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Could the eventual results of the NSABP 39/RTOG 0413 trial for partial breast irradiation (PBI) be improved by combining spherical applicators and whole breast irradiation? Radiobiology suggests it may

B.J. Smit - Tygerberg, SOUTH AFRICA
Combining spherical application and whole breast irradiation may improve outcome of partial breast irradiation.

Management of abnormal cytological findings
P. Bösze - Budapest, HUNGARY
Point of view of experts on management of abnormal cytological findings is discussed.

Hyperthermic intraperitoneal chemotherapy added to the treatment of ovarian cancer. A review of achieved results and complications
A review of achieved results and complications of hyperthermic intraperitoneal chemotherapy added to the treatment of ovarian cancer.

Hormone therapy/adjuvant chemotherapy induced deleterious effects on the bone mass of breast cancer patients and the intervention of physiotherapy: a literature review
T. Tonezzer, C.M.A.P. Pereira, U.P. Filho, A. Marx - São Paulo, BRAZIL
The aim of this study was a literature review. Twenty four articles were selected to analyze the possible deleterious effects caused by adjuvant chemotherapy and hormone therapy on the bone mass of patients diagnosed with breast cancer and the physiotherapy intervention.

Molecular markers in epithelial ovarian cancer: Their role in prognosis and therapy
F. Zagouri, M.A. Dimopoulos, E. Bournakis, C.A. Papadimitriou - Athens, GREECE
The use of molecular markers in epithelial ovarian cancer in common clinical practice seems promising for the diagnosis and prognostication. It is tempting to anticipate the gradual integration of molecular profiling in clinical practice.

Liquid based cytology improves the positive predictive value of glandular smears compared to conventional cytology
R.J. Edmondson, C.A. Errington, D.J.A. Mansour - Newcastle upon Tyne, UK
A database review suggests that LBC increases the positive predictive value of cervical sampling to detect preinvasive and invasive cancer.
Increase of Mcm3 and Mcm4 expression in cervical squamous cell carcinomas
N. Gan, Y. Du, W. Zhang, J. Zhou - Zhejiang Province, CHINA
Mcm3 and Mcm4 were highly expressed in cervical squamous carcinoma and these two proteins might be useful as biomarkers in clinical diagnosis.

Repeated chemosensitivity testing in patients with epithelial ovarian carcinoma
M. Pospíšková, M. Špenerová, R. Pilka, M. Kudela, M. Hajdúch, V. Śrámek, B. Melichar, K. Cwiertka - Olomouc, CZECH REPUBLIC
Changes of chemosensitivity were repeatedly evaluated in a cohort of patients with epithelial ovarian carcinoma. Limited, but statistical increase in chemoresistance was observed for paclitaxel and carboplatin.

Non-hormonal treatment of vasomotor symptoms in gynecological cancer patients
L. Del Pup, T. Maggino - Mestre-Venice, ITALY
Non-hormonal therapies such as venlafaxine, paroxetine, gabapentin and clonidine have been shown to reduce hot flashes in randomized trials compared to placebo.

Analysis of clinical and molecular associations of triple negative breast cancers in node-negative patients
Immunohistochemical expression of patients with node-negative invasive breast carcinomas was compared. The TN phenotype is associated with aggressive histology.

Bevacizumab, paclitaxel and carboplatin for advanced ovarian cancer: low risk of gastrointestinal and cardiovascular toxicity
Despite concerns for hypertension and bowel perforations, bevacizumab can be safely administered during induction therapy for ovarian carcinoma.

Expression of p16 in serous ovarian neoplasms
H.O. Nazlioglu, I. Ercan, T. Bilgin, S. Ozuysal - Bursa, TURKEY
P16 expression of benign, borderline and malignant serous ovarian neoplasms was immunohistochemically evaluated, and was found to be strong in serous ovarian carcinomas.

Distribution of HPV genotypes in uterine cervical lesions among the Uighur women in Xinjiang province of China
A. Abudukadeer, Y. Ding, M. Niyazi, A. Ababaikeli, A. Abudula - Urumqi, P.R. CHINA
HPV16 was the most common type found in Uighur patients with cervical cancer while HPV18 and 58 types were relatively low and other types were absent.

Intestinal-type metaplasia in the original squamous epithelium of the cervix
E. Sivridis, G. Karpathiou, V. Malamou-Mitsi, A. Giatromanolaki - Ioannina, GREECE
The expression of mucin-distended goblet cells in the normal/original squamous epithelium of the cervix is described for the first time.

Evaluation of preoperative diagnosis with results of histopathological examinations of ovarian tumors in women of reproductive age
D. Samulak, S. Sajdak, M. Wilczak, M. Michalska, B. Pi ta, M. Englert-Golon - Poznan, POLAND
Gynecological, ultrasonographic and Doppler examinations as well as determination of CA-125 antigen provide an efficient research panel in the preoperative diagnosis of ovarian tumors.

Management of recurrence from a retroperitoneal xanthogranuloma: case report
D. Salehin, C. Haugk, A. Stricker, R. Triefenbach, M. Friedrich - Krefeld, GERMANY
A rare case of a xanthogranuloma from the retroperitoneal space diagnosed in a 24-year-old woman is presented.
A benign metastasizing leiomyoma involving a nodule in the pulmonary artery: case and literature review
O. Poujade, A.S. Genin, M. Dhouha, D. Luton - Paris, FRANCE
A benign metastasizing leiomyoma with local recurrence and metastasis growing into the left pulmonary artery was managed by surgery.

Metastatic and recurrent adenocarcinoma of the uterine cervix: a long-term survival of 16 years
A multi-modal salvage approach may achieve long-term survival in rare cases of recurrent metastatic cervical adenocarcinoma.

Three-dimensional power Doppler color ultrasonographic features of a minimal deviation adenocarcinoma of the uterine cervix
L. Hereter, F. Tresserra, B. Graupera, M.A. Pascual, M.A. Martinez, A. Úbeda - Barcelona, SPAIN
A case of minimal deviation adenocarcinoma of the cervix in a 34-year-old female is presented.

Ovarian metastasis of a primary renal cell carcinoma: case report and review of literature
S. Guney, N. Guney, D. Özcan, T. Sayilgan, E. Özakin - Istanbul, TURKEY
A case of ovarian metastasis of a primary renal cell carcinoma in a 29-year-old patient is reported.

Liver resection for metastases arising from recurrent granulosa cell tumour of the ovary - a case series
T.K. Madhuri, S. Butler-Manuel, N. Karanjia, A. Tailor - Guildford, UK
This series discusses three recurrent cases of GCT of the ovary successfully managed with resection of hepatic metastases improving patient quality of life.

Ovarian carcinomatosis presenting as bilateral inguinal hernia: a brief report
Y.H. Hung, C.T. Hsu, C.C. Chang - Taiwan, R.O.C.
Gynecologists should be aware of the difficulties associated with a delay in diagnosis of ovarian carcinomatosis presenting as inguinal hernias.

Serous ovarian cystadenocarcinoma incidentally discovered in a 29-year-old patient: case report
N. Cutura, V. Soldo, M. Vasiljevic - Belgrade, SERBIA
A case report of a serous ovarian cystadenocarcinoma incidentally discovered in a 29-year-old women is presented.

Chemotherapy with low-dose bevacizumab and carboplatin in the treatment of a patient with recurrent cervical cancer
The case of patient with platinum-resistant recurrent cervical cancer treated with low-dose bevacizumab and carboplatin with resultant improved disease progression is presented.

Groin recurrence following Stage IA squamous cell carcinoma of the vulva with negative nodes on superficial inguinal lymphadenectomy
A.C. Iyibozkurt, O.C. Dural, S. Topuz, S. Berkman, E. Bengisu - Istanbul, TURKEY
Stage 1A vulvar cancer may recur in the groin despite finding negative nodes in superficial inguinal lymph node dissection.
Could the eventual results of the NSABP* 39/RTOG** 0413 trial for partial breast irradiation (PBI) be improved by combining spherical applicators and whole breast irradiation? Radiobiology suggests it may

B.J. Smit
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Summary
There may be unacceptable risks associated with the relatively large single doses of irradiation prescribed over five days instead of over six weeks for three of the four trial arms of the NSABP39/RTOG 0413 clinical trial seeking to enlist 4,300 patients. The first arm prescribes 60 Gray (Gy) in two Gy fractions over six weeks, which is the present standard. The dose implications of the other three arms with reference to this standard were examined using the ID2 formalism. Particularly poor (non-homogeneous) dose distributions characterise spherical applicators like “MammoSite™” used as a sole device for accelerated partial breast irradiation (APBI). The alternative treatment, APBI done by 3-D conformal radiation, may also have a drawback, namely a sudden sharp cut-off in dose which may cause cosmetic problems due to circumscribed fibrosis and edema. Some recently published results from this trial reveal an alarming level of complications. The possible causes of these complications and poor cosmetic outcomes and how to avoid them are examined. An obstacle to the more widespread use of the “MammoSite™” type of device is that the device is not allowed closer than 5-7 mm from the skin or ribs; a possible remedy for this restriction is offered. It is also intended to make the relevant radiobiological principles usable for surgical oncologists.

Key words: APBI; 3-D conformal radiotherapy; NSABP29/RTOG 0431; MammoSite™; Complications; ID2; Alpha/beta ratios; Radiobiology.

Introduction
The use of radiotherapy as an adjuvant to lumpectomy for patients with breast cancer is established as an alternative to mastectomy. Mature 20-year-old data show clear equivalence of local excision plus radiotherapy versus mastectomy alone [1, 2].

The next question: Is it necessary to irradiate the entire breast? A review of data showed that the majority of recurrences occurred near the original tumor bed. Moreover, whole breast irradiation (WBI) did not stop recurrences remote from the primary lesion [3-5].

If partial breast irradiation (PBI) would work, then perhaps giving the treatment in a shorter time (accelerated partial breast irradiation or APBI) may also work, as argued by several investigators [6-9]. This untested idea was enthusiastically embraced by surgeons, vendors and radiation oncologists alike and a spate of radio-therapeutic devices and -options followed to test the principle, like the “Mammosite™”, “MammaSphere” and later a multichannel “MammoSite™”, followed by the Strut-Adjusted Volume Implant (SAVI) [10].

The Alpha/Beta ratio is a figure which is an indicator of the sensitivity of a particular type of tissue to the size of the dose per fraction. Alpha/ beta ratios for various tissues and some tumors have been determined. For example, early or acutely reacting tissues: skin, $\alpha/\beta = 9-12$ Gy; late reacting tissues: spinal cord $\alpha/\beta = 1.7-4.9$ Gy; kidney, $\alpha/\beta = 1.0-2.4$ Gy; etc. There is some evidence that the alpha-beta ratio for breast cancer is 4 Gy. The term “Gy” here is a mathematical oddity, and can be ignored in the application of the ID2 formula as the term in the numerator and denominator will always cancel out. By way of example, an $\alpha/\beta$ ratio of 4 Gy for breast cancer and an $\alpha/\beta$ ratio of 2 Gy for the “late reacting tissues” responsible for late complications, i.e., normal breast tissue were used. Because different tissues have different $\alpha/\beta$ ratios the prescribed dose will not reflect the real damage to a particular tissue type; this damage is quantified by incorporating the $\alpha/\beta$ ratios into the equation.

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There is therefore an unavoidable trade-off between large doses per fraction, with respect to effectiveness on the one hand and an increased risk of complications on the other. The design of the clinical trial NSABP 39/RTOG 0413 will serve to discuss this dilemma, to highlight possible dangers associated with this trial, and to explore safer treatment approaches based on radiobiological principles. Some results of the early 3-D conformal arm of the NSABP 39/RTOG 0413 trial in the first 60 patients caused concern as at 15 months of follow-up, an unexpectedly high percentage of the patients developed severe and early complications [11]. These results inspired a closer look at the radiobiology of the trial.

Examination of dose distributions

The dose distribution and impact around the spherical applicators used in NSABP 39/RTOG 0413 were reconstructed in relation to the radiobiological “reference regimen”, the so-called “ID2” based on the linear quadratic model of cell kill (to be found in any modern textbook on radiobiology). It should be clearly understood, that at the outset, the “dose” as shown in Table 1 of the NSABP 39/ RTOG 0413 trial, should be seen as the nominal, prescribed dose in Gray. However, for each nominal dose prescribed there is at least one other, or are often two, simultaneously relevant doses operative depending on the alpha-beta ratios chosen (see above). Firstly there is the biologically equivalent dose for tumor kill, usually with a large $\alpha/\beta$ ratio, and secondly the biologically relevant dose, determining the complication rate, received by the “late reacting tissues” (normal breast tissue), usually with a small $\alpha/\beta$ ratio. Complications which may occur include edema, necrosis and fibrosis. The prescribed dose does therefore not give accurate information about the efficacy and safety of a regimen because different tissue types have different sensitivities to the effects of single large doses of radiation. “Large” for the purpose of this paper, is a dose larger than 2 Gy per fraction, given six hours or more apart.

The physics and radiobiology of dose distribution around point sources of radiation

The late reacting tissues are particularly vulnerable to fraction size, which is large near the surface of spherical devices due to the “inverse square law”, which states that the dose is inversely related to the square of the distance from a point source. This implies a very rapid drop in dose with distance. This problem is complicated by the fact that the dose per fraction is increased at the same time therefore the effects of the inverse square law are amplified in the areas of high dose. Spherical applicators therefore have a “double negative whammy”. Below is a summary of the four arms of the NSABP39/ RTOG 0413 breast cancer therapy protocol, designed to test whether APBI applied by various means can be as effective as the usual six-week course of tele-therapy by linear accelerator. Some early results claim equivalence for APBI brachytherapy and whole breast irradiation (WBI) [12-14]. The brachytherapy used however, was done by volume implants and not with the totally different, spherical balloon type of device and are therefore hardly relevant.

NSABP 39/RTOG 0413 APBI protocol

Table 1. Prescribed doses (nominal) for each arm of the NSABP 39/RTOG 0413 protocol are as follows:

1) Whole breast irradiation to 50 Gy with a 10 -15 Gy “boost”, all delivered in 2 Gy fractions over five to six weeks (Comment: This is a “standard” or reference regimen, also designated the “active comparator”). The following arms are “experimental”.

2) Accelerated partial breast irradiation by MammoSite™, 34 Gy in ten fractions of 3.4 Gy each 10 mm from the surface of the sphere, over five days, two fractions per day, minimum six hours apart.

3) Accelerated partial breast irradiation by multi-catheter type of spherical device 34 Gy in 3.4 Gy prescribed at 10 mm from the sphere in ten fractions over five days, minimum six hours apart.

4) 3-D conformal radiotherapy 38.5 Gy in ten fractions of 3.85 Gy each over five days, 10 mm from the marked lumpectomy cavity, minimum six hours apart.

These nominal doses of the four arms will be converted by means of the ID2 (see below) to illustrate the biologically effective doses for tumor and normal breast tissue complications relative to the expected effects induced by a standard course of radiotherapy of 60 Gy delivered in 30 fractions of 2 Gy each daily, resting week-ends = ID2.

The ID2 formula

The formula for calculating the ID2 can easily be used by oncology surgeons:

$$ID2 = \frac{nd (d + \alpha/\beta)}{(2 + \alpha/\beta)}$$

Where the ID2 is the identical dose relative to a 2Gy per fraction course of 60 Gy, n is the number of fractions, d is size of the dose per fraction in Gray, 2 is a constant and $\alpha/\beta$ is a factor which quantifies the sensitivity of the various tissues to dose per fraction, and nd is the total dose.
Implications of the $\alpha/\beta$ ratio for the four trial arms of NSABP 39/RTOG 0413

Arm 1: Whole breast irradiation: The standard of care

Tissue specific, $\alpha/\beta$ dependent doses for a nominal (prescribed) dose of: 50 Gy plus 10 Gy “boost” in 2 Gy fractions:

$$\text{ID}_2 = 30 \times 2 \text{ Gy} \times \frac{2 + 4}{2 + 4} = 60 \text{ Gy} \quad \text{for an} \quad \alpha/\beta \quad \text{ratio of} \quad 6 \text{ Gy, 4 Gy, 2 Gy or any other } \alpha/\beta.$$

In this particular case, the nominal dose = dose to tumor = dose to late reacting tissues that will cause the predictable effects of 60 Gy in 30 fractions of 2 Gy each on tumor tissue and “late reacting” normal breast tissue alike.

(Dose = vertical axis, distance = horizontal axis)

Arms 2 and 3: Accelerated partial breast irradiation (APBI) by spherical applicators like the “MammoSite™” balloon.

If we assume that an equivalent dose of 80 Gy in 2 Gy fractions will induce necrosis, then it can be shown by simple calculation that this dose (and the risk) extends up to 4.0 mm from the surface of a 5 cm diameter sphere for a prescribed dose of 34 Gy at 10 mm from the balloon’s surface. This means that a volume of 36.7 cc or 32% of the 114 cc of tissue treated to receive the minimum of 34 Gy, will be at risk of necrosis. Keeping in mind that necrotic tissue is an excellent medium for bacterial growth, then one could anticipate an unnecessarily increased incidence of infection with spherical applicators, apart form the fact that the cavity may not be perfectly closed.
Arm 3 is basically similar with a multi-cannel device, but the dose distribution is more complex and will not be discussed.

Arm 4: “3-D conformal irradiation” prescribes a homogeneous dose of 38.5 Gy to be delivered in ten fractions of 3.85 Gy each over five days at the lumpectomy cavity and 10 mm beyond the cavity. Table 3 and Figure 4 illustrate the relatively poor dose distributions (compare with Figure 3). Although the dose is “homogeneous” it is not iso-effective vis a vis the different tissue types irradiated because the fraction of 3.85 Gy is larger than the 2 Gy of the reference regimen; 3-D conformal therapy implies a very strictly defined partial volume of breast irradiated, and the edge of the irradiated volume will have a sharp zone of transition between the irradiated volume and the normal un-irradiated breast tissue in contrast to whole breast irradiation. The “tumor effective dose” at 10 mm is given in bold = 50.4 Gy.

Table 2. — Shows the dose distribution for a spherical applicator with a central point source of radiation energy. The combined detrimental effects of the inverse square law plus the effects of large doses per fraction especially at the surface of the applicator are shown. It emphasizes the dangerously high doses at the surface of the sphere (*) and the simultaneous relatively low doses for tumor control at 10 mm and 20 mm distant from the surface of the sphere (**). Bold: tumor effective dose at 10 mm from the surface of the balloon. Alternative schedules where spherical applicators are involved will be normalized to this dose of 41.9 Gy.

<table>
<thead>
<tr>
<th>Method/Dose (Gy)</th>
<th>Nominal</th>
<th>ID2 (α/β = 4)</th>
<th>ID2 (α/β = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sphere surface</td>
<td>66.6 Gy</td>
<td>118.3 Gy*</td>
<td>144.2 Gy*</td>
</tr>
<tr>
<td>2. At 10 mm</td>
<td>34.0 Gy</td>
<td><strong>41.9 Gy</strong></td>
<td>45.9 Gy**</td>
</tr>
<tr>
<td>3. At 20 mm</td>
<td>20.56 Gy</td>
<td>20.8 Gy**</td>
<td>21.2 Gy**</td>
</tr>
</tbody>
</table>

Table 3.

<table>
<thead>
<tr>
<th>3-D conformal</th>
<th>Nominal</th>
<th>ID2 (α/β = 4)</th>
<th>ID2 (α/β = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>38.5 Gy</td>
<td>50.4 Gy</td>
<td>56.3 Gy</td>
</tr>
<tr>
<td>At 10 mm</td>
<td>38.5</td>
<td><strong>50.4</strong></td>
<td>56.3</td>
</tr>
<tr>
<td>At any mm</td>
<td>38.5</td>
<td>50.4</td>
<td>56.3</td>
</tr>
</tbody>
</table>

Figure 3. — Graph illustrating the contents of Table 2 and showing the very rapid drop of the “three doses” with distance, for 34 Gy prescribed at 10 mm. Ideally the dose should not vary more than 10% across a treated volume; here it varies by 95% for the nominal dose (black column) and by 244% for the late reacting (normal breast) tissues (light-grey column).

Figure 4. — Graph of 3-D conformal radiotherapy illustrates the dosage distribution of a 38.5 Gy nominal dose given in ten relatively large fractions of 3.85 Gy each (compared to the standard of 2 Gy per fraction) on the tumor and late reacting (normal breast) tissues. The dose to the “late reacting tissues” is effectively 11.7% higher than the dose affecting the tumor tissue. The dose would terminate abruptly at the edge of the irradiated volume and may leave a “fibrotic ball” in the breast of susceptible patients. See text.
Problems with Spherical (balloon) type applicators.

There are two possible sources of trouble with spherical applicators: 1) The very poor dose distribution with the risk of tissue necrosis near the surface of the applicator. 2) The danger of placing a spherical applicator closer than 7 mm to the skin and ribs.

Solutions to the problems:

1. Methods to reduce the very sharp drop in dose around the spherical type of applicator.

Combining a spherical applicator with 3-D conformal therapy or whole breast irradiation.

The result of such a combination will be to “smear out” the dose so that the surface dose will be reduced and the dose at 10 mm will be the same or improved and beyond 10 mm a better tumor-killing dose will result. The combination will work better if the total dose is given in smaller fractions over a longer period of time. The proposal is to combine these mechanisms by giving the MammoSite-type dose in the first five days and to follow this by a second five-day course with 3-D conformal, or 3-D conformal “shrinking field” technique or with whole breast irradiation over the next five days in order to reduce the daily dose per fraction. The tumor-killing, ID2 = α/β ratio linked dose at 10 mm from the spherical applicator surface is normalized to 50.4 Gy, the same as for 3.85 Gy in ten fractions.

Dose distribution of a combination of MammoSite and 3-D conformal or whole breast dose to avoid a sharp drop in dose.

Table 4. — Shows the calculations for the tissue specific dose distributions illustrated in Figure 5 for 17 Gy given in ten fractions of 1.7 Gy each over five days, two fractions per day minimum six hours apart, plus, from the sixth day on, 24 Gy by 3-D conformal or whole breast irradiation (WBI) in 2 Gy fractions twice a day over five days, total nominal dose Gy at 10 mm, and a tumoricidal dose at 10 mm of 41.7 Gy.

<table>
<thead>
<tr>
<th>Sphere</th>
<th>Nominal</th>
<th>ID2 (α/β = 4)</th>
<th>ID2 (α/β = 2)</th>
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<tr>
<td>Surface</td>
<td>33.3</td>
<td>40.7</td>
<td>44.4</td>
</tr>
<tr>
<td>At 10 mm</td>
<td>17</td>
<td>16.2</td>
<td>8.6</td>
</tr>
<tr>
<td>At 20 mm</td>
<td>10.3</td>
<td>15.7</td>
<td>7.5</td>
</tr>
<tr>
<td>By 3-D conformal</td>
<td>24</td>
<td>25.7</td>
<td>26.4</td>
</tr>
</tbody>
</table>

Summated dose:

<table>
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<th>Sphere</th>
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<th>ID2 (α/β = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>57.3</td>
<td>68.4</td>
<td>70.8</td>
</tr>
<tr>
<td>At 10 mm</td>
<td>41.0</td>
<td>41.7</td>
<td>35.0</td>
</tr>
<tr>
<td>At 20 mm</td>
<td>34.3</td>
<td>41.4</td>
<td>33.9</td>
</tr>
</tbody>
</table>

Figure 5. — Graph illustrating the tissue specific dose distributions of Table 4. (The combination of 17 Gy given in five days by the MammoSite or similar device, followed by five days of 3-D conformal treatment or whole breast irradiation, 24 Gy in five days, total time 10 days). This shows a clear improvement in dose distribution; compare this dose distribution to that in Figure 3. The tumor killing ability is very nearly 42 Gy in both cases at 10 mm.

2. The skin and rib proximity problem

The recommendation to MammoSite users is to get no closer than 7 mm from the ribs or the skin, as this will lead to dangerously high doses to these structures (also due to the extremely rapid drop of dose with distance and the very high doses on the surface of the applicator). Reports of skin necrosis and rib fractures are on record. Will the combination approach allow the MammoSite type of device to be deployed closer to the skin and the ribcage than the recommended 7 mm?
The Problem with Arm 4 of NSABP 39/RTOG 0413 trial (3-D conformal APBI): The sharp cut-off of dose around a small defined volume of breast irradiated to 38.5 Gy in ten fractions

A combined approach of 3-D conformal therapy and whole breast irradiation could likewise alleviate the problem of the sharp transition between high dose at the edge of a 3-D irradiated volume and the normal breast tissue surrounding this volume which may cause an unsightly “fibrotic, edematous ball”. Here again a combined approach with a dose of 24 Gy delivered in ten fractions by 3-D, followed by 24 Gy in ten fractions wider field or even whole breast irradiation over five days, will have significant benefits. The ID2 for an α/β ratio of 4 Gy will be 50.4 Gy which is normalized and equal to the ID2 for 38.5 Gy 3-D conformal in ten fractions over five days, however, the complication risk may be less because an ultra-sharp cut-off is avoided.

Three-dimensional treatment by intensity modulated radiotherapy (IMRT) may be even better for the 3-D half the treatment protocol, because Saibishkumar et al. [29] showed that planning with IMRT can be so fine that the skin can be designated as an “organ at risk” and thus be spared.

Discussion

Dissatisfaction with the dose distribution around the MammoSite type device may have stimulated the development of a multi-strut device [10] which has a less severe dose drop than a single point source in the center of a balloon and has some ability to adjust and optimize the dose distribution. Some good preliminary data have been reported by Yashar et al. [15]. The claim is that this device may extend the utility of this sort of device to women with smaller breasts. It has been shown however, that by combining the MammoSite and similar devices with 3-D conformal IMRT or even whole breast irradiation, this objective could also be achieved using spherical applicators, plus the added benefit of a reduced risk of complications such as edema, fat necrosis, infection and rib fractures. The clinical results of PBI and APBI to date appear to be reasonable, but the trials are not mature and many are not randomized and controlled. The aim should also be not only to get results equivalent to historical results, but to improve on the older techniques in terms of survival, freedom from recurrence, patient comfort, improved cosmetic results and cost effectiveness.
Mature data cited in Donegan and Spratt [16] quote the first Milan trial (1973-1980) which showed that a “quadrantectomy” followed by 50 Gy radiation plus a 10 Gy “boost” gave results equal to a mastectomy for TNM Stage 1 lesions – the QUART trial. The rate of recurrences or new tumors in the breast was 6.5%, that of mastectomy 2.3% (4.3% less). In the second Milan trial (1985-1987) Veronesi found recurrences in 7.5% vs 2.2% (5.3% less) of patients if tumors up to 2.5 cm were allowed into the trial [4]. It is clear that the larger the tumor, the bigger is the risk of recurrence.

Local recurrence should be treated seriously as salvage mastectomy defeats the object of breast conservation and subjects the patient to additional mutilating procedures. Despite statements that “patients with breast recurrence after surgery fare surprisingly well” by having 5-year survivals of about 50%, Kurz et al. [17, 18] and, Osborne and colleagues [19] found that survivors are inferior to those that remain well. Fischer et al. [20] determined that recurrent breast cancer was associated with a three- to fourfold risk of distant dissemination but considered it to be a sign of existing dissemination rather than its cause. The possibility remains however, that recurrence of breast cancer may offer some cancers a second opportunity to disseminate.

Can some patients with lumpectomy skip radiotherapy? The question is unsettled, but the NSABP trial B06 showed that small tumor size did not prevent substantial recurrences in patients with tumors as small as 1 cm, as 25% of unirradiated patients with tumor diameter < 1 cm developed recurrence by eight years post treatment compared to 10% of radiated patients [21]. It seems that there are many factors determining whether a recurrence will happen or not, but the most important preventive action after lumpectomy is adequate irradiation.

The very low recurrence rates presently reported could be attributed to the stringent selection criteria. In this regard, a recent communication at ASCO 2009 (American Society of Clinical Oncology) by Mauri et al. [22] discussed a meta-analysis of 1,140 patients that had undergone PBI vs patients who had whole breast radiotherapy (WBRT). Women who had PBI were twice as likely to have cancer recur in the same breast and three times as likely to have recurrences in the lymph nodes, yet the survival as well as the development of distant metastases were the same. He also warned that PBI “is still experimental” and that “marketing should not be confused with robust medical evidence”. Also with regard to the ASTRO Guidelines of July 2009, very strict criteria were put for APBI: Age > 60 years, T ≤ 2 cm, margins clear by at least 2 mm, the estrogen receptors should be positive (ER+) lymphovascular invasion must be absent, ductal-carcinoma-in-situ was not allowed, and there should be no sign of multi-centricity. Consensus statements like these have a quasi-legal ring and the advice to oncologists is not to deviate from the recommendations. Likewise Sauter-Bihlet et al. [23] warn that “Partial breast irradiation is still experimental and should be discouraged outside of clinical trials”. The implications of the dose distributions illustrated above support these concerns and emphasize the fact that the trial designs should be optimal and very carefully contemplated.

Cosmesis: Clark et al. reported that 50 Gy of radiotherapy will produce breast edema and progressive shrinkage of the breast, as “frequent and obvious side effects” [24]. Less frequent complications include excessive fibrosis and fat necrosis [25]. The doses employed with the “3-D conformal” arm of the NSABP 39/ RTOG 0413 trial with tumor related doses of 50.4 Gy are therefore capable of causing a localized “fibrotic ball” which may be unsightly.

Clinical experience with PBI and APBI:

MammoSite results

Uncontrolled Trials

Benitez et al. [26] from the Department of Surgery, William Beaumont Hospital reported on 5-year results of the MammoSite RTS balloon brachytherapy (RTS CyTyc Corp, Malborough, MA) From May 2000 to 2001, 70 patients were enrolled in this prospective study; 62% completed the treatment. The catheter could not be implanted in 21.1%; the cavity was “not amenable to balloon placement” in 14.2%, patients were ineligible by criteria in 5.7% and skin spacing was problematic in two, or 2.8%. The infection rate was 9.3%. A seroma occurred in 32.6% of patients. Asymptomatic fat necrosis was seen in 9.3%. Good cosmetic results were seen in 83.3%; 5.6% had serious complications: mastitis and abscess. No local recurrences were seen in for a median follow-up of 5.5 years.

Controlled trials comparing PBI and WBI

Interstitial volume implants, electron therapy and whole breast irradiation compared

Polgár et al. [27] reported on the results of a randomized trial comparing the survival and cosmetic results of breast conserving therapy by PBI by volume implant or electron therapy with conventional WBI. The authors concluded that survival was the same, but cosmesis was better with the HDR–PBI implants than with electron therapy. These results are of interest, but do not address the issue of dose distribution around spherical applicators.

3-D conformal or results from NSABP 39/RTOG 0413

The preliminary results from this randomised, controlled trial by Hepel et al. [28] are given for the first 60 patients. At a mean follow-up period of 15 months, moderate to severe complications occurred in six (10%) patients, mainly subcutaneous fibrosis. This was a rather high complication rate for such a short follow-up period. The authors appeared surprised at the high complication rate so early on in the course of follow-up results. This technique sounds simple, but it requires that the heart, lung, opposite breast and so on must be protected from too large doses, also the skin.

As shown, a combined approach of 3-D conformal radiotherapy in two stages will avoid the sharp cut-off as well as the effects of the large doses per fraction, thus allowing the total dose to be delivered in ten days instead of five, which will have immediate benefits. A simpler option would be to give the non-3-D portion by a larger 3-D conformal field.
i.e., to use a “shrinking field” technique. This would also avoid the sharp edge. Alternatively, whole breast irradiation with two simple tangential fields could be employed.

The combined approach may, in addition, also remove the problems with 3-D conformal radiation, namely large doses of radiation to the heart and lungs, especially with IMRT.

A possible critique of the data presented above is that the $\alpha/\beta$ ratios for breast cancer tissue and that of the late reacting tissues may be similar or identical. This is unlikely, since well differentiated tumors have better prognoses than poorly differentiated tumors with expected differences in radio-sensitivity and different alpha-beta ratios. Even if the late reacting tissue and the breast tumor tissue $\alpha/\beta$ ratios are identical at 4 Gy, then the dark grey column of each graph will reflect the situation and it will not remove the detriment of the dose distribution associated with spherical applicators.

The benefit of small doses per fraction is one of the few lessons to have emerged from the discipline of radiobiology; that other concepts have had a lesser impact reflects perhaps more on the attitude of physicians than on the scientific prowess of the radiobiologist. Radiobiology taught us that there is, in general, no way to escape the detrimental effects of large doses per fraction, and elegant formulae have been developed over time to address the impact of total dose, overall time, and dose per fraction for the completion of a course of radiotherapy. The laws of physics dictate that the dose distribution from point sources within spherical applicators (“balloons”) are bound to be bad.

Conclusions

Despite good results reported from non-randomized trials with the MammoSite type of applicator, it seems prudent to be skeptical. It is likely, based on radiobiological grounds, that there will be substantial volumes of tissue bound to undergo necrosis and fibrosis with the current doses prescribed around spherical applicators. This may lead to infection, poor cosmesis or both. At the distance of 10 mm away from a spherical applicator, the tumoricidal dose is barely sufficient, and beyond this zone, if any malignant cells are about, they are unlikely to be killed. Recurrence rates are higher with APBI, and recurrences are dangerous. The ultimate goal to strive for is a shorter (less than 6 weeks), but more effective course, not too short a course with inferior results. Ten days instead of six weeks seems like a good compromise.

With 3-D conformal therapy, the risk of unsightly localized fibrosis is evident and may be avoided by combining spherical applicators with either whole breast irradiation, “shrinking field” 3-D conformal or IMRT irradiation and by giving the combined dose over ten instead of over five days.

References


Could the eventual results of the NSABP 39/RTOG 0413 trial for partial breast irradiation (PBI) be improved by combining etc.--


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Management of abnormal cytological findings

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Introduction
The intention of this new series of EJGO is to publish the view of experts in controversial issues. The major aim is to find out the personal approaches rather than reaching consensus. Only a few personal comments will be made. The first set of questions and answers are related to cervical cancer screening, including management of abnormal findings.

1) Atypical squamous cells of undetermined significance (ASC-US) smear

Your patient with ASC-US is 21 years old. What is your first step in management?

Bornstein: Repeat cytology in six months.
Jones: Do reflex HPV testing. Colposcopy only if high-risk HPV+.
Leeson: Colposcopy and punch biopsy of any abnormality.
Ng: Repeat Pap in six months; if the repeat smear is ASC-US/LSIL, routine screening every six months instead of one year for two years.

What is your approach if:

a) satisfactory colposcopy, no abnormal findings

Bornstein: Repeat cytology in six months.
Jones: Repeat cytology in 12 months.
Leeson: Repeat cytology in six months.
Prendiville: I would reassure the patient and advise a repeat smear in two or three years.

b) satisfactory colposcopy, fine mosaic pattern and punctuation with smooth surface and slight aceto-whiteness

Bornstein: Cervical biopsy.
Jones: No biopsy, repeat cytology in one year.
Leeson: Multiple punch biopsies.
Prendiville: I would reassure the patient and advise a follow-up smear in one year and a follow-up colposcopic examination or consultation in 15 to 18 months.

c) satisfactory colposcopy, high-grade findings, but no atypical vessels

Bornstein: Cervical biopsy.
Jones: Colposcopically directed biopsy.
Leeson: As above in such a young woman.
Prendiville: If I suspected CIN3, I would take a colposcopically directed biopsy.

d) unsatisfactory colposcopy

Bornstein: Repeat cytology in six months.
Jones: Minor lesion externally - cytology in 12 months; high-grade lesion externally - biopsy and endocervical curettage (ECC).
Leeson: Six weeks local estrogen and repeat colposcopy.
Prendiville: Why, do you mean because the transformation zone (TZ) is type 3 or infective or for some other reason? Whatever the situation I would try to correct it and I would try very hard not to treat this young girl.
Your patient with ASC-US is a 37-year-old. What is your first step in management?
Bornstein: HPV test.
Jones: Refer to colposcopy.
Leeson: As above.
Ng: Repeat Pap in six months; if the repeat smear is ASC-US/LSIL, routine screening every six months instead of one year for two years.

What is your approach if:
a) satisfactory colposcopy, no abnormal findings
Bornstein: HPV test and ECC.
Jones: Repeat cytology in 12 months.
Leeson: As above.
Prendiville: Reassure, probably do a hybrid capture 2 (HC2) but either way, reassure with follow-up cytology, if HC2 is not available, in one year.

b) satisfactory colposcopy, fine mosaic pattern and punctation with smooth surface and slight acetowhiteness
Bornstein: Cervical biopsy.
Jones: Colposcopically directed biopsy.
Leeson: As above.
Prendiville: Probably similar approach to above.

c) high-grade findings, but no atypical vessels
Bornstein: Cervical biopsy.
Jones: Colposcopically directed biopsy.
Leeson: Discuss with patient but may consider loop excision. Alternative – multiple punch biopsies.
Prendiville: If I suspected a CIN2+ lesion I would have a low threshold for LLETZ. I might take a directed biopsy, if there was a clear area of CIN2+.

d) unsatisfactory colposcopy
Bornstein: Colposcopy-directed biopsy and ECC.
Jones: Biopsy any external lesion and ECC.
Leeson: Six weeks local estrogen as above and reexamine.
Prendiville: Why, do you mean because the TZ is type 3 or infective or for some other reason? Whatever the situation I would try to correct it and I would try very hard not to treat this woman.

2. Low-grade squamous epithelial lesion (LSIL) smear
Your patient with a LSIL is 21 years old. What is your first step in management?
Bornstein: Colposcopy.
Jones: Refer to colposcopy.
Leeson: Repeat cytology in six months.
Ng: Repeat Pap in six months; if the repeat smear is ASCUS/LSIL, routine screening every six months instead of one year for two years.

What is your approach if:
a) satisfactory colposcopy, no abnormal findings
Bornstein: ECC.
Jones: Repeat cytology in 12 months.
Leeson: If a patient has persistent low-grade changes only then would I perform colposcopy. Then in this situation I would arrange repeat cytology in six months.
Prendiville: Reassure; Pap in one or two years.

b) satisfactory colposcopy, fine mosaic pattern and punctation with smooth surface and slight acetowhiteness
Bornstein: Cervical biopsy, ECC.
Jones: No biopsy, repeat cytology in one year.
Leeson: Multiple punch biopsies.
Prendiville: Reassure; Pap in one or two years.

c) pronounced atypical changes, but no atypical vessels
Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Multiple punch biopsies.
Prendiville: If I suspect CIN 2 then I would still try not to treat her and might take a colposcopically directed biopsy but might also just follow her in the short term.

d) unsatisfactory colposcopy
Bornstein: Colposcopy-directed biopsy and ECC.
Jones: Minor lesion externally – cytology in 12 months. High-risk grade lesion externally – biopsy and ECC.
Leeson: Six weeks local estrogen and reexamine.
Prendiville: As before, try to work out why the colposcopy is unsatisfactory – it’s very rarely a permanent problem in a young 21-year-old.

Your patient with LSIL is 37 years old. What is your first step in management?
Bornstein: Colposcopy.
Jones: Refer to colposcopy.
Leeson: Six weeks local estrogen and reexamine.
Ng: Repeat Pap in six months, if the repeat smear is ASC-US/LSIL, routine screening every six months instead of one year for two years.

What is your approach if:
a) satisfactory colposcopy, no abnormal findings
Bornstein: ECC.
Jones: HPV testing in 12 months.
Leeson: Repeat cytology in six months.
Prendiville: Reassure routine cytology follow-up.

b) satisfactory colposcopy, fine mosaic pattern and punctuation with smooth surface and slight acetowhiteness
Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Multiple punch biopsies.
Prendiville: Reassure routine cytology follow-up, follow-up colposcopy in about a year.

c) pronounced atypical changes, but no atypical vessels
Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Multiple punch biopsies.
Prendiville: Depends a little on parity and future fertility aspirations but I would have a low threshold for treatment if I suspected CIN 2+.

d) unsatisfactory colposcopy
Bornstein: Colposcopy-directed biopsy, If no lesions - random cervical biopsies, ECC.
Jones: Biopsy any external lesion and ECC.
Leeson: Six weeks local estrogen and reexamine.
Prendiville: Correct the reason for unsatisfactory colposcopy and repeat the exam.

3. High-grade squamous epithelial lesion (HSIL) smear

Your patient with HSIL is 21 years old. What is your first step in management?
Bornstein: Colposcopy.
Jones: Refer to colposcopy.
Leeson: Colposcopy.
Ng: Refer to colposcopy.

What is your approach if:
a) satisfactory colposcopy, no abnormal findings
Bornstein: Review cytology, random cervical biopsies and ECC.
Jones: Review cytology with pathologist. If confirmed high-grade, repeat cytology and colposcopy in four to six months. (This assumes good colposcopy of the cervix, vagina and introitus by an experienced colposcopist. Otherwise consider immediate referral to an expert colposcopist).
Leeson: Colposcopy and refer to the colposcopic multidisciplinary team (MDT).
Ng: Biopsy and HPV testing.
Prendiville: Review cytology, repeat colposcopy.
b) satisfactory colposcopy, fine mosaic pattern and punctation with smooth surface and slight acetowhiteness

Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Colposcopy and multiple punch biopsy.
Ng: Biopsy and HPV testing.
Prendiville: Take directed biopsy and review cytology.

c) pronounced atypical changes, but no atypical vessels

Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Loop excision.
Ng: Biopsy and HPV testing.
Prendiville: Take directed biopsy and review cytology.

d) unsatisfactory colposcopy

Bornstein: Random cervical biopsies, ECC.
Jones: Biopsy any external lesion and ECC.
Leeson: If abnormality is suggestive of high-grade disease then loop. If not then give six weeks local estrogen and refer to colposcopic MDT with a view to repeat the colposcopy.
Ng: Biopsy, ECC, and HPV testing.

Your patient with HSIL is 37 years old. What is your first step in management?

Bornstein: Colposcopy.
Jones: Refer to colposcopy.
Leeson: Loop excision.
Ng: Refer to colposcopy.

What is your approach if:

a) satisfactory colposcopy, no abnormal findings

Bornstein: Review cytology, then random cervical biopsies and ECC.
Jones: Review cytology with a pathologist. If confirmed high-grade, repeat cytology and colposcopy in four to six months. (This assumes good colposcopy of the cervix, vagina and introitus by an experienced colposcopist. Otherwise consider immediate referral to an expert colposcopist).
Leeson: Loop excision.
Ng: Excise the abnormal area.
Prendiville: Review cytology; if cytology opinion persists in suspecting high-grade disease and the vagina is normal then I would do a LLETZ.

b) satisfactory colposcopy, fine mosaic pattern and punctation with smooth surface and slight acetowhiteness

Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Loop excision.
Ng: Excise the abnormal area.
Prendiville: LLETZ.

c) pronounced atypical changes, but no atypical vessels

Bornstein: Cervical biopsy, ECC.
Jones: Colposcopically directed biopsy.
Leeson: Loop excision.
Ng: Excise the abnormal area.
Prendiville: LLETZ.

d) unsatisfactory colposcopy

Bornstein: Random cervical biopsies and ECC.
Jones: Biopsy any external lesion and ECC.
Leeson: Loop excision.
Ng: Excise the abnormal area and ECC.
Prendiville: Correct the unsatisfactory colposcopy with LLETZ or LLETZ cone.
4. Is your policy the same in case of atypical squamous cells possible high lesion (ASC-H) smear as with HSIL? If not what is the difference?

Bornstein: Same policy.
Jones: No, manage ASC-H like LSIL. Epidemiology is the same as LSIL.
Leeson: No. Manage similar to LSIL with punch biopsies if abnormality and MDT review if normal. If high-grade abnormality for loop.
Ng: Colposcopy and biopsy if needed and HPV testing.
Prendiville: Completely different; depends on the colposcopic findings, my cytologist and how well I know him or her, etc.

5. Atypical glandular cells (AGC)

Your patient with AGC is 21 years old. What is your first step in management?

Bornstein: Colposcopy, ECC.
Jones: Refer to colposcopy.
Leeson: Unusual. If no abnormality at colposcopy for the colposcopic MDT, check finding and if confirmed perform loop.
Ng: Refer to colposcopy.

What is your approach if:

a) satisfactory colposcopy, no abnormal findings

Bornstein: ECC.
Jones: Repeat cytology in 12 months.
Leeson: If 21 then after MDT confirmation for loop.
Ng: Biopsy and ECC.
Prendiville: Reassure, repeat cytology review.

b) satisfactory colposcopy, fine mosaic pattern and punctation with smooth surface and slight acetowhiteness

Bornstein: Cervical biopsy and ECC.
Jones: Repeat cytology in 12 months.
Leeson: Loop excision.
Ng: Biopsy and ECC.
Prendiville: Reassure, repeat cytology, review.

c) pronounced atypical changes, but no atypical vessels

Bornstein: Cervical biopsy, ECC.
Jones: Biopsy external lesion and ECC.
Leeson: Loop excision.
Ng: Biopsy and ECC.
Prendiville: Where are these changes - in the TZ or above it? I would try not to treat this young girl.

d) unsatisfactory colposcopy

Bornstein: Biopsy cervical lesions and ECC.
Jones: Biopsy any external lesion and ECC.
Leeson: If 21 then after MDT confirmation for loop.
Ng: Cone biopsy and ECC. If the histology of the cone biopsy is AIS and/or CIN3 or carcinoma Stage A1, what is your further strategy? Observe if the margin is free, wide excision if the margin is not free.
Prendiville: Correct the reason for unsatisfactory colposcopy.

Your patient with AGC is 37 years old. What is your first step in management?

Bornstein: Colposcopy, ECC, endometrial biopsy.
Jones: Refer to colposcopy.
Leeson: Colposcopy and loop.
Ng: Refer to colposcopy.
What is your approach if:

a) satisfactory colposcopy, no abnormal findings

**Bornstein:** ECC and endometrial biopsy.
**Jones:** ECC and endometrial biopsy.
**Ng:** Cone biopsy, ECC, and endometrium curettage.
**Prendiville:** I would consult with the cytologist grade of AGC, site of suspected abnormality.

b) satisfactory colposcopy, fine mosaic pattern and punctuation with smooth surface and slight acetowhiteness

**Bornstein:** Cervical biopsy, ECC and endometrial biopsy.
**Jones:** Colposcopically directed biopsy of external lesion, ECC and endometrial biopsy.
**Leeson:** Loop excision.
**Ng:** Cone biopsy, ECC, and endometrium curettage.
**Prendiville:** I would consult with the cytologist grade of AGC, site of suspected abnormality.

c) pronounced atypical changes, but no atypical vessels

**Bornstein:** Cervical biopsy, ECC, consider conization, endometrial biopsy.
**Jones:** Colposcopically directed biopsy of external lesion, ECC and endometrial biopsy.
**Leeson:** Loop excision.
**Ng:** Cone biopsy, ECC, and endometrium curettage.
**Prendiville:** If I suspected high-grade glandular abnormality I would do a cone biopsy using a LLETZ or straight-wire excision (SWETZ) technique.

d) unsatisfactory colposcopy

**Bornstein:** Biopsy cervical lesions if they exist, ECC, endometrial biopsy.
**Jones:** Colposcopically directed biopsy of the external lesion, ECC and endometrial biopsy.
**Leeson:** Loop excision.
**Ng:** Cone biopsy, ECC, and endometrium curettage. If the histology of cone biopsy is AIS and/or CIN3, a further strategy would be to observe if the margin is free - cervical amputation or hysterectomy if the margin is not free.
**Prendiville:** I would correct the reason for the unsatisfactory colposcopy.

The way I do colposcopically directed biopsy and ECC

**Bornstein:** Explain to the patient and make sure the pain is minimal so she does not refuse to return. If needed, inject local anesthetic. Do the posterior lip biopsies first. Gentle with ECC. Apply Silver-Nitrate to stop bleeding. The technique itself is standard.

**Jones:** I usually do the colposcopy-directed biopsy first unless I really suspect an endocervical lesion. Then I would do the ECC first. My cervical biopsy technique is standard. I use Burke or Tischler biopsy instrument. For the ECC I use a small Kevorkian or Townsend endocervical curette with a basket.

**Ng:** ECC to scrape lightly on the inner cervix using a brush.

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Hyperthermic intraperitoneal chemotherapy added to the treatment of ovarian cancer. A review of achieved results and complications

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Summary

Objective: The late revelation of ovarian cancer ensures it as the leading cause of death among gynecologic cancers. Cytoreductive surgery (CRS) and intravenous (IV) chemotherapy have been the cornerstone for a long time to treat this disease. More recently, the modality of intraperitoneal administration of chemotherapy under hyperthermic conditions (HIPEC) has been added. This review surveys the results of HIPEC added to CRS in ovarian cancer. Methods: A multi-database search was conducted focusing on mortality, morbidity and overall and disease-free (DF) survival rates. Results: 16 studies were identified reporting the results of CRS followed by HIPEC of 546 patients with advanced ovarian cancer. Postoperative mortality was reported for 14 out of 481 patients in total (2.9%). The major morbidity rate varied between 3.4 and 50.0%. In all but one study (533 patients), 185 events were reported (34.5%) and 21 re-interventions after 476 operations (4.4%). Survival data ranged from 10.0 to 57.1 months for the DF survival and from 19.0 to 76.1 months for the overall survival. Optimal cytoreduction and recurrent disease were associated with a better outcome in selected cases. Conclusions: Adding HIPEC to the current treatment modalities for ovarian cancer seems to be feasible. Improved survival rates have been reported at the cost of acceptable mortality rates. Nevertheless, there was a selection bias, the morbidity should not be underestimated and it is unclear yet which patient will benefit most from this treatment. Randomized controlled trials will provide an answer to this question.

Key words:
2. To determine if there are some patients with ovarian cancer who are more or less likely to benefit from this treatment.

Patients and Methods

A search was conducted in PubMed, Embase, CINAHL and the Cochrane Library to identify articles reporting results of HIPEC in ovarian cancer treatment. An example of the search in PubMed is given.

#1 Search “Ovarian Neoplasms” [Mesh]
#2 Search ovar* cancer
#3 Search ovarian
#4 Search cancer
#5 Search peritoneal carcinomatosis
#6 Search #1 OR #2 OR (#3 AND (#4 OR #5))
#7 Search “Hyperthermia, Induced” [Mesh]
#8 Search Hypertherm*
#9 Search heated
#10 Search #7 OR #8 OR #9
#11 Search #6 AND #10

The search was carried out from 1998 until October 2008. Only studies in the English language with an abstract were incorporated in the analysis. All types of study designs were included. Studies of primary and recurrent ovarian cancer were included if results in terms of mortality, morbidity or survival were reported separately. Studies with less than ten patients were excluded as well as pure-dose finding studies. The bibliography of all selected articles was hand searched to identify additional articles that met the inclusion criteria.

The published version of each article was then analyzed by two authors. In case of incongruity a third author was consulted. The following data were extracted if reported: type of study, duration of follow-up, criteria used to define cytoreduction, and the percentage of patients successfully cytoreduced, major morbidity and mortality. A meta-analysis was not appropriate due to heterogeneity of patient populations and differences in treatment regimens.

Results

Sixteen studies met the inclusion criteria and were identified after review of 270 abstracts retrieved from the search. A total of 546 patients were included in the studies which were published between 2001 and March 2008. Mean age of patients varied from 44 to 65 years and patients had primary advanced, persistent or recurrent ovarian cancer. Mean follow-up time ranged from 13.7 to 73 months (Table 1).

Survival

Reported mean or median survival ranged from 19.0 to 76.1 month. Five-year survival and mean or median DF survival varied from 15.0 to 63.4% and from 10.0 to 57.1 months, respectively (Table 2).

Completeness of cytoreduction-specific survival was reported in seven of the 16 articles (Table 3). In most articles the completeness of cytoreduction (CC) was scored as proposed by Sugarbaker [30]: CC0: no residual disease; CC1: residual disease less than 2.5 mm; CC2: residual nodules between 2.5 mm and 2.5 cm; CC3: residual disease greater than 2.5 cm. Optimal cytoreduction is defined as CC0 or CC1. Mean and median survival was longer in patients in whom optimal cytoreduction was achieved.

Analysis of the difference between survival of primary and recurrent disease was given in five articles (Table 4).
There is clinical heterogeneity between the studies. Not only in definitions of recurrent disease also in the variety of additional treatments. No relevant evidence was found showing that HIPEC is more efficient in one of the two groups. However, a tendency was found suggesting that HIPEC might be more efficient in the treatment of recurrent ovarian cancer.

**Mortality**

Mortality rates within 30 days after the surgery varied from 0.0 to 10.5%. Causes of death were pulmonary embolism, anastomotic leakage, sepsis, myocardial infarction, renal failure and diffuse intravascular coagulation (Table 5). In total, postoperative mortality was reported for 14 out of 481 patients (2.9%).

**Major morbidity**

Major morbidity rates ranged from 3.4-50.0% whereas in most studies the number of events of major morbidity was counted and not the number of patients experiencing one or more types of major morbidity. In total 184 events were reported for 533 patients (34.5%). Types of morbidity are further outlined in Table 6. Re-operation was required in 0.0 to 16.6% of patients and in total 21 times for 476 patients (4.4%). The reported mean or median hospital stay varied from 8-25 days.

**Discussion**

The results presented in this review suggest that administration of chemotherapy intraperitoneally under hyperthermic conditions may further improve survival of patients with advanced ovarian cancer. Our findings report a mean DF survival and a mean overall survival ranging from 15.0 to 63.4 and from 19.0 to 76.1 months, respectively. Included were patients with primary advanced and recurrent ovarian cancer. The extent of benefit could not be extracted due to the heterogeneous designs of the individual trials. For the same reason it is not clear which patient would benefit most from this treatment modality.

The rationale for the idea that IP administration of chemotherapy is associated with an increased survival rate lies in its direct cytotoxic effect. Presumably intra-
venous therapy reaches tumors through their vascular beds whereas the IP component diffuses through the surface of peritoneal disease. Adding the component of hyperthermia is thought to be even more beneficial as hyperthermia enhances drug penetration into the tumor cells and has direct cytotoxic effects itself [31].

The treatment regimen of unheated cyclic IP chemotherapy through a catheter has already been shown superior to IV chemotherapy in a meta-analysis of seven RCTs with a hazard ratio of 0.8 [13]. However, there has been some criticism about these results arguing that the benefit that is seen in the IP-arm may be misleading as some of the trials included in the meta-analysis, compared IP chemotherapy to a control arm which is at present not the standard anymore as no taxanes were included. It remains difficult to set a standard survival rate for advanced ovarian cancer following the current standard treatment regimen of CRS and IP chemotherapy. Bristow et al. [32] report a mean overall survival of 30.3 (10-62) months for patients with recurrent ovarian cancer that were treated with cytoreductive surgery and IV systemic chemotherapy. Studies specifying a disease-free interval of at least six months prior to attempted cytoreductive surgery for recurrent ovarian cancer were included. In a recently updated review [33] on primary advanced ovarian cancer, the treatment regimen of CRS and IP chemotherapy resulted in a mean overall survival of 25 (23-27) months and a median DF survival of 14.2 months.

The results of the present review on both primary and recurrent disease tend to show longer survival rates than the above-mentioned reviews. Undoubtedly, patient selection has influenced these results, however, they show the possibilities of this treatment adjustment.

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<th>Author</th>
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perioperative mortality rate varied between 0 and 10.5%. Regarding the total of 14 out of 481 patient (2.9%) this is comparable to a mortality rate of 3.7% after primary cytoreductive surgery for advanced stages recently found in a systematic review [34]. Therefore, it seems reasonable to attempt to integrate heated intraperitoneal chemotherapy into the management of ovarian cancer.

Previously, Bijelic et al. [10] reported comparable results on this topic in their review. They ended their search in 2006 and analyzed 14 studies of which eight [17-20, 23-25] were also included in our study. In the present review, we added more recent studies [26-29] with larger patient groups and focused on morbidity as well. In here, slightly more positive results in terms of overall and DF survival (19.0-76.1 and 15.0-63.4 vs 22.0-54.0 and 10-26.0 months) were found implying that the effects of heated IP chemotherapy could be effective.

It is still a controversial issue which patients are most likely to benefit from HIPEC. The importance of optimal debulking surgery is well known from other studies with (heated) IP chemotherapy as optimal cytoreduction has been proven to be the most important positive prognostic factor. Therefore, the administration of heated IP chemotherapy may be less effective in patients in whom optimal debulking cannot be achieved. In this review we analyzed seven studies that reported a CC-specific survival. Consistent with previous findings, we found that overall survival was roughly better in patients with optimal cytoreduction compared to patients with suboptimal debulking (13.0-54.9 vs 5.1-25.0 months).

Interestingly, we showed that there might be a tendency towards recurrent disease to be more likely to benefit from this treatment. Only five studies reported results from primary and recurrent advanced ovarian cancer separately. These results showed a longer disease-free period after surgery in patients with recurrent disease in comparison to patients with primary advanced disease. The mean progression-free interval was not stated in all studies. Mostly it exceeded the period of six months as this period differs between chemo-resistant or not. A part of the primary advanced stages treated could have been chemo-resistant which might be an explanation for the tendency for longer disease-free survival for recurrences. Future studies should include the disease-free interval to sustain the extent of sensitivity for chemotherapy.

With regard to morbidity of IP administration it is argued that it is associated with an almost unacceptable high, mostly catheter-related rate of major morbidity. However, we also report major morbidity rates ranging from 3.4 to 50.0% for HIPEC without the use of catheters. It is difficult to interpret with this wide range. Rarely was a common toxicity criteria grade noted, postoperative complications were not graded at all and patient and event numbers were reported. Nevertheless, an impression is given with up to 184 reported events in 533 patients (34.5%). This high rate of morbidity is probably an overestimation due to the fact that in most studies the amount of events was counted rather than the number of patients experiencing major morbidity. Furthermore, the great variety may be a result of different definitions for major morbidity by the different authors. A great diversity in treatment regimens, the experience of the surgeon and the extent of the surgical procedure may also contribute to the wide range.

In summary, the addition of HIPEC to the modalities of treatment for ovarian cancer seems to be feasible. Good results in survival have been reported at the cost of reasonable mortality rates. Nevertheless, there was a selection bias, the morbidity should not be underestimated and it is unclear yet which patient will benefit most from this treatment. As stated by Markman recently [35], a randomized trial is needed to define whether OVIPEIC improves survival of patients with ovarian cancer and which patients benefit most. Such a trial is currently being carried out at the Netherlands Cancer Institute, which randomizes patients during interval debulking between surgery alone or surgery plus OVHIPEC. At present nine centers in various countries are participating in this trial. The results of that study will clarify the actual survival benefit that derives from the combination of maximum surgical treatment in combination with administration of intraperitoneal chemotherapy under hyperthermic conditions.

References

Hyperthermic intraperitoneal chemotherapy added to the treatment of ovarian cancer. A review of achieved results and complications


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Hormone therapy/adjuvant chemotherapy induced deleterious effects on the bone mass of breast cancer patients and the intervention of physiotherapy: a literature review

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Summary
In recent years, breast cancer has witnessed some notable improvements regarding early diagnosis and new therapeutical strategies, mainly because of the utilization of new drugs and systemic treatment protocols, which have had a direct impact in the increase of these patients' global survival rate. At the same time, it is an ever-growing concern among oncology professionals to identify and minimize as much as possible the effects of long-term toxicity resulting from cancer therapies. Within this context, physiotherapy fits as a preventive and rehabilitating factor regarding functional and skeletal alterations, deriving not only from the direct action of breast cancer, but also from the treatment to which these patients are submitted. Objectives: The aim of this study was to revise the scientific literature on possible adjuvant chemotherapy-induced secondary deleterious effects on the bone mass of patients diagnosed with breast cancer, and also to revise the literature on the intervention of physiotherapy in cases of secondary bone mass loss caused by adjuvant chemotherapy in patients suffering from breast cancer. Methodology: The research was carried out by consulting the following medical websites: Medicus Medline Index, Lilacs, Scielo, PubMed (National Library of Medicine), Google Academic and Capes (a Brazilian website for scientific information). The selection gathers articles written in different languages, English in special, published from January 1998 to October 2008. Results: 24 studies explicitly mention chemotherapy-induced direct and/or indirect effects upon bone mass. Different authors refer to bone mass loss as one possible secondary deleterious effect resulting from adjuvant chemotherapy applied in breast cancer treatment. Nonetheless, no scientific articles were found on the subject of physiotherapy intervention aimed at patients in this specific condition. Conclusion: the results achieved in this revision study point out the possible chemotherapy-induced late deleterious effects on patients diagnosed with breast cancer, as well as the additional risks for the development of further osteoporotic conditions. Hormone therapy and adjuvant chemotherapy treatments may in fact augment and accelerate the loss of bone mass, be it directly, through the action of chemotherapeutical drugs, or indirectly, through the reduction of estrogenic levels and precocious menopause. The scarce material on the rehabilitation of bone mass loss deriving from adjuvant treatments reveals, as it seems, a strong need for new studies on the subject.

Key words: Breast cancer; Physiotherapy; Hormone therapy; Adjuvant chemotherapy; Osteoporosis; Bone mass.

Introduction
Occurrences of malignant neoplasias are a Brazilian and a worldwide spreading phenomenon. At the same time, the treatment of oncological patients itself has witnessed some notable improvements, originating from new approaches and from more accurate cancer prognoses. Among all malignant tumors, breast disease is the second most frequent type of neoplasia all over the world, totaling 22% of new occurrences of women’s cancer [1]. Recent reports from the INCA (Instituto Nacional do Câncer) regarding new cases of breast cancer in Brazil, show that occurrences amounted to 49,000 cases in 2008, with an expected risk of 51 cases for every 100,000 women [1]. The global survival rate of women diagnosed with breast cancer has been augmenting considerably in recent years, mainly due to early diagnosis and more efficient therapies aimed at cancer treatment [2]. Nevertheless, patients who have survived breast cancer manifest greater risks of developing chronic-degenerative diseases, such as osteopenia and osteoporosis, induced by the secondary action of antineoplastic drugs (combined in the chemotherapy process) on the bone mass, and by the absence of hormonal reposition schemes during menopause [3]. Osteoporosis is today considered, especially in developed countries, one of the most serious problems to affect the elderly population, chiefly women in the postmenopause period. It is characterized by low bone density and the subsequent degeneration of the microstructure, responsible as it is for increased bone fragility, which may result in the occurrence of fractures. Estimations show that one in every two women may suffer at least one osteoporotic fracture during their lifetime, which makes us more concerned about public health [4]. Moreover, the occurrences of osteoporosis are rising not only among the elderly population, as a result of the natural aging process, but also among those who have overcome cancer in their lives, chiefly breast cancer, as a result of their crescent survival rate, and also the chemotherapy-induced secondary effects which many of them are subjected to [5].
The female population is exposed to a greater risk of developing long-term osteoporosis, especially in the postmenopause stage of life, due to the systemic reduction of estrogen levels. Notwithstanding, if subjected to adjuvant chemotherapeutic treatment, patients who have overcome breast aplasia develop an additional factor which favors the pathological evolution, even before menopause. Some of the substances employed in the adjuvant chemotherapeutic schemes induce ovarian failure and the anticipation of menopause [3]. This paper aims at revising the medical literature produced throughout the last decade, in order to identify the possible secondary effects deriving from adjuvant antineoplastic chemotherapy on the bone mass of patients diagnosed with breast cancer. The goal is to discuss the potential risks, regarding this specific population, of future osteoporotic developments, and the consequences implied in this morbidity: the increase of bone fragility and fracture risks. Most of these fractures will result in several skeletal alterations, such as deformities and stature reduction, accompanied by serious – at times chronic – algetic processes, disability, hospital commitments and even death [6].

Method

This study was based on revision of the recent literature concerning chemotheraphy-induced deleterious effects on the bone mass of patients with breast cancer. The primary criterion observed was to select the sources of information: Medicus Medline Index, Lilacs, Sciello, PubMed (National Library of Medicine), Google Academic and Capes (a Brazilian website for scientific information). In their respective search-engines, the following keywords were used: physiotherapy, cancer, breast cancer, adjuvant chemotherapy, bone loss, secondary effects, osteopenia, hormone therapy, fatigue, and bone densitometry. The selection encompasses articles written in different languages published from January 1998 to October 2008.

The criteria of inclusion were: female patients diagnosed with breast cancer; patients with breast cancer subjected to adjuvant chemotherapy; women in the pre-, peri-, and postmenopause periods, regardless of their age; physiotherapy and physical exercise programs for osteoporosis; physiotherapy and/or physical activity for patients suffering from breast cancer and subjected to adjuvant chemotherapy treatments. Patients diagnosed with breast cancer but not subjected to adjuvant chemotherapy treatments were not considered. Thirty articles, all of which follow these criteria, were gathered.

Results

In this literature review we selected 24 studies approaching the proposed subject, with explicit references to direct or indirect chemotheraphy-induced effects on the bone mass of patients. Different authors point out, among the possible deleterious effects induced by adjuvant chemotherapy, the diminution of bone mass in patients with breast cancer. Twenty-four of these studies made explicit reference to direct and indirect effects induced by chemotherapy on the bone mass of patients [5, 6, 14-32, 34, 43, 44]. No specific references were identified, however, on the physiotherapeutic rehabilitation of breast cancer patients who experience bone mass diminution. Furthermore, no scientific studies were found that mention physiotherapeutic intervention in patients going through adjuvant chemotherapy and who manifest bone mass diminution.

Discussion

Intending not only to improve the local control of the disease, but also to augment the survival rate of patients with breast cancer, several clinical tests based on neoadjuvant chemotherapy took place as a means of treatment, at times followed by radiotherapy [7].

The main goal of adjuvant chemotherapy is to diminish the chances of local and systemic relapse of the cancer, through the long-range elimination of micrometastasis, since it is a matter of clinically occult microscopic focus of the disease [7, 8].

The understanding of molecular and biological processes concerning breast cancer has produced some major improvements regarding therapeutic intervention strategies, such as the introduction of novel antineoplastic agents and the reformulation of breast cancer-aimed systemic treatment protocols. Such strategies have optimized the interaction among systemic, radiotherapeutic and surgical treatments, increasing the disease-free period as well as the global survival rate [9, 10].

Today, it can be assumed that adjuvant systemic treatment is indicated for each and every patient who conforms to “average and high risks” [9-11]. Table 1 below features the most commonly employed chemotherapy schemes aimed at breast cancer.

| Table 1. — Chemotherapy schemes applicable in breast cancer. |
| CMF - cyclophosphamide, methotrexate, 5-fluorouracil (5FU) |
| FAC/CAF-5FU, doxorubicin (adriamycin), cyclophosphamide |
| CMF±VP - cyclophosphamide, methotrexate, 5FU, vincristine, prednisone |
| AC - doxorubicin, cyclophosphamide with or without sequential paclitaxel |
| AC-CMF - doxorubicin, cyclophosphamide/cyclophosphamide, methotrextate 5FM |
| AC-T - doxorubicin, cyclophosphamide, paclitaxel (Taxol) or docetaxel |

More recently, in the sphere of clinical oncology, hormone therapy came to aid the adjuvant treatment of women suffering from invasive breast cancer, being prescribed exclusively or sequentially in the chemotherapeutic treatment of women (either pre or postmenopausal), “whose tumors manifest the presence of hormonal receptors for estrogen and/or progesterone” [9].

The goal of this kind of therapy is to saturate the estrogen receptors located in the cancerous cell, preventing – through multiple events – cell duplication induced by the action of estradiol. Such pharmacons are called selective estrogen receptor modulators (SERMs). SERMs induce
estrogenic agonism on specific tissues (such as the bone and liver) while acting antagonistically on breast and uterine tissues.

Among the most commonly adopted pharmacons are tamoxifen, raloxifene and aromatase inhibitors [12]. Tamoxifen’s performance is equivalent to 70% of estrogen’s action in terms of bone mass increase. According to Murrad [10], “tamoxifen is a non-steroid antiestrogenic featuring agonistic and antagonistic properties that prevent the linkage between estradiol and estrogen receptors, being considered the standard pharmacon in hormone therapy”. Furthermore, it can be prescribed to women either in the pre- or postmenopause period for a 5-year term.

Ovarian ablation (surgical, radiotherapeutical, or chemical oophorectomy accompanied by LH-Rh antagonistic substances, such as gosereline or leuprolide) has also been prescribed, isolatedly or associated to tamoxifen, to women in the premenopause period, having achieved some notable improvements on their survival rate [10].

As for women in the post-menopause phase, it has been used for endocrinal therapy purposes, either tamoxifen or aromatase inhibitors, such as letrozole (Femara), anastrozol (Arimidex) and exemestane. Aromatase inhibitors prevent the conversion of testosterone and androstenedione (adrenal androgens) into estradiol and estrone on these patients’ tissue level. However, they should not be utilized in women who manifest ongoing ovarian function, since they do not block the estrogen and the progesterone produced by the ovaries [7, 13].

Recently achieved improvements concerning early diagnosis and new possibilities on cancer treatment have had a direct impact on the patient survival rate. Consequently, it has become an ever-growing concern among oncology professionals to identify and minimize long-term toxicity effects induced by antineoplastic therapies. Cancer-treatment-induced bone loss (CTIBL) is a well-known late effect that manifests itself in a large number of breast cancer patients. Antineoplastic therapies, such as chemotherapy, radiotherapy, hormone therapy and surgical castration, may cause direct or indirect bone damage, inducing additional bone mass loss and, at times, anticipating and intensifying osteopenia and osteoporosis conditions. The primary causes of CTIBL are chemotherapy-induced, radiotherapy-induced, hormone-therapy-induced (SERMs and aromatase inhibitors) and surgical-castration-induced (oophorectomy) hypogonadism. Other factors directly or indirectly linked to decreased bone mass are physical inactivity and inadequate ingestion of calcium and vitamin D5 [14-21]. Thus, one can assume that bone loss occurs more rapidly and more acutely in women going through chemotherapy than healthy women of the same age. That is to say, adjuvant chemotherapeutic treatment is an additional risk factor for osteoporosis that should not be underestimated, considering that it adds to the genetic and constitutional ones, such as race and low body mass rate, estrogenic deficit and lifestyle, to name a few. Different studies assert that chemotherapy-induced effects on the gonadal hormones are the most common causes of bone mass loss in women suffering from breast cancer in the premenopause period, since the treatment schemes that include cyclophosphamide (FAC, CMF, AC) and/or taxanes damage the ovaries, drastically diminishing estrogen levels and thus inducing precocious menopause [19, 22-24].

Ramaswamy and Shapiro [23] confirm that various antineoplastic drugs applied to breast cancer treatment have a straight impact on bone loss, independent of their effects on gonadal hormones. Among those are methotrexate, cyclophosphamide, ifosfamide and doxorubicin. From the tests conducted on animals, it has been ascertained that methotrexate increases bone resorption and decreases its formation, leading to intense bone loss. This particular drug reduces the production of osteoblasts through the inhibiting mechanism of DNA synthesis, just as it seems to debilitate the bone’s mineralizing matrix. Cyclophosphamide and its metabolites prevent both bone formation and resorption, while keeping osteoblast and osteoclast cells from dividing, thus leading to their shortening on the bone’s surface. According to these authors and others, in vitro studies have verified that doxorubicin inhibits both the proliferation and the differentiation of osteoblasts, selectively reducing bone formation rates while interfering in the action mechanisms between PTH and the osteoblastic receptors [19, 23, 25].

The frequency of CTIBL on patients with breast cancer is yet to be understood, since the extension in which the bone loss occurs depends directly on the type and on the combination of the antineoplastic drugs employed, as well as on the ovarian function rate. According to many authors, women who prematurely experience chemotherapy-induced menopause display, in the following 12 months after treatment, considerable bone loss in the vertebral column (4% to 6%), the femur head and the hip (2%). Furthermore, these women keep on losing bone mass up to four or five years after the treatment has terminated. If the ovarian function is not reactivated, this may certainly be extended to over five years [17, 26, 43, 44].

Exposing similar ideas, Adler [27] and Greenspan et al. [28] attest that osteoporotic fractures are indeed the potential late effect induced by adjuvant chemotherapy on bone tissue. According to recent studies, breast cancer survivors who have undergone chemotherapy would be more exposed to future osteoporosis developments and even fracture risks, especially the vertebral column and the hip [27, 28]. Other works report that the employment of tamoxifen could increase bone loss in women with breast cancer during their premenopause period. The very treatment that frequently precedes chemotherapy may have both an intensifying effect on bone mass loss and an opposite action, reducing the loss and increasing bone density over approximately 2.4% in one year (for women in the postmenopause period), thus depending on the menopausal status of the patient [27, 29-31].

Hirbe [32] and others [26, 33] suggest that tamoxifen can reduce bone loss up to 50% in patients precociously in menopause as a result of chemotherapy treatment.
The studies of Maxwell and Vialle [17] and Gralow and Bone [34] suggest that patients undergoing hormone therapy with tamoxifen display additional risks of CTIBL. The reason is that the effects induced by this particular drug, during both the pre and postmenopausal periods, are opposite: in postmenopause it preserves the bone mineral density, increasing it between 0.6 and 1.2% approximately in one year. As for women in premenopause, the bone mineral density decreases approximately 1.4% [17, 34].

According to Ramaswamy and Shapiro [23] (an important reference in most articles selected on the theme), aromatase inhibitor-based therapies, such as anastrozol and letrozol, may also induce intense bone mass loss. Therefore, the caretaking of these patients should include early preventive action chiefly regarding everyday life style, physical exercise, calcium consumption and specific medications (biphosphonate, raloxifene, calcitonin), among others [23].

Different authors refer to the various benefits derived from the intervention of physiotherapy for treating osteoporosis in menopausal women. According to Nogueira et al. [35], physical exercise can help women with osteoporosis during menopause to relieve pain, increase bone mass and muscular resistance, improve their articulation mobility as well as their posture. Furthermore, physiotherapy is designed to orient and educate these women, and also to prevent immobility. However, it should not be neglected that, in the case of women subjected to cancer treatment, chemotherapy in particular, states of inactivity, fatigue and pain are frequently increased, which makes both the evaluation and safety margins of these patients’ rehabilitation very important. After all, more accurate parameters applicable in the physical exercise of oncological patients, regarding intensity, frequency, strength and resistance-training, are yet to be established.

The possibilities of caretaking and preventing osteoporosis within specific populations, such as the cancer-diagnosed, should include the adaptation of protocols, parameters and safety margins aimed at improving the rehabilitation of elderly people and the treatment of chronic diseases, for example. In these cases, high intensity and impact exercises should be avoided after cancer treatments, since they could eventually induce stress augmentation and immunosuppressant effects. Low and moderate intensity exercises should be chosen instead. The goals and modalities of physiotherapeutic procedures should be based on a detailed evaluation of these patients, including: the kind and the condition of the tumor, the treatment protocol (surgical approach, chemotherapy, number of cycles and kinds of chemotherapeutic drugs, and radiotherapy), a report on physical activity or inactivity, favorite physical activities, basal aptitude, co-morbidities, and also the personal answers given during treatment (nausea, extreme fatigue, cardiotoxicity, neuropenia, peripheral sensorial neuropathy, among others).

LeMura and Duvillard [36], and Spínola et al. [37], have revised the specialized literature searching for the influences of physical exercise and/or activity on cancer. They compiled the main recommendations made by Courneya et al. [38], and by the American College of Sports Medicine concerning the prescription of aerobic exercises for cancer survivors. They also gathered some suggestive data, taken from Schwartz and cohorts [39], attesting that the practice of physical exercises may significantly reduce fatigue levels as well as maintain functional ability of women with breast cancer who have undergone chemotherapy treatment.

Spínola et al. [37] have revised the recent literature for the influences of physical exercise and/or activity on cancer, and they have gathered some meaningful data from different authors, confirming that the practice of physical exercises may significantly reduce fatigue levels and maintain the functional ability of women with breast cancer.

This literature review has not found any specific reference about physiotherapeutical intervention on patients with breast cancer suffering from bone mass loss. Neither were there any scientific articles with specific references to physiotherapeutical rehabilitation applying to breast cancer patients who present adjuvant chemotherapy-induced bone mass loss.

Even though there are several articles referring to the role of physiotherapy regarding osteoporosis treatment for women going through their pre or postmenopause period, and regardless of the vast literature concerning breast cancer-oriented physiotherapy, no studies were found that correlate the intervention of physiotherapy aiming at decreased bone mass loss in patients with breast cancer subjected to adjuvant chemotherapy.

Albeit many studies, such as Navega’s and cohorts, [40] point out the benefits implied in physiotherapy as a means to prevent and to minimize osteoporosis-induced deleterious effects, none of these authors have specifically correlated them to adjuvant chemotherapy-induced late effects on breast cancer patients. This attests to the need for new researches that are able to more deeply explore the theme proposed herein, since these women accumulate additional risks of osteoporosis, and given the scarcity of specific cancer-oriented protocols and lines of direction [35, 40].

The physiotherapist should know the cancer patients well, and also the specific aspects involved in their disease and its treatment, so that they are able to promote preventive and rehabilitating actions to improve these women’s quality of life and to prevent future morbidities and hospital commitments.

Physiotherapy and the breast cancer patient

It is common sense among specialists today that breast cancer induces a considerable decline in the majority of these patients’ quality of life – a setting that favors critique functional losses (cardiovascular and lung, weakness and muscle atrophy), fatigue, sleep and weight alterations, not to mention its role in the diminution of physical activity and exercises. According to LeMura and Duvillard [36], it is not clear yet as to what extent this
decreased physical function is a direct consequence induced by cancer and the treatment, or if it is a result of the secondary inactivity induced by the latter. Even if the side-effects are more intense during the treatment, the late or chronic effects may manifest themselves months and even years after the therapies were ceased [36].

The main goal of physiotherapy in breast cancer patients is to prevent and rehabilitate the complications induced by this disease and eventually originated by the treatments themselves – chiefly the surgical approach, chemotherapy and radiotherapy.

The most common complications are pain and edema, especially in the surgical incision and adjacent areas, scar adherence, retractions and fibrosis, decreased movement amplitude, fatigue, shortening of muscles, lymphatic disorders such as lymphedema, and also sensitivity, posture, self-image and respiratory alterations. After the administration of chemotherapeutic agents, vascular alterations of the superior limbs may occur as well [36, 41, 42].

**Conclusion**

It can be verified, based on the results achieved in this literature review, that there are several scientific articles approaching the theme herein proposed. Different authors have shown the possible chemotherapy-induced late deleterious effects on the bone mass of breast cancer patients, and the additional risks of future osteoporotic developments, since chemotherapeutic and therapeutic hormone (SERMs) treatments may indeed increase and accelerate bone mass loss, either directly through the action of some specific drugs, or indirectly through the decrease of estrogen levels and precocious menopause. Considering the lack of literature on physiotherapeutic intervention in breast cancer patients who suffer from chemotherapeutic-induced secondary bone loss, the effort made herein was to contribute to future study advances in this direction. The collected data reinforce our view that new studies are needed to establish specific rehabilitation protocols and exercises, so that they can reach maximum efficacy and maximum safety in the treatment of osteoporosis within special populations, such as cancer patients.

**References**


Hormone therapy/adjuvant chemotherapy induced deleterious effects on the bone mass of breast cancer patients and the etc.


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Molecular markers in epithelial ovarian cancer: Their role in prognosis and therapy

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Summary

Epithelial cancer of the ovary is the most lethal malignancy of all gynaecological cancers. Various clinical and pathological features of ovarian cancer are used as predictors of clinical outcome. The use of molecular markers in common clinical practice seems promising for the diagnosis and prognostication. The aim of this review article is to describe current theories regarding the pathogenesis and molecular evolution of epithelial ovarian cancer. With respect to the molecules involved, this article focuses on whether they are associated with poor prognosis or not. This evaluation is performed in light of the progress made and the potential usefulness in treatment decisions without overlooking existing controversies that should be further studied. It is tempting to anticipate the gradual integration of molecular profiling in clinical practice.

Key words: Molecular markers; Epithelial ovarian cancer; Prognosis; Targeted therapies.

Introduction

Epithelial cancer of the ovary is the most lethal malignancy of all gynaecological cancers and is the fourth most frequent cause of death from cancer in women [1, 2], with approximately 22,000 new cases and 16,000 deaths occurring annually [2]. The greatest incidence rates are reported in North America and Northern Europe, particularly in Scandinavia [3-5]. The disease predominantly affects elderly or middle-aged women; it is relatively rare in women younger than 30 years, with only 1.5 new cancers/100,000 women per year in the 20-29 year age group, but beyond this age the incidence reaches a rate of 49/100,000 women in the 60-69 year age group [1-6]. Typically, this cancer has an insidious onset, and consequently, 70% of women present in International Federation of Gynecology and Obstetrics (FIGO) Stages III and IV, resulting in a high mortality rate despite optimal surgery and aggressive chemotherapy. The prognosis of these patients remains poor, with a 5-year actuarial survival of 23% and 14% for FIGO Stages III and IV, respectively [1, 2, 7].

Various clinical and pathological features of ovarian cancer are used as predictors of clinical outcome, of which the volume of postoperative residual disease and FIGO stage are the most important [8, 9]. The use of molecular markers in common clinical practice seems promising for the diagnosis and prognostication.

The aim of this review article is to describe the current theories regarding the pathogenesis and molecular evolution of epithelial ovarian cancer. With respect to the molecules involved, this article focuses on whether they are associated with poor prognosis or not. Moreover, the evaluation of molecular markers is performed under the light of their potential usefulness in treatment decisions in common clinical practice.

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Pathological types

Epithelial tumours of the ovary, which account for 90% of malignant ovarian tumours, are further divided into subgroups taking into consideration traditional histomorphologic features [1, 2, 10]. The World Health Organization’s classification of ovarian tumours recognises serous, mucinous, endometrioid, clear cell, transitional cell, mixed and undifferentiated ovarian neoplasms [10]. It is worth mentioning that a reproducible subclassification of ovarian carcinomas is biologically and increasingly therapeutically important. Examples include the biologic distinctiveness of low- and high-grade serous carcinomas, common molecular genetic pathways and etiologic relationships between the different subtypes of epithelial ovarian carcinomas [11-20]. Although specific therapies for each disease entity do not yet exist, standardising diagnostic criteria will become essential as effective regimens are developed.

Serous tumours: Serous tumours are high-grade tumours, comprising approximately 80% to 85% of all ovarian carcinomas [1, 21]. Up to 95% of patients presenting with FIGO Stages III-IV disease have serous carcinomas, while FIGO Stage I serous carcinomas are very uncommon. Serous carcinomas display a very broad spectrum of pathologic appearances, in contrast with most other primary ovarian carcinomas. This suggests that some tumours currently diagnosed as serous carcinomas represent transformation or progression from other tumour types. Serous carcinomas, usually, exhibit papillary and micropapillary architecture with evident slit-like spaces at least focally, but glandular, cribriform [22-24], solid, microcystic [25] and trabecular architecture may predominate. Cytologically, they typically contain columnar cells with pink cytoplasm, but polygonal eosinophilic cells, clear cells, signet ring cells [25], and spindle cells may also be present. Focal squamous differentiation [26] and elements resembling choriocarcinoma [27] may be detected.
Intestinal mucinous tumours: Primary ovarian mucinous carcinomas (POMCs), are very uncommon, comprising less than 3% of all ovarian carcinomas [21, 28]. Although the identification of intracytoplasmic mucin is mandatory for a POMC diagnosis, many mucinous tumours lack obvious apical mucin in large parts of the tumour, thereby conveying an endometrioid appearance. Mucinous borderline tumours that lack goblet cells are classified separately from intestinal mucinous neoplasms and are referred to as Mullerian mucinous or endocervical mucinous or seromucinous or mixed epithelial neoplasms with a mucinous component [29-31].

Endometrioid tumours: Endometrioid carcinoma accounts for approximately 10% of all ovarian carcinomas and represents the second most common ovarian carcinoma subtype [21]. It is the most common of FIGO Stage I carcinomas, approximately constituting at least 50% of such cases [19]. They are associated with endometriosis, endometrioid borderline tumour, or a synchronous endometrial neoplasm of endometrioid type [17, 18, 20]. Architectural patterns containing tubules, cribriform structures, solid, sheetlike growth, and papillae are typically present in the context of an easily recognised endometrial-like background. The majority of endometrioid carcinomas contain either squamous or mucinous differentiation and may show secretory features [22].

Clear cell tumours: Clear cell carcinoma is the third most common subtype of epithelial ovarian carcinoma, accounting for approximately 5% of all ovarian tumours [21]. As in endometrioid carcinomas, it is predominantly represented in FIGO Stages I and II [32-34]. The majority of clear cell carcinomas are associated with endometriosis, particularly atypical endometriosis or endometriosis-associated tumours, while many contain a tubulocystic adenofibromatous component with a range of cytologic atypia. It has recently been reported [35] that clear cell adenocarcinomas associated with clear cell adenofibromatous components may be a subgroup of ovarian clear cell adenocarcinomas. Clear cell carcinomas exhibit a rather restricted architectural inventory: only papillary, tubulocystic, and solid architectural varieties are recognised. The typical clear cell carcinoma is composed of hobnail cells with clear cytoplasm; the nuclei although large, atypical, and frequently featuring a large nucleolus, do not often show striking pleomorphism. Clear cell carcinoma papillae are short and round and may display eosinophilic and hyalinised stroma. There are generally only one or two layers of cells lining the papillae, in contrast to the prominent tufting usually seen in serous carcinomas [22].

Transitional cell tumours: Transitional carcinomas are very uncommon, although their true prevalence is difficult to assess. Resembling urothelial carcinomas, they are composed of cytologically low-grade cells with longitudinal nuclear grooves, arranged in broad papillae. Cytologically, high-grade tumours forming broad papillae frequently also contain microcysts, slit-like fenestrations and small, filiform papillae, rendering distinction from serous carcinoma virtually impossible. Squamous differentiation and psammoma bodies may also be identified [22].

Mixed epithelial ovarian tumours: The diagnosis of mixed epithelial ovarian tumour (MOT) is established when at least two pathologically distinctive elements are present, each constituting at least 10% of the tumour [22].

Undifferentiated carcinomas: The absence of pathologic differentiating features has been deemed as sufficient to establish the diagnosis of undifferentiated carcinoma [22].

Molecular markers

Many molecules have been investigated as potentially biologic factors involved in the complex mechanism of cancer progression. These include factors involved in mitogenesis, cell cycle regulation, intercellular interaction, extracellular matrix regulation and degradation, and angiogenesis.

WT1: A characteristic feature of ovarian serous carcinoma is the widespread WT1 expression [36-38]. Endometrioid carcinomas lack WT1 expression [39, 40]. Clear cell carcinomas tend to lack WT1 expression [41-43]. Transitional cell tumours express WT1 [44] and frequently overexpress p53 [45].

WT1 is the main immunohistochemical molecular marker to contribute to the differential diagnosis of serous subtype.

p53: p53 is a tumour suppressor gene, mutations of which are associated with accumulation or overexpression of this protein [12]. Loss of p53 function is thought to be an early molecular event associated with de novo carcinogenesis of high-grade serous, endometrioid, and clear cell carcinoma [22, 46]. Borderline tumours frequently contain wild-type p53 and may serve as precursor lesions for low-grade and mucinous EOC [47-50]. Low-grade epithelial ovarian cancer, arising from borderline tumours, frequently maintains wild-type p53 phenotype while acquiring unique genetic alterations [47-50].

A characteristic feature of ovarian serous carcinoma is p53 overexpression, while p53 mutation is usually found in high-grade varieties [51-55]. Endometrioid carcinomas lack p53 overexpression, although this has been described in purported poorly differentiated varieties [56]. P53 expression can be encountered in clear cell carcinomas, but diffuse and strong overexpression of the sort seen in most high-grade serous carcinomas is not characteristic [57-59]. Transitional cell tumours frequently overexpress p53 [45].

Positive p53 staining, has been correlated with poor survival [60, 61], with a propensity to develop recurrent disease and in time to distant recurrence [46, 61-63]. However, other studies conducted either in early [46] or in advanced stage [64], failed to correlate p53 dysfunction with disease-specific survival.

BRCA genes: BRCA genes are tumour suppressor genes which inhibit the growth of cancer cells through their role in the maintenance of genomic integrity, DNA repair, cell cycle control and apoptosis [65]. BRCA dysfunction is also considered to be an early event associated
with de novo carcinogenesis of high-grade serous EOC [48, 50, 66]. The association between BRCA1 or BRCA2 mutations and familial high-grade serous carcinomas is characteristic [67-69], as is the loss of BRCA1 expression in many high-grade serous carcinomas [70].

Ras: Ras proteins are GTPases which when activated influence growth and differentiation, cell cycle regulation, cell survival and angiogenesis [71].

K-Ras mutations are common in ovarian mucinous carcinomas [66, 72, 73] and in clear cell carcinoma [74, 75], but not in serous tumours [66, 76]. Serous borderline tumours frequently contain K-Ras mutations [77], as do mucinous borderline tumours [47, 77-80]. Deregulation of Ras in serous carcinoma has been associated with poor prognosis in patients with incomplete response to platinum-based chemotherapy [81].

Targeting the Ras/Raf/MAPK pathway, BAY 43-9006, a specific Raf-1 kinase inhibitor, has been studied in phase I trials, with limited efficacy reported [82]. ISIS 5132, a DNA oligonucleotide against human c-raf kinase, has been tested [83]. R115777 and SCH66336, two small molecules that inhibit farnesyldtransferase of Ras, are currently being evaluated in clinical trials [84].

BRAF: Serous borderline tumours and low-grade carcinomas frequently contain BRAF mutations [47, 77, 79, 80].

CK7: POMCs preferentially express CK7 over CK20 [85-88].

Beta-catenin: POMCs are negative to nuclear beta-catenin, a feature that helps their distinction from colorectal carcinomas [89], whereas endometrioid carcinomas and clear cell borderline tumours do express beta-catenin [50, 90-93]. Deregulation of beta-catenin in serous carcinoma has been associated with poor prognosis in patients with incomplete response to platinum-based chemotherapy [81].

p16: p16 is a tumour suppressor gene that belongs to INK4 gene family. Low p16 expression is more common in mucinous and endometrioid, while overexpression of p16 is more common in high-grade serous cancers [94]. There is no evidence to suggest that aberrant expression of p16 is associated with patient outcome in ovarian cancer [62, 95, 96].

p21: p21, a tumour suppressor gene, plays a functional role in cell differentiation [97]. Elevated p21 protein has been correlated with well-differentiated serous epithelial cancer [62, 98]. In the majority of the studies, this molecule has not been proven as an independent prognostic factor [46]. However, in other studies decreased p21 expression has been associated with aggressiveness of the tumours and/or poor patient prognosis defined by response to chemotherapy, disease-free interval and/or overall survival [99-105]. Moreover, loss of p21 combined with overexpression of cyclin D1, in the presence of p53 overexpression, has been shown as a predictor of overall survival and a shorter progression-free survival [62].

p27: p27 is a tumour suppressor gene. In some studies, p27 has been correlated as an independent factor in determining the clinical outcome in patients with ovarian cancer [106-108]. However, in others this was not shown [62, 109].

ERs: ER immunopositivity is a characteristic trait of endometrioid carcinomas [90-93], whereas POMCs and clear cell carcinomas tend to lack nuclear estrogen receptor (ER) expression [41-43, 110]. Consistent with the above finding, Fujimura et al. reported ER-alpha immunostaining in 97% of serous adenocarcinomas, 100% of endometrioid carcinomas, and 70% of mucinous adenocarcinomas and none in the clear cell carcinoma specimens [111]. It has been shown that levels of ERa mRNA are similar or slightly higher in cancer samples, compared to those in normal biopsies [112-116]. In contrast, all ovarian cancer subtypes have been found to express low levels of ER-beta; this observation suggests the possible tumour-suppressor role of ER-beta in ovarian carcinogenesis, rendering this receptor an indisputably interesting target for cancer therapy [111-116].

Moreover, it has been speculated that the loss of ER expression in ovarian cancer may explain the disappointing responses to antiestrogen therapy [117, 118].

Although receptor studies have shown that not only normal ovaries but also many malignant ovarian tumours can be considered as endocrine-related and hormone-dependent, the place of hormonal therapy in the management of patients with ovarian cancer remains unsettled (reviewed in [6]). A literature review shows that response to hormonal therapy even in a preterminal patient is modest, with about 8% objective response but almost no side-effects [6, 119, 120]. However, the majority of trials of hormonal treatment in ovarian cancer have been retrospective, involved only limited numbers of patients, and lacked important patient-related data and information pertaining to tumour characteristics (reviewed in [6]). Consequently, the place of hormonal therapy in the management of patients with epithelial ovarian cancer needs more thorough evaluation.

PR: While PR immunopositivity has been observed in the majority of borderline tumours, it is documented as ranging from 0-50% in different types of ovarian cancer [111, 121, 122]. It is worth mentioning, that the nuclear expression of progesterone receptor (PR) is a characteristic feature of endometrioid carcinomas [90-93].

As far as, PR-isoforms are concerned, PR-A isoform has been found to be expressed in normal and benign ovarian tissues, and to exhibit a marked reduction in malignant cancer specimens. On the other hand, no significant difference has been noted in the expression of PR-B among normal/benign ovarian tissues and cancer specimens [111]. It has been said that a loss of PR-A is associated with ovarian malignancy [111, 118]. Another study showed that PR-B immunopositivity is an independent prognostic factor for ovarian cancer [123].

SMAD4: SMAD4 expression is retained in POMCs [86].

DPC4: POMCs retain DPC4 expression [86].

PTEN: Mutations in PTEN have been reported in clear cell carcinoma [13]. Moreover, endometrioid and clear
Molecular markers in epithelial ovarian cancer: Their role in prognosis and therapy

Cell borderline tumours arising in endometriosis display PTEN mutations and microsatellite instability [50]. On the other hand, PTEN mutations have not been found in serous or mucinous types [124, 125].

**VEGF/VEGFR:** VEGF is the most important driving factor behind angiogenesis and is expressed in most ovarian cancers [126]. Gootdheart et al. [46] demonstrated that at the molecular level in Stage I ovarian cancer, high levels of VEGF staining increased the risk of death by the disease by a factor of 6.5; a result consistent with the one previously reported [127]. However, this phenomenon did not persist in the multivariate analysis [46]. Also worthy of mention, VEGFs are overexpressed in metastases compared to primary tumours [128].

Different agents have been developed to inhibit VEGF or its receptors (VEGFR and VEGFR2) [129]. Bevacizumab, a recombinant humanised monoclonal antibody against VEGF, has shown activity in ovarian cancer (reviewed in [130] and in [131]). Specifically, bevacizumab was given as a single agent in a GOG170 phase II study (women with recurrent ovarian or primary peritoneal cancer). The response rate was 21% (13 of 62 women enrolled), and 40% of patients had a progression-free survival of six months or more [132]. Similar results were reported by Cannistra et al. [133]. Another study showed a 28% response rate to bevacizumab in combination with low-dose metronomic oral cyclophosphamide in patients with recurrent ovarian/peritoneal cancer [134]. The weekly administration of a combination of taxane and bevacizumab [135, 136] appeared to achieve a temporary improvement in cancer-related symptoms. However, bowel perforation has been described as a very serious side-effect [130, 131, 137]; in a retrospective analysis Wright et al. [138] reported that eight of 158 (5%) patients treated with bevacizumab developed bowel perforation. An ongoing GOG phase III trial is comparing standard carboplatin and paclitaxel with either placebo or bevacizumab in patients with suboptimal Stage III and Stage IV disease. Thalidomide [139] and carboxamidotriazole [140] have been tested in phase II trials, with less encouraging results as compared to bevacizumab.

**ErbB receptors:** This receptor family comprises the following four related receptors: the EGFR itself (ErbB1/EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER 3) and ErbB4 (HER4) [141-144]. Overexpression of EGFR has been shown to be associated with platinum resistance and consequent poor prognosis in ovarian

<table>
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<th>Molecule</th>
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Table 1.
cancer [137], though it has to be said that this finding was not proven in other studies [61].

Overexpression of the proto-oncogene HER2 has been correlated with poor survival [61, 145, 146]. However, only 10% of ovarian cancer patients display an overexpression of HER2 in their tumour [147].

Inhibitors of the ErbB family of receptor tyrosine kinases have been studied in ovarian cancer. However, the results are not promising; trastuzumab, a humanised monoclonal antibody against HER-2/ErbB2, has been shown to have a limited response rate [147] (reviewed in [148]). Small molecule inhibitors (gefitinib, erlotinib HC1) of the epidermal growth factor receptor (EGFR, ErbB1) have displayed minimal activity [149, 150]. Lapatinib, a dual inhibitor of EGFR and HER-2/ErbB2, in a recent phase II study showed no objective responses [151]. Additionally, pertuzumab has not demonstrated any effectiveness [152, 153] and similarly matuzumab [154] failed to show promising results.

Endothelin: Endothelin (ET)-1 mRNA is expressed in more than 90% of primary tumours and 100% of metastatic ovarian cancers [137, 155]. ET-1 mRNA expression has been found to be significantly higher in tumours than in normal ovarian tissue [128, 137, 156]. It has been shown that this molecule is relevant to the progression of ovarian cancer [128, 156]. The recent identification of highly-selective small molecules (ZD4054, ABT-627) that inhibit the ligand-induced activation of ETAR leads itself to possible testing in clinical practice [155, 157-159].

HSP90: Heat shock protein (HSP90) is a chaperone protein required for the function of several protein kinases [160, 161]. Inhibition of HSP90 leads to degradation of oncogenic proteins, including RAF-1 and mutant p53, causing cell-cycle arrest [160-162]; 17AAG, an inhibitor of HSP90, has been tested [163, 164].

Metalloproteinase: Metalloproteinase (MMP) is overexpressed in metastases compared to primary tumours [128]. More specifically, MMP-9, but not MMP-2, has been associated with poor prognosis [165].

Src: Deregulation of Src in serous carcinoma, is associated with poor prognosis in patients with incomplete response to platinum-based chemotherapy [81]. SU6656, a small molecule inhibitor of Src, has proven effective in preclinical studies in ovarian cancer [81, 166].

Hypoxia-inducible factor 1 alpha: Hypoxia-inducible factor 1 alpha has been tested as a positive marker of clear cell differentiation [167].

Human kidney injury molecule-1: Human kidney injury molecule-1 has been tested as a positive marker of clear cell differentiation [168].

Hepatocyte nuclear factor-1 beta: Hepatocyte nuclear factor-1 beta has been tested as a positive marker of clear cell differentiation [15, 169, 170].

Glypican-3: Glypican-3 has been tested as a positive marker of clear cell differentiation [171].

Plasminogen activator: Plasminogen activator is overexpressed in metastases compared to primary tumours [128].

Collagen: Collagen is overexpressed in metastases compared to primary tumours [128].

Fibroblast growth factor: Fibroblast growth factor is overexpressed in metastases compared to primary tumours [128].

Thrombospondins: Thrombospondins are overexpressed in metastases compared to primary tumours [128].

Integrins: Integrins are overexpressed in metastases compared to primary tumours [128].

Table 1 summarises the expression of the main aforementioned molecules in the different pathological types, their prognostic role, as well as their potential usefulness in treatment decisions.

Conclusion

As more effective and less toxic cancer drugs reach patients, the need for accurate and reliable cancer diagnostics and prognostics have become widely appreciated. The evaluation of the molecules, whose importance today has been elucidated, could prove equally useful in the therapeutic decision. The clinician should be aware of recent advancements in molecular biology, to enable them to tailor their approach to each patient according to her individual risk factors, tumour markers and histological profile.

With respect to the molecular markers presented above, this article reviews the progress made and their potential usefulness in treatment decisions without overlooking the existing controversies which should be further studied.

It is tempting to anticipate the gradual integration of the molecular profiling in clinical practice. The simultaneous evaluation of multiple genes will be of special interest in the context of the development of new biological therapies. Nowhere is there more dire need than in ovarian cancer. Ovarian cancer represents an attractive target for these therapies by virtue of the biology of the disease and the means by which it disseminates.

References

Molecular markers in epithelial ovarian cancer: Their role in prognosis and therapy


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Human papillomavirus (HPV)-type distribution in relation to oral contraceptive use in women with cervical intraepithelial neoplasia, Durban, South Africa

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Introduction
The annual worldwide number of new cases of cervical cancer is estimated to be about 493,000 with a mortality of 274,000 [1]. In South Africa the risk of a woman developing invasive cervical cancer is one in 26 [2]. It is also the commonest cancer among Black South African women. Human papillomavirus (HPV) is causally linked to the pathogenesis of cervical dysplasia, neoplasia and invasive cervical neoplasia [3]. HPV is reported to be detected in 99.7% of cervical cancers [3]. In high-grade cervical intraepithelial lesions (HGSIL) the presence of high-risk HPV varies according to the HPV type. HPV types 16, 18, 31, 45, 52, 58 and 33 are present in 45%, 7.1%, 8.8%, 2.3%, 5.2%, 6.9% and 7.2% of women with high-grade squamous intraepithelial lesions, respectively [4]. Although HPV is necessary for the genesis of cervical neoplasia, co-factors are required. Possible co-factors include steroid contraception, smoking, infective agents, amongst others. Epidemiological studies have reported that steroids are associated with the development of cervical cancer, so much so, that in 2005 the World Health Organization (WHO) labelled oral contraception as a group 1 cancer-causing agent. The odds ratios of developing cervical cancer with the use of oral contraceptive use of less than five years duration, between five and ten years duration and more than ten years duration are 0.77, 2.72 and 4.48, respectively [4]. However, the risk-benefit ratio of steroid contraception, especially in the developing world needs to be borne in mind. HPV-type distribution in women with cervical dysplasia in relation to oral contraceptive usage in South Africa has not been reported. Oral contraception is postulated to increase the acquisition [5] and persistence [6] of high-risk HPV . The aim of the study was to determine the HPV-type distribution in women with cervical dysplasia according to oral contraceptive usage and human immunodeficiency virus (HIV) status.

Patients and Methods
After institutional ethical approval was granted, patients were prospectively recruited from the colposcopy clinics of King Edward VIII and Inkosi Albert Luthuli Central hospitals, Durban, South Africa. These are the two tertiary referral clinics for the Durban area. The study was conducted over an 18-month period. Patients were divided into four groups and HPV DNA typing was performed for each group: group 1 no contraceptive usage, group 2 oral contraceptive use of less than five years duration, group 3 oral contraceptive use of between five years and ten years, and group 4 oral contraceptive use of more than ten years. Swabs of the cervix were analysed for HPV DNA using the polymerase chain reaction method. Results: A total of 124 women were recruited for the study. There were 75 patients who were HIV-infected (seroprevalence 61%). There were 102 patients who were HPV positive (82%), of which 79 patients had high-risk HPV DNA (78%). In terms of the four oral contraceptive groups, high-risk HPV DNA was detected in 70% (n = 21), 79% (n = 22), 90% (n = 21) and 71% (n = 15) of patient, respectively. The odds of having HPV DNA was six times higher for the combination of contraceptive users of less than five years duration/non-users (OR 5.9, 95% CI: 1.87-18.77). There was no change when adjustment was made for age (OR 6.1, 95% CI: 1.9-19.4). HPV DNA type 16 and/or 18 was present in a total of 21 patients (49%) (non-contraceptive users and users < 5 years duration) versus 15 patients (42%) (oral contraceptive users of more than 5 years duration) (p = 0.524). HPV type 16 was the commonest HPV type detected (20.2%) and HPV type 58 was the next commonest high-risk HPV type (16.1%). HPV types 58 and 33 were detected in a much greater percentage of our population and HPV 16 in a much smaller percentage of our population compared with a non-South African population. Conclusion: The findings of this study demonstrate an interesting distribution of HPV types in a South African population.

Summary
Objective: To determine HPV-type distribution among women with cervical dysplasia in relation to oral contraceptive usage. Methods: Prospective cross-sectional study of four groups of patients according to oral contraceptive usage: non-users, users of less than five years duration, users of between five years and ten years, and users of more than ten years duration. Swabs of the cervix were analysed for HPV DNA using the polymerase chain reaction method. Results: A total of 124 women were recruited for the study. There were 75 patients who were HIV-infected (seroprevalence 61%). There were 102 patients who were HPV positive (82%), of which 79 patients had high-risk HPV DNA (78%). In terms of the four oral contraceptive groups, high-risk HPV DNA was detected in 70% (n = 21), 79% (n = 22), 90% (n = 21) and 71% (n = 15) of patient, respectively. The odds of having HPV DNA was six times higher for the combination of contraceptive users of less than five years duration/non-users (OR 5.9, 95% CI: 1.87-18.77). There was no change when adjustment was made for age (OR 6.1, 95% CI: 1.9-19.4). HPV DNA type 16 and/or 18 was present in a total of 21 patients (49%) (non-contraceptive users and users < 5 years duration) versus 15 patients (42%) (oral contraceptive users of more than 5 years duration) (p = 0.524). HPV type 16 was the commonest HPV type detected (20.2%) and HPV type 58 was the next commonest high-risk HPV type (16.1%). HPV types 58 and 33 were detected in a much greater percentage of our population and HPV 16 in a much smaller percentage of our population compared with a non-South African population. Conclusion: The findings of this study demonstrate an interesting distribution of HPV types in a South African population.

Key words: Oral contraceptives; HIV; Cervical dysplasia; HPV.
ten years duration. All data including nature of the abnormal Papanicolaou smear findings were recorded on a questionnaire. After counseling, blood was taken for HIV status. Prior to colposcopy examination, a swab was taken of the ectocervix and endocervix for HPV DNA typing. Colposcopy and treatment in the form of large loop excision of the transformation zone (LLETZ) was performed. At the follow-up visit patients were counseled regarding HPV status, and blood was taken for CD4 counts where relevant. This technique allows the detection of 37 high- and low-risk HPV types. HPV DNA typing was performed using the Roche Linear Array polymerase chain reaction (PCR) genotyping test (Roche Molecular diagnostics, Pleasanton, CA). Correlations were made regarding the Papanicolaou smear result, histology findings, contraceptive usage, HPV DNA status and HIV serostatus.

Statistical Methods

An association between contraception use and high-risk HPV DNA types with other variables was evaluated by calculating odds ratios and by chi-square tests or Fisher’s exact tests, where appropriate. Logistic regression was then used where required to adjust the association for confounding by age. Statistical analysis was done using Stats Statistical Software: Release 10.

Results

A total of 124 women were recruited for the study. There were four groups of patients according to oral contraceptive usage: non-users, users of less than five years duration, users of between five and ten years duration and users of more than ten years duration. The total number of patients per group was: 32, 30, 31, and 31 patients, respectively. High-dose oral contraceptives were used by the contraceptive-users. The mean age of all patients was 39.2 years (range 22-78 years). The mean ages of patients according to these four groups were: 38.3 years, 39 years, 36.3 years and 44 years, respectively. Table 1 illustrates the demographic factors for all 124 patients. There were 104 patients (84%) with a high-grade squamous intraepithelial lesion (HGSIL) detected on Papanicolaou smear findings, while 20 patients (16%) had a low-grade squamous intraepithelial lesion (LGSIL). The distribution of patients with HGSILs according to the four contraceptive groups was: 28 (88%), 24 (80%), 26 (84%) and 26 (84%), respectively. HGSIL included both cervical intraepithelial neoplasia types 2 and 3 since the treatment was the same in the institution for both types of intraepithelial neoplasia.

Of the total of 124 patients, there were 75 patients who were HIV-infected resulting in a HIV-seroprevalence of 61%. Two patients declined HIV testing. For each of the four contraceptive groups, the HIV seroprevalence was 78% (n = 25), 55% (n = 16), 67% (n = 20) and 45% (n = 14), respectively. The CD4 counts for the 75 patients are illustrated in Table 1. Of these patients, 56% (n = 42) had CD4 counts less than 200 cells/µl. All these patients were receiving anti-retroviral therapy. The majority of patients (n = 123; 99%) were treated with LLETZ as a single-step procedure at the time of colposcopy. One patient had biopsy-confirmed HGSIL followed by hysterectomy as per patient request and history of menorrha-gia. There were eight patients (6%) whose histology results revealed no dysplasia and 14 patients who had dysplasia, the grade of which could not be determined on histology due to cautery artefact and/or traction distortion. All 22 patients were followed-up with repeat Papanicolaou smears with no evidence of dysplasia at the 6-month follow-up visit.

With regards to HPV DNA detection, there were 102 patients who were HPV positive (82%). All 22 patients who were HPV DNA negative were retested and remained negative. Of the 102 HPV-positive patients there were 79 patients who had high-risk HPV DNA (78%). The prevalence of high-risk and low-risk HPV DNA is illustrated in Figures 1 and 2. In terms of the four oral contraceptive groups, high-risk HPV DNA was detected in 70% (n = 21), 79% (n = 22), 90% (n = 21) and 71% (n = 15) of patients, respectively. The distribution of HPV DNA negative results for the four contraceptive groups was: two, two, eight and ten patients. High-risk and low-risk HPV DNA was detected in 52 and 23 HIV-infected patients compared with 26 and 21 HIV non-infected patients, respectively. There was no difference in the detection of high-risk HPV DNA between HIV-infected and HIV non-infected women (p = 0.1). There was also no difference in the detection of high-risk HPV DNA and low-risk HPV DNA according to CD4 counts (p = 0.9).

If the group of non-contraceptive users and users of less than five years duration are combined and compared with users of between five and ten years and more than ten years duration, then the distribution of high-risk HPV DNA was 74.1% (43/58) and 82% (36/44), respectively (p = 0.4). The odds of having HPV DNA was six times higher for the combination of contraceptive users of less than five years duration/non-users (OR 5.9, 95% CI: 1.87-18.77). Using logistic regression analysis and adjusting for age, there was no change when adjustment was made for age (OR 6.1, 95% CI: 1.9-19.4). The distribution of HPV DNA versus age and high-risk HPV DNA versus age is illustrated in Table 2 and Table 3, respectively. HPV DNA type 16 and/or 18 was present in a total of 21 patients (49%) (non-contraceptive users and users < 5 years duration) versus 15 patients (42%) who used oral contraceptives of more than five years duration (p = 0.524). In other words, the odds of having HPV DNA types 16 and 18 were similar by duration of contraceptive use. Only three patients had HPV DNA type 16 alone while five patients had HPV DNA 16 in association with a second HPV type. There were 36 patients who had HPV 16 and 18 in addition to other HPV types. There were 13 patients with only two HPV types other than types 16 and 18. The majority of patients therefore had multiple HPV types. The distribution of high-risk and low-risk HPV DNA according to age is illustrated in Table 4.

There were 20 patients who had a single HPV type detected. These single types included types 35, 16, 6, 45, 62, 33, 69, 81, 58, 72, 83, 54, 51, 31, 82 and 52. The prevalence of HPV types 31, 33 and 45 was 7%, 16% and 12%, respectively. Although HPV type 16 was the commonest HPV type detected (20.2%), HPV type 58 was the
Table 1. — Demographic data for 124 patients.

<table>
<thead>
<tr>
<th></th>
<th>No contraception</th>
<th>Contraception</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Age (mean)</td>
<td></td>
<td></td>
<td>38.3 (7.8)</td>
</tr>
<tr>
<td>Parity &lt; 3</td>
<td>23</td>
<td>72%</td>
<td>19</td>
</tr>
<tr>
<td>Parity &gt; = 3</td>
<td>9</td>
<td>28%</td>
<td>11</td>
</tr>
<tr>
<td>Number of partners &lt; 3</td>
<td>21</td>
<td>66%</td>
<td>20</td>
</tr>
<tr>
<td>Number of partners &gt; = 3</td>
<td>11</td>
<td>34%</td>
<td>10</td>
</tr>
<tr>
<td>Marital Status Married</td>
<td>5</td>
<td>16%</td>
<td>6</td>
</tr>
<tr>
<td>Marital Status Not married</td>
<td>27</td>
<td>84%</td>
<td>24</td>
</tr>
<tr>
<td>Partner Circumcised Yes</td>
<td>6</td>
<td>19%</td>
<td>9</td>
</tr>
<tr>
<td>Partner Circumcised No</td>
<td>26</td>
<td>81%</td>
<td>21</td>
</tr>
<tr>
<td>HIV Positive</td>
<td>25</td>
<td>78%</td>
<td>16</td>
</tr>
<tr>
<td>HIV Negative</td>
<td>7</td>
<td>22%</td>
<td>13</td>
</tr>
<tr>
<td>CD4 group &lt; 200</td>
<td>16</td>
<td>64%</td>
<td>9</td>
</tr>
<tr>
<td>CD4 group 200-350</td>
<td>7</td>
<td>28%</td>
<td>2</td>
</tr>
<tr>
<td>CD4 group &gt; 350</td>
<td>2</td>
<td>8%</td>
<td>5</td>
</tr>
<tr>
<td>Pap smear HGSIL</td>
<td>28</td>
<td>88%</td>
<td>24</td>
</tr>
<tr>
<td>Pap smear Other</td>
<td>4</td>
<td>13%</td>
<td>6</td>
</tr>
<tr>
<td>Smoking Yes</td>
<td>1</td>
<td>3%</td>
<td>4</td>
</tr>
<tr>
<td>Smoking No</td>
<td>31</td>
<td>97%</td>
<td>26</td>
</tr>
<tr>
<td>Warts Yes</td>
<td>4</td>
<td>13%</td>
<td>4</td>
</tr>
<tr>
<td>Warts No</td>
<td>28</td>
<td>88%</td>
<td>26</td>
</tr>
<tr>
<td>Colposcopy HGSIL</td>
<td>30</td>
<td>94%</td>
<td>29</td>
</tr>
<tr>
<td>Colposcopy Other</td>
<td>2</td>
<td>6%</td>
<td>1</td>
</tr>
<tr>
<td>Treatment Dysplasia</td>
<td>32</td>
<td>100%</td>
<td>30</td>
</tr>
<tr>
<td>LLETZ</td>
<td>0</td>
<td>0%</td>
<td>0</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>23</td>
<td>72%</td>
<td>21</td>
</tr>
<tr>
<td>Histology Results HGSIL</td>
<td>7</td>
<td>22%</td>
<td>7</td>
</tr>
<tr>
<td>Histology Results Other</td>
<td>2</td>
<td>6%</td>
<td>1</td>
</tr>
<tr>
<td>Histology Results Not abnormality</td>
<td>0</td>
<td>0%</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. — CD4 counts versus high-risk and low-risk HPV status.

<table>
<thead>
<tr>
<th>CD group</th>
<th>Low-risk HPVs</th>
<th>High-risk HPVs</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>29</td>
<td>81%</td>
<td>7</td>
<td>19%</td>
</tr>
<tr>
<td>200-350</td>
<td>12</td>
<td>80%</td>
<td>3</td>
<td>20%</td>
</tr>
<tr>
<td>&gt; 350</td>
<td>11</td>
<td>85%</td>
<td>2</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 3. — HPV DNA status versus age.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>HPV negative</th>
<th>HPV positive</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>5 (33%)</td>
<td>10 (67%)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>6 (11%)</td>
<td>48 (89%)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>≥ 40</td>
<td>11 (20%)</td>
<td>44 (80%)</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>102</td>
<td>122</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — High-risk and low-risk HPV versus age.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Low-risk HPVs</th>
<th>High-risk HPVs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>2 (20%)</td>
<td>8 (80%)</td>
<td>10</td>
</tr>
<tr>
<td>30-39</td>
<td>9 (19%)</td>
<td>39 (81%)</td>
<td>48</td>
</tr>
<tr>
<td>≥ 40</td>
<td>12 (27%)</td>
<td>32 (73%)</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>79</td>
<td>102</td>
</tr>
</tbody>
</table>

Table 5. — Comparison of high-risk HPV types worldwide (ref 23) and index study.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Review paper Sub Saharan Africa Percentage</th>
<th>% of HPV+ n = 102 Percentage</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>48%</td>
<td>25%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>18</td>
<td>19%</td>
<td>13%</td>
<td>0.03</td>
</tr>
<tr>
<td>45</td>
<td>15%</td>
<td>12%</td>
<td>0.2</td>
</tr>
<tr>
<td>33</td>
<td>3%</td>
<td>16%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>58</td>
<td>3%</td>
<td>20%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Sign rank test p = 0.14.
next commonest high-risk HPV type and was detected in 16.1% of patients. The odds of having HPV type 33 was 2-fold if the patient was HIV-infected in comparison to non-infected HIV patients (OR 2.04, CI: 0.6-9.2).

**Discussion**

Epidemiological research has supported the role of HPV as a necessary causal agent in the development of cervical dysplasia and cervical cancer [3, 7]. Further, HPV is also implicated in the development of intraepithelial lesions and invasive cancers of the lower female genital tract, and genital and anal cancers in males and females [8-10]. The evidence for the role of HPV is also borne out in HPV vaccine trials which have demonstrated 100% efficacy in protection against HPV type 6, 11, 16, and 18-related vulval and vaginal intraepithelial neoplasias [11]. It is reported that at any given point in time about 10.4% of women across the world with normal cervical cytology will harbour HPV [12].

Steroid contraception has been epidemiologically linked as a co-factor in the development of cervical cancer and preneoplastic cervical lesions. Although these data have been inconsistent, many studies have reported an increased risk [13, 14]. However, epidemiological studies have provided a strong and statistically significant association, especially among long-term users of oral contraception [15, 16]. There is a consistent association between long-term oral contraceptive users of more than five years duration and cervical neoplasia [15]. Increased risk for the use of oral contraception and cervical cancer is also reported for adenocarcinoma in-situ and invasive adenocarcinomas. Oral contraceptive users of six years or more duration are reported to have 6-fold increased risk of adenocarcinoma in-situ [17]. Data from the IARC consisting of pooled analysis of case-control studies have reported that HPV-positive women who have used oral contraception were 42% more likely to develop carcinoma in-situ and cervical cancer than never users (OR 1.42; 95% CI: 1.0-2.0) [18]. Biological plausibility has been provided because of increased acquisition, persistence and progression of HPV-infected epithelia to cervical cancer as well as the presence of hormone receptors in the cervix [12, 19]. It is postulated that steroid contraceptives increase the expression of the E6/E7 oncogenic HPV genes of the high-risk HPV viruses, which in turn enhance the degradation of the p53 tumour suppressor gene product [12].

In contrast, the report from the Royal College of General Practitioner’s oral contraception study found no association with cervical cancer and that steroids may in fact have a net public gain [20]. This study had commenced in 1968 and included datasets of cancers and observation of women by their general practitioners. In comparison to never users, users had a statistically significant reduction in the risk of any cancer (adjusted relative risk 0.88, CI: 0.83-0.94). Statistical significant reductions were found for cancers of the large bowel or rectum, uterine body and ovaries. A small non-significant increase was found for cancers of the lung, cervix and central nervous system. A molecular study of the interaction between steroid contraception, HPV, HPV 16 E6 gene expression and cervical cancer in our setting revealed no increase in expression between steroid users and controls [21].

If steroid contraception influences the development of
cervical neoplasias via HPV or high-risk HPV, then an analysis of the type distribution of HPV among oral contraceptive users of varying duration would be relevant. To our knowledge, no such report has been documented in South Africa. The high HIV sero-prevalence of 61% in this study is reflective of a sexually active younger population of women in the study and the fact the majority of patients with cervical dysplasia are referred from HIV treatment clinics. Just over half of the patients had CD4 counts below 200 cells/μl but were receiving anti-retroviral therapy.

All patients in this study had some degree of cervical intraepithelial neoplasia. While it is accepted that patients with cervical intraepithelial neoplasia would harbour HPV, the emphasis of the study was to determine the HPV-type distribution according to the duration of oral contraceptive usage. The finding of 82% HPV prevalence in this study among women with abnormal cytology is similar to that reported for Africa (85%), America (83%), Europe (88%) [22]. In contrast, HPV prevalence among cytologically normal women across the world include reports of 22% in African women, 20.4% in women from America, 11.3% among North American women, 8.0% in Asian women and 8.1% in European women [22]. In a meta-analysis of HPV in 3,230 HIV-infected women with normal cytology, HPV prevalence was reported to be 57% in African women, compared with 31% in Asian women, 32% in European women, 31% in North American women and 57% in women from South/Central America [23]. In a study to determine the natural history of high-risk HPV and cervical disease in Cape Town, South Africa, the prevalence of high-risk HPV in a cohort of HIV-infected women was 68% (n = 400) [24]. The prevalence of high-risk HPV among the HIV-infected women was 51% (52/102) and 78% among all women in our study. We did not find a difference in the detection of high-risk HPV DNA if HIV status or CD4 counts were compared, perhaps because of the smaller number of women who had HPV (n = 102). The five most prevalent HPV types and high-risk HPV types in this study were HPV 54, 62, 16, 58, 72 and HR-HPV 16, 58, 33, 18 and 35, respectively, in order of decreasing frequency. In contrast, the five most common high-risk HPV types from the study in Cape Town, South Africa were HPV 16, 52, 53, 35 and 18, in order of decreasing frequency. In a meta-analysis of high-risk HPV types among African women with HGSIL, HPV type 16 was the commonest (41%) followed by HPV type 31 (10.1%). Our study showed that HPV 16 was the commonest high-risk HPV type detected, but in only 20.2% of women, followed by HPV 58 (16.1%). HPV type 31 occurred in 5.6% of women in our study. Figure 3 illustrates a comparison of high-risk HPV types in our population of women with that of non-South Africans [25]. Table 5 reflects the differences in high-risk HPV distribution between these two populations and it can be noted that HPV types 58 and 33 occur in a much greater percentage of our population and HPV 16 in a much smaller percentage of our population compared with the non-South African population.

It would have been expected that the reason for finding a greater percentage of high-risk HPV in the combined groups of women who did not use oral contraception and users of less than five years duration was due to an age factor. However, logistic regression analysis considering the age factor revealed that age did not account for the observed difference. HPV type 16 alone was detected in only three women, while HPV types 16 and 18 were detected in 36 women in this study. However, the occurrence of multiple HPV types among most women is similar to that described in many other studies [23, 24]. HPV DNA is detected in virtually 100% of women with cervical carcinomas [3]. The type specific distribution among African women with carcinomas include HPV 16 (55%), 18 (16%), 33 (7.6%), 45 (6.5%), 31 (2.9%), 58 (2.7%) and 52 (1.5%). This study did not include women with cervical cancers and it is possible that HPV 16 and 18 in cervical cancers might still reflect types 16 and 18 to be the commonest types in our population.

In conclusion, although the number of patients in this study is small, the information on HPV-type distribution adds to the database of HPV-type distribution in an African population. It is accepted that HPV would have been present and driven the process of dysplasia in the women in this study. It is also accepted that the HPV types detected among women with established cervical intraepithelial lesions may not reflect the HPV types which were initially present and promoted the development of such lesions. The role of steroid contraception in relation to HPV remains to be proven by prospective laboratory-based research.

References


Human papillomavirus (HPV)-type distribution in relation to oral contraceptive use in women with cervical intraepithelial etc.


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A comparison of outcome in patients with Stage 1 clear cell and grade 3 endometrioid adenocarcinoma of the endometrium with and without adjuvant therapy

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Summary

Objective: To determine the outcomes in patients with Stage I uterine clear cell carcinoma (UCCC) treated with and without adjuvant therapy, and to compare the outcomes in these patients to that of matched controls, patients with Stage I, grade 3, endometrioid adenocarcinoma of the endometrium (EC). Methods: Patients with FIGO Stage I UCCC who underwent comprehensive surgical staging between January 1996 and January 2007 were identified. Cases (UCCC) were matched by age, stage, adjuvant therapy, and year of diagnosis to controls consisting of patients with grade 3 EC. Recurrence and survival were analyzed using the Kaplan-Meier method. Results: 25 patients with Stage I UCCC were identified of whom 13 (52%) received no adjuvant therapy and 12 (48%) received adjuvant radiation therapy (XRT). The 5-year disease-free survival and overall survival rates for the observation and the XRT groups were 78% and 75%, (p = 0.7) and 85% and 82% (p = 0.1), respectively. When compared to controls, the 5-year disease-free survival rates and overall survival rates of patients with Stage I UCCC were not significantly different, 77% vs 75% (p = 0.8) and 84% vs 88% (p = 0.5), respectively. Conclusions: In patients with Stage I UCCC tumors there was no clear benefit to adjuvant radiation given the absence of improvement in recurrence risk or any survival benefit. These data question the benefit of radiation therapy in UCCC patients with disease confined to the uterus.

Key words: Uterine cancer; Uterine clear cell carcinoma; Endometrioid adenocarcinoma of the endometrium; Radiotherapy.

Introduction

Endometrial cancer is the fourth most common malignancy in women and the most common gynecologic cancer in the United States [1]. In 2008, an estimated 40,100 cases of uterine corpus malignancies were diagnosed in the United States, with approximately 7,470 deaths from the disease [1]. The majority of endometrial cancers are early-stage, low-grade, endometrioid tumors with a favorable prognosis. On the other hand, uterine clear cell carcinoma (UCCC) has been identified as a high-risk endometrial cancer. Its poor prognosis was recognized in 1976 in a series of 21 cases reported by Kurman and Scully [2].

UCCC is a rare histologic variant of adenocarcinoma of the uterus, comprising only about 5% of all endometrial carcinomas [3-5]. The optimal adjuvant management in Stage I UCCC remains controversial. Multiple authors agree on the need for comprehensive surgical staging in order to appropriately define the extent of disease [6]. However, the published experience describing the management of patients with surgical Stage I UCCC remains scant, with few reports evaluating the role of radiotherapy in adequately staged UCCC tumors [6]. In addition, it also remains unclear as to whether or not Stage I UCCC and Stage I, grade 3 endometrioid adenocarcinoma of the endometrium (EC) share similar prognosis and clinical outcome, and if similar adjuvant management strategy should be adopted for both. Our aim was to determine the outcomes in patients with Stage I UCCC tumors with and without adjuvant therapy after comprehensive surgical staging, as well as to compare this group’s outcome to that of patients with Stage I, grade 3, EC.

Materials and Methods

Patients with FIGO Stage I UCCC who underwent surgical staging at the Massachusetts General Hospital and Brigham and Women’s Hospital between January 1, 1996 to January 1, 2007 were identified from the tumor registry database. The primary inclusion criterion was primary uterine tumors where at least 50% of the cells had clear cell histologic features [4]. Assignment to stage was in accordance with the 1989 International Federation of Gynecology and Obstetrics (FIGO) [7] criteria for staging and included: peritoneal washings for cytology, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic/paraaortic lymphadenectomy. In every case, the surgery was performed by a gynecologic oncologist. Patients were excluded from the study for the following reasons: histology consistent with mixed papillary serous and clear cell carcinoma, surgical exploration at another institution, incomplete clinicopathologic data, history of an additional primary tumor within the five years before or after endometrial cancer diagnosis, pre-operative radiation, incomplete surgical staging, coexisting second primary gynecologic cancer, and advanced-stage disease.
Thirty-three patients determined to have Stage I UCCC following comprehensive surgical staging were identified as cases. In each case, the diagnosis was confirmed by a dedicated gynecologic pathologist following post-surgery pathology review. The histopathologic diagnosis of clear cell carcinoma was rendered based on the criteria described by Kurman and Scully [2]. We excluded eight patients from our final analysis. Five patients were excluded because their tumors showed mixed papillary and clear cell histology. Two patients had a history of breast cancer, and one patient had a synchronous double primary cancer of the endometrium and ovary. A total of 25 patients were included in the final analysis. The clinical records were reviewed for demographics, treatment, and outcome parameters.

Cases (UCCC) were matched by age (± 5), stage (Stage IA vs IB vs IC), adjuvant therapy, and year of diagnosis (± 3) to controls consisting of patients with Stage I grade 3 EC at a ratio of two controls to one case. Of note, all controls required comprehensive surgical staging to be included in the study. Controls were selected without knowledge of patient outcome.

Statistical analysis

Continuous variables were evaluated by the Student’s t-test or Wilcoxon-Mann-Whitney test for normally or non-normally distributed variables, respectively. Categorical variables were evaluated by \( \chi^2 \) analysis or Fisher’s exact test as appropriate for category size. Survival estimates were plotted utilizing the Kaplan-Meier method. The log-rank test was utilized to statistically quantify these survival differences on univariate analysis. Length of survival was calculated from the date of initial surgery to the date of death; surviving patients were censored at the date of last contact. All statistical tests were 2-sided and differences were considered statistically significant at \( p < 0.05 \).

Statistical analyses including Kaplan-Meier curves were plotted using SPSS statistical software (version 16.0, SPSS, Inc, Chicago, IL). All other data analyses were performed with Stata statistical software (version 9.2, Stata Corp LP, College Station, TX).

Results

Twenty-five patients with FIGO surgical Stage I UCCC were identified. All patients underwent surgical staging as previously outlined. Median age was 68 years (range, 48-82 years); 44% of cases had mixed endometrioid histology. However, in all cases the predominant histology was clear cell and therefore qualified to be labeled UCCC according to the GOG pathology committee manual. Eleven patients had Stage IA disease, ten had Stage IB disease, and four were diagnosed with Stage IC disease. The median number of nodes removed by lymphadenectomy was 11 (range, 2-26 nodes). All the patients underwent pelvic lymph node dissection; in addition, five patients underwent paraaortic lymph node dissection. The median follow-up in this study was 31 months (range, 1-90 months). Thirteen patients (52%) received no adjuvant therapy and 12 (48%) received adjuvant radiation therapy (XRT) (external beam radiation therapy to the pelvis or brachytherapy). None of the patients received adjuvant chemotherapy as part of primary therapy. The groups did not differ statistically in age.

There was a statistically significant difference between the treatment and the adjuvant therapy in sub-stage distribution and the median number of nodal count excised (Table 1).

Table 1. — Patient and treatment data (Stage I uterine clear cell carcinoma).

<table>
<thead>
<tr>
<th></th>
<th>Adjuvant Treatment Group*</th>
<th>Observation Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 (± 7)</td>
<td>70 (± 8)</td>
<td>0.07</td>
</tr>
<tr>
<td>Nodal Count (N)</td>
<td>13 (± 6)</td>
<td>8 (± 4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IA [N(%)]</td>
<td>11 (17%)</td>
<td>9 (69%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Stage IB [N(%)]</td>
<td>10 (58%)</td>
<td>3 (23%)</td>
<td></td>
</tr>
<tr>
<td>Stage IC [N(%)]</td>
<td>4 (25%)</td>
<td>1 (8%)</td>
<td></td>
</tr>
</tbody>
</table>

* Radiotherapy, brachytherapy alone or whole pelvic radiation and brachytherapy. Values for continuous measurements are means, unless otherwise specified.

Table 2. — Treatment data and site of recurrence (Stage I uterine clear cell carcinoma).

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>Site of recurrence</th>
<th>Type of radiation</th>
<th>Site of recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>9</td>
<td>Vaginal cuff (2)</td>
<td>BT (2)</td>
<td>Vagina and upper abdomen (1)</td>
</tr>
<tr>
<td>IB</td>
<td>3</td>
<td></td>
<td>BT (7)</td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>1</td>
<td></td>
<td>BT (1)</td>
<td>BT + EBRT (2)</td>
</tr>
</tbody>
</table>

BT = brachytherapy alone; EBRT = external beam radiotherapy.

Of the 13 patients who did not receive adjuvant therapy, nine were diagnosed with Stage IA disease, three patients were diagnosed with Stage IB disease, and one patient was diagnosed with Stage IC disease. There were two recurrences (15%) in the observation group; these patients were originally diagnosed with Stage IA disease. Both recurrences occurred at the vaginal cuff. They were both salvaged with whole pelvic radiation and brachytherapy. One of these patients ultimately developed distant metastatic disease, treated with chemotherapy, and ultimately died from her recurrence.

In the group of patients who received adjuvant radiation therapy, two patients were diagnosed with Stage IA disease, seven patients were diagnosed with Stage IB disease, and three patients were diagnosed with Stage IC disease. Ten patients (83%) received brachytherapy alone (mean dose 21 Gy), two patients (17%) received whole pelvic radiation (mean dose 45 Gy) and brachytherapy. The two patients who received whole pelvic radiation and brachytherapy were originally diagnosed with Stage IC disease (Table 2). There was one recurrence (9%) in this cohort of 12 radiated patients. This patient was originally diagnosed with Stage IA disease and received brachytherapy alone after her surgery. She had a recurrence in the vagina and upper abdomen, and ultimately died from her disease despite additional treatment with chemotherapy. Adjuvant RT was well tolerated with acute side-effects controlled with medications, and all patients received all their planned doses without interruption.

The median follow-up for the observation group was 34 months (range 1 to 117 months) and 26.5 months.
The fact that 50-60% of patients with UCCC have Stage II-IV disease compared to only 21% for all endometrial cancer histologies suggests that the poorer prognosis associated with UCCC may be due to advanced disease at the time of diagnosis [5, 6]. However, such a comparison raises the question of UCCC histology being more aggressive, stage for stage, when compared to endometrioid adenocarcinoma. Our findings suggest that patients with Stage I UCCC have a similar overall and disease-free survival to patients with Stage I grade 3 EC. Similar results have been reported by other authors following comprehensive surgical staging. Cirisano et al. [5] described by de Boneville in 1911 [3], it did not appear in the English literature until 1957 when Kay reported two cases [9]. Subsequently, other investigators have published the outcome series of women with clear-cell histology [6, 10-12]. A significant amount of controversy has surrounded the optimal surgical and adjuvant management of patients with Stage I UCCC. The rarity of this histology has made it particularly difficult to identify the optimal management of patients with Stage I UCCC.

The benefit of adjuvant radiation therapy in the management of patients with early-stage UCCC remains undefined, primarily because of the rarity of these tumors and lack of large scale prospective data available to guide recommendations for adjuvant therapy. Some of the larger series have grouped UCCC together with tumors of serous histology further limiting the generalizability of their results [13, 14]. Our analysis showed no demonstrable improvement in overall survival in patients who were treated with adjuvant postoperative radiation therapy compared to those who were closely followed. In a study by Creasman et al. [7] adjuvant radiation therapy after surgical staging was evaluated with regards to impact upon survival, in all instances, survival was better in those who received radiation therapy but the difference in survival was not statistically significant.

The results of this work demonstrate that the recurrence rates for patients who were managed with observation are similar to those of patients treated with postoperative radiation therapy. In agreement with our findings that recurrence in Stage I UCCC is uncommon, Thomas et al. [6] reported that in 22 patients with Stage I or II disease confirmed by systematic lymphadenectomy (half treated with radiation/half received no treatment), only one patient had a vaginal recurrence. However, an OS and DFS comparison between those who received adjuvant therapy and those who did not was not reported in their study. In contrast to our findings, Murphy et al. [10] found that pelvic failure rates in patients with Stage IA-IIB treated with and without adjuvant radiotherapy were 0% (0/16) and 83% (5/6), respectively (p < 0.0001). Among the patients who did not receive adjuvant radiation therapy, five developed pelvic recurrences, Stage IA (1), Stage IB (1), Stage IC (1), and Stage IIA (2). Four of these patients had recurrences in the upper vagina. These results suggest that in patients with Stage I UCCC, adjuvant brachytherapy may play a role in reducing the risk of vaginal cuff recurrences.

Case-control analysis

In the case-control analysis the criteria for which the groups had been matched: age, sub-stage, and percentage of patients who received adjuvant therapy were similar between cases and controls. In addition, there was no significant difference between median lymph node count (Table 3). There were three recurrences in the UCCC group vs five recurrence in the stage 1 grade 3 EC group (12% vs 10%, p = 0.3). Of the five recurrences in the Stage I grade 3 EC group, three patients did not receive adjuvant RXT and two did receive adjuvant therapy. Table 4 summarizes the treatment data and site of recurrence in the Stage I grade 3 endometrioid adenocarcinoma group. The 5-year disease-free survival rates were not significantly different in Stage I UCCC compared to controls (Stage I grade 3 EC), 77% vs 75% (p = 0.8), respectively. In addition, the 5-year overall survival rates for the Stage I UCCC and control group were 84% vs 88% (p = 0.5), respectively.

Discussion

UCCC is a relatively rare tumor, representing less than 5% of all endometrial carcinomas [2-5, 8]. Originally
reported on 564 patients with clinical Stage I-II endometrial cancer: 44 (8%) with papillary serous (PS), 12 (2%) with UCCC, and 509 (90%) with endometrioid adenocarcinoma. In the endometrioid group, 59 patients (13%) had FIGO grade 3. When PS/UCCC was compared with endometrioid adenocarcinoma, the difference in survival was significant, with a RR of 2.8 (p < 0.001). However, when patients with grade 3 endometrioid carcinoma were compared to those with serous/UCCC malignancies, (p = 0.11), this difference was no longer seen. In addition, Alektiar et al. [15] found no significant difference in OS between grade 3 endometrioid cancer and serous/UCCC (71% vs 79% respectively, p = 0.3). Nor was a difference in local control or DFS found.

We acknowledge that the small cohort size, the inherent biases of this being a retrospective study, and varied adjuvant treatment schedules prohibit definitive conclusions. Furthermore, only five patients in our series underwent a paraaortic lymph node dissection as part of the staging procedure, reflecting differences in surgeons’ practices at the time, across two different institutions. However, it is important to note that this is one of the largest series of patients with surgically Stage I UCCC tumors reviewed to date. Previously published studies have been limited to small numbers of patients, most with tumors not surgically staged, and inclusive of other histologies such as PS. We believe that the standardization of surgical staging, including an anatomic lymphadenectomy, is a particular strength of this investigation.

In summary, in surgical Stage I UCCC there is no clear benefit to adjuvant radiation given the absence of improvement in recurrence risk or any survival benefit. These data question the benefit of radiation therapy in UCCC patients with disease confined to the uterus. In addition, no significant difference in outcome exists between Stage I grade 3 endometrioid adenocarcinoma and UCCC cancer. Additional studies will be necessary to confirm these findings. The lack of consensus regarding the role of adjuvant radiation and chemotherapy in early-stage UCCC highlights the compelling need for prospective studies to establish optimal therapy for these women.

References

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Liquid based cytology improves the positive predictive value of glandular smears compared to conventional cytology

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Summary

Purpose: To investigate whether the introduction of liquid-based cytology (LBC) in an urban setting decreases the diagnosis of glandular neoplasia (grade 6) and improves the positive predictive value (PPV) of cervical cytological screening. Methods: A retrospective database review was conducted identifying women with cervical cytological abnormalities including glandular neoplasia (grade 6) before and after the introduction of LBC. Results: Following the introduction of LBC the rate of glandular neoplasia (grade 6) referrals fell from 1.08% to 0.69% of all cervical cytological abnormalities. There was a significant reduction in ‘abnormal’ cytological samples subsequently found to be associated with no invasive or preinvasive disease but no decrease in the number showing preinvasive or invasive disease. A significant decrease in number of patients having a final diagnosis of normal/inflammatory or wart changes was seen in those patients referred during the LBC period (p < 0.01). Conclusion: The introduction of LBC in an urban setting decreased cytological glandular neoplasia referrals but not at the expense of missing preinvasive and invasive cancers. It has also increased the PPV of cervical sampling to detect preinvasive and invasive cancer from 59.6% to 76.0%.

Key words: Liquid based cytology; LBC; CIN; CGIN; Glandular neoplasia.

Introduction

The use of LBC has recently been recommended by the National Institute for Health and Clinical Excellence (NICE) as the method of choice for collecting and preparing cervical cytology specimens in England and Wales [1]. All English cytology screening laboratories have now changed to using LBC technology. The recommendation by NICE was based, at least in part, on the results of a pilot project involving three cytology laboratories in England [2]. The pilot study found a clear reduction in the rate of glandular neoplasia (grade 6) detected on routine cytology, with the rate of glandular neoplasia falling from 0.08% to 0.04%. This change in detection rate was similar in all three pilot sites.

This change in rate of detection of glandular neoplasia may be a cause for concern. One study suggested that the finding of glandular neoplasia on cytology is associated with an invasive cancer in 36% of cases while a further 63% of cases have cervical intraepithelial neoplasia (CIN) or cervical glandular intraepithelial neoplasia (CGIN) found at biopsy. In the remaining 44% of cases no evidence of invasive or preinvasive disease was found [3]. In a second study 59% of women had CGIN, 5.3% had endocervical adenocarcinoma and 31.6% had endometrial adenocarcinoma when ‘glandular neoplasia’ was the cytological diagnosis [4].

There are several possible explanations for this decreased rate of diagnosis of glandular abnormalities on cervical cytology. Firstly that these cervical samples are now being reported as normal and therefore some cases with preinvasive or invasive disease are being missed. Secondly it is possible that these cases are being reported as another form of cervical abnormality, such as squamous dyskaryosis or borderline glandular abnormality, and are still therefore being referred for colposcopy. Thirdly it is possible that LBC has a greater specificity than conventional cytology and is able to differentiate those cases in which there is no proven abnormality from those cases with preinvasive or invasive disease.

In this study we attempted to investigate whether the decreased rate of reporting of glandular neoplasia associated with LBC reflects a global decrease in the subsequent colposcopic and histological diagnoses or whether it represents a specific decrease in one histological diagnostic group.

Methods

This study was performed in the colposcopy department of one of the three units taking part in the English Human Papilloma Virus/LBC pilot project. LBC was introduced into the cytology laboratory on 1 July 2001 following which all samples were reported using this technology. On completion of the pilot study the laboratory has continued using LBC employing the Surepath system. Data were extracted from the colposcopy database – a fully computerised system which records details of all patients referred for colposcopic opinion including details of referral cervical cytology and subsequent histological diagnosis. Patient information was collated over 81 months including a 51-month period when cytology was collected in the traditional manner and 30 months using LBC technology.

All patients referred with cytological abnormalities including glandular neoplasia (grade 6) were identified and their outcome
was recorded including colposcopic opinion and histological diagnoses from either punch or loop biopsies. All patients referred with glandular neoplasia (grade 6) underwent colposcopic assessment by a British Society for Colposcopy and Cervical Pathology (BSCCP) accredited colposcopist with loop excision of the transformation zone or punch biopsy taken of any abnormality seen. Endometrial sampling using a Pipelle sampler was performed in those who were postmenopausal where no cervical abnormality was seen. All patients, irrespective of whether they underwent treatment, were followed up in the colposcopy clinic until both colposcopy and cervical cytology were reported as negative.

**Results**

During the preLBC period there were 4,787 referrals of women with abnormal cytology to the colposcopy unit, of which 53 (1.1%) were reported as showing a glandular neoplasia (grade 6), compared with 3,318 referrals during the LBC period, of which 23 (0.69%) were reported as showing glandular neoplasia. This fall does not achieve statistical significance (chi-square test).

All patients with the cytological diagnosis of glandular neoplasia were seen in the colposcopy clinic. Of the 76 patients referred 73 underwent biopsy (with 51 having a large excision of the transformation zone and 22 punch biopsies of the cervix) and histological examination. The remaining three had no colposcopic abnormality and were followed-up with repeat cytology and colposcopy at six months and one year. In these cases both cytology and colposcopy were normal at these subsequent visits. The final diagnoses for 76 patients are shown in Table 1.

A significant decrease in number of patients having a final diagnosis of normal/inflammatory or wart changes was seen in those patients referred during the LBC period (4/3,318 for the LBC period compared with 22/4,787 for the preLBC period (p < 0.01; chi-square test). No significant change was seen in the rates of patients subsequently found to have preinvasive (18/3,318 for the LBC period compared with 29/4,787 for the preLBC period) or invasive disease (1/3,318 for the LBC period compared with 2/4,787 for the preLBC period (chi-square test).

When the figures are corrected for time, to give an average number of cases seen per year, this finding corresponds to a decrease in cases of no preinvasive or invasive disease from 5.2 cases per year in the preLBC group to 1.5 cases per year in the LBC group. There was no change in the number of cases per year of preinvasive or invasive disease between the two groups. There were three cases of invasive cancer diagnosed during the study period, two cases of adenocarcinoma in the preLBC period and one case of squamous cell carcinoma in the LBC period. Calculating positive predictive values (PPV) for the ability of cervical cytology to predict preinvasive or invasive disease gives a PPV of 59.6% for the preLBC period and a PPV of 76.0% for the LBC period.

**Discussion**

Although the numbers of women referred to colposcopy units with ‘glandular neoplasia’ are small they remain an important group because of the high incidence of invasive and high-grade preinvasive disease [3-5]. It is important therefore that any change to current cervical cytology screening programmes does not deleteriously affect the ability to detect this group of patients.

Study of glandular neoplasia is difficult because of the relative rarity of cytological specimens showing this abnormality along with the challenges of converting from conventional to LBC [6]. This study has used data from a six-year period in an attempt to increase the validity of its findings. Our findings show that following the introduction of LBC the numbers of women referred with cytology suggesting glandular neoplasia (grade 6) fell from 1.1% to 0.69% but not at the expense of missing pre-invasive and invasive cancers. While it is possible that there has been a real change in the incidence of invasive and preinvasive disease in this time it seems unlikely and therefore it is reasonable to conclude that the introduction of LBC has been associated with a true increase in the PPV with no deterioration in the sensitivity of the test.

This study does however suffer from the limitation that it compares a study group with a historical control and it is possible that other factors were responsible for the change in rates of diagnosing glandular abnormalities. In particular, guidance from the National Health Service Cervical Screening Programme (NHSCSP) and the BSCCP may have had an effect on the diagnostic criteria used to determine glandular neoplasia and influenced patterns of cytological reporting.

This retrospective analysis also suggests that LBC has increased the positive predictive value of cervical sampling to detect preinvasive and invasive cancer from 59.6% to 76.0%. PPVs quoted here are for the ability of cervical cytology to detect both CIN and CGIN, and not cervical cytological glandular abnormalities, to predict glandular neoplasia. However from the pragmatic view of the colposcopist and the patient the importance of abnormal cytology is its ability to detect all premalignant lesions which require treatment.

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**Table 1. — Comparison of outcomes for women referred with glandular abnormalities diagnosed on conventional cytology (preLBC) and using LBC technology (LBC).**

<table>
<thead>
<tr>
<th></th>
<th>PreLBC (n = 4,787)</th>
<th>LBC (n = 3,318)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glandular referrals</td>
<td>Number per year</td>
<td>Number per year</td>
</tr>
<tr>
<td>Histological outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No invasive or</td>
<td>22 (42)</td>
<td>5.2 4 (17)</td>
</tr>
<tr>
<td>preinvasive disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN/CGIN</td>
<td>29 (55)</td>
<td>6.8 18 (78)</td>
</tr>
<tr>
<td>Cancer of cervix</td>
<td>2 (3)</td>
<td>0.47 1 (5)</td>
</tr>
</tbody>
</table>

* referrals corrected for time to give average number of patients per year; n.s.: not significant.
Conclusion

LBC has been introduced into the United Kingdom because of its important advantages over conventional cytology, principally in the reduction in the number of smears reported as unsatisfactory [7]. The finding from the pilot studies suggesting that LBC is associated with a decrease in the rate of glandular abnormalities noted was initially a concern. This study helps to allay those fears by demonstrating that the reduction in number of smears reported as showing glandular abnormalities is principally in the group of patients who were subsequently found to have neither invasive nor preinvasive disease and therefore the introduction of LBC appears to be associated with a decrease in the false-positive rate for cytology. Importantly the introduction of LBC does not appear to affect the rate of diagnosis of invasive or preinvasive disease. This leads to a substantial increase in the PPV of cytology to predict invasive and preinvasive disease of the cervix.

References


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Increase of Mcm3 and Mcm4 expression in cervical squamous cell carcinomas

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Summary

Purpose: To examine the relevance of expression of two proteins essential for DNA replication initiation, Mcm3 and Mcm4, in cervical squamous cell carcinoma (CSCC). Methods: 53 cases of cervical squamous cell carcinoma, 35 cervical intraepithelial neoplasias (CIN) grade 2-3, 11 CIN I and 26 normal controls were studied. Immunohistochemistry was performed with anti-Mcm3 and anti-Mcm4 antibodies. Results: We found Mcm3 and Mcm4 protein expression had a tendency to be stronger from control to CSCC. Both Mcm3 and Mcm4 were significantly upregulated in squamous cervical carcinoma compared to the control, CIN grade 1 and grade 2-3 (p < 0.001), and Mcm3 expression was correlated with CSCC cell differentiation. However there were no independent prognosis correlations between Mcm3 and Mcm4 and clinicopathological parameters including age, stage, tumor size, invasive depth and lymph node metastasis. Conclusions: Mcm3 and Mcm4 were highly expressed in CSCC, and these two proteins might be useful as biomolecular markers in clinical diagnosis.

Key words: Mcm3; Mcm4; Cervical squamous cell carcinoma.

Introduction

Cervical cancer was the second leading cause of cancer death in the 1920’s, 30’s and 40’s [1], and it is the principal cancer of women in most developing countries, where 80% of cases occur [2, 3]. Cervical cancer comes in two types; squamous cell carcinoma accounts for 80-90% of all cervical cancers, with adenocarcinoma making up 10-20% [4]. Biomolecular factors for early-stage cervical cancer are important in the clinical diagnosis in order for patients to have the best chance of being cured. Promising biomarkers for cervical squamous cell carcinoma (CSCC) are under development [5-7].

Minichromosome maintenance (Mcm) proteins are essential for the key regulatory step of initiation of DNA replication in all eukaryotes investigated. The Mcm complex is made up of six highly conserved proteins, Mcm2 through Mcm7, all of which are necessary for replication-fork progression [8, 9]. Pervious experiments have demonstrated that Mcm proteins 2, 4, 5, 6 and 7 are associated with cervical cancer [10]. Immunohistochemical studies of the human uterine cervix showed that Mcm3 and Mcm4 are ubiquitously expressed in cancer cells. The positive rate and level of Mcm3 and Mcm4 expression have appeared to be higher in cancer cells than in normal proliferating cells of the uterine cervix and dysplastic cells, suggesting that they can be useful markers to distinguish these cells [11]. However, expression level and pathophysiological significance of Mcm3 and Mcm4 in patients with squamous cervical carcinoma remain to be clarified.

Materials and Methods

Tissue samples

Tissue samples (n = 125) were obtained from the Second Affiliated Hospital, School of Medicine, Zhejiang University and Huzhou Maternity and Child Care Hospital of Zhejiang Province from July 2004 to May 2007. The specimens consisted of cervical intraepithelial neoplasia grade 1 (CIN I, n = 11), grade 2-3 (CIN II-III, n = 35; including CIN II, CIN III and carcinoma in situ) and invasive cervical squamous cell carcinomas (CSCC, n = 53). Normal cervical squamous epithelium (n = 26) was obtained from patients who underwent hysterectomy due to diseases other than cancer. No CSCC patient received any chemotherapy or radiotherapy. Complete clinical pathologic parameters were obtained including age, clinical stage of the patient, tumor size, invasive depth, lymph node metastasis and differentiation of tumor cells. Informed consent was obtained from each patient. This study was approved by the Hospital Ethics Committee.

Immunohistochemistry

The sections were incubated with primary antibodies; goat anti-Mcm3 polyclonal antibody (1:10000) and mouse anti-Mcm4 monoclonal antibody (1:50) (Santa Cruz, CA), and then were visualized using an HRP-conjugated polymer detection system (Zymed, Canada). All slides were counterstained with hematoxylin. The negative control was subjected to the same technique except that the primary antibody was replaced by phosphate-buffered saline.
Positive cells were indicated by the presence of brown staining. Immunohistochemical results were evaluated under a light microscope and scored as follows: negative (–) for no detectable staining (< 5%), (+) for weak but definitely detectable staining (≥ 5% and ≤ 25%), (++) for moderate staining (> 25% and ≤ 75%), and (+++) for abundant staining (> 75%). All the evaluations were made by two independent pathologists, unaware of the clinical data.

Statistical analysis

Statistical analysis was carried out using SPSS version 12.0. Immunohistochemical staining of Mcm3 and Mcm4 was analyzed using the Kruskal-Wallis test. Spearman and Kendall analyses were used to evaluate correlations with clinicopathological parameters. Differences were considered to be statistically significant at $p < 0.05$.

Results

Expression of Mcm3 and Mcm4 proteins using immunohistochemical staining

The staining results in patients from the control, CIN I to CIN II-III and CSCC are summarized in Figure 1, Table 1. Expression of Mcm3 and Mcm4 was found in squamous epithelial cells in all cases of CSCC, CIN II-III and CIN I. In controls, Mcm3 expression was absent in 24 cases and present (+) in two cases, while Mcm4 expression was absent in 25 cases and present (+) in one case. A significant statistical difference in Mcm3 and Mcm4 expression was observed between the normal control group and tumor groups. Mcm3 and Mcm4 expression increased gradually, but became significantly stronger from intraepithelial neoplasia grade I through to cervical squamous cell carcinomas ($p < 0.001$), except there was no significant difference between CIN I and CIN II-III.

Mcm3 and Mcm4 protein expression in relation to clinicopathological parameters

Immunostaining results of the different proteins (Mcm3 and Mcm4) in relation to clinicopathological parameters including age, stage, tumor size, invasive depth, lymph nodes metastasis and differentiation are shown in Table 2. The only significant association was between Mcm3 expression and differentiation ($p = 0.024$). However, Mcm3 and Mcm4 were not associated with any other parameters.

Discussion

Cervical cancer is a leading cause of death in women but it does not have to be. About one-third of the cancer burden could be decreased if cases were detected and treated early. When caught early, the chances of being cured are very good. Therefore, biomarkers for clinical diagnostics are of vital importance.

The entire Mcm family (Mcm2-7) plays an essential role in eukaryotic DNA replication. Several reports have indicated the usefulness of Mcm proteins as markers of cancer cells in histopathological diagnosis. A linear increase in Mcm5 mRNA expression has been observed in the normal cervix, CIN III and invasive cervical carcinoma [13]. Diffuse and full epithelium thickness staining for Mcm7 was observed in high-grade cervical epithelial lesions and in invasive cervical carcinoma [14]. Gene-expression profiling experiments have demonstrated...
upregulation of Mcm proteins 2, 4, 5 and 6 in cervical cancer cells when compared with normal cervical keratinocytes [15]. More frequent and higher-level expression of Mcm3 and Mcm4 were found in cancer cells than in normal proliferating cells of human uterine cervices and dysplastic cells [11]. An essential role in proliferation for Mcms and their regulators makes them potentially important biomarkers for routine clinical use in cancer detection and prognosis [16, 17].

In the present study, we observed that Mcm3 and Mcm4 were expressed in cervical squamous epithelium in all CSCC, CIN II-III, CIN I (except 1 case) cases. Both Mcm3 and Mcm4 expression showed a tendency to be stronger from normal cervical epithelium through to invasive squamous cervical carcinomas, suggesting a potential marker in diagnosis of CSCC in clinics. Mcm3 expression was significantly associated with tumor cell differentiation, although there was no association between Mcm3 and Mcm4 and any other clinicopathological parameters (age, stage, tumor size, invasive depth and lymph node metastasis). Thus, Mcm3 and Mcm4 could be more relevant to progression rather than prognosis.

In conclusion, our study demonstrated that the two proteins, Mcm3 and Mcm4, which are essential for DNA replication in eukaryotes, had diagnostic significance for CSCC, and might be used as biomarkers in clinical diagnosis.

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Free of charge
Repeated chemosensitivity testing in patients with epithelial ovarian carcinoma

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Palacký University Medical School & Teaching Hospital, Olomouc (Czech Republic)

Summary
Epithelial ovarian carcinoma (EOC) is a highly chemosensitive tumor, but most patients with advanced EOC initially responding to first-line chemotherapy will eventually relapse. Chemosensitivity testing may offer an opportunity for the optimal selection of chemotherapeutic agents for individual patients. In the present retrospective analysis we have examined the changes in chemosensitivity profiles during the course of the disease. Chemosensitivity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test. Two or more samples at least 14 days apart were obtained from 34 patients with ovarian cancer. Chemoresistance increased significantly at the second measurement only for paclitaxel and carboplatin, the most frequently used cytotoxic drugs. No significant difference compared to baseline was observed at subsequent measurements for any other cytotoxic agent studied, although a non-significant trend for increased chemoresistance was observed. In conclusion, in the present cohort only paclitaxel and carboplatin chemosensitivity changed significantly, although to a limited extent, during the course of the disease. In contrast to a limited increase of paclitaxel and carboplatin chemoresistance, no significant changes were observed for other cytotoxic agents examined. The present data indicate that chemoresistance increases, to a modest extent, against the drug most frequently used, but remains relatively stable during the course of disease, especially for agents that are not used in the therapeutic regimen.

Key words: Chemosensitivity; Epithelial ovarian carcinoma; Paclitaxel; Platinum.

Introduction
Epithelial ovarian carcinoma (EOC) is the leading cause of death from gynecological cancer [1]. At the same time, EOC is a highly chemosensitive tumor. Currently, the standard first-line regimen in advanced EOC is the combination of paclitaxel and platinum (cisplatin or carboplatin), and the response rate to this combination is about 60-70% [2, 3]. Although the complete response rate is also relatively high, the tumor recurs in a vast majority of patients after a median of 16 to 18 months. The response rate to agents used in the second line of therapy, including topotecan, gemcitabine, or liposomal doxorubicin, is substantially lower, and these responses are usually of limited duration. The response rate to combinations of second-line agents is also relatively low [4]. Recurrent EOC responds to repeat administration of paclitaxel/platinum combination or platinum monotherapy [5, 6]. The probability of response to repeat administration of platinum-based regimens increases with the time from the last platinum administration (platinum-free interval), and, based on platinum-free interval, recurrent EOC may be classified as refractory, resistant or sensitive [7].

Although the probability of response to paclitaxel/platinum combination in platinum-sensitive recurrent EOC is relatively high, the optimal management of recurrent EOC is still a matter of dispute, and there is no universally accepted standard of care in these patients [8, 9]. The choice of therapeutic agents in patients with second or subsequent recurrences is even less clear. The activity of second line chemotherapeutic agents in EOC, e.g. topotecan, gemcitabine, etoposide, or fluoropyrimidines, alone or in combination with platinum, is compromised by considerable toxicity [4]. In many patients the limited benefit of administration cytotoxic agents in patients with recurrent or refractory EOC may not justify the risk of, sometimes life-threatening, toxicity. In individual patients, the decision whether or not to start treatment with second-line chemotherapy could be difficult.

Chemosensitivity testing offers a potential to predict efficacy of cytotoxic agents [10, 11]. Earlier studies using different methods for the assessment of chemosensitivity have reported that chemosensitivity may predict therapeutic response to conventional chemotherapeutic regimens [11-13] and overall or recurrence-free survival [13-15], but these results were not reproduced in other studies [16]. In a randomized trial in patients with recurrent EOC, only a non-significant trend in response rate and survival was observed in favor of patients who had chemotherapy selected based on chemosensitivity testing compared to patients treated by chemotherapy selected by the physicians [17].

There is currently limited information on how chemosensitivity changes during the course of therapy and recurrence in EOC. We present here a retrospective analysis of changes in chemosensitivity during the course of disease in EOC patients.
Patients and Methods

From the database of patients treated at the Palacký University Medical School Teaching Hospital and tested for chemosensitivity, patients who had chemosensitivity testing performed repetitively were identified through the retrospective search. Thirty-four such patients aged 54 ± 12 (range 33-82 years) with histologically verified EOC were identified. The patients underwent standard diagnostic workup, surgery and systemic chemotherapy. Twenty-one patients were examined at the diagnosis. The stage distribution in these patients was as follows: Stage Ic one patient, Stage IIb two patients, Stage IIIc 14 patients and Stage IV four patients. Thirteen patients had recurrent EOC and received one or more lines of chemotherapy prior to chemosensitivity testing.

Chemosensitivity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test as described previously [18, 19]. Briefly, the suspension of tumor cells was obtained from solid tissue samples or ascites obtained during surgery or paracentesis performed for therapeutic purposes. Solid tumor samples were first digested enzymatically and ascitic fluid was processed directly. The tumor cell suspension was then washed twice and centrifuged (1600 rpm, 7 min). After obtaining a cell count, 50-80x10³ cell aliquots were added to each well of 96-well plate. The cells were then diluted overnight after adding 10 μl of 10% sodium dodecylsulphate. The absorption was then read at 540 nm, and the IC₅₀ values was observed.

Two or more samples at least 14 days apart and with at least one chemotherapy cycle administered inbetween were obtained from 34 patients with EOC. Because of limited material in some samples, the determination of chemosensitivity across the whole panel of cytotoxic agents was not possible in every sample. The median time between the measurements was ten months (range 14 days to 57 months), the median time from the last chemotherapy was one (range 0-15) month. No significant differences in baseline chemosensitivity parameters were observed among 21 patients examined at the time of diagnosis compared to 13 patients with recurrent disease who had received prior chemotherapy (data not shown), and therefore both groups were pooled for further analyses. Complete response was obtained in 19 patients. No significant differences were observed in chemosensitivity at the first or second determination between patients who had or did not have complete response, with the exception of lower IC₅₀ for doxorubicin at the second determination in patients with complete response (mean ± standard deviation: 1.5 ± 0.5 vs 1.8 ± 0.5 μg/ml, p = 0.03). Twenty-six patients received platinum-based therapy (carboplatin in 16 patients) and 14 patients received paclitaxel after the first chemosensitivity testing. Chemoresistance increased significantly at the second measurement only for paclitaxel and carboplatin (Table 1). No significant differences were observed based on the interval from the last therapy (less than 6 months vs 6 months and more, data not shown). Also no significant difference was observed compared to baseline at subsequent measurements for any of the cytotoxic agents studied, although a general trend for higher IC₅₀ values was observed.

Discussion

In the present cohort, a relatively limited but statistically significant increase in paclitaxel and carboplatin chemoresistance has been observed. This finding is not surprising as paclitaxel and carboplatin are standard front-line agents, and the increase in chemoresistance may be explained by the exposition to these agents during therapy and the selection of drug-resistant cells. The changes of chemosensitivity were relatively limited, reflecting a relatively long interval between the tests. Longer interval between the tests is obviously associated with prolongation of platinum-free interval. Moreover, a selection bias has to be presumed, with surviving patients more likely to have chemosensitive disease. Third or fourth determination of chemosensitivity was available only for a limited number of patients, and the interpretation of negative results later in the course of follow-up could be more difficult.

EOC represents the leading cause of mortality for gynecologic cancer [1]. Surgery alone can be curative for a minority of patients who present with early disease. The use of platinum and taxane combination chemotherapies

<table>
<thead>
<tr>
<th>Agent</th>
<th>Baseline IC₅₀ (μg/ml)</th>
<th>Second determination</th>
<th>Third determination</th>
<th>Fourth determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etoposide</td>
<td>40 ± 14</td>
<td>47 ± 10</td>
<td>46 ± 12</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>1.6 ± 1.3</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 0.4</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>16 ± 14</td>
<td>19 ± 14</td>
<td>24 ± 25</td>
<td>39 ± 41**</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>16 ± 15</td>
<td>23 ± 14*</td>
<td>23 ± 15</td>
<td>25 ± 21</td>
</tr>
<tr>
<td>Vinorelbine</td>
<td>26 ± 23</td>
<td>35 ± 21</td>
<td>37 ± 18</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.7 ± 0.5</td>
<td>1.7 ± 0.6</td>
<td>1.9 ± 0.2</td>
<td>2.0 ± 0.04</td>
</tr>
<tr>
<td>Topotecan</td>
<td>5.6 ± 10.3</td>
<td>8.6 ± 16.4</td>
<td>6.8 ± 15</td>
<td>11.2 ± 19.6</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>41 ± 35</td>
<td>55 ± 32*</td>
<td>49 ± 36</td>
<td>70 ± 28</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>173 ± 126</td>
<td>136 ± 136</td>
<td>176 ± 124</td>
<td>221 ± 122</td>
</tr>
</tbody>
</table>

*Indicates statistical significance compared to control wells.

Table 1. Changes of chemosensitivity during the course of disease.

*p = 0.05; **p = 0.08.
has contributed to improved survival. However, since 80% of patients in Stages III and IV will progress or relapse within five years, interest remains in the development of new therapeutic strategies. Despite aggressive surgery and chemotherapy, most patients with advanced disease will ultimately relapse and die. On the other hand, the survival of EOC patients has improved substantially over the recent decades. The combination of platinum derivatives (cisplatin or carboplatin) with paclitaxel currently represents the standard front-line regimen for patients with advanced disease after demonstration of superior survival in randomized clinical trials [2, 3], and new therapeutic options have emerged in patients with recurrent disease.

The efficacy of drugs used for second-line therapy of EOC, including topotecan, gemcitabine, etoposide, or fluoropyrimidines is limited [4]. Moreover, the potential benefit of second-line therapy is offset by considerable toxicity. Chemosensitivity testing may offer a solution for helping in the selection of cytotoxic agents for individual patients. However, conflicting results have been reported regarding the prognostic and predictive value of chemosensitivity testing in EOC [11-17, 20, 21]. This could be due to differences in patient populations, therapeutic regimens or methods of chemosensitivity assessment. Different methods of measuring chemosensitivity have been reported, including extreme drug resistance assay [16, 20, 21], adenosine triphosphate assay [10, 15, 17], 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium (MTS) assay [11] or the MTT test used in the present study [12, 14]. A modification of the MTT test using histoculture in collagen gel sponge has also been utilized [13]. Some reports have indicated significant heterogeneity in chemosensitivity between samples obtained from different tumor locations [21]. Differences in chemosensitivity were found among tumors of different histology [20]. On the other hand, little is known about changes of chemosensitivity during the course of the disease.

The present data indicate that chemosensitivity in EOC measured by the MTT test is relatively stable throughout the course of the disease. This is, in general, in agreement with clinical experience that EOC responds repeatedly to paclitaxel and platinum, especially after prolonged intervals since the last chemotherapeutic regimen [22]. The chemosensitivity to second-line agents is not affected by paclitaxel/platinum chemotherapy indicating lack of cross-resistance. As mentioned above, the retrospective design of the study probably selected patients with more chemosensitive disease. From the point of view of clinical practice, these findings may be reassuring. Although a limited number of patients were examined, the present data seem to justify the repeat administration of cytotoxic drugs in multiple lines of chemotherapy. On the other hand, the present retrospective data also demonstrate limitations of chemosensitivity testing using the current method. Chemosensitivity was not associated with the response to chemotherapy in this cohort of patients. Utilization of the present chemosensitivity assay for therapeutic decisions should remain experimental.

In conclusion, in the present cohort the paclitaxel and carboplatin chemosensitivity changed significantly during the course of the disease. In contrast to a limited increase of paclitaxel and carboplatin chemoresistance, no changes were observed for other cytotoxic agents examined. The present data indicate that chemosensitivity remains stable during the course of disease, especially for agents that are not used in the therapeutic regimen.

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Non-hormonal treatment of vasomotor symptoms in gynecological cancer patients

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Introduction

Younger women who undergo bilateral oophorectomy for gynecological cancers have a precipitous drop in estrogen levels, generally causing earlier, more severe and long-lasting hot flashes than women entering menopause spontaneously [1]. The quality of life of oncologic patients is further reduced by the effects of surgery, chemotherapy or radiotherapy. Climacteric symptoms (Table 1) are mixed with psychological symptoms caused by genital mutilation and by the knowledge of having a neoplasm, often with a poor prognosis. The most effective means of relieving hot flashes is hormone replacement therapy (HRT) which will reduce their frequency by 80-90%. Unfortunately, HRT is contraindicated in estrogen-sensitive gynecological neoplasms like endometrial or endocervical adenocarcinomas. Furthermore, because of the presence of estrogen or progesterin receptors in ovarian cancers and in the epithelium of the lower genital tract, the risk of cancer cell growth promotion cannot be fully dismissed even in these so-called non-estrogen sensitive cancers.

To try to avoid hormonal therapy, every woman has to be informed about all the strategies to reduce hot flashes. Women who actively participate in the decision-making process regarding their health care are more satisfied with their ultimate choices, especially if the approach is tailored to the woman’s individual situation and reflects her priorities and values. Exercise, lighter clothing, sleeping in a cooler room and reducing stress may be sufficient for many women. Avoidance of possible triggers, including spicy foods, caffeine, smoking and alcohol may also help.

If the non-pharmacological treatment is not sufficient, there are non-hormonal therapies but most of these trials suffer from methodological shortcomings [2]. Almost all data come from studies performed on breast cancer patients, and the number of patients is small, follow-up is too short and the placebo effect ranges from 20%-40%. The best evidence available today that confirms the significantly higher efficacy of non-hormonal treatments over placebo will be reviewed and discussed [3-7].

Summary

Gynaecological cancer patients generally suffer from an earlier and more severe menopausal syndrome than the general female population. Hormone replacement therapy is often contraindicated and there are non-hormonal treatments that are proven to be more effective than placebo in randomized controlled trials, e.g., some antidepressants, gabapentin and clonidine. The main limits to the use of these drugs in controlling hot flashes are the off-label use for this purpose, the very short follow-up and the fact that data come from studies performed on breast cancer, not on gynaecological cancer patients. Patients believe that drugs derived from plants could be effective in relieving hot flashes and that they are harmless. Evidence is contrary to this belief and estrogen-sensitive cancer patients should be warned of the potential, though very weak, estrogenic effect of phytoestrogens and other “natural” drugs, and that their efficacy is close to that of a placebo.

Key words: Gynecological cancers; Menopausal syndrome; Antidepressants; Gabapentin; Clonidine; Phytoestrogens.

Table 1. — Menopausal symptoms in gynecological cancer patients.

<table>
<thead>
<tr>
<th>Categories of symptoms</th>
<th>Climacteric symptoms</th>
<th>Usual presentation of menopausal syndrome in gynecological oncologic patients *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychological</td>
<td>Mood swings, depression, anxiety, irritability, insomnia.</td>
<td>Earlier. Suddenly induced by surgery, chemotherapy and/or radiotherapy.</td>
</tr>
<tr>
<td>Sexual</td>
<td>Vaginal dryness, dyspareunia, decreased libido, sexual dysfunction.</td>
<td>Mixed with psychological symptoms caused by genital mutilation and by the knowledge of having a neoplasm often with a poor prognosis.</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>Urinary frequency or incontinence.</td>
<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>Myalgia or arthralgia, weakness, paresthesias.</td>
<td></td>
</tr>
</tbody>
</table>

*Versus spontaneous menopause in non-oncologic women.
Serotonin reuptake inhibitors (SRI)

Serotonin reuptake inhibitors (SRI) can be divided in two groups: selective serotonin noradrenalin reuptake inhibitors (SNRIs) including venlafaxine, and selective serotonin reuptake inhibitors (SSRIs) such as paroxetine and fluoxetine. These newer antidepressants provide almost immediate relief from hot flashes, as opposed to the higher doses and much longer time frame needed to treat depression. Two-thirds of women derive some benefit from these agents, compared to approximately one-third who may typically respond to placebo. Evidence is fairly good for venlafaxine and paroxetine.

Venlafaxine for the relief of vasomotor symptoms has been compared to placebo in randomized controlled trials which reported a short-term significant reduction in the frequency and severity of hot flashes in cancer patients. The initial daily dose of venlafaxine is 37.5 mg. If this is not effective after 7-14 days, it could be increased to 75 mg/day. The most frequent side-effects are dry mouth, insomnia, sedation, decreased appetite and constipation. Venlafaxine could interact with MAO inhibitors, tioridazine and warfarin [8-11].

Paroxetine is more effective than placebo in reducing the frequency and severity of hot flashes. The lower dose (10 mg/day) is generally well tolerated and associated with fewer side-effects: headache, nausea, insomnia, anxiety, and loss of libido. Paroxetine has similar side effects to those of venlafaxine, but it could also interact with tamoxifen [12-15].

The effect of fluoxetine is still controversial: it seemed useful for hot flashes but it was not effective in a more recent placebo-controlled randomized study at the dose of 20-30 mg/day [16, 17].

Citalopram showed a trend towards an improvement in vasomotor symptoms which did not however reach statistical significance at the dose of 20-60 mg/day, perhaps because of a significant drop-out rate [18].

Gabapentin

Gabapentin is an anticonvulsant which can alleviate hot flashes. In a few randomized controlled trials at a dose of 900 mg/day divided in three doses, it was found to be more effective than placebo in reducing the frequency and severity of hot flashes in postmenopausal women, including those with breast cancer. Gabapentin may cause weight gain, drowsiness, fatigue, somnolence, dizziness and palpitations [19-21].

Clonidine

Clonidine is an anti-hypertensive agent which acts as an alpha-2 agonist by reducing vascular reactivity. Oral clonidine is used at the dose of 0.1-0.4 mg/day, while the clonidine patch applied weekly releases 0.1 mg/day. A meta-analysis of the four most recently published placebo-controlled randomized trials showed a moderate, but statistically significant reduction in hot flash frequency when used for between eight and 12 weeks [5]. It may cause sedation, drowsiness and dry mouth. When discontinuing, the dose should be gradually tapered to avoid adverse reactions [22-30].

Phytoestrogens

Phytoestrogens can function as selective estrogen receptor modulators (SERMs) and they are thought to reduce the severity and frequency of hot flashes, but there is conflicting and poor evidence of this. To date, studies have been small and lack statistical power. These studies cannot be readily aggregated to increase the statistical power due to differences in their methodologies. A systematic review and meta-analysis of 18 small randomized controlled trials found that phytoestrogens were not clinically superior to placebo [31]. There is also some concern about phytoestrogens in that they should not be used in women with estrogen-sensitive cancers.

Black cohosh (Cimicifuga racemosa)

Black cohosh appeared to be more effective than placebo only in cases of mild to moderate vasomotor symptoms. It is no longer used in Italy and it could be harmful in estrogen-sensitive neoplasms [32].

Red clover (Trifolium pratense)

Red clover is thought to act as an estrogen receptor agonist. A meta-analysis of seven randomized studies did not show red clover to be clinically superior to placebo for the relief of vasomotor symptoms, while its long-term safety and efficacy are still unknown [31].

Ginseng (Panax ginseng)

Ginseng showed no significant benefit over placebo in reducing hot flash frequency after four months of use in a placebo-controlled double-blind multi-centre randomized trial [33]. Ginseng is also not effective in improving cognitive and psychosomatic symptoms during menopause [34].

Evening primrose (Oenothera biennis)

Evening primrose is popular as a rich source of gamma linolenic acid, believed to treat mood swings, irritability, and breast tenderness associated with premenstrual syndrome and menopause. In a double-blind, placebo-controlled randomized trial, results proved to be worse than placebo [35].

Dong-Quai (Angelica sinensis)

Dong-quai is considered to be a tonic in traditional Chinese medicine, but the essential oil in dong-quai contains a carcinogenic substance. Its effectiveness in reducing postmenopausal vasomotor symptoms was not confirmed in a double-blind, placebo-controlled randomized trial [36].

Vitamin E

Vitamin E is used for treating hot flashes. A randomized crossover trial found that 800 IU of Vitamin E resulted in one less hot flash per day than the placebo, therefore of no significance [37].
Non-hormonal treatment of vasomotor symptoms in gynecological cancer patients

Drugs Under Evaluation

Mirtazapine at the dose of 15-30 mg daily resulted in almost complete resolution of hot flashes within one week of treatment initiation [38].

Propranolol in a randomized, placebo-controlled study showed a significant reduction in the frequency and severity of vasomotor symptoms [39].

Conclusion and clinical practice suggestions

Gynecological cancer patients who seek relief from their menopausal symptoms should be fully informed about the benefits and risks of the different non-hormonal treatments (Table 2). Active listening by the physician, collaborative decision-making and adequate support should be offered to the patient. Individual values and pri-
orities should also be considered. A healthy lifestyle and/or lifestyle modifications are the first most important step in both menopausal symptom relief and disease prevention.

Non-hormonal therapies such as venlafaxine, paroxetine, gabapentin and clonidine have been evaluated in randomized trials and have been shown to reduce the frequency and/or severity of hot flashes better than placebo. To date, there have been no direct head-to-head randomized comparisons between the various effective non-hormonal therapies for the relief of menopausal vasomotor symptoms.

A brief trial of a week’s treatment with an SSRI or SNRI is worth a try as a first choice. They may demonstrate effectiveness in one week. If this does not work, doses could be increased or another antidepressant could be tried. There is currently insufficient evidence to recommend the use of fluoxetine. None of the above-mentioned treatments is licensed for the treatment of vasomotor symptoms. However, a large part of gynecological oncological patients have some degree of depression or hypertension and, in these cases, the use of an antidepressant or clonidine, respectively, could be justified. Gabapentin is an effective alternative but its main problem is the off-label use for this purpose.

Phytoestrogens and red clover seem to be as effective as placebo, but much larger and longer studies are needed to detect infrequent, but potentially serious, adverse events. Inconsistencies among studies to date may be explained by different doses, sources and production methods.

Black cohosh is no longer available due to its potential risks. Non prescription medications like ginseng, evening primrose, Dong-Quai and vitamin E are not effective and could even be harmful: patients must be adequately informed about this.

Larger studies using standardized definitions of the treated population and outcome measures together with a high quality methodology and longer follow-up duration are still needed to confirm the optimal non-hormonal treatment options. Because of the heterogeneity in the neoplastic cell receptors and the interindividual metabolic variability, population studies are difficult to project on a personal level. The only way to determine efficacy and tolerability is to test the drug and to closely monitor its effects on each particular subject.

References


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Analysis of clinical and molecular associations of triple negative breast cancers in node-negative patients

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J. Chrysikos³, G. Xepapadakis³, A. Manouras¹

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Summary

Introduction: Therapeutic modalities in node-negative breast cancer patients remain a matter of controversy. Various prognostic factors have been proposed to help select those patients that would most likely benefit from adjuvant therapy. In view of this notion the triple negative phenotype (hormone receptors and HER2 negative tumors) has gained increasing attention. Aim: To evaluate the clinicopathologic characteristics of triple negative (TN) tumors in node-negative invasive breast carcinomas. Methods: We retrospectively analyzed the archival pathology tissues of 160 patients with node-negative invasive carcinomas, diagnosed and treated in two surgical departments in Greece from 1999 to 2006. Statistical analysis was used to examine the association between TN tumors and other clinicopathological factors. Results: Triple negative breast cancers correlated with higher histologic grade, mitotic activation index and Ki-67 expression (p < 0.05). Moreover TN tumors were correlated with negative staining for bcl-2 (p < 0.05). Conclusion: In node-negative breast cancer patients, triple negativity is associated with aggressive biologic behavior. Further studies are required to better understand the clinical implications of these findings.

Key words: Triple negative breast cancer; Molecular profile; EGFR expression.

Introduction

Breast cancer is one of the most common malignances worldwide. According to the 2008 cancer statistics, in the United States breast cancer is the most common female cancer and the second frequent cause of cancer death in women [1]. Nonetheless overall breast cancer mortality rates have declined – a phenomenon that is partly attributed to the widespread application of adjuvant systemic therapy [2]. Adjuvant therapy refers to the use of endocrine therapy, chemotherapy, and/or trastuzumab (a humanized monoclonal antibody directed against HER2) after primary surgery. The choice regarding which of the aforementioned therapies will be used/combined depends on both clinicopathologic factors as well as tumor hormone receptors and HER2 status [3]. Although the use of adjuvant therapy is fully recommended for patients with lymph node involvement, its efficacy on node-negative tumors remains controversial [4, 5]. The need for more reliable prognostic factors that could help select those patients with early-stage breast cancer, who would most likely benefit from adjuvant therapy, triggered the onset of various studies [6]. During the last few years the use of genomic technology for the analysis of breast tumor samples has gained increasing attention [7, 8]. As a result of gene-expression analyses, four major molecular classes have emerged: luminal-A, luminal-B, basal-like, and human epidermal growth factor receptor (HER)-positive cancers [9]. Immunohistochemistry appears to be an acceptable method, at least partly, able to discriminate between these molecular subclasses. The basal-like subtype is characterized by negativity for estrogen receptor (ER), progesterone receptor (PR), and HER2 (triple-negative TN) and is associated with aggressive histology and poor prognosis [10, 11]. Although only about 85% of TN breast cancers are deemed to be basal-like, the TN phenotype has become a commonly used proxy for this subtype [10]. During the last decade various researchers have commented on the possible clinical implications of this certain phenotype; nevertheless, current data remain inconclusive [12-14]. We conducted a study in order to further elucidate the association of triple negativity with patients’ and tumor characteristics in node-negative invasive breast carcinomas.

Methods

We retrospectively analyzed the archival pathology tissues of 529 patients with breast cancer diagnosed and treated at the First Department of Surgery of the Hippokration Hospital, the Second Department of Surgery of the 417 Military Veterans’ Fund Hospital and the Breast Surgical Unit of the IASO General Clinic between 1999 and 2006. All patients with node-negative invasive mammary carcinomas and adequate results of immunohistochemistry for hormone receptors and HER2 were included in the study.

Patients having node-positive disease (n = 209) or being histologically diagnosed with in situ carcinomas (n = 27) or sarcomas (n = 2) were excluded. From the remaining 291 patients, 131 were excluded because of insufficient data and the remaining 160 patients constituted our study group. The following factors were evaluated: patient age at diagnosis, tumor size, and
grade, as well as ER, PR and HER2 status. Furthermore, in a subgroup of patients we were able to collect data regarding tumor mitotic activity (93 patients) and expression of the epidermal growth factor receptor (EGFR) (50 patients), bcl-2 (69 patients), p53 (90 patients) and Ki-67 (115 patients). The study protocol was approved by the participating hospitals’ ethics committee. Immunohistochemistry was carried out on 5 μm tissue sections from paraffin blocks using the avidin-biotin immunoperoxidase method. Briefly paraffin was removed from the slides by heating them at 60°C for 10 min, followed by three washes in xylene. After gradual hydration through graded alcohol, the slides were incubated for 30 min in 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase activity. The sections were incubated in citrate buffer (0.01M: pH 6.0) for two cycles of 5 min in a microwave oven for antigen retrieval. The following monoclonal antibodies were used: primary antibodies directed against ER (NCL-ER-6F11), PR (NCL-PGR), c-erbB-2 oncoprotein internal domain (NCL-CB11), p53 (NCL-CMI) [Novocstra, Newcastle upon Tyne, UK] and Ki-67 (clone MIB-1), bcl-2 (clone 124) and EGFR (clone H11) [Dako, Glostrup, Denmark] were applied in a dilution of 1/50, 1/50, 1/100, 1/500, 1/800, 1/50 and 1/50, respectively. For statistical analysis purposes, the sections were scored as either negative or positive. Staining for p53, ER, PR, Bcl-2 and EGFR was graded as positive if 10% or more of the tumor cells were stained. Ki-67 staining of 20% or more was used to define a high level of cell proliferation [15]. The HER2 membrane staining was scored as 0, 1+, 2+ or 3+. Tumors with 0, 1+ or 2+ staining were defined as negative and with 3+ as positive. Mitotic activity was evaluated as the number of mitotic figures per ten high-power fields (HPF) [mitotic activity index (MAI)]. For clinical analysis, three mitotic activity index subgroups were considered: low (MAI < 10/HPF), medium (10 ≤ MAI ≤ 15) and high (MAI > 15). Tumor grading was performed according to the Ellis and Elston grading system [16].

Statistics
A standard statistical software package SPSS (SPSS Inc., Chicago IL) was used in the analysis. Descriptive statistics were calculated for all variables. The chi-square test or Fisher’s exact test was used; while in the absence of normal distribution the Mann-Whitney Smirnov test was used to test if a variable was normally distributed. Normally distributed data were analyzed with the T-test; 0.05) and higher Ki-67 expression (100% of the TN tumors had 20% or more Ki-67 staining compared to 58.5% of the non-TN tumors, p < 0.05). Moreover TN tumors were associated with negative staining for bcl-2 expression (85.7% of the TN tumors vs 35.5% of the non-TN tumors, p < 0.05).

Although TN tumors were associated with positive staining for EGFR (50% of the TN tumors vs 6.3% of the non-TN tumors), this correlation was not statistically significant. Furthermore TN patients had a higher percent of p53 positive tumors, compared to non-TN (52% vs 34.4%), nevertheless also this relationship did not reach statistical significance.

Lastly p53 staining was associated with higher histologic grade (61.8% of the p53 positive tumors compared to 33.3% of the p53 negative, p < 0.05), higher MAI (53.8% vs 22.2%, p < 0.05) and higher Ki-67 expression (76.6% vs 51.5, p < 0.05).

Discussion
One of the most intriguing areas of research in breast cancer is the study of the different subtypes that have emerged with the help of molecular biology. Among them the basal-like molecular subtype has been characterized by the lack of expression of hormone receptors, and the absence of HER2 protein overexpression, features which render these tumors ineffective to targeted therapies.

This study showed that TN tumors were associated with higher histological grade, MAI and Ki-67 expression, markers that have been correlated with poor prognosis [17-19]. The biologic behavior of TN tumors has been a subject of evolving research during the last decade. A review of the current literature reveals that the majority of

<table>
<thead>
<tr>
<th>Table 1. — Tumor characteristics of the patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (mean ± SD)</td>
</tr>
<tr>
<td><strong>Histological type, number (%)</strong></td>
</tr>
<tr>
<td>Ductal</td>
</tr>
<tr>
<td>Lobular</td>
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<tr>
<td>Ductal/lobular</td>
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<tr>
<td>Colloid</td>
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<tr>
<td>Tubular</td>
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<tr>
<td>Medullary</td>
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<tr>
<td><strong>T stage, number (%)</strong></td>
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<tr>
<td>T1</td>
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<tr>
<td>T2</td>
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<tr>
<td>T3</td>
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<tr>
<td><strong>Tumor grade, number (%)</strong></td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td><strong>Positive tumors for [number (%)]</strong></td>
</tr>
<tr>
<td>ER</td>
</tr>
<tr>
<td>PR</td>
</tr>
<tr>
<td>HER2</td>
</tr>
<tr>
<td>TN tumors</td>
</tr>
</tbody>
</table>

ER: estrogen receptors; PR: progesterone receptors; TN: triple negative tumors (ER, PR and HER2 negative).
published studies describe TN breast cancers as tumors of high histologic grade [20]. In the same manner a recent study by Rhee et al., in node-negative breast cancer patients, showed that TN breast cancer had a higher relapse rate and more aggressive clinicopathologic characteristics than non-TN tumors [21].

Furthermore, we showed that TN tumors were correlated with negative staining for bcl-2 expression. The bcl-2 protein is a member of the bcl family that regulates apoptosis and its expression exerts a protective effect in breast cancer [22]. A recent meta-analysis concluded that bcl-2 is an independent prognostic factor with possible significant clinical implications [23].

Previous studies on TN tumors have shown that they are closely correlated with the expression of the EGFR [21]. EGFR plays an important role in breast carcinogenesis and its potential role as prognostic indicator has been documented by previous researchers, while EGFR-targeted therapies have proved quite valuable [24,25]. Considering the lack of targeted therapies in TN breast cancer patients, the use of EGFR-targeted agents has been proposed as an attractive alternative [26]. According to our results, although TN tumors were associated with positive staining for EGFR, this correlation was not statistically significant, a finding that may be attributed to the low statistical power of this study (data regarding the EGFR status were available in a subgroup of 50 patients).

In the current study, TN patients had a higher percent of p53 positive tumors, compared to non-TN patients, nevertheless this correlation was not statistically significant. Published data by larger series regarding the clinicopathologic features of TN breast tumors have shown a significant association with the expression of the p53 marker [27-29]. Nonetheless the clinical significance of this marker in breast cancer remains a matter of ongoing debate. A meta-analysis by Pharough et al. showed that p53 could serve as an independent predictor of decreased disease-free and overall survival in both node-positive and node-negative patients [30]. On the other hand other studies have failed to show any prognostic value of the p53 expression [31, 32]. Analysis of our data showed that p53 staining was associated with higher histologic grade, MAI and Ki-67 expression, markers which, as previously mentioned, are associated with adverse outcome. This controversy regarding the significance of p53 may be attributed to a number of factors such as: a) the low statistical power of some studies and the differences between the applied detection methods (immunohistochemistry or molecular techniques) and b) the use of adjuvant chemotherapy, since p53 gene alteration may be associated with resistance or sensitivity to different therapeutic agents [33].

This study has some limitations. Although there is general agreement that HER2 0 and 1+ are clinically negative and HER2 3+ is clinically positive, there is uncertainty about the appropriate classification of HER2 2+ patients. In the current study due to the lack of fluorescent in situ hybridization (FISH) information on the HER2 2+ patients, they were regarded as HER2 negative during analysis. Furthermore data regarding the status of some immunohistochemical markers were available in a small number of patients.

In summary, we showed that in node-negative breast cancer patients triple negativity is associated with aggressive biologic behavior. The use of EGFR-targeted agents has been proposed as an attractive alternative for this particular subtype of breast cancer, nevertheless this study failed to show a significant association between TN cancer and EGFR expression. Further studies are warranted to better understand the biologic behavior of triple negative breast cancers, so as to develop better treatment strategies.

References

Analysis of clinical and molecular associations of triple negative breast cancers in node-negative patients


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Bevacizumab, paclitaxel and carboplatin for advanced ovarian cancer: low risk of gastrointestinal and cardiovascular toxicity

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Summary

The purpose of this preliminary study was to retrospectively assess the incidence of bowel perforation and hypertension in two separate advanced ovarian cancer patient populations following first-line therapy, comprising paclitaxel, carboplatin and bevacizumab. The first 20 patients were treated with six cycles of paclitaxel (175 mg/m²), carboplatin (AUC of 5 IV), and bevacizumab (15 mg/kg of body weight); q21 days per an independent protocol. The subsequent patients (n = 12) were administered weekly paclitaxel (80 mg/m²), carboplatin (AUC of 5 IV) every four weeks, and bevacizumab (10 mg/kg of body weight) every two weeks for six cycles according to a separate, independent protocol. Bevacizumab was not added to either chemotherapy regimen until cycle 2. In both groups patients who achieved a complete response, partial response or stable disease at the conclusion of induction therapy received bevacizumab (10 mg/kg) and paclitaxel (135 mg/m²) q21 days as maintenance therapy. A total of 170 cycles (median = 6; range 3-6) of primary induction chemotherapy, 140 of which contained bevacizumab, were administered. Moreover, 206 cycles (median = 9; range 1-12) of maintenance chemotherapy have been delivered to 28 patients thus far. There was no incidence of GI perforation and only two patients demonstrated clinically significant hypertension. Previous studies involving bevacizumab have reported varying rates approaching 11% [6, 9, 11]. Thus, we did not encounter difficulties with either of these complications. While we recognize that the risk for bowel perforation remains in the 5-11% range, the study’s preliminary results suggest that first-line treatment of advanced stage ovarian carcinoma with bevacizumab can be safely administered.

Key words: Bowel perforation; Gynecologic oncology; Ovarian cancer; Bevacizumab.

Introduction

Ovarian cancer is the fifth most common cancer in women, accounting for nearly 15,280 deaths annually [1]. While cytoreductive surgery and adjuvant chemotherapy have been effective in treating ovarian cancer, most patients eventually exhibit disease recurrence. Consequently, several investigators have studied the activity of novel non-cross resistant agents in combination with standard therapy to further stave off disease progression and mitigate patient toxicity [2].

Bevacizumab (Genentech, Inc.; San Francisco, CA) is a recombinant humanized monoclonal antibody that binds to and thereby, inhibits the biologic activity of human vascular endothelial growth factor (VEGF) [3]. VEGF is an essential component of angiogenesis in ovarian cancer and has been measured in several tumors, including lung, breast, thyroid, gastrointestinal tract, kidney, bladder, and cervix [4]. An increase in VEGF expression strongly correlates with a poor ovarian cancer prognosis [5]. Therefore, targeting the VEGF receptor ligand in an attempt to inhibit angiogenesis may be an effective strategy for treating epithelial ovarian carcinoma [6, 7].

Bevacizumab has been studied in several clinical trials, demonstrating promising results in patients with colorectal, lung, ovarian and breast carcinoma [7, 8]. Prior studies have discussed the safety and toxicity profile of bevacizumab, reporting mild to moderate cases of hypertension, proteinuria, hemorrhage, and uncommon but manageable wound healing complications [8, 9]. In particular, hypertension has been observed in several clinical trials [6, 9].

Gastrointestinal (GI) perforations also appear to be a direct consequence of bevacizumab [8-11]. In ovarian cancer, patients with diffuse abdominal carcinomatosis are already at significant risk for bowel obstruction and perforation. Moreover, bevacizumab potentially impedes new blood vessel formation and thus, patients with an already compromised blood supply to the bowel may be more susceptible to gastrointestinal (GI) perforation [6, 9].

Bevacizumab also appears to cause tumor necrosis in the bowel, predisposing patients to a GI perforation [10, 11]. Wright et al. reported a 9% incidence of GI perforation in their population of 23 platinum-refractory ovarian cancer patients treated with bevacizumab [10]. Similarly, Cannistra et al. reported an 11% incidence of bowel perforation following bevacizumab in their study comprised of 44 advanced platinum-resistant ovarian cancer patients [11], formally raising a safety concern in treating ovarian cancer [12].

Despite the apparent risk of GI perforation with bevacizumab in treating recurrent ovarian cancer, studies have reported varying rates approaching 11% [6, 9, 11]. Thus, the true incidence of bowel perforation, particularly in advanced stage ovarian carcinoma, remains indeterminate. The goal of this preliminary study was to retrospectively document the occurrence of GI perforation and hypertension following two distinct primary induction chemotherapy regimens for the treatment of advanced stage ovarian carcinoma.
Patients and Methods

Patient population and treatment regimen

Advanced stage ovarian carcinoma patients who received first-line paclitaxel, carboplatin and bevacizumab chemotherapy via an IRB approved monthly or weekly regimen were eligible for this retrospective evaluation. Every patient signed a consent form prior to enrollment. The intent of both protocols was to commence initial chemotherapy for all subjects within three weeks of cytoreductive surgery. Optimal cytoreduction was defined as tumor debulking with residual tumor ≤ 1 cm [13].

The first protocol was approved by Genentech, Inc. for 20 patients in March 2005 and closed in March 2006. The study participants all received standard induction chemotherapy with paclitaxel (175 mg/m²) and carboplatin (AUC = 5). Bevacizumab (15 mg/kg) was delivered over 90 min per study protocol. The dose of bevacizumab was determined by Genentech, Inc. based on data from their previous clinical studies [14]. The regimen was repeated every 21 days for six cycles. The primary endpoints of this paclitaxel, carboplatin and bevacizumab chemotherapy regimen were safety and progression-free survival. The secondary endpoints were objective response rate and patient overall survival. The results from this study have been previously reported [7].

The second protocol was approved by Genentech, Inc. for 20 patients in November 2007 and remains open to enrollment while the study data approach maturation. Currently, 12 patients have received primary induction chemotherapy comprising weekly paclitaxel (80 mg/m²), carboplatin (AUC of 5 IV) every four weeks, and bevacizumab (10 mg/kg) every two weeks for six cycles per study protocol. The dose of bevacizumab was determined by Genentech, Inc. based on their prior clinical data [15]. The primary intent of this weekly regimen was also to evaluate safety and progression-free survival. Similarly, the secondary endpoints were patient objective response rate and overall survival.

Since weekly and monthly chemotherapy regimens appear to have a similar toxicity profile and analogous efficacy [16], we elected to combine the two patient groups’ incidence of bowel perforation and hypertension. In order to be included in the toxicity analysis, patients had to complete ≥ 2 cycles of bevacizumab chemotherapy. Toxicity was graded using the National Cancer Institute criteria [17]. Clinical response was assessed by clinical, serologic, and radiographic means according to the RECIST criteria [18]. Chest X-ray and abdominal/pelvic computed tomography (CT) scans were performed prior to initial treatment, before cycles 1 and 2, and following the completion of cycle 6. In both regimens, patients who achieved a complete response, partial response or stable disease at the conclusion of induction therapy, received maintenance therapy encompassing bevacizumab (10 mg/kg) followed by paclitaxel (135 mg/m²) every 21 days for up to 12 cycles.

Eligible patients had adequate bone marrow, renal, and hepatic function, white-cell count of at least 3,000 per ml², platelets of at least 100,000 per ml², creatinine clearance ≥ 50 ml per minute, serum bilirubin no greater than 1.5 times normal, serum aspartate aminotransferase no greater than three times normal, no previous history of chemotherapy or irradiation, and an ECOG ≤ 2.

Exclusion criteria included septicemia, severe infection, borderline tumor diagnosis, acute hepatitis, severe gastrointestinal bleeding, history of congestive heart failure, angina, or myocardial infarction within the past six months. Patients removed from the study may have received additional treatment according to their physician’s discretion.

Results

From March 2005 until December 2008, 32 Stage III/IV ovarian carcinoma patients received their first course of chemotherapy within three weeks of cytoreductive surgery. Twenty-six women underwent optimal debulking surgery and six had suboptimal debulking surgery. Twenty-one patients underwent bowel resection with a subsequent colostomy (n = 5) or primary anastomosis (n = 16). A total of 170 cycles (median = 6; range 3-6) of chemotherapy were administered, 140 of which contained bevacizumab.

Bevacizumab was delayed until cycle 3 in one patient who underwent a bowel resection at the treating physician’s discretion. In addition to induction chemotherapy, there were 206 cycles (median = 9; range 1-12) of maintenance chemotherapy administered to 28 patients in the two study populations. Patient characteristics are exhibited in Table 1.

Table 1. — Patient characteristics (n = 32).

<table>
<thead>
<tr>
<th>Mean age</th>
<th>58.0 (range 41-76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Type (%)</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>26 (81.3)</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Fallopian</td>
<td>2 (6.2)</td>
</tr>
<tr>
<td>Histology type (%)</td>
<td></td>
</tr>
<tr>
<td>Papillary Serous</td>
<td>26 (81.3)</td>
</tr>
<tr>
<td>Mixed</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Stage (%)</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>IIIB</td>
<td>2 (6.2)</td>
</tr>
<tr>
<td>IIIC</td>
<td>25 (78.2)</td>
</tr>
<tr>
<td>IV</td>
<td>2 (6.2)</td>
</tr>
<tr>
<td>Grade (%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27 (84.4)</td>
</tr>
<tr>
<td>2</td>
<td>3 (9.4)</td>
</tr>
<tr>
<td>1</td>
<td>2 (6.2)</td>
</tr>
<tr>
<td>Residual disease (%)</td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>26 (81.3)</td>
</tr>
<tr>
<td>Suboptimal</td>
<td>6 (18.7)</td>
</tr>
</tbody>
</table>

Bowel perforation. There were no GI perforations associated with either induction or maintenance chemotherapy. One patient completed her sixth cycle of chemotherapy and had confirmatory scans; she was later hospitalized due to a bowel obstruction and removed from the study. The obstruction resolved with conservative management involving naso-gastric suctioning and total parenteral nutrition. The bowel obstruction did not recur.

Hypertension. Two patients developed grade 3 hypertension (HTN). They were subsequently managed medically by their primary care physician and remained on the study. Grade 2 HTN developed in three patients. Nine patients exhibited grade 1 HTN, although in one case, the study participant elected to stop taking her antihypertensives. She resumed her medication and the HTN was resolved.

Disease-free survival and overall survival. Currently, one patient has exhibited disease progression and none of the subjects have expired.
Discussion and Conclusions

Since nearly 80% of advanced ovarian carcinoma patients eventually exhibit disease recurrence, innovative therapeutic agents are continually studied in an attempt to improve patient response rates and overall survival. Bevacizumab is one of the first anti-neoplastic agents to exhibit significant activity in cancer patients by inhibiting the biologic activity of human VEGF [3]. Targeting the VEGF with bevacizumab appears to be a viable strategy for bolstering standard chemotherapy because the therapy has a novel mechanism of action with a distinct toxicity profile [6, 7].

Bevacizumab has been studied in several patient populations including colorectal, lung, and breast carcinoma [7, 8]. While there have been several investigations evaluating bevacizumab for the treatment of ovarian carcinoma, they have primarily involved recurrent disease populations [8, 9, 19, 20]. These studies have reported concerns with non-hematologic toxicity, particularly hypertension and GI perforation [8-11]. In particular, Cannistra et al. evaluated 44 recurrent ovarian cancer patients who were treated with single agent bevacizumab (15 mg/kg), but the study was prematurely closed because of an 11% incidence of bowel perforation [11]. Wright et al. documented that GI perforations occurred in 9% of their recurrent, refractory ovarian carcinoma patients following treatment with bevacizumab [10]. Burger et al. reported that six (9.7%) recurrent ovarian cancer patients experienced grade 3 hypertension following treatment with bevacizumab [9]. Similarly, Cannistra et al. documented a 9.1% rate of grade 3 hypertension in their bevacizumab study [11].

In the current investigation, we retrospectively examined the incidence of GI perforation and hypertension in two advanced ovarian carcinoma populations who were treated with a monthly or weekly paclitaxel, carboplatin, and bevacizumab primary induction therapy regimen. Although the study’s findings were preliminary, we did not encounter any GI perforations and the incidence (6.3%) of grade 3 hypertension was reasonably low. Penson et al. [21] likewise, reported a low incidence of grade 3 hypertension and no bowel perforations in their study of 30 gynecologic cancer patients who received first line treatment with carboplatin, paclitaxel, and bevacizumab.

Bowel perforations have frequently been documented in heavily pretreated cancer patients and attributed to intraabdominal inflammation, tumor necrosis and chemotherapy-induced colitis [8, 22]. For example, in the Cannistra et al. study, their patients with GI perforations were heavily pretreated and all had radiologic evidence of bowel involvement [11]. Transmural tumor burden, inflamed bowel response and impaired bowel mucosal renewal or neovascularization may also be contributing factors to the manifestation of GI perforation [19].

We suggest that the development of GI perforation with bevacizumab may be mitigated with careful patient selection and appropriate management [20]. In particular, the risk of bevacizumab related GI toxicity is potentially reduced in patients who are optimally debulked and treated in a first-line setting (i.e., not heavily pretreated) [8, 22, 23]. For example, Simpkins et al. suggested that bevacizumab is potentially contraindicated in patients with bowel obstruction, bowel involvement, or any evidence of rectosigmoid involvement [19].

In the current study, we also elected to initiate bevacizumab at cycle 2 to minimize the risk of surgical wound or bowel anastomotic healing complications. Delaying the bevacizumab until cycle 2 is similar to the approach used routinely for colon cancer patients following surgery [7]. Furthermore, one may consider postponing the bevacizumab until cycle 3 in patients with severe bowel healing complications.

While we cannot derive any definitive conclusions regarding the risk of GI toxicity and hypertension associated with bevacizumab, the lack of bowel perforation and reasonable cardiovascular toxicity are encouraging. Consequently, we felt compelled to assess and report the cumulative data prior to the second study’s conclusion. Conversely, since data collection from the second chemotherapy trial has not been completed, we do not preclude the possibility that some of those patients may eventually develop a bowel perforation.

We recognize that our relatively small sample size may be insufficient to exclude any risk for bowel perforation, particularly since the documented rates range from 5-11% [8, 9, 11]. Moreover, the concern for selection bias is noteworthy. The preliminary results from this retrospective study would also be strengthened had both protocols been completed and data collection finalized. However, as the data mature, we can comment further on response evaluation, toxicity, disease-free survival and overall survival. Additional study of bevacizumab incorporated into larger first-line clinical trials for the treatment of advanced stage ovarian carcinoma is warranted.

Acknowledgement

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Bevacizumab, paclitaxel and carboplatin for advanced ovarian cancer: low risk of gastrointestinal and cardiovascular toxicity


Expression of p16 in serous ovarian neoplasms

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Summary

**Purpose:** We aimed to examine p16 protein expression in ovarian serous neoplasms along with normal ovarian tissues. **Materials and Methods:** p16 expression was immunohistochemically evaluated in 86 ovarian serous neoplasms (21 cystadenomas, 20 borderline tumors and 45 carcinomas) and 21 non-neoplastic ovarian tissue. The results were also compared with histopathological grade in serous adenocarcinomas. **Results:** p16 expression rates for benign, borderline ovarian tumors and ovarian cancer were 14.2%, 85% and 86.6%, respectively. It was significantly higher in carcinomas ($p < 0.001$) and borderline tumors ($p < 0.001$) compared to cystadenomas. No immunoreactivity was found in the non-neoplastic ovarian surface epithelial cells. The percentage of p16 expression did not change significantly with histological grade in carcinomas. **Conclusion:** p16 expression is strong and widespread involving most tumor cells in serous papillary ovarian carcinomas, and is probably an early event.

**Key words:** p16; Immunohistochemistry; Ovarian serous carcinoma; Borderline tumor; Cystadenoma.

Introduction

p16 is an important tumor suppressor gene located on chromosome 9p21. The p16 protein binds specifically to CDK4 and CDK6 inhibiting the formation of the CDK/cyclin D complex and resulting in cell cycle arrest at the G1 phase [1, 2]. The aberrant p16 protein caused by point mutations, small deletions, large hetero- and homozygous deletions, and silencing by methylation of CpG islands in the promoter region has been found in many kinds of human tumors, indicating that these factors are closely related to tumorigenesis [3-5].

In the ovary, as in other human tumors, accumulation of genetic alterations occur during malignant transformation [6]. p16 expression has not been extensively investigated in ovarian neoplasms. Serous type ovarian carcinomas appear to express p16 more commonly than other morphologic subtypes. This is analogous to the situation in uterine serous carcinomas, and suggests that p16 may be involved in the pathogenesis of serous carcinomas within the female genital tract [7].

The aim of our study was to investigate p16 expression in ovarian serous tumors. We attempted to determine the p16 expression among benign, borderline, and malignant ovarian serous tumors; and the correlation between p16 expression and tumor grade in ovarian serous carcinomas.

Materials and Methods

**Tissue Specimens**

A total of 86 ovarian serous tumor specimens (21 cystadenomas, 20 borderline tumors, and 45 carcinomas) and 21 non-neoplastic normal ovaries were evaluated for p16 expression by immunohistochemistry. Formalin-fixed and paraffin-embedded tissues were retrieved from the files of the Department of Pathology, Uludag University Hospital during the period of 1997-2007. The cases were reviewed by two pathologists involved in the study and the histological diagnoses were confirmed. The tissues were used with the approval of the ethical committee of Uludag University.

**Grading of tumors**

Malignant tumors were graded according to the World Health Organization (WHO) criteria [8]. Using both architectural and cytological features, tumors were graded as 1, 2, or 3 corresponding to well, moderately or poorly differentiated.

**Immunohistochemical staining and analysis**

Formalin-fixed, paraffin-embedded tissue blocks from each case were cut in 4 μm sections. Antigen retrieval was performed by incubating the sections in EDTA buffer (pH: 9) in a microwave oven (800 W for 5 min + 400 W for 15 min). Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 15 min at room temperature. Primary antibody for p16 (p16INK4a Ab-7, clone 16P04, Neomarkers, Lab Vision, Fremont, CA, USA) was diluted at 1:100. The streptavidin-biotin peroxidase complex kit (Lab Vision, Fremont, CA, USA) was used for antibody detection. DAB was used as the chromogen. Cells with brown-colored nuclear or cytoplasmic staining were considered positive. Human cervical adenocarcinoma sections were used as positive controls. For negative controls, sections were treated similarly with the exception of the primary antibody. Scoring of immunohistochemistry results was performed on the basis of both the staining intensity and the percentage of immunoreactive epithelial cells. The scoring criteria for p16, as shown in percentages (%): no expression (–); < 20, weak staining (+); 20-30, weak or moderate staining (++); 31-50, moderate or strong staining (+++); > 50 strong staining (+++). The scores +, ++ and +++ were considered positive for p16 [9]. Kruskal-Wallis and Mann-Whitney tests were used to compare the p16 stainings of benign, borderline and malignant tumor groups. Spearman’s rho correlation coefficient was used to determine the association between tumor grades and p16 staining.

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Results

Using a cutoff value of ≥ 20% positivity, p16 expression was detected in 14.2% (3/21) of benign tumors, 85% (17/20) of borderline tumors, and 86.6% (39/45) of ovarian carcinomas. In general, all the p16 immunoreactive cells exhibited both nuclear and cytoplasmic staining. In normal ovaries, no immunoreactivity was found. In serous cystadenomas, only three cases showed positivity; expression was weak in 33.3% (1/3) and moderate in 66.7% (2/3). In borderline tumors, 17/20 showed positivity, and the percentage of weak-moderate and strong expression was 29.4% (5/17), 52.9% (9/17), and 17.6% (3/17), respectively. In serous carcinomas, 39/45 showed positivity, and the majority (33/39, 84.6%) were strongly positive (Figure 1, Table 1).

Table 1. — Expression of p16 in serous ovarian neoplasms compared to normal ovaries.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ovary</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cystadeno- ma</td>
<td>21</td>
<td>18</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Borderline</td>
<td>20</td>
<td>3</td>
<td>15</td>
<td>5</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>45</td>
<td>6</td>
<td>13</td>
<td>3</td>
<td>13</td>
<td>45</td>
</tr>
<tr>
<td>Normal ovary</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total staining: 107 48 10 15 36

Significantly higher p16 levels were detected in all tumors compared to the control group (p < 0.001). Significantly high p16 expression was observed in serous carcinomas compared to the normal ovarian tissues, benign and borderline tumors (p < 0.001); and in borderline tumors compared to the benign group (p < 0.001).

Using a cutoff value of > 50% positivity, p16 expression was detected in 15% (3/20) of borderline tumors, and 73.3% (33/45) of ovarian carcinomas, and the difference between the borderline tumors and ovarian carcinomas was statistically significant (p < 0.001).

Of the 45 serous carcinomas, two were well (G1), 30 moderately (G2), and 13 poorly differentiated (G3). Grade 1 tumors were very few in number (2 cases), so they were taken into account together with Grade 2 tumors. No significant correlation was found between the grades of the malignant tumors and p16 expression (r = 0.084; p = 0.582). p16 protein expression in ovarian serous carcinomas by tumor grade is shown in Table 2.

Table 2. — p16 immunopositivity in ovarian serous carcinomas compared to tumor grade.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total</th>
<th>p16 IHC immunostaining pattern Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+ 2+ 3+ Total</td>
</tr>
<tr>
<td>1-2</td>
<td>12</td>
<td>4 3 2 23 28 87.5%</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>2 1 10 11 84.6%</td>
</tr>
</tbody>
</table>

Discussion

The p16 gene is considered to be a potential tumor-suppressor gene [1]. There have been relatively few studies documenting p16 expression in ovarian cancers and conflicting results have been published. The loss of p16 expression has been reported in 26-37% of ovarian cancers [10, 11]. However, some studies indicate that p16 overexpression is relatively common in malignancies occurring at this site [12-14]. Armes et al. [7] found serous papillary carcinoma immunopositivity for p16 in 9/10 cases using a tissue microarray. Dong et al. [15] and Milde-Langosch et al. [16] detected the immunohistochemical expression of p16 protein in 89 and 80% of ovarian tumors, and 96% and 90% of serous carcinomas, respectively. Our findings also confirm that a large proportion of ovarian serous carcinomas have strong p16 protein expression. We observed p16 expression in 86.6% of ovarian carcinomas, and noticed that many of these invasive carcinomas had uniform and strongly intense p16 overexpression throughout the majority of tumor cells.

Ovarian cancer is a heterogeneous group of neoplasms with several different histologic types, each with its own underlying molecular genetic mechanism [17]. Therefore, immunohistochemical expression of proteins as well as molecular analyses should be evaluated separately by histologic type. However, the expression of cell cycle regulatory proteins according to histologic subtypes has received little attention. Recent evidence demonstrated that high p16 expression was associated with serous histology and loss of p16 expression was detected mainly in mucinous and endometrioid types [7, 15-19]. Increased p16 expression is observed in high-grade serous and undifferentiated carcinomas compared with other morphologic types of ovarian carcinoma, and critical molecular abnormalities are present in high-grade serous carcinoma of the ovary [20, 21]. Forty-six ovarian serous papillary carcinomas examined by whole section immunohistochemistry revealed that 31 cases (67.4%) displayed strong nuclear and cytoplasmic staining for p16 in more than 80% of tumor cells [18]. It has been shown that a large proportion of high-grade ovarian serous carcinomas and also uterine serous carcinomas have strong diffuse p16 expression [18]. These findings suggest that p16 expression may be one of the histological type-specific events in ovarian tumorigenesis.
There have been only limited studies concerning p16 expression in serous borderline and benign neoplasms. Armes et al. reported 90% of serous adenocarcinomas were positive for p16, while borderline serous tumors showed negative staining, where there were only three borderline cases [7]. In another study, most benign ovarian neoplasms in contrast to 11% of malignant tumors were negative [15], p16 expression was reported to be decreased from benign and borderline to malignant tumors, and it was concluded that p16 protein was down-regulated in ovarian carcinoma [22]; however in the two latter studies, histopathologic subtypes were not evaluated separately [15, 22]. In another study including only serous type carcinoma and borderline neoplasms, p16 positivity was reported in 93.5% (43/46) of invasive tumors and 88.9% (16/18) of borderline tumors [23]. In the current study, we observed overall positive p16 expression in 85% and 86.6%, with high and widespread strong expression of 15% and 73.3% of serous borderline tumors and carcinomas, respectively.

Studies investigating p16 expression of normal ovarian surface epithelia are few in number with strikingly conflicting results, which may be caused by the use of different p16 antibodies [10, 24]. Many commercially available p16 antibodies show substantial nonspecific binding in normal epithelial cells [25]. We also did not observe any expression in normal ovarian surface epithelium.

In summary, we have demonstrated increased expression of p16 in serous adenocarcinomas compared with serous borderline tumors and serous cystadenomas, and no expression in normal epithelial cells; and confirmed the strong and widespread expression in serous ovarian carcinomas.

References


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Distribution of HPV genotypes in uterine cervical lesions among the Uighur women in Xinjiang province of China

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Summary

Objective: The aim of this study was to investigate the distribution of HPV genotypes in uterine cervical lesions of Uighur women in the Xinjiang province of China. Methods: A total of 223 formalin-fixed paraffin-embedded cervical tissue specimens from Uighur patients with squamous cell carcinoma (SCC) and cervical intraepithelial neoplasia (CIN) were analyzed with HPV specific general primer pairs MY09/11 by PCR amplification and HPV chip. Results: Among 223 cases, HPV-positive samples accounted for 58.7% (131/223). HPV infection rate increased along with the pathological grade of the specimen, with a clear tendency of normal < CIN 1 < CIN 2 < CIN 3 < SCC. HPV16 infection was the predominant one and reached the highest level in SCC with 96%. HPV18 and 58 were detected only in some specimens as a second infection in addition to HPV16. The infection rate and type of HPV was not closely associated with the histological differentiation of the cervical cancer. Conclusion: HPV16 was the most common type detected in Uighur women with SCC and CIN in the Xinjiang area of China. Together with the high infection rate, this may be the reason for the four-fold higher cervical cancer incidence in this province and in this population, when compared to total China. The prevalence of HPV18 and 58 was relatively low.

Key words: HPV-genotypes; Cervical cancer; CIN; Uighur population of Xinjiang.

Introduction

Cervical cancer continues to be one of the leading female genital cancers worldwide. About 80% of cases occur in developing countries [1-3]. In China, there is an annual incidence of about 46,000 cases, and cervical cancer presents a major health problem [4]. The Xinjiang province has one of the highest incidence (590/100,000) and mortality rate of cervical cancer in China, especially the south of Xinjiang. Thus the incidence of cervical cancer among the Uighur women is four times higher than the mean incidence of China (138/100,000) [5, 6]. Molecular epidemiologic evidence clearly indicates that certain types of HPV are the principal cause of invasive cervical cancer and cervical intraepithelial neoplasia (CIN). It is widely believed that persistent infection with high-risk human papillomavirus (HPV) represents the prime risk factor in cervical carcinogenesis [7, 8]. Of a total of approximately 40 mucosal HPV types [9], 15-18 types are currently considered ‘high-risk’ with variable oncogenic potential [10, 11]. Squamous cervical cancer (SCC) is strongly associated with HPV infections and the prevalence of HPV is about 95%, whereas HPV DNA cannot be identified in the same proportion of adenocarcinomas [12]. Since the distribution of HPV genotypes in various geographical areas and populations varies widely [13, 14], development of effective vaccines would require a comprehensive study of the HPV genotypes in different regions of the world.

With regard to these facts, the aim of the present study was to evaluate the frequency and distribution of high-risk HPV (HR-HPV) types in a series of paraffin-embedded tissues from SCC and CIN among the Uighur women in Xinjiang. This seems to us important for guiding the selection of vaccine candidates and studying the pathogenesis of cervical cancer.

Methods and Materials

Cervical tissue specimen

A total of 223 formalin-fixed paraffin-embedded (FFPE) cervical tissue specimens from Uighur patients who had been diagnosed or hospitalized at the Department of Gynecology of both the First Affiliated Hospital of Xinjiang Medical University and the Hospital of Xinjiang Uighur Autonomous Region between February 2006 and June 2007 were analyzed. Cervical tissue specimens were derived from punch biopsies, loop electrosurgical excisions, cone biopsies, and hysterectomies. The pathology slides were reviewed and original histological diagnoses of samples were confirmed by experienced pathologists. The diagnoses were as follows: non-neoplastic cervix, n = 38; CIN, n = 94 (CIN 1, n = 36; CIN 2, n = 28; CIN 3, n = 30); and squamous cell carcinoma (SCC), n = 91, of which 15 were well differentiated (grade 1), 49 were moderately differentiated (grade 2), and 27 were poorly differentiated (grade 3). Patient age ranged from 18 to 78 years, with a mean of 48 years.

DNA extraction

The paraffin-embedded tissue sections were deparaffinized with xylene, rehydrated by decreasing concentrations of ethanol and double distilled water (ddH2O) followed by digestion with...
100 mg/ml proteinase K. The genomic DNA was extracted by the standard phenol-chloroform (1:1) extraction and ethanol precipitation. Purified DNA was then quantified using a Gene Quant II spectrophotometer (GE) and stored at -20°C until further use.

Screening and genotyping of HPV in tissue specimens

To screen HPV-positive samples, the genomic DNA was analyzed with HPV specific general primer pairs MY09/11 (MY09: 5’-GTCCMARRGGAWACTGATC-3’; MY11: 5’-GCMCAGGGWCATAAYAATGG-3’) by PCR amplification, followed by electrophoresis on 2% agarose gel labeled with ethidium bromide and ultraviolet visualization (Gel Doc XR, Biorad, Germany). The HPV genotyping of 23 common subtypes (HPV 6, 11, 16, 18, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 56, 58, 44, 59, 66, 68, 73, 83 and MM4) was carried out using HPV chip kit (Y ANENG Biotech, PR China) according to the manufacturer’s instruction.

Statistical analysis

Statistical analysis was performed by SPSS statistical software package version 11. The chi-square test was applied for comparing results of HPV and other parameters. Statistical significance was assumed at a value of < 0.01.

Results

HPV infection was highly associated with cervical cancer in Uighur women. Among 223 cases in this study, 58.7% (131/223) were positive for HPV, and the highest HPV positivity rate was observed in patients aged between 35 to 44 (data not shown). HPV infection increased along with the pathological grade of the specimens, with a clear tendency of normal < CIN 1 < CIN 2 < CIN 3 < SCC (Table 1).

Table 1. — Incidence of positive HPV tests in different types of cervical lesions.

<table>
<thead>
<tr>
<th>Pathologic examination</th>
<th>HPV test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Normal</td>
<td>38</td>
</tr>
<tr>
<td>CIN 1</td>
<td>36</td>
</tr>
<tr>
<td>CIN 2</td>
<td>28</td>
</tr>
<tr>
<td>CIN 3</td>
<td>30</td>
</tr>
<tr>
<td>SCC</td>
<td>91</td>
</tr>
</tbody>
</table>

HPV typing of HPV-positive samples using HPV chips containing 23 common HPV types showed that the HPV16 infection predominated with an infection rate of 33% in normal individuals. The rate gradually increased with the pathological grade of CIN and reached the highest level in squamous cell cervical cancer with 96% (Table 2). Interestingly, we detected HPV 18 and 58 only in some specimens as a second infection in addition to HPV 16.

We further evaluated the association of histological differentiation (grade) of cervical cancer with the HPV infection. HPV 16 infection occurred in equally high rates in every grade of cancer. The infection with HPV 18 or 58 seems to be associated with cancer progression, as positivity rate of these high-risk HPVs increased gradually with cancer grade (Table 3). However this was not statistically significant.

Discussion

Cervical cancer is a potentially preventable disease; it is a common cancer and a serious threat to women’s lives in developing countries. Squamous cell carcinoma of the cervix remains the most predominant phenotype. Persistent infection with high-risk HPV is the main etiological factor in the development of cervical cancer and may depend on HPV genotypes and variants. HPV 16 and HPV 18 are the most prevalent genotypes associated with cervical carcinomas globally [14, 15].

To evaluate the health impact of HPV infections and in order to design HPV vaccines, it is necessary to know the distribution of oncogenic HPV in cervical cancer and precancerous disease among the different geographical regions and different populations. In the present study, among 91 cervical cancer patients the overall HPV positive rate detected by MY09/11 PCR was 87.9% in SCC (80/91), which is close to the value obtained in a meta-analysis on HPV prevalence in 5,954 ICC cases in Asia (85.9%) [16], and higher than the rate reported in a meta-analysis for China only (83.7%) [17]. In this study it was shown that HPV 16 is the most common high-risk HPV detected in Uighur cancer patients in the Xinjiang region. HPV 16 was found in 84.6% (77/91) of all cervical cancer cases and 96.3% (77/80) of all HPV-positive SCCs. This is higher than the results of a meta-analysis comprising all China (58.7%) [17]. Detection rate of HPV 18 (15.4%) was similar to the results reported for Asia (14.9%) [16]. HPV 58 was found in 2.2% (2/91) of all cervical cancer cases.

The overall rate of HPV positivity in CIN cases was 51.1% (48/94), whereby the rates for HPV 16, 18 and 58 were 43.6% (41/94), 8.5% (8/94), or 5.3% (5/94), respectively. Results obtained from HPV high-risk typing
in SCC and CIN have shown that HPV 16 was the most frequent type also in the Uighur patients, which is inconsistent with the results of other authors who have described HPV16 as the main oncogenic type of HPV associated with cervical cancer in Japanese, Latvian and Chinese women [18-20]. In this study we identified HPV 18 followed by HPV 58 as the second and third prevalent types after HPV 16. HPV 18, and HPV 58 may be more important in glandular lesions and adenocarcinoma, but this will be analyzed in more detail in further studies. Other HPV types could not be detected in the present study.

HPV 16 infection may also play a central role in the pathogenesis and development of cervical squamous cancer and precancerous lesions in Uighur women and contribute to the high mortality from cervical cancer. Many reports have demonstrated that persistent HPV 16 infection could induce oncogenic potency and proposed to use it as a marker, in addition to morphology, for progression of cervical precancerous lesions [21-24]. However, the high-risk HPV types did not show any significant association with histological grading of squamous cervical cancer. Preliminary clinical trials in humans demonstrated that HPV vaccine can prevent HPV infection and precancerous lesions [25]. Recently, vaccines designed to protect against the worldwide most common HPV high-risk types HPV 16 and HPV 18 have become available [26]. As these two types of HPV are the most prominent in the Uighur population we assume that this vaccination could be of great benefit. Therefore, in screening programs HPV DNA testing and cervical cytology are very important to identify women at risk for cervical cancer. HPV DNA testing is a promising alternative or complementary test to improve the efficacy of cervical cytology, to reduce cervical cancer incidence and mortality. Prospective studies have shown that HPV DNA-positive women are significantly more likely to develop high-grade squamous intraepithelial lesions within ten years than women with a negative HPV DNA test [27, 28]. Fortunately, the transition to cancer usually takes years or decades, thus allowing the opportunity for detection by a cervical screening test.

Our study showed that women between the ages of 35 and 44 years had the highest number of HPV infections and CIN. Similar results have been reported in other studies [29, 30]. Education of women about HPV genital infections and the performance of Pap smear screening and HPV testing for all women 35 years of age and older is critical.

Conclusions

HPV 16 is the most common HPV type detected in Uighur patients while the prevalence of HPV 18 and 58 was relatively low, and all other types were absent. These results could explain the high cervical cancer incidence and mortality. They also could be the basis for the development of prevention measures against cervical cancer e.g., HPV vaccination. This study also shows the necessity of epidemiological studies and the performance of further research on the molecular variants of HPV 16 in Uighur population.

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Intestinal-type metaplasia in the original squamous epithelium of the cervix

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Summary

There have been a number of reports on cervical carcinomas, both invasive and intraepithelial (CIN III), indicating the presence of intracellular mucins in the absence of glandular differentiation. Yet, the expression of such cells in the normal/original squamous epithelium of the cervix remains unexplored. We investigated the presence of mucin-distended goblet cells at this site, after examining retrospectively normal cervices from 250 hysterectomy specimens. Goblet cells were detected in 3.2% (8/250) of the cervices examined using haematoxylin and eosin stained sections and confirmed by mucin histochemistry: alcian blue (AB) pH 2.5, periodic acid-Schiff reaction with and without diastase digestion (PAS-d, PAS) and the combined AB/PAS. Additional sections were stained with Diazo and Masson-Fontana for argentaffin granules and Grimelius for argyrophil cells, but were all negative and no other cell types were identified. It is believed that this incomplete type of intestinal metaplasia is an acquired change in the cervix, derived from multi-potential stem cells of Müllerian duct origin.

Key words: Intestinal metaplasia; Goblet cells; Intracellular mucin; Squamous metaplasia; Uterine cervix.

Introduction

Mucin secretion is not a function of the normal stratified squamous epithelium of the cervix. Nevertheless, there have been a number of reports recently of cervical carcinomas containing intracellular mucin [1-3] and, indeed, it has been claimed that as many as 39% of invasive squamous cell carcinomas of the cervix [4], and 14% of cervical intraepithelial neoplasias grade 3 (CIN 3) [5, 6] express mucins in the absence of glandular differentiation [1]. Park et al. investigating mucin secretion in stratified epithelia resembling CIN, were able to demonstrate areas of discrete cytoplasmic vacuoles, mucicarmine positive, justifying the designation “stratified mucin-producing intraepithelial lesions” (“SMILE”) [7]. Earlier, Trowell and Azzopardi and Hou identified goblet cells in the normal/original and the neoplastic endocervical (columnar) epithelium, respectively [8, 9]. Kurman described goblet cells in the native endocervical glands [10], and Fox et al. in foci of immature squamous metaplasia [11]. Yet, similar cells have not been described in the original cervical squamous epithelium. This stimulated a study for the re-evaluation of the presence or otherwise of goblet cells in the original squamous epithelium of the uterine cervix.

Material and Methods

The material used for this study was drawn from the files of the Department of Pathology, Democritus University of Thrace and the University General Hospital, Alexandroupolis, Greece.

The tissues comprised 250 hysterectomy specimens, removed for reasons other than neoplasia, usually uterine prolapse or leiomyomas. The cervical epithelium had been previously described as unremarkable, within normal limits or with no specific features. They had been fixed in formalin and embedded in paraffin wax.

Haematoxylin and eosin (H & E) stained sections at 5 μm were examined for the presence of goblet cells; these were recognised as discrete mucin-secreting vacuoles among the squamous cells of the stratified epithelium of the uterine cervix, whether normal or metaplastic. Two paraffin blocks were selected from each case for further histochemical analysis.

Cases harbouring goblet cells were additionally stained with periodic acid-Schiff reaction, with and without diastase digestion (PAS-D, PAS), the alcian blue (AB) at pH 2.6 and the combined AB/PAS. Alcian blue identifies acid mucins, PAS-D identifies neutral mucins, while the combined AB/PAS demonstrates acidic and neutral mucins [12]. Additional sections were stained with the Masson-Fontana and the Diazo reaction for argentaffin granules and the Grimelius method for argyrophil cells.

Results

Among the 250 normal cervices examined, there were 81 cases which harbouried foci of squamous metaplasia. Goblet cells, recognised as large epithelial cells distended by a single clear vacuole (Figure 1a), were identified on H and E stained sections both in the original squamous epithelium of the cervix (5 of 250 cases, 2%) and in foci of immature squamous metaplasia (6 of 81 cases, 7.4%). More specifically, in three cases, goblet cells were seen within groups of both original and metaplastic squamous cells; in another three cases, such cells were detected in foci of squamous metaplasia at the transformation zone; and in two cases, goblet cells were present only in
the original/surface epithelium (Table 1). Overall, goblet cells were detected in eight of the 250 cases studied (3.2%). Absorptive cells or Paneth cells were not seen.

Further mucin histochemistry confirmed the intestinal differentiation of the lesions: the goblet cells were stained positively with alcian blue (AB) pH 2.5, periodic acid-Schiff (PAS) reaction with and without diastase digestion, and the combined AB/PAS for the demonstration of acidic and neutral mucins (Figure 1b-1d).

However, sections stained with the Masson-Fontana and the Diaz reaction for argentaffin granules and the Grimelius method for argyrophil cells were all negative.

The goblet cells tended to occupy the intermediate zone and the superficial layer of the surface squamous epithelium, whether original or metaplastic, which was, in general, non-keratinizing and thinned. There was no morphological evidence of glandular differentiation.

It is of interest that the seven women in the series, having goblet cells in the cervix, were all young, aged 45 or less. They had been subjected to hysterectomy for recurrent vaginal bleeding and anaemia, apparently due to submucosal “fibroids”. There was no history of medications.

Table 1. — Goblet cell metaplasia in the original and metaplastic squamous epithelium of the cervix (among 250 surgical specimens examined).

| Original squamous epithelium (exclusively) | 2 |
| Metaplastic squamous epithelium/ transformation zone (exclusively) | 3 |
| Original squamous epithelium and metaplastic squamous epithelium | 3 |
| Native endocervical glandular epithelium | Not assessed |

Discussion

This study indicates that the stratified squamous epithelium in the cervix, whether metaplastic or normal/original, express, albeit rarely, goblet cells. Earlier studies have shown that mucin expression, in the form of indistinct cytoplasmic clearing, is not an unusual finding in the neoplastic squamous epithelium [1-3] and, indeed, it has been
claimed that between 35 and 39% of invasive squamous cell carcinomas of the cervix contain intracytoplasmic mucin without evidence of glandular differentiation [4, 5, 13, 14]. Such mucin-containing squamous neoplasms were designated “muco-epidermoid” or “adenosqua-
mous” (WHO classification) carcinomas – a diagnosis, which should be always confirmed by mucin histochemistry [15, 16].

Less commonly (in 14% of cases), mucin expression, of a similar morphological and histochemical pattern (indistinct cytoplasmic clearing with mucin positivity), develops in preinvasive lesions of the cervix, namely the cervical intraepithelial neoplasia grade 3 (CIN 3) [5, 6] and the stratified squamous epithelia resembling CIN lesions [7]. Interestingly, among 18 such CIN-like lesions reported by Park et al. as SMILE, there were a few (number of cases not specified) with areas showing discrete cytoplasmic vacuoles, giving rise to speculation about a greater invasive potential. Others have reported goblet cell metaplasia in native endocervical glands [10] and in foci of immature squamous metaplasia [11], but not in the original squamous epithelium.

The role of mucins in squamous lesions of the cervix remains controversial and, certainly, is not confined to preinvasive disease. Thus, in some series, mucin production by invasive squamous cell carcinomas was associated with a higher incidence of lymph node metastasis [4, 5, 15, 17], but in others mucin detection had little influence on prognosis [3, 13, 14].

The situation is totally obscure in preinvasive lesions of the cervix for complete paucity of information exists. However, in light of our current work, the presence of cytoplasmic mucin in CIN or CIN-like lesions should not be taken as an indication of aggressive behaviour, i.e. increased risk of invasion, since similar mucin-positive cytoplasmic vacuoles and goblet cells occur, albeit rarely, in normal/original and metaplastic epithelia and are not the exclusive finding of cervical intraepithelial carcinomas. Yet, the possibility that such mucin-containing cytoplasmic vacuoles and goblet cells are part of a stepwise sequence of alterations in the cervical epithelium leading ultimately to cancer, in a manner analogous to that occurring in the gastric or oesophageal mucosa [18], can not be excluded.

With regard to the intestinal nature of the metaplasia described in this study, this was readily recognised on H and E stained sections as goblet cells and was further confirmed by mucin histochemistry. For, indeed, mucin distended goblet cells stained positively with the most widely used techniques, i.e., the alcian blue (AB) pH 2.5, the periodic acid-Schiff reaction with and without diastase pretreatment (PAS-D and PAS) and the combined AB/PAS demonstrating acidic and neutral mucins [3, 5, 6, 8, 13-15]. Others found equally useful markers for intestinal differentiation the histochemical stains high iron diamine-
alcian blue stain (HID-AB) for the demonstration of sulpho- and sialomucins, and the periodate borohydrate/potassium hydroxide/PAS (PB/KOH/PAS) technique for the demonstration of O-acetylated sialomucins [19, 20].

This multiplicity of staining reactions reflects the immense heterogeneity of mucins (usually found as mixtures rather than in pure form) within the phenomenon of intestinal metaplasia [21]. The fact that the metaplastic epithelium contained goblet cells, but not columnar cells with a brush border (absorptive cells), argentaffin and argentophil cells or Paneth cells, classifies our cases of intestinal metaplasia as incomplete (type II) as opposed to complete intestinal differentiation (type I) having the full complement of intestinal cell population [21].

The presence of goblet cells in the normal and meta-
plastic cervical epithelium, though by no means common, is in many ways explicable. The cervix, as, indeed, the upper third of the vagina [22], is a Müllerian-derived tissue [23] and there is now convincing evidence that intes-
tinal metaplasia can develop from pluripotential epithelial stem cells in tissues of Müllerian origin. Thus, in addition to the endocervical canal [8, 9, 19, 20], further examples of intestinal (goblet cell) metaplasia in Müllerian-derived tissues include the endometrial cavity [19, 24], the vagina [19, 25] and the ovarian surface, although the intestinal type mucinous tumours of the ovarian surface may well be teratogenous rather than metastatic in origin [26]. Other, less well recognised, forms of metaplastic change that may develop at this site include sebaceous [27] and transitional cell metaplasia of the epithelium [28]. That goblet cell metaplasia is not a congenital heterotopia, was supported by the failure to demonstrate a full thickness structured intestinal mucosa or more than one type of intestinal epithelial cells within the normal stratified squamous epithelium [21].

In conclusion, the presence of mucin-distended goblet cells, as a defining feature of goblet cell metaplasia, is well-documented in the neoplastic and metaplastic squamous epithelium of the cervix, and its description in the original squamous epithelium is only an example of the explicit histogenetic plasticity of the Müllerian epithelium.

References


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Evaluation of preoperative diagnosis with results of histopathological examinations of ovarian tumors in women of reproductive age

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Summary

Ovarian tumors are the most frequent lesions encountered by gynecologists. Ovarian carcinoma most often develops asymptomatically and until now no sufficient screening diagnostic methods have been developed, which is why various diagnostic methods are being tried concurrently to increase diagnostic sensitivity. The aim of this paper was to evaluate the compliance of the preoperative diagnoses with the results of histopathological examinations of ovarian tumors and to determine the usefulness of simultaneous application of gynecological, ultrasonographic, and Doppler examinations together with determination of CA-125 antigen in the diagnostic process of ovarian tumors. The study comprised a group of 250 women in reproductive age who were operated on for tumors of the ovary. Results of histopathological examinations were compared with the preoperative diagnosis based on the above-mentioned examinations and prognostic indicators: sensitivity, specificity, negative and positive prediction value as well as accuracy were determined. The results showed that combining the four diagnostic methods is a useful research panel in the preoperative diagnostic process of ovarian tumors and makes selecting the appropriate procedure and surgical treatment viable.

Key words: Ovarian carcinoma; Morphological index; Doppler examination; CA-125 antigen.

Introduction

Ovarian tumors are the most frequent lesions encountered by gynecologists in daily medical practice. Differentiation between benign ovarian tumors and ovarian carcinoma, especially in early stage, before the operation is difficult because ovarian carcinoma most often develops asymptomatically and until now no sufficient diagnostic screening methods have been developed. Today joining various diagnostic methods in order to increase diagnostic sensitivity is being attempted [1].

Gynecological examination together with a precise interview is the basis of the diagnostic process for ovarian carcinoma patients. Also ultrasonographic examination, by means of transvaginal probe (TVS) is a diagnostic standard in cases of suspicion of tumor changes within the ovary [2]. Great precision in assessment of the size and location of the tumor in the pelvis minor, the velocity, simplicity and low price make ultrasonography (US) a very useful screening examination, especially when it is extended with the Doppler examination. Another standard used in the diagnostic process of ovarian carcinoma since the beginning of the 1980s is determination of CA-125 antigen.

None of the above-mentioned methods has 100% sensitivity or specificity and the final diagnosis is possible only after obtaining the results of the histopathological examination.

The aim of the paper was to evaluate the compliance of the preoperative diagnosis with results of the histopathological examination of ovarian tumors in women in reproductive age and to determine the usefulness of simultaneous appliance of the above-mentioned examinations in the diagnostic process of the tumor.

Material and Methods

The study covered a group of 250 women aged from 18 to 48 years (mean 36 years) who were diagnosed and operated on in the Clinic of Gynecological Surgery at the Karol Marcinkowski University of Medical Sciences in Poznan in the years 2006-2008. Prior to surgery, after a detailed interview, each patient underwent a gynecological examination, US examination by means of Aloka apparatus (model 5500 with a TVS probe at a frequency of 5.0-6.5 MHz), Doppler examination of the detected ovarian tumor and blood analysis to determine the concentration of CA-125 antigen. During the gynecological examination the following features were examined: the shape of the tumor, its consistency, mobility, location (one- or two-sided) as well as the occurrence of ascites – the presence of which means greater progression of carcinoma, at least Stage Ic in FIGO (the International Federation of Gynecology and Obstetrics) classification. During US the morphological index was determined according to Szpurek et al. [3] - (the capacity of the tumor – calculated on the basis of the ellipse formula: length x width x height x 0.523, the structure of the inner wall, its thickness, the structure of the septum, echogenicity and the presence of ascites), assuming a cut-off point of 7 points out of 17 possible. By means of the color Doppler technique the wave shape of the velocity of blood flow in tumor vessels was analyzed and the
following features were examined: location of the vessels, pulsation index (PI), resistance index (RI) and systolic-diastolic indexes (S/D). On the basis of the results the patients were divided into three groups: I – no suspicion that the detected ovarian tumor might be of a malignant character, II – doubtful results, did not determine unambiguously the character of the change, III – the tumor had features of a malignant neoplasm. Women were placed in particular groups depending on how many examinations of four types (gynecological examination, US, Doppler, and CA-125 marker) indicated the malignant character of the tumor. In case of more than two indications – a patient was placed in group III, and in case of fewer than two – in group I. Finally, these results were compared with the results of histopathological examinations. On this basis, after determining prognostic indicators: sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV), as well as accuracy the usefulness of a simultaneous appliance of the above-mentioned methods in the diagnostic process of the tumor were assessed. Histopathological examinations were carried out in the Gynecological-Obstetrics Clinical Hospital Histopathological Laboratory of Poznan University of Medical Sciences.

Results

On the basis of the preoperative examinations, out of 250 examined patients, 152 met the criteria for group I, 12 for group II and 86 patients for group III. The following results were obtained after comparing the preoperative diagnosis with the results of the histopathological examinations: in 145 patients from group I (benign tumors) the preoperative diagnosis was confirmed with the histopathological result and in seven women of this group ovarian carcinoma was diagnosed in the final examination, mainly in low clinical stage. In group III, 64 patients were diagnosed with a malignant ovarian neoplasm in the histopathological examination, including one case of metastatic neoplasm from gastric carcinoma, and in 22 women carcinoma was not confirmed with the results of the histopathological examination. However in group II, ten patients had benign tumors and two were diagnosed with malignant changes (Table 1).

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Group</th>
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<tbody>
<tr>
<td></td>
<td>I (N 152)</td>
</tr>
<tr>
<td>Benign neoplasm</td>
<td>145 (95.4%)</td>
</tr>
<tr>
<td>Malignant neoplasm</td>
<td>7 (4.6%)</td>
</tr>
</tbody>
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The most frequent diagnosis among benign changes in group I included serous cystadenoma of the ovary (37), simple cyst (35) and endometrial cyst (27). Among false-negative diagnoses in this group, it was primary ovarian carcinomas of low clinical stage that were predominant (FIGO IA, Ib). In such cases the suspicion of a malignant change emerged only either by US TVS or the color Doppler examination.

In group III the most frequently diagnosed carcinoma was primary ovarian carcinoma. However, tumors in this group that were false-positive in the preoperative examinations turned out to be mainly teratomas (15) and inflammatory ovarian-tubal tumors (5). In these cases the gynecological examination, the US examination and most often Doppler examination were incorrect, more rarely for CA-125 marker levels.

In group II, in which the preoperative diagnoses were most doubtful and generated difficulties, the majority of tumors constituted endometrial tumors (8) – malignant neoplasms included granuloma cell tumor and borderline serous adenocarcinoma. In cases of endometrial tumors the suspicion of malignant changes emerged during the gynecological and US examinations or US examination and the increased level of CA-125 marker.

Prognostic indicators were as follows: sensitivity of the methods used together amounted to 90.14%, specificity – 86.83%, PPV – 74.42%, NPV – 95.39%, accuracy in the prediction of the tumor character – 87.81%.

These indicators were calculated on the basis of groups I and III in which the preoperative diagnoses were unambiguous.

On the basis of group II it was found that the probability of occurrence of ovarian carcinoma in women in reproductive age whose results of the preoperative examinations were doubtful was 16.66% which means that 1.7 in ten of these patients would have malignant ovarian carcinoma. However the simultaneous use of the gynecological, US-TVS, color Doppler and CA-125 examinations in the preoperative diagnostic process indicated that doubtful diagnoses amounted to 4.8% of all diagnoses and ovarian carcinoma in this group would constitute 0.8% of all examined tumors.

Discussion

The lack of unambiguous diagnostic screening methods for ovarian carcinoma has brought about the search for new methods with higher sensitivity. At the same time researches in which sensitivity and specificity obtained through the conjunction of contemporarily known diagnostic methods are determined are being carried out in an attempt to find an appropriate research panel which would make it possible to diagnose ovarian carcinoma before surgery [4, 5].

In our study after combining four basic diagnostic methods (gynecological examination, US-TVS examination, Doppler examination and CA-125 level) high sensitivity (90.14%), specificity (86.83%), NPV (95.39%), as well as accuracy of the method (87.81%) were obtained, which means that the conjunction of these methods gives good results as far as the assessment of tumor character is concerned.

Similar results were obtained by Varras [6] and Marret [2]. They found higher sensitivity and specificity when combining several diagnostic methods (the gynecological examination, the US-TVS examination, the Doppler examination and CA-125 examination) than when using each method separately. Varras [6] drew attention to the possibility of a diagnostic mistake during color Doppler examination of changes in the ovaries in women in pre-
menopausal age due to the presence of corpus luteum and a rich flow visible within it.

Antonič et al. [7] examined the usefulness of the simultaneous application of assessment of blood flow in ovarian tumors by means of color Doppler and power Doppler as well as CA-125 marker levels in the process of differentiating ovarian benign changes from malignant ones in women above 34 years old. The results showed that lack of blood flow during the color Doppler examination and CA-125 level under 35 U/ml could exclude the malignant character of the tumor in a reliable way.

In their papers Sawicki et al. [8, 9] proved that US examination of ovarian tumors with a TVS probe should be carried out together with assessment of blood flow because the evaluation of RI and intensification of vascularization improve the accuracy of the methods used in determining the character of a tumor in a significant way.

Assessing the usefulness of time-averaged maximum velocity of blood flow (TAMXV), peak systolic velocity of blood flow (PSV), RI and PI in differentiating benign changes from malignant ones, Tailor et al. [10] came to the conclusion that the highest diagnostic sensitivity and specificity can be obtained by examining the parameters of resistance and flow capacity simultaneously rather than only resistance indicators. However, Szpurek et al. [11] proved that PI and maximum end-diastolic velocity of blood flow (MEDV) are the best prediction indicators in women in premenopausal age and RI and MEDV in women after menopause.

Moszyński et al. [12] proved limited usefulness of the US index itself in assessing the character of ovarian tumors and found that it was always necessary to consider combining this diagnostic method with blood flow assessment by means of color Doppler and the examination of biochemical markers.

Conclusions

1. The combination of the four diagnostic methods (the gynecological examination, US-TVS examination, Doppler examination, CA-125 marker) is a useful research panel in the preoperative diagnostic assessment of ovarian tumors. This panel has high sensitivity, specificity, accuracy and NPV, which proves its advantage in assessing the character of ovarian tumors over each of these methods used separately.

2. Determining the preoperative diagnosis on the basis of the four above-mentioned diagnostic methods makes it possible to select an appropriate procedure and operational treatment and the high NPV of the panel allows unnecessary surgical treatment to be avoided or the use of less invasive procedures (laparoscopy instead of laparotomy).

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Management of recurrence from a retroperitoneal xanthogranuloma: case report

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Summary

Xanthogranulomas from the retroperitoneal space are rare. To our knowledge only a few cases have been reported in the literature in the retroperitoneal space. In this report, the authors present the case of a 24-year-old woman with a recurrence of this rare tumor. Two years after resection, the mass showed rapid local recurrence. Attention should be paid to the possibility of the transformation into a fibroxanthosarcoma, which could have an aggressive clinical course.

Key words: Xanthogranuloma; Retroperitoneal space; Fibroxanthosarcoma; Malignant histiocytoma.

Introduction

The retroperitoneal xanthogranuloma reflects the morphology of a fibrotic tumor of unknown etiology and pathogenesis. It is a histiocytic disorder, primarily but not exclusively seen through the first two decades of life [1].

It is an uncommon process best known to occur in the kidney [2].

In 1935 Oberling described lesions composed of varying proportions of fibrous tissue, foamy histiocytes and other inflammatory cells in the retroperitoneal soft tissues of three young adults [3, 4].

This diagnosis has been applied to a variety of benign and malignant inflammatory lesions in the retroperitoneum. Russack et al. [5] described cases which were characterized by foamy histiocytes with variable amounts of giant cells and other chronic inflammatory cells.

In general it is uncertain whether to classify the xanthogranuloma as a benign inflammatory process or a low-grade malignant process. In a review of the literature there are cases that have had a fatal outcome while transferring into a carcinoma or sarcoma.

Case Report

A case of a 24-year-old woman with retroperitoneal xanthogranuloma is presented.

The patient was admitted to our hospital. Two years ago she was operated and a retroperitoneal xanthogranuloma was removed. Then, the magnetic resonance imaging (MRI) scan indicated an increasing retroperitoneal tumor with a diameter of 7 cm. The patient complained of dyspareunia and contact bleeding. Vaginal examination revealed a large solid displacing tumor.

Colonoscopy revealed a regular state of the colon, rectum and terminal ileum. Ultrasound (US) showed regular kidneys with a smooth surface and no obstruction of the renal pelvis.

Histopathology showed fibrolipomatic tissue with infiltration of chronic inflammatory cells such as cystic tissue.

Mainly the cells were foamly histiocytes and lymphocytes. The histiocytes were immunoreactive for CD 68 and thus the diagnosis of retroperitoneal xanthogranuloma was confirmed.

The cystic tumor was 5.8 x 3.5 x 3 cm in size. There was a hemorrhagic membrane adjacent to the inner wall of the cyst. Histologically the cyst wall consisted of fibrous tissue with a large number of myofibroblasts. There was dense mononuclear infiltration with many foamy histiocytes. Furthermore, there was sparse infiltration with lymphocytes, multinucleated giant cells and hemosiderin-laden macrophages. A xanthogranuloma was diagnosed.
Management of recurrence from a retroperitoneal xanthogranuloma: case report

Discussion

In 1965 Armstrong described retroperitoneal tumors as uncommon with a frequency of 0.04% of all hospital admissions and of 0.1% of total malignancies [6]. According to this 0.04% retroperitoneal xanthogranuloma is a rare member of the group.

Xanthogranulomas are benign tumors. On the other end of the spectrum, retroperitoneal fibroxanthosarcoma are associated with aggressive behavior with high recurrence rates and rapid progression of disease.

Pack et al. reported that 91% of retroperitoneal tumors are malignant and among the benign ones the retroperitoneal xanthogranuloma and cysts are most frequently encountered.

Rapid transformation into a fibroxanthosarcoma is also described in literature as well as the transformation into a storiform fibrous xanthoma or a malignant histiocytoma [7, 8].

Reports of all these rare tumors have suggested a high-incidence of recurrence following local excision and wide excisional surgery offers the best chance of cure in these tumors [9].

Conclusion

The etiology of retroperitoneal xanthogranuloma is unclear. Khan believes it is an inflammatory process [10] but that there is presence of histiocytes in the retroperitoneal xanthogranuloma that probably become malignant.

Usually the clinical presentation is late so that there is a large intraabdominal mass.

In some cases these tumors may present early when involving the urinary tract but in general the management should be surgical removal to the extent possible. There is always doubt concerning the transformation into malignancy and there is benefit described when most of the tumor mass is removed [11].

Lastly, our case supports the feasibility of complete surgical resection. Although our patient’s recurrence was identified prior to the primary place in the pelvis, she ultimately underwent complete resection in this area.

References


Figure 2. — Pathologic findings with fibrotic tissue with embedded CD-68 positive histiozytic cells.


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A benign metastasizing leiomyoma involving a nodule in the pulmonary artery: case and literature review

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Summary
Benign metastasizing leiomyoma (BML) is a rare disease defined as a primary benign uterine tumor with "metastatic" lesions preferentially occurring in the lung, pelvis and lymph nodes. There are few reports about local recurrence after initial surgery. We report a case of a BML with local recurrence and metastasis growing into the wall of the left pulmonary artery, diagnosed 11 years after initial hysterectomy. A 55-year-old woman complaining of abdominal discomfort, heaviness and asthenia was admitted to our hospital for investigation of a voluminous uterine mass with high vascularization and three pulmonary nodules. The resection of the mass by laparotomy was complicated by uncontrolled severe hemorrhage due to vascular proliferation, requiring multiple transfusions, packing the cavity and postoperative uterine artery embolization. Three months later the patient underwent a left upper lobe lobectomy with the aim of removing the largest pulmonary nodule, a nodule located in the lingular branch of the left pulmonary artery. The comparison of hysterectomy and lobectomy pieces showed a similar aspect, leading thus to the diagnosis of BML. Awareness of this rare entity should potentially avoid under-diagnosis and difficulties due to hemorrhage during surgery.

Key words: Benign metastasizing leiomyoma.

Introduction
Benign metastasizing leiomyoma (BML) is a rare disease characterized by a primary benign uterine smooth muscle tumor and “metastatic” lesions occurring preferentially in the lung, pelvis and lymph nodes [1, 2]. Although pulmonary lesions have been described years after initial hysterectomy, there are few reports about local recurrence after initial surgery. Despite a benign pathological appearance, uterine leiomyomas potentially metastasize to various organs. The metastasizing process usually occurs ten years after hysterectomy [3], with a range from three months [4] to 20 years [3]. In most cases BML is not responsible for local recurrence. The commonest sites of metastasis are the lung, lymph nodes, deep soft tissues, heart, mesentery and bones [3, 5-12].

We report the case of a patient with hypervascularized BML involving pelvic recurrence and metastasis in the wall of the left pulmonary artery, with extremely severe hemorrhage during laparotomy.

Case Report
A 55-year-old woman complaining of abdominal discomfort, heaviness and asthenia was admitted to our hospital for investigation of a voluminous pelvic mass. Eleven years before she had undergone a sub-total hysterectomy for multiple uterine benign fibromas. The uterus measured 14 cm x 18 cm x 8 cm and weighed 850 g with very vascular fibroids. Initial surgery was complicated by severe hemorrhage due to uterine vessel injury that required packing the cavity. The follow-up cervical smears were normal.

Computed tomography (CT) scan and magnetic resonance imaging (MRI) at entrance showed a voluminous pelvic mass with high vascularization and compression of the urinary bladder (Figure 1). MRI and CT also revealed three pulmonary nodules, two of 6 mm and 10 mm in the right inferior lobe and one of 15 mm in the left upper lobe just adjacent to the lingular branch of the left pulmonary artery (Figure 2); no adenomegaly was visualized in the pelvic and pulmonary areas. Blood chemistry and tumoral markers CEA, CA 125 and CA15-3 were within the normal ranges.

The patient underwent explorative laparotomy which revealed a multilobulated hypervascularized pelvic mass adhering to the adjacent organs. No peritoneal carcinosis or clinically abnormal lymph node was found. The resection of the mass was complicated by uncontrolled severe hemorrhage due to vascular proliferation, responsible for an acute coronary ischemia requiring multiple transfusions, packing the cavity to stop peripertative bleedings and postoperative uterine arteries embolization.

Macroscopically the tumor measured 14 cm x 9 cm x 4 cm, was regular, soft and white, well-circumscribed and arose from the uterus. Histopathological examination revealed an encapsulated vascular proliferation of thick-walled blood vessels in collagen fibers sometimes with myxoid and spindle cells, with no abnormal mitosis, atypical nuclei or necrosis. Immunohistochemistry of spindle cells was positive for smooth muscle actin, BCL2, and negative for cytokeratin (AE1/AE3), PS 100, CD34 and HMB-45.

A diagnostic intervention on the pulmonary area was necessary in order to obtain a correct diagnosis and exclude another metastasizing disease. Thus, three months after pelvic surgery, the patient underwent a left upper lobe lobectomy with the aim of removing the largest pulmonary nodule. Histopathological examination revealed an intra-arterial-wall nodule located in the lingular branch of the left pulmonary artery, measuring 1 cm in diameter, composed of spindles of smooth muscle cells in a fibro-hyalin matrix, with highly vascularized areas composed of large thick-walled vessels (Figure 3). In particular, no mitoses of less than 1 per 10 HPF, necrosis, or inflammatory responses of the host tissue were detected. This nodule was well circum-
scribed by a fibrous capsule and did not extend to the normal pulmonary tissue. Immunohistochemistry was positive for actin, desmin, h-Caldesmon, BCL2, estrogen and progesterone receptors and negative for HMB-45 and CD34. The comparison of hysterectomy and lobectomy specimens showed a similar aspect, leading thus to the diagnosis of BML. No further treatment was done and the patient was controlled by CT scan six months later.

Discussion

First reported 70 years ago by Steiner in 1939, BML remains a controversial disease consisting of benign smooth muscle cells with intervening connective tissue [1]. BML is characterized by a histologically benign smooth muscle tumor originating from a uterine leiomyoma, with the development of similar tumors (metastasis) in distant locations, generally occurring in the premenopausal period. This pathology concerns mainly premenopausal women aged 35-55 years, although cases of elderly women over 70 years old have been reported [3]. In most previously reported cases of BML, there is an interval of several years between the initial uterine surgery (including myomectomy, subtotal or total hysterectomy) and the diagnosis. The metastasizing process may occur three months [4] to 20 years after hystere-
tomy [3], with an average ranging from ten [3] to 14.9 years [2].

The physiopathology remains controversial, nevertheless vascular dissemination and translocation of leiomyomatous cells involving many organs is the most widely accepted. This vascular spread of leiomyomatous cells may be induced by curettage, hysterectomy or myomectomy. In our case, the initial subtotal hysterectomy performed 11 years before had been extremely hemorrhagic, which argues that peroperative vessel injury may have induced vascular dissemination of leiomyomatous cells. Knowing the problems of the previous surgery, we might have considered a preoperative artery catheter placement and a suitable vascular surgeon. Other hypotheses concerning the pathogenesis have been suggested. Kayser et al. [2] argued that BML is a slow-growing variant of leiomyosarcoma of the uterus, whereas Cho et al. [5] advocated that the initial lesions could be low-grade sarcomas with metastatic potential. Lastly Wolff and colleagues [6] considered that these metastases from smooth muscle tumors may have undergone maturation.

The commonest sites of metastasis due to BML are the lung and lymph nodes. Jautzke et al. [3] reviewed 74 cases of BML and noted that extraterine tumors principally localize in the lung. Moreover, BML has been described in the deep soft tissues [5, 7-9], the heart [10], omentum and mesentery [5, 7], bones [11, 12], spine [12] and the central nervous system [11].

In comparison with other cases of BML, the histological examination of the pelvic tumor from our patient showed a hypervascularized tumor with thick-walled vessels within the proliferation. Immunohistochemistry and histopathologic examination proved the lung tumor was similar to the previously resected pelvic mass. Curiously the histological examination of the lung nodule revealed an unusual aspect of metastasis due to BML. Indeed the nodule was found to be part of the lingular branch of the left pulmonary artery, with an arterial aneurysmal dilatation close to this lesion.

Only a few articles have reported local recurrence of BML [13, 14], often requiring iterative laparotomy. Spontaneous regression of pulmonary leiomyomas has been described due to estrogen levels decreasing either in case of menopause [3, 15], termination of pregnancy [16] after the withdrawal of hormonal contraception [17] or ovarian suppression [18].

There is no standard treatment for BML due to the limited number of reported cases. Recent studies have proved that raloxifene induces a significant reduction in leiomyoma size in postmenopausal women [19]. Moreover a randomized placebo-controlled trial demonstrated that the association of raloxifene and LHRH agonist induces a greater reduction of leiomyoma sizes [20].

Interestingly our case of BML presented two specific aspects: 1) a pulmonary nodule localized in the wall of the pulmonary artery and 2) a hypervascularized pelvic tumor responsible for a severe preoperative hemorrhage requiring packing the cavity and postoperative uterine arterial embolization. Awareness of this extremely rare entity can potentially avoid under-diagnosis by radiologists and difficulties due to hemorrhage during surgery.

References
Benign metastasizing leiomyoma.


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Metastatic and recurrent adenocarcinoma of the uterine cervix: a long-term survival of 16 years

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Summary

Purpose of investigation: Recurrent metastatic adenocarcinoma of the cervix is associated with an extremely poor prognosis. Treatment options for recurrent disease are limited and cure is extremely rare. Case report: We report a case of a 43-year-old patient with Stage IB adenocarcinoma of the cervix. She had multiple metastatic recurrence episodes salvaged with several radical surgeries, external and intraoperative irradiation, and chemotherapy over a survival period of 16 years. Conclusion: We conclude that long-term multi-modal salvage treatment may achieve longer survival in rare cases with recurrent metastatic adenocarcinoma of the cervix.

Key words: Metastatic adenocarcinoma; Recurrent adenocarcinoma; Long-term survival.

Introduction

Cervical cancer is the third leading cause of cancer-related death in women worldwide. The incidence of cervical adenocarcinoma has increased relatively compared to squamous cell carcinoma [1]. The prognostic significance of the adenocarcinoma cell type is still controversial. Chemotherapy remains the recommended treatment for recurrent or metastatic adenocarcinoma of the cervix that is not amendable to surgical resection or salvage radiation therapy. We present a case of cervical adenocarcinoma, with multiple recurrences salvaged with multiple multi-modal approaches over 16 years.

Case Report

A 43-year-old patient with Stage IB adenocarcinoma of the cervix underwent radical hysterectomy, bilateral salpingo-oophorectomy and bilateral pelvic lymphadenectomy in 1990. Pathology revealed moderately differentiated endocervical adenocarcinoma with microscopic parametrial invasion, and negative surgical margins and lymph nodes. She was randomized to the chemotherapy arm of a study comparing chemotherapy alone versus chemotherapy and pelvic irradiation. She received two cycles of bleomycin (32U daily day 1-4) and cisplatin (50 mg/m² day 4) followed by cisplatin (50 mg/m²) every three weeks for two cycles. The patient remained disease-free for three years until 1993 when she was diagnosed with a small recurrence in the right upper vaginal wall without paravaginal extension. She was treated with surgical excision through a vaginal approach followed by external beam irradiation (4500 cGy) and vaginal brachytherapy (7352 cGy). Tumor margins were negative.

In 1995 she developed a recurrence in the same location but with paravaginal invasion. She underwent radical upper vaginectomy, partial resection of the bladder and right ureter with reimplantation. Tumor margins were negative for malignancy. She received postoperative brachytherapy to the pelvic sidewall (iridium catheters, 3000 cGy) and pelvic external beam radiation (4000 cGy).

In 1997 she experienced a third vaginal recurrence in the same location. She underwent total infrarelevator pelvic exenteration with creation of a neovagina, ileal conduit, and sigmoid colostomy. Periaortic lymph nodes were excised and found to be negative. Tumor margins were negative for tumor. The patient remained disease-free for four years until 2001 when she had a recurrence in the anterior wall of the neovagina extending to the retropubic space, levator ani muscle and cecum. This was treated by radical resection of the neovagina, partial pubectomy and ileocecal resection. Margins were negative for tumor.

In 2002 she had an elevated CA-125 level (101 U/ml). PET scan revealed recurrence in the right inguinal lymph nodes. She underwent a right inguinofemoral lymphadenectomy. Pathology revealed nine out of 13 lymph nodes involved with adenocarcinoma. The patient received postoperative external beam radiation therapy (4860 cGy) to the right inguinal region. A few months later in 2002, she was diagnosed with metastasis to the left inguinal lymph nodes by PET scan. She underwent left inguinofemoral lymphadenectomy. Pathology revealed 13 out of 15 positive lymph nodes and she received postoperative external beam radiation therapy (5100 cGy) to the left groin.

In 2004 she was diagnosed with metastases in the mesentery of the sigmoid by PET scan after detection of an elevated CA-125 (192 U/ml). She received nine cycles of topotecan (0.75 mg/m² day 1-3) and cisplatin (50 mg/m²) every three weeks. Subsequently, she had a normal CA-125 level (11 U/ml) and a negative PET scan.

In 2005 she developed recurrence in the aortic nodes and right ischial area diagnosed by PET scan. She received external beam radiation to the periaortic nodes and perineum (4000 cGy) with concurrent cisplatin (40 mg/m²/week). Subsequently, a follow-up PET scan showed two suspicious areas around the ischial tuberosity. Both lesions were surgically excised. She received intraoperative radiation therapy (1250 cGy) and postoperative cisplatin (50 mg/m²) for 3 cycles.

In 2006 the PET scan revealed multiple positive metastases in the retroperitoneal nodes, pubic bone, groin area and mediastinum. The patient received two courses of mitomycin (10

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mg/m², day 1) and irinotecan (100 mg/m²/week), on a clinical trial, with improvement of metastatic lesions on a follow-up PET scan. A few months later in 2006, she died from a pulmonary embolus, 16 years after her initial diagnosis, but with extensive pulmonary metastatic disease.

Discussion

The management of recurrent and metastatic adenocarcinoma of the cervix remains a challenging problem. The relative infrequent occurrence of cervical adenocarcinoma has made the evaluation of different types of salvage therapy very difficult. Despite the multiple recurrences and surgical interventions, cycles of chemotherapy, overdosed pelvic irradiation, and aortic irradiation, we were able to achieve control of the recurrent sites over a long period of time.

Surgical excision in combination with pelvic irradiation appeared to be a viable option for the first recurrence due to being superficial and small in size. At the time of the second recurrence the patient declined pelvic exenteration, in spite of the increased risks for fistula formation with local radical excision after previous irradiation. She agreed to pelvic exenteration in 1997 after her third vaginal recurrence, and because there were no other viable alternatives. Additional surgical excisions in irradiated areas remained as the only option to maintain control of her disease. It is of interest, that in spite of multiple bilateral groin nodal metastases, she had no groin recurrences.

Chemotherapy is the mainstay treatment of recurrent and metastatic cervical adenocarcinoma not treatable by radiation or surgically not resectable. Previous studies on single agent chemotherapy for recurrent or metastatic cervical adenocarcinoma have shown response rates between 11% and 31% [2]. There are very limited data on combination chemotherapy for recurrent adenocarcinoma of the cervix. Recent studies have compared the response of squamous cell carcinoma and nonsquamous cell type to combination chemotherapy (Table 1). The authors noticed a lower response rate for the squamous cell carcinoma group compared to the nonsquamous cell type. The median survival of patients with squamous cancers was inferior compared with that of patients with other histologies [3-10].

Our patient was randomized to bleomycin and cisplatin after her initial surgery in 1990 and remained disease-free for three years. In 2004 she received nine cycles of topotecan and cisplatin and experienced clinical and radiographic evidence of disease remission. In 2005 she responded to concurrent chemoradiation with cisplatin, and in 2006 she had a partial response to mitomycin and irinotecan. CA-125 levels and fusion CAT/PET scan remained predictable and reliable in detecting recurrences and metastatic lesions during her disease.

Conclusion

Although recurrent and metastatic adenocarcinoma of the cervix is essentially an incurable disease, a multimodal salvage approach depending on the site of recurrence or metastasis may achieve long-term survival in rare cases.
References


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Three-dimensional power Doppler color ultrasonographic features of a minimal deviation adenocarcinoma of the uterine cervix

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Introduction

Minimal deviation adenocarcinoma (MDA) is an uncommon histological type of endocervical cancer. It was first described by Goodlin in 1963 [1] who named it adenoma malignum due to its differentiation mimicking a benign lesion. It represents about 1-3% of the cervical adenocarcinomas [2] and the reports of this entity are mainly isolated cases or small series [3-5], thus the descriptions of its appearance by diagnostic imaging techniques are very unknown [6, 7]. The aim of this report is to illustrate the ultrasonographic features of MDA, particularly those expressed by three-dimensional power Doppler color ultrasonographic study.

Case Report

A 34-year-old female with no relevant gynecological history was seen at our Institution for post coital hemorrhage and spotting for two months. The patient had two children. Clinical exploration revealed a nodular lesion in the posterior cervical lip which bled when it was touched, but clinical characteristics suggested a benign lesion such as a myoma or an adenomyoma.

Ultrasonographic study showed an enlarged uterus with regular structure and preserved myometrial pattern. Both ovaries and tubes were normal. In the posterior cervical lip a well delimited nodular lesion was seen. It measured 28 x 27 mm and the Doppler color study depicted a peripheral vascular rim deforming the uterine shape. Central vascularization was also seen (Figure 1). In 3-D technique the vessels were regular with scanty ramifications more prominent inside the tumor but also present at the periphery (Figure 2) without any dominant vascular pedicle. The vascularization was seen by a histogram representing a vascularization index (VI) of 2.049, a flow index (FI) of 32.025 and a vascularization flow index (VFI) of 0.656. The volume of the lesion was of 23.946 cc calculated by VOCAL™. The ultrasonographic features suggested the diagnosis of a myoma but with more vascularization than a conventional one.

The patient was surgically treated by local excision of the lesion. The intraoperative histological study by frozen section was suggestive of an adenomyoma. The resected specimen measured 4 x 3 cm and sections studied in paraffin-embedded tissue demonstrated a tumor infiltrating the whole thickness of the cervical wall composed by glands of tubular or tortuous configurations lined by a very well differentiated epithelium of endometrioid type (Figure 3). No endometrial stroma was seen surrounding these glands. No lymphovascular involvement was detected. The pathologic diagnosis was of minimal deviation adenocarcinoma, endometrioid type, of the cervix and the surgical margins were involved by the lesion.

Tumor was staged as IB1 endocervical adenocarcinoma according FIGO classification and a radical hysterectomy with pelvic and aortic lymphadenectomy was performed. In the surgical specimen residual tumor was seen with the same histological characteristics of the lesion previously resected. The lymph node did not show metastatic involvement. The patient underwent radiotherapy and she was free of disease after one year of follow-up.

Discussion

Presurgical diagnosis of MDA by imaging techniques is often difficult and sometimes it looks like a benign lesion [7]. The diagnosis can only be established in the surgically resected specimen where the well differentiated lesion mimicking a benign endocervical tumor can also induce mistakes in the diagnosis [3, 5]. In some cases cytologic features detected in Pap smears of MDA have suggested well differentiated adenocarcinoma or atypical glandular endocervical cells [8].

Sometimes MDA has been associated with Peutz-Jeghers syndrome which is an autosomal dominant disorder characterized by gastrointestinal polyps and mucocu-
taneous melanic pigmentation with a higher incidence of MDA [9, 10]. Moreover a higher incidence of mucinous ovarian tumors associated with MDA has been described – these tumors can be benign, borderline or malignant type, and also be related to sex cord tumors with annular structures [11].

Ultrasonography (US), MRI and CT usually show fluid accumulation in the endometrial cavity and/or vagina in those cases of MDA. Both CT and US also show a multicystic lesion located at the cervical region [9] but the study performed by T2-weighted MRI is the most accurate in the diagnosis showing a non-cystic fine villous tumor or a multicystic lesion depending on the case [7]. This multicystic appearance can be due to dilatation of some well differentiated glands of MDA that display a microcystic pattern [10]. The 3-D power Doppler US findings of MDA have not been previously described, and with this technique the presence of vascularization in the center and periphery of a solid lesion is the main feature, indicating the increase in vessels of the tumor. Alterations found with 3-D US in the vascular indices have been previously described in conventional cervical carcinoma [12].

The elective treatment is hysterectomy with bilateral salpingo-oophorectomy; although MDA is more frequent in young women, the association with ovarian tumors does not allow conservative surgery [11].

Classically the prognosis of MDA has been considered to be poor, but its association to Peutz-Jeghers syndrome and to ovarian tumors can influence this evolution [3, 9, 10, 11]. The prognosis of MDA seems to be the same as for conventional adenocarcinoma at the same stage [2].

Figure 1. — Power Doppler ultrasound showing a nodular lesion with mainly central vascularization.

Figure 2. — Three-dimensional ultrasonographic study with vascular subtraction revealing a non branching regular vascular structure.

Figure 3. — Microscopic appearance of a very well differentiated adenocarcinoma of endometrioid type (hematoxylin and eosin X 40).
References


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Ovarian metastasis of a primary renal cell carcinoma: case report and review of literature

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Summary
Ovarian metastases from renal cell carcinoma (RCC) are very rare, with only 23 cases reported in the literature. We report a case of 54-year-old women who developed bilateral ovarian metastasis 39 months after diagnosis of clear cell carcinoma. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was carried out. Subsequently she was treated with sunitinib. The patient is still alive four years after the initial diagnosis of the renal primary, and disease has stabilized on sunitinib. We conclude that, although rare, the possibility of metastatic RCC should be considered in the differential diagnosis of clear cell tumors of the ovary.

Key words: Ovarian metastasis; Renal cell carcinoma.

Introduction
Renal cell carcinoma (RCC) rarely metastasizes to the ovary and in some instances it is the ovarian metastasis that results in the patient coming to medical attention [1]. RCC represents 3% of adult tumors and it usually appears in 50-to 70-year-old individuals [2]. In most cases renal tumors grow symptomless in the retroperitoneal area and metastases already exist in 30% of patients at the time of diagnosis. RCC most frequently metastasizes to the lung, bones, adrenals, liver and skin, but is well known for its propensity to metastasize to unusual sites via hematogenous spread [2]. Ovarian metastases from RCC are rare, but can be mistaken with primary clear cell ovarian carcinoma due to the histological similarity. Clear cell tumors of the ovary may pose a diagnostic dilemma because of the variety of primary and metastatic clear cell tumors that may occur there. It is very important to differentiate between the two because of the therapeutic and prognostic implications. We report a case of bilateral ovarian metastases from renal clear cell carcinoma and a review of the literature.

Case Report
A 54-year-old woman was referred to the Oncology Unit for scheduled postoperative imaging. Four years ago she underwent radical right nephrectomy for renal cell carcinoma of clear cell type. The tumor (size 10 x 7 x 6.5 cm) involved the lower pole of the right kidney. Histology revealed adenocarcinoma of clear cell type. There was no direct capsular invasion and the renal vein was not involved. The patient was symptom-free. No lymphadenopathy, or evidence of intraperitoneal, pulmonary or other metastatic disease was detected. No secondaries were identified on bone scintigraphy. Serum levels of carcinoembryonic antigen (CEA), CA12-5, CA19-9 and alpha-fetoprotein were within normal limits. Three years later she developed a recurrence of the tumor. The recurrent tumor involved the right renal bed, and the right and left suprarenal glands. The tumor involving the suprarenal gland could right not be distinguished clearly from the borders of the liver. She was treated with interferon. While undergoing treatment with interferon, a bilateral adnexal mass was detected at the end of the 3-month control. A pelvic ultrasound (US) showed a bilateral heterogeneous adnexal mass. On examination there was a firm bilateral fixed mass in the left iliac fossa about 7.5 x 6 cm in size and in the right iliac fossa a mass about 8 x 10 in size. Bimanual examination revealed a mass in the right and left fornix which was fixed and firm in consistency. Full blood count and renal function tests were done and CA12-5 was 25 IU/ml. Pelvic US revealed bilateral solid adnexal masses (right 11.6 x 11.8 x 9.7 cm and left 7.4 x 6.7 x 7.3 cm). There was no ascites. Laparotomy showed no bilateral ovarian tumors. Total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAH+BSO) was carried out. Subsequently she was treated with sunitinib. The patient is still alive four years after the initial diagnosis of the renal primary, and her disease has stabilized on sunitinib.

Macroscopically, the frozen specimen of the left ovary measured 7 x 5.5 x 5 cm and was 6.5 cm long with a 0.7 cm wide tubal segment over it. The external ovarian external surface contained multiple cysts with diameters of 0.5-1 cm. On the cut surface of the ovarian tissue several hemorrhagic cysts 0.5-2 cm in diameter, separated from each other by fibrous tissue, were observed. The size of the right ovary was 9 x 5 x 4 cm and 4 cm long with a 0.8 cm wide tubal segment over it. On cut sections, multiple cysts with diameters ranging between 0.5 cm and 4 cm were observed; almost all parts of both ovaries consisted of these cystic spaces (Figure 1).

Frozen section examination did not yield any certain results. The specimen was prepared under routine procedures. Tissues from both ovaries were fixed in 10% formalin, paraffin-embedded and stained with hematoxylin eosin.
Microscopic examination of multiple sections from all over the ovaries revealed that each of the cyst walls were lined by a flattened or variably stratified cuboidal epithelia with abundant clear or granular eosinophylic cytoplasm and low grade nuclei (Figure 2). Mitotic figures were very rare. Small epithelial islands or narrow cords consisting of similar epithelial cells were demonstrated in the loose fibromyxoid stroma between the cyst walls. The lumina of the cysts contained faint eosinophylic material or blood. Immunohistochemically these epithelial cells showed diffuse and strong EMA, vimentin and CD10 expression. The cells did not stain with CA12-5 or inhibin. The rest of the TAH+BSO specimen and resected omentum material demonstrated no considerable pathological features except an ordinary intramural small leiomyoma nodule.

The patient had undergone a right radical nephrectomy four years before. The slides of the kidney specimen were revised and conformed as grade 2, Stage pT2 renal clear cell carcinoma. Based on the morphological, clinical and immunohistochemical findings, the tumors in both ovaries were diagnosed as clear cell RCC metastases in an extraordinary multicystic architecture.

Discussion

Ovaries are a common site for distant metastases from non genital tumors, especially stomach, colon and breast carcinomas. Approximately 7% of ovarian tumors presenting clinically as ovarian primaries are identified as secondaries on histological examination [3]. However, ovarian metastasis from renal cell carcinoma is rare. This may be due to the fact that renal cell carcinoma predominates in males, vascular sclerosis of postmenopausal ovaries in which age group RCC is common, and some of the metastatic lesions being mistaken for primary ovarian tumors [4]. In one autopsy study, ovarian metastasis was found in 0.5% of cases of RCC [1]. Metastasis to the ovaries is thought to occur by retrograde venous embolization through the renal vein to the ovarian vessels [1, 4]. Clear cell carcinoma of renal origin may pose a diagnostic challenge to the pathologist, especially when there are metastases to sites at which other clear cell tumors have the potential to arise, such as the thyroid, liver, and female genital tract.

In the ovary, the differential diagnosis of clear cell neoplasms includes primary clear cell carcinoma, steroid cell tumor, and dysgerminoma. Metastatic clear cell carcinoma of renal origin may mimic these entities. However, careful gross and microscopic examination combined with immunohistochemical analyses can aid in the distinction of this metastatic lesion [5].

Primary clear cell carcinoma of the ovary occurs in women between 50 and 70 years of age. Bilateral involvement is unusual and is seen in only 2%-4% of patients with Stage I disease [3]. The gross appearance of this tumor may be identical to that of metastatic renal cell carcinoma, with a predominantly cystic mass and focal solid areas. Like RCC, the cysts in the tubulocystic variant of clear cell carcinoma may contain hemorrhagic or dark brown fluid if the carcinoma is arising from an endometriotic cyst. The microscopic appearance of clear cell carcinoma is most often clear cells or hobnail cells lining the cysts and tubules. The clear cells may line complex papillae variably containing periodic acid-Schiff-positive hyaline basement membrane material expanding the papillary cores. Clear cells may also be arranged in solid nests or masses, closely resembling the pattern of a clear cell carcinoma of renal origin. In our case, histology revealed glandular structures containing erythrocytes and was consistent with a RCC origin.

Immunohistochemically stained tumor cells of primary ovarian clear cell carcinomas express cytokeratin, EMA,
and CA12-5 [6]. CA-125 is commonly expressed in ovarian adenocarcinomas but is typically absent in renal cell carcinomas [5]. Nolan et al. proposed an immunohistochemical panel to aid the differential diagnosis between primary ovarian clear cell carcinoma and metastatic RCC consisting of CA12-5, estrogen receptor, progesterone receptor, the anti-cytokeratin antibody 34 E12 and vimentin. Vimentin is an intermediate filament protein typically present in cells of mesenchymal origin and is commonly identified in renal cell carcinomas [7]. The majority of clear cell RCCs show a restricted expression of low molecular weight cytokeratins and vimentin [8]. High molecular weight cytokeratin (HMWCK) and cytokeratin 7 (CK7) are rarely expressed in clear cell RCCs [8], whereas clear cell ovarian carcinomas are almost always positive for CK7 [6]. The typical immunohistochemical profile of our case (vimentin+, low molecular weight cytokeratins+, EMA+) further supported the diagnosis of a metastatic RCC.

Bilateral involvement of the ovaries by tumor should always raise the possibility of metastatic disease. Nearly 10% of bilateral ovarian tumors are metastatic, most commonly from the gastrointestinal tract and breast [3]. Recognition of the metastatic nature of an ovarian tumor depends on an adequate clinical history, but a panel of immunohistochemical markers may play an important role if the primary lesion is unknown.

RCC rarely metastasizes to the ovaries, with only 23 cases reported in the literature from 1937 onwards [9]. The age group ranged from 17 to 68 years. The left kidney was the site of the primary tumor in 11 cases (48%), in two cases primary laterality was not listed, in one case bilateral RCC was diagnosed [10] and in the remaining ten cases including the present case (39%), the right kidney was the site of the primary tumor.

Left ovarian metastases were detected in ten cases (43%), the right ovary was involved in four cases (17%), ovarian laterality was not listed in one case and bilateral ovarian tumors were detected in eight cases (35%). Four of the right-sided RCCs metastasized to the contralateral ovary (44.4%), two to the ipsilateral ovary (22.2%) and two gave bilateral ovarian metastases (22.2%). Five of the left-sided RCCs metastasized to the left ovary (45.4%), two to the right ovary (18%) and in four cases (36.3%) similarly to our case, bilateral ovarian metastases were detected. Usually, secondary ovarian tumors are bilateral. Sixty to 70% of secondary ovarian tumors were bilateral in two published series of ovarian metastasis [11, 12].

In 15 cases (65.2%), the diagnosis of RCC preceded the detection of ovarian metastases with a time interval ranging from three months to 14 years. Renal and ovarian tumors were mainly detected within the first four years after a RCC diagnosis. In the present case, bilateral ovarian metastases were detected 39 months after RCC diagnosis. In four cases (17%), the ovarian tumors were detected before the diagnosis of RCC. Young et al. reported an interesting case of a renal primary detected eight years after ovarian tumor diagnosis [1]. Surgery was the main treatment with radiation, chemotherapy and interferon used in several cases. Information on long-term survival is limited. However, in well documented cases overall survival ranged from three months to 16 years [1, 13, 14].

In summary, ovarian metastasis appears early in the metastatic pathway of RCC. The possibility of disease progression involving the ovaries should be considered in women with a history of RCC. Although rare, the possibility of metastatic RCC should be considered in the differential diagnosis of clear cell tumors of the ovary. Careful attention should be paid to characteristic morphologic patterns and the immunohistochemical profile. Early diagnosis of this rare metastatic tumor results in prompt treatment and prolonged patient survival.

References


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Liver resection for metastases arising from recurrent granulosa cell tumour of the ovary - a case series

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Summary
Ovarian granulosa cell tumours (GCT) occur rarely and represent 2-3% of all ovarian tumours. Regarded as tumours with low malignant potential and renowned for late recurrences, these tumours occasionally metastasize to the liver. We present our experience with three patients who underwent secondary cytoreductive surgery including liver resection for recurrence of the disease resulting in greatly improved quality of life and disease-free interval.

Key words: Granulosa cell tumours; Liver metastases; Hepatectomy.

Introduction
Granulosa cell tumours (GCT) of the ovary are rare sex cord stromal tumours with low malignant potential. Their long natural history and notoriety for late recurrences is a prominent feature but, few patients are offered surgical resection to debulk the extensive recurrences.

We present our series of three patients with late recurrence to the liver from GCT. All three patients had their primary surgery more than ten years prior to their recurrence. Two had been referred from other centres for further management. All three underwent liver resection with significant improvement in their performance status and quality of life.

Case Summaries

Case 1
The patient initially presented in 1986 at 49 years of age and underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH +BSO) for Stage 1 grade 1 adult GCT of the ovary. No adjuvant chemotherapy was given and she remained disease-free until 2003. She then attended with acute shortness of breath following recurrent chest infections and a computed tomography (CT) scan showed right-sided pleural effusion, a 3 cm left paraaortic mass and a 7 cm cystic lesion in the pelvis. Histology from the chest and abdominal biopsies confirmed metastatic disease. Optimal surgical cytoreduction of the pelvis was carried out in October 2003. Note was made of a multiloculated cystic lesion in segments 7 and 8 of the liver, with further abnormality in the hepatorenal pouch, together with a confluent subhepatic mass. Due to the extent of disease the hepato-biliary (HPB) team declined to operate in favour of continued surveillance. In 2004 the patient developed acute shortness of breath following recurrent chest infections and a computed tomography (CT) scan showed right-sided pleural effusion, a 3 cm left paraaortic mass and a 7 cm cystic lesion in the pelvis. Histology from the chest and abdominal biopsies confirmed metastatic disease. Optimal surgical cytoreduction of the pelvis was carried out in October 2003. Note was made of a multiloculated cystic lesion in segments 7 and 8 of the liver, with further abnormality in the hepatorenal pouch, together with a confluent subhepatic mass. Due to the extent of disease the hepato-biliary (HPB) team declined to operate in favour of continued surveillance. In 2004 the patient developed acute shortness of breath following recurrent chest infections and a computed tomography (CT) scan showed right-sided pleural effusion, a 3 cm left paraaortic mass and a 7 cm cystic lesion in the pelvis. Histology from the chest and abdominal biopsies confirmed metastatic disease.

Case 2
The patient underwent laparotomy + TAH+BSO + omentectomy in 1996 at her local hospital which confirmed an adult GCT of the right ovary at the age of 62. She remained disease-free until 2002 and was referred following dysuria and frequency and underwent further debulking of a pelvic mass which was causing ureteric obstruction. Further debulking in 2005 was attempted but abandoned due to extensive recurrence and palliative management was adopted. She was subsequently referred to our centre in December 2006 with increasing abdominal pain and discomfort, frequency and nocturia. CT scan showed extensive sub-capsular metastasis in the right lobe of the liver. There was a recurrence in the caecal area of the bowel mesentery and some large necrotic looking deposits of tumour in the pelvis. Surgical resection with assistance from the HPB team was performed. Recurrences were present on the liver, right hemi-diaphragm, gall bladder, ascending colon, dome of the bladder, the pelvic colon and meso-rectum and the subcutaneous fat of the mons pubis. Extensive surgery included hepatectomy involving segments 2/3, stripping of the right hemi-diaphragm, cholecystectomy, right hemicolectomy and side-to-side anastomosis and anterior resection and end-to-end anastomosis. Following review by the HPB team, repeated drainage of the cystic fluid from the liver was carried out. Adjuvant chemotherapy in the form of weekly carboplatin was given totalling 12 cycles.

Case 3
The patient was diagnosed with GCT of the left ovary in 2001 at 51 years of age. She underwent a left salpingo-oophorectomy (LSO) at her local hospital and was discharged with no follow-up being falsely reassured it was benign. She was referred to our centre six years later with abdominal distension and consti-
Liver resection for metastases arising from recurrent granulosa cell tumour of the ovary - a case series

Imaging showed multiple large pelvic recurrences and an 8 cm subcapsular liver lesion. At surgery, multiple, large fluctuant and vascular nodules were seen attached to the uterus, bladder and sigmoid bowel with sparse nodules on the mesentery, large nodules on the anterior abdominal wall and multiple, small (2-3 cm) nodules on the omentum.

She underwent a complete resection of her pelvic disease and wedge hepatic resection of segments 2, 6 and 7, complete bilateral diaphragmatic stripping with no residual macroscopic disease. Her recovery was complete apart from a ureterovaginal fistula which was repaired successfully and remains disease free at 2 years post-operatively.

Discussion

Ovarian cancers are the fifth common cancers in women after breast, bowel, lung and uterus representing 5-6% of cancer deaths in women [1]. Eighty-five percent arise from the ovarian surface epithelium while sex-cord stromal tumours account for 2-5% overall, with GCT being the commonest of these [2]. Based on clinical presentation and histological differentiation, they are classified into adult and juvenile GCT and the median age at presentation of the adult form is 50 years in the peri/postmenopausal period [3].

GCTs are generally low-grade neoplasms, presenting at an early stage with symptoms of abnormal uterine bleeding and pain. Pressure symptoms due to large tumour volume are also reported with a palpable abdominal mass [3].

Most patients present with Stage I disease (i.e., disease confined to one or both ovaries). Distant metastatic deposits at first presentation are rare [4]. Treatment for young women keen to preserve their fertility is unilateral salpingo-oophorectomy plus staging biopsies including paraaortic node sampling. Five-year survival for Stage I disease is 85-90%. For women who have completed their childbearing, definitive surgery with TAH + BSO + staging biopsies is more appropriate [5]. None of our three patients had surgical staging initially. One had fertility sparing surgery in the form of LSO and then had two subsequent pregnancies. The two postmenopausal patients underwent TAH+BSO and Case 1 alone had chemotherapy with little beneficial effect. Adjuvant treatments may be reserved for patients with large residual or inoperable tumours as the degree of radiosensitivity of GCT is variable and adjuvant chemotherapy extends the disease-free interval by only a few months [6].

Knowledge about the indolent nature of the disease has prompted expectant management policy as these tumours are well known for their late recurrences with an incidence of 25-30% [7]. In our series, Case 1 presented 18 years following her primary surgery. Some advocate follow-up with serial inhibin levels. Rising levels may herald recurrence and may tend to cause anxiety.

Recurrences of GCT tend to occur within the pelvis in 5-10% of cases with the majority in the abdomen. Hepatic metastases are rare and reported to occur in 5-6% of all recurrences [1, 8]. The metastases are rarely limited to one segment and almost invariably are multifocal, large and occupy a wide area of the liver parenchyma [9, 10]. The first case series in the English literature was reported by Margolin et al. [9] in 1985.

Pelvic metastases are often resected surgically as the disease-free interval is long with good prognosis [11, 12] but management of upper abdominal recurrence has traditionally been considered to be palliative and is often managed by either chemotherapy or radiotherapy. Radiofrequency ablation of hepatic metastases from GCT has been more commonly advocated and reported in the literature [13-15]. Historically, surgical resection of liver metastases for GCT was not considered and was performed only as a palliative procedure rather than as a planned therapeutic cytoreductive intervention with demonstrable increase in the disease-free interval.

Figure 1. — Extensive liver metastases is seen (case 2).
Figure 2. — Extensive abdomino-pelvic metastases arising from GCT of the ovary post primary surgery (case 2).
Figure 3. — No macroscopic disease is seen post secondary debulking surgery (case 2).
In our series, all three patients presented with distressing abdominal distension, changes in bowel habit and hepatomegaly. They underwent successful partial hepatectomy and resection of large metastases involving more than three liver segments. The decision to resect the liver metastases was made with the awareness that surgery could significantly improve survival, disease-free interval, symptom control and quality of life in these patients.

Our series is small but demonstrates the role for considering surgical resection of hepatic metastases for GCT especially in patients who present with long disease-free survival after primary surgery. We hope to encourage more liver surgeons to consider hepatic resection for GCT when approached by gynaecological-oncology colleagues for these rare ovarian tumours as liver resection significantly improves both survival and quality of life.

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References

Ovarian carcinomatosis presenting as bilateral inguinal hernia: a brief report

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Summary

The differential diagnosis for what may seem an inguinal hernia may be complex, as lateral pain may be of many types of origin. We report the case of a 48-year-old female patient who presented with a history of painful, progressively protruding soft bulging masses over the bilateral inguinal area and a 20-year history of head cancer and hepatitis B virus. Pathological analysis, gynecological ultrasound and abdominal computed tomography scan were required to make final determination. Final diagnosis was Stage IV ovarian carcinomatosis, which responded to chemotherapy. Initial diagnosis of inguinal hernia should not rule out other potential diagnoses, particularly in complex cases with other risk factors.

Key words: Bilateral; Differential diagnosis; Inguinal hernia; Ovarian carcinomatosis.

Introduction

The differential diagnosis for inguinal hernia is complex because the lateral pain may originate from the bowel, appendix, ovary, pancreas, spleen, or elsewhere [1]. Unusual ovarian presentations of inguinal hernia include an ovulating ovary within an inguinal hernia [2] and a misplaced ovary and fallopian tube [3], but such cases are rare.

Case Report

In July, 2008, a 48-year-old female patient presented with complaints of soft bulging masses over the bilateral inguinal area. The masses protruded while straining or standing and reduced with bed rest. The masses progressed over some weeks in size and pain. The patient was of average height and build (166.2 cm, 76.5 kg). She had hepatitis B virus but denied other systemic disease. She had undergone benign scalp tumor surgery 20 years before.

After an initial diagnosis of bilateral inguinal hernia, a herniorrhaphy was performed by a urologist. Both bilateral inguinal herniated sacs were resected with high ligation and mesh placed on each side. The herniated right inguinal sac with a mass inside yielded a tissue sample which was sent for pathological analysis. The pathology report indicated a mucinous tumor in the right frozen section of the soft tissue but the final pathology report revealed adenocarcinoma, primary site unknown, in both the right and left inguinal excision of the soft tissue (Figure 1). Two weeks later, laboratory results indicated CEA was 18.01 ng/ml and CA-125 was 57.5 ng/ml.

At the same time, abdominal computed tomography (CT) showed no definite space-occupying lesion in the liver, spleen and pancreas, however ascites with scalloping of the liver surface, ascites in the right lower abdomen with septum and small soft tissue density, and an enlarged uterus with a 7.7 cm hypodense mass and a heterogeneously enhanced mass were present (Figure 2A). There was no definite retroperitoneal lymphadenopathy or hydroureteronephrosis and the bilateral adrenal glands were unremarkable. The clinical impression was uterine tumor with ascites, suspected of being a uterine malignancy.

Three weeks after this observation, the patient was admitted for further surveillance. Colonoscopy was negative for malignancy. D&C indicated an endometrial secretory phase, but was negative for malignancy. One week later, gynecological ultrasound found a right ovarian cyst 7.5 x 5.3 cm in size and an enlarged uterus with multiple myomas and mild cul-de-sac fluid accumulation. Abdominal sonography revealed mild ascites; an attempted aspiration failed. The patient was advised to undergo a course of chemotherapy but was hesitant.

She returned in two months (November, 2008), with a distended abdomen, feeling of fullness, bilateral lower abdominal pain off and on, and constipation lasting days. Laparotomy by a gynecological surgeon revealed a jelly-like mass filling the pelvic cavity (1300 cc), an enlarged uterus with multiple myomas, and bilateral ovarian tumors (right, 25 cm in diameter; left, 12 cm in diameter). The patient was diagnosed with ovarian carcinoma Stage IV with carcinomatosis. She subsequently underwent four courses of chemotherapy (Taxol + cis-
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Follow-up after five months showed the CA-125 level had declined significantly from 72.7 ng/ml to 35.7 ng/ml. Abdominal CT scan of the whole abdomen showed a heterogeneously enhanced mass about 7.4 cm in diameter over the uterus, with size unchanged from the previous CT study, normal liver, pancreas, and kidneys, and liver and spleen characteristics consistent with peritoneal pseudomyxoma (Figure 2B).

Discussion

Ovarian carcinomatosis presenting as an inguinal hernia has been reported [4], but it is rare. Díaz-Montes posited that difficulty in diagnosis may contribute to this rarity [5]. Such cancers may present as a chest wall nodule [6], pancreatic pseudocyst [7] or be discovered by accident [8]. As in this case, the carcinomatosis may present as an inguinal hernia [5]. CA-125 levels are used to screen for cancer recurrence, and have recently been shown to correlate with tumor stage, particularly in the presence of ascites, as in this case [9]. CT provides good sensitivity, specificity and accuracy, as well as anatomical detail to guide surgical treatment [10]. In summary, ovarian carcinomatosis presenting as a bilateral inguinal hernia is rare, and the differential diagnosis is required to sort out the inguinal hernia is often not straightforward.

References


Serous ovarian cystadenocarcinoma incidentally discovered in a 29-year-old patient: case report

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Summary

This is a review of a case of Stage IA serous ovarian cystadenocarcinoma in a 29-year-old patient. The patient had no symptoms of illness. She underwent a surgical intervention because of cysts in both of her ovaries. By histopathological examination of the tissue sample taken during the surgical intervention, it was discovered that the patient suffered from a serous cystadenocarcinoma in her left ovary. Five years after the surgical intervention there have been no symptoms of relapse.

Key words: Ovarian cancer; Diagnosis; Symptoms; Survey.

Introduction

Ovarian cancer is the most frequent cause of death in women suffering from malignant gynaecological tumours. It is rarely found in women younger than 40. The occurrence is influenced the most by low parity, positive family history and risk factors. Due to the lack of early or specific symptoms of this disease, as well as to the lack of generally accepted/undisputed screening tests in healthy populations, this carcinoma is usually detected in advanced stage disease [1, 2]. In 65% of women with diagnosed ovarian carcinoma, the disease occurs in FIGO Stage III or IV. The treatment includes surgical intervention as well as chemotherapy [3, 4]. The five-year survival rate in patients with ovarian cancer in FIGO Stage I or II was reported to be 84% and 76%, respectively [5].

Case Report

A 29-year-old nulliparous patient with no symptoms of disease came to our clinic because of cysts in both of her ovaries detected during a regular gynaecological examination. The patient had not suffered from pelvic inflammatory diseases nor had she used oral contraception. Her body weight was normal, and the results of her blood and urine tests were also normal. Her sedimentation rate was 36. The X-ray results of her heart and lungs were normal. Pap smear test results were normal and no data about malignant diseases in the family history were noted. On vaginal examination the uterus was of a normal size, and a palpable cystic formation approximately 3-6 cm in diameter was discovered in the area of the left ovary, and a palpable cystic formation approximately 3 x 3 cm in diameter was discovered in the region of the right ovary. By transvaginal ultrasound (TVS), a unilocular cystic formation with transonic areas, 6 x 6 cm in size, was detected on the left ovary and unilocular cystic formation with transonic areas, 3 x 4 cm in size, was detected on the right ovary. A resistance index flow of 0.48 was registered in the pericystic blood vessels. There was no ascitic liquid in the peritoneal cavity. Results of the ultrasound (US) examination of the abdominal organs were normal.

The values of the tumor marker CA125 were slightly elevated – 40 mlU/ml. The patient underwent laparotomy. Cystectomy was performed on both ovaries. Histological results showed serous cystadenocarcinoma on the left ovary and serous cystadenoma on the right ovary. The histological finding of the neoplasm is presented in Figure 1. Stage of the histological malignity was G1, NG1, and stage of the disease was FIGO I A. The patient’s case was shown to the consulting body in charge of treating malignant diseases. In compliance with the decision of the consulting body, the patient underwent a relaparotomy with salpingo-oophorectomy on the left side, biopsy of the right ovary, partial omentectomy, selective lymphadenectomy and lavage of the abdominal cavity followed by cytological analysis. The final histopathological diagnosis was a right ovarian follicular cyst and left foreign body ovarian granuloma. The omentum showed no significant morphological changes, and secondary metastatic deposits were not found in the nine lymph nodes that were tested. Cytological analysis of the peritoneal wash showed no malignant cells. The patient’s case was shown to the consulting body who decided that the patient should undergo regular gynaecological control examinations every two months during the first two years. The results of the US were normal at all of the control examinations. The values of tumor marker CA125 were normal. Results of the magnetic resonance imaging (MRI) scan of the abdominal cavity and pelvis were normal with no secondary deposits. The patient’s general condition was good.

Discussion

The incidence of ovarian cancer is approximately one per 1,000 women in the UK, although in Japan the incidence is 0.38-0.74 per 1,000 women [2]. It has the greatest mortality of all malignant gynaecological diseases, occurs more often in younger women, and its growth is linear-type in women aged between 30 and 50. Serous histological type accounts for approximately 75% of epithelial ovarian carcinomas. In our patient, it was a well differentiated serous cystadenocarcinoma (G1). Our patient had no history of previous pregnancies. Hereditary factors, toxic chemical agents, irradiation, virus infections, hormonal factors, environmental factors and nulliparity are considered predisposing factors for
occurrence of the disease. Lately, certain authors have started to consider hereditary factors as very important for the occurrence of ovarian carcinoma [6, 7]. Two genotypes of hereditary ovarian carcinoma have been determined. One includes mutations of the BRCA gene, and the other is Lynch cancer family syndrome II. The risk of suffering from an epithelial ovarian carcinoma is higher in persons with positive family history [8]. Certain epidemiological studies show that environmental factors are of great importance for the occurrence of this cancer and that it occurs far more often in highly industrialised environments [9]. The total number of ovulations in a woman’s lifetime is a risk factor for this carcinoma [10]. Pregnancy and oral contraception are considered to have a protective effect against this cancer [1]. Ovarian cancer in early stages usually remains unnoticed for a long time, with patients sometimes experiencing irregular menstrual bleeding [11, 12]. The most important symptom of ovarian cancer is the presence of a pelvic mass at physical examination [13]. Our patient showed no symptoms and the cysts in her ovaries were detected incidentally during a regular examination. Symptoms occur when the disease has already advanced and are the result of pressure of the tumour on its environment or complications [11]. Diagnosing ovarian carcinoma is rather difficult. Several diagnostic procedures should be applied in the diagnosis of the carcinoma consisting of anamnesis, physical examination, gynaecological and rectal examination, laboratory tests, cytological examination, measurement of tumour markers, TVS colour Doppler examination, 3-D US, laparoscopy and endoscopic examinations, X-ray, CT and MRI [14]. In our patient, serum levels of CA-125 were slightly elevated. Approximately 75% of patients suffering from nonmucinous epithelial ovarian cancer have slightly elevated serum levels. A cyst was diagnosed in the ovary by US, with no elements indicating that it was a potential malignant cystic tumour. When US detects a cystic mass more than 8 cm in diameter in an ovary, it always arouses suspicion of being a potential malignant tumour, with the exception of patients using ovulation-inducing agents [15]. In our patient, the diameter of the cyst was approximately 6 cm. In the treatment of the ovarian carcinoma, surgery is of the utmost importance. Adjuvant therapy, e.g. chemotherapy, radiotherapy and immunotherapy should be carried out following surgery [16]. Surgical treatment of Stage I epithelial ovarian cancer comprises total abdominal hysterectomy and bilateral salpingo-oophorectomy. Unilateral ovariectomy is a conservative surgical approach that can be applied only in strictly defined cases [3, 17]. Our patient underwent unilateral salpingo-oophorectomy because she was a young patient with no prior deliveries who desired pregnancy. During the relaparotomy, no elements of malignancy were found on the surgically removed ovary so adjuvant therapy was not induced. The patient attended regular check-ups after surgical treatment. Today, five years after the surgical intervention, she is in good health with no symptoms of relapse and is in the process of preparing for infertility examinations and treatment. Patients with Stage I cancers have a 5-year disease-specific survival of 84% compared with 76% in those with Stage II disease [5].

References


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Chemotherapy with low-dose bevacizumab and carboplatin in the treatment of a patient with recurrent cervical cancer

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Summary

Management of patients with recurrent or advanced cervical cancer is a challenge. Concurrent chemotherapy has become the mainstay of treatment and platinum remains the most effective single agent. Combinations of other agents have not demonstrated significant advantages. The application of angiogenesis inhibitors such as bevacizumab, an antibody inhibiting vascular endothelial growth factor, in metastatic or advanced cervical cancer remains to be evaluated. We present the case of a patient with platinum-resistant recurrent cervical cancer treated with low-dose bevacizumab and carboplatin, with resultant improved disease progression and tolerable toxicity profiles.

Key words: Bevacizumab; Cervical cancer; Metronomic.

Introduction

Carcinoma of the cervix is the second most common cancer among women worldwide, and was responsible for over 250,000 deaths in 2005 [1]. Surgery remains the primary treatment of early-stage cervical cancer, and locally advanced lesions are managed with concurrent chemoradiation. The recurrence rate of cervical cancer is 10% to 20% for FIGO Stages IB-IIA, and 50% to 70% in locally advanced Stage IIB-IVA disease [2]. Only 12% to 45% of patients with advanced stage (IIIA-IVB) disease completely respond to the primary treatment described above, compared to a complete response rate of 70% to 90% in early stages [2]. A low response rate and poor prognosis with a 1-year survival rate between 15% and 20% in patients with recurrent disease or pelvic metastases [3] remain ongoing problems in clinical practice.

The role of chemotherapy in cervical cancer has been proven [3], and concurrent chemoradiation therapy has become the standard treatment of patients with locally advanced or metastatic cervical cancer [4]. Of all the drugs tested, cisplatin is the only drug with sufficient response, and has been suggested as the standard for treatment [5]. There have also been many trials with combination therapies, but the results are not satisfactory. Thus, the development of new drugs is imperative. With an improved understanding of molecular events in tumor cells, targeted therapies have become an important modality in cancer treatment. Angiogenesis has been reported to be an important mechanism in cancer development, including cervical cancer [6-8].

Bevacizumab is a monoclonal antibody targeting vascular endothelial growth factor (VEGF) and has been shown to be effective in many solid malignancies [7]. Anti-angiogenesis is an appealing strategy for cervical cancer treatment, and there are several ongoing clinical trials. We herein report the case of a patient with advanced cervical cancer who was treated by the combination of low-dose bevacizumab and carboplatin, with encouraging clinical results.

Case Report

A 37-year-old Taiwanese woman (G2, P1) who worked in Korea had an abnormal Pap smear with atypical cells of undetermined significance in August 2006. Cervical biopsy revealed carcinoma in situ. Conization of the cervix done in September 2006 showed squamous cell carcinoma with invasion to 8 mm in depth, compatible with Stage IB1 disease. She underwent radical hysterectomy with bilateral pelvic lymph node dissection at a tertiary medical center in Taiwan in October 2006. Pathological examination demonstrated no lymph node involvement, but positive lymph-vascular space invasion and close margins. Thus, postoperative radiotherapy including whole pelvic radiation with a total dose of 5400 cGy and intravaginal brachytherapy with a total dose of 3000 cGy was delivered beginning November 2006. She received regular follow-up after treatment.

She developed low back pain in June 2007. A pap smear did not disclose evidence of recurrence; however, elevated anti-SCC (2.3 ng/ml) was noted. Computed tomography (CT) of the abdomen revealed paraaortic soft tissue masses and destruction of the L4 vertebra, Metastasis was proven by CT-guided biopsy, followed by palliative surgery with pedicle fixation, L3/4 laminectomy, and partial tumor excision for symptom relief in September 2007. Palliative radiotherapy for the residual metastatic lesions was advised.

The patient was then seen at our hospital for further management. Chemotherapy with weekly cisplatin (40 mg/m²) was administered from October to November 2007. Her lower back pain improved and serum anti-SCC levels declined as shown in Figure 1.

Four months later, lower back pain and sciatica associated with elevation of anti-SCC recurred. CT of the abdomen and pelvis showed paraaortic soft tissue masses causing bony destruction of the L4 vertebra, suggesting recurrence. Weekly

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cisplatin for six cycles was administered again, with resultant mildly improved symptoms and declining anti-SCC (Figure 1). Disease progression with worsening lower back pain, further destruction of L4 and L5 vertebrae, and elevation of anti-SCC was noted two months later. A histone deacetylase inhibitor, valproic acid, coupled with cisplatin for sensitization of chemoresistance was used for four weeks, which was ineffective as evidenced by a rising level of the tumor marker (Figure 1). A combination of low dose bevacizumab (3 mg/kg) and carboplatin (AUC = 5) every three weeks was tried. The response was dramatic with declining anti-SCC (Figure 1) and stable symptoms. Shrinkage of the paraaortic soft tissue mass was noted by CT four months later (Figure 2). Toxicity was assessed according to the National Cancer Institute’s Common Toxicity Criteria, version 3.0, grade 1-2 leukopenia, grade 1-2 thrombocytopenia, and grade 2-3 anemia were noted, and overall, the regimen was well tolerated. However, disease progression with elevated anti-SCC, followed by worsening of pain developed again three months after the therapy. The patient received hospice care thereafter.

Discussion
Chemotherapy with single agent cisplatin at 50 mg/m² every three weeks has been used for advanced or recurrent cervical cancer with a response rate from 20% to 30% and overall survival of seven months [3]. No other single regimen has documented greater benefits than cisplatin. Carboplatin has also been tested in a phase II trial for recurrent or metastatic squamous carcinoma of the cervix, and showed a response rate of 15% and median progression-free survival of 3.4 months [9]. Combination therapy with cisplatin and topotecan was reported superior to single-agent cisplatin with response rates of 27% and 13%, median overall survival of 9.4 and 6.5 months, and median progression-free survival of 4.6 and 2.9 months, respectively [10]. Despite more common bone marrow suppression, this doublet did not worsen the quality of life [10]. Even so, the prognosis remains poor and identifying active chemotherapy regimens with tolerable adverse effects is of great importance to maximize the length and quality of life of these patients.

Angiogenesis has been proven to play an important role in solid malignancies. Increased angiogenesis contributes to the development and progression of cervical cancer, and is also associated with advanced disease and poor prognosis [8, 11]. The VEGF pathway is one of the major pathways involved in angiogenesis, and high VEGF expression was found to be associated with deep tumor invasion, pelvic node metastases, pelvic and distant failure, and impaired survival in cervical cancer patients [11].

With angiogenesis being a crucial pathway in cervical carcinogenesis and disease progression, the therapeutic strategy using the anti-VEGF antibody bevacizumab is theoretically rational. A number of phase II and III trials have documented the efficacy of bevacizumab for a variety of solid tumors [7]. In 2006, a retrospective study of bevacizumab combined with 5-fluorouracil or capecitabine in heavily pretreated patients with recurrent cervical cancer showed clinical benefit in 67% of the subjects, including one complete response, one partial response, and two patients with stable disease [12]. The progression-free interval of these four patients ranged from 2.5 to 5.9 months, suggesting potential activity in pretreated recurrent cervical cancer [12]. Since then, several phase II clinical trials of monotherapy or combination therapy with bevacizumab in cervical cancer have been initiated.
A recent report demonstrated an encouraging result using single agent bevacizumab at 15 mg/kg every 21 days in persistent or recurrent squamous cell carcinoma of the cervix [13]. Among the 46 patients enrolled, 23% survived without disease progression for at least six months, and 10.9% had a partial response. The median progression-free survival was 3.4 months, with a range of 2.53 to 4.53 months. However, many grade 3 or 4 adverse events were noted, including hematologic toxicity, hypertension, deep venous thrombosis, pulmonary embolus, and gastrointestinal and genitourinary/renal events. Additionally, one patient died of grade 5 infection, possibly due to the therapy [13].

Preclinical data have shown that bevacizumab not only has an anti-angiogenic effect, but may normalize tumor vasculature, thereby diminishing tumor hypoxia and promoting drug delivery [14]. Thus, combining an anti-angiogenic agent with cytotoxic chemotherapy is believed to enhance antitumor activity. Additionally, a new approach, metronomic chemotherapy, with low doses on a frequent schedule has demonstrated benefits in the treatment of several kinds of tumors. Unlike traditional chemotherapy using the ‘maximum tolerated dose’, this strategy provides not only efficacy, but reduced toxicity and improved quality of life.

In the present report, the combination of low-dose bevacizumab and carboplatin provided significant positive effects on symptoms, level of anti-SCC, and tumor size. The progression-free interval was approximately three months, comparable with the previously reported data of the trial, and this regimen was well tolerated with only minor hematologic toxicity. Recently, doubt has been cast on the application of traditional ways of measuring therapeutic responses in newly developed drugs [8, 15]. Surrogate biomarkers for therapeutic response may be needed for guidance regarding dose and schedule. Our report demonstrates that lower doses of bevacizumab in combination with carboplatin may work well in cervical cancer, an important implication considering the lower economic burden and toxicity.

With limited treatment options for advanced cervical cancer, we found low-dose bevacizumab combined with carboplatin to be possibly effective, with lower costs and side-effects than other options. Optimal dosing and markers for monitoring the treatment response using bevacizumab have not been well established. Further studies are warranted to investigate metronomic therapy using lower doses of bevacizumab and carboplatin in the setting of recurrent and advanced cervical cancer. The lower dose and lower cost of such a newly developed target therapy may help patients with cervical cancer, especially in developing countries.

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Chemotherapy with low-dose bevacizumab and carboplatin in the treatment of a patient with recurrent cervical cancer


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Groin recurrence following Stage IA squamous cell carcinoma of the vulva with negative nodes on superficial inguinal lymphadenectomy

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Summary

Purpose of Investigation: Stage IA vulvar cancer with a depth of stromal invasion less than 1 mm is generally managed by wide local excision alone since there is less than 1% risk of lymph node involvement. Case: A 62-year-old patient was admitted to a university hospital with a suspicious vulvar lesion. Results: We present the first case of inguinal node and a possible contralateral pubic ramus recurrence following bilateral superficial inguinal lymphadenectomy and wide local resection for Stage IA vulvar cancer. Conclusion: There is no evidence that extended radical surgery provides a better overall survival or reduces recurrence rate in Stage IA vulvar carcinomas. Conservative vulvar resection and sentinel node dissection seem to be a rational choice. Nevertheless the disease may recur in the inguinal areas and frequently be lethal, therefore close surveillance and early attempts to treat the recurrent disease before infection and inflammation ensues should be the aim of current treatment strategies.

Key words: Carcinoma; Groin recurrence; Inguinal lymphadenectomy; Lymphatic metastasis; Vulvar cancer; Squamous cell.

Introduction

Carcinoma of the vulva is a rare neoplasm accounting for about 5% of gynaecological malignancies [1]. The majority are squamous cell carcinoma. It primarily affects women in their sixth and seventh decades of life. Radical resection of the vulva in conjunction with bilateral groin dissection has been the standard surgical therapy for this cancer since the 1930s [2, 3]. However, this operative procedure is accompanied by substantial morbidity. Hence a more individualised treatment comprising fewer radical procedures for vulvar cancer patients has been developed in the past 30 years [4]. In 1979, DiSaia et al. combined some of these innovations by proposing radical local excision and superficial inguinal lymph node dissection for selected patients [5].

Stage IA tumours of the vulva and depth of stromal invasion less than 1 mm are candidates for this less radical surgery since they have less than a 1% risk of lymph node involvement [6, 7]. As a result, they are generally managed by wide local excision alone. However, eight case reports reporting lymph node metastasis in Stage IA vulvar cancer patients treated by wide local excision alone have been published [8-14]. In this report, we will describe inguinal lymph node metastasis in a Stage IA patient treated by wide local excision and bilateral superficial lymph node dissection.

Case Report

A 62-year-old patient presented with a 2 x 1 cm suspicious vulvar lesion on the inner aspect of the right labium majus near the clitoris. Biopsy revealed a well differentiated keratinising squamous cell carcinoma with a depth of invasion of less than 1 mm. The tumour also had radial margin involvement. Local radical excision with bilateral superficial inguinal lymph node dissection was performed. Histopathologic evaluation of the specimen revealed no residual invasive carcinoma. There was also no evidence of metastasis in any of the harvested lymph nodes (Stage IA). No further treatment was administered.

The patient’s follow-up was unremarkable for the next 27 months until she presented to the emergency clinic with a painful and infected cystic swelling of 2 cm in the right inguinal area which had begun around two months before. Immediate drainage of the cystic mass along with culture was performed and the patient was internalised for administration of systemic antibiotics. K. pneumoniae and P. aeruginosa were isolated from the lesion. Magnetic resonance imaging of the pelvis showed a 2 cm lymphadenopathy in the right groin and a 1.5 x 2 cm lesion on the acetabular joint of the left pubic ramus displaying high-signal intensity on post contrast axial T2-weighted images. Both lesions were suspicious for metastasis and inflammatory connective tissue was noted. The patient therefore underwent an excisional biopsy of the right groin lymph node, but it was incomplete due to extranodal spread of disease – especially towards the external iliac vessels – and ongoing infection. Pathology confirmed metastatic squamous cell carcinoma.

Hence, her lesion in the acetabular joint was not biopsied and she was treated with palliative radiation therapy. The patient deteriorated gradually and died within four months.

Discussion

A single vulvar cancer lesion measuring 2 cm or less in diameter with a depth of stromal invasion less than 1 mm is defined as Stage IA tumour [15]. This stromal distance is measured from the base of the epithelium at the nearest most superficial dermal papilla to the deepest point of tumour penetration [16]. It has been reported that these tumours have a very low risk of lymph node involvement.
Groin recurrence following Stage IA squamous cell carcinoma of the vulva with negative nodes on superficial inguinal lymphadenectomy

There is no evidence in the literature that sentinel lymph node biopsy in vulvar cancer [28]. Groin relapse in patients who had negative nodes at superficial inguinal lymphadenectomy is uncommon, but when it occurs, it carries a very poor prognosis as in our patient [29]. Stehman et al. reported a 7.4% recurrence in the inguinal region after superficial inguinal lymphadenectomy [30]. Burke et al. reported unexpected groin relapses in 5.8% of patients with negative nodes on superficial inguinal lymphadenectomy [31]. Kirby et al. found an inguinal recurrence rate of 4.6% – similar to other studies evaluating superficial inguinal lymphadenectomy [32]. It is important to emphasise once again that this kind of relapse, even in patients with Stage 1A disease, has a grave prognosis. Of the reported eight cases only one patient is alive and the others died of disease. Surgery and postoperative radiotherapy was the treatment modality for the only survivor of this metastasis [14]. Unfortunately previous surgery of the inguinal area along with profuse infection and inflammation hampered our efforts of a similar type of optimal surgery for the right side. In addition, metastasis to the contralateral left pubic ramus made it impossible. This type of metastasis, as far as we know, is reported for the first time in the literature for Stage 1A patients.

Conclusion

To conclude, there is no evidence in the literature that extended radical surgery provides better overall survival, significantly improves the disease-free survival, or reduce recurrence rate in Stage 1A vulvar carcinomas. A return to radical vulvectomy with inguinal lymph node dissection may not be confirmed as it significantly increases surgical morbidity. Conservative vulvar resection and sentinel node dissection seem to be a rational choice in these patients. Nevertheless, the disease may recur in the inguinal areas and frequently be lethal. Therefore close surveillance and an early attempt to treat the recurrent disease before infection and inflammation ensues should be the aim of current treatment strategies.
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