issue in a gynaecological pathology setting. Both cases were sent for consultation on account of a previous diagnosis of metastatic carcinoma and subsequent failure to identify the primary tumour.

Case Reports

Case 1

A 39-year-old woman with no significant past or current medical history presented with menorrhagia and abdominal swelling. She was found to have multiple uterine fibroids on ultrasound scan. Abdominal myomectomy was performed and three fibroids were removed.

Pathological findings revealed the specimen consisted of three firm circumscribed nodules with diameter ranging from 17 mm to 70 mm. The cut surface all three lesions showed a pale tan whorled appearance characteristic of leiomyomas. The two larger nodules were composed of fascicles of benign smooth muscle, in keeping with leiomyomas. The smallest of the nodules was described by the referring pathologist as having ‘nests of epithelial cells in a cellular stroma’ (Figure 1) with ‘positive expression for cytokeratin, oestrogen and progesterone receptors’ (Figure 2). A metastatic carcinoma with a possible primary origin from the breast was indicated based on the morphology and immunoprofile.

Case 2

A 52-year-old woman presented with a 30-week in size abdominal mass. Ultrasound (US) and computed tomography (CT) scan revealed a right ovarian mass with likely mucinous contents. A total abdominal hysterectomy with bilateral salpingo-oophorectomy, pelvic lymphadenectomy and omentectomy was performed. At laparotomy, a smooth surfaced, intact, mobile right ovarian cystic mass was noted. A normal appendix was also visualised.

Pathological findings showed the right ovary contained a 210 mm intact cystic mass with mucinous contents. The cyst was described as multiloculated with a single focus of solid whitish nodule (25 mm). The left adnexae, omentum and uterus were unremarkable except for the presence of a soft polyp in the endometrial cavity.

The cystic mass was described as containing ‘mucinous columnar epithelium with atypia and an infiltrative growth pattern’. The ‘glands were positive for CK20, CDX2 and CEA and negative for CK7’: the left ovary was normal as were the uterus and cervix. Based on these observations, a diagnosis of metastatic mucinous adenocarcinoma was made. The large intestine including the appendix was cited as a possible site of the primary lesion.

Case Review

Case 1: The smallest nodule had a well circumscribed margin and contained a mixture of smooth muscle cells and epithelioid cell nests with the latter being the predominant component. Further immunohistochemistry revealed positive expression for calretinin, inhibit in (Figures 3, 4, 5) CD10, CD99, melan A and CD56 in the epithelioid nests. H-caldesmon staining showed diffuse strong expression in the entrapped smooth muscle elements and patchy positivity in the epithelioid cells. The epithelioid elements were negative for CK7, CK20, CEA and S100 protein. A diagnosis of uterine tumour resembling ovarian sex cord tumour (UTROSCT) was made.

The patient is well with no evidence of recurrence 24 months following the procedure.

Case 2: The sections revealed a mucinous neoplasm of
enteric type with epithelial stratification and moderate to severe cytological atypia. Also noted, were the prominent presence of mucin pools dissecting the tumour stroma (pseudomyxoma ovarii) (Figure 5). The epithelial atypia amounted to intraepithelial carcinoma and there were focal areas with stromal infiltration in keeping with the original diagnosis of mucinous carcinoma. The tumour was diffusely positive for CK20, CEA and CDX-2 and negative for CK7 (Figures 6a, 6b). An additional observation in two of the 20 tumour sections included presence of keratin and hair shafts and parts of a cyst wall lined by squamous epithelium, indicating a co-existing teratoma. A florid lipogranulomatous reaction was also noted adjacent to the teratomatous elements (Figure 7). In the absence of a demonstrable extra ovarian pathology, a diagnosis of primary ovarian mucinous adenocarcinoma (enteric type) arising in a teratoma was made. Postoperative CT scan of the pelvis and abdomen was normal. The patient is well and free of symptoms 12 months following laparotomy.

**Discussion**

**Case 1:** Uterine tumours resembling ovarian sex cord tumours (UTROSCTs) were originally described by Clement and Scully more than 30 years ago [1]. However they still offer a particular challenge owing to the relative rarity, a complex and varied immunophenotype [2, 3], and appearances that show a considerable degree of overlap with endometrial stromal tumours (EST) [4]. They occasionally get thrown into the path of an unsuspecting histopathologist as an incidental finding in a fibroid uterus, as happened in our case.

UTROSCTs were originally described as tumours with prominent sex cord-like differentiation in which there is no conspicuous endometrial stromal background [1]. In the recent version of the World Health Organisation classification of tumours, this is recognised as an entity separate from EST [5].
Primary gynaecological tumours mistaken for metastases: report of two cases with review of literature

Primary gynaecological tumours mistaken for metastases have been reported [8, 11]. In the absence of clear evidence from long-term follow-up studies, the current view is to consider these as tumours to be of uncertain, but low malignant potential [2, 8].

Case 2: Distinction between primary ovarian mucinous epithelial neoplasms and metastatic involvement of ovaries by primary gastrointestinal tumours is a well recognised problem. In response to this awareness, several studies have been published focussing on the differences in morphological patterns and immunohistochemical profiles between the two [12-14]. As a result there has been greater clarity on the subject and pathologists have a better understanding than ever of the histological differences and the role that immunohistochemistry can play in resolving this issue.

It is well established now that CK7 and CK20 expression profiles are helpful in differentiating primary ovarian mucinous tumours from tumour metastases from the lower gastrointestinal tract including the appendix [15, 16]. Although primary ovarian tumours are known to show expression with CK7 and CK20, the staining is more diffuse with the former and patchy with variable intensity with the latter. Metastatic tumours from the large intestine and appendix including ovarian involvement by low-grade appendiceal mucinous tumours show a diffuse expression with CK20 and negative to focal faint positivity with CK7. CK7/CK20 profiles are of limited value in differentiating primary mucinous tumours from metastases from the stomach (intestinal type adenocarcinomas), pancreas, gall bladder and breast.

There is also ample published evidence to support an appendiceal origin of ovarian mucinous tumours associated with pseudomyxoma peritonei (PMP) [17, 18].

This greater awareness about metastatic mimics in the ovary is reflected in the dramatic drop in the incidence of primary ovarian mucinous carcinomas over the years [19, 20]. In a study looking at the relative incidences of various types of ovarian carcinomas, Seidman et al noted that primary ovarian mucinous carcinomas constituted < 3% of all carcinomas [19]. Previous studies had quoted an incidence ranging from 6% to 25% with a mean of 12%. Another study looking at a series of 52 consecutive ovar-

Figures 3a, 3b, 3c, 3d. — Epithelioid cells with positive expression for CAM 5.2, ER and PgR and mostly negative expression with caldesmon (case 1).
ian mucinous carcinomas identified 40 (77%) of them to be metastatic. Gastrointestinal tract (45%) was the single most common site of primary origin in this series [21].

Thus in light of all the statistical evidence, an ovarian mucinous tumour with CK20+/CK7-ve profile is most likely to be a metastasis from a primary tumour in the appendix or large intestine. It is essential to indicate this in a report so that all appropriate measures can be taken to track down the extra-ovarian source. In this context, we would like to highlight the occurrence of a rare converse scenario that is also worth consideration. As illustrated by this case and several other publications including studies on two major series [22-28], primary ovarian mucinous carcinomas with lower gastrointestinal phenotype and immunoprofile (CK20+, CK7-ve) are known to exist. These tumours of teratomatous origin are much rarer than their surface epithelial counterparts and metastatic tumours [22]. These are also known to be heterogeneous depending on the chosen line of germ cell differentiation. Teratomatous mucinous tumours showing sinonasal or upper gastrointestinal line of differentiation share many features that overlap with primary mucinous surface epithelial tumours including a CK7+/CK20-ve profile. Whereas mucinous tumours derived from lower intestinal germ cell lines display histological features and immunoprofiles that are indistinguishable from metastatic colorectal and appendiceal mucinous neoplasms [22-24]. It is also evident that this subset of ovarian mucinous neoplasms is more frequently associated with the clinical syndrome of PMP, another feature that is shared by appendiceal mucinous tumours but not tumours of surface epithelial origin [22-28].

There have been studies on two major series (44 and 42 patients) [22, 23] and one short series of three cases (24) of ovarian teratomatous mucinous tumours focussing on the histomorphological features, immunohistochemical spectrum and clinical association with PMP. Emerging evidence has indicated that these neoplasms are morphologically and immunohistochemically diverse with benign (cystadenomatous), borderline (low malignant potential) and malignant (intraepithelial and invasive) histology and varied patterns of CK7 and CK20 expression. In both series, cystadenomas and proliferative (borderline) mucinous tumours comprised the majority with invasive carcinomas forming 12%-14% [22, 23]. It has also been observed that compared with adenomas a greater proportion of borderline tumours (with or without intraepithelial carcinomas) and invasive carcinomas express an enteric immunoprofile (CK20+, CK7-ve). Pseudomyxoma ovarii (dissecting pools of extracellular mucin within the stroma) is also described as a recurring feature in these neoplasms. This has been observed with greater frequency in borderline tumours and invasive carcinomas, and an association with a CK20+, CK7-ve profile is also described. The aforementioned studies failed to establish any predictive link between pseudomyxoma ovarii and PMP [22, 23].

As pointed out by Vang et al., the germ cell components in these mucinous tumours tend to get overgrown by mucinous areas resulting in a need for careful sampling to
demonstrate the teratomatous origin [22]. Cases have been described in the literature where evidence of teratomatous origin was limited to finding keratin flakes and fragments of squamous epithelium-lined cysts in one or two sections [23], an observation identical to our case.

In a young woman with unilateral ovarian mucinous tumour with prominent pseudomyxoma ovarii and a lower gastrointestinal type immunoprofile (CK7-,

CK20+ve), the pragmatic approach should include a detailed examination of sections and generous sampling of the tumour with the aim of identifying a possible teratomatous origin. The positive identification of teroma-
tous elements in the absence of clinical or pathological evidence of a nonovarian (appendiceal) primary tumour is sufficient to confirm the primary nature of this neoplasm. Although it is theoretically possible to have a collision tumour comprising a teratoma and a metastatic adenocarcinoma, the evidence from previous studies does not support this. The majority of the cases from the previously quoted series were accompanied by histologically confirmed normal appendices [22-24].

A teratoma may not be uncovered in spite of extensive sampling in a scenario when the tumour has arisen in a monodermal teratoma that has been completely replaced and overgrown by it [22]. In such an event, if an extra ovarian primary is absent, it is worth stating in the report that a primary tumour is still a possibility [23, 24].

In view of the more common occurrence, every effort should be made to exclude a possible metastasis from the lower gastrointestinal tract including appendix. This should ideally involve a detailed clinical and intraoperative evaluation, an appendectomy and thorough histological sampling of the specimen [22]. However due consideration should be given to the alternate possibility of a primary ovarian origin in a teratoma. This will reduce the element of uncertainty and distress for the patient. From the surgeon's perspective, this provides the much needed assurance for not pursuing further investigative procedures such as a surgical re-exploration at significant expense, which some of the patients in the previous studies [23] had to endure.

Invasive mucinous adenocarcinomas arising in teratomas have the potential to metastasise and hence need consideration for adjuvant chemotherapy and close clinical follow-up [22, 23]. A close watch is equally warranted for borderline mucinous neoplasms of teratomatous origin because of their increased predisposition to PMP [24-28].

Conclusion

In summary, we have described two distinctive cases with rare histological diagnoses in a gynaecological pathology setting. Both cases were mistakenly diagnosed as metastatic carcinomas for various reasons. In recent years, much attention has been given to metastatic tumours mimicking primary tumours. These two cases illustrate that pathologists should equally well guard themselves against the reverse scenario.

Acknowledgements

We would like to thank Miss Shanti Raju (Guy's & St. Thomas' Hospital (GSTT), London) and Mr. Frank Lawton (Kings College Hospital & GSTT, London) for referring the cases and providing clinical follow-up.

References


Primary gynaecological tumours mistaken for metastases: report of two cases with review of literature


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Rare metastases of pancreatic tail carcinoma in female genital organs

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Summary

A case is presented of pancreatic tail carcinoma metastasizing to the uterus, right ovary and right sacrouterine ligament 2.5 years after the primary tumor had been detected and treated. During explorative laparotomy, performed after 3D color Doppler ultrasonographic visualization of a suspected finding in the right adnexal region, metastatic deposits in the uterus, right ovary, right sacrouterine ligament and right ureter originating from the primary adenocarcinoma of the tail of the pancreas were detected and surgically removed.

Key words: Pancreatic tail carcinoma; Urogenital metastases; Pancreatic carcinoma metastases.

Introduction

Primary carcinoma of the pancreas accounts for 1-2% of all carcinomas. It may appear at any age, however, it is most frequent in the period between the sixth and eighth decade of life [1], while according to some authors, two-thirds of the patients are between 40 and 60 years old [2]. It is two times more frequent in men than in women. The incidence of pancreatic carcinoma is constantly increasing, but early diagnosis of this tumor is still a difficult issue. It is usually detected late, when it is impossible to undertake radical treatment.

Pancreatic carcinoma usually occurs in the head of the pancreas (60-70%), followed by the body (20-30%), while it is least frequent in the tail of the pancreas (5-10%).

One of the characteristics of pancreatic carcinoma is its tendency to spread along the perineural lymph spaces, followed by invasion into the surrounding tissues, such as the duodenum, choledochus, stomach, inferior vena cava, upper mesenteric and portal vein, and celiac trunk. Thus, early metastases to the liver usually result as a consequence of tumor spread by direct invasion, while hematogenous and lymphogenous metastases are more frequently found in carcinomas of the body and tail of the pancreas. These carcinomas spread into the retroperitoneal space, while deep invasion of the tumor into the perineural lymph spaces as well as infiltration into the celiac plexus causes deep pain [3].

Almost as a rule, carcinoma of the head of the pancreas metastasizes to the liver early, while carcinoma of the tail of the pancreas spreads to the peritoneum. Metastases to the brain, heart, pericardium, skin and subcutaneous tissue has been described in the literature, but it is rare, extremely rare in the ovary, uterus, bladder and thyroid gland, testicle, prostate and rectum. It is, however, interesting to note that, in a noticeable percentage of cases (even up to 15%) autopsy has not detected metastases to other organs [2].

Case Report

The case of a 50-year-old patient, whose primary carcinoma of the tail of the pancreas was verified, surgically and chemotherapeutically treated and, 2.5 years after detection and treatment metastasized to the uterus and ovary is presented.

Discomfort started six years earlier when the patient went to a gastroenterologist due to problems with irregular bowel movements (constipation followed by diarrhea, etc.). Irritable colon followed by a gastroenterologist due to problems with irregular bowel movements (constipation followed by diarrhea, etc.). Irritable colon was diagnosed. Administered therapy only partially reduced the discomfort. During ultrasonographic (US) examination, a small cystic tumefaction, localized in the tail of the pancreas, towards the spleen, was visualized. Three years after discomfort started, when the patient was undergoing regular check-ups, US examination visualized first small and later significant growth of the previously mentioned cystic tumefaction. The cyst had grown from an initial 4 x 4 cm to 8 x 9 cm. The patient complained only about sporadic weak, dull pain in the left hypochondrium, which occasionally spread towards the left scapula. At that time the value of tumor marker CA 19.9 was over 8000 IU/ml. Surgical treatment was indicated. Existence of a malignant pancreatic tail tumor with infiltration in the left adrenal gland was intraoperatively confirmed. Resection of the distal part of the pancreas and splenectomy were performed and histopathological findings verified invasive pancreatic ductal adenocarcinoma with caudal localization. Chemotherapy was administered over 12 cycles, followed by regular US and computed tomography (CT) check-ups and monitoring of the values of tumor marker CA 19.9, which gradually decreased to normal. Two years later values of the tumor marker started to increase again. The patient had no discomfort, and US and CT findings were without signs of disease. Six months later dull pain in the pelvis appeared, accompanied by an increase in the value of tumor marker CA 19.9. None of the applied diagnostic methods, including CT, nuclear magnetic resonance imaging, digestive endoscopic methods, irigography, scintigraphy, nor a complete neurological examination were able to find the cause of the discomfort nor the reason for the increase of tumor marker value, i.e., non of...
them were able to pinpoint the primary disease. The patient was sent to the University Clinic, Gynecology and Obstetrics Department, for a gynecological exam. Bimanual exam determined a slight sensitivity in the right adnexal region. Colposcopic findings and Pap smear were normal. A slight lesion in the right adnexal region approximately 25 mm in size was visualized by transvaginal color Doppler US examination. It was irregular in shape with flow magnification and low resistance in blood vessels. Exploratory laparotomy was suggested. Exploration of the abdomen revealed the existence of a malignant tumor of the genital organs. Tumor tissue slightly infiltrated the uterine cervix, right sacrouterine ligament, right parametrial region and pelvic blood vessels, and furthermore infiltrated the distal part of the right ureter. At about 2 cm from the ostium of the right ureter into the bladder tissue had formed a stricture of the ureter. Total hysterectomy with bilateral salpingo-oophorectomy, deliberation and resection of the distal part of the right ureter and its implantation into the bladder were performed, as well as lymphadenectomy of the right inguinal region. Histopathological findings verified metastases of the seromucinous adenocarcinoma originating from the primary adenocarcinoma of the tail of the pancreas.

Discussion
Pancreatic carcinoma has been ranked as the fifth cause of death for patients suffering from malignant diseases, even though it accounts for only 1-2% of all primary carcinomas, considering the fact that it results in lethal outcome in over 98% of cases [4]. High pancreatic carcinoma mortality can be explained by the fact that it is usually detected in advanced stage, i.e., it metastasizes to the liver early, either by direct invasion into the surrounding organs and large blood vessels - lienal, mesenteric, and portal vein, or even by lymphogenous or hematogenous spread. Rare and non-specific forms of this carcinoma also exist, significantly complicating already difficult diagnostics [5]. Thus, the time period between the time of definite diagnosis and death is relatively short [1].

Distant metastases are a rare finding. According to reports from the literature, incidence of hematogenous metastases in the liver is 31.3%, lungs 1.8%, adrenal gland 1.1% and the navel 0.4%. Other localizations of secondary deposits, developed either by lymphogenous or hematogenous spread, are very rare and present in less than 0.01% of cases [6].

Metastases of pancreatic carcinoma to the organs of the urogenital tract are extremely rare. Up to now only two cases of secondary deposits in the spermatic cord and prostate gland have been described. In both cases the primary tumor was discovered only after secondary deposits were detected [7]. In addition to this, one case of metastatic paratesticular mucinous adenocarcinoma with invasion to the testicle and epididymis was described, whose primary origin was in the pancreas [8].

The literature contains data of a total of 342 cases of metastases to the ureters, whereas only in two cases the primary tumor was in the pancreas, more precisely in the head of the pancreas [9].

Metastases of pancreatic carcinoma in the female genital system (in the ovary) have been described only a few times. In two cases metastatic carcinomas of the ovary were detected before detection of the primary pancreatic carcinoma [10]. Other cases of pancreatic carcinoma metastases in the ovary were detected in the autopsy material [6].

Metastases of carcinoma of the tail of the pancreas into the ovary, uterus, sacrouterine ligament and the ureter are extremely rare events, especially considering the fact that in the described case there were no metastatic changes in the liver, which would represent an expected finding, as well as the fact that more than 2.5 years had passed since the discovery of the primary tumor, and that survival rate in this period is very low.

References

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Atypical polypoid adenomyoma of the uterus.  
A case report and a review of the literature

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Summary

Atypical polypoid adenomyoma (APA) is a rare, benign lesion. The tumor occurs in nulliparous women aged 22-48 years (average 33 years) and it has been suggested as being related to prolonged estrogenic stimulation. We describe a case of a 72-year-old woman who presented at our hospital with persistent, worsening urinary incontinence and pelvic pain. Physical examination and pelvic ultrasound disclosed uterine enlargement, a mass in the endometrial cavity and multiple small myomas. Total hysterectomy with bilateral salpingo-oophorectomy was performed. The histological diagnosis for the mass of the endometrial cavity was atypical polypoid adenomyoma. APA should be distinguished from endometrial carcinoma and other malignant uterine neoplasms such as adenofibroma, adenosarcoma and malignant mixed Mullerian tumor. The immunohistochemical panel which usually includes alpha smooth muscle actin, desmin, Ki67 and recently CD10 is often helpful in establishing the diagnosis. The treatment may vary depending on the patient’s age, her desire to preserve fertility, and the severity of her symptoms.

Key words: Uterus; Atypical polypoid adenomyoma; Endometrial stromal cells; Immunohistochemistry.

Introduction

The term atypical polypoid adenomyoma (APA) was first described by Mazur in 1981 [1]. This entity is a rare, benign, polypoid tumor of the uterus and it is composed of endometrial glands admixed with a stromal component of interlacing bundles of smooth muscle [2]. The tumor occurs in premenopausal women, usually in their fourth and fifth decades, and rarely after menopause. In addition APA has been seen in patients with Turner syndrome [3]. Although the lesion is benign, it should be distinguished from hyperplasia, endometrial carcinomas and other malignant uterine neoplasms with which it is often confused, particularly in curettage specimens [4, 5]. The published series indicate an average risk of endometrial carcinoma of 8.8% in women with a history of polypoid adenomyoma [6]. We report a case of APA of the uterus in a postmenopausal woman, discuss the histogenesis of this tumor, and review the English literature.

Case Report

A 72-year-old woman, gravida 2, para 2, presented with a 4-month history of lower abdominal pain and abnormal uterine bleeding of about three weeks duration. She had completed menopause at the age of 53. Gynecological and medical history were uneventful. On physical examination an enlarged, irregular uterus was found. No other masses were detected anywhere else. Laboratory findings were within normal limits, including tumor markers CEA and CA125. Pelvic ultrasound (US) disclosed uterine enlargement, a mass in the endometrial cavity and multiple myomas. A total hysterectomy with bilateral salpingo-oophorectomy was performed. On gross examination the tumor was composed of widely spaced proliferative glands with slightly irregular spaces which were lined by atypical cells showing occasional mitoses (Figure 1). The stroma was composed of short interlacing bundles of smooth muscle cells which appeared benign (Figure 2). Mitoses were rare. Foci of adenomyosis were seen at the margins of the glands. The tumor did not invade the myometrium. The uninvolved endometrium was atrophic.

Immunohistochemistry the myofibromatous mesenchymal cells were strongly positive for SMA (Figure 4) weakly positive for desmin and negative for CD10, oestrogen, progesterone. The Ki67 proliferative antigen was low (Figure 5). The tumors in the myometrium were leiomyomata.

Discussion

The term APA of the uterus was first described in 1981 by Mazur [1] and approximately less than 200 cases in the uterus have been reported in the English literature ever since. He defined it as an uncommon focal polypoid lesion of the uterus featuring the proliferation of irregular endometrial glands with squamous metaplasia embedded within a prominent cellular smooth-muscle stroma.

APA is an uncommon endometrial tumor that typically occurs in women of reproductive age. Average age of occurrence is 39 years, but ages have ranged from 21 to 73 years. Rarely, affected patients are postmenopausal [7]. In addition, APA has been seen in patients with Turner syndrome, possibly representing a complication of long-term estrogen therapy in these patients [3]. The
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The main clinical symptom is abnormal uterine bleeding. Vaginal discharge, pelvic pain or postcoital spotting may be seen [8-10]. Most lesions are detected on endometrial biopsy specimens or dilation and curettage specimens obtained for workup of the abnormal bleeding. However, APA has been discovered incidentally during routine hysteroscopy and biopsy for infertility or, when the uterus is removed for other reasons, such as leiomyomata [2, 5, 9]. It arises most frequently in the lower uterine segments but has also been reported to involve the uterine fundus, as in our case, or endocervical canal. Grossly APA presents as a pedunculated or sessile polypoid mass with bulging, lobulated or bosselated, firm or rubbery sectioned surfaces. The greatest dimension ranges from 0.1 to 6 cm [9-11]. The size of the tumor in the present case was 12 cm in the longest diameter.

Microscopically APA is a biphasic tumor, consisting of atypical endometrial glands separated by intersecting fascicles of smooth muscle cells.

The glands vary considerably in size and frequently have irregular shapes and are lined by cuboidal to low columnar to pseudostratified columnar epithelium with varying degrees of cytologic atypia and mitoses [2, 6, 8, 9].

Longacre et al. reported that if the APA contains markedly complex glands and glandular proliferation, the lesion should be designated as “APA of low malignant potential” [10].
The glandular components may be focally obliterated by the metaplastic squamous elements. In some cases the squamous elements seem to blend almost imperceptibly with the stroma [2]. Foci of mucinous metaplasia are occasionally seen and an Arias-Stella-like change may be evident in pregnant patients [11].

The stromal component, typically appears benign and predominantly consists of interlacing bundles of smooth muscle. The stroma of APA differs from normal myometrium by exhibiting increased cellularity, short interlacing fascicles rather than elongated muscle bundles and a minor component of fibrous tissue [2, 11]. Hyalinization may be present. The uninvolved endometrium is typically benign and rarely the adjacent endometrium may be secretory or hyperplastic [2]. Minor foci of superficial adenomyosis may be seen in the tumor. Longacre et al. proposed an alternative term “atypical polypoid adenofibroma”, as they demonstrated that the stroma in these lesions contained a mixture of smooth-muscle cells, fibrous tissue and endometrial stromal cells [10]. The differential diagnosis of APA includes endometrial carcinoma with invasion of the myometrium [12, 13], adenofibroma, adenosarcoma and malignant mullerian mixed tumor [1, 2].

The young age of patients with APA (average 39 years), should be a clue to the diagnosis because adenofibroma, adenosarcoma and malignant mullerian mixed tumor (MMMT) all typically occur in postmenopausal women. On gross examination small, solid, polypoid, well circumscribed, lobulated APA differs in appearance from large exophytic masses seen in most adenosarcomas and MMMTs.

In addition APA differs from tumors in the adenofibroma-adenosarcoma category in the muscular nature of its stromal component. The latter category almost always resembles that of endometrial stroma and its fibrous stroma. The stromal component of adenosarcomas often forms periglandular cuffs. Distinction between APA and MMMT should not be difficult because both the glandular and stromal components of the latter are highly malignant [2].

The most important differential diagnostic problem presented by APA is exclusion of well differentiated endometrial carcinoma invading the endometrium [12-14]. Although APA architecturally may resemble endometrial adenocarcinoma it lacks stromal desmoplasia and usually affects young women. The distinction between them is very important but sometimes very difficult. In these cases the immunohistochemical panel, which usually includes alpha smooth-muscle actin and desmin [14, 15], and recently CD10 is often helpful in establishing the diagnosis [16].

It is acknowledged in the field of gynecologic pathology that CD10 is a sensitive and diagnostically useful marker of neoplastic and non neoplastic endometrial stromal cells [15]. According to recent studies CD10 immunostaining pattern demonstrated a significant difference in the stromal components between microinvasive carcinoma and APA. The microfibromatous stromal component of APA is completely negative for CD10 in most cases, whereas mesenchymal cells immediately surrounding the myoinvasive carcinoma were positive (fringe-like staining pattern) [16-19].

Immunohistochemical studies for Ki67 have demonstrated that proliferative activity of the glands in APA is lower when compared with glands of usual endometrial adenocarcinoma.

A few molecular studies for DNA ploidy analysis demonstrated that APA had a diploid DNA content and that the S phase fraction was relatively low [20].

The histogenesis of APA remains uncertain. The intimate relation between its glands and the myofibromatous stromal component suggests that the myofibromatous stromal component of APA may be explained by the histogenesis of the “myofibromatous metaplasia” of the endometrial stromal cells [18]. Moreover, Clement and Young believe that the estrogen-related factor may play an important role in the development of atypical polypoid adenomyoma due to the fact that the lesion mainly occurs in premenopausal patients and the uninvolved endometrium exhibits an estrogen phase appearance [3].

Since APA generally occurs in premenopausal women and as conserving fertility potential may be an important consideration, hysteroscopic resection of such tumors may be a therapeutic option in women who wish to retain their uterus or who would be at high medical risk for hysterectomy [21].

In conclusion APA is a rare benign tumor of the uterus that could appear in postmenopausal women. The fact that in our case the uninvolved endometrium was atrophic shows that this tumor was not related to estrogenic conditions.

References
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