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Breast cancer - new aspects of tumor biology: are calcitriol and cyclooxygenase-2 possible targets for breast cancer?
M. Thill, A. Terjung, M. Friedrich - Krefeld, Germany
The importance of cyclooxygenase-2 inhibitors in the preventive as well as in the adjuvant setting in breast cancer is evaluated with a wide literature review.

Review Articles

Brain metastases from gestational trophoblastic neoplasia: review of pertinent literature
E. Piura, B. Piura - Beer-Sheva, Israel
The rarity of brain metastases from gestational trophoblastic disease is revised with the aim to encourage the reporting of all small series or singular cases to improve knowledge and analysis.

The importance of alpha/beta (α/β) interferon receptors and signaling pathways for the treatment of cervical intraepithelial neoplasias
L. Montes, C.M.R. Andrade, M.A. Michelin E.F.C. Murta - Uberaba, MG, Brazil
Action of interferon-α/β receptors and signaling pathways are described, as well as the clinical importance against cervical lesions.

Fertility preservation in women with early stage cervical cancer. Review of the literature
I. Boutas, C. Sofoudis, E. Kalampokas, C. Anastasopoulos, T. Kalampokas, N. Salakos - Athens, Greece
In early stages of cervical cancer, especially in young women, the preservation of fertility is one of the most important targets for the gynecologist.

Original Articles

Relationship of human papilloma virus multiple genotype infection with patient’s age and type of cervical lesion
E. Mazarico, M.D. Gómez-Roig, J. Miñano, L. Cortes, E. Gonzalez-Bosquet - Barcelona, Spain
Mean age of patient with single genotype HPV infection was higher than patients with multiple genotype HPV infections, and no evidence of prevalence of multiple HPV infections was found in patients with carcinoma.

Predictors of malignancy in endometrial polyps: a multi-institutional cohort study
P. Litta, J. Di Giuseppe, L. Moriconi, G. Delli Carpini, M.G. Piermartiri, A. Ciavattini - Padua, Italy
Older patients with abnormal uterine bleeding, a high body mass index, and hypertension are at higher risk for premalignant and malignant polyps.

An in vivo model for the study of ovarian cancer and the persistence of characteristic mutations in xenografts
Y. Li, Y.J. Gu, C.N. Liu, T.F. Yue - Tianjin, China
Advanced stage serous epithelial ovarian cancer and early stage non-serous epithelial ovarian cancer were easy to grow in nude mice, and xenografts maintained the characteristic mutation.
Toxicity of concurrent chemoradiotherapy for locally advanced cervical cancer
J. Jakubowicz, P. Blecharz, P. Skotnicki, M. Reinfuss, T. Walasek, E. Luczynska - Krakow, Poland
The most frequent acute toxic effect of concurrent chemoradiotherapy in patients with locally advanced cervical cancer are: haematological, vulvo-vaginal, and gastrointestinal disorders, while the late effects are: rectal bleeding, bowel complication, stenosis or recto-vaginal fistula.

En-bloc pelvic resection with concomitant rectosigmoid colectomy and immediate anastomosis as part of primary cytoreductive surgery for patients with advanced ovarian cancer
Surgical cytoreduction, in cases of advanced ovarian cancer, is required despite a relatively high general complication rate, that can be reduced with personalized surgical strategies.

Apparent diffusion coefficient on 3.0 Tesla magnetic resonance imaging and prognostic factors in breast cancer
C. De Felice, V. Cipolla, D. Guerrieri, D. Santucci, A. Musella, L.M. Porfiri, M.L. Meggiorini - Rome, Italy
The 3.0 Tesla apparent diffusion coefficient may be a helpful tool for identifying high-grade invasive breast carcinoma.

Contrast-enhanced ultrasonography in diagnosis of benign and malignant breast lesions
X.Y. Wang, L.K. Kang, C.Y. Lan - Nanning, China
Accuracy of differential diagnosis of breast pathology is presented.

Comparison of Pelvic Masses Score (PMS) and Risk of Malignancy Index (RMI 3) in the evaluation of pelvic masses
A. Rossi, L. Forzano, I. Romanello, G. Ambrosini, V. Iuri, D. Marchesoni - Udine, Italy
Pelvic Masses Score is an evaluating system that is more reliable than risk malignancy index 3 to assess pelvic masses in the clinical practice.

Prognostic factors affecting lymph node involvement in cervical cancer
I.K. Koleli, E. Ozdogan, B. Sarıibrahim, L.B. Ozturk, A. Karateke - Istanbul, Turkey
Knowledge of prognostic factors in cervical cancer plays an important role in morbidity and mortality rate.

A survey of Jordanian obstetricians and gynecologists' knowledge and attitudes toward human papillomavirus infection and vaccination
A survey of the knowledge about the HPV vaccine in Jordan gynecologist is not satisfactory, even if they are willing to preseibe the vaccination.

Curcumin induces human SKOV3 cell apoptosis via the activation of Rho-kinase
Z. Yin, J. Sun - Linyi, China
Curcumin induces human SKOV3 cell apoptosis in a dose-dependent effect, mediated by Rho-kinase.

Is HE4 a useful endometrioma marker?
C. Leggieri, G. D’Agostino, L. Tommasi, M. Plebani, L. Conte - Padua, Italy
The trend of tumor marker levels CA125 and HE4 in fertile women affected by ovarian cyst before and after a laparoscopic surgery was assessed.

Therapy-related myelodysplastic syndrome and acute myeloid leukemia following chemotherapy (paclitaxel and carboplatin) and radiation therapy in ovarian cancer: a case report
The management of a case of advanced ovarian cancer with myelodysplastic syndrome following chemotherapy is discussed.
Rectus abdominis muscle resection and fascial reconstruction for the treatment of uterine leiomyosarcoma invading the abdominal wall: a case report


The surgical strategy and reconstructive method in case of advanced leiomyosarcoma are discussed.

A case of accessory mammary cancer in a male patient and a literature review

Y.G. Gao, S.H. Zhang, Y. Wang - Beijing, China

A case of primary breast cancer, originating from accessory axillary mammary tissue in a male, is reported.

Successful treatment of a large symptomatic lymphocyst with percutaneous drainage and repeated iodopovidone sclerotherapy

M. Stukan, M. Dudziak - Gdynia, Poland

Symptomatic lymphocyst after lymphadenectomy, for cervical neoplasia, was successfully treated by drainage and sclerotherapy.

Heterologous type of malignant mixed Müllerian tumor of the uterus presenting as a vulvar mass

M. Grigore, C. Ilea, C. Terinte, A. Sava, R. Popovici - Iasi, Romania

An unusual clinical form of uterine mixed Müllerian tumor is described.

Uterine endometrial carcinoma with trophoblastic differentiation: a case report with literature review

T. Seki, N. Yanaihara, Y. Hirata, M. Fukunaga, T. Tanaka, A. Okamoto - Tokyo, Japan

A rare endometrial carcinoma with trophoblastic differentiation is reported.

Aggressive angiomyxoma of the female genital tract: report of two cases

E.Y. Ki, J.S. Park, A. Lee, S.Y. Hur - Seoul, Korea

Preoperative investigations, treatment, and postoperative management of a case of aggressive angiomyxoma are discussed.

Rupture of an endometrioma with extremely high serum CA-125 level (>10,000 IU/ml) and ascites resembling ovarian cancer

C.M. Park, S.Y. Kim - Jeju City, Republic of Korea

High level of CA-125 requires careful differential diagnosis between benign and malignant abdominal masses, as in this case report.

Adenosarcoma of the uterine body initially presenting as an interstitial small tumor of the uterus: a case report

H. Miyata, N. Tsuji, T. Jimi, Y. Butsuhara, K. Terakawa, T. Nagano - Osaka, Japan

A case of prodromal signs of adenosarcoma detected by transvaginal ultrasound and magnetic resonance is reported.
Breast cancer - new aspects of tumor biology: are calcitriol and cyclooxygenase-2 possible targets for breast cancer?

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Summary
Up until now there have been many advances in treatment options for breast cancers such as targeted therapies like monoclonal antibodies, tyrosine kinase inhibitors, mTOR antagonists, and vaccines. Despite these advances, there are still many more that warrant further exploration. Two of these targets might be the cyclooxygenase-2 (COX-2), the key enzyme required to convert arachidonic acid to prostaglandins, and calcitriol [1,25(OH)₂D₃] which is the biologically active form of vitamin D. Both calcitriol and the inhibition of COX-2 have shown antiproliferative and prodifferentiation, as well as pro-apoptotic effects in different malignancies in vitro and in vivo, and the key prostaglandin catabolic enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is known to have tumor suppressor activity. Furthermore, the combination of calcitriol and nonsteroidal anti-inflammatory drugs (NSAIDs), such as non-selective and selective COX-2 inhibitors, acting synergistically to achieve significant cell growth inhibition in prostate cancer. Some epidemiological studies suggest that vitamin D confers a moderate benefit against breast cancer while most epidemiological studies presume that NSAIDs confer the same. Nevertheless there is growing body of evidence that COX-2 expression is a fundamental step in breast cancer carcinogenesis. To date, clinical trials have been conducted in patients with different malignancies using treatment strategies including COX-2 inhibitors and calcitriol and are showing partially encouraging results. The goal of this review is to shed light on the association between the prostaglandin as well as vitamin D metabolism relating to the incidence and therapy of breast cancers. Moreover, this review will also highlight potential treatment options, as well as extract any existing interactions between the two metabolisms.

Key words: Prostaglandin; Vitamin D; Calcitriol; Cyclooxygenase-2 (COX-2); Breast cancer.

Introduction
Currently, breast cancer is the most common malignancy in women. In the U.S. in 2005, approximately 211,240 patients were newly diagnosed with primary breast cancer and 58,490 women were diagnosed with ductal carcinoma in situ (DCIS). Of these, 58,490 deaths are estimated. Therefore breast cancer takes second place following only behind lung cancer [1-3]. Because of this, it is necessary to develop new strategies and treatment options that may improve the prognosis.

Besides the classic histo-pathological parameters used to estimate the prognosis of malignant diseases, the identification of additional molecular prognostic parameters would be very helpful in planning treatment by evaluating protein or messenger ribonucleic acid (mRNA) expression in tumor tissue. One of these potential molecular prognostic parameters might be the cyclooxygenase-2 (COX-2) [4, 5]. New treatment strategies using compounds that attack well defined proteins in the tumor require verification of the expression of these target proteins. Many similarities exist between tumor tissue and inflammatory modified tissue and normally, inflammatory reaction is self-limiting, however, in tumor tissue the inflammatory reaction is persistent. An increased angiogenesis and an elevated production of cytokines, chemokines, and proteases lead to good conditions for cell proliferation and invasion in the tumor tissue [6].

Targeted strategies might eliminate this inflammatory reaction that promotes tumor growth and tumorigenesis and there is already promising data regarding the use of COX-2-inhibitors. The antiproliferative effects of vitamin D may be another starting point; however the data on vitamin D intake or on the exertion of vitamin D analogs is occasionally inconsistent.

The important role that vitamin D and calcium adopt in the human metabolism was recognized as early as the 1920s as it was used to prevent bone disease and rickets which was widespread in children at that time [7]. In the last 20 years non-classical effects of vitamin D and its influence on physiology followed because it is potentially anticarcinogen impacts made it more and more interesting. Besides stable calcium-homeostasis by the renal expressed 1-a-hydroxylase functionality, extra-renal expressed 1-a-hydroxylase also is also known to have antiproliferative and immune-modulating features [8-10]. This fact has led to the development of new treatment strategies in the clinical use of 1,25(OH)₂D₃ (calcitriol). The goal was to affect and treat cancer, psoriasis, autoimmune diseases, and host-graft-rejection [11-14]. Implementation of these
new treatment options in vivo was conspicuously hindered as 1,25(OH)₂D₃ has a potentially hypercalcaemic side effect. Finally the application of synthetic 1,25(OH)₂D₃ analogs led to several successful results due to its less calciotropic effects [15, 16]. The implementation of vitamin D, primarily in cancer and autoimmune diseases, appears to play a more preventative role as opposed to therapeutic [17, 18].

Observational studies showed an association between vitamin D intake and 25(OH)₂D₃ plasma levels, as well as a reduced risk of breast cancer [19, 20]. Studies that tried to elucidate the correlation between sunlight and cancer prevention demonstrated that long sunlight exposure was associated with a low rate of primary breast cancer and consecutively a low mortality rate [21-24]. 1,25(OH)₂D₃ is the biologically active form of vitamin D that binds as a ligand to the nuclear vitamin D receptor (VDR) of the genes that are important for vitamin D metabolism (1-a-hydroxylase, 24-hydroxylase) [25]. 1,25(OH)₂D₃ and its analogs are able to inhibit the proliferation of breast cancer cells in vitro and in vivo [26-29].

**Prostaglandin metabolism**

The COX system consists of two different isoenzymes, COX-1 and COX-2. This system is an integral part of the prostaglandin synthetase complex and is involved controlling inflammatory processes (Figure 1). After transformation of arachidonic acid to prostaglandin G₂ (PGG₂), a glutathione dependent peroxidase converts PGG₂ to PGH₂ by an oxi- and peroxidation. PGH₂ acts as basic substrate for the synthesis of different prostaglandins by the microsomal and cytosolic prostaglandin synthase, which are tissue- and cell-specific. Based on the cellular enzyme setting, different prostaglandins are synthesized in different tissues where they act in an auto- or paracrine manner [30]. Prostaglandin E₂ (PGE₂) is one of the best known prostaglandins and is generated by the prostaglandin E synthase. These consist of three different forms: two microsomal prostaglandin E synthases and the cytosolic prostaglandin synthase E [31].

The 15-hydroxyprostaglandin dehydrogenase (15-PGDH) belonging to the oxidoreductases family, inactivates all generated prostaglandins by oxidation to 15-keto metabolites which then have greatly reduced biological activity [32].
PG-receptors

The physiological effects of many prostaglandins are mediated by binding to G protein coupled receptors. These effects regulate inflammatory mediations, control hormone regulation, constrict or dilate in vascular smooth muscle cells, and regulate calcium movement and their specific receptors activate signal transduction pathways which could induce chronic processes like angiogenesis. For example, PGE2 interacts with four cell surface receptors - EP1-4 and the EP2 receptor subtype is involved in the Gs/cAMP/proteinkinase which is a-signalling pathway leading to an increased vascular endothelial growth factor (VEGF) expression. PGJ2 and PGA2 interact with nuclear receptors, belonging to the peroxisome proliferator-activated receptors (PPARs) family. After dimerization with the 9-cis-Retinoid receptor (RXR) and then binding to a sequence specific responsive element located at the promoter of its target gene, they directly induce gene expression [33].

Isoenzymes COX-1 and COX-2

COX-1 is ubiquitary and not a relevant prognostic factor [34]. In contrast, the COX-2 enzyme is not constitutively expressed. The COX-2 gene expression is stimulated by many growth factors, cytokines, and prostaglandins and is associated with inflammation [35]. COX-2 is predominantly a proinflammatory enzyme but late in the inflammatory phase, the enzyme is involved in limiting inflammation.

Studies with COX-1 and COX-2 knockout mice lead to new consolidated findings about the function of these enzymes concerning ovarian functionality and reproduction as well as cardiovascular development [36-39]. The COX enzymes are the main target of non-steroidal anti inflammatory drugs (NSAID) where isoenzymes specifically inhibit the biological activity of COX enzymes. Celecoxib and rofecoxib are selective COX-2 inhibitors whereas acetylsalicylic acid, ibuprofen, and indomethacin are non-specific and target both isoenzymes.

Role of COX-2 in carcinogenesis

The COXs, especially COX-2, play an important role in the development and progression of malignant tumours. The over expression of COX-2 is associated with the differentiation of tumor cells by several mechanisms [40] and can be detected in various epithelial carcinomas such as in colon [41, 42], gastric [43], and esophageal cancers [44], as well as in prostate [45], liver, pancreas, and lung cancers [46]. One of the mechanisms that are modulated during carcinogenesis is neoangiogenesis [47-55].

Epidemiological studies have shown that a continuous intake of NSAIDs protects against the incidence of breast cancer [56-58].

Increased PGE2 levels can be detected in cultivated human breast cancer cell lines as well as in invasive human breast cancer cells [59-62] and are associated with both a negative hormone receptor status and an escalated metastatic potential [59].

As mentioned previously, PGE2 is the ligand for at least four cell surface receptors - EP1-4 and several studies have presented the impact of the EP1-receptor in carcinogenesis of colon and breast cancer [63]. A blockage of the EP2-receptor leads to a reduction and a diminishment of intestinal polyposis in APC^{570E}-knock-out mice [64]. There was an increased detection of EP2- and EP4-receptors in the breast tumors of COX-2-MMTV mice; therefore, it appears that the EP-receptors play an important role in mediating PG functions and in promoting carcinogenesis.

Role of 15-PGDH in carcinogenesis

Increased PGE2 levels in context to mammary carcinomas are associated with an enhanced cell proliferation, invasiveness, resistance to apoptosis, and angiogenesis [65, 66]. The regulation of plasma PGE2 level results from its synthesis and its biological inactivation through 15-PGDH, the key enzyme for the biological inactivation of PGs [32]. Recent studies hypothesized 15-PGDH as a tumor suppressor gene in correlation to colon, bladder, and bronchial carcinomas [67-69]. Wolf et al. [70] assumed antiproliferative effects of 15-PGDH in breast cancer cells. The estrogen receptor (ER) positive and well differentiated MCF-7 breast cancer cell line had an increased 15-PGDH expression compared to poorly differentiated, ER negative MDA-MB-231 cells, which express COX-2 and lead to primary breast cancer. Different studies reported that MCF-7 cells are the only breast cancer cell line with an enhanced 15-PGDH expression and low levels of 15-PGDH are accompanied by poorly prognostic factors [70]. This data attended by a microarray analysis of van’t Veer et al. [71] supports the advice that a loss of 15-PGDH expression plays a pivotal role in the development of poorly differentiated mammary carcinomas. Data generated from genetically modified MDA-MB-231 cells that over-express the enzyme and MCF-7
where 15-PGDH was knockout, corroborates the hypothesis that 15-PGDH acts as a tumor suppressor gene in breast cancer [70]. MDA-MB-231 cells showed a decreased invasiveness similar to studies in colon [67] and bronchial carcinomas [68]. Yan et al. [67] reported that 15-PGDH is naturally expressed in colon tissues and was dramatically reduced in colon carcinomas. The reconstitution of 15-PGDH in immunodeficiency mice prevented the colon cancer cells from generating tumors and so the authors concluded, that 15-PGDH acts as tumor suppressor and inhibits the angiogenic and proliferative effects of COX-2 in vivo.

**COX-2 expression in breast cancer**

Experimental immunochemical studies of COX-2 expression in breast cancer have produced varying and sometimes controversial and inconsistent data. Generally the consensus is that COX-2 is expressed by invasive ductal and lobular carcinoma and that the proportion of COX-2 positive tumors varies between studies (Table 1). In studies where poor prognostic tumor characteristics were examined, a correlation was found between prognostic parameters such as hormone receptor negativity, human epidermal growth factor receptor 2 (HER2) positivity, increased tumor size, high nuclear grade, development of distant metastases, and a reduced survival rate (Table 1) [5]. Moreover COX-2 expression correlates with aromatase expression. An explanation for the variable findings of COX-2 protein expression may be caused by the different scoring systems and cut-offs used for COX-2 immunoreactivity.

Half et al. [72] examined immunochemical human breast cell lines of normal and neoplastic breast tissue and detected a COX-2 expression in breast cancer cells in 43%, in DCIS in 62.5% and benign breast cells had a COX-2 expression in 81%. The more elevated COX-2 expression in DCIS in terms of a premalignant lesion might mean that an up-regulation or over-expression of COX-2 occurs relatively early in the carcinogenesis of breast cancer [72]. Contrary to Half et al. [72], Denkert et al. [73] could not detect a COX-2 expression in benign breast tissue and this may support the partially conflicting data. Denkert et al. [73] detected a COX-2 expression in 41% in invasive ductal breast cancer, however detected it in only 14% of invasive lobular tumors and 21% in other breast carcinomas (Table 1). The COX-2 expression was associated with positive axillary lymph nodes (> 50% node positive, just 16% in node negative breast cancer), extensive tumor growth (58% in tumors > 20 mm, in 24% in tumors < 20 mm), poor nuclear grading, vascular invasion, and hormone receptor negativity.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N =</th>
<th>COX-2 positive (%)</th>
<th>Benign tissue</th>
<th>Correlation of COX-2 expression and clinicopathological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carcinoma</td>
<td>DCIS</td>
<td></td>
</tr>
<tr>
<td>[77]</td>
<td>44</td>
<td>2/44 (4.5%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[78]</td>
<td>27</td>
<td>7/17 (42%)</td>
<td>8/10 (80%)</td>
<td></td>
</tr>
<tr>
<td>[79]</td>
<td>221</td>
<td>80/221 (36%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[70]</td>
<td>106</td>
<td>18/42 (43%)</td>
<td>10/16 (63%)</td>
<td>39/48 (81%)</td>
</tr>
<tr>
<td>[71]</td>
<td>46</td>
<td>50%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[74]</td>
<td>1576</td>
<td>589/1576 (37.4%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[75]</td>
<td>106</td>
<td>90/106 (85%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[76]</td>
<td>128</td>
<td>41%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[77]</td>
<td>192</td>
<td>40.6%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[78]</td>
<td>65</td>
<td>41/65 (63%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>[79]</td>
<td>43</td>
<td>41/43 (95%)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. — Immunochemical examinations of COX-2 expression and correlation with selected clinicopathological parameters in breast tissue.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N =</th>
<th>COX-2-mRNA positive (%)</th>
<th>Clinicopathological correlation of COX-2 with Angio- HR- HER2 Grading Age Node + Big tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>[86]</td>
<td>40</td>
<td>40/40 (100%)</td>
<td>Not examined</td>
</tr>
<tr>
<td>[72]</td>
<td>9</td>
<td>9/9 (100%)</td>
<td>Not examined</td>
</tr>
<tr>
<td>[87]</td>
<td>7</td>
<td>7/7 (100%)</td>
<td>-</td>
</tr>
<tr>
<td>[88]</td>
<td>20</td>
<td>10/20 (50%)</td>
<td>-</td>
</tr>
<tr>
<td>[85]</td>
<td>18</td>
<td>18/18 (100%)</td>
<td>Not examined Yes (PR)</td>
</tr>
<tr>
<td>[84]</td>
<td>30</td>
<td>27/30 (90%)</td>
<td>Yes (ER+)</td>
</tr>
</tbody>
</table>

Table 2. — COX-2 mRNA expression and correlation with selected clinicopathological parameters in breast cancer.
Not all the studies have determined a correlation between COX-2 expression and clinicopathological parameters. Half et al. [72] could not demonstrate a significant correlation but Ristimäki et al. [74] certainly did show a significant correlation between COX-2 expression and hormone receptor negativity, extensive tumor growth, high nuclear grading, and HER2 positivity. In a recently published paper by Singh-Ranger et al. [75], a correlation to distant metastases was described and Nassar et al. [76] demonstrated a correlation to nuclear grading and tumor size; however a correlation to important clinical goals such as eradicating the disease and enhancing overall survival rate have not yet been found.

Therefore COX-2 over-expression correlates in a different manner depending upon its aggressiveness the invasive potential of tumor cells, and then consequently exhibiting a higher incidence of distant metastases [40].

Transcriptional studies have also revealed a distinct variation in their results regarding COX-2 expression. The detection rate varies between 50% and 100% in the literature (Table 2).

There is a comparable relationship between COX-2 immunoreactivity and mRNA expression in tumor tissue [77]. Zhao et al. [84] demonstrated an increased mRNA expression in hormone receptor positive breast cancer; a result that was confirmed by Singh et al. [85] in breast cancer with positive progesterone receptors. However, only a small number of studies have examined the correlation between mRNA expression and clinico-pathological parameters. These results are summarized in Table 2.

These results are contrary to the immunochemically evaluated data, which show an association to hormone receptor negative tumors. This could be explained because before the genetic information of COX-2 is translated into a biologically active protein, COX-2 mRNA is post-transcriptionally modified in the nucleus. Thus, we speculate that the COX-2 mRNA is destabilised by its AU rich sequences and no COX-2 protein is generated. Therefore, the correlation between the hormone receptor status and COX-2 mRNA levels is not obvious in studies where the COX-2 protein expression was investigated [5].

There are some well known factors which affect the COX-2 mRNA levels like interleukin-1 (IL-1) stabilises the highly unstable COX-2 mRNA transcript [89], however steroids may destabilise the COX-2 mRNA [90]. Furthermore, it might be possible that genetically different subtypes of breast cancer express COX-2 and are then associated with both hormone receptor-negative and receptor-positive tumors [91]. Additionally, Ristimäki et al. [74] reported that hormone receptor-positive patients who express COX-2 had a poor survival rate.

**COX-2 and hormone receptors**

There is concurrent evidence regarding the interaction of PGE_2/COX-2 and the ER signalling pathway. For example, COX-2 expression is correlated with the expression of the aromatase [92] and in vitro studies support this data. It has been shown that COX-2 promotes the aromatase transcription, whereas COX-2 inhibitors diminish it [93]. Based on the elevated synthesis of prostaglandins in cells that express COX-2, the aromatase expression and activity is increased in breast cells [94, 95]. Expression of aromatase leads to estrogen production and from cell line studies; we know that hormone receptor expression can be induced by sex steroid hormones [96]. All the data supports the close correlation between COX-2 and hormone receptors. Wolf et al. [70] reported a link between the estrogen signalling pathway and 15-PGDH by a negative feedback mechanism. High levels of this hormone reduced the 15-PGDH expression but the activity of the estrogen responsive element (ERE) and the activity of the aromatase increased. New studies suggest a synergism between selective COX-2 and aromatase inhibitors.

**Results from in vivo studies**

The impact of COX-2 in carcinogenesis of breast tumors has been shown in transgenic mice models [97]. It has been reported that the over-expression of COX-2 in breast tissues is associated with decreased BAX and Bcl-xL (pro-apoptotic) and increased Bcl-2 (anti-apoptotic) protein levels. Therefore, the authors suggested that induction of carcinogenesis is COX-2 dependent [97]. In contrast, the resistance to apoptosis is associated with increased COX-2 levels [98]. The importance of COX-2 in correlation to the tumor formation has been investigated in COX-2 knockout mice. The COX-2 knockout mice lead to an 86% reduction of intestinal adenoids [99].

**COX-2 and tumorigenesis**

The expression of COX-2 is regulated by post-transcriptional and post-translational mechanisms. Different cytokines, growth factors and oncop genes have been shown to induce the COX-2 expression which is associated with carcinogenesis [46, 100].

**Influence of COX-2 on angiogenesis and apoptosis**

Angiogenesis is the development of new blood vessels and is an important factor in tumor proliferation, invasion, and metastasis. Davies et al. [101] showed a significant positive correlation between COX-2 expression and the endothelial surface marker CD31. Other reports confirmed a positive correlation between COX-2 and the VEGF [102, 103]. During carcinogenesis, COX-2 modulates neoangiogenesis and seems to stimulate the production of proangiogenic factors such as VEGF, basic fibroblast growth factor (bFGF), transforming growth factor 1 (TGF1), platelet derived growth factor
apoptosis significantly [119]. Yoshinaka tumors (46.3%) in comparison to the control group. Therefore, celecoxib dropped the COX-2 expression and enhanced the application of celecoxib resulted in a significant growth reduction of the MCF7/HER-18 tumors (58.7%) and the MDAMB231 tumors in mice by injecting estrogen-positive MCF7/HER2-18- and estrogen-negative MDAMB231 breast cancer cells. The described food was continued for another 105 days. A distinct reduction in tumor incidence, variety, and tumor volume was either ibuprofen or nothing. After seven days 7,12-Dimethylbenz(a)anthracene (DMBA) was applied intragastrically and the rats. Three groups of rats were formed. In one group the food was enriched with celecoxib. The other two groups obtained
tumor enlargement of 518% was observed in the control group [117]. Harris the use of celecoxib in rats led to an averaged downsizing of breast tumor volume by 32%, however, a tumor volume en-
ductive Canadian study including 5,882 patients reported a reduction of breast cancer incidence by 24% due to the NSAIDs intake for two to five years [58]. Another case control study demonstrated a 40% reduction after five years of NSAIDs in-
take [57]. These results seemingly justify the preventive use of NSAIDs, however, contrary results were delivered by the Nurses Health Study. This trial showed no difference during the intake of ASS (100 mg) in neither women with breast cancer nor in healthy women [114]. On the contrary it was in patients with colon cancer who led the continuous intake of NSAIDs to a reduction of incidence in 40-50% [56, 115, 116].

Breast cancer and NSAIDs
The rationale for using NSAIDs is their non-selective (ASS, ibuprofen, etc.) or selective (COX-2 inhibitors such as celecoxib) suppression of the COX-system. In a meta-analysis consisting of 14 epidemiological studies (six cohort studies, and eight case control studies), breast cancer risk was reduced by 18% due to constant intake of NSAIDs [113]. An extensive Canadian study including 5,882 patients reported a reduction of breast cancer incidence by 24% due to the NSAIDs intake for two to five years [58]. Another case control study demonstrated a 40% reduction after five years of NSAIDs in-
take [57]. These results seemingly justify the preventive use of NSAIDs, however, contrary results were delivered by the Nurses Health Study. This trial showed no difference during the intake of ASS (100 mg) in neither women with breast cancer nor in healthy women [114]. On the contrary it was in patients with colon cancer who led the continuous intake of NSAIDs to a reduction of incidence in 40-50% [56, 115, 116].

Data of animal models supports the use of selective COX-2 inhibitors for both therapeutic and preventive uses. For instance, the use of celecoxib in rats led to an averaged downsizing of breast tumor volume by 32%, however, a tumor volume en-
largement of 518% was observed in the control group [117]. Harris et al. [118] examined the influence of celecoxib in 120 rats. Three groups of rats were formed. In one group the food was enriched with celecoxib. The other two groups obtained either ibuprofen or nothing. After seven days 7,12-Dimethylbenz(a)anthracene (DMBA) was applied intra-gastrically and the described food was continued for another 105 days. A distinct reduction in tumor incidence, variety, and tumor volume was shown in the celecoxib treated group [118]. In a recently published paper Barnes and co-workers [119] could induce breast tumors in mice by injecting estrogen-positive MCF7/HER2-18- and estrogen-negative MDAMB231 breast cancer cells. The application of celecoxib resulted in a significant growth reduction of the MCF7/HER-18 tumors (58.7%) and the MDAMB231 tumors (46.3%) in comparison to the control group. Therefore, celecoxib dropped the COX-2 expression and enhanced the apoptosis significantly [119]. Yoshinaka et al. [120] also showed that the use of celecoxib significantly reduced tumor sizes, increased apoptosis, and that a reduced DNA synthesis in the tumor tissue of mice induced breast carcinomas. Moreover the neoangiogenesis was influenced as VEGF-A-mRNA levels were found to be reduced [120].

COX-2-inhibitors in systemic treatment
Several studies have evaluated the significance of COX-2 inhibitors in combination with systemic treatment. A phase II study observed a clinical benefit of 47.5% for the combination of capecitabine and celecoxib in patients with metastatic breast cancer. The combination was well-tolerated [121].

Recently published data about COX-2 and its significance on the aromatase and influence on the female hormonal balance are of strong interest. Besides finding an increased effect on estrogen synthesis in malignant breast tissue, a strong correlation between COX-2 and aromatase mRNA expression were found. This data supports the assumption that COX-2 is able to regulate aromatase activity in breast tissue [92]. A possible synergism between COX-2 and aromatase-inhibitors is even more interesting and hence a prospective randomised phase III multicenter trial (REACT-trial) was conducted that included primary breast cancer patients in order to evaluate the combination of celecoxib and exemestane, an aromatase inhibitor, in an adjuvant setting. The combination of celecoxib and exemestane was already well-tolerated and had shown a clinical benefit of 74% [122] or had led to a benefit extension (median 96.6 weeks vs. 49.1 weeks) in pa-
ents with metastatic breast cancer [123].

Other malignancies were also proven on the benefit of selective and non-selective COX-2 inhibitors in combination with other compounds such as chemotherapy [124, 125], tyrosinekinase inhibitors [126], and other new approaches [127]. Some of them are encouraging, like the results of the ASCENT trial [124] and some are disappointing. Further work is required to establish how NSAIDs can be best applied for therapeutic benefit.
Vitamin D

Vitamin D metabolism

Vitamin D, a secosteroid hormone, is assimilated by food (milk, fish, liver), multi-vitamin preparations, and dietary supplements [128]. Vitamin D is also synthesised from 7-dehydrocholesterol and provitamin D3 after skin exposure with sunlight (ultraviolet spectrum 290-315 nm) [129]. Based on its animal or herbal origin, there are two existing vitamin D metabolites: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) [17], which is less efficient in increasing the 25-hydroxyvitamin D [25(OH)2D3] serum levels [130]. Cholecalciferol attains to the liver through the bloodstream and is transformed to 25(OH)D3 (25-hydroxyvitamin D3, calcidiol, 25-hydroxycholecalciferol) by a hydroxylation on the C25 position [131, 132]. 25(OH)D3, a circulating metabolite, correlates with the vitamin D balance. The hydroxylation of cholecalciferol on the C25 position is inadequately regulated. 25(OH)2D3 level increased with the vitamin D intake, therefore, the 25(OH)2D3 serum level is normally used as an indicator of the vitamin D balance [133]. The serum level range of 25(OH)2D3 is between 10 and 50 ng/ml and round about 30 pg/ml for 1,25(OH)2D3 [134]. 25(OH)2D3 is renally converted to the biologically active metabolite 1,25-dihydroxycholecalciferol [1,25(OH)2D3] by the 1-a-hydroxylase (CYP27B1). 1,25(OH)2D3 is 100-1,000 fold more active than the other natural metabolites [135]. The 1-a-hydroxylase, a mitochondrial enzyme, which belongs to the P450 enzyme family, is located in the renal proximal tubule. Besides the renal expression of the enzyme, many studies reported an extra-renal expression of 1-a-hydroxylase and thus an extra-renal synthesis of 1,25(OH)2D3. This enzyme has been detected in many cell types and tissues, e.g. prostate, breast, lung, pancreas, parathyroid, and monocytes [136]. The extra-renal synthesised 1,25(OH)2D3 has cell specific functions and as a result acts as local auto- and paracrine factors. In this context, many extra-renal effects of 1,25(OH)2D3, e.g. cell cycle arrest, induction of apoptosis, and cell differentiation, have been reported [136]. The fine tuned activity of 1-a-hydroxylase correlates inversely with the calcium metabolism and thus the circulating levels of 1,25(OH)2D3 correlates inversely with the ingested amount of calcium [137]. 1,25(OH)2D3 serum levels are maintained in pmol/l range by a classic negative feedback mechanism. The decrease of calcium or phosphate levels leads to an increase of the 1-a-hydroxylase activity and an enhanced synthesis of 1,25(OH)2D3 which in turn promotes the intestinal resorption of calcium and phosphate and the calcium mobilisation from the bones. The activity of 1-a-hydroxylase decreased with increasing 1,25(OH)2D3 levels, which leads to 24 hydroxylase activation. This enzyme degrades 1,25(OH)2D3 to its inactive metabolite 24,25(OH)2D3 [138, 139], which is subsequently converted to calcitroic acid and excreted. Hence, the nutritive intake of calcium directly regulates 1-a-hydroxylase activity and indirectly modifies parathormone levels. This hormone produced in the parathyroids increases the phosphate excretion in the proximal tubule but promotes sodium, potassium, and calcium resorption in the distal tubule. Under normocalcaemic conditions, the activity of 1-a-hydroxylase is inhibited. These regulations are necessary to synthesize 1,25(OH)2D3 although much is needed to cover the calcium and phosphate demand and to avoid a 1,25 (OH)2D3 intoxication [139]. The circulating vitamin D level depends on many different factors such as: the vitamin D content in either the ingested nutrition or the dietary supplements, and the endogenous production and degeneration via vitamin D metabolising enzymes. A simplified scheme of vitamin D metabolism is presented in Figure 2.

Extra-renal vitamin D metabolizing enzymes

The biologically active metabolite is produced after a series of hydroxylations through cytochrome P450 enzymes which belong to the cytochrome p450 super family. The different enzymes are handled as follows:

1-a-hydroxylase (CYP27B1)

The 25-hydroxyvitamin-D3-[25(OH)2D3]-1-a-hydroxylase (1-a-hydroxylase) is encoded by the CYP27b1 gene and catalyzes the synthesis of 1,25(OH)2D3 from 25(OH)2D3. 1,25(OH)2D3 is the most important regulator of the enzyme that leads to a decreased enzyme expression. The regulation of the extra renal 1-a-hydroxylase depends on local factors like cytokines (ILs, interferones, and tumor necrosis) and growth. The optimal 1,25(OH)2D3-level tuning mechanism is not yet completely understood [139]. The reduced expression of the enzyme suggests the involvement of a negative vitamin D responsive element (VDRE) and Turunen et al. [140] showed that the enzyme’s response to 1,25(OH)2D3 is a cell specific event with participation of many VDREs. The suppression of cell proliferation, the induction of apoptotic events, and the modulation of immune responses are counted among the classical features of 1,25(OH)2D3. After binding to the vitamin D receptor, 1,25(OH)2D3 is able to arrest the cell cycle of a tumor cell in the G1-G0 phase via specific mechanisms [139]. In prostate and colon cancer the tumor protective effects of vitamin D is correlated to vitamin D deficiency [141]. Much data reports that both the renal and extra renal 1-a-hydroxylase are based on the expression of the same gene product. In contrast to the renal 1-a-hydroxylase, the extra-renal enzyme is not subjected to the autoregulation as mentioned above [136, 142]. Therefore the enzyme’s tissue specific expression might be a key mechanism in connecting the vitamin D metabolism to the anticarcinogenic effects of 1,25(OH)2D3.
Although the enzyme’s cytokine and growth factor related regulation is not completely understood, it has been shown that different cytokines stimulate the 1-α-hydroxylase in different cell types [139, 143-146]. Another potential mechanism of gene regulation is the incidence of different gene polymorphisms [147] and inactive variants due to alternative splicing of the 1-α-hydroxylase mRNA, but this mechanism’s function is not completely clarified. Alternative splicing within the post-transcriptional modification is a normal process of gene expression in breast cancer cells and based on the pre mRNA, different mature mRNAs are generated when introns or exons are deleted or added. Thus, the translation of these mRNAs leads to different enzyme proteins, however, mis-spliced mRNAs are usually quickly degraded although it appears that this mechanism has failed in various cells. It has been reported that different protein variants of 1-α-hydroxylase might have diverse biological functions. Fischer et al. [148] showed six different variants of the enzyme in MCF10F via nested touchdown polymerase chain reaction (PCR), but in MCF-7, these variants appeared weakly expressed. Based on this data, the authors concluded that because alternative splicing regulates the level of the active enzyme extra-renal, it therefore regulates the local production of 1,25(OH)2D3 [149]. The activity of the extra-renal expressed 1-α-hydroxylase is an important factor of the tumor pathophysiology because of an accumulation of 1,25(OH)2D3 in many tissues. Studies of prostate [150, 151], colon [152-154], and breast cancer [148, 155, 156] have shown the expression of 1-α-hydroxylase in healthy as well as in malignant tissues. Thus, 1,25(OH)2D3, which is produced extra-renal might have autocrine behaviour to protect cells against transformation and supports the suggestion of its carcinoprotective effects. Accordingly, low 1-α-hydroxylase levels correlate with the risk of prostate-, colon- [157] or breast cancer [158, 159]. Moreover, the extra-renal production of 1,25(OH)2D3 inhibits cell proliferation and

Figure 2. — Simplified scheme of vitamin D metabolism. Vitamin D (food intake, synthesis in skin) is metabolised in liver to 25(OH)2D3, then via the renal 1-α-hydroxylase (endocrine signalling pathway or extra-renal in tissues (autocrine/paracrine signalling pathway) to 1,25(OH)2D3.
promotes cell differentiation in xenograft models [160]. Besides the expression of the 1-a-hydroxylase in breast [155, 161], endometrial [162], cervical, and ovarian carcinomas [163], the induction of the enzyme has also been shown in lymphomas [164] and dysgerminomas [165]. In these reports, the local synthesis of 1,25(OH)_{2}D_{3} was mediated by the 1-a-hydroxylase expression of tumor-associated macrophages. The expression of the enzyme mammary gland tissue occurs in lobules and ductus, primarily in the cancer tissue and invasive tumor cells and inflammatory infiltrate. Thus, it might be possible that the enzyme activity and the vitamin D receptor (VDR) expression are considerably higher than in the benign tissue compared to aggressive tumor cell lines (MCF-7res, MDA-MB231) [166]. Townsend et al. [166] compared breast cancer and benign tissue samples via reverse transcription PCR. They reported a 27-fold induction of the 1-a-hydroxylase expression and seven-fold induction of the VDR expression in tumor samples. Because 80% of the tumor tissues had an increased 1-a-hydroxylase and VDR, they concluded that there was a closed coupling of both gene products. These results are in compliance with Segersten et al. [161]. The capacity of 1-a-hydroxylase to synthesize 1,25(OH)_{2}D_{3} within the mammary gland parenchyma results in, on the one hand, the available amount of 25(OH)_{2}D_{3}, and is dependent on sunlight exposure and the season [167-170] – normally there is no definite correlation between 25(OH)_{2}D_{3} and 1,25(OH)_{2}D_{3} – yet on the other hand, the level of the extra-renal production of 1,25(OH)_{2}D_{3} is limited by the expression of the 1,25(OH)_{2}D_{3} – decomposing enzyme 24-hydroxylase, which is stimulated by 1,25(OH)_{2}D_{3} in VDR expressing tissues. Based on the missing correlation of 24-hydroxylase and VDR or the 1-a-hydroxylase in breast cancer tissues, it seems that 24-hydroxylase is independently regulated. Kemmis et al. [171] demonstrated the expression of a functioning VDR and an inhibition of proliferation via 1,25(OH)_{2}D_{3} in benign breast cells and MCF-7. The VDR expression in human mammary epithelial cells (HMEC) breast cells was higher than in MCF7 cells. Furthermore, the authors showed an expression of 25(OH)_{2}D_{3} metabolizing 1-a-hydroxylase and 24-hydroxylase in these cell types, whereas the 1-a-hydroxylase expression was higher in MCF-7. In contrast to renal HKC8 cells, the expression of 1-a-hydroxylase was not inhibited by 1,25(OH)_{2}D_{3}. Based on the strong induction of the 24-hydroxylase through the 1,25(OH)_{2}D_{3} application, the authors showed that MCF7 cells were more sensitive in response to 1,25(OH)_{2}D_{3} compared to HKC-8 and HMEC cells. From this data, they concluded that there is a functional vitamin D receptor as well as intact signalling transduction pathways in MCF-7 cells. The data suggests that the synthesis of 1,25(OH)_{2}D_{3} and the activation of the VDR inhibits the cell proliferation in breast cells. Thus, the treatment of benign breast cells with 25(OH)_{2}D_{3} leads to an activation of the VDR transcription and the regulation of its target genes (CYP27B1, CYP24), and finally to an inhibition of cell proliferation. According to that, CYP27B1 lords it over CYP24 which means a transformation of 25(OH)_{2}D_{3} to 1,25(OH)_{2}D_{3}. Kemmis et al. [171] have shown for the first time that physiological 1,25(OH)_{2}D_{3} levels (30-100 nmol/L) are able to inhibit cell proliferation in benign HMEC cells and in MCF7 breast cancer cells. Interestingly, aging process and the associated lack of estrogens correlate with decreased 25(OH)_{2}D_{3} levels. The reason is that the ability of estrogen to stimulate the renal CYP27B1 activity [172]. Accordingly, the lack of estrogens leads to decreased 1,25(OH)_{2}D_{3} levels and presents the highest risk for breast cancer in postmenopausal women [273].

24-hydroxylase (CYP24)

The 25-hydroxyvitamine D_{3}-24 hydroxylase (24-OHase, 24-hydroxylase) encoded by the CYP224 gene is induced by 1,25(OH)_{2}D_{3} in breast cell lines where the enzyme is time and dose dependently stimulated by 1,25(OH)_{2}D_{3} [174]. An increased enzyme expression in ovarian, cervical, and breast cancer compared to healthy tissue samples has been shown by immunochemistry and real time PCR [163]. In contrast, Townsend et al. [166] showed a four-fold increase of the enzyme expression in malignant breast tissues compared to healthy tissue samples using the same technique. Additionally, the expression of 24 hydroxylase increased in breast cancer cells foremost in hormone resistant MCF-7 Res and the aggressive MDA-MB231 cells compared to benign MCF-12A cells [166]. Kemmis et al. [171] reported the highest 24 hydroxylase expression in MCF-7 cells and Segersten et al. [161] showed a two-fold enzyme expression in tumor tissues compared to benign tissue samples. The authors concluded that the conversion of 1,25(OH)_{2}D_{3} into the inactive metabolite 1,24,25(OH)_{3}D_{3} is significantly higher in malignant tissues. Furthermore Townsend et al. [166] detected the enzyme only in breast cancers with an increased 1-a-hydroxylase and VDR expression. Further analysis showed that in a healthy tissue sample expression of 24-hydroxylase correlated with both 1a-hydroxylase and VDR. There was no such correlation in breast tumors. Hypothetically, the 24 hydroxylase acts as a part of a well-organized feedback mechanism and is transcriptionally modulated to increase the local 1,25(OH)_{2}D_{3} and VDR level [166]. The synthesis of 1,25(OH)_{2}D_{3} via the 1-a-hydroxylase has been shown in benign and malignant mammary gland tissues but this mechanism’s efficiency in tumor tissues might be affected by a dysregulated 24 hydroxylase expression.

Vitamin D-receptor (VDR / mVDR)

The vitamin D receptor (VDR) is an ubiquitary expressed steroid hormone receptor. Like other steroid, thyroid, and retinoid receptors, the VDR is a member of the nuclear hormone receptor family. The receptor binds to its ligand 1,25(OH)_{2}D_{3}, interacts with other receptors by dimerization, and binds as homodimers or heterodimers to specific DNA
sequences. So called VDRE recruit additional co-activators (such as SRC-1, GRIP-1/TIF2, ACTR) and interact with the transcriptional processing order to initiate or inhibit the transcription of its target genes [25]. It is well known, that steroid receptors consist of different variants with distinct specificities. Sunn et al. [175] described an N-terminal variant of the VDR. 1,25(OH)₂D₃ mediates its genomic effects as a VDR ligand and via the directed binding to the VDRE [176]. Besides its function in bone metabolism and in the calcium/phosphate balance, the VDR interacts with different signalling pathways, e.g. with p21, a cyclin dependent kinase inhibitor which is involved in cell cycle regulation and inhibition of the cancer cell proliferation [26]. There are some suggestions about the existence of a membrane VDR (mVDR) [177]. The mVDR mediates its signals through the change of the intracellular calcium concentrations and through interactions with the protein kinase C and enzymes of the mitogen-activated protein kinases (MAPK) family [178-183]. Although the mVDR seems unrelated to the nuclear VDR, Marcinkowska et al. [184] reported an interaction of both receptors. The function of this mechanism is not clearly defined and the cloning of the mVDR has failed until today.

Many studies reported that extra-renal VDR expression is associated with the non-classical effects of 1,25(OH)₂D₃. The VDR expression has been shown in healthy breast tissues and in more than 80% of the breast cancer tissues [185]. The natural ligand of the VDR, 1,25(OH)₂D₃ and many new developed synthetic vitamin D analogues inhibit cell proliferation and induce apoptosis in breast cancer cell lines [186, 187]. Furthermore, in animal models, vitamin D analogues retard the tumor growth and lead to a regression of breast tumors [12].

**Vitamin D-receptor gene polymorphism**

The gene that encodes the VDR has various polymorphisms. It has been hypothesised that the genetic VDR polymorphism influences the breast cancer risk due to its potential effects on VDR gene expression and protein function [188, 189]. Many polymorphisms of the VDR gene have been identified and several, such as FOK1, Bsm1, APA1, TAQ1, and Poly(A) are well analysed [190, 191]. The studies that were conducted had conflicting results [191]. Curran et al. [192] showed a significant association of the VDR polymorphism APA1 and TAQ with the breast cancer risk. A significant increased breast cancer risk in women with the ff genotype FOK1 was observed by Chen et al. [193]. Sinotte et al. [194] detected a significant link between familial breast cancer disposition and FOK1. Other data came from Trabert et al. [195] who found a correlation between a higher breast cancer risk and the genotype Bsm1 bb in postmenopausal women, although there is also published data without any evidence for a link between VDR polymorphisms and breast cancer risk [196-199]. An analysis of the last 13 published studies in which different VDR polymorphisms and its relation to breast cancer were examined, leads to the suggestion that the modification of breast cancer risk is associated with certain VDR polymorphisms and therefore 1,25(OH)₂D₃ might modify the risk of breast cancer [200]. A recently published paper by McCullough et al. [201] presented certain VDR gene polymorphisms associated with a decreased breast cancer risk in women who ingested high doses of calcium (no calcitriol), concluding that nutritive influences might modify the link between gene polymorphisms and breast cancer. This data could shed light on breast cancer risk evaluation or could even be used in a predictive manner to answer the question about which women are strongly endangered to develop distant metastases.

**Calcium**

Like vitamin D, humans ingest calcium through food or dietary supplements; 99% of calcium is bound as hydroxyl phosphatide in bones and teeth [202]. Only 1% calcium is extracellularly located. Plasma levels of calcium (Ca²⁺) are limited by intestinal absorption, renal secretion, and reabsorption. Additionally, the skeletal calcium storage and resorption keep the plasma levels of calcium in a closed range (3.5–5 mmol/L) [202].

**Vitamin D, calcium, and breast cancer risk**

**Dietary and supplemental vitamin D intake**

For 1,25(OH)₂D₃ several studies have shown both an antiproliferative effect and an inhibition of angiogenesis in malignant and healthy breast cancer cells [17, 185, 203-206]. In mouse models, an increased intake of vitamin D led to the suppression of epithelial hyperproliferation and tumorigenesis of the mammary gland that was caused by rich nutrition [207, 208].

Last but not least, it has been proven by the First National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study that sunlight exposure is inversely correlated with breast cancer risk [209, 210]. In this study, the female population in the north-eastern parts of the U.S. have a higher risk of contracting breast cancer compared to the other states of the U.S. This leads to the suggestion that sunlight induced vitamin D production has a positive influence in avoiding breast cancer [20].

In contrast, in the Nurses’ Health Study, there was an inverse association between vitamin D intake and breast cancer risk among premenopausal women, but no association among postmenopausal women [20]. Consistent with this observation, a study published a few years ago was based on the Cancer Prevention Study II Nutrition Cohort and observed no
associations between breast cancer and total and dietary vitamin D intakes among postmenopausal women [211]. Another Italian study recently showed an inverse association between vitamin D intake (in the study > 143 IU) and breast cancer in 2,569 breast cancer patients [212]. Two other studies that concentrated on vitamin D deficiency and its susceptibility for breast cancer incidence approved that a deficiency conditional on nutrition in adolescence does not lead to an increased breast cancer risk [213, 214].

The proper dose of vitamin D remains unclear and a recommendation does not exist, however a meta-analysis gives evidence towards a dose of > 400 IU per day to reduce breast cancer risk [213].

Role of vitamin D in breast cancer

To date, there have been several epidemiologic studies of the association between vitamin D and breast cancer risk, however, their results have not been consistent. Several studies observed an association between 25(OH)2D3 plasma levels and breast cancer incidence [19, 215-217]. The predictive value of 25(OH)2D3 plasma levels depends upon the time they have been measured. Plasma levels that have been measured within a few years before breast cancer diagnosis are less predictive than plasma level measured many years before [217]. Furthermore, plasma levels that have been measured around 15 years before diagnosis do not have any aetiological value for the genesis of breast cancer [216].

Bertone-Johnson et al. [217] found a marginally significant reduction of breast cancer risk in women > 60 years who had elevated 25(OH)2D3 and 1,25(OH)2D3 plasma levels. In contrast, published data by Shin et al. [20] demonstrated a significantly decreased breast cancer incidence in premenopausal, but not in postmenopausal women, who had continuous vitamin D intake.

Furthermore, a case control study observed that women with plasma 25(OH)2D3 concentration <5 0 nmol/L had > five times higher risk of breast cancer than those with plasma concentrations exceeding > 150 nmol/l [158]. Janowsky et al. [19] also showed an inverse association between 1,25(OH)2D3 plasma levels to the point of diagnosis and breast cancer risk in patients with breast cancer. However, there was no difference in 1,25(OH)2D3 plasma levels between patients with breast cancer and those with DCIS. The authors suggested that the grade of invasion was not correlated with the extent of 1,25(OH)2D3 level. Another nested case-control study with 96 breast cancer cases and 96 controls found no association between prediagnostic 1,25(OH)2D3 levels and levels at the time of diagnosis and breast cancer risk among postmenopausal women [216].

The circulating concentration of 25(OH)2D3 is considered to be an excellent measure of the availability of vitamin D from the diet, supplements, and from synthesis in the skin [218]. Its potential importance in breast carcinogenesis is due to the fact that 25(OH)2D3 can be metabolised to 1,25(OH)2D3 by 1-a-hydroxylase in breast tissue [155]. Thus, 25(OH)2D3 levels may be more representative of intracellular levels of 1,25(OH)2D3 than circulating levels of 1,25(OH)2D3 [217]. To date, no studies have been published investigating intracellular or tissue levels of 1,25(OH)2D3 and 25(OH)2D3 in association with breast cancer risk.

Dietary and supplemental calcium intake

Many studies about the importance of calcium and its association to breast cancer have already been published. Most of them are case-control studies and nearly all of them are relatively small, and there is insufficient documentation regarding risk factors for breast cancer in multivariate analyses.

Calcium is participating on carcinogenesis via regulation of cell proliferation, differentiation, and apoptosis [219-221]. Cell proliferation and differentiation of breast cells can be increased by elevated calcium levels [208, 222, 223]. Boyapati et al. [224] observed a non-significant inverse association between calcium intake and breast cancer risk among pre- and postmenopausal women but the Nurses’ Health Study has shown this association only for premenopausal women [20]. The anti-carcinogenic effects of calcium are last but not least, mediated by vitamin D, therefore calcium is one of the key mediators of the vitamin D induced apoptosis in breast cancer cells [208].

Calcitriol and prostaglandins in cancer

The stimulation of the renal calcitriol [1,25(OH)2D3] synthesis in vitro is well known as well as the inhibition of acetosalicylic acid as a non-selective NSAID [225]. This justifies the clinical use of NSAIDs in treating arthritis for example. Hayes et al. [226] observed an inhibition of calcitriol synthesis caused by PGE1 and PGE2 in synovial fluid macrophages from arthritic joints and with that they proved the link between vitamin D and prostaglandin metabolism. Several published studies have proven the anti-carcinogenic effects shown in different signalling pathways on prostate cancer cells [227-229]. The team around David Feldman examined the influence of calcitriol in established human prostate cancer cell lines (androgen dependent LNCaP cells and androgen independent PC-3 cells) and in primary normal prostatic epithelial cells derived from normal and cancerous human prostate tissue. They showed that calcitriol
regulates biologically active prostaglandin levels and prostaglandin actions by three mechanisms: calcitriol suppresses the COX-2 expression and moreover it up-regulates the expression of 15-PGDH. This dual influence of calcitriol was associated with a decrease of PGE$_2$ secretion in prostate cancer cells. Calcitriol reduces the mRNA expression of prostaglandin receptors EP$_2$ and FP, additionally a mechanism to inhibit the biological activity of prostaglandins.

The combination of calcitriol and NSAIDs led to a significant growth inhibition in prostate cancer cells via its synergistic effects. These findings might postulate that calcitriol and NSAIDs are definitely a useful combination in chemotherapeutic settings in breast cancers and finally that will evaluate the promising importance in the neoangiogenesis in cancer? Does a link exist between vitamin D and prostaglandin metabolism in breast cancers? These questions have to be evaluated in order to clear the safety of a celecoxib treatment in metastatic breast cancer. Furthermore, calcitriol and calcium have shown anti-carcinogenic effects in experimental studies and several epidemiological studies have demonstrated an inverse association between vitamin D and calcium intake and breast cancer. Other studies have detected an inverse association between plasma and serum levels and breast cancer risk. Experimental studies support the hypothesis that the reduction of breast cancer risk is more significant among premenopausal women than among postmenopausal women and microsomal prostaglandin E synthase-1 (mPGES-1) and EP receptors might be important targets for the development of new anti-inflammatory and anti-proliferative tumor therapies.

Questions that remain unanswered are: has calcitriol as anti proliferative effects in breast cancer as was proven in prostate cancer? Does a link exist between vitamin D and prostaglandin metabolism in breast cancers? These questions have to be answered as the increasing incidence of breast cancer have yet to be solved. Innovative treatment strategies fall on fruitful ground. Thus we need further studies that elucidate the importance of COX-2 inhibitors in the preventive as well as in the adjuvant settings in breast cancers and finally that will evaluate the promising importance in the neoangiogenesis in detail.

Concluding remarks

In conclusion, there is promising preclinical data inhibiting COX-2 in breast cancers, therefore the chance exists to innovatively disturb carcinogenesis of those gynecological oncological neoplasms Phase II trials have already been conducted to clear the safety of a celecoxib treatment in metastatic breast cancer. Furthermore, calcitriol and calcium have shown anti carcinogenic effects in experimental studies and several epidemiological studies have demonstrated an inverse association between vitamin D and calcium intake and breast cancer. Other studies have detected an inverse association between plasma and serum levels and breast cancer risk. Experimental studies support the hypothesis that the reduction of breast cancer risk is more significant among premenopausal women than among postmenopausal women and microsomal prostaglandin E synthase-1 (mPGES-1) and EP receptors might be important targets for the development of new anti-inflammatory and anti proliferative tumor therapies.

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Brain metastases from gestational trophoblastic neoplasia: review of pertinent literature

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Summary

Brain metastasis from gestational trophoblastic neoplasia (GTN) is rare with about 222 cases documented in the literature and an incidence of about 11% in living GTN patients. Brain metastasis from GTN was part of a disseminated disease in 90% of patients, single metastases in the brain – 80% and located in the cerebrum – 90%. Brain metastasis was the only manifestation of metastatic GTN in 11.3% of patients, appeared synchronously with metastatic GTN in other sites of the body – 30.6% and was diagnosed from 0.3 to 60 months after diagnosis of metastatic GTN in other sites (most often in the lung) – 58.1%. Overall, 83.9% of patients with brain metastases from GTN had also lung metastases from GTN. Brain metastases from GTN showed a greater tendency to be hemorrhagic compared to brain metastases from other primaries. In patients with brain metastases from GTN, the best outcome was achieved with multimodal therapy including craniotomy, whole brain radiotherapy, and EP-EMA or EMA-CO chemotherapy. Nonetheless, brain metastasis from GTN is a grave disease with a median survival time from diagnosis of brain metastasis of about 12 months.

Key words: Brain; Choriocarcinoma; GTD; GTN; Metastases.

Introduction

Gestational trophoblastic neoplasia (GTN) represents diseases that form the malignant end of the gestational trophoblastic disease (GTD) spectrum, i.e., invasive mole, choriocarcinoma, and placental-site trophoblastic tumor. GTN is divided into non-metastatic GTN (disease confined to uterus, FIGO Stage I) and metastatic GTN (disease extends outside uterus but is limited to genital structures, FIGO Stage II; disease extends to lungs with or without genital tract involvement, FIGO Stage III; disease involves other metastatic sites, FIGO Stage IV) [1, 2]. Thus, in presence of brain metastases, the GTN is automatically allocated FIGO Stage IV. According to the modified WHO prognostic scoring system for GTN as adapted by FIGO in 2000, GTN is divided into low-risk GTN (sum of scores < 7) and high-risk GTN (sum of scores ≥ 7) [3]. Patients with non-metastatic (Stage I) and low-risk metastatic (Stages II and III – score < 7) GTN are treated with single-agent chemotherapy (methotrexate or actinomycin D) with resulting survival rates approaching 100%. Patients with high-risk metastatic (Stage IV – any score, and Stages II-III – score ≥ 7) GTN should be treated with multi-agent chemotherapy (EMA-CO, combination of etoposide, methotrexate, actinomycin D, cyclophosphamide, and vin-cristine; or EP-EMA, combination of etoposide, cisplatin, methotrexate and actinomycin D) with or without adjuvant radiotherapy or surgery to achieve cure rates of 80% - 90% [2]. In presence of brain metastases, GTN of any WHO/FIGO score is automatically allocated high-risk metastatic GTN. Since the vast majority of invasive moles are non-metastatic (confined to the uterus) and placental site trophoblastic tumor is an extremely rare condition, the term metastatic GTN has become synonymous to choriocarcinoma. Therefore, in this article, the terms metastatic GTN and choriocarcinoma are used interchangeably.

GTN is preceded by hydatidiform mole in 50% of cases, abortion: 25%, full-term pregnancy: 23%, and ectopic pregnancy: 2%. [1,2,4]. There is considerable variation in the incidence of molar pregnancy in different geographic regions and ethnic groups, with the highest rates in the Far East (Japan and the Philippines; two to three per 1,000 pregnancies) and the lowest rates in western countries (0.6 – 1.1 per 1,000 pregnancies). The estimated incidence of choriocarcinoma in North America and Europe is one per 40,000 (0.0025%) pregnancies and one per 40 (2.5%) hydatidiform moles, whereas in Southeast Asia and Japan metastatic GTN rates are higher at 9.2 and 3.3 per 40,000 pregnancies, respectively [1, 5, 6]. In recent years, however, the widespread use of ultrasound in early pregnancy has led to earlier recognition of molar pregnancy and, thus, the ominous consequences of molar pregnancy can be avoided or significantly reduced if, after evacuation of the molar pregnancy, women are registered for follow-up with assay of beta-human choriionic gonadotropin (β-hCG) and promptly treated with chemotherapy if needed. It seems that the incidence rates of both hydatidiform mole and choriocarcinoma have declined over the past 30 years in all populations [1, 4, 7].
The brain, along with the bone, liver, and lung, is one of the most common sites of metastases. About 170,000 patients are newly diagnosed with brain metastases each year in the USA, a figure which is ten-fold higher than that of patients newly diagnosed with primary malignancy of the brain [8-10]. Brain metastases most commonly arise from lung carcinoma (~ 25% – 50% of lung carcinoma patients develop brain metastases), breast carcinoma (~ 15% – 30% of breast carcinoma patients develop brain metastases) and malignant melanoma (~ 30% – 70% of malignant melanoma patients develop brain metastases), and occur at a reduced frequency in patients with renal and colorectal carcinoma and other cancer types [8,10-12]. Female genital tract cancers, however, are considered “neurophobic” since brain metastases from female genital tract malignancies, apart from metastatic GTN, are rare with only about one percent of ovarian carcinoma, endometrial carcinoma, and cervical carcinoma patients developing brain metastases in the course of their disease [13-15]. Notwithstanding, brain metastasis occur in ~ 10% - 20% of metastatic GTN patients and represents the major cause of death from metastatic GTN [16, 17]. Choriocarcinoma has a tendency to metastasize rapidly by blood-borne dissemination; the most common site of distant metastasis of choriocarcinoma is the lung followed by the brain, liver, gastrointestinal tract, spleen, and kidney [18]. Brain metastases from choriocarcinoma are frequently associated with lung metastases from choriocarcinoma and it seems that in a substantial amount of patients, brain metastasis is secondary to the lung metastasis. This provides an evidence that the hematogenous route of dissemination of blood-borne malignant trophoblastic cells from the uterus to the brain is through the pelvic veins, inferior vena cava, right atrium, right ventricle, pulmonary artery, lungs, pulmonary veins, left atrium, left ventricle, aorta, carotid arteries, into the brain arterial circulation, and then to the brain parenchyma.

Until more than three decades ago, brain metastases from GTN had rarely been documented in the literature. Vaughan and Howard [19] in 1962 documented a case of intracranial hemorrhage due to metastatic choriocarcinoma. Stilp et al. [20] in 1972 reported three women with brain metastases from choriocarcinoma who had been successfully treated. Weed and Hammond [21] in 1980 documented 14 patients with brain metastases from choriocarcinoma and showed that in some circumstances brain metastases can be eradicated. Since then, 36 papers (single case reports and series of patients) on brain metastasis from GTN in living patients have been published in the literature, totaling 222 patients [16, 17, 22-55]. This review summarizes these papers and focuses on the following topics: incidence of GTN as source of brain metastases in patients with brain metastases from GTN, incidence of brain metastases in patients with GTN, age of patients with brain metastases from GTN, type of antecedent pregnancy, interval from antecedent pregnancy to diagnosis of metastatic GTN, interval from diagnosis of metastatic GTN to diagnosis of brain metastases, symptoms and signs of brain metastases from GTN, type, amount and site of brain metastases from GTN, treatment of brain metastases from GTN, and survival after diagnosis of brain metastases from GTN.

Incidence of GTN as source of brain metastases in patients with brain metastases

Tom [56] in 1946 reviewed 33 women with brain metastases and found that the breast was the source of brain metastases in 13 (39.4%), gastrointestinal tract: seven (21.2%), lung: three (9.1%), uterine cervix: two (6.1%), malignant melanoma: one (3%), uterus: one (3%), ovary: one (3%), thyroid: one (3%), and undetermined: four (12.1%); in none of the cases, GTN was source of brain metastases. Chason et al. [57] in 1963 found brain metastases in 200 (18.3%) of 1,096 autopsies of cancer patients and observed that choriocarcinoma was source of brain metastases in only one (0.5%) of 200 cases with brain metastases. Hunter and Rewcastle [58] in 1968 reviewed 393 autopsies of patients with brain metastases and showed that lung carcinoma was by far the commonest source of brain metastases (34.1%) followed by breast carcinoma (18.6%) and malignant melanoma (6.1%); they could not demonstrate even one case of brain metastases from GTN. Zimm et al. [59] in 1981 reviewed 191 patients with brain metastases and did not observe cases of brain metastases from GTN. LeChevalier et al. [60] in 1985 reviewed 31 women with brain metastases from various primary tumors and did not find cases of brain metastases from GTN. Nussbaum et al. [61] in 1996 reviewed 729 patients with brain metastases and did not specify whether there were cases of brain metastases from GTN. Lagerwaard et al. [62] in 1999 surveyed 1,292 patients with brain metastases and observed that lung carcinoma was source of brain metastases in 721 (55.8%), breast: 213 (16.5%), kidney: 48 (3.7%), other sources (not specified): 208 (16.1%), and unknown: 102 (7.9%). The authors [62] did not specify whether there were cases of brain metastases from GTN. Apparently, since metastatic GTN is a rare disease entity, its presentation in the general population of patients with brain metastases is extremely rare.

Incidence of brain metastases in patients with GTN

Kobayashi et al. [63] in 1982 surveyed 87 patients with histologically-verified choriocarcinoma treated in their institution during 1965 - 1977 and found that 24 (27.6%) developed brain metastases. Thirty-three (38%) of the 87 patients died of choriocarcinoma; all 33 patients underwent autopsy and brain metastasis was confirmed in 22 (66.7%) of the autopsies. Notably, the lung and brain were the most common sites of metastatic choriocarcinoma followed by the liver, kidney, spleen, pelvis, bowel, and urinary bladder [63]. Ishizuka et al. [17] in 1983 surveyed 168 patients with choriocarcinoma treated at their institution over a 24-year period (1957 – 1980) and found that brain metastases
developed in 36 (21.4%) patients. A comparative study of the differences in the rate of developing brain metastases from GTN before and after the introduction of chemotherapy with actinomycin-D (Act D) in 1965 revealed a remarkable decrease in the development of brain metastases. Before 1964, the incidence of brain metastases in choriocarcinoma patients was 37.5% (9/24 patients), and after 1965 the incidence dropped to 18.8% (27/144 patients) ($p < 0.05$) [17]. Athanassiou et al. [64] in 1983 surveyed 782 patients who had chemotherapy for GTN at their institution during 1957 – 1981 and revealed that 69 (8.8%) had brain metastases; 33/69 (48%) presented with brain metastases prior to chemotherapy, and 36/69 (52%) developed brain metastases while on chemotherapy or relapsed in the brain after an initial complete or partial remission. The periods 1957 – 1973 and 1974 – 1980 were compared. During 1957 – 1973, 42/367 (11.4%) patients with GTN had brain metastases; 20 (5.4%) presented with brain metastases prior to chemotherapy, and 22 (6%) developed brain metastases while on chemotherapy or relapsed in the brain after an initial complete or partial remission. During 1974 – 1980, 26/402 (6.4%) patients with GTN had brain metastases; 13 (3.2%) presented with brain metastases prior to chemotherapy and 13 (3.2%) developed brain metastases while on chemotherapy or relapsed in the brain after an initial complete or partial remission [64]. Kikuchi et al. [30] in 1990 stated that the frequency of cerebral metastases in choriocarcinoma patients varied from 7% to 22%, with an average prevalence at diagnosis of 20%. Evans et al. [34] in 1995 reviewed 454 patients with metastatic GTN treated at their institution during 1966 – 1992 and identified 42 (9.3%) patients with brain metastases; 16 patients presented with brain metastases before primary therapy and 27 patients received significant therapy prior to presentation with brain metastases. In summary, 171 (11.4%) of 1,491 women with metastatic GTN collated from large series in the literature had brain metastases from GTN (Table 1) [17, 34, 63, 64].

### Table 1. — Incidence of brain metastases in patients with GTN.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study period</th>
<th>No. of women with GTN</th>
<th>No. of women with BM</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kobayashi et al.</td>
<td>1965 – 1977</td>
<td>87 (33/87 patients died of disease and had autopsy)</td>
<td>24 (BM was found in 22/33 autopsies)</td>
<td>27.6 (at autopsy: 66.7)</td>
</tr>
<tr>
<td>Ishizuka et al.</td>
<td>1957 – 1980</td>
<td>168</td>
<td>36</td>
<td>21.4</td>
</tr>
<tr>
<td>Athanassiou et al.</td>
<td>1957 – 1981</td>
<td>782</td>
<td>69</td>
<td>8.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>1,491</strong></td>
<td><strong>171</strong></td>
<td><strong>11.4</strong></td>
</tr>
</tbody>
</table>

BM: brain metastases.

**Type of antecedent pregnancy**

Type of antecedent pregnancy was available in 170/222 (76.5%) patients and was hydatidiform mole in 72 (42.3%), term pregnancy in 56 (32.9%), miscarriage in 31 (18.2%), stillbirth in seven (4.1%), artificial abortion in two (1.2%), invasive mole in one (0.6%), and tubal pregnancy in one (0.6%) (Table 2). Thus, hydatidiform mole was the most common type of antecedent pregnancy.

**Interval from antecedent pregnancy to diagnosis of metastatic GTN**

The interval from antecedent pregnancy to diagnosis of metastatic GTN was available in 61/222 (27.4%) patients and ranged from 0 to 240 months (median, 11 months) (Table 2). In 14/61 (23%) patients, metastatic GTN was diagnosed ≥ four months from index pregnancy (in six of the 14 patients, metastatic GTN, and the index pregnancy were diagnosed synchronously). In 12/61 (19.7%) patients, metastatic GTN was diagnosed seven to <seven months from index pregnancy. In 9/61 (14.7%) patients, metastatic GTN was diagnosed 7 months to <13 months from index pregnancy. In 26/61 (42.6%) metastatic GTN was diagnosed 13 months from index pregnancy. Thus, metastatic GTN was most commonly diagnosed after more than one year from antecedent pregnancy.

**Interval from diagnosis of metastatic GTN to diagnosis of brain metastases**

The interval from diagnosis of metastatic GTN to diagnosis of brain metastases was available in 124/222 (55.8%) patients and ranged from 0 to 60 months (median, one month) (Table 2). In 14/124 (11.3%) patients, brain metastases from GTN was first and only manifestation of metastatic GTN. In 38/124 (30.6%) patients, brain metastases appeared synchronously with metastatic GTN in other sites of the body. The other sites were: lung – 20, lung and pelvis – four, lung and liver – two, lung, liver and bowel – two, lung and bowel – one, lung and neck – one, lung and kidney – one, lung and vertebra – one, lung, liver, spleen, and kidney – one, lung, spleen, kidney and skin – one, bowel – one, liver and bowel – one, liver and pelvis – one, mediastial and mesenterial lymph nodes – one. In 72/124 (58.1%) patients, brain metastasis was diagnosed from 0.3 to 60 months after diagnosis of metastatic GTN in other
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Age (yrs)</th>
<th>Type of AP</th>
<th>Time from AP to GTN (mon)</th>
<th>Surgery of uterine tumor</th>
<th>Time from GTN to BM (months)</th>
<th>Other sites of metastases</th>
<th>Treatment of BM</th>
<th>Survival after BM (mon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnes 1982 [22]</td>
<td>17</td>
<td>SB</td>
<td>0</td>
<td>No</td>
<td>0</td>
<td>Lung</td>
<td>Craniotomy, Act D → MAC, WBRT</td>
<td>D 90</td>
</tr>
<tr>
<td>Ishizuka 1983 [17]</td>
<td>NR</td>
<td>16 mole 1 term 5 misc. 1 TP 3 term 1 SB</td>
<td>1 – 240 (15)</td>
<td>20 hyster. 7 no</td>
<td>0 – 47 (8)</td>
<td>27 Lung</td>
<td>17 Act D 5 Act D, craniotomy 3 craniotomy, Act D 2 Act D, shunt</td>
<td>26 D 0.1 – 50 (1.6) 1 AW 54</td>
</tr>
<tr>
<td>Liu 1983 [23]</td>
<td>30 – 50 (33.7)</td>
<td>NR</td>
<td>NR</td>
<td>Some had hyster.</td>
<td>0.3-1</td>
<td>Lung, Vagina</td>
<td>6-MP, MTX, Act D, 5-FU</td>
<td>7 AW 12-120</td>
</tr>
<tr>
<td>van den Doel 1985 [24]</td>
<td>42</td>
<td>Term</td>
<td>NR</td>
<td>Hyst. 2 yrs before</td>
<td>0</td>
<td>Mediastinal and mesenterial lymph nodes</td>
<td>Craniotomy x 2 to evacuate hematoma</td>
<td>D 1</td>
</tr>
<tr>
<td>Momma 1986 [25]</td>
<td>29</td>
<td>Mole</td>
<td>20</td>
<td>NR</td>
<td>0</td>
<td>Bowel, liver</td>
<td>Craniotomy for clot evacuation and OA resection, shunt</td>
<td>D 1</td>
</tr>
<tr>
<td>Illancheran 1988 [26]</td>
<td>26 – 30</td>
<td>1 mole 1 term 2 misc.</td>
<td>5 – 21</td>
<td>2 no</td>
<td>0</td>
<td>1 Lung 1 No</td>
<td>2 Craniotomy, MTX + Act D</td>
<td>2 AW 39 – 108</td>
</tr>
<tr>
<td>Mates 1988 [28]</td>
<td>17 – 26</td>
<td>1 mole 1 term</td>
<td>4 – 5</td>
<td>2 no</td>
<td>0</td>
<td>1 No 1 lung, liver, b</td>
<td>1 Craniotomy to evacuate hematoma - 1 MTX, Act D</td>
<td>2 D 0.1 – 0.5</td>
</tr>
<tr>
<td>Rustin 1989 [29]</td>
<td>25 pts. treated with EMA-CO</td>
<td>9 mole 8 term 6 misc. 2 SB</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>23 lung 2 no</td>
<td>25 EMA-CO. Part of pts. had also craniotomy and/or WBRT</td>
<td>15 pts. had a CR to EMA-CO lasting 4 – 74 (33) months</td>
</tr>
<tr>
<td>Kikuchi 1990 [30]</td>
<td>36 – 37</td>
<td>2 mole 1 term</td>
<td>36 – 45</td>
<td>2 NR</td>
<td>0</td>
<td>1 lung, 1 bowel</td>
<td>2 Craniotomy, MAC, WBRT</td>
<td>1 AW 60, 1 AD 9</td>
</tr>
<tr>
<td>Jones 1990 [31]</td>
<td>19 – 54 (32)</td>
<td>9 mole 5 term 2 misc. 1 SB 1 inv. M 1 NR</td>
<td>NR</td>
<td>6 hyster. 1 hyster-o 4 D&amp;C 1 SA 7 NR</td>
<td>0 – 60 (11)</td>
<td>8 lung, liver 4 lung 3 lung, liver, b. 1 lung, neck 1 lung, mediast. 1 lung, liver, sp. 1 No</td>
<td>16 MAC, WBRT 1 Act D, WBRT 2 MAC, WBRT, Cran.</td>
<td>14 D 0.1 – 24 (5.5) 5 AW 48 – 180 (96)</td>
</tr>
<tr>
<td>Wilkinson 1991 [32]</td>
<td>36</td>
<td>Term</td>
<td>12</td>
<td>NR</td>
<td>NR</td>
<td>Liver, pelvis, heart</td>
<td>Craniotomy, C</td>
<td>D 1</td>
</tr>
<tr>
<td>Giannakopoulos 1992 [33]</td>
<td>30</td>
<td>Term</td>
<td>24</td>
<td>No</td>
<td>0</td>
<td>Lung, kidney</td>
<td>Craniotomy to evacuate hematoma</td>
<td>D 0.5</td>
</tr>
<tr>
<td>Evans 1995 [34]</td>
<td>16 mole 15 term 8 Misc.</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Leslie 1996 [35]</td>
<td>26</td>
<td>Term</td>
<td>0</td>
<td>CS</td>
<td>18</td>
<td>Lung</td>
<td>EMA-CO, SRS → EMA-CO, intrathecal MTX</td>
<td>AW 24+</td>
</tr>
<tr>
<td>Schechter 1998 [36]</td>
<td>19 – 54 (35)</td>
<td>10 mole 5 misc. 3 term 1 SB 1 NR 1 none</td>
<td>NA</td>
<td>8 D&amp;C 4 hist. 1 his-o 2 SA 4 No 2 NR</td>
<td>21 NR</td>
<td>21 NR</td>
<td>All patients: WBRT Most patients: MTX and Act D-based chemotheraphy. Some patients: EMA-CO.</td>
<td>13 D 0.1 – 44 (8) 7 AW 15 – 170 (77) 1 AD 11</td>
</tr>
<tr>
<td>Nozue 2000 [37]</td>
<td>25</td>
<td>Term</td>
<td>5</td>
<td>No</td>
<td>0</td>
<td>Lung</td>
<td>EMA-CO</td>
<td>AW 10+</td>
</tr>
<tr>
<td>Suresh 2001 [38]</td>
<td>17-35</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>10 no</td>
<td>Craniotomy to evacuate hemorrhage, 6 pts. referred to chemotherapy</td>
<td>6 AD NA 4 D 1</td>
</tr>
<tr>
<td>Mamelak 2002 [39]</td>
<td>27</td>
<td>Term</td>
<td>0</td>
<td>CS</td>
<td>0</td>
<td>No</td>
<td>Delivery at 30W and then craniotomy, EMA-CO, WBRT</td>
<td>AW 12+</td>
</tr>
<tr>
<td>Balagopal 2003 [40]</td>
<td>24</td>
<td>Mole</td>
<td>96</td>
<td>NR</td>
<td>0</td>
<td>Bowel, lung</td>
<td>EMA-CO → BEP</td>
<td>Lost</td>
</tr>
</tbody>
</table>
sites, most often in the lung. Overall, 104/124 (83.9%) patients with brain metastases from GTN had also lung metastases from GTN.

**Type, amount and site of brain metastases from GTN**

Type of brain metastases of GTN with respect to whether the metastasis is confined to the brain only (isolated brain metastases) or is part of a disseminated disease affecting also other parts of the body was available in 157/222 (70.7%) patients (Table 2). Brain metastasis was an isolated disease confined to the brain in 16/157 (10.2%) patients whereas brain metastasis was part of a disseminated disease in 141/157 (89.8%) patients. Amount of brain metastases with respect to whether the metastasis is single (solitary) brain metastases or multiple brain metastases was available in 46/222 (20.7%) patients. Brain metastasis was single brain metastases (one metastases) in 37/46 (80.4%) patients whereas brain metastasis was multiple brain metastases (two or more metastases) in 9/46 (19.6%) patients. Site of metastasis in the brain with respect to whether the metastasis is supratentorial (cerebrum) or infratentorial (cerebellum) or both was available in 78/222 (35.1%) patients. Brain metastasis was located in the cerebrum in 69/78 (88.5%) patients, cerebellum in 5/78 (6.4%) patients, and both cerebrum and cerebellum in 4/78 (5.1%) patients. Thus, brain metastasis from GTN is part of a disseminated disease in ~90% of patients, single metastases in the brain in ~80% of patients and supratentorial in ~90% of patients.

**Symptoms and signs of brain metastases from GTN**

Symptoms and signs of brain metastases from GTN are not different from symptoms and signs of other space occupying lesions of the brain. Common presenting symptoms and signs of brain metastases from GTN include headache, confusion, dizziness, decreased mental status, consciousness disturbance, general weakness, extremity weakness, gait disturbance, neurological motor deficit, hemiparesis, hemiplegia, ataxia, visual disturbance, incontinence, nausea, vomiting, speech impairment (aphasia), parasthesias, syncope, seizure, and raised intracranial pressure manifested by papilledema. Nevertheless, brain metastases from GTN are characterized by their greater tendency to be hemorrhagic and associated with brain oncocytic aneurism and intracranial hemorrhage. In a series of 34 pa-
tients with brain metastases from GTN reported by Liu et al. [23], 18 patients presented with headache, ten: hemiparesis, seven: vomiting, six: dizziness, five: blurred vision, three: nausea, two: depression, two: insomnia, and two: seizure (the number of patients sums up to more than 34 since some patients had more than one symptom). In 19 patients with brain metastases of GTN reported by Jones et al. [31], headache, seizure, and dizziness were the most common symptoms. In ten patients with brain metastases from GTN reported by Suresh et al. [38], sudden onset of headache, vomiting, and convulsion were the most frequent initial symptoms (seven patients) followed by hemiparesis and sensory disturbances (three patients). In the majority of patients (8/10), the brain lesions seen at cranial CT scan were interpreted as hemorrhagic masses [38]. Zairi et al. [16] in 2011 reported two patients in whom intracranial hemorrhage due to ruptured oncocytic aneurysm was the first manifestation of metastatic GTN. It has been concluded that metastatic GTN must be considered in the differential diagnosis of any intracranial hemorrhage in women of childbearing age.

**Treatment of brain metastases from GTN**

Data with respect to treatment modality of brain metastases of GTN was available in 175/222 (78.8%) patients collated from literature (Table 2). Of the 175 patients, 140 (80%) had chemotherapy, 56 (32%) had WBRT, 42 (24%) had craniotomy, four (2.3%) had SRS, two (1.1%) had a shunt, and one (0.6%) had no treatment (number of patients adds up to more than 175 since some patients received more than one treatment modality). The most common unimodal treatment was chemotherapy and the most common multimodal treatment was craniotomy in combination with other treatments such as chemotherapy, WBRT, and SRS. Noteworthy, of the 42 craniotomies performed in patients with brain metastases from GTN, 30 (71.4%) craniotomies were performed for resection of brain metastases and 12 (28.6%) were performed for evacuation of intracranial hematoma and resection of brain oncocytic aneurism.

Because of the rarity of metastatic GTN, the accrual of patients with brain metastases from GTN occurred over prolonged periods of time during which treatment approaches and modalities changed. In presence of brain metastases the GTN is allocated Stage IV and if untreated may be rapidly fatal. Over the years, the multi-agent chemotherapy regimen of choice for patients with high-risk metastatic GTN (including brain metastases) changed. In the 1970s and 1980s, the combination of methotrexate, actinomycin D, and cyclophosphamide, and vincristine (EMA-CO) resulted in improved remission and survival rates of 80 - 90% [2]. Of the 140 patients who had chemo-therapy for brain metastases from GTN collated from literature (Table 2), the vast majority of patients treated before 1990 had actinomycin D- and methotrexate-based chemotherapy such as MAC, whereas almost all patients treated after 1990 had etoposide-based multi-agent combination chemotherapy, mainly EMA-CO or EMA-EP. At least one patient had also intrathecal chemotherapy with methotrexate [35].

**Survival after diagnosis of brain metastases from GTN**

Data with respect to patient status (alive without disease or alive with disease or dead) at the end of follow-up was available for 171/222 (77.3%) patients with brain metastases from GTN documented in the literature (Table 2). Of these 171 patients, 57 (33.3%) were alive without disease at follow-up of two to 192 months, nine (5.2%) were alive with disease at follow-up of four to 11 months, and 105 (61.4%) died of disease from 0.1 to 90 months (median, one month) after diagnosis of brain metastases. Overall, the survival time until the end of follow-up or death in these 171 patients ranged from 0.1 to 192 months (median, 12 months).

The survival after diagnosis of brain metastases from GTN according to mode of therapy of brain metastases was assessed in details in series of more than three patients. Of 27 patients with brain metastases from GTN reported in 1983 by Ishizuka et al. [17], 17 (62.9%) had chemotherapy with single-agent actinomycin D (dactinomycin) alone (all died of disease from 0.1 to 46 months [median, 0.9 month] after diagnosis of brain metastases), five (18.5%) had chemotherapy with single-agent actinomycin D followed by craniotomy (all died of disease from 1.2 to 50 months [median, four months] after diagnosis of brain metastases), three (11.1%) had craniotomy followed by chemotherapy with single-agent actinomycin D (two died of disease eight and 18 months, respectively, after diagnosis of brain metastases and one was alive without disease at follow-up of 54 months) and two (7.4%) had chemotherapy with single-agent actinomycin D followed by shunt (both died of disease 0.8 and 21 months, respectively, after diagnosis of brain metastases). Overall, 26 patients died of disease from 0.1 to 50 months (median, 1.6 months) after diagnosis of brain metastases and one patient was alive without disease four months after diagnosis of brain metastases [17]. Liu et al. [23] in 1983 reported 34 patients who had chemotherapy (6-MP, MTX, Act D, 5-FU, some had also Chinese herbs) for brain metastases from GTN; seven (20.6%) patients were alive and well at follow-up of 12 to 120 months and 27 (79.4%) patients died of disease from less than one to five months (median, < one month) after diagnosis of brain metastases. Rustin et al. [29] in 1989 reported 25 patients who had EMA-CO multi-drug chemotherapy (part of patients had also craniotomy and/or WBRT) for brain
metastases from GTN; 15 patients had a complete response to EMA-CO lasting from 33 to 74 (median, 33) months. Of note, 18 of the 25 patients presented with brain metastases before EMA-CO whereas seven developed brain metastases on or after EMA-CO [29]. Jones et al. [31] in 1990 reported 18 patients who had MAC and one patient who had actinomycin-D chemotherapy followed by WBRT (two patients had also craniotomy) for brain metastases from GTN; 14 patients died of disease from 0.1 to 24 (median, 5.5) months after diagnosis of brain metastases and five patients were alive without disease at follow-up of 48 to 180 (median, 96) months. Overall, median survival time until end of follow-up or death was seven months (range, 0.1 to 180 months) [31]. Schechter et al. [36] in 1998 reported 21 patients with brain metastases from GTN who had WBRT (most patients had also MTX and Act D-based chemotherapy); 13 patients died of disease from 0.1 to 44 (median, eight) months after diagnosis of brain metastases, seven patients were alive without disease at follow-up of 15 to 170 (median, 77) months, and one patient was alive with disease at follow-up of 11 months. Suresh et al. [38] in 2001 reported ten patients with brain metastases from GTN who had craniotomy to evacuate brain hemorrhage (six patients were also referred to chemotherapy); six patients were alive with disease at follow-up of unknown duration and four patients died of disease within one month after diagnosis of brain metastases. Ghaemmaghami et al. [42] in 2004 reported nine patients with brain metastases from GTN who had EMA-EP (eight patients) or EMA-CO (one patient) multi-agent chemotherapy followed by WBRT; five patients were alive without disease and four patients died of disease at follow-up of nine to 50 (median, 24) months. Soper et al. [45] in 2007 documented four patients with brain metastases from GTN; three had craniotomy followed by EMA-EP multi-agent chemotherapy and were alive without disease at follow-up of 12, 18, and 24 months (median, 18), respectively, after diagnosis of brain metastases and one patient had brain stereotactic radiosurgery (SRS) followed by EMA-CO and was alive without disease at follow-up of 15 months.

Survival according to mode of therapy of brain metastases in all patients collated from literature (case reports and series of patients) in whom data with respect to mode of therapy and survival was available is displayed in Table 3. The evolution of chemotherapy for high-risk metastatic GTN over the years from single-agent chemotherapy (such as methotrexate or actinomycin-D) through multidrug chemotherapy composed of MAC and eventually multidrug chemotherapy composed of EP-EMA or EMA-CO has improved the outcome of patients with high-risk metastatic GTN including patients with brain metastases from GTN. Thus, it seems that in patients in whom it is feasible, the best results may be achieved with multimodal therapy including craniotomy, WBRT, and EP-EMA or EMA-CO chemotherapy.

**Conclusion**

Brain metastasis from GTN is rare with about 222 cases documented in the literature and an estimated incidence of about 11% in living GTN patients. Age at diagnosis of brain metastases from GTN ranged from 17 to 54 years (median, 34 years). Antecedent pregnancy before GTN was hydatidiform mole in 42.3% of patients, term pregnancy: 32.9%, miscarriage: 18.2%, stillbirth: 4.1%, termination of pregnancy: 1.2%, invasive mole: 0.6% and tubal pregnancy: 0.6%. Time from antecedent pregnancy to GTN ranged from 0 to 240 months (median, 11 months). Time from

<table>
<thead>
<tr>
<th>Mode of therapy</th>
<th>Survival range (months)</th>
<th>Median (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-agent chemotherapy: actinomycin D or methotrexate (either alone or combined with other treatment modality/ies)</td>
<td>0.1 – 170</td>
<td>2</td>
</tr>
<tr>
<td>Multidrug chemotherapy: MAC (either alone or combined with other treatment modality/ies)</td>
<td>0.1 – 180</td>
<td>8.5</td>
</tr>
<tr>
<td>Multidrug chemotherapy: EP-EMA or EMA-CO (either alone or combined with other treatment modality/ies)</td>
<td>0.1 – 192</td>
<td>26.5</td>
</tr>
<tr>
<td>Bimodal therapy: EP-EMA or EMA-CO chemotherapy and WBRT</td>
<td>4 – 50</td>
<td>24</td>
</tr>
<tr>
<td>Bimodal therapy: EP-EMA or EMA-CO chemotherapy and craniotomy</td>
<td>1 – 72</td>
<td>12</td>
</tr>
<tr>
<td>Triple modal therapy: craniotomy, chemotherapy and WBRT</td>
<td>4 – 90</td>
<td>34</td>
</tr>
<tr>
<td>Craniotomy (either alone or combined with other treatment modality/ies)</td>
<td>0.1 – 108</td>
<td>12</td>
</tr>
<tr>
<td>WBRT (either alone or combined with other treatment modality/ies)</td>
<td>0.1 – 180</td>
<td>24</td>
</tr>
<tr>
<td>SRS (either alone or combined with other treatment modality/ies)</td>
<td>1 – 24</td>
<td>13.5</td>
</tr>
<tr>
<td>Shunt (either alone or combined with other treatment modality/ies)</td>
<td>0.8 – 21</td>
<td>1</td>
</tr>
<tr>
<td>Craniotomy alone to evacuate an intracranial hematoma</td>
<td>0.1 – 1</td>
<td>0.3</td>
</tr>
<tr>
<td>No treatment</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>All modes of therapy</td>
<td>0.1 – 192</td>
<td>12</td>
</tr>
</tbody>
</table>

WBRT: whole brain radiotherapy, SRS: stereotactic radiosurgery.
GTN to diagnosis of brain metastases ranged from 0 to 60 months (median, one month). Brain metastasis from GTN was part of a disseminated disease in ~90% of patients, single metastases in the brain in ~80% of patients, and located in the cerebrum in ~90% of patients. Brain metastasis was first and only manifestation of metastatic GTN in 11.3% of the patients, appeared synchronously with metastatic GTN in other sites of the body in 30.6%, and was diagnosed from 0.3 to 60 months after diagnosis of metastatic GTN in other sites, most often in the lung, in 58.1%. Overall, 83.9% of patients with brain metastases from GTN had also lung metastases from GTN. Symptoms and signs of brain metastases from GTN are not different from symptoms and signs of other space occupying lesions of the brain. Nevertheless, brain metastases from GTN are characterized by their greater tendency to be hemorrhagic and associated with brain oncotic aneurism and intracranial hemorrhage. Chemotherapy for high-risk metastatic GTN has evolved over the years from single-agent chemotherapy to multidrug chemotherapy composed of EP-EMA or EMA-CO. It seems that in patients with brain metastases from GTN, the best outcome may be achieved with multimodal therapy including craniotomy, WBRT, and EP-EMA or EMA-CO chemotherapy. Nonetheless, brain metastasis from GTN is a grave disease with a median survival time overall from diagnosis of brain metastasis of only about 12 months. Current knowledge of brain metastases from GTN is based on the experience of authors who reported series of patients or singular cases. Because of the rarity of brain metastases from GTN, patient accrual occurred over prolonged periods during which treatment approaches and modalities changed. Consequently, very few individuals or even referral centers can build up an adequate experience of handing this disease and, thus, the understanding of the variable biologic behavior and treatment alternatives of brain metastases from GTN is still limited. Hence, the reporting of further cases of brain metastases from GTN should be encouraged since analysis of information from even small series or singular cases may form an important source of knowledge for future research and treatment recommendations.

References


Brain metastases from gestational trophoblastic neoplasia: review of pertinent literature

367
The importance of alpha/beta (α/β) interferon receptors and signaling pathways for the treatment of cervical intraepithelial neoplasias

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Summary

Introduction: Immunotherapies have been effective in treating various forms of cancer, including cervical intraepithelial neoplasias (CINs) predominantly caused by human papilloma virus (HPV). Development: To establish persistent infections in stratified epithelia, HPV induces proliferative lesions. Viral gene products are able to change gene expression and cellular proteins. Interferons (IFNs) are inducible glycoproteins that have immunomodulatory, antiviral, antiproliferative, and antiangiogenic effects. In particular, interferon-alpha (IFN-α) has been shown to inhibit the development and progression of cervical cancer. In this review, actions of interferons α/beta (α/β), including their receptors and signaling pathways, are described, as well as their clinical importance in the immune response against cervical lesions. Conclusion: The interaction of IFN-α/β with its receptor results in a series of phosphorylation events. These mechanisms can be ineffective in IFN response, then it can also compromise the therapeutic effects of immunotherapy.

Key words: Type I Interferons; Interferon receptors; Cervical neoplasia.

Introduction

Human papilloma virus (HPV) has a great affinity for squamous epithelial cells. As a result, HPV has been associated with the formation of cervical lesions, referred to as cervical intraepithelial neoplasias (CINs). These lesions are characterized by the formation of an acetowhite epithelium in the cervix [1], and are classified as slight, moderate, or severe neoplasias - CIN I, II, and III, respectively. As these lesions are considered the precursors of cervical cancer, their early detection, and subsequent intervention, can potentially prevent tumor development [2].

HPV establishes persistent infections in stratified epithelial cells by inducing proliferative lesions and maintaining a low number of epimal copies in infected basal cells. Viral gene products have been shown to interfere with the expression of native genes and cell proteins, such as Rb and p53, thereby altering cell cycle progression. Persistent infections are induced by high risk HPV, which are also associated with high oncological risk due to their ability to escape an immune response. For example, HPV types 16, 18, and 31 are able to block interferon-stimulated gene (ISG) expression and compromise the antiviral function of cytokines involved in an immune response, including the function of interferons (IFNs) [3].

Interferon-alpha/beta (α/β) has been shown to play a key role in mediating both innate and adaptive immune responses. Furthermore, there are the possibility of IFNs in the clinical treatment of cancer, particularly for neoplasias that are a precursor to cervical cancer. However, in order for interferon-α/β to be used effectively as an immunotherapy, the components of its signaling pathways need to be expressed and functionally characterised in target cells.

Therefore, the goal of this review is to present current evidence regarding the actions of IFN-α/β and its associated signaling pathways, and their role in the immune response against cervical intraepithelial lesions. In addition, the clinical importance of treating these lesions is highlighted, to elucidate the systemic response to endogenous IFN-α, for further studies on the efficacy of treatment with IFN-α in these patients are necessary.

The importance of IFNs in CINs

In Table 1, the expression, functions, and receptors associated with the three main types of IFNs are presented. For type I IFNs, their direct antiviral and antitumor properties are complemented by their role in immunovigilance [4]. For example, type 1 IFNs (e.g., IFN-c/β) induce the expression of hundreds of ISGs which regulate antiviral effects, cell growth, and affect immunomodulation. Correspondingly, when epithelial cells are infected with HPV, production of IFN-α/β is induced, and this can lead to an inhibition of viral replication, the induction of cytotoxic...
and antiproliferative functions, and negative regulation of angiogenesis [4]. However, the viral proteins, E6 and E7, can block downstream targets of IFN-α/β, thereby providing a mechanism for evasion of the immune system. Moreover, when HPV episomes are lost, proliferation of cells that have the virus integrated into their genome is enhanced [5].

While characterizing the signaling pathways activated by IFN-α, including those involving gene expression, a family of transcription factors was discovered that links cell surface receptors with nuclear events. These proteins were referred to as, signal transducers and activators of transcription (STAT), and they were found to localise outside the nucleus; however, following stimulation with IFN, they are activated by tyrosine phosphorylation, undergo multimerization, and migrate to the nucleus where they recognize regulatory sequences in DNA [6]. Using mouse models, genetic experiments have been performed to define the crucial roles that each STAT has in mammals, thereby characterizing this family of latent cytoplasmic proteins that upon activation, affect gene expression in response to extracellular polypeptides [7].

The interaction of IFN-α/β with its receptor results in a series of phosphorylation events. First, binding of the tyrosine kinases, TYK2 and JAK1, occurs, followed by their binding of the IFN I receptors, IFNAR1 and IFNAR2. The receptors are then activated by phosphorylation of tyrosine residues present in the intracellular subunits of each receptor, and these phosphorylated sites become binding sites for phosphorylate STATs. STAT1 binds STAT2 to form a heterodimer that subsequently binds a regulatory factor of IFN (IRF) to form interferon-stimulated gene factor 3 (ISGF3). ISGF3 then localizes to the nucleus and promotes the transcription of specific genes, interferon stimulation response element (ISRE) which participate in IFN stimulation events. This signaling pathway of type I IFN activation is referred to as the JAK-STAT pathway [8,9] (Figure 1).

### Table 1. — The three main types of IFNs and their characteristics.

<table>
<thead>
<tr>
<th>IFN type</th>
<th>Main subtypes of IFNs</th>
<th>Expressed by:</th>
<th>Primary Functions</th>
<th>Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IFN-α</td>
<td>Leukocytes</td>
<td>- Mainly acts on innate immunity</td>
<td>IFNAR1</td>
</tr>
<tr>
<td></td>
<td>IFN-β</td>
<td>Fibroblasts</td>
<td>- Predominantly mediates antiviral activities</td>
<td>IFNAR2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cells infected by virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>IFN-γ</td>
<td>Activated T cells</td>
<td>- Acts with IL-12 on acquired immunity to differentiate into Th1 cells</td>
<td>IFNGR1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Exhibits antiviral and antitumor activity via activation of macrophages to eliminate pathogens via phagocytosis</td>
<td>IFNGR2</td>
</tr>
<tr>
<td>III</td>
<td>IFN-λ1</td>
<td>Various cell lineages, except for non-infected cells</td>
<td>- Induction of antiviral protection</td>
<td>IL28Rα</td>
</tr>
<tr>
<td></td>
<td>IFN-λ2</td>
<td></td>
<td>- Augments expression of MHC I</td>
<td>IL10R2</td>
</tr>
<tr>
<td></td>
<td>IFN-λ3</td>
<td></td>
<td>- Its action mechanisms remains to be fully characterized</td>
<td></td>
</tr>
</tbody>
</table>

Sources: Hsieh et al. [31]; Müller et al. [32]; Kotenko et al. [33].

### Type I interferon receptors

IFN-α/β receptors are made up of two subunits, IFNAR1 and IFNAR2, with the latter having three distinct forms: IFNAR-2a (short form), IFNAR-2b (soluble form), and IFNAR-2c (long form). Both IFN-α and IFN-β bind the same receptors, and these receptors are expressed by many different types of cells [10, 11] (Table 1).

For type I IFNs to function, an initial binding event between these cytokines and their specific receptor must occur. Following binding, a chain of intracellular signaling events is induced which results in the responses mediated by IFNs [12]. However, variations in the concentration and the number of receptors expressed by a cell, can affect the intensity of the responses generated by the stimulation to IFN-α/β [13]. In addition, it has been suggested that when IFNAR1 is highly expressed on the cell surface, it undergoes phosphorylation, ubiquitination, and degradation via mechanisms independent of its binding by TYK2 [14]. While this mechanism can be effective in avoiding an excessive IFN response, it can also compromise the therapeutic effects of immunotherapy.

Over the years, the present research group has studied the applicability of IFN-α to the treatment of CINs, with significant clinical results. For example, in approximately 60% of patients who received immunotherapy and have changed a Th1 cytokine profile (e.g., IFN-γ, TNF-α, IL-2) in the stroma, a response to IFN-α treatment was observed [15], with a reduction in HPV load detected. In contrast, patients that did not respond to IFN-α were found to have a Th2 cytokine profile (e.g., IFN-γ, TNF-α, IL-2) in the stroma, a response to IFN-α treatment was observed [15], with a reduction in HPV load detected. In contrast, patients that did not respond to IFN-α were found to have a Th2 cytokine profile, IL-4, or Treg (e.g., transforming growth factor beta 2 and 3 - TGF-β2, TGF-β3). The latter group also had a history of smoking. In another study which evaluated vaginal secretion in patients with CIN treated with IFN, a reduced viral load and lower levels of the inflammatory cytokines, IL-6 and TNF-α, were detected concomitant with neoplasia regression [16].
When expression of receptors in patients with different grades of CIN were analysed versus a healthy control group, both lower local levels of IFN-α mRNA and reduced expression of IFN-α receptors were detected in the patients with CIN. Moreover, simultaneous expression of IFNAR1/IFNAR2 was not detected in the former group, yet was in the latter [17]. Taken together, these findings suggest that IFN-α immunotherapy can be ineffective if there is an insufficient number of receptors present on the cell surface, and may be represents a mechanism by which HPV and neoplastic cells can evade the immune response.

The presence of IFNR has been associated with an improved response to immunotherapies involving IFN-α. For example, patients with hepatocellular carcinoma who were treated with IFN-α were subsequently found to have a greater number of cells expressing the IFNAR-2 receptor, and this expression was proportional to the treatment response observed [18]. In addition, studies of pancreatic cell lines have demonstrated that expression of the IFNR receptors facilitates the apoptotic and antiproliferative effects of IFN-α/β, thereby disrupting the cell cycle of these the cell lines [19].

Activation of the JAK/STAT signaling pathways is an important regulatory mechanism through which host cells are able to inhibit viral infections provoked by a variety of viral RNA and DNAs. Moreover, the STAT proteins, 1, 2, and 3, are the central transcriptional activators of this pathway. HPV has been found to suppress the constitutive expression of STAT-1 at the transcriptional level, while levels of STAT-2, IRF-9, and STAT-3 remain unaffected [3, 20]. In addition, the HPV oncoproteins, E6 and E7, independently suppress the expression of STAT-1, which is necessary for amplification of the HPV genome and maintenance of its episomes. In combination, these results suggest that STAT1 has an important role in viral pathogenesis [3].
After an infection is established, production of IFN-α/β depends on activation of IRF-3 and IRF-7 via phosphorylation by TBK-1 and IKKe kinases, respectively. Upon phosphorylation, IRF-3 and IRF-7 dimerize, then undergo nuclear translocation to activate type I IFN promoter genes [4]. The transcriptional activity of IRF-7 is dependent on phosphorylation of its C-terminus, and expression of this multifunctional protein is restricted to B lymphocytes and dendritic cells. However, in other cell types, IRF-7 production can be induced by virus or IFN [21]. When toll-like receptors (TLRs) in endosomes recognize an accumulation of viral DNA, they activate signaling pathways that result in the phosphorylation of transcription factors and the induction of IFN genes as well. Figure 1 schematically represented the modes of activation for type I IFNs.

Final considerations

Based on the antiviral and antitumor potential of IFNs, various studies have explored their effects on the immune system, especially the ability of IFNs to enhance the treatment of many different types of cancer. For example, IFN-α/β has been shown to effectively inhibit tumor cell proliferation, induce cell apoptosis, and increase expression of main histocompatibility complex (MHC) class I molecules [23]. Furthermore, treatment with IFNs has yielded good results in the treatment of patients with neoplasias that are precursors of cervical cancer [15,16], vaginal cancer [24] and pancreatic cancer [19]. In addition, IFN has been shown to interfere with the viral transcription of HPV16 and HPV18 [25], partly by reducing the expression of E6 and E7 proteins in cells infected by HPV [26]. However, a deficiency, or absence of some of the elements involved in the JAK-STAT signaling pathway can result in a loss of responsiveness by IFN-α/β. For example, in recent studies using mouse models, deletion or inactivity of TYK2 did not impede the activation of JAK1, yet, phosphorylation of STATs 1 and 2 were affected both in vitro and in vivo. With the final step of signaling compromised, effector functions of IFN-α/β were disrupted [27]. Furthermore, STAT needs to be functional in order to achieve adequate gene transcription and signaling [28].

Changes or deformities in IFNAR-1/IFNAR-2, particularly an absence of one of the receptor chains, can also damage the JAK-STAT signaling pathway, and consequently, inhibit its effects [29]. For example, certain types of genetic polymorphisms in one of the subunits can prevent an adequate response from IFN-α/β. This has been demonstrated in chronic hepatitis B carriers where the presence of these changes has been implicated in the elimination of hepatitis B infection in its early stages, and this can also affect the long-term course of the infection [30].

Conclusions

Type I IFNs (IFN-α/β) have important biological functions, from development and activation of cells of the immune system to destroy tumor cells to the inhibition of viral replication. After the viral infection or activation of toll-like receptors (TLR), promotes IFN gene and enhances the production of responsive genes important for the development of an effective antiviral immune response [8]. Therefore, future studies of immunotherapies involving IFN-α/β should monitor the functionality of the elements involved in IFN-α/β signaling pathways in order to better understand the mechanisms involved in the immune response of patients with HPV and CINs. In addition, this would help identify the main obstacles for maintaining a treatment’s effectiveness.

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The importance of alpha/beta (α/β) interferon receptors and signaling pathways for the treatment of cervical intraepithelial neoplasias


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Fertility preservation in women with early stage cervical cancer.
Review of the literature

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Summary
Within the last decades, the percentage of diagnosed cervical cancer in women of reproductive age has increased. The possibility of diagnosing small cervical tumors (≤ two cm) in childbearing age, can be explained due to the fact that many women, are aware of the benefits of Pap smear or colposcopy examination. Many demand a more conservative policy to handle such lesions in order to have an uneventful pregnancy in the near future.

Key words: Cervical cancer; Trachelectomy; Fertility.

Introduction
Although it can be perceived that cancer is the disease of elderly women, a significant percentage reveals the opposite. Forty percent of women with diagnosed cervical cancer are in the reproductive age [1]. Nowadays, cervical cancer is considered to be the second most common malignancy in women in developing countries and the seventh in developed countries [2]. Twenty-two percent of the new diagnosed cases each year are indicated in women under the age of forty-five years.

In order to maintain life quality, women with cervical cancer, especially in early ages, focus on the preservation of fertility and by extension childbearing.

Worthy mentioning indeed, the frequency of diagnosed cervical cancer in women of reproductive age has become very conventional in the last two decades. The frequency of diagnosed cervical cancer in women of reproductive age has become very conventional in the last two decades. The increased diagnosis of small cervical tumors (≤ two cm) in childbearing age, even in nulliparous patients, can be explained due to the fact that many women, nowadays, are aware of the benefits of Pap smear or colposcopy examination and of the early symptoms of this disease. As a conclusion, many of them demand a more conservative treatment to handle such lesions.

In modern societies even more women are postponing maternity until their mid and late 30s, owed to social and personal reasons such as career achievements. This leads to a considerable number of women who will develop early invasive cancer of the cervix without fulfilling their personal thoughts of maternity. Therefore gynecologists should take under consideration the need of fertility sparing surgery in suitable cases.

In 1948, Franz Novak in Ljubljana apprehended a vaginal trachelectomy. The word trachelectomy has its roots in the ancient Greek idiom from the word “trachelos” meaning cervix [3]. Later in 1956, Aburel [4] circumscribed an abdominal approach with the removal of cervix. In the 1970s, however, Burghardt and Holzer [5] perceived that the removal of the corpus uteri was not indispensable in all cases of small early invasive cancer.

Dargent et al. [6] presented in 1994 more references for uterine conservation. Through the vaginal route they excised the cervix with the para-cervical and upper vaginal tissues and simultaneously a laparoscopic pelvic-node dissection was executed.

Shephert et al. [7] and Roy and Plant [8] amended this approach with felicitous outcomes. The abdominal approach in combination with a pelvic-node dissection was re-introduced in 2005 by Ungar et al. [9].

The traditional therapeutic choice for cervical cancer is either surgically with radical hysterectomy or with radiation. Neither of these options preserves the function of the utero-ovarian system, which is requisite for reproduction. As far as the more advanced stage disease is concerned, the management remains the same. On the contrary, for the early-staged cervical cancer it has been concluded in the past two decades, that fertility preservation can be achieved without compromising oncologic outcomes [10-15].

Staging of cervical cancer
The procedure of staging cervical cancer is performed under anaesthesia with accessional data from imaging techniques including chest radiography and an intravenous program computerized axial tomography (CT). In order to detect para-aortic lymph nodes abdominal and cervical scanning are widely used. This scanning offers a high specificity and low sensitivity [16].

In assessing pelvic nodes disease, magnetic resonance imaging (MRI) has demonstrated to be as precise as CT, and the results for

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primary tumours within the cervix with MRI are more accurate and concrete. Moreover it evaluates the parametral tumour spread by different imaging [17, 18] and also permits the assessment of pelvic lymph nodes [19, 20].

MRI imaging provides several benefits such as the measurement of the size of the tumour, the location and the distance from the isthmus, and the assessment of the endocervical canal and the uterine cavity. The available accuracy of the endovaginal coil with MRI imaging permits the acquisition of better definition of the tumour and the involvement of the cervix with the inner portion of the paracervical and paravaginal tissues [19].

A specific examination under anaesthesia and an updated patient’s history as far as the histology obtained from previous biopsies are required for the evaluation of the fertility sparing surgery. MRI assists in the selection of the patient by careful assessment of the cervix and the paracervical tissues [21] for the precise staging of the tumour [22].

The detection of pelvic lymph node metastases remains laborious and lymphangiography which was common for many years, has been abandoned. Some gynaecologists advocate the sentinel node detection [23, 24]. Immunocytochemistry [25] performs the detection of circulating tumours cells and micrometastases in sentinel nodes. Worth mentioning are the accurate results that the combining laparoscopy with lymphoscintigraphy offers in sentinel node mapping [26]. Furthermore, molecular imaging has been described and shown to have enthralling possibilities in the detection of micrometastases by the use of ultrasmall iron oxide particles [27].

Patients’ characteristics

The management of fertility sparing surgery must subsume a special group of patients carefully chosen and a well-informed background on them. The briefing about the preoperative examinations, the surgery, the postoperative complications, and especially about the risk of a premature delivery is indeed necessary. The patient must be aware of the risk that she will probably face in future pregnancies and about the need of alteration of her daily lifestyle activities [28-31].

The main criterion in all health centers remains the strong desire of fertility and maternity. The preservation of the uterus is controversial in patients who are not willing a future pregnancy and they ask for the preservation only for personal reasons [32, 33].

Surgical methods for fertility preservation

The primary lesion as well as the potential metastases must be involved in treatment for invasive cancer. The initial treatment varies, due to the extent of the disease and the diversity of the patients.

The most common management for early stage cancer is the definitive surgery. However, in terms of primary treatment radiation therapy may also be used. Both these management offers equal effectiveness in the treatment of early cervical malignancies. More advanced cases are usually treated with combination radio-chemotherapy [34].

The option of the fertility preservation depends on the staging of the cancer. Stage IA1 cervical cancer, which defined as an invasion of less than three-mm depth of stromal invasion and less than seven-mm horizontally, is considered as the earliest stage of cancer [35].

The treatment suitable for young women wishing to preserve fertility is a cone biopsy. Cone biopsy is considered as the most established method due to the accurate assessing of the cervical tumour. It can be performed either by cold knife or diathermy, or by large loop excision of the transformation zone or loop electrosurgical procedure.

The surgical guidelines can be given by the depth and the diameter of the lesion with a 3D measurement. The therapeutic choice for small, superficially invasive stages, Stage IA1 and even IAII, is considered to be cone biopsy. Therefore no further treatment is required. The margins of excision must be clear in both invasive and high-grade pre-invasive intraepithelial neoplasia.

A simple hysterectomy, namely the removal of the uterus and the cervix without the ovaries and the fallopian tubes, is mostly performed for this stage of cancer. However, if the patient desires to preserve fertility, she must be aware that the approach of cone biopsy is not the standard one.

During the cone biopsy an endocervical sampling is conducted. It is very useful that the surgery confirms the depth of the invasion (less than three mm of stromal invasion and less than seven mm horizontally) in order to dodge more extensive surgeries. The patient can be followed closely, only if the endocervical canal curettage sample is negative, as well as all the margins [36].

In order to preserve an ensuing pregnancy and avoid premature delivery, a cervical suture may be necessary, although it may increase the risk of cervical incompetence, depending on the risk of the internal orifice and on how high the excision has been carried out.

There are options for women with microinvasive disease, classified as IAII to IB1, willing to preserve fertility. Radical hysterectomy is the main therapeutic management for patients following lymph node dissection. A radical hysterectomy removes the uterus and the cervix as well as the surrounding parametrial tissue, due to the pattern of the spread of cervical cancer. It tends to extend to the sidewall of the pelvic horizontally [37].

Radical vaginal trachelectomy

Radical vaginal trachelectomy (RVT) with laparoscopic lymphadenectomy is an alternative procedure for the preservation of fertility. It has gained worldwide acceptance as a method of surgically treatment of small invasive cervical cancers.

This procedure has been first described in 1994 by Daniel-Dargent et al. [38]. Since then over 1,000 cases have been reported to undergo a RVT and over 250 live births have been reported in women treated with this procedure [39, 40]. The success of this procedure is due to the strict criteria for patient selection. It has been estimated that about half of all women diagnosed with cervical cancer under the age of 40 are eligible for RVT [41-43].

Candidates for RVT

RVT includes the distal resection of the cervix, paracervical tissue, and upper vagina as in a Schauta vaginal hysterectomy (Table 1). The procedure of the resection of the tissue is the same as the distal part of a Wertheim’s radical abdominal hysterectomy. Laparoscopically, a pelvic node dissection is performed.
Candidates suitable for RVT

1. Patients wishing to preserve their fertility,
2. Patients younger than 45 years,
3. Patients with cervical cancer Stage IA1, L1V0, IA2V0, IB1V0 according to FIGO. The tumour histological diagnosis should be referred as squamous cell or adenocarcinoma. Patients with neuroendocrine tumours are not suitable for RVT, because neuroendocrine tumours or small cell cancers of the cervix are more likely to be associated with lymph node metastases, lymphovascular space invasion (LVSI), and local and distant failure.
4. According to the MRI or colposcopy examination, cervical lesion less than two or 2.5 cm.

RVT was very beneficial in the 40% of patients who underwent this procedure, taking into consideration the above criteria. On the other hand, the probability of wrong patient’s selection for RVT is about 10-12%. In this regard, it is possible that the patient will be treated with a definitive radical hysterectomy or adjuvant treatment as radiotherapy, due to extensive endocervical margins or lymphatic involvement [44]. The Outcomes following RVT for early stage cervical cancer are shown in Table 2.

Pre-operative assessment

Preoperative assessment must take into consideration several criteria, as the size of the tumour, the exact location and the distance of the isthmus and therefore the upper endometrial canal, as well as the probability of obtaining at least one cm of normal tissue surrounding the residual carcinoma.

An MRI in patients with pelvic mass can be useful [50], due to the importance of the accurate size and location of the mass, the severity of the endocervical involvement, the length of the cervical canal, and the distance between superior edges of the mass to isthmus [51].

The adequate clearance of the normal cervical stroma beyond the tumour is given by ensuring the exact length that the cervix is being resected under this procedure.

Technique of RVT

1. In the beginning a laparoscopic pelvic lymph node dissection takes place [52, 53]. The pelvic side walls are exposed, external, internal, lower common iliac, and obturator lymph nodes are taken. Before proceeding to the vaginal part of the surgery, any suspicious nodes are collected and sent for a frozen section analysis. Infiltration of the paracervical and vaginal tissues using a 0.25% marcaine with one in 200,000 adrenaline is performed.
2. Preparatory phase: a two-cm cuff of the vagina is outlined with cutting diathermy.
3. Anterior phase: due to find dissection of cervix without any injury to bladder, vesicovaginal space in anterior of cervix and also paracervical spaces in both lateral sides determine the bladder pillars and ureters.
4. Posterior phase: determination of the pararectal space in order to clamp and cut sacral part and the ligament.
5. Lateral phase: assessing the ureters, the proximal part of parametria in the level of the isthmus is removed. The importance of the main uterus artery is salvaged, because it supplies the uterus circulation during pregnancy.
6. Cervix removal phase: one cm of isthmus is removed followed by endocervical curettage.
7. Cerclage phase.

Table 1. — Candidates For RVT [46-47].

<table>
<thead>
<tr>
<th>Author (ref)</th>
<th>No. of patients</th>
<th>No of pregnancies</th>
<th>Outcomes Relapses/death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shepherd et al. [45]</td>
<td>26</td>
<td>14 in 8</td>
<td>0/0</td>
</tr>
<tr>
<td>Dargent et al. [46]</td>
<td>82</td>
<td>22 in 18</td>
<td>3/2</td>
</tr>
<tr>
<td>Burnett et al. [47]</td>
<td>68</td>
<td>3 in 3</td>
<td>0/0</td>
</tr>
<tr>
<td>Plante et al. [48]</td>
<td>68</td>
<td>33 in 23</td>
<td>1/1</td>
</tr>
<tr>
<td>Bernadini et al. [49]</td>
<td>80</td>
<td>22 in 18</td>
<td>7/4</td>
</tr>
</tbody>
</table>

Recurrence risk factors

1. Size: lesions more than two cm [44, 54, 55]. The rate for recurrence probability is up to 29% in comparison with 1.6% for lesions less than two cm.
2. Lymphovascular invasion: the rate for lesions more than two cm is 12% compared to 2% for lesions less than two cm [55]. This rate is considered as exclusion criteria for RVT [56].
3. Pathologic type: although it is not clarified yet, nosquamous cell tumours are associated with higher recurrence. Also, neuroendocrine tumours are related with faster invasion and higher probability of recurrence, even with intact margin and no lymph node involvement [44, 55].

Complications in RVT

Some obstetrical complications associated with the surgery appear for women who undergo a RVT and subsequently have a pregnancy. The risks of RVT during pregnancy include:

- Cervical incompetence
- Miscarriage
- Premature delivery
- Low birth weight

It should be mentioned that a loss rate up to 17% during the first trimester of pregnancy almost equals the loss rate of the general population, but in the second trimester the rate remains significantly high (up to 12%) [56].

Pregnancy-related outcomes after RVT (Table 3)

It is a high possibility that these patients, who are facing these complications, may require assistance of advanced reproductive technology to conceive [61].
Table 3. — Pregnancy-related outcomes after RVT.

<table>
<thead>
<tr>
<th>Study</th>
<th>No of patients attempted pregnancy</th>
<th>No of patients conceived (%)</th>
<th>Total pregnancies</th>
<th>Losses (%)</th>
<th>Pre-term (%)</th>
<th>Full-term (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covens et al. [57]</td>
<td>32</td>
<td>13</td>
<td>4 (31)</td>
<td>5</td>
<td>2 (40)</td>
<td>0</td>
</tr>
<tr>
<td>Shepherd et al. [45]</td>
<td>26</td>
<td>13</td>
<td>8 (62)</td>
<td>14</td>
<td>5 (36)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>Burnett et al. [47]</td>
<td>19</td>
<td>19</td>
<td>3 (16)</td>
<td>3</td>
<td>1 (33)</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Schlaerth et al. [58]</td>
<td>10</td>
<td>10</td>
<td>4 (40)</td>
<td>4</td>
<td>2 (50)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Mathievet et al. [59]</td>
<td>95</td>
<td>43</td>
<td>34 (79)</td>
<td>56</td>
<td>22 (39)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Bernadini et al. [49]</td>
<td>80</td>
<td>39</td>
<td>18 (46)</td>
<td>22</td>
<td>4 (18)</td>
<td>6 (27)</td>
</tr>
<tr>
<td>Plante et al. [60]</td>
<td>72</td>
<td>31</td>
<td>31 (50)</td>
<td>50</td>
<td>10 (20)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Total</td>
<td>214</td>
<td>137</td>
<td>71 (52)</td>
<td>104</td>
<td>36 (35)</td>
<td>20 (19)</td>
</tr>
</tbody>
</table>

Conclusions

Nowadays, more and more women decide to become pregnant after the age of thirty. The screening tests’ success has led to an increased detection of early stage cervical cancer. In order to preserve fertility, these women demand more conservative surgical techniques, such as RVT.

References


Introduction

Human papillomavirus (HPV) is one of the main risk factors for invasive cervical cancer [1-3]. The genotypes involved most frequently are related with HPV 16 and HPV 18 [4-6] and are associated with approximately 70% of all cancers of the cervix, 50% of all high-grade intraepithelial neoplasias of the cervix, and 25% of all low-grade intraepithelial neoplasias of the cervix. However, other HPV genotypes are associated with high risk (HPV 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 70, and 85) or probable high risk (HPV 53, 66), and also play an important role in cervical pathology and cervical cancer [7]. The prevalence of simultaneous infection by more than one genotype varies widely from 10% to 80% depending on the population studied [8-13].

It is currently unclear whether multiple HPV genotype infection is predictive of the severity of the cervical lesion. Controversy continues regarding the possible mechanisms through which multiple genotype infection might increase the risk of cervical intraepithelial neoplasia grade 2 or 3 (CIN 2-3) lesions or carcinoma [10, 11].

The aims of this study were to document the prevalence of infection by multiple HPV genotypes in patients with cervical pathology in a study population, and to shed light on how multiple genotype infection is related with the patient’s age and with type of cervical pathology. Its interest remains in describing the authors’ own population and own prevalence rates.

Materials and Methods

Study population

Information for this prospective cross-sectional descriptive study was gathered for a total of 1,007 patients, with a mean age of 35.8 years (range 14–73), seen at the cervical pathology clinic of Sant Joan de Déu University Hospital in Barcelona (Spain) between January 2003 and March 2011. Statistical analyses were done with SPSS v.19 software. Differences between groups were considered statistically significant at $p < 0.05$.

Results: There was 28.3% of the women (286 cases) that were infected by multiple HPV genotypes. The mean number of genotypes identified was 2.52 (range 2 to 8). Mean age of the patients with multiple genotype infection was 32.31 years, and mean age of the patients with single genotype infection was 37.27 years ($p < 0.001$). The prevalence of infection by multiple HPV genotypes was 28% in patients with cervical intraepithelial neoplasia grade 1 (CIN 1) and 33% in patients with grade CIN 2-3 lesions, and both prevalence rates were significantly higher than in patients with carcinoma (20%) ($p=0.03$).

Conclusions: In the present study population the authors found no evidence of higher prevalence of multiple HPV genotype infection in women with carcinoma. Age of women with multiple infection was lower than those with single infection.

Key words: Human papillomavirus infection; Cervical cancer; Cervical intraepithelial neoplasia; Grade 1, 2, 3; Multiple infection.
punctuation or mosaicism, iodine-negative, atypical vessels), findings suggestive of invasive cancer, and unsatisfactory colposcopic examination. In all the women the diagnosis was confirmed histologically. Samples were obtained by colposcopically-guided punch biopsy from all areas of the cervix with atypical colposcopic findings. The biopsy specimens were fixed in formalin, analyzed by a pathologist, and classified as follows: negative, CIN 1, 2 or 3, carcinoma, and adenocarcinoma.

**HPV genotyping by PCR**

Cervical scrapes were obtained with a cotton brush and transported at room temperature to the molecular microbiology department for HPV genotyping. Cytology was conventional and no medium was used to transport the cervical specimens. During the study period two techniques—line probe assay (LiPA) and microarray—were used consecutively. For LiPA assays, cervical swabs for DNA extraction were obtained with a commercial kit and eluted to a final volume of 200 µl. For microchip array assays, DNA was extracted with a proteinase K lysis solution (20 mg/ml). The purified DNA extracts were stored at −20 °C.

The LiPA assay was based on the reverse hybridization principle and provides type-specific genotype information for 25 different HPV genotypes (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74) simultaneously. Amplification of HPV DNA was based on the SPF10 PCR primer set, which amplifies a fragment of only 65 bp within the L1 open reading frame (ORF) region. Part of the human beta-globin gene (268 bp) was amplified in each sample as a control. Line probe assays with SPF10 were done with 10 µl of the DNA extract in a final reaction volume of 100 µl.

The microchip array assay detected infections and coinfections by up to 35 of the most relevant HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74) in different sample types. The system was based on a low-density microarray attached to the bottom of a classical 2-ml Eppendorf tube. For DNA amplification a reaction mixture was used which amplifies a 450-bp fragment within the L1 ORF region. A 892-bp fragment of the human CFTR gene was amplified in each sample as a genomic DNA control. To avoid false negative results, an amplification control was added to the reaction mixture. The control used for the genotyping was the control of each of the kits.

In the present study HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 70, and 85 genotypes were considered high-risk and HPV 53 and 66 probable high risk, on the basis of recently published studies. However, the present sample included no women infected with large loop excision of the transformation zone, and 54.9% underwent conization (cone biopsy); 9.3% underwent hysterectomy. A total of 740 women (73.2%) had HPV infection, among whom 86.4% (639) had a high-risk HPV genotype.

In the present sample, 28.3% of the women (286 cases) were infected by multiple HPV genotypes (included high-risk and low-risk HPV). Many patients with high-risk HPV genotype infection were infected with multiple HPV genotypes (43.7%), whereas among patients with low-risk HPV genotype infection, only 1.9% (nine cases) were infected with more than one genotype ($p < 0.001$). Among women with multiple HPV genotype infection, the mean number of genotypes per patient was 2.52 (range two to eight). Two-thirds of multiple infections (66.1%) involved two genotypes, 23.4% involved three, 5.25% involved four, 3.5% involved five, 1% involved six, 0.3% involved seven, and 0.3% involved eight genotypes.

**Statistical analysis**

All data were analyzed with SPSS software (v. 19). The authors used Student’s $t$ test for quantitative variables when the data were distributed normally, and the Mann–Whitney U test when normal distribution could not be confirmed. Comparisons for qualitative variables were analyzed with the chi-squared test. Analysis of variance was used for comparisons involving more than two samples. The results were considered statistically significant if the $p$ value was $< 0.05$.

**Results**

Of the patients included in this study, 48.7% (486) of them were diagnosed as having CIN 1, 47.3% (476) as CIN 2/3, and 3.7% (37) had carcinoma. The high percentage of patients with CIN 2/3 is due to all women that were referred to the present hospital due to cytological alterations from their primary health centers and most patients with transitory CIN 1 were not referred. The age of the patients is considered at the time of diagnosis of cervical pathology. Mean age in women with HPV infection by Table 1. — Mean age of patients infected by different numbers of HPV genotypes.

<table>
<thead>
<tr>
<th>Number of genotypes</th>
<th>Mean age of patients (years)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>32.90</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>32.03</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31.87</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26.80</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>28.67</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Frequency of multiple HPV genotype infection depending on the type of lesion.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Infection by one HPV genotype (number of patients and percentage)</th>
<th>Infection by multiple HPV genotypes (number of patients and percentage)</th>
<th>Total</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 1</td>
<td>275 (72%)</td>
<td>107 (28%)</td>
<td>382</td>
<td></td>
</tr>
<tr>
<td>CIN 2-3</td>
<td>309 (67%)</td>
<td>150 (33%)</td>
<td>459</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>30 (80%)</td>
<td>7 (20%)</td>
<td>37</td>
<td>0.03</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2 (66.6%)</td>
<td>1 (33.3%)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>619</td>
<td>265</td>
<td>881</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. — Frequencies of patients infected by different numbers of HPV genotypes depending on the type of lesion, considering only patients with coinfection.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN 1</td>
<td>63.4%</td>
<td>24.8%</td>
<td>8.9%</td>
<td>3%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0.98</td>
</tr>
<tr>
<td>CIN 2-3</td>
<td>64.7%</td>
<td>25.3%</td>
<td>3.3%</td>
<td>3.3%</td>
<td>2%</td>
<td>0.4%</td>
<td>0.4%</td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>71.4%</td>
<td>14.2%</td>
<td>14.2%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>172 (65.2%)</td>
<td>65 (24.6%)</td>
<td>14 (5.3%)</td>
<td>8 (3%)</td>
<td>3 (1.1%)</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In the population studied, the prevalence of infection by multiple HPV genotypes was 28%, a lower figure than in earlier studies [8-13]. Variability in the prevalence of multiple HPV genome infection may be explained by methodological differences between studies. Because different tests are used to detect HPV, comparisons across studies are problematic. In addition, differences in the characteristics of study populations may account for the variability, because infection by multiple genotypes is influenced by differences in geographic, demographic, and clinical factors [11, 12].

The prevalence of coinfection was higher among women infected with high-risk HPV genotypes (43.7%) than with low-risk HPV genotypes (1.9%). This finding is consistent with earlier results, as most series have reported a very low prevalence of multiple infection by low-risk genotypes, whereas most multiple infections involve high-risk HPV genotypes [8, 12-16].

The authors found a clearly higher prevalence of coinfection in younger women, and coinfection became less frequent as age increased. In addition, mean age in patients with coinfection decreased as the number of coinfecting genotypes increased. These results are fully consistent with those of earlier studies of the relationship between coinfection and age of the patients [8, 11, 13, 17-19]. All reports have noted a trend towards a higher prevalence of coinfection in younger patients. Young women are more likely to be infected with HPV per se, and to be infected by multiple genotypes, because multiple HPV genotype infections are closely related to sexual behavior [17]. Moreover, the inverse relationship between the prevalence of coinfection and the patients’ age can also be attributed to acquired immunity which develops with the duration of exposure to HPV. This process may also explain why coinfection by a larger number of genotypes is more frequent at younger ages [20-25].

In connection with the relationship between coinfection and the type of lesion, a population-based study in Madrid (Spain) by Martin et al. [26] found that coinfection was more closely associated with grade CIN 1 lesions (45%) than with grade CIN 2-3 lesions (20%). These authors postulated that as the lesion progresses from low grade to high grade, genotypes that bear a higher oncological risk persist while those with low oncological risk are eliminated. A study in an Italian population by Gargiulo et al. [16] also found a higher prevalence of coinfection among women with grade CIN 1 lesions (6.5%) than grade CIN 2-3 lesions (2.3%) or carcinoma (3.2%). Rousseau et al. [19] obtained similar findings: coinfection appeared in 23% of the women with grade CIN 1 lesions and 7% of the women with grade CIN 2-3 lesions. Muñoz et al. [27], in an international case-control study of cervical cancer, reported that multiple HPV genotype infections were not associated with a higher risk of carcinoma than single-genotype infections. Similarly, the SUCCEED study by Wetzensen et al. [18] at the University of Oklahoma found a higher percentage of single-genotype infection among cases of carcinoma (66%) than among lesions diagnosed as grade CIN 1 (24.7%). In the present population sample, patients with carcinoma had a lower prevalence of multiple infection than those with diagnosis of CIN 2-3.

It is currently unclear whether multiple HPV genotype infection is predictive of the severity of the cervical lesion. Moreover, the possible mechanisms by which multiple genotype infection may increase the risk of grade CIN 2-3 lesions or carcinoma are controversial [10, 11]. Some authors [8, 10, 11, 17] have suggested that compared to single-genotype infections, multiple HPV genotype infections are associated with an increased risk of grade CIN 2-3 lesions and carcinoma. These researchers have postulated that multiple-genotype infections may increase the risk of grade CIN 2-3
lesions or carcinoma because they are associated with a notable increase in the duration of HPV infection. The present authors observed no relationship between the number of genotypes involved in coinfection and the type of lesion, and coinfection by two genotypes was the most frequent type of HPV infection regardless of the type of cervical lesion. These results are consistent with the findings of some earlier studies [11, 16, 18]. Additional studies are needed to evaluate the possible effects of multiple-genotype HPV infection on the risk of progression of cervical intraepithelial lesions, and to shed light on the mechanisms involved in their progression.

Acknowledgments

The authors thank K. Shashok for translating parts of the original manuscript into English.

References


Introduction

The introduction of routine ultrasound and office hysteroscopy in the evaluation of dysfunctional or organic lesions of uterine cavity have increased the number of diagnosis of uterine polyps [1]. Endometrial polyps (EPs) are a localized overgrowth of endometrial tissue and may contain varying amounts of stroma and blood vessels covered by pseudostratified epithelium [2]. The diagnosis of EPs occur in 10% to 40% in women with abnormal uterine bleeding (AUB) and obesity, postmenopausal state, abnormal uterine bleeding (AUB), hypertension, and risk of malignant EPs. On multivariable analysis, the correlation remained only for age (OR 1.08, 95% CI 1.03 - 1.14) and AUB (OR 3.53, 95% CI 1.87 - 6.65). Conclusion: Older patients in postmenopausal status with AUB, a high BMI, and hypertension are at higher risk for premalignant and malignant polyps. In these patients a surgical approach should be used, consisting in hysteroscopic removing of the polyp.

Materials and Methods

The medical records of premenopausal and postmenopausal women consecutively undergoing operative hysteroscopy for endometrial polypectomy between February 2010 and December 2012 at the Department of Gynaecological Sciences and Human Reproduction, University of Padua (Padua, Italy) and at the Woman’s Health Sciences Department, Università Politecnica delle Marche (Ancona, Italy) were retrospectively analyzed in an observational multi-institutional cohort study (Canadian Task Force II-2). An informed consent was obtained from all patients, which explained the involved side effects, risks, and benefits of medical and surgical management [8, 9].

Previous studies have demonstrated an increased rate of premalignant and malignant lesions in patients postmenopausal status with EPs who have associated vaginal bleeding [10, 11]. Furthermore, some authors reported the onset of endometrial malignant polyps exclusively in symptomatic or postmenopausal women [12]. Others described risk factors are obesity, arterial hypertension, and use of hormonal and tamoxifen therapies [13, 14]. Therefore, the correct management of asymptomatic women with EPs is actually unclear. In fact, gynecologists must balance the risk of malignant progression with the risk of complications of hysteroscopy and analgesia/anesthesia and the costs of the intervention [15-17]. Currently, the management of the EPs either asymptomatic or symptomatic is the hysteroscopic resection in women of any age.

The aim of this study was to determine the prevalence of atypical lesions on EPs removed by hysteroscopic procedures. Furthermore, the authors wanted to evaluate the association between clinical parameters and demographical characteristics as well as the histopathological features of these lesions.

Key words: Endometrial polyps; Body mass index; Hypertension; Endometrial cancer; Hysteroscopic polypectomy.
All women underwent presurgical evaluation with physical examination, transvaginal ultrasound, and office hysteroscopy. Diagnostic outpatient hysteroscopy was performed using saline solution as a distention medium, and an endoscope with a five-mm diagnostic sheath. The vaginoscopic approach (without speculum or tenaculum) was used in all cases to avoid patient discomfort or pain not directly related to uterine examination. Neither analgesia nor local anesthesia were administered to any patient.

Demographic characteristics and data on diabetes, hypertension, and menopausal status were collected, and anthropometric parameters were analyzed. Patients were considered postmenopausal if they reported a period of at least 12 months of amenorrhea. AUB was defined as any vaginal bleeding in postmenopausal women not receiving hormonal replacement therapy (HRT) or in premenopausal woman with not regular bleeding or in treatment with HRT. Women in treatment with tamoxifen in adjuvant therapy for breast cancer were also included in the study group. Arterial hypertension (diastolic pressure $>$90 mmHg and/or systolic pressure $>$140 mmHg), body mass index (BMI) (women with BMI more than 30 were considered obese), diabetes mellitus (fasting glucose $>$126 mg/dl), presence or absence of symptoms, hormonal and tamoxifen therapy, parity, and history of previous diagnosis of breast cancer were recorded. Exclusion criteria were: cervical cancer, complex adnexal pathology, severe liver pathology, and pregnancy.

The diagnosis of EPs was histologically made after a hysteroscopic polypectomy, carried out rarely under spinal anesthesia [18] or usually under general anesthesia. Procedures were performed using a nine-mm resectoscope 12° forward-oblique lens with a monocolor loop 90°, and glycine as distension medium or a ten-mm resectoscope 0° forward lens with a 2.5 mm twistle electrode. The electrode worked on bipolar energy, so saline was used as the distension media. Myomas or polyps were hysteroscopically distinguished and additional information about surrounding endometrium was obtained. The aim of the resection was the complete removal of the EP. Evaluation of the endocervical canal, endometrial surface, vascularity, tubal ostia or synechiae was performed.

In premenopausal women the procedure was performed in proliferative phase of the menstrual cycle. In perimenopausal women, with heavy bleeding, a transcervical endometrial resection was associated [19]. Hysteroscopic polypectomies were performed by senior gynaecologist surgeons. No intraoperative or postoperative complications were recorded.

Specimens removed by hysteroscopic resection (EPs and endometrial areas) were sent for histopathological examination to the Institute of Pathological Anatomy of the University of Padua and the Institute of Pathological Anatomy of the Ospedali Riuniti, Ancona, Italy. Cases of submucous leiomyoma or uterine adenomyoma were excluded by the analysis.

Diagnosis distinguished between polyps that were recognized as benign (atrophic, proliferative, or hyperplastic polyps and simple hyperplasia and complex hyperplasia without atypia), premalignant (complex hyperplasia with atypia), and those harboring carcinoma [20]. The histopathologic definitions of endometrial hyperplasia and adenocarcinoma were according to the following definitions [21]. Endometrial simple hyperplasia was defined by the endometrial architecture that was moderately distorted. The lining epithelium of the glands was pseudostratified showing mitotic activity with no atypia of cells. Atypical simple hyperplasia was defined by architecture similar to simple hyperplasia, but the glands were more irregular. The glands were lined by atypical cells. Endometrial carcinoma was defined by crowded malignant tubular glands varying in size and invading the stroma.

<table>
<thead>
<tr>
<th>Histology</th>
<th>N. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign polyps and polyps with hyperplasia</td>
<td>766</td>
<td>94.2</td>
</tr>
<tr>
<td>without atypia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyps with hyperplasia with atypia</td>
<td>23</td>
<td>2.8</td>
</tr>
<tr>
<td>Cancerous polyps</td>
<td>24</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The statistical analysis was performed with Medcalc 13.1. Student’s $t$-test was applied, as appropriate, to compare continuous variables. Proportion were compared with Chi-squared test. Statistical significance was considered achieved when $p < 0.05$. Univariable and multivariable logistic regressions were performed to verify the presence of statistically significant correlation among age, BMI, menopause, AUB hypertension, (independent variables), and the presence of EPs or adenocarcinoma.

**Results**

The main demographic and clinical characteristics of the study population (813 cases) are shown in Table 1. The mean age was 52.5 years (range: 22-87) and 392 (48.2%) patients were in postmenopausal status. Mean BMI was 25.1 ± 5.2 (16.7-58.6) for the study population (813 cases).

Table 1. — Demographic and clinical characteristics of the study population (813 cases).

<table>
<thead>
<tr>
<th>Age (mean ± DS, range)</th>
<th>52.5 ± 13.1 (22-87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menopausal status</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>421 (51.8%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>392 (48.2%)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>Nulliparity</td>
<td>222 (27.3%)</td>
</tr>
<tr>
<td>Multiparity</td>
<td>591 (72.7%)</td>
</tr>
<tr>
<td>AUB</td>
<td>267 (32.8%)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1 ± 5.2 (16.7-58.6)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>206 (25.3%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>25 (3.1%)</td>
</tr>
<tr>
<td>History of breast cancer</td>
<td>34 (4.2%)</td>
</tr>
<tr>
<td>HRT</td>
<td>84 (10.3%)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>22 (2.7%)</td>
</tr>
</tbody>
</table>

The statistical analysis was performed with Medcalc 13.1. Student’s $t$-test was applied, as appropriate, to compare continuous variables. Proportion were compared with Chi-squared test. Statistical significance was considered achieved when $p < 0.05$. Univariable and multivariable logistic regressions were performed to verify the presence of statistically significant correlation among age, BMI, menopause, AUB hypertension, (independent variables), and the presence of EPs or adenocarcinoma.

Table 2. — Histopathological diagnosis of the resected lesions.

<table>
<thead>
<tr>
<th>Histology</th>
<th>N. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign polyps and polyps with hyperplasia</td>
<td>766</td>
<td>94.2</td>
</tr>
<tr>
<td>without atypia</td>
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<td>2.8</td>
</tr>
<tr>
<td>Cancerous polyps</td>
<td>24</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The statistical analysis was performed with Medcalc 13.1. Student’s $t$-test was applied, as appropriate, to compare continuous variables. Proportion were compared with Chi-squared test. Statistical significance was considered achieved when $p < 0.05$. Univariable and multivariable logistic regressions were performed to verify the presence of statistically significant correlation among age, BMI, menopause, AUB hypertension, (independent variables), and the presence of EPs or adenocarcinoma.

**Results**

The main demographic and clinical characteristics of the study population (813 cases) are shown in Table 1. The mean age was 52.5 years (range: 22-87) and 392 (48.2%) patients were in postmenopausal status. Mean BMI was 25.1 (5.2 SD) with a 16.7% of obese patients.

Table 2 shows the histopathological diagnosis of the resected lesions. Forty-seven (5.8%) premalignant and malignant lesions were found, consisting of 23 (2.8%) polyps with complex hyperplasia with atypia and 24 (3.0%) carcinomas.

Association between clinical parameters and histopathologic results are shown in Table 3. Of these factors, age ($p < 0.001$), BMI ($p < 0.001$), menopause ($p < 0.001$), AUB ($p < 0.001$), and hypertension ($p < 0.001$) showed any significant association. In particular, older women (>60 years) had a statistically significant higher risk of premalignant and malignant lesions, while younger women (<40 years and 40-50 years) had more frequently benign polyps. Higher BMI values were correlated with higher risk of malignant lesion, with a specific attention to BMI values in the range of obesity (>
with the increased use of diagnostic tools for the study of the uterine cavity, such as ultrasounds, hysterosonography and office hysteroscopy, the diagnosis of EPs are increas-

Table 3. — Association between clinical parameters and histologic results.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Benign polyps and hyperplasia without atypia (766 cases)</th>
<th>Preneoplastic and neoplastic lesions (47)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±DS)</td>
<td>51.8 ± 12.8</td>
<td>64.1 ± 12.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&lt; 40 years</td>
<td>132 (17.2)</td>
<td>2 (4.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>40-50 years</td>
<td>238 (31.1)</td>
<td>4 (8.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>50-60 years</td>
<td>176 (23)</td>
<td>10 (21.3)</td>
<td>0.9</td>
</tr>
<tr>
<td>&gt; 60 years</td>
<td>220 (28.7)</td>
<td>31 (65.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (mean±DS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI &lt; 25</td>
<td>457 (59.7)</td>
<td>15 (31.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI 25-30</td>
<td>187 (24.4)</td>
<td>18 (38.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI &gt; 30</td>
<td>122 (15.9)</td>
<td>14 (27.8)</td>
<td>0.05</td>
</tr>
<tr>
<td>Menopause</td>
<td>354 (46.2)</td>
<td>38 (80.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AUB</td>
<td>239 (31.2)</td>
<td>28 (59.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>210 (27.4)</td>
<td>12 (25.6)</td>
<td>0.9</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>22 (2.9)</td>
<td>3 (6.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>183 (23.9)</td>
<td>23 (48.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>32 (4.2)</td>
<td>2 (4.3)</td>
<td>0.7</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>22 (2.9)</td>
<td>0 (-)</td>
<td>0.5</td>
</tr>
<tr>
<td>HRT</td>
<td>79 (10.3)</td>
<td>5 (10.6)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

All values are n (%) unless otherwise specified;

Table 4. — Multivariable logistic regression, of age, menopause, AUB, hypertension, BMI, and the presence of preneoplastic and neoplastic lesions.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Preneoplastic and neoplastic lesions</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;0.001</td>
<td>1.08</td>
<td>1.03 - 1.14</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.3</td>
<td>1.03</td>
<td>0.97 - 1.10</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>0.8</td>
<td>1.18</td>
<td>0.41 - 3.37</td>
<td></td>
</tr>
<tr>
<td>AUB</td>
<td>&lt;0.001</td>
<td>3.53</td>
<td>1.87 - 6.65</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.6</td>
<td>0.84</td>
<td>0.39 - 1.76</td>
<td></td>
</tr>
</tbody>
</table>

30) and overweight (25-30). Among the other clinical variables, menopause, hypertension and the presence of AUB were statistically found to be more frequent in patients with preneoplastic and neoplastic changes of the EPs. Other clinical data, such as nulliparity, presence of diabetes mellitus, history of breast cancer, tamoxifen therapy or HRT were not significantly correlated with malignant progression of those lesions. The variables with a significant association with cancer progression were included in a multivariable logistic regression model. On multiple regression analysis (Table 4), all the independent variables lost their statistical significance, except for age and AUB with a OR of 1.08 (95% CI 1.03-1.14) and 1.87 (95% CI 1.87-6-65), respectively.

Discussion

With the increased use of diagnostic tools for the study of the uterine cavity, such as ultrasounds, hysterosonography and office hysteroscopy, the diagnosis of EPs are increas-

EPs are often a benign lesion, but a risk of malignant progression was described that varies from 0.3% to 4.8% [5, 6, 22-25]. It might be useful to identify clinical, hysteroscopical, and demographical characteristics correlated with a high risk of malignant progression. In this study, the authors attempted to correlate some demographic and clinical factors with the rate of progression.

The present results show a high prevalence of premalignant and malignant lesions (5.8%), probably because the population had an gradually increasing average age compared to older studies. In univariate analysis, older age and in particular age over 60 years, hypertension, postmenopausal status, and AUB were identified as statistically significant factors associated with premalignancy and malignancy in EPs (Table 3). These results appear similar to the others published in literature. In fact, Costa-Paiva et al. and Antunes et al. identified age as a risk factor for malignant polyps [3, 9]. The same relationship was identified by the group of Baiocchi et al. [21]. Accordingly to the studies of Baiocchi et al., Giordano et al., Costa-Paiva et al., and Savelli et al., hypertension and postmenopausal status were also predictive of malignancy in women with EPs [3, 21, 22, 26]. In the present univariate analysis, AUB was identified as a risk factor for endometrial cancer in women with EPs. This association has been reported in most studies in the literature [3,23]. However, the present study showed a linear relationship between BMI and risk of endometrial malignancy. A recent study reports the same correlation between BMI >25 and endometrial cancer [27]. Many epidemiological studies show that overweight (BMI 25-29.9) and obese (BMI >30) patients have a higher tumor general risk, and also for endometrial neoplasia [28]. In a meta-analysis from WCRF/AICR (2007) from 28 case-control studies, the authors estimated a relative risk of 1.56 for endometrial cancer (95% CI: 1.45 – 1.66) for increments of five kg weight. Same results has been reported in two other meta-analysis by Renehan et al. and Antunes et al. and the others published in literature. In fact, Costa-Paiva et al., Giordano et al., Costa-Paiva et al., and Savelli et al., hypertension and postmenopausal status were also predictive of malignancy in women with EPs [3, 21, 22, 26]. In the present univariate analysis, AUB was identified as a risk factor for endometrial cancer in women with EPs. This association has been reported in many studies in the literature [3,23]. However, the present study showed a linear relationship between BMI and risk of endometrial malignancy. A recent study reports the same correlation between BMI >25 and endometrial cancer [27]. Many epidemiological studies show that overweight (BMI 25-29.9) and obese (BMI >30) patients have a higher tumor general risk, and also for endometrial neoplasia [28]. In a meta-analysis from WCRF/AICR (2007) from 28 case-control studies, the authors estimated a relative risk of 1.56 for endometrial cancer (95% CI: 1.45 – 1.66) for increments of five kg weight. Same results has been reported in two other meta-analysis by Renehan et al. and Antunes et al. It can be assumed that multiple factors lead to carcinogenesis. This factors probably involve insulin growth factor (IGF) -1 [23] and hyperestrogenism. Chronic hyperinsulinemia could provoke the estrogen-dependent tumors also by inhibiting the synthesis of sex hormone binding globulin and increase the bioavailability of estrogens [31]. In women with AUB, both in pre- and postmenopausal status, the surgical approach is often advocated [6, 23, 24]. However, data is limited on the management of asymptomatic women with an incidental diagnosis of EP [32]. Therefore, gynecologists must balance between the risk of endometrial cancer and the risk of complications of hysteroscopy and analgesia/anesthesia and the costs of the intervention [15, 16]. In fact, this procedure that often entails a hospital stay with an amount...
healthcare costs, can involve a high risk of surgical and anesthesiologic complications much more in elderly women, and has to be performed by skilled gynecologist. Removing polyps is necessary because they are abnormal lesions. They have to be ruled out to exclude a malign lesion, by histological evaluation, nevertheless they have a low-risk of malignancy [22] and they could resolve spontaneously [12,33].

While some authors have recommended that all women with polyps undergo surgical evaluation, it may be better to evaluate each individual case. In fact, patients are subjected to the risks of surgery and intervention in a large number of cases and are associated with substantial healthcare costs. In general, asymptomatic premenopausal women are at low risk but should be observed carefully as occasionally cancer is detected [5]. While the present study benefits from the inclusion of a relatively large number of patients, the authors recognize some limitations. Because of the retrospective design of this study, severity and time lapsed from the onset of diabetes, hypertension, AUB, and obesity were not checked for risk assessment of EPs. Another limit of this study is that BMI, glucose levels, and blood pressure were examined as dichotomous variables and not as continuous variables. Moreover, the data were collected exclusively from medical charts that may be responsible for incomplete or inconsistent information.

Conclusion

Older symptomatic women with a diagnosis of EP need to have this lesion removed considering the higher risk of premalignant and malignant changes. Other coexisting factors, such as obesity, menopause, and hypertension must be taken into account because they may represent additional risk factors to cancerization.

References


Predictors of malignancy in endometrial polyps: a multi-institutional cohort study


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An in vivo model for the study of ovarian cancer and the persistence of characteristic mutations in xenografts

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Department of Obstetrics and Gynaecology, Tianjin Union Medical Center, Tianjin (China)

Summary

Objective: To identify factors affecting xenograft growth of epithelial ovarian cancer (EOC) cells in nude mice and to detect characteristic mutations occurring in the xenografts following serial passage. Materials and Methods: A total of 64 human EOCs were subcutaneously inoculated in Balb/c nude mice in order to obtain a series of xenografts. Whole-exome sequencing was analyzed with Agilent SureSelect targeted enrichment capture system and Illumina Solexa Hiseq 2000 sequencing platform. Mutations were confirmed by comparison against the reference genome build 37.3. Results: The tumor take rate was 50% (32/64). TP53 mutation was detected in nine of ten Type II tumors. BRAF and CTNNB1 were not mutated in any of the samples, and PTEN mutation occurred in only one sample. The present data indicate that advanced stage serous EOCs and early stage non-serous EOCs were easy to grow in nude mice, and xenografts maintained the characteristic mutations. Conclusions: Advanced stage serous EOCs and early stage non-serous EOCs were easy to grow in nude mice, and xenografts maintained the characteristic mutations. Xenografts in nude mice are useful in vivo models for the study of human EOCs.

Key words: Ovarian epithelial cancer; Nude mice; Heterologous transplantation; Mutation.

Introduction

Ovarian cancer is the most lethal cancer of the female reproductive system. Approximately 70% of ovarian cancers are diagnosed at an advanced stage, and only 30% of women with such cancers can expect to survive five years [1, 2]. Hence, it is important to develop new and effective treatments. Unfortunately, only limited progress has been achieved largely because of the lack of suitable laboratory animal model systems of ovarian cancer.

Animal models of cancer provide an alternative means to determine the causes of and treatments for malignancy [3]. Currently, there are several different laboratory models of ovarian cancer [4]. The development of nude mice has provided a host that supports growth of human tumors in vivo. Almost all human malignant tumors can grow in nude mice after implantation of the original tumor from the patient and the tumor xenografts largely reflect the features of the donor tumor, including pathological characteristics, biological behavior, and sensitivity to chemotherapy drugs [5-7]. However, heterogeneity is the key and dominant feature of human cancers. Xenografts from one or even several patients do not represent the whole picture of a certain type of malignancy. Hence, it is essential to establish large samples of tumor xenograft models.

At present, collective analyses of the clinicopathological and molecular features of the major types of ovarian carcinomas have proposed that surface epithelial tumors can be divided into two categories, designated Type I and Type II tumors [8, 9]. These two types of tumors refer to tumorigenic pathways and are not specific histopathologic terms. Type I tumors often have KRAS, BRAF, PIK3CA, CTNNB1, and PTEN mutations. In contrast, Type II tumors generally lack these mutations, but are characterized by a high frequency of TP53 mutations.

Currently, there are many methods for detecting mutations associated with diseases. Next-generation sequencing, in particular whole-exome sequencing, which allows the global analysis of protein coding sequences in the human genome, has become an effective and affordable approach in detecting causative genetic mutations in disease [10-12]. Several platforms for human exome capture for massively parallel sequencing have been developed and marketed to date. Agilent SureSelect Target Enrichment System is one of the platforms and has proven to be a useful method for exome capture [13, 14]. Hence, it’s a very useful tool to detect mutation.

Although human xenografts grown in mice showed high similarity to the primary tumor, there have been few reports describing the occurrence of mutation after multiple passages in vivo. The aim of this study was to provide a platform for studying epithelial ovarian cancers (EOCs) by establishing more than thirty tumor xenografts in Balb/c nude mice and to confirm the genetic stability after multiple passages in vivo by detecting characteristic mutations related to Type I and Type II tumors using the Agilent SureSelect platform.
Materials and Methods

Animals

Six- to eight-week old female Balb/c nude mice weighing 18-22 grams were used in this experiment. These mice were raised in specific pathogen-free (SPF) conditions with a 12-12 hour day-night cycle. All mice received appropriate anesthetics before they were sacrificed. This study protocol was approved by the Animal Ethics Committee of National Research Institute for Family Planning Beijing. The health of the animals was properly monitored during the experimental period.

Tumor collection

This study collected 64 cases of EOCs received in the Department of Obstetrics and Gynecology in the General Hospital of Tianjin Medical University from January 2010 to December 2011. Informed consent was obtained from all the participants. All the tumors were diagnosed by a pathologist examining frozen sections and confirmed with neutral-buffered formalin-fixed, paraffin-embedded tissue sections. Staging of the collected tumors was done according to the International Federation of Gynaecologists and Obstetricians (FIGO 2009) staging system for primary ovarian carcinomas. In brief, fresh, live primary or peritoneal metastatic ovarian solid tumor tissues were collected immediately after ablation in the operation room under aseptic conditions, placed in RPMI 1640 medium containing 10% fetal calf serum and inoculated into Balb/c nude mice within six hours. The archived SKOV3 tumor xenograft was previously established from the cell line by subcutaneous injection in the scapular region of Balb/c nude mice.

Tumor xenograft nude mouse model

Tumor tissue was mechanically dissected into fragments measuring approximately 2.0 mm in diameter using eye scissors and placed in the paracentesis trocar (two to three fragments per trocar). The fragments in the trocar were subcutaneously inoculated into both sides of the scapular and buttocks region of Balb/c nude mice (four inoculated sites per mice) after sterilizing with 75% alcohol. For each case, depending on the size of the obtained tumor sample, three to five mice were used to try to establish xenografts. For serially passaged tumors, five mice were used for each tumor and one side of the capular region was chosen as the inoculated site.

Tumor volume and body weight were measured twice per week, and tumor volume was calculated with the equation $V = \frac{1}{2}ab^2$ (a and b representing the short and long diameter, respectively, measured by caliper). A tumor take was defined as a tumor, which grew progressively after inoculation and could be serially passaged. If no growth occurred within six months of implantation of the original tumor, the mice were sacrificed, and this was defined as a negative take. The xenograft was serially passaged by s.c. implantation when the volume of the first xenograft reached approximately 500-600 mm³ or the subsequent xenograft volume reached 800-1,000 mm³. Tumor growth curves were expressed as tumor volume versus the day after implantation.

The harvested tumor tissues were frozen in liquid nitrogen for molecular biology detection and fixed in 10% neutral-buffered formalin for subsequent histological examination. The vital organs (liver, lung, and kidney) were also macroscopically and microscopically examined for evidence of tumor metastasis. Haematoxylin and eosin (H&E) stained paraffin-embedded sections (four µm) of each xenograft and internal organ were reviewed by a pathologist. The xenograft tissues of passage 5 were used for mutation detection.

Mutation detection

Ten xenografts of the fifth-passage were used for mutation detection. The whole-exome sequencing was analyzed with Agilent SureSelect targeted enrichment capture system and Illumina Solexa Hiseq 2000 sequencing platform. The mutations were confirmed by comparing against the reference genome build 37.3. The procedure was conducted according to the manufacturer’s instructions. Briefly, the authors used the Qubit dsDNA BR Assay to determine the concentration of the extracted DNA sample. The DNA was sheared with a target peak for base pair size 150-200 bp. The samples were purified using the Agencourt AMPure XP beads and their quality was assessed with the Agilent 2100 Bioanalyzer. They used the Paired-End Sample Preparation Kit (p/n PE-102-1001) to repair the ends. After being purified again, ‘A’ Bases were added to the 3’ end of the DNA fragments, and then indexing-specific paired-end adapters were ligated and amplified to create the adapter-ligated library. Samples were purified with the Agencourt AMPure SPRIXP beads. The quality and quantity were assessed with the Agilent 2100 Bioanalyzer. The SureSelect capture library was enriched by PCR and hybrid capture was performed with SureSelect. The authors amplified the captured library to add index-barcode tags. They used the Agilent QPCR NGS Library Quantification Kit (for Illumina) to determine the concentration of each index-tagged captured library. The final samples were sequenced with Illumina Solexa Hiseq 2000 sequencing platform.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0. Chi-square and Fish’s accurate inspection were used to compare the influence of various clinic-pathologic factors on the tumor take rate. For all tests, a $p$-value < 0.05 was considered statistically significant.

Results

Xenograft formation in nude mice

Sixty-four EOCs were implanted in nude mice, including serous adenocarcinoma (SAC, 40 cases), endometrioid adenocarcinoma (EAC, eight cases), clear cell carcinoma (CCC, seven cases), poorly differentiated adenocarcinoma (PAC, five cases), and mucous adenocarcinoma (MAC, four cases). Persistent growth were observed in 34 xenografts (53.13%), of which 32 tumors were serially passaged giving an overall successful take rate of 50%. Advanced stage carcinomas (FIGO Stage III and IV) had a higher tumor take rate than early stage carcinomas (FIGO Stage I and II) for serous adenocarcinoma ($p = 0.018$), but not for non-serous cancer. However, for all the carcinomas with FIGO Stage I and II, non-serous cancer had a higher tumor take rate than serous adenocarcinoma ($p = 0.044$). Data are shown in Table 1. No statistically significant differences in tumor take rate were found among different FIGO stages ($\chi^2 = 3.022; \ p = 0.388$), nor among different tumor types, excluding mucinous adenocarcinoma ($\chi^2 = \ldots$).
growth was initially observed. However, soon after, the serially inoculated tumor grew faster, and cachexia occurred when the tumor volume reached 1,000 mm³. None of the nude mice died over the experimental period.

**Xenograft growth curves**

Tumor growth curves were generated at the fifth passage. Different growth characteristics were associated with each of the xenografts originating from different patients. The tumor could grow stably, rapidly, slowly or with an accelerated phase (Figure 3). Most xenografts grew at a stable rate.

**Characteristic mutation in the xenografts**

In order to confirm the genetic stability of the xenografts, the authors measured characteristic gene mutations in ten xenografts at passage 5, including Type II tumor-associated genes (TP53) and Type I tumor-associated genes (BRAF, CTNNB1, PTEN, PI3KCA, and KRAS). All ten xenografts were categorized as Type II ovarian cancers. The results coincided with the criteria for classification. TP53 mutations were detected in nine of ten xenografts. BRAF and CTNNB1 were wild type in all samples, and PTEN mutation occurred in only one sample. Data are shown in Table 3.

**Discussion**

EOC comprises approximately 90% of ovarian cancers. It can be divided into four major histopathological groups: serous, endometrioid, mucinous, and clear cell tumors [15]. Of these tumors, the most prevalent subtype is serous ade-
nocarcinoma [16]. Tumors develop and progress as a result of accumulated molecular genetic changes, such as point mutations and gene amplification. Importantly, six to eight key mutations in oncogenes or tumor suppressor genes are the drivers of tumorigenesis [17].

According to the pattern of tumor progression and molecular genetic changes, EOCs can be divided into Type I and Type II tumors. As described above, Type I tumors and Type II tumors have different somatic mutations. This classification is very useful for biological study. Whether or not these characteristic mutations are associated with either Type I or Type II tumors change following serial passage in nude mouse models has not been reported. In this study, the authors detected whole-exome mutations in xenografts after being passaged five times using the Agilent SureSelect Target Enrichment System. Mutations related to Type I tumors seldom occurred in the ten xenografts. For example, BRAF and CTTNB1 were wild type in all samples, and PTEN mutation occurred in only one sample. In contrast, mutations related to Type II tumors were detected in nine of the ten xenografts. This suggests that the xenografts maintained the characteristic mutations even after serial passages, and they might not undergo too much genetic change with each passage.
Tumor models are very useful for cancer research. The first in vivo tumor models were established in the mid-1960s [18]. Currently, there are a variety of animal models, and each model has its advantages and disadvantages [19, 20]. The advantages of human tumor xenograft models are as follows: (1) the malignant cells are human; (2) the resultant xenografts can maintain their original architecture; and (3) many of these models are quite reproducible. The disadvantages are that most of the tumors are grown in a non-natural site (i.e. subcutaneously), and that xenografts fail to reflect the patterns of tumor growth and spreading that are observed in cancer patients [18]. The models could be used in many fields, such as preclinical testing of drugs, determination of the optimal combination chemotherapy regimen for treatment of platinum-resistant ovarian cancer, and discovering new serum biomarkers [14, 21]. Importantly, patient-specific models have recently been proposed as a means to prospectively personalize treatment regimens.

Subcutaneous tumor models in nude mice are traditional models for the study of human malignant tumors. Currently, the most used xenograft models have originated from cell lines. The main limitation of this model is the loss of heterogeneity. Concerning the heterogeneity of human cancers, the authors collected 64 cases of human EOCs of different subtypes to establish subcutaneous tumor models in Balb/c nude mice. Xenograft formation was observed in 34 cases. However, two of them failed to grow in subsequent passages, and the xenograft growth in the first passage was quite slow. Hence, the total tumor take rate was 50% (32/64). It suggests that xenografts that initially grow slowly are more prone to fail in subsequent passages. These results were consistent with those of Friedlander, who reported that the overall successful take rate of human EOCs was 54% [22]. Many factors may influence the tumor take rate, such as tumor stage and histologic type. In this study, Stage III/IV serous adenocarcinomas were more likely to grow in nude mice compared to Stage I/II serous tumors. Non-serous adenocarcinoma, including endometrial carcinoma, clear cell cancer, and low differential adenocarcinoma, were more likely to grow in nude mice compared to serous adenocarcinoma. No mucinous adenocarcinoma xenograft formation was observed in nude mice six months after inoculation. Friedlander et al. [22] also found that the tumor take rate was higher in late stage than early stage tumors.

Figure 3. — Growth curves of the xenografts. The growth of the xenografts originated from different patients show different character. Most xenografts grew at a stable rate and were passaged at five weeks after inoculation (3A). Some xenografts grew very quickly and the tumor volume reached 800 mm\(^3\) approximately ten days after inoculation (3B). Some xenografts grew slowly, and it took about 50 days to reach 800 mm\(^3\) (3C). Some xenografts had an accelerated phase from 20 days post-inoculation (3D).
Xenografts have similar characteristics with the originating human tumor tissues. In this study, the primary human tumor and xenografts of passage 3 and passage 5 showed similar microscopic characteristics. This is in stark contrast to xenografts originating from the human ovarian cancer cell line SKOV3. Tumor tissue xenografts maintained the architecture of the primary tumor. Bankert et al. [18] reported that human lymphocytes, leukocytes, and fibroblasts were present in the microenvironment of tumor xenografts. However, the metastatic rate in this study was only 8.82% (3/34), which is in accordance with other studies [23]. No ascite formation was observed in any of the xenograft models. The model did not accurately reflect the patterns of growth and metastasis observed in cancer patients. This was mainly because these tumor xenografts were not established orthotopically.

In conclusion, the results indicate that advanced stage serous adenocarcinoma has a high tumor take rate. The xenografts maintained the characteristic mutations even after serial passage in nude mice. The established mouse models could serve as powerful platforms for further study of EOCs.

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References

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Toxicity of concurrent chemoradiotherapy for locally advanced cervical cancer

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Summary

Aim of the study: The analysis of acute and late toxicity of concurrent chemoradiotherapy (CCRT) for locally advanced cervical cancer (LACC) based on review of 120 patients treated in Centre of Oncology in Krakow between 2001 and 2007. Materials and Methods: Medium age of the patients was 52 years (43-66). Overall, 12 patients (10.0%) were in Stage IB2, 54 (45.0%) in Stage II, 43 (35.8%) in Stage III, and 11 (9.2%) in Stage IV A. Squamous cell carcinoma was present in 114 (95.0%) patients. Well-differentiated (grade 1) tumour was found in 39 (32.5%) patients, moderately differentiated (grade 2) in 41 (34.2%), and poorly differentiated (grade 3) in 40 (33.3%). Karnofsky performance status score was 70 in 72 (60.0%) patients, and 80-90 in 48 (40%). External radiation therapy was delivered with high-energy six to 15 MV photon beams using four-field brick technique. The total dose of 40 Gy was given in 25 fractions within five weeks using standard fractionation. Concurrently with external radiotherapy, six cycles of chemotherapy were administered to all the patients as an intravenous infusion of once-weekly cisplatin 40 mg/m². On completion of external beam radiotherapy, low-dose rate brachytherapy with tandem and two colpostats was performed to deliver the dose of 40 Gy to point A in two 20 Gy insertions at weekly intervals. Results: Of the 120 patients in the investigated group, 78 (65%) were disease-free for five years. Symptoms of acute treatment-related toxicity grade 3 or 4 (WHO) occurred in 21.6% of patients including leucopenia in 7.5%, anaemia in 5.0%, nausea and vomiting in 3.3%, diarrhea in 5.0%, and urinary tract infection in 0.8%. Full planned treatment (teleradiotherapy + chemotherapy + brachytherapy) completed 78.3% of the group; full planned radiotherapy without full chemotherapy completed 20% of the patients. Late treatment complications of grade 3 or 4 were observed in two (1.6%) patients (narrowing of large intestine requiring surgery and recto-vaginal fistula). Conclusions: In patients with LACC treated with CCRT, the most frequent acute toxic effects include: haematological disorders (leucopenia, anaemia), gastrointestinal disorders (nausea and vomiting, diarrhea), vulvo-vaginal disorders, and urinary tract infection. The most frequent late toxic effects included: rectal bleeding, bowel complications requiring surgery, stenosis or recto-vaginal fistula, and haematuria.

Key words: Locally advanced cervical carcinoma; Chemoradiotherapy; Toxicity.

Introduction

Concurrent chemoradiotherapy (CCRT) is nowadays the standard treatment for patients with locally advanced cervical carcinoma (LACC) [1-13]. The introduction of CCRT for the radical treatment of LACC resulted in an improvement in local control, progression-free survival, and overall survival [14-23]. A systematic review by the Meta-Analysis Group, Medical Research Council Clinical Trials Unit (London), of individual patient data from 13 randomized trials showed a six-percent increase in five-year survival with chemoradiotherapy versus the same radiotherapy alone [24]. Vale et al. presented a Royal College Radiologists audit of patients treated with radiotherapy in 42 UK cancer centres in 2001-2002. Overall, five-year survival with radiotherapy and chemoradiotherapy was 44% and 55%, respectively. For women treated with radiotherapy, overall survival at five years was 59% (Stage IB), 44% (Stage IIB) and 24% (Stage IIIB); for those treated with chemoradiotherapy, it was 65%, 61%, and 44%, respectively [21]. Although the survival gains are significant, there is concern about the acute and late toxicity of CCRT [24-30]. The audit by Vale et al. showed that the addition of chemotherapy to radiotherapy had improved survival compared with radiotherapy alone without an apparent rise in late treatment complications [21]. Some authors conclude that in view of the consistency and extent of the survival benefit for CCRT, the additional acute toxicity appears to be acceptable [3, 7, 8, 10, 23]. Most of the authors consider that serious morbidity is higher in patients treated with CCRT than in those treated with radiotherapy alone [3, 5, 8, 10, 20, 22, 27, 28]. The study by Tan and Zahra has shown that the addition of chemotherapy to radiotherapy for cervical cancer probably improves the survival of patients treated outside research settings, but the benefit may not be as large as that obtained in clinical trials and the risk of serious late toxicity is increased [22]. In the authors’ opinion of Klopp and Eifel, the success of CCRT in cervical cancer patients has been limited in part because the side-effects of standard platinum–based chemoradiation regimens already approach the limits of tolerability [14]. Aim
of this study was to analyse the acute and late toxicity of CCRT in patients with LACC, based on review of the authors’ clinical material and as well as literature data.

**Materials and Methods**

Between January 2001 and June 2007, 120 LACC patients in Stage IB2-IVA were treated with CCRT at the Centre of Oncology in Krakow. Patients were staged according to the International Federation of Gynaecology and Obstetrics (FIGO) staging system [31]. Two toxicity grading systems for reporting complications of treatment were used: Franco Italian glossary [32] and the National Cancer Institute / Common Toxicity Criteria (1988) (NCI, CTC) [33, 34]. The first one describes toxicity associated with radiation therapy and the second is an elaboration by the World Health Organisation (WHO) for reporting chemotherapy toxicity. Study group patients were chosen using the following inclusion criteria: FIGO Stage IB2-IVA, serum haemoglobin levels > 10 g/dl, white blood cell count > 3,000/µl, platelet count 100,000/µl, and normal renal and hepatic function. Median age of the patients was 52 (range: 43 to 66 years). Overall, 12 patients (10.0%) were in Stage IB2, six (5.0%) in IIA, 48 (40.0%) in IIB, four (3.3%) in IIIA, 39 (32.5%) in IIIB, and 11 (9.2%) in IVA. Squamous cell carcinoma was present in 114 (95.0%) patients whereas adenocarcinoma was observed only in six (5.0%) patients. Well-differentiated (grade 1) tumour was found in 39 (32.5%) patients, moderately differentiated (grade 2) in 41 (34.2%), and poorly differentiated (grade 3) in 40 (33.3%). Karnofsky performance status (KPS) score was 70 in 72 (60.0%) patients, and 80-90 in 48 (40%). Fifty-two (43.3%) patients had haemoglobin level < 12 g/dl before starting CCRT, 72 (60.0%) patients, and 80-90 in 48 (40%). Fifty-two (43.3%) patients had haemoglobin level < 12 g/dl before starting CCRT, 16 of whom received pre-treatment blood transfusions.

External radiation therapy was delivered with high-energy six- to 15-MV photon beams using four-field brick technique (anterior, posterior, left, and right lateral fields). Irradiated volume included whole pelvis. The following field borders were used: superior - sacral promontory; inferior - inferior edge of obturator foramina; lateral - one cm off pelvic sidewall; anterior - centre of symphys pubis; and posterior - lower border of S2 vertebra. Before starting radiotherapy, conventional planning based on orthogonal films was performed for 50 patients and three-dimensional virtual computed tomography (CT) simulation for 70, in order to define target volume and organs at risk (rectum, bladder, and femoral head). The total dose of 50 Gy was given in 25 fractions within five weeks using standard fractionation. Concurrently with external radiotherapy, six cycles of chemotheraphy were administered to the patients as an intravenous infusion of once-weekly cisplatin 40 mg/m². On completion of external beam radiotherapy, low-dose rate brachytherapy with tandem and two colpostats to deliver the dose of 40 Gy to point A in two 20 Gy insertions at weekly intervals. After the treatment, patients were followed-up every three to six weeks and subsequently every three months for five years. In case of clinical suspicion of recurrence, additional investigation included biopsy, magnetic resonance imaging (MRI) or CT scan. Median follow-up was seven years. Survival time was counted starting from the first day of radiotherapy.

**Results**

The fate of patients in the investigated group is presented in Table 1. Of the 120 patients in the investigated group, 78 (65%) were disease-free for five years. One patient died of cerebral haemorrhage with no evidence of recurrent disease in the third year after treatment; the second died due to acute sepsis and recto-vaginal fistula occurrence. In 40 (33.4%) patients, the cause of death was uncured cervical cancer. The primary cause of chemoradiotherapy failure in the investigated group of patients was pelvic recurrence, which amounted to 77.5% (31 patients) of treatment failures. Thirteen, i.e. 32.5% of uncured patients, developed distant metastases in lungs (six patients), bones (three patients), liver (three patients), and brain (one patient); in nine (22.5%) cases, it was the only cause of treatment failure.

Symptoms of acute treatment-related toxicity occurred in 112 (93.3%) patients of the study group, as shown in Table 2. Seventy-two (60%) patients developed leukopenia, in 63 (52.5%) of whom of grade 1 or 2, and in nine (7.5%) of grade 3. Anaemia occurred in 33 (27.5%) patients, including 27 (22.5%) cases of grade 1 or 2, four (3.3%) of grade 3, and two (1.7%) of grade 4. Thrombocytopenia of grade 1 or 2 occurred in 14 (11.7%) patients. In total, acute haematological toxicity was observed in 84 (70%) patients with 74 cases of grade 1 or 2 and ten of grade 3 or 4. Among these patients, 56 developed one type of haematological toxicity and 28 two or three types.

Depending on severity of haematological complications, patients were managed with typical procedures including transfusions, administration of erythropoietin derivatives, haemopoietic agents, etc.

### Table 1 – The fate of 120 patients in the investigated group.

<table>
<thead>
<tr>
<th>Fate of patients</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived five years disease-free (five years NED)</td>
<td>78</td>
<td>65.0</td>
</tr>
<tr>
<td>Died of other causes</td>
<td>2</td>
<td>1.6</td>
</tr>
<tr>
<td>Died of cervical cancer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- pelvic recurrence</td>
<td>27</td>
<td>22.5</td>
</tr>
<tr>
<td>- pelvic recurrence+distant metastases</td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td>- distant metastases</td>
<td>9</td>
<td>7.5</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 2 – Acute treatment-related toxicity in the investigated group of patients.

<table>
<thead>
<tr>
<th>Acute toxicity</th>
<th>Toxicity grade (WHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematological toxicity:</td>
<td></td>
</tr>
<tr>
<td>- leucopenia</td>
<td>52.5%</td>
</tr>
<tr>
<td>- anaemia</td>
<td>22.5%</td>
</tr>
<tr>
<td>- thrombocytopenia</td>
<td>11.7%</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>16.7%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>55.0%</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>10.8%</td>
</tr>
<tr>
<td>Acute vaginal mucositis</td>
<td>1.7%</td>
</tr>
</tbody>
</table>
Nausea and vomiting occurred in 24 (20%) cases, 20 (16.7%) of which were of grade 1 or 2, and four (3.3%) of grade 3 or 4. Patients were given typical antiemetic agents including ondansetron and thiethylperazine.

Diarrhoea occurred in 72 (60%) patients; 66 (55%) cases were of grade 1 or 2, and six (5%) of grade 3 or 4. Diﬀerentiation of treatment and typical anti diarrheal drugs were advised; in six cases, severity of rectal reaction was the cause of bleeding.

Urinary tract infection, manifested by pollakiuria, burning sensation, and pain with urination, and even modest haematuria in one case, was observed in 14 (11.6%) patients; treatment-related toxicity was graded 1 or 2 in 13 (10.8%) patients, and 3 in one case.

Two (1.7%) patients developed acute vaginal mucositis of grade 2 toxicity accompanied by slight bleeding from genital tract.

Course of treatment of 120 patients in the investigated group is presented in Table 3. Full-planned treatment was completed in 94 (78.3%) patients of the investigated group.

Twenty-four patients, i.e. 20% of the group, completed full-planned radiotherapy, but were given only three or four cycles of cisplatin. Reduced number of chemotherapy cycles was caused by acute haematological toxicity (ten patients), gastrointestinal disorders of grade 3 or 4 (six patients), disease progression (two patients), signiﬁcant deterioration of patient performance status with exacerbation of accompanying diseases, such as bronchial asthma, diabetes (four patients), further chemotherapy refusal (two patients).

Full planned radiotherapy (tele- + brachytherapy) was delivered to 118 patients, i.e. 98.3% of the investigated group, 114 (95%) of whom completed the treatment without any interruptions within expected time, and four (3.3%) with one- to two-week break in external beam radiotherapy caused by excessive postradiation reaction in pelvis minor.

In two cases, i.e. 1.7% of the patients, teleradiotherapy was discontinued after delivering 40 Gy due to intensiﬁed symptoms of intestine postradiation reaction (bothersome diarrhea). The two patients were given ﬁve cycles of cisplatin and four weeks after teleradiotherapy termination when gastrointestinal reaction abated, full-planned brachytherapy was performed.

Late treatment complications (occurring three months after completion or later) were observed in 18 (15%) patients, including 16 (13.3%) cases of mild complications (grade 1 or 2) and two (1.7%) severe (grade 3 or 4). Table 4 presents late treatment complications observed.

Data presented in Table 4 show that the most frequent late complications of grades 1 or 2 occurring in the investigated group were postradiation changes in large intestine and rectum manifested in persistent diarrhea and bleeding. These symptoms were reported in 11 patients (9.2%) and amounted to over two-thirds of all grade 1 or 2 complications. Three patients (2.5%) developed chronic cystitis and two (1.7%) vagina narrowing.

All of the complications were managed with conservative treatment and, in the majority of cases, the symptoms subsided within few months or were considerably reduced (e.g. vagina narrowing).

Severe (grade 3 or 4) late complications were observed in two patients (1.6%) of the investigated group. In one case, it was narrowing of large intestine requiring surgery, which occurred in the 16th month after the treatment; resection of the narrowed section restored normal intestinal passage. The patient survived three years with no evidence of disease. One patient developed recto-vaginal fistula while local disease progression continued (initially in stage IVA). The patient died showing symptoms of acute sepsis.

Discussion

Of the 120 patients treated in Centre of Oncology in Krakow, ﬁve-year NED was recorded in 78 (65.5%) cases, including 83.3% (55/66) of patients in FIGO Stage IB2 - II, 48.8% (21/43) in Stage III, and 18.2% (2/11) in Stage IVA. The results are generally in line with the literature reports in which long-term survival [4-8 years] of chemoradiotherapy patients in study groups of similar clinical proﬁle varies between 47% and 83% with the range of 70-
Acute toxicity

In 112 of 120 patients, i.e. in 93.3% of the investigated group, acute treatment-related toxicity occurred; 14 (11.6%) of the cases were assigned grade 3 or 4, and the remaining 98 (81.7%) grade 1 or 2. The most frequent was haematological toxicity (84 patients, i.e. 70.0%), manifested particularly often with leucopenia, less frequently with anaemia, and significantly rarer with thrombocytopenia. Acute gastrointestinal complications (nausea, vomiting, and diarrhoea) were observed in 72 (60%) patients with only six (5%) cases of grade 3 or 4 toxicity. Additionally, patients in the investigated group developed urinary tract infection and acute vaginal mucositis, their severity, however, was graded only 1 or 2.

Ninety-four (78.3%) of the patients completed full planned chemoradiotherapy. Twenty-four (20%) completed full-planned radiotherapy without completing full chemotherapy, with three or four cycles of cisplatin instead of five to six cycles. In two (1.7%) patients, teletherap-apy dose was limited to 40 Gy instead of the planned 50 Gy. The reason for limiting the dose of chemoradiotherapy was mostly acute haematological toxicity (ten patients) and then gastrointestinal disorders (eight patients), disease progression (two patients), exacerbation of accompanying diseases (four patients), and further chemoradiotherapy refusal (two patients).

The aforementioned results present the current authors’ observations regarding early chemoradiation toxicity in cervical cancer patients and are entirely consistent with literature reports in terms of type as well as incidence rate. The majority of authors emphasize that haematological, gastrointestinal, and urinary tract disorders constitute an overwhelming part of acute complications and most of them are of grade 1 or 2. In terms of incidence and causes, limitations introduced to the course of planned treatment are also in line with literature data. The authors underscore that in 25% up to 33% of patients, it is not possible to complete full chemoradiotherapy [3, 7, 10, 11, 13, 14, 20-25, 35-39].

In the material analysed by Reig et al. [23], almost all 56 patients developed acute haematological toxicity including anaemia of grade 1-2 in 94.5% of cases and of grade 3-4 in 5.2%; leucopenia of grade 1-2 in 49.9% of cases, and of grade 3 in 30.3%. Acute gastrointestinal toxicity of grade 1-2 was observed in 89.2% of the group and of grade 3 in 10.7%. Acute urinary tract infections of grade 1-2 were reported in 49.1% and of grade 3 in 25.0% of the patients. Acute vaginal mucositis of grade 1-2 occurred in 64.2% of the women and of grade 3 in 16.0% of them. Six cycles of chemotherapy was given to 67.8% of patients, five cycles to 19.6%, and four cycles to 13.5%; the main reason for reducing the number of cycles was leucopenia.

In the 74-patient study group presented by Tana et al., (25) the most common adverse side-effects were: diarrhea in 80.6% of patients, malaise in 66.7%, and nausea in 62.5%. Anaemia of grade 1-2 occurred in 41.7% of the patients and of grade 3-4 in 42%. One patient developed grade 3 thrombocytopenia and another one neutropenia of grade 4. In the group, 97.3% of patients completed external beam radiotherapy and 70.2% of them completed the planned number of chemotherapy cycles.

Late toxicity

Late treatment complications were observed in 18 (15%) cases, 16 (13.3%) of which were mild complications (grade 1 or 2) including persistent diarrhea, rectal bleeding, chronic cystitis, and vagina narrowing. The complications were managed with conservative treatment and, in the majority of patients, the symptoms subsided within few months or were considerably reduced (e.g. vagina narrowing).

In the Lukka et al., meta-analysis of randomized trials of cisplatin-based chemoradiotherapy [8], the reported rates of acute grade 3 or 4 toxicity ranged from four to 47% (mean: 23%) for haematological toxicity, zero to 15% (mean: 9%) for gastrointestinal toxicity, and one to eight percent (mean: 2%) for genitourinary toxicity.

<table>
<thead>
<tr>
<th>Authors, reference entry number</th>
<th>Year of publication</th>
<th>Late toxicity of grade 3 or 4 [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eifel et al. (53)</td>
<td>2004</td>
<td>12.6%</td>
</tr>
<tr>
<td>Totta et al. (50)</td>
<td>2005</td>
<td>2.5%</td>
</tr>
<tr>
<td>King et al. (47)</td>
<td>2006</td>
<td>12.7%</td>
</tr>
<tr>
<td>Chen et al. (54)</td>
<td>2006</td>
<td>14.3%</td>
</tr>
<tr>
<td>Novetsky et al. (49)</td>
<td>2007</td>
<td>6.0%</td>
</tr>
<tr>
<td>Rose et al. (52)</td>
<td>2007</td>
<td>2.8%</td>
</tr>
<tr>
<td>Atahan et al. (51)</td>
<td>2007</td>
<td>8.0%</td>
</tr>
<tr>
<td>Tan and Zahra (22)</td>
<td>2008</td>
<td>18.3%</td>
</tr>
<tr>
<td>Parker et al. (3)</td>
<td>2009</td>
<td>4.0%</td>
</tr>
<tr>
<td>Spensley et al. (20)</td>
<td>2009</td>
<td>9.3%</td>
</tr>
<tr>
<td>Vale et al. (21)</td>
<td>2010</td>
<td>10.0%</td>
</tr>
</tbody>
</table>
A meta-analysis of 19 trials by Kirwan et al. reported 11 toxic deaths, eight acute (sepsis), and three late toxicities (small bowel obstruction, ureteral fibrosis, and pulmonary embolus) [10]. A recent UK series presented by Tan and Zahra reported late grade 3 and 4 toxicity rate of 18.3%, with three toxic deaths. Thirteen of 71 patients (18.3%) had one complication that was classified as grade 3 or 4: 8.5% had urinary complications (frequency, haematuria, and cystostomy), 70% bowel complications (diarrhea, rectal bleeding, ileus, and colostomy), and 8.5% complications affecting other organs (cervix ulcer, sensory or motor neuropathy, vascular necrosis of hips). Five patients had grade 3 or 4 complications affecting more than one organ (22). In the analysis of 75 patients by Spensley et al., late toxicity was reported in seven (9.3%), including three patients with bowel toxicity requiring surgery (rectal fistula, sigmoid stricture, and small bowel obstruction), three patients with bladder toxicity, and one patient with vaginal stenosis [20]. In the Vale et al. study to present the results of Royal College of Radiologists audit (2001-2002), grade 3/4 late toxicity was observed in ten percent of 471 patients treated with CCRT + RT; the complications occurred in vagina (5%), rectum (3%), colon (1.5%), small bowel (1%), and bladder (2%) [21]. Of the 92 patients presented by Parker et al., four (4%) had late toxicities of grade 3 or 4 (recto-vaginal fistula requiring colostomy, delayed osteonecrosis of the hips requiring total hip replacement, vaginal bleeding requiring hysterectomy, and vesico-vaginal fistula requiring the formation of an artificial bladder) [3].

CCRT vs. RT alone (acute and late toxicity)

In the literature, the discussion is held whether CCRT does increase severity of acute and late toxicity [3, 5, 7-10, 13, 14, 20-25, 27-30].

In the meta-analysis presented by Green et al. (2001) and Lukka et al. (2002), late toxicity of CCRT was examined; no differences were found in the rates of bowel or bladder toxicities between patients treated with RT alone and those treated with CCRT [7, 8].

In 2003, Kirwan et al. showed that grade 1 and 2 acute haematological toxicities were higher in CCRT than in RT alone group and significant differences were seen in grade 3 and 4 acute haematological and gastrointestinal toxicities; however, the authors concluded that “in view of the consistency and extent of the survival benefit for CCRT, the additional acute toxicity appears to be acceptable” [10].

Published in 2008, meta-analysis from Meta-Analysis Group, Medical Research Council Clinical Trials Unit (Vale et al.), showed that acute haematological and G1 toxicity was increased with CCRT, but data was too sparse for an analysis of late toxicity [24].

The study presented by Tan and Zahra showed that the addition of chemotherapy to radiotherapy for cervical cancer increased risk of serious late toxicity [22], while Parker et al. reported in their study that the presented regimen (CCRT + high-dose rate brachytherapy) is effective with acceptable long-term side effects [3].

Spensley et al. established in their study that late toxicity rate increased to 9.3% compared with 3.4% reported by Denton et al. in 2000 [48] for national audit patients treated in 1993; the increase, however, was not statistically significant. Acute toxicity is increased in CCRT “but with careful monitoring and evaluation of the patient during treatment is manageable” [20].

In 2010, Vale et al. presented results of a Royal College of Radiologists audit. In the group of 355 patients treated with RT only, late complications of grade 1 and 2 were found in 43% of women, and of grade 3 and 4 in eight percent; whereas in the CCRT group, the rate was 47% and 10%, respectively, “without an apparent rise in late complications” [21].

The acute and long-term toxic effect of CCRT is one of major challenges in LACC patients. Attempts to limit the toxic effect are currently focused on three major fields: investigation of new cytotoxic chemotherapy agents (gemcitabine and topotecan) and biologically targeted agents (3-AP, tirazapamina, and avastin); more sophisticated radiology for radiotherapy planning (CT, MRI, positron emission tomography - PET), as well as advances in radiotherapy technique (intensity-modulated radiation therapy – IMRT, image-guided brachytherapy), and improved supportive care (antiemetic, growth factors) [5, 14, 20-22, 30].

Conclusions

In patients with LACC treated with CCRT, the most frequent acute toxic effects include: haematological disorders (anaemia, neutropenia, thrombocytopenia), gastrointestinal disorders (nausea and vomiting, and diarrhea), vulvovaginal disorders, and urinary tract infection; the most frequent late toxic effects include: gastrointestinal disorders (diarrhea, rectal bleeding, bowel complications requiring surgery), vaginal disorders (fibrosis, stenosis, recto–vaginal fistula), urological disorders (frequency, haematuria, vesico-vaginal fistula, cystectomy), and cervix ulcer. Rate and severity of CCRT toxicity may be reduced by investigation of new agents, advances in radiation therapy, and optimal supportive care.

References


Toxicity of concurrent chemoradiotherapy for locally advanced cervical cancer


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En-bloc pelvic resection with concomitant rectosigmoid colectomy and immediate anastomosis as part of primary cytoreductive surgery for patients with advanced ovarian cancer

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Summary

Objective: To assess the authors’ experiences in en bloc pelvic resection with concomitant rectosigmoid colectomy and primary anastomosis as a part of primary cytoreductive surgery for patients with advanced ovarian cancer. Materials and Methods: A total of 22 patients with FIGO Stage IIB-IV epithelial ovarian cancer who underwent en bloc pelvic resection with anastomosis were retrospectively reviewed. Data analyses were carried out using SPSS 10.0 and descriptive statistics, Kaplan-Meier survival curves, and Log Rank (Mantel-Cox) test were used for statistical estimations. Results: Median age was 58.8 years. FIGO stage distribution of the patients was; one (4.5%) IIB, three (13.7%) IIC, three (13.7%) IIIA, six (27.3%) IIIB, and nine (40.9%) IIIC. Median peritoneal cancer index (PCI) was 8 (range 5-22) and optimal cytoreduction was achieved in 18 patients (81.8%) of whom 13 (59.1%) had no macroscopic residual disease (complete cytoreduction). There was no perioperative mortality. A total of nine complications occurred in seven (31.8%) patients. Anastomotic leakage was observed in one (4.5%) patient. There was no re-laparotomy. Mean follow-up time was 60 months. There were 15 (68.2%) recurrences of which 12 (80%) presented in extra-pelvic localizations. Mean disease-free survival (DFS) and overall survival (OVS) were estimated as 43.6 and 50.5 months, respectively. Patients with complete cytoreduction had a better DFS ($p = 0.006$) and OVS ($p = 0.003$) than those with incomplete cytoreduction. Conclusion: En bloc pelvic resection, as a part of surgical cytoreduction, seems to be a safe and effective procedure in many patients with advanced ovarian cancer if required. Despite relatively high general complication rate, anastomosis-related morbidity of this procedure is low as 0.8%. Nevertheless, surgical plan and perioperative care should be personalized according to medical and surgical conditions of the patient.

Key words: Ovarian cancer; Cytoreductive surgery; En-bloc pelvic resection; Rectosigmoid colectomy; Primary anastomosis.

Introduction

Surgical cytoreduction is the cornerstone in the treatment of ovarian cancer. In addition to directly eliminating tumor burden, it has indirect contributions to chemotherapy by means of improving drug delivery, increasing the growth fraction (rate of tumor cells in the proliferative phase), reducing and reversing drug resistance, and improving immunologic, intestinal, and metabolic functions. Ovarian cancer is the prototype of tumors in which maximal/optimal cytoreduction is one of the major and most powerful determinations of survival [1-8]. In a meta-analysis, Bristow et al. reported that each 10% of increasing in optimal cytoreduction rate resulted in two months of prolonging in overall survival (OVS) (22.7 months vs. 33.9 months) [8]. With these perspectives, in the last two decades, for achieving optimal cytoreduction, significant changes took place in surgical paradigm of ovarian cancer treatment by shifting from conservative surgical techniques to more complex, comprehensive, and ultra-radical procedures. Chi et al. from MSKCC reported about two-fold increased optimal cytoreduction rates (46% vs 80%) and 11 months median OVS advantage by means of performing extensive upper abdominal and pelvic surgical procedures in years 2001-2004 compared to 1996-1999 [9].

En-bloc pelvic resection, which is also known as “Hudson’s pelvic deperitonealization procedure”, “modified posterior exenteration”, “reverse hysterocolposigmoidectomy” or “radical oophorectomy”, is an old technique first described by Hudson in 1968 for eliminating gross pelvic disease in locally advanced ovarian cancer. This technique, of which majority similar to radical hysterectomy, removes entire pelvic tumor together with adjunctive structures including internal genitalia, rectosigmoid colon, “cul de sac” of Douglas, and bladder peritoneum by using retroperitoneal and retrograde approach. It is usually considered that it allows safe, prompt, and bloodless tumor removal with the assistance of using “cul de sac” as a pseudo-capsule. In Hudson’s series, between 1965 and 1972, a total of 25 cases were treated with this method. There were only one (4%) intraoperative and five (20%) disease-related deaths. At the end of study period, 17 patients (68%) were alive and dis-
ease-free. In light of these results, the authors concluded that only time will show whether or not this procedure has a salvage effect, but it is clear that the results are not worse than those removing the tumor partially with cutting the tumor tissue [10, 11].

During second half of last century and 2000s, many studies have focused on the technical details, feasibility, morbidity, and long-term (survival) effect of this procedure in patients with advanced ovarian cancer [12-30]. In the present study, the authors aimed to report their experiences in en bloc pelvic resection with concomitant rectosigmoid colectomy and immediate (primary) anastomosis as a part of primary cytoreductive surgery for patients with advanced ovarian cancer.

Materials and Methods

The authors retrospectively reviewed medical charts of a total of 426 patients who underwent cytoreductive surgery for FIGO Stage IIIB-IV epithelial ovarian cancer at Ege Gynecology & Obstetrics Training and Research Hospital and Erzincan University School of Medicine between 2003 and 2013; for identifying en bloc pelvic resection cases. Patients who had borderline ovarian tumor, neoadjuvant chemotherapy, prior radiation therapy, prior hysterectomy, Hartmann’s procedure, and en bloc pelvic resection for secondary or tertiary cytoreduction were excluded. A total of 22 patients who had been performed en bloc resection and primary anastomosis performed for upfront surgery consisted of final material of the study. Surgical and pathologic reports of these cases were re-reviewed for disease-related, surgical, postoperative factors, and long-term results.

The patients received liquid diet one day prior to surgery and mechanical bowel preparation (MBP) was not used except in two patients. No antibiotic bowel preparation was used. For infection prophylaxis, a combination of cefuroxime axetil 0.75 gr iv and metronidazole 0.5 gr iv one hour before surgery was administered, and this regimen was continued to use during postoperative five days in case of a gross colonic contamination was present. All patients also received low molecular weight heparin enoxaparin 400 anti-Xa/0.4 ml 1x1 or 2x1 sc for the prevention of venous thrombosis for three to 15 days following surgery depending on their thrombosis risk categories. The patients were encouraged for early mobilization at first day of surgery if not contraindicated and early oral intake was also started as soon as possible when bowel movement returned.

All surgical operations were carried out by experienced surgeons in the fields of general or gynecological oncologic surgery. After midline vertical incision, firstly the authors evaluated the resectability of disease especially located at upper abdomen and small bowel and its mesentery. Surgical effort was first directed to the resection of upper abdominal disease and if an optimal resection in this area had been achieved, pelvic procedure was then begun. Optimal cytoreduction was accepted as ≤ one cm maximum residual tumor size in accordance with Gynecologic Oncology Group (GOG) definition [6]. En bloc pelvic resection retrieving en bloc specimen of uterus, ovaries, rectosigmoid, cul de sac of Douglas, and anterior pelvic and bladder peritoneum was planned in only cases of pan pelvic disease with en bloc pelvic viscera involvement (Figure 1). In all cases the procedure was performed as described by McCartney and Hudson [12] and all en bloc resections were of type II radical oophorectomy (Figure 2) according to Bristow et al.’s classification [13]. During the preparation of rectosigmoid colon for resection, in all cases except two, inferior mesenteric artery (IMA) was preserved with low ligation of left colic artery branch. Colorectal/ileoanal anastomoses were carried out in hand-sewn manner using 3/0 silk and single layer closure. Because of routine omentectomy, an omental J flap could not have been used for additional support to anastomoses. After controlling anastomosis patency with both finger examination and bubble test, a pelvic drain was placed into Douglas and abdomen was closed.

Data evaluation was carried out using SPSS 10.0. Descriptive statistics, Kaplan-Meier survival curves and Log Rank (Mantel-Cox) test were used for statistical analyses.
Table 1. — Pre-treatment and disease-related characteristics of the patients (n = 22).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients (%)</th>
</tr>
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<tbody>
<tr>
<td>Median age (range)</td>
<td>58.8 yrs (35-69)</td>
</tr>
<tr>
<td>Median BMI (range)</td>
<td>25.3 kg/m² (21-30)</td>
</tr>
<tr>
<td>Median albumin (range)</td>
<td>3.8 gr/dl (3.4-4.2)</td>
</tr>
<tr>
<td>Median Hb (range)</td>
<td>12.6 gr/dl (12.1-13.9)</td>
</tr>
<tr>
<td>Co-morbidity</td>
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<tr>
<td>Hypertension</td>
<td>7 (31.8%)</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>2 (9.0%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (9.0%)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1 (4.5%)</td>
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<td>ECOG performance*</td>
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<tr>
<td>0</td>
<td>9 (40.9%)</td>
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<tr>
<td>1</td>
<td>9 (40.9%)</td>
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<tr>
<td>2</td>
<td>4 (18.2%)</td>
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<td>ASA class **</td>
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<tr>
<td>1</td>
<td>7 (31.8%)</td>
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<tr>
<td>2</td>
<td>11 (50.0%)</td>
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<td>3</td>
<td>4 (18.2%)</td>
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<td>Tumor histology</td>
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<tr>
<td>Serous</td>
<td>17 (77.3%)</td>
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<tr>
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<tr>
<td>Clear cell</td>
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<td>Mucinous</td>
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<tr>
<td>Grade</td>
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<tr>
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<td>1 (4.5%)</td>
</tr>
<tr>
<td>2</td>
<td>6 (27.3%)</td>
</tr>
<tr>
<td>3</td>
<td>15 (68.2%)</td>
</tr>
<tr>
<td>FIGO stage***</td>
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<tr>
<td>IIIB</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>IIC</td>
<td>3 (13.7%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>3 (13.7%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>6 (27.3%)</td>
</tr>
<tr>
<td>IIIC</td>
<td>7 (31.8%)</td>
</tr>
<tr>
<td>IVA</td>
<td>2 (9.0%)</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10 (45.5%)</td>
</tr>
<tr>
<td>Present &lt; 1000 ml</td>
<td>5 (22.7%)</td>
</tr>
<tr>
<td>Present ≥ 1000 ml</td>
<td>7 (31.8%)</td>
</tr>
</tbody>
</table>

* Eastern Cooperative Oncology Group (ECOG)
** American Society of Anesthesiologists Classification of Physical Status (ASA)
*** International Federation of Gynecology and Obstetrics (FIGO)

Results

1. Patients and Disease Characteristics

    Median age was 58.8 years with the range of 35-69. There was no any patients with history of previous intestinal surgery, inflammatory bowel disease, and immunosuppressive or corticosteroid medication. Median pre-operative serum albumin value was 3.8 gr/dl and median pre-operative Hb level was 12.6 gr/dl. FIGO stage distribution of the patients was; one (4.5%) IIIB, three (13.7%) IIC, three (13.7%) IIIA, six (27.3%) IIIB, and nine (40.9%) IIIC. The most common histological type (17/22: 77.3%) was serous. Ascites were observed in 12 cases (54.5%). Demographic, preoperative, and disease-related characteristics are summarized in Table 1.

2. Surgical Results

    Median duration of surgery was 244 minutes (range: 135-420) and median estimated blood loss (EBL) was 800 ml (range: 200-2400). Five patients (22.7%) required blood (packed red blood cell-PRBC) transfusion and mean unit of PRBC transfused was 1.6 units (0-7). Median peritoneal cancer index (PCI) was 8 (5-22). Optimal cytoreduction was achieved in 18 (81.8%) of whom 13 (59.1%) had no macroscopic residual disease (complete cytoreduction). In eight patients (36.4%), at least one upper abdominal surgical procedure other than omentectomy was performed. Retroperitoneal lymph node dissection (RPLND) was performed in all patients (n = 13) who underwent a complete cytoreduction for their intra-peritoneal disease. Anastomosis types were colo-rectal in 20 (91.0%) patients and ileo-rectal in two (9.0%) patients. Two patients (9.0%) had also small bowel anastomosis in addition to...
their colo-rectal anastomoses. All colo-rectal or ileo-rectal anastomoses were below the peritoneal reflection and in 12 patients (54.5%) anastomosis was within the seven-cm to anal verge. Median anastomosis distance to anal verge was 7.5 cm with the range of six and ten cm. In pathologic evaluation, muscular layer involvement of rectosigmoid with or without mucosal extension was described in 19 of 22 patients (86.4%). Table 2 shows surgery-related factors and operative details of the patients.

3. Post-operative Characteristics and Complications

Median length of intensive care unit (ICU) stay was one day (range: 0-3). Two patients (9%) needed ICU stay of >48 hours. Median duration of nasogastric suction was 1.6 days and median time to tolerance of diet was 2.8 days. There was no perioperative mortality within 30 days of surgery. A total of nine complications occurred in seven (31.8%) patients (Table 3). There was no post-operative intraperitoneal hemorrhage, intestinal fistula, and septicemia. Clinical apparent anastomotic leakage was observed in only one (4.5%) patient who had a rectosigmoid anastomosis. The patient presented with fever, leucocytosis, and fistula (a stream of bowel content from abdominal incision) at seventh day of surgery. Due to low out-put of fistula (daily output was about 150 ml) and the absence of septicemia findings the patient was managed conservatively with using board spectrum systemic antibiotics, regulating fluid and electrolyte balance, and administrating parenteral and enteral nutrition (two gr/kg/d amino acid and 1,500 kcal/day calories) under close clinical observation. She was discharged at 13th day of surgery after removing her pelvic drain. Total time to completely closure of fistula was 30 days. There was no re-laparotomy for any complication in this series. Median length of hospitalization was eight days ranging from six to 13 days.

4. Follow-up and Long-term Outcomes

All patients received adjuvant chemotherapy in a combination of paclitaxel (175 mg/m²) and carboplatin (5 AUC) with ranging from five to nine cycles. All chemotherapies were begun after third week of surgery and there was no chemotherapy administration during hospitalization period. Monthly tumor marker (Ca 125) test and three-month clinical and radiological examinations were performed during follow-up period. Mean follow-up time was 60 months (range: six to 120 months). There was no fecal incontinence or anastomosis stenosis. There were 15 (68.2%) recurrences of which 12 (80%) presented in extra-pelvic localizations. A secondary cytoreductive surgery was performed for eight of these 15 recurrences (53.3%) and an optimal cytoreduction was achieved in four (50%) of them. At the end of study period, 11 patients were alive and disease-free, seven

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomosis leak</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Prolonged (≥10 days) ileus</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Wound infection</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>2 (9.0%)</td>
</tr>
<tr>
<td>Deep venous thrombosis</td>
<td>1 (4.5%)</td>
</tr>
</tbody>
</table>

Figure 3. — Disease-free survival (DFS) according to completeness of cytoreduction of 22 patients who underwent en bloc pelvic resection and primary anastomosis (complete vs incomplete)

Figure 4. — Overall survival (OVS) according to completeness of cytoreduction (complete vs incomplete)
patients were alive with a recurrence, and four patients died from disease. Mean disease-free survival (DFS) and overall survival (OVS) were estimated as 43.6 and 50.5 months, respectively. Patients with complete cytoreduction (n = 13) had a better DFS (52.7 months vs. 30.5 months; \( p = 0.006 \)) and OVS (59.3 months vs. 37.8 months; \( p = 0.003 \)) than those with having any amount of macroscopic residual disease (n = 9) (Figures 3 and 4).

Discussion

Initial treatment of epithelial ovarian cancer is generally upfront (primary) cytoreductive surgery. In the authors’ clinical practice, especially since 2008, they aim to perform primary cytoreductive surgery in all patients if the patient does not meet neoadjuvant chemotherapy use criteria [31] and her general condition is appropriate for comprehensive surgical procedures and multi-visceral organ resections. Despite the lack of prospective data, several studies have suggested that major determination of long-term survival in patients with advanced ovarian cancer is whether primary surgery is optimal regardless of the definition used for “optimal” [2-9]. Nevertheless, recent reports have noted the importance of optimal reductions (complete) cytoreduction and suggested the status of “no visible disease” as “optimal” [5, 6, 32]. In the present study, optimal (≤ one cm maximum size of residual tumor) and complete cytoreductions were about 82% and 59%, respectively. The authors found that, in accordance with previous two reports [27, 28], patients underwent en bloc resection with a complete cytoreduction (vs. incomplete cytoreduction) had significantly better disease-free and overall survival curves.

It is well known that en bloc resection is clearly effective in providing complete clearance of pan pelvic disease in patients with advanced ovarian cancer. It also seems to be useful to prevent loco-regional (pelvic) recurrence with the increasing evidence of tendency of recurrences to have extra-pelvic location in patients who experience any recurrence after en bloc resection [13, 28]. Bristow et al. performed second-look surgery [14 (laparoscopy and one laparotomy)] in patients with radical oophorectomy and noted that 46.6% rate of persistent disease but no patient was found to have persistent pelvic disease [13]. Also, Sainz de la Cuesta et al. reported that 62% of relapses following en bloc resection were found in extra-peritoneal locations [19]. In the present series, at the end of study period, 68.2% of the patients developed a recurrence of which only 20% was in the pelvis. The present findings support results of previous reports regarding recurrence pattern after en bloc resection of ovarian cancer. On the other hand, some reports comparing en bloc resection and pelvic peritoneal stripping claimed the lack of advantage of en bloc resection in terms of long-term oncologic results in case of optimal cytoreduction. In an Italian study, Gallotta et al. performed recto-sigmoid resection (RR) in 71 patients (38%) and Douglas (pelvic) peritomeum (PP) in 116 (62%). In their series, the estimated mean DFS was 30.7 months (95% CI 24.6-36.8) in the RR arm and vs. 25.9 months in the PP arm (95% CI 33.4-44.2) (\( p = 0.29 \)). Similarly, no statistically significant difference was found in terms of OS (\( p = 0.12 \)) [30]. Aletti et al., in a study with 209 patients, reported five-year survival rates of 39%, 37%, and 6% for rectosigmoidectomy (RS), stripping of the peritoneum (SoP), and neither subgroups; respectively (\( p = 0.02 \)). When evaluating patients with no macroscopic residual disease, a survival advantage for patients managed with RS compared with SoP was observed (five-year overall survival, 89% (RS) vs 50% (SoP) (\( p = 0.04 \)) [22].

Intraoperatively, especially in young women with advanced disease, it can be difficult to decide to perform en bloc resection because of the possibility of co-existing non-cancer conditions that involve pelvic viscera together as in cases of co-existent deeply infiltrating endometriosis and dense inflammatory (fibrous) adhesions. In different series, a histopathological discrepancy (co-existing benign adhesive diseases in surgical specimens) after en bloc pelvic resection was reported between the rates of 20% and 27% [27, 33]. Also, in a previous study it was reported that true (beyond the serosa/subserosa) rectosigmoid colon involvement was detected in about 28.3% in patients who underwent en bloc resection [27]. In the present study, a histologically confirmed muscular or deeper involvement was as high as 86.4%. Three patients (13.7%) had serosal/subserosal disease alone and there were no false-positive cases and co-existing benign inflammatory processes. In the present authors’ opinion, pelvic surgeon should not hesitate to perform en bloc resection in patients with advanced ovarian cancer with pelvic disseminated disease, even in cases of suspicious tumoral invasion of rectosigmoid because of a general low rates of false-negative specimen and a high rate of secondary problems (such as pain and obstruction) owing to co-existing severe inflammatory pelvic disease if present.

Looking at the literature of both ovarian and colorectal cancers, the authors have seen a generally low and acceptable morbidity rates after anterior and low anterior resections of rectosigmoid colon with concomitant colorectal anastomosis [13, 18, 20, 21, 27-29, 34]. In Table 4, the authors summarize recent literature on the morbidity of en bloc resection and primary anastomosis for ovarian cancer. An anastomotic leakage, which is the major complication of the procedure, was reported in between the rate of 0.8% and 8.7% [13, 18, 20, 21, 27, 28] with the accordance of the present 4.5% of leakage rate.

The reported major determinants of anastomotic leakage in previous studies were preoperative nutritional status (albumin level < 3.0 gr/dl vs ≥ 3.0 gr/dl) and distance between anastomosis and anal verge (< 7.0 cm vs ≥ 7.0 cm) [17, 20, 27, 34]. The roles of age, body mass index, perioperative hemoglobin level, ascite, peritoneal disease vol-
ume (intra-abdominal tumor burden), diabetes mellitus, duration of surgery, estimated blood loss, additional bowel surgery, pelvic drain use, protective ostomy, technique of anastomosis (stapled vs hand-sewn), and type of mesenteric dissection (high ligation of inferior mesenteric artery-IMA vs left colic branch ligation) are unclear [21, 27, 34].

In the present study, the authors were able to perform a tension-free anastomosis without the need for high ligation of IMA in 20 of the patients (90.9%). They are generally trying to avoid any type of protective ostomy as much as possible unless there is a history of pelvic radiation therapy and peritonitis because it cannot reduce anastomotic complication rates despite of increasing operative time, having high frequency of ostomy-related early and long-term problems, decreasing quality of life, and indicating the need a second surgery to closure [21, 27, 34]. In this series, due to the relatively small sample size, the authors were not able to test the role of several factors on anastomotic leakage. Nevertheless; considering high rate of low level of anastomosis (54.5%) and none of patients with a protective ileostomy or colostomy, an anastomotic leakage seems to be a safe and effective procedure in many patients with advanced ovarian cancer after en bloc resection of ovarian cancer. In this series, due to the relatively small sample size, the authors were not able to test the role of several factors on anastomotic leakage. Nevertheless; considering high rate of low level of anastomosis (54.5%) and none of patients with a protective ileostomy or colostomy, an anastomotic leakage rate of 4.5% can be considered to be too ambitious. The authors believe that meticulous surgical technique allowing a tension-free, water-tight, and suitable vascularized anastomosis, peri-operative nutritional and blood product support, and early out of bed are the crucial factors and the best ways in avoiding from anastomotic complications regardless of other surgical, technical, and medical factors.

As a result, definitive conclusion based on the present findings is not possible because the study has the major limitation of its retrospective nature and small sample size. With the evaluation of literature data, however, as a part of surgical effort for optimal cytoreduction with macroscopic clearance, en bloc pelvic resection together with re-anastomosis seems to be a safe and effective procedure in many patients with advanced ovarian cancer if required. In fact,
there are no differences between colonic re-anastomosis and Hartmann’s procedure in aspect of quantity of complications, estimated blood loss, and duration of surgery in advanced ovarian cancer patients undergoing bowel resection for surgical cytoreduction [38]. In spite of slightly increased general post-operative complication, anastomosis-related morbidity of this procedure is as low as 0.8%. En bloc resection when needed, the pelvic surgeon should not only be responsible for adhering to the fundamental surgical principles, but also personalize surgical technique and perioperative care according to medical and surgical conditions of the patient.

References


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Apparent diffusion coefficient on 3.0 Tesla magnetic resonance imaging and prognostic factors in breast cancer

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Summary

Aim: The aim of the study was to evaluate whether the apparent diffusion coefficient (ADC) provided by 3.0 Tesla diffusion-weighted imaging (3T DWI) varies with the prognostic factors Ki67 and grading in invasive breast cancer. Materials and Methods: Seventy-three patients with 75 invasive breast cancer lesions who had undergone 3.0 Tesla magnetic resonance imaging (MRI) for local staging were enrolled. All lesions were confirmed by histologic and immunohistochemical analysis. MRI included both dynamic contrast-enhanced and DWI sequences. ADC value was obtained for each lesion. Histologic tumor grade was established according to the Nottingham Grading System (NGS), while Ki67 expression was evaluated by MM1 clone IgG1 mouse anti-human monoclonal antibody. Patients were divided into the following groups: grade 1 (G1), grade 2 (G2), grade 1 plus grade 2 (G1+G2) and grade 3 (G3), and low Ki67 (≤ 14%), intermediate Ki67 (15%-30%), and high Ki67 (≥30%). ADC values were compared with the G and Ki67 groups. Statistical comparison was carried out using the Mann-Whitney U and the Kruskal-Wallis H test. Results: ADC values were significantly higher in G3 than in G1+G2 tumors; no significant difference was observed when G1, G2, and G3 were compared. There was no statistically significant correlation between ADC values and Ki67 percentage (p > 0.05). Discussion: ADC values obtained on 3T DWI correlate with low (G1+G2) and high-grade (G3) invasive breast carcinomas. Conclusion: ADC may be a helpful tool for identifying high-grade invasive breast carcinoma.

Key words: Apparent diffusion coefficient; Diffusion-weighted imaging; Invasive breast cancer; Grading; Ki67.

Introduction

Breast cancer is a heterogeneous group of diseases with various morphologies, clinical courses, and response to treatment [1,2]. Biological markers such as hormone receptor (HR) expression, human epidermal growth factor receptor 2 (HER2) status, and proliferation defined by Ki67 labeling index have been used together with traditional parameters such as tumor size, grade, and axillary lymph-node status to estimate prognosis and to predict the efficacy of adjuvant post-surgical treatment [3, 4]. Histologic grade which is currently evaluated according to the Nottingham Grading System (NGS), is an accurate and reproducible method for establishing breast cancer prognosis [5, 6].

In breast cancer, the proliferation marker Ki67 is commonly used as a complement to grading systems that include mitotic counting as a sign of proliferation [7]. Ki67 index may provide additional prognostic information in grade 2-intermediate risk tumors, which may be subdivided on the basis of Ki67 expression into low and high-risk populations. Evaluation of Ki67 is also useful for guiding management decisions [8–10]. To improve the accuracy of magnetic resonance imaging (MRI) in breast lesions, diffusion-weighted pulse sequences have been investigated by many authors [11,12]. Diffusion-weighted imaging (DWI) detects the random free water motion of molecules due to kinetic energy (brownian motion), which is influenced by tissue cellularity and cellular membrane permeability. Signal intensity in DWI is inversely proportional to the degree of water molecular diffusion, which is influenced by histological features; in other words, DWI signal intensity reflects the histological structure of the tissues [13–16].

In addition to an eye-based qualitative evaluation of images, it is possible to quantify the diffusion coefficient of water by measuring the apparent diffusion coefficient (ADC). The ADC value depends on many factors, but first of all on tissue cellularity, and ADC values can therefore provide information which would otherwise not be available [14, 17].

The aim of this study was to evaluate whether ADC values vary with Ki67 labeling index and grading in invasive breast cancer.

Materials and Methods

In this retrospective study, all MRI examinations performed at the Department of Radiological Sciences for local staging of breast cancer between April 2011 and February 2013 were reviewed. Only patients who met the following inclusion criteria were enrolled in the study: (a) MRI was performed using a 3.0 Tesla magnet, (b) both dynamic contrast-enhanced MRI and DWI sequences were performed, (c) diagnosis was confirmed by pathological

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doi: 10.12892/ejgo24792014
analysis after surgery or core biopsy; (d) all histologic analyses comprised immunohistochemical Ki67 assessment.

Patients whose images were not of a good diagnostic quality were excluded from the study.

All MRI examinations were performed on a 3T system using a dedicated eight channel breast coil and with the patient in the prone position.

After localizer sequences in three orthogonal planes, the following sequences were acquired:
1) Axial T2-weighted single shot fast spin echo sequence using Dixon technique for separation of intravoxel water from fat [18] (TR/TE 3500 - 5200 / 120-135 ms, matrix 352 x 224, FOV 370 x 370, NEX 1, slice thickness 3.5 mm); 2) Axial single shot fat suppressed echo-planar diffusion weighted sequence (TR/TE 2700 / 58 ms, matrix 100 x 120, FOV 360 x 360, NEX 6, slice thickness 5 mm) with sensitizing diffusion gradient applied along x, y, z axis, and with a b value of 0 and 1000 sec / mm²; 3) Axial T1-weighted 3D gradient echo fat suppressed (TR/TE 6.6/4.3 ms, flip angle 10°, matrix 512 x 256, NEX 1, slice thickness 2.4 mm); before and five times after contrast administration.

Contrast medium was gadobenate-dimeglumine administered in a concentration of 0.2 mmol/kg injected through a 20 G intravenous cannula at the rate of two ml/sec using an automatic injector and followed by infusion of 20 ml saline solution at the same speed. Subtracted images were obtained from DCE-MRI (early post-contrast minus pre-contrast). All MR pulse sequence parameters are shown in table 1.

Images were transferred to a workstation for post-processing. For a quantitative analysis of the data obtained at DWI, parametric ADC maps were generated by Functool software.

ADC value was obtained by tracing a region of interest (ROI) within the lesion margins and calculated according to the following equation: ADC= (-1/b) ln (S0/S1), where b is the diffusion factor, S0 is the attenuated signal (b value of 1000 s/mm²) and S1 is the full spin-echo signal without diffusion gradient (b value of 0 s/mm²) [13].

MRI images were reviewed in consensus by two radiologists with nine and four years’ experience with breast MRI; both were blinded to clinicopathological findings except diagnosis of invasive breast cancer.

In order to standardize image analysis as much as possible, the radiologists reviewed first subtracted images analyzing:
1) Shape (lobulated, regular, irregular, spiculated) of the main index lesion and possible additional foci (iso-hyperintense compared to the glandular parenchyma);
2) Enhancement pattern (homogeneous, inhomogeneous);
3) Enhancement kinetics assessed by intensity/time curve;
4) Size of the index lesion defined as the greatest diameter of the lesion on subtracted images;

Additional foci were considered only if > five mm. Multifocality was diagnosed in the presence of multiple foci of malignancy in the same breast quadrant. Multicentricity was diagnosed when two or more foci of malignancy were found in more than one quadrant. Bilaterality was diagnosed if neoplastic lesions were found in both breasts (bilateral synchronous breast cancer) [7].

Subtracted images were subsequently superimposed on DWI images (b = 1000 s/mm²) and ADC maps for cancer lesion detection, and ROI was manually drawn on the slice in which the lesion reached the greatest diameter. In all cases ROI was circular and measured three to six mm in diameter. In case of heterogeneous tumors, ROI was drawn to avoid necrotic areas; in case of multifocal or multicentric lesions, ROI was positioned in the largest malignant lesion. A general pathologist performed histopathologic analysis.

Table 1.— MR pulse sequence parameters.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial T2 IDEAL</td>
<td>TR/TE (ms)</td>
</tr>
<tr>
<td></td>
<td>3500-5200 / 120-135</td>
</tr>
<tr>
<td>Matrix</td>
<td>352 x 224</td>
</tr>
<tr>
<td>FOV (mm)</td>
<td>370 x 370</td>
</tr>
<tr>
<td>NEX</td>
<td>1</td>
</tr>
<tr>
<td>Slice thickness (mm)</td>
<td>3.5</td>
</tr>
<tr>
<td>Axial T2 EPI DW</td>
<td>TR/TE (ms)</td>
</tr>
<tr>
<td></td>
<td>2700 / 58</td>
</tr>
<tr>
<td>Matrix</td>
<td>100 x 120</td>
</tr>
<tr>
<td>FOV (mm)</td>
<td>360 x 360</td>
</tr>
<tr>
<td>NEX</td>
<td>6</td>
</tr>
<tr>
<td>B-values (s/mm²)</td>
<td>0-1000</td>
</tr>
<tr>
<td>Slice thickness (mm)</td>
<td>5</td>
</tr>
<tr>
<td>Axial T1VIBRANT</td>
<td>TR/TE (ms)</td>
</tr>
<tr>
<td></td>
<td>6.6 / 4.3</td>
</tr>
<tr>
<td>Flip angle</td>
<td>10°</td>
</tr>
<tr>
<td>FOV (mm)</td>
<td>380 x 380</td>
</tr>
<tr>
<td>Matrix</td>
<td>512 x 256</td>
</tr>
<tr>
<td>NEX</td>
<td>1</td>
</tr>
<tr>
<td>Slice Thickness (mm)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Morphological features were evaluated using slices stained with hematoxylin-eosin. Cellular density was determined by counting nuclei of cancer cells in ten high-power fields (x 400) for each lesion and the mean value was recorded.

Histologic tumor grade was established according to the NGS and patients were subdivided into four groups: grade 1 (G1), grade 2 (G2), grade 1 plus grade 2 (G1+G2) and grade 3 (G3).

MM1 clone IgG1 mouse anti-human monoclonal antibody was used to evaluate Ki67 percent expression. The staining identified the Ag cellular membrane expression; 200 neoplastic cells from at least two randomly chosen high power fields were manually counted [1, 19].

Patients were divided into three groups also on the basis of percentage of Ki67 expression: low Ki67: ≤ 14%; intermediate Ki67: 15% - 30%; high Ki67: > 30%.

ADC was considered as a continuous dependent variable, while independent variables were tumor grade (G1 vs G2 + G3 and G1+G2 vs G3) and Ki67 percentage (≤ 14% vs 15% - 30% vs >30%). ADC values, tumor grade and Ki67 did not follow a normal distribution, hence median and ranges were therefore chosen to calculate summary statistics. Statistical comparison was carried out using the Mann–Whitney U test (two-group comparisons) and the Kruskal-Wallis H test (multiple-group comparisons). The relationship between ADC values, grading, and percentage of Ki67 expression was examined. A p-value of < 0.05 was considered to be statistically significant. The data are expressed as medians. All statistical analyses were performed using R for Windows 2.15.3 version (R Foundation for Statistical Computing, http://www.r-project.org).

Results

Of the 302 pre-surgical breast MR examinations which were reviewed, 114 were excluded because they were performed on 1.5 T, 30 were excluded as no suspicious abnormality was visible on the dynamic images, 70 were excluded
because the patients were lost to the present institution; seven were excluded because Ki67 labeling index was not established at histologic analysis; three were excluded because DWI sequences showed motion or distortion artifacts, and five were excluded because the lesions were not visible on DWI sequences.

A total of 73 MRI examinations identifying 75 breast lesions were analyzed; two patients had two lesions. Median age of patients was 56 years (range 29-88); 51 patients (69.8%) were in menopause, 22 (30.2%) were pre-menopausal. Lesions features are summarized in Table 2.

At MRI, margins were irregular in 32 (42.6%) lesions, lobular in eight (10.6%) lesions, spiculated in 34 (45.3%) lesions, and regular in one lesion.

In two (2.6%) lesions enhancement was homogeneous, in 52 (69.3%) enhancement was inhomogeneous, and in 21 (28%) there was rim enhancement. This resulted in a type I dynamic curve in four (5.3%) lesions, type II in 39 (55%) lesions, and type III in 32 (42.6%) lesions. Median diameter was 20 mm (range 5-90 mm).

There were 40 (53.3%) unifocal, 20 (26.6%) multifocal and 6 (8%) multicentric tumors, while 12 tumors (16%) were bilateral. Histologic analysis showed 13 (17.3%) lobular infiltrating tumors, 61 (81.3%) ductal tumors, and one (1.3%) tubular tumor. In total eight lesions were G1, 33 were G2 and 34 were G3.

At immunohistochemical analysis, Ki67 was <15% in 25 lesions, 15% - 30% in 30 lesions, and >30% in 20 lesions.

ADC values were not significantly different (p = 0.09) in subgroups G1, G2, and G3 as median ADC values were 1.17, 1.07, and 1.03 x 10^{-3} mm²/s, respectively. However, G3 tumors showed significantly lower ADC values if compared to subgroup G1+G2 (p < 0.05), which presented median ADC values of 1.05 and 1.17, respectively.

ADC values of lesions grouped by Ki67 expression did not show statistically significant differences, even though there was a trend of higher ADC values in malignant lesions with Ki67 > 50%.

High grade lesions (G3) were significantly larger than low grade lesions (G1) with a median diameter of 21 mm vs nine mm, respectively (p < 0.05). Lesions with high Ki67 (>30%) were also larger than those with low Ki67 (<30%), with a median diameter of 24 mm vs 16 mm (p < 0.05).

Discussion

Contrast enhanced breast MRI is currently accepted as the most sensitive imaging technique for the diagnosis and staging of breast cancer. However, specificity of breast MRI including T2-weighted imaging and contrast-enhanced T1-weighted imaging, is low in the assessment of breast tumors [13, 20]. Consequently, there is considerable interest in the development of adjunct MRI methods to improve the specificity of breast MRI, and DWI is therefore being investigated for its potentials.

DWI is used to visualize the degree of water molecule diffusion in vivo. Signal intensity at DWI is inversely proportional to the degree of water molecule diffusion. The degree of water diffusion in tissue is inversely correlated to tissue cellularity and the integrity of cell membranes. Thus DWI is highly sensitive to changes in the microscopic cellular environment without requiring intravenous contrast material injection. ADC is a quantitative measurement of diffusion and this parameter defines the average area covered by a molecule per unit time.

Signal intensity on DWI depends on the histologic structure, hence lesions with high cell density show high signal intensity on DWI and low ADC value. Cellular density and histologic architectural variation of breast cancer nests and stroma may be affected by the growth pattern, which can be expressed by histological grade and proliferation rate.

In the last decade, histologic grading has become widely accepted as a powerful prognostic indicator in breast cancer. The NGS is recommended by the National Cancer Comprehensive Network (NCCN) Guidelines of 2013 [21].
and is being incorporated into treatment algorithms for the management of patients with breast cancer. The system considers three variables for tumor grading: nuclear grade, tubule formation, and mitotic rate. Each component is given a score of 1 to 3 (1 being the best and 3 the worst). The sum of the three scores provides the “grade”. The lowest possible score is 3, obtained by 1+1+1 indicating a well differentiated tumor, well formed tubules and a low mitotic rate, whereas the highest is 9 obtained by 3+3+3, indicating that the tumor presents little or no differentiation, high pleomorphism and high mitotic rate.

The prognostic relevance of the NGS has been validated in numerous studies [6]. Histological grade can predict tumor behavior: low grade tumors have a good prognosis, whereas high grade tumors require prompt adjuvant chemotherapy because of the risk of early recurrence and death.

Grade 2 tumors present intermediate outcome in the initial follow up but tend to recur over time.

Some authors argued that the mitotic rate is the most important of these features and can sustain the grading system alone [22]. More recently, other authors have demonstrated that mitotic rate does not necessarily correlate with proliferation rate [23] and that more accurate methods of assessing proliferation based on the detection of nuclear antigens and mainly relying on Ki67 proteins have been investigated.

Figure 1. — In a 49-year-old woman an eight-mm mass is detected at mammography and US; a) subtracted DCE-MRI identifies a small mass with irregular margins and heterogeneous enhancement; b) kinetic pattern is of Ib type; c) in DWI it is hyperintense with d) an ADC of $1.13 \times 10^{-3}$ mm$^2$/sec. After that a quadrantectomy was performed, histopathology confirmed a lobular invasive cancer (grade 2, Stage T1b), hormone-receptor positive, HER2 negative, with a Ki67 of 10%.
Ki67 is the proliferation marker used to determine the growth fraction of a given cell population since it is a protein expressed in the nucleus during all the active phases of the cell cycle. In breast cancer, Ki67 is commonly used as a complement to grading systems to determine mitotic and proliferation rate.

Although Ki67 has been extensively studied and multivariate analysis studies have shown its role as an independent prognostic factor for disease-free survival, this biomarker is not currently accepted as a standard and it is not included in the list of recommended routine biological markers in the guidelines of the American Society of Clinical Oncology [24] nor in NCCN guidelines [21].

On the other hand, the St. Gallen International Expert Consensus [1] recommends Ki67 proliferation index for sub-classification of grade 2 patients and patients with hormone positive tumors: women with a Ki67 value >30% should receive neoadjuvant chemotherapy and hormonal therapy, while those with Ki67 <15% should receive hormone therapy alone. When Ki67 values range from 16% to 30%, other prognostic indexes should be used.

The aim of this study is to evaluate if there is a relationship between ADC values and proliferation markers such as grading and Ki67. To the authors’ knowledge, only a few reports in the literature [25–28] correlate ADC and prognostic factors and most of them are based on 1.5T MRI, except one study in which patients were randomly assigned to a 1.5T or a 3T unit [29].

In the present study only patients who had undergone 3T MRI were included, as a higher magnetic field yields a greater signal-to-noise and contrast-to-noise ratio, as well
as higher quality DWI despite a shorter acquisition time. Only in three cases of this series, DWI images were not of a good diagnostic quality due to motion artifacts. It has furthermore been observed that small lesions are more clearly visible at 3T [30] (Figure 1).

To standardize image acquisition and analysis as much as possible, only a b-value of 1000 sec/mm² was used in all patients.

Pereira et al. found that it is not necessary to use multiple b values [31] because the sensitivity of an ADC value using two b values is equivalent to that of multiple b values. Given the time constraints of clinical practice, analysis of an ADC value with two b values may be considered reasonable and acceptable.

In order to minimize the effect of interobserver variation and to simulate what might happen in a real clinical setting, all ROIs were selected on an ADC map positioned in the portion of the lesion which to the clinical eye appeared most vascularized on subtracted images (Figure 2).

A significant difference in ADC values was observed only between the two groups G1+G2 and G3. This result suggests a possible correlation between ADC values and some histological features of the tumors, meaning that a rich proliferation and mitotic rate and tubular formation reflect a greater cellularity of the parenchyma which results in higher signal intensity on DWI. The quantitative parameter ADC reflects this cellularity, and the values decrease in proportion to the grading. The results obtained in this study are similar to those reported by Martineich et al. and Razek et al. [25,28] who observed the same difference related to low/intermediate and high grade tumors.

No statistically significant differences were found in ADC values when grading groups were considered separately (G1 vs G2 vs G3), neither was found a statistically significant difference between ADC values in different Ki67 groups.

Some factors may have influenced the present results. It should be kept in mind that Ki67 markers vary not only according to cellular proliferation but also to aggressiveness which is influenced by some factors, such as neoformation of blood vessels, hypercellularity, and necrosis. These factors lead to changes in motility of water molecules and thus in signal intensity on DWI. In living tissue, DWI is influenced by microperfusion or blood flow which is an important potential competitor in the previously defined diffusion phenomenon. This signifies that the DWI signal may be mixed with a perfusion signal thereby increasing the net ADC value. Although the effect of microperfusion in normal fibro-glandular breast tissue is not considered significant because of the low vascularity of breast tissue [32], this effect could be more pronounced in breast cancer because of increased vascularity, although this has not yet been proved.

ROI selection is another important factor that may have influenced the present results. When the ROI is positioned in the most vascularized portion of the lesion, the ADC value may be significantly influenced by microperfusion and it may therefore not reliably reflect the cellularity of the lesion. In other previous studies [25], the ROI was manually drawn on the slice in which the lesion showed the greatest diameter, carefully avoiding large or predominantly necrotic areas. To obtain clinically useful quantitative ADC values, the best measuring method should be found for each type of lesion, especially when the lesions present a mixed pattern.

Finally, the present results may be influenced by the relatively small number of lesions included in the study.

Conclusion

The present findings suggest that the ADC values obtained on 3T DWI correlate with low and high-grade invasive breast carcinomas (G1+G2 and G3). This study particularly demonstrated that a low ADC value is associated with high histologic tumor grades. It also demonstrated that 3T ADC may be a helpful tool for identifying high-grade invasive breast carcinoma.

References


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Contrast-enhanced ultrasonography in diagnosis of benign and malignant breast lesions

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Department of Ultrasound, People’s Hospital of Guangxi Zhuang Autonomous Region, Nanning (China)

Summary
Purpose: This study aims to investigate the value of real-time contrast-enhanced ultrasonography (US) in differentiating benign and malignant breast lumps. Materials and Methods: Patients with breast lesions were observed. The enhancement form, intensity, and time-intensity curve were classified, and the characteristics of all the lesions were analyzed. Results: Inhomogeneous partial enhancement and entire enhancement were exhibited in most of the malignant lesions. High enhancements were observed in malignant lesions, whereas lower enhancement and no enhancement were exhibited in the benign tumors. The peak value and regression time were significantly different between the two groups. Conclusion: There’s a significant difference regarding the results of real-time contrast-enhanced ultrasound between the benign tumor and malignant tumor which would help to improve the diagnostic accuracy of breast neoplasms.

Key words: Breast neoplasms; Ultrasonography; Time-intensity curve.

Introduction
Breast carcinoma is one of the most common forms of malignant tumors in women worldwide. With the increasing global prevalence of breast carcinoma, it is becoming increasingly difficult to diagnose breast cancer because different types of breast cancers have similar signs, making it difficult to be classified. Currently, ultrasonography (US) has been regarded as a major technology for the diagnosis of breast cancer, however the accuracy of ultrasound evaluation is poor detection in size change of breast cancer caused by fibrosis and necrosis after chemoradiotherapy treatment. Currently, contrast-enhanced ultrasound (CEUS) has made further improvement on microbubble stability with much more advances.

Blood flow distribution, form, type, enhancement pattern, and duration time in different kinds of breast tumors present various characteristics. As a result, systematic observation of morphological characteristic in high frequency imaging and contrast perfusion mode in breast tumors is in popular demand.

The present study used systematic analysis for determining the morphologic feature gathered in high-frequency ultrasound (HFU), color blood flow imaging, and contrast perfusion mode in breast lesions. This process focused particularly in malignant lesions that were then compared with actual pathological results, revealing the characteristic of US and contrast perfusion mode in different breast tumors. The present study as a whole, contributes to the generation of new ideas and in the advancement of diagnostic criteria, in the hope of providing basis for continuous research on tumor angiogenesis and ultrasound imaging. The knowledge and new discoveries in differential diagnosis obtained in this study may be valuable for improving the accuracy in breast lesion diagnosis.

Materials and Methods
Patients
A total of 752 patients with breast tumor underwent color Doppler contrast-enhanced US, from March 2007 to June 2011. All patients were female, in which 108 of them (mean age 50.17 ± 11.54) were proven to have malignant lesions by pathological examination or operation. The diameters of the lesions were four to 54 mm. No treatments were provided before operation. There were no complications in all the patients included. Specimens of the lesions were collected and sent for pathological examination. This study was conducted in accordance with the declaration of Helsinki, and with approval from the Ethics Committee of the People’s Hospital of Guangxi Zhuang Autonomous Region. Written informed consents were obtained from all participants.

Ultrasoundography procedures
An ultrasound scanner, with a linear-array probe (12.0 MHz) and a contrast probe (9.0 MHz), was used in the experiments. The contrast agent used was SonoVue. The SF6 microbubble suspension was prepared using SonoVue, with the addition of five ml of sterile saline solution.

Conventional two-dimensional gray-scale ultrasound examination was first conducted to record the size, location, shape, margins, internal echo, and the relationship with peripheral tissue of the lesions. Compressed probe was used to find any change in lesion morphology and size. Color Doppler flow imaging and color Doppler energy were used to observe the flow distribution and to measure the peak systolic velocity, minimum diastolic velocity, and resistance index. Richest blood flow cut surface of the lesions was selected, then subjected into the real-time ultrasound imaging mode with the contrast probe. SonoVue (2.4 ml) was injected via the elbow vein in a bolus manner, and the distribution and location of the microbubble contrast agents inside the lesion were then dynamically observed. Real-time dynamic images were stored for four minutes, and then were saved for further offline analysis. One region of interest (ROI) of the lesion was se-
lected to analyze the time-intensity curve, recording the initial time of perfusion (ITP), time to peak (TTP), peak intensity (PI), and the washout time (WOT) (1/2 WOT).

**Imaging analysis**

The entire malignant lesion was classified in detail based on the pathological results, and the feature of the perfusion mode inside and around of the lesions were recorded. Classification was based on the enhancement, intensity, and time-intensity curve [1]. The types of enhancement pattern (EP) can be divided into the following: pin point enhancement, ring or semi-ring enhancement, dendrites enhancement, partial enhancement, and global enhancement. The intensity criterion indicated that the normal tissue around the lesion was with moderate-enhancement. Thus, with this standard, the intensity of the lesion was divided into hyper-enhancement, moderate-enhancement, hypoenhancement, and no enhancement [2, 3].

**Statistical analysis**

Data were analyzed by using SPSS 11.5. All the measured data were presented as mean ± standard deviation (x ± s). T-test was used with measurement data and the chi-square test was used with enumeration data.

**Results**

**General information**

Of the total 108 patients with malignant cases, 82 were with invasive ductal carcinoma, eight with intraductal carcinoma (three carcinoma in situ with microinvasive), seven with invasive lobular carcinoma, three with phyllodes carcinoma, one with intraductal papilloma with local canceration, five mucinous carcinoma, and one clear cell carcinoma (Table 1).

Of the total 120 benign lesions, 49 were fibroadenoma and 58 were cyclomastopathy and 13 were papilloma. The

<table>
<thead>
<tr>
<th>Type of malignant tumor</th>
<th>n</th>
<th>Contrast-enhanced manifestation (major)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ductal carcinoma</td>
<td>82</td>
<td>Hyperenhancement</td>
</tr>
<tr>
<td>Intraductal carcinoma</td>
<td>8</td>
<td>Hyperenhancement</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>7</td>
<td>Moderate enhancement</td>
</tr>
<tr>
<td>Phyllodes carcinoma</td>
<td>3</td>
<td>Hyperenhancement</td>
</tr>
<tr>
<td>Intraductal papilloma with local canceration</td>
<td>1</td>
<td>Moderate enhancement</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>5</td>
<td>Hypoenhancement</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>1</td>
<td>Moderate enhancement</td>
</tr>
</tbody>
</table>

**Table 1. — The major contrast-enhanced manifestation of different types of malignant tumor.**

---

Figure 1. — A) Contrast-enhanced US shows a partial enhancement mass by heterogeneous enhancement; B) Contrast-enhanced US shows a mass with the features of heterogeneous enhancement and relatively rich perfusion.

Figure 2. — Imagings of gray scale US and contrast-enhanced US of the same patient having invasive ductal carcinoma. A) Gray scale US shows the mass with irregular margin. B) Contrast-enhanced US shows the mass with features of global enhancement and rich perfusion.
Contrast-enhanced ultrasonography in diagnosis of benign and malignant breast lesions

Figure 3. — Imagings of gray scale US and contrast-enhanced US of the same patient. A) Gray scale US shows enhancement around the mass; B) Contrast-enhanced US shows the perfusion area is obviously larger compared to the mass size showing at gray scale US.

Figure 4. — Imagings of gray scale US and contrast-enhanced US of the same patient. A) Gray scale US shows a mass of poorly-defined margin, with spiculate sign; B) Contrast-enhanced US shows the mass perfusion area obviously enlarged, within the feature of peripheral radial enhancement.

Figure 5. — Imaging of Gray scale US and Contrast-enhanced US of the same patient having clear cell carcinoma. A) Gray scale US shows a mass with the features of relatively regular margin; B) Contrast-enhanced US shows the mass perfusion area is the same to that at gray scale US.

Figure 6. — The time-intensity curve of breast mass. A) The curve of breast cancer displayed as “fast washin and slow washout”; B) The curve of fibroadenoma displayed as “fast washin and washout” (yellow for mass, green for the tissue around).
fibroadenoma lesions consisted of 33 cases of simple fibroadenoma, ten fibroadenoma with hyperplasia and duct ectasia, six fibroadenoma with extensively hyaline degeneration and calcium deposition. Among the 58 cyclomastopathy, 14 were cystic hyperplasia, 19 were with adenomatous hyperplasia, 12 were with lobular hyperplasia, five were sclerosing adenosis hyperplasia, and nine were with papilloma hyperplasia and mild to severe atypical hyperplasia.

The internal features of malignant breast lesion by CEUS were as follows: 1) most enhancement pattern showed heterogeneous enhancement (Figure 1) and whole enhancement (Figure 2), but less local or pinpoint enhancement; 2) majority of the intensity displayed as hyperenhancement, whereas hypoenhancement was less. Most of the invasive ductal carcinoma, intraductal carcinoma, and phyllodes carcinoma showed hyperenhancement, whereas mucinous carcinoma showed hypoenhancement. Hyperenhancement, moderate-enhancement, and hypoenhancement showed in 91, ten, and seven cases, respectively (Table 1). Meanwhile, the following observations were recorded for the surrounding manifestation of malignant lesions: 1) Most malignant lesions showed the perfused areas were enlarger than that in the two-dimensional US (Figure 3); 2) contrast agent perfused into the lump via several vessels showed a radial enhancement around the lesion (Figure 4); 3) It was hardly observed that the perfusion area was consistent with the two-dimensional imaging (Figure 5).

The contrast-enhanced internal manifestation of benign lesions were as follows: 1) benign lesions showed scattered punctiform enhancement, heterogeneous enhancement, dendritic enhancement, and whole enhancement (Figure 6). 2) Most benign lesions showed moderate enhancement and hypoenhancement. Hyperenhancement, moderate hypoenhancement, hypoenhancement, and non-enhancement, are showed in Table 2, respectively. Otherwise the surrounding manifestation of benign lesions were observed as follows: 1) it was shown that the perfusion area was consistent with the two-dimensional imaging; 2) the periphery of the lesions always showed as annular or semi-annular enhancement with thick or thin wall; 3) the perfusion area beyond that on the two-dimensional US was hardly shown.

### Table 2. CEUS results of breast neoplasms (%.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hyper-enhancement</th>
<th>Moderate-enhancement</th>
<th>Hypo-enhancement</th>
<th>No-enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>84.26 (91)</td>
<td>9.26 (10)</td>
<td>6.48 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>28.57 (14)</td>
<td>24.49 (12)</td>
<td>32.65 (16)</td>
<td>14.29 (7)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>24.14 (14)</td>
<td>27.59 (16)</td>
<td>31.03 (18)</td>
<td>17.24 (10)</td>
</tr>
<tr>
<td>Papilloma</td>
<td>61.54 (8)</td>
<td>15.38 (2)</td>
<td>23.08 (3)</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3. Comparison of contrast filling models in lesions (± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>ITP (s)</th>
<th>TTP (s)</th>
<th>WOT (s)</th>
<th>PI (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>11.25±3.68</td>
<td>17.20±4.53</td>
<td>29.22±5.71</td>
<td>8.16±3.05*</td>
</tr>
<tr>
<td>Benign</td>
<td>10.56±3.68</td>
<td>16.33±3.45</td>
<td>53.13±10.71</td>
<td>15.22±4.01</td>
</tr>
</tbody>
</table>

*Compared with malignant lesion, p < 0.05.

### Table 4. CEUS of breast cancer with different histopathologic types (cases).

<table>
<thead>
<tr>
<th>CEUS Finding</th>
<th>Histopathologic classification</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>106 (a)</td>
<td>125 (a+b)</td>
</tr>
<tr>
<td>Benign lesions</td>
<td>2 (c)</td>
<td>103 (c+d)</td>
</tr>
<tr>
<td>Total</td>
<td>108 (a+c)</td>
<td>228 (a+b+c+d)</td>
</tr>
</tbody>
</table>

The characteristic of time-intensity curve: the benign group displayed as “slow washin and fast washout” and “fast washin and fast washout”, while “fast washin and slow washout” was shown in the malignant group. The present study showed that there are no significant differences (p > 0.05) in terms of ITP and TTP, although they seem to be faster in malignant lesions than in benign lesions. However, the PI and WOT are significant differences between benign lesions and malignant lesions (p < 0.05, Table 3).

The result of CEUS examination (with pathological diagnosis as the gold standard): the sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of diagnosis in malignant lesions were 98.5%, 86.13%, 91.34%, 88.33%, 97.75%, respectively (Table 4).

Nineteen cases were misdiagnosed in 120 breast cancer by using the contrast-enhanced US: five fibroadenoma, nine cyclomastopathy, meanwhile five cases were papilloma, and two cases misdiagnosed as adenopathy hyperplasia proved to be intraductal carcinoma and invasive ductal carcinoma.

### Discussion

Breast tumor is a vascular-dependent lesion, and its growth, invasion, and metastasis is on the premise of angiogenesis. CEUS improves the sensitivity of color Doppler, making it possible to detect even at low blood flow, and displays the characteristics of blood supply by enhancing the contrast between lesions and the surrounding normal tissue, which is not achievable with traditional ultrasound due to the abnormal perfusion areas caused by the damaged vessels or diseased tissue. CEUS is valuable in early diagnosis, understanding blood perfusion, especially in differential diagnosis, which led to its popularity in clinical practice and gained increased attention in the tumor.
Histopathologically shows single or small mass of tumor. Mucinous carcinoma is a pure mucinous carcinoma that displayed by the patient, and also histopathology. Mucinous carcinoma can be diagnosed by simply looking into the characteristics of the tumor cell, hence, perfusion was observed. Mucinous carcinoma were characterized with hypoenhancement, thus, was the opposite on the low-grade. Five cases of mucinous carcinoma were observed to have larger enhancement on contrast-enhanced US images. The possible reason for this observation may be the angiogenesis of the benign tumor that shows normal proliferation and thickening, and have the same size and homogenous distribution. Meanwhile, the vessels of malignant lesion are repaired and randomly distributed, forming arteriovenous fistula or vessel caecum for thrombus. All these pathological changes led to the difference in PT and TTP values between the benign and malignant lesions.

For the pathological basis of contrast-enhanced US images, the observed perfusion area was larger than that of the two-dimensional imaging, which was probably due to the malignant lesion infiltrating the peripheral normal tissue and adipose tissue. Intraductal carcinoma spread with crab-like invasion in the ducts, resulting in a radial enhancement on contrast-enhanced US images.

Applying the pathological basis in contrast-enhanced ultrasonography, the difference of the vessel anatomy and hemodynamics between benign and malignant lesions can be shown by the shape, continuity, trace, and shunt of the vessels. Real-time ultrasound showed the microcirculation of breast lesion, making the enhancement mode helpful in the differential diagnosis of benign and malignant tumors, especially in some substantial mass with small and weak enhancement. Even in some mass that was difficult to name the substantial and liquid phases, the diagnosis was made easier by observing the enhancement mode. Contrast-enhanced US is not the most basic diagnostic tool of all technology, however, it is a useful adjunct to conventional ultrasound in improving diagnostic accuracy and help to diagnose the benign and malignant tumor. When lesions are dubious malignant on two-dimensional ultrasound, contrast-enhanced US can be performed. If the lump is observed to be rich in perfusion, it is likely to be malignant. In addition, if a typical be-
nign lesion has the enhancement mode of spotty distribution, or no enhancement, breast lesion can be concluded to be benign. One case that cannot be expected as malignant is the poor perfusion on uncertain lesions. Moreover, two cases cannot match the explanation, perhaps due to the small shape, poorly-defined margin, weak enhancement, and poor perfusion of the lesions. Therefore, when dealing with old aged patients with atypical masses and poor perfusion of lesions, periodic follow-up, biopsy, and even operation are necessary.

The present study discovered some enhancement features on different breast masses, particularly in malignant lesions. Via the systematical observation, contrast-enhanced US may be helpful in improving the sensitivity of the velocity blood flow with its especial imaging. It was useful in characterizing malignant lesions and was able to improve diagnostic accuracy in differentiating malignant and benign breast tumors.

Acknowledgements

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References


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Comparison of Pelvic Masses Score (PMS) and Risk of Malignancy Index (RMI 3) in the evaluation of pelvic masses

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² Department of Obstetrics and Gynecology, University of Padua, Padua (Italy)

Summary

Purpose: Ovarian cancer is the fourth cause of death from cancer in women worldwide and the majority of its diagnoses is made in an advanced stage of the disease. Several sonographic scoring systems have been created for a better preoperative discrimination between benign and malignant pelvic masses. The aim of this study was to evaluate the performances of the Risk of the Malignancy Index 3 (RMI 3) and the Pelvic Masses Score (PMS). Materials and Methods: This retrospective study was performed in 55 women admitted to the department of Obstetrics and Gynecology of University of Udine for surgical exploration of pelvic masses between 2009 and 2012. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for both the scores. Results: PMS showed a sensitivity of 100%, a specificity of 93.8%, a PPV of 70%, and a NPV of 100%, while RMI 3 yielded a sensitivity of 85%, a specificity of 91%, a PPV of 60%, and a NPV of 97.8%. Conclusion: The authors found that, in discriminating between benign and malignant pelvic disease, the PMS method was more reliable than RMI 3. PMS is a simple scoring system which can be used in clinical practice.

Key words: Ovarian cancer; Pelvic mass; Risk of malignancy index; Pelvic masses score.

Introduction

Ovarian cancer is the fourth cause of death from cancer in women worldwide. In Italy, the incidence of ovarian tumors is 13:100,000 females, with a total of approximately 4,150 new diagnoses and 2,700 deaths per year. Despite significant advances in the treatment of ovarian cancer, the five-year survival rate has not yet improved in the last 30 years [1, 2].

As far as 24% of all the ovarian tumors diagnosed in premenopausal women result to be malignant, while this percentage increases up to 60% in postmenopausal state [3].

The poor prognosis for women suffering from ovarian cancer is due to the fact that in 75% of women the diagnosis is made in an advanced stage of the disease (FIGO Stage III-IV), as in the early phases of this pathology signs and symptoms are non-specific or completely missing [4, 5].

The accurate diagnosis of a pelvic mass is a challenge to the gynecologist: a correct method for better preoperative discrimination of pelvic masses would increase the number of women receiving the most suitable first-line therapy. [6]

Biochemical markers and ultrasound examination, considered alone, are not accurate for a proper diagnosis of a pelvic mass. Transvaginal sonography (TVS) with color Doppler ultrasound may be useful in detecting and describing pelvic neoplasms, but even for an experienced ultrasound examiner, the correct echographic diagnosis of a pelvic mass may be difficult [2, 7]. Mathematical models that take into account the ultrasound image, color Doppler ultrasound examination, and clinical and biochemical parameters of the patient, may be helpful in calculating the individual malignancy risk for an adnexal mass [8].

In the 1990, Jacobs et al. developed a score, called Risk of Malignancy Index (RMI 1) later modified as RMI 3 [9, 10].

In particular, RMI 3 takes into account the ultrasound appearance of the mass, menopausal status, and serum concentration of CA125 [6].

Recently a new echographic score, named Pelvic Masses Score (PMS) has been created. It considers the CA125 serum concentration, the patient’s menopausal state, the mass’ ultrasound morphological pattern, the vascular distribution of the examined pelvic mass, and the resistance index (RI) of its vascular system [11].

The aim of this study was to evaluate the ability of RMI 3 and PMS to discriminate a benign from a malignant pelvic mass and to evaluate the validity of these two scores.

Materials and Methods

This is a retrospective study. The clinical data were obtained from 55 women aged 19 to 84 years (mean age 54.22, standard deviation ± 18.57) admitted to the Department of Obstetrics and Gynecology of University of Udine for surgical exploration of pelvic masses between March 2009 and December 2012. This study was approved by the local institutional review board and all subjects provided written consent before entering the study.

Ultrasound examination and preoperative serum CA125 levels were noted. All cases were evaluated by transvaginal ultrasonography with a 9.0 MHz transducer by a single operator (A.R.).

On the basis of data obtained, the RMI 3 and PMS were calculated for all patients together with the sensitivity, specificity, posit...
A. Rossi, L. Forzano, I. Romanello, G. Ambrosini, V. Iuri, D. Marchesoni

The positive predictive value (PPV) and negative predictive value (NPV) of the two methods. RMI 3 = U x M x CA125; ultrasound scans were scored as one point for each of the following characteristics: multilocular cyst, solid areas, intra-abdominal metastases, ascites, and bilateral lesion. A total ultrasound score of 0 or 1 yielded $U = 1$ and a score of 2 or more yielded $U = 3$; premenopausal status yielded $M = 1$, while postmenopausal status $M = 3$. The blood concentration of CA125 was directly applied to the calculation $[3, 6, 10, 12, 13]$. According to the literature, RMI 3 cut-off value for the best discrimination between benign and malignant pelvic masses was 200 $[3, 6, 13-15]$. Then Pelvic Masses Score (PMS) was applied for each case:

$$
PMS = SASS \times \log(\text{CA125}) \times \text{VAS} \times MS / RI;$$

where SASS is the numeric value of the Sassone’s score (considering the thickness of the mass, the presence/absence of internal septa, and the echogenic pattern), $\log$ CA125 is the base 10 logarithm of the CA125 serum levels, VAS is the type of vascularization (peripheral = 1; central/septal = 2), MS is the menopausal state (premenopausal = 1; postmenopausal = 2) and RI is the numeric value of the RI of the pelvic mass. PMS cut-off value was 29. Sassone’s score system is depicted in Table 1 $[11, 15]$.

The histopathological diagnosis of samples and surgical specimens was considered as the definite outcome. In case of malig-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Benign masses</th>
<th>Malignant masses</th>
<th>$p$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.19 (SD ± 18.37)</td>
<td>64 (SD ± 15.56)</td>
<td>0.065</td>
<td>55</td>
</tr>
<tr>
<td>Volume (cm$^3$)</td>
<td>891.83 (SD ± 2,072.14)</td>
<td>974.61 (SD ± 1834.56)</td>
<td>0.8</td>
<td>55</td>
</tr>
<tr>
<td>Resistance index</td>
<td>0.98 (SD ± 1.5)</td>
<td>0.39 (SD ± 0.1)</td>
<td>0.04</td>
<td>55</td>
</tr>
<tr>
<td>CA125 (UI/ml)</td>
<td>35.16 (SD ± 85.15)</td>
<td>277.89 (SD ± 326.95)</td>
<td>0.02</td>
<td>55</td>
</tr>
<tr>
<td>Sassone score</td>
<td>5.64 (SD ± 1.44)</td>
<td>11 (SD ± 2.73)</td>
<td>0.04</td>
<td>55</td>
</tr>
<tr>
<td>Central/septal vascularisation</td>
<td>5.45%</td>
<td>12.73%</td>
<td>0.055</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 1. — Sassone’s scoring system.

<table>
<thead>
<tr>
<th>Score</th>
<th>Wall structure</th>
<th>Wall thickness</th>
<th>Septa</th>
<th>Ultrasound pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Smooth</td>
<td>&lt; 3 mm</td>
<td>Absent</td>
<td>Anechoic</td>
</tr>
<tr>
<td>2</td>
<td>Irregular &lt; 3 mm</td>
<td>&gt; 3 mm</td>
<td>&lt; 3 mm</td>
<td>Iperechoic</td>
</tr>
<tr>
<td>3</td>
<td>Papilla &gt; 3 mm</td>
<td>Solid</td>
<td>&gt; 3 mm</td>
<td>Iperechoic with iperechoic spots</td>
</tr>
<tr>
<td>4</td>
<td>Solid</td>
<td>Mixed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Iperechoic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Population characteristics.

Table 3. — Comparison between scoring systems.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMI 3 index</td>
<td>85</td>
<td>91</td>
<td>60</td>
<td>97.8</td>
</tr>
<tr>
<td>PMS index</td>
<td>100</td>
<td>93.8</td>
<td>70</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ROC PMS</th>
<th>ROC RMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under the ROC curve (AUC)</td>
<td>Area under the ROC curve (AUC)</td>
</tr>
<tr>
<td>0.997</td>
<td>0.869</td>
</tr>
<tr>
<td>Standard error</td>
<td>Standard error</td>
</tr>
<tr>
<td>0.00412</td>
<td>0.106</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>95% Confidence interval</td>
</tr>
<tr>
<td>0.931 to 1.000</td>
<td>0.752 to 0.944</td>
</tr>
<tr>
<td>z statistic</td>
<td>z statistic</td>
</tr>
<tr>
<td>120.562</td>
<td>3.482</td>
</tr>
<tr>
<td>Significance level $p$ (area = 0.5)</td>
<td>Significance level $p$ (area = 0.5)</td>
</tr>
<tr>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 4. — Receiver operating characteristics (ROC) of PMS compared to RMI 3.
Comparison of Pelvic Masses Score (PMS) and Risk of Malignancy Index (RMI 3) in the evaluation of pelvic masses

Discussion

The majority of malignant ovarian neoplasms are diagnosed in post-menopausal women, with a peak at age of 62 years [17-19].

An accurate method for preoperative prediction of the nature and the origin of a pelvic mass is essential in order to an optimal surgical management, which, in case of malignancy, would provide the best chance of a long disease-free survival period or of therapy. This study has confirmed that both RMI 3 and PMS were able to preoperatively discriminate between non-invasive lesions and invasive malignant masses.

L.R. Medeiros et al. [20], in their review concerning the accuracy of CA125 assay in the diagnosis of ovarian tumors, showed that levels of CA125 ≥35 UI/ml might detect malignant and borderline ovarian tumors with 80% sensitivity and 75% specificity. The major limitation of CA125 is that it may be elevated also in many benign diseases, such as ovarian cysts, inflammatory pelvic disease, uterine myomas, and endometriosis. This is the reason why the combination of CA125 serum levels with the ultrasound parameters, other tumor markers, and the patient’s menopausal status increases the discriminating power of both PMS and RMI between benign and malignant masses. [20-22]

Considering sensitivity, specificity, PPV and NPV of the two methods, the superiority of PMS over RMI 3 can be confirmed.

PMS is a simple scoring system which uses inexpensive tests and which can be used routinely in the clinical practice by an experienced sonographer in the evaluation and the management of pelvic masses. Further larger prospective studies are required to permit a better evaluation of PMS.

References


Prognostic factors affecting lymph node involvement in cervical cancer

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Summary

Aim: Clinical and histopathological factors that affect lymph node involvement in cervical cancer and the prognostic importance of these factors were evaluated in this study. Materials and Methods: A total of 179 patients were diagnosed with cervical cancer between January 2001 and June 2010 and were included in this study. The patients’ charts were evaluated retrospectively and information was collected by reaching 89 patients and asking questions. Results: When the prognostic factors that affect pelvic lymph node involvement were evaluated, increased tumor size and increased invasion depth, presence of lymphovascular area involvement, and an advanced stage were observed to statistically significantly increase pelvic lymph node involvement. No relationship was found between tumor histology and grade; parametrial, endometrial, vaginal involvement, and pelvic lymph node involvement. Conclusion: Knowledge of prognostic factors in cervical cancer plays an important role in determining the morbidity and mortality and the treatment strategies.

Key words: Cervical cancer; Lymph node involvement.

Introduction

The response of patients with cervical cancer to the treatment depends on the presence of prognostic factors. As with other cancers, the most important factor affecting the prognosis in cervical cancer is the stage of the disease. Other clinicopathological prognostic factors are lymphatic invasion, tumor size, depth of stromal invasion, parametrial invasion, lymphovascular area invasion (lvai), histological type, close surgical border, positive surgical border, age, and HPV type.

Materials and Methods

A total of 179 patients who had presented at the Zeynep Kamil Training and Research Hospital Gynecologic Oncology Clinic and were diagnosed with cervical cancer between January 2001 and June 2010 and were included in this study. The authors applied to the hospital ethics committee and obtained consent following study evaluation. Patients’ charts were assessed retrospectively and information was collected by reaching and asking questions to 89 patients. The patients were staged clinically. Radical hysterectomy and pelvic/para-aortic lymph node dissection was performed in patients with FIGO Stage Ia-IIa disease. Adjuvant radiotherapy or chemoradiotherapy was administered to the patients according to the prognostic factors and stage. As Stage Ia cannot be divided into clinical subtypes, differentiation was carried out pathologically. Patient age, gravidity, parity, number of miscarriages, number of abortions, smoking, tumor size, histological type, grade, FIGO stage, presence of vaginal, endometrial, ovarian, parametrial, lvai, pelvic lymph node, para-aortic lymph node involvement, tumor-free area, stromal invasion depth, year and type of surgery performed, whether complications developed during surgery, and whether the patient received radiotherapy after the surgery were investigated. The effects of these parameters on pelvic and para-aortic lymph node involvement, postoperative prognosis, and five-year survival were then investigated. Statistical analyses were performed with the NCSS 2007 software package.

Data were evaluated using descriptive statistical methods (mean, standard deviation), as well as the t-test for the comparison of two independent groups, and the chi-square test for the comparison of qualitative data. Survival rates were determined with the Kaplan-Meier test. The cutoff point for tumor diameter in pelvic lymph node involvement was determined and the sensitivity, specificity, positive cutoff point, negative cutoff point, and likelihood ratio (LR) were calculated. The results were evaluated at the p < 0.05 significance level and 95% confidence range.

Results

The age of the patients ranged from 20 to 84 years. The mean age was 51 years, mean gravidity 5, and mean parity 3. There were 32 patients in Stage Ia, 55 patients in Stage Ib1, 26 patients in Stage Ib2, 14 patients in Stage IIa, 44 patients in Stage IIb, five patients in Stage IIIa, and five patients in Stage 3b. Of the 127 total Stage 1a-2a patients treated, 115 patients underwent pelvic/para-aortic lymph node dissection. Five patients underwent trachelectomy and 12 patients type 1 (extrafascial) hysterectomy, while 60 patients did not undergo surgery due to their advanced stage and four patients due to their medical condition. Seventy-six patients received radiation therapy and 46 patients received chemoradiotherapy.
The first year survival rate in this study group was 98%, second year 94%, third year 88%, and fifth year 81%. Median survival period was 99.53 months (Table 1).

No statistically significant difference was observed between PLN (-) and PLN (+) groups’ mean age, gravidity, parity, miscarriage, abortion, number of dissected nodes, or tumor-free area (cm).

The PLN (+) group’s mean tumor size (cm) was found to be statistically significantly higher than the PLN (-) group (\(p = 0.009\)). The PLN (+) group’s mean invasion depth was also found to be statistically significantly higher than the PLN (-) group (\(p = 0.006\) (Tables 2 and 3).

No statistically significant difference was observed between PLN (-) and PLN (+) groups for parametrial, endometrial, or vaginal positivity, and presence of complications.

A statistically significant difference was observed between PLN (-) and PLN (+) groups’ lvai distributions, FIGO distribu-

---

**Table 1. — Survival rates in the patients.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Survival Rate ± SE</th>
<th>Median Survival Duration ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>0.985 ± 0.015</td>
<td>99.53 ± 4.99 (89.75 - 109.32)</td>
</tr>
<tr>
<td>Second</td>
<td>0.949 ± 0.029</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>0.885 ± 0.045</td>
<td></td>
</tr>
<tr>
<td>Fifth</td>
<td>0.817 ± 0.077</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. — Recurrence rates in the patients.**

<table>
<thead>
<tr>
<th>Year</th>
<th>Recurrence Rate ± SE</th>
<th>Median Survival Duration ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>0.945 ± 0.027</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>0.909 ± 0.036</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>0.847 ± 0.048</td>
<td></td>
</tr>
<tr>
<td>Fifth</td>
<td>0.782 ± 0.077</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. — The effect of age, gravidity, parity, miscarriage, abortion, tumor size, depth of invasion, number of lymph nodes dissected and tumor-free area on pelvic lymph node involvement.**

<table>
<thead>
<tr>
<th>Factor</th>
<th>PLN Involvement (-)</th>
<th>PLN Involvement (+)</th>
<th>(\chi^2)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50.03±11.24</td>
<td>46.79±11.16</td>
<td>1.01</td>
<td>0.314</td>
</tr>
<tr>
<td>Gravida</td>
<td>4.92±2.89</td>
<td>6.14±2.93</td>
<td>-1.48</td>
<td>0.14T</td>
</tr>
<tr>
<td>Parity</td>
<td>3.61±2</td>
<td>3.38±1.66</td>
<td>0.39</td>
<td>0.698</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>1.65±1.3</td>
<td>3±2.31</td>
<td>-2.21</td>
<td>0.033</td>
</tr>
<tr>
<td>Abortion</td>
<td>2.41±2.09</td>
<td>2.5±1.73</td>
<td>-0.09</td>
<td>0.932</td>
</tr>
<tr>
<td>Tumor Size (Cm)</td>
<td>2.82±1.71</td>
<td>4.06±0.91</td>
<td>-2.67</td>
<td>0.009</td>
</tr>
<tr>
<td>Invasion Depth</td>
<td>1.31±0.98</td>
<td>2.1±1.12</td>
<td>-2.78</td>
<td>0.006</td>
</tr>
<tr>
<td>Number of Nodes</td>
<td>26.39±16.23</td>
<td>28.07±14.75</td>
<td>-0.37</td>
<td>0.713</td>
</tr>
<tr>
<td>Tumor-free Area Cm</td>
<td>0.46±0.37</td>
<td>0.26±0.18</td>
<td>1.62</td>
<td>0.109</td>
</tr>
</tbody>
</table>

**Table 4. — Factors affecting pelvic lymph node involvement.**

<table>
<thead>
<tr>
<th>Factor</th>
<th>PLN Involvement (-)</th>
<th>PLN Involvement (+)</th>
<th>(\chi^2)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVAI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>0</td>
<td>10</td>
<td>0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>56</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parametrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>96</td>
<td>13</td>
<td>12</td>
<td>0.006</td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>1</td>
<td>1.94</td>
<td>0.163</td>
</tr>
<tr>
<td>Endometrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>94</td>
<td>14</td>
<td>8.75</td>
<td>0.003</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>93</td>
<td>12</td>
<td>6.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>2</td>
<td>6.6</td>
<td>0.01</td>
</tr>
<tr>
<td>FIGO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>20</td>
<td>0</td>
<td>1.24</td>
<td>0.001</td>
</tr>
<tr>
<td>1B1</td>
<td>51</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1B2</td>
<td>18</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>12</td>
<td>2</td>
<td>8.75</td>
<td>0.003</td>
</tr>
<tr>
<td>Complication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>60</td>
<td>7</td>
<td>6.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>2</td>
<td>1.24</td>
<td>0.001</td>
</tr>
<tr>
<td>Uremia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>62</td>
<td>6</td>
<td>8.75</td>
<td>0.003</td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>0</td>
<td>6.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Yes</td>
<td>33</td>
<td>11</td>
<td>6.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>1</td>
<td>5.39</td>
<td>0.02</td>
</tr>
<tr>
<td>Yes</td>
<td>24</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>59</td>
<td>6</td>
<td>4.29</td>
<td>0.038</td>
</tr>
<tr>
<td>Recurrence</td>
<td>6</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>3</td>
<td>3</td>
<td>8.75</td>
<td>0.003</td>
</tr>
<tr>
<td>Alive</td>
<td>62</td>
<td>6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(PLN: pelvic lymph node, Ca: cancer, lvai: lymphovascular area invasion)
Table 5. — Factors affecting survival.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Death n: 7</th>
<th>Alive n: 82</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.43±11.56</td>
<td>49.09±11.24</td>
<td>0.30</td>
<td>0.763</td>
</tr>
<tr>
<td>Parity</td>
<td>5.43±3.16</td>
<td>3.41±1.97</td>
<td>2.47</td>
<td>0.016</td>
</tr>
<tr>
<td>Follow up duration (years)</td>
<td>30.57±15.32</td>
<td>34.55±26.84</td>
<td>-0.39</td>
<td>0.701</td>
</tr>
</tbody>
</table>

Buttons, uremia presence, recurrence, and survival status. Lvai positivity was seen in all patients in the PLN (+) group. PLN positivity was found to be high in the Stage 1B2 and 2A groups. Uremia positivity was found to be high in the PLN (+) group. Recurrence presence was found to be high in the PLN (+) group. The death rate was also found to be high in the PLN (+) group (Table 4).

No statistically significant difference was observed between the mean age, gravida, abortion, tumor size, invasion depth, number of lymph nodes dissected, and tumor-free area of the dead and living groups.

The mean parity of the deceased group was found to be statistically significantly higher than the living group (p = 0.016) (Table 5).

**Discussion**

As with other cancers, the most important factor affecting the prognosis in cervical cancer is the stage of the disease. While five-year survival in Stage 1 cervical cancer is 80%, it is reduced to 10% in Stage 4. This study also found decreased survival with increasing stage. One-year survival is 98% and five-year survival 81% in patients with FIGO Stage 1a-4 while five-year survival is 82% in Stage 1b1 and 66% in Stage 3b [1].

One of the most important prognostic factors in cervical cancer is lymphatic spread. The mean number of lymph nodes dissected in this study was 26.3. Park et al. [2] reported the mean number of dissected lymph nodes as 32 and Pikaart et al. [3] as 21. The pelvic/para-aortic lymph node metastasis rate in FIGO Stage I-IIa was 10.4% in Pikaart et al.’s study. They found no patient with para-aortic involvement in their study of 200 patients. Costin et al. found a lymphatic metastasis rate of 50% in 266 Stage I-IV cervical cancer cases who underwent retroperitoneal lymphadenectomy in 1998. They found only pelvic node involvement in 65% of 133 cases, pelvic + para-aortic lymph node involvement in 33% and only para-aortic lymph node involvement in 1.5% [4]. The present authors found only pelvic lymph node involvement in 11 patients, pelvic + para-aortic lymph node involvement in two patients, and only para-aortic lymph node involvement in one patient among the 119 Stage I-IIa patients in this study.

Various studies have reported five-year survival as 51% to 78% in the presence of lymph node involvement in cervical cancer [5, 6]. Hyun Nam et al. in 2010 evaluated 1,828 Stage Ia2-IIa patients and found a five-year survival in the presence of lymph node involvement to be 76% [2]. A study carried on lymph node involvement in cervical cancer found a five-year survival of 92% in patients without lymph node involvement and 50% in patients with node involvement [1]. Fuller et al. found a five-year survival of 85-90% in patients without lymph node involvement [7]. The present authors found a five-year survival to be 80% in patients without pelvic lymph node involvement, and 58% in patients with pelvic lymph node involvement. The difference was found to be statistically significant and these results are consistent with the literature. A statistically significant difference was observed between the uremia development rate of the pelvic lymph node involvement (-) and pelvic lymph node involvement (+) groups (p = 0.003) with a higher uremia development rate in the pelvic lymph node involvement (+) group. Pelvic lymph node involvement affects survival negatively.

There are studies that show a significant relationship between the number of metastatic lymph nodes and recurrence in cervical cancer [8]. In the present study, only 9% of the patients with negative pelvic lymph nodes developed recurrence while this rate was 33% in patients with pelvic lymph node involvement. The difference was found to be statistically significant (p = 0.08).

Tumor size is an important factor in the staging of cervical cancer. Salmal et al. investigated the relationship between the tumor size and recurrence of the disease in 1997 by dividing cervical cancer cases without lymphatic spread into two groups according to tumor size as those with a size of three cm and ≥ three cm. There was tumor recurrence in 4% of 120 cases smaller than three cm and in 13% of 76 cases greater than three cm. The difference was found to be statistically significant and they concluded that the tumor size has a negative effect on the prognosis by itself [9]. In 2010, Turan et al. investigated the relationship between tumor size and lymph node involvement in 174 Stage 1b patients and found lymph node involvement in 30% when tumor size was smaller than four cm and 58% when the tumor was larger than four cm and the difference was statistically significant (p = 0.013) [10]. The mean tumor size in the group with pelvic lymph node involvement was four cm and in patients without pelvic lymph node involvement 2.8 cm in the present study. Tumor size was statistically significantly higher in patients with pelvic lymph node involvement (p = 0.009). The highest risk of pelvic lymph node involvement was found when the tumor diameter was 2.5 cm.

Some articles state that tumor volume also affects the prognosis in addition to tumor size. Salmal et al. examined 179 Stage Ib-IV cervical cancer cases and found a relationship between the tumor size and tumor volume with univariate
survival analysis. In the multivariate analysis of the same study, the tumor volume and not the tumor size was found to be the independent prognostic factor [1, 11].

The prognosis worsens with increasing stromal invasion depth of the tumor [7, 12, 13]. Factors such as tumor size, IvaI, and deep stromal invasion increase the possibility of lymphatic invasion (1). In the present study, the mean invasion depth of the pelvic lymph node involvement (+) group was found to be statistically significantly higher than = the lymph node involvement (-) group ($p = 0.006$).

The possibility of parametrical invasion increases with increased tumor size and in turn increases the possibility of lymphatic metastasis. A study conducted in Stage Ia-Ib1 patients found parametrical spread in 5% of the cases. The same study found lymphatic metastases in 80% of cases with parametrical spread and in 10% of patients without spread. The present authors found a relationship between parametrical spread and tumor recurrence as 40% of patients with parametrical spread and 4% without parametrical spread were observed to have tumor recurrence [1]. Hyun Nam et al. identified the rate of parametrical involvement as 30% and reported parametrical involvement to worsen the prognosis [6].

The present authors found a parametrical involvement rate of 6% in Stage 1a-2a patients in this study. There was no statistically significant difference between the distribution of uremia development in pelvic lymph node involvement (-) and pelvic lymph node involvement (+) groups ($p = 0.730$). LvaI significantly affects the prognosis [14, 15]. Several studies have reported a five-year survival rate of 90% when there is no LvaI, decreasing to 50-70% in the presence of LvaI. Studies have reported a five-year survival rate of 90% when Lvai significantly affects the prognosis [14, 15]. Several studies have reported a five-year survival rate of 90% when there is no Lvai, decreasing to 50-70% in the presence of Lvai. In the present study, the mean invasion depth of the pelvic lymph node involvement (+) group was found to be statistically significantly higher than = the lymph node involvement (-) group ($p = 0.006$).

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The present authors found a parametrical involvement rate of 6% in Stage Ia-2a patients in this study. There was no statistically significant difference between the distribution of uremia development in pelvic lymph node involvement (-) and pelvic lymph node involvement (+) groups ($p = 0.730$). LvaI significantly affects the prognosis [14, 15]. Several studies have reported a five-year survival rate of 90% when there is no LvaI, decreasing to 50-70% in the presence of LvaI [16-18]. Pikaart et al. found LvaI in 33% of Stage Ia-IIa patients while Hyun Nam et al. reported a rate of 31%. The lymphovascular area invasion rate was 55% in the present study. The high rate is possibly associated with the higher rate of Stage Ia patients in other studies.

**Conclusion**

Knowledge of prognostic factors in cervical cancer plays an important role in determining the morbidity and mortality and the treatment strategies. When the prognostic factors that affect pelvic lymph node involvement were evaluated, increased tumor size and increased invasion depth, presence of LvaI, and an advanced stage were observed to statistically significantly increase pelvic lymph node involvement.

**References**


A survey of Jordanian obstetricians and gynecologists' knowledge and attitudes toward human papillomavirus infection and vaccination

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⁵Private Practice, Amman (Jordan)

Summary

Objective: To assess the knowledge and attitudes of Jordanian obstetricians and gynecologists toward human papillomavirus (HPV) infection and its vaccine. Materials and Methods: A self-administered, anonymous questionnaire was distributed to 400 participants attending scientific meetings. The survey focused on three areas: knowledge of HPV infection, vaccine, and attitude toward vaccination of female adolescents. Results: Survey response rate was of 72.3%. The vast majority knew most of the statements related to knowledge of HPV infection, 66% thought that conventional screening Pap test have a sensitivity of >75%, and only 44% of them knew that there are 13 to 17 HPV types that cause cervical cancer. The majority of the respondents (79%) knew that the vaccine would lead to long lasting immunity and 45% of the respondents thought that the vaccination would eliminate the need for regular Pap test. The majority (78%) indicated that the vaccine should be given to girls before the beginning of sexually active life. Overall, 67.5% of respondents intend to prescribe HPV vaccines and 79.6% of the respondents intend to recommend the vaccine if it is publicly funded. Conclusion: Most of the gynecologists in Jordan have the intention to recommend HPV vaccine, the deficit in their knowledge of HPV infection and vaccine must be corrected to assure acceptability of the vaccine.

Key words: Attitudes; Gynecologists; Human papillomavirus; Knowledge; Vaccine.

Introduction

Despite being a theoretically preventable disease, cervical cancer is the most common malignancy in women of developing countries [1], and second only to breast cancer worldwide [2]. There is now consistent and convincing evidence that cervical cancer is in fact a rare consequence of infection of the genital tract by some mucosatropic types of human papillomavirus (HPV) [3].

HPV is one of the most common sexually transmitted infections [4], with prevalence rate of 30-50% in sexually experienced young women [5,6]. Genital HPV types are categorized as low-risk types (e.g. 6 and 11), which may cause genital warts, or high risk/oncogenic types (e.g. 16 and 18), which cause virtually all cases of cervical cancerous and precancerous intraepithelial lesions [6-10]. In placebo-controlled trials, two prophylactic vaccines, a bivalent (types 16 and 18) [11,12] and a quadrivalent (types 6,11,16, and 18) [13, 14], have demonstrated almost 100% efficacy in preventing anogenital warts, persistent infection, and the development of precancerous lesions caused by the most prevalent HPV types (6, 11, 16, and 18).

It is well known that physicians can significantly influence patients’ and parents’ immunization decisions [15, 16]. In Jordan, obstetricians/gynecologists seem to have an important role in promoting HPV vaccination, given that adolescents and young females are more likely to be seen by them than any other healthcare provider. In order to do so, they should have appropriate knowledge of HPV-related diseases and HPV vaccines. It is anticipated that obstetricians/gynecologists attitudes influence their communication to patients and parents. The willingness of gynecologists to recommend HPV vaccination will be one essential step for successful implementation of HPV immunization program.

This study was the first of its kind in Jordan and Middle East region overall. It provides the first estimate of Jordanian obstetrician/gynecologists’ knowledge and attitudes about HPV infection and its prevention, as well as issues associated with willingness to prescribe HPV vaccines.

The objective of this study was to assess the knowledge and attitudes of a national representative sample of Jordanian obstetricians and gynecologists toward HPV infection and its vaccine.

Materials and Methods

From February to August 2012, a survey regarding knowledge and attitude toward HPV infection and vaccination was conducted among a national sample of obstetricians and gynecologists in Jordan. The study was approved by the institutional review board committee of the Jordan University of Science and Technology.
Table 1. — The percentage of Jordanian gynecologist who answered “yes” with the statements related to human papillomavirus (HPV) infection.

<table>
<thead>
<tr>
<th>Statement</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV is the most common sexual transmitted infection</td>
<td>267</td>
<td>92.4</td>
</tr>
<tr>
<td>&gt;70% of the people is infected with the HPV at some point in their life</td>
<td>266</td>
<td>92.0</td>
</tr>
<tr>
<td>Persistent HPV infection with the high risk subtypes is necessary cause of cervical cancer</td>
<td>260</td>
<td>90.0</td>
</tr>
<tr>
<td>Conventional screening Pap test have a sensitivity of &gt;75%</td>
<td>191</td>
<td>66.1</td>
</tr>
<tr>
<td>There are 13 to 17 HPV types that cause cervical cancer</td>
<td>122</td>
<td>42.2</td>
</tr>
<tr>
<td>HPV 6 and 11 are responsible for &gt;90% of anogenital warts</td>
<td>274</td>
<td>94.8</td>
</tr>
<tr>
<td>Anogenital warts induced by HPV 6 and 11 are not cervical cancer precursors</td>
<td>272</td>
<td>94.1</td>
</tr>
<tr>
<td>A regular Pap test with a frequency of ≤ 3 years reduces the life time risk of cervical cancer by: 71-90%</td>
<td>260</td>
<td>90.0</td>
</tr>
<tr>
<td>The proportion of cervical cancer related to HPV-16 and HPV-18 types is: 61-80%</td>
<td>273</td>
<td>94.5</td>
</tr>
</tbody>
</table>

Survey population and administration

The survey was distributed to 400 obstetricians and gynecologists attending one international conference and five national scientific meetings in obstetrics and gynecology held in different cities of Jordan that covered the whole country. According to the Jordanian Society of Obstetrics and Gynecology records in 2012, the total number of registered members was 580. The authors undertook the distribution and collection of the questionnaires to those eligible. The questionnaire was distributed to gynecologists of all health sectors (Ministry of Health, military medical services, university hospital, private practice, and others) in Jordan. In the first national scientific meeting, all those with at least six months experience in obstetrics were invited to participate, while at the following meetings only those who did not receive the questionnaire previously were invited to participate.

Survey design

The English-language structured questionnaire was anonymous and self-administered to protect confidentiality. Thus, non-responders were not identified. To establish the content validity of the questionnaire, the survey was piloted on 30 gynecologists who were excluded from the subsequent study. The two-page questionnaire focused on three conceptual areas: knowledge of HPV infection, knowledge of HPV vaccine, and attitude toward HPV vaccination of female adolescents.

The authors took the permission of Gilca et al. [17], to use their questionnaire for this study. Almost all items of knowledge of HPV infection and most of the items of attitude toward HPV vaccination were taken from the mentioned study and modified for the present population.

Nine questions of the survey focused on knowledge about HPV infection, four questions focused on knowledge about HPV vaccine, and seven questions focused on attitude toward HPV vaccination.

Nominal variables were numerically coded and entered into a database prior to analysis using Microsoft Excel program 2010. Percentages were based on the number of respondents for each variable.

Table 2. — The percentage of Jordanian gynecologist who answered “yes” with the statements related to human papillomavirus (HPV) vaccines.

<table>
<thead>
<tr>
<th>Statement</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine would lead to long lasting immunity</td>
<td>229</td>
<td>79.2</td>
</tr>
<tr>
<td>Vaccine would not cause adverse side effects</td>
<td>190</td>
<td>65.7</td>
</tr>
<tr>
<td>Vaccine would protect against genital warts in addition to cervical cancer</td>
<td>188</td>
<td>65.1</td>
</tr>
<tr>
<td>Vaccination would eliminate the need for regular Pap test</td>
<td>130</td>
<td>45.0</td>
</tr>
</tbody>
</table>

Results

Of the 400 obstetricians and gynecologists who received the questionnaire and agreed to participate in this study, 378 participants returned the surveys and 22 did not. Of those 289 surveys were complete and included in the analysis, giving a response rate of 72.3% and 91 surveys were excluded as incomplete.

Knowledge about HPV infection

Respondents’ answers for items concerning the area of knowledge of HPV infection and screening of cervical cancer are shown in Table 1. The vast majority of the respondents (90% or more) knew the following items: HPV is the most common sexual transmitted infection, >70% of the people is infected with the HPV at some point in their life, persistent HPV infection with the high risk subtypes is necessary cause of cervical cancer, HPV 6 and 11 are responsible for >90% of anogenital warts, anogenital warts induced by HPV 6 and 11 are not cervical cancer precursors, a regular Pap test with a frequency of ≤ 3 years reduces the life time risk of cervical cancer by 71-90%, and the proportion of cervical cancer related to HPV-16 and HPV-18 types is 61-80%. Sixty-six percent of respondents thought that conventional screening Pap test have a sensitivity of >75% and only 44% of them knew that there are 13 to 17 HPV types that cause cervical cancer.

Knowledge about HPV vaccines

Respondents’ answers for knowledge about HPV vaccines are reported in Table 2.

The majority of the respondents (79%) knew that the vaccine would lead to long lasting immunity. Sixty-five percent of the respondents knew that the vaccine would protect against genital warts in addition to cervical cancer and it would not cause adverse side effects. Forty-five percent of the respondents thought that the vaccination would eliminate the need for regular Pap test.

Attitudes toward HPV vaccines

Table 3 shows the respondents opinions and attitudes toward HPV vaccine use. The majority of the respondents (78%) agreed with statement that HPV vaccines should
be given to girls before the beginning of sexually active life, and 64% of them agreed with statement that the best age for a universal immunization program would be below 14 years. Sixty-seven percent of the gynecologists agreed to recommend HPV vaccination to their patients.

With regard to respondents opinion on parents and female acceptance of the vaccine: Fifty-five percent of the respondents thought that parents will accept the HPV vaccination for their daughters below 14 years of age. However, an important minority (21.8%) preferred immunization at an older age. This is in line with the results of other surveys of gynecologists, pediatricians, and family physicians in Western countries [16-22]. In order to obtain the best protection against HPV diseases; immunization is required before the beginning of sexual activity. This finding might be explained through socio-cultural perspective; in Jordan it is not accepted and unusual for females to have sexual activity before marriage.

The present findings show that HPV vaccines will be reasonably accepted (67.1%) for use by gynecologists. In general, respondents did not strongly believe that HPV vaccines will be accepted by parents, adolescents, and young adults. This might be explained by the clinicians’ concern about negative parental reaction to a discussion of STIs with their daughter or their hesitancy to discuss issues related to sexuality with preadolescent girls.

The majority of the respondents intend to prescribe HPV vaccines either for protection against both cervical cancer and anogenital warts or for protection against cervical cancer only. It indicates that the gynecologists’ knowledge and their theoretical attitude toward HPV vaccines were congruent. This confirmed that respondents’ practice was a reflection of their belief and knowledge. It is therefore suggested

Table 3. — The percentage of Jordanian’ gynecologists who agreed with the statements related to human papillomavirus (HPV) vaccines

<table>
<thead>
<tr>
<th>Statement</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV vaccines should be given to girls before the beginning of sexually</td>
<td>226</td>
<td>78.2</td>
</tr>
<tr>
<td>active life.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The best age for a universal immunization program would be: &lt;14 years</td>
<td>185</td>
<td>64.0</td>
</tr>
<tr>
<td>In your opinion most:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gynecologists will recommend HPV vaccination to their patients</td>
<td>194</td>
<td>67.1</td>
</tr>
<tr>
<td>Parents will accept the HPV vaccination for their daughters &lt; 14 years of</td>
<td>159</td>
<td>55.0</td>
</tr>
<tr>
<td>age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescents and young adults will accept the HPV vaccination</td>
<td>181</td>
<td>62.6</td>
</tr>
<tr>
<td>Your patients will comply with the counsel regarding HPV vaccination</td>
<td>193</td>
<td>66.8</td>
</tr>
<tr>
<td>A vaccination program would eventually permit the reduction of the</td>
<td>188</td>
<td>65.1</td>
</tr>
<tr>
<td>frequency of screening interventions in vaccinated females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I will recommend the vaccine to my patients:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If it is publicly funded</td>
<td>230</td>
<td>79.6</td>
</tr>
<tr>
<td>Even if the patients have to pay for the vaccine (70 JD per dose)</td>
<td>140</td>
<td>48.4</td>
</tr>
<tr>
<td>If it protects against both cervical cancer and anogenital warts</td>
<td>233</td>
<td>80.6</td>
</tr>
<tr>
<td>If it protects against cervical cancer only</td>
<td>238</td>
<td>82.4</td>
</tr>
<tr>
<td>I will prescribe HPV vaccines</td>
<td>195</td>
<td>67.5</td>
</tr>
</tbody>
</table>

Discussion

The survey had an adequate response rate (72.5%) for a national sample of obstetricians and gynecologists. The findings of this study demonstrate that Jordanian gynecologists have excellent knowledge of HPV infection, general characteristics of HPV, and its role in causing diseases. The vast majority of the respondents correctly identified the vital role of infection with HPV in the development of pre-invasive and invasive cervical cancer. Lack of precise knowledge of 66.1% of the respondents, of the sensitivity of conventional screening Pap test, and poor knowledge (42.2% of them) concerning the number of oncogenic types of HPV may have only marginal effect on the use of the vaccine.

This study found that the majority of respondents have a good knowledge about HPV vaccine. Most of the respondents (79.2%) knew that the vaccine would lead to long lasting immunity; 65.7% of them knew that the vaccine would protect against genital warts in addition to cervical cancer and it would not cause adverse side effects. Forty-five percent of the respondents thought that the vaccination would eliminate the need for regular Pap test. This represented a sound knowledge of the vaccine immunity, side effects, and protection against HPV infection. The authors suggest that it is still important that educational efforts in Jordan should maintain and improve the knowledge in this area.

Independent of the knowledge level, the majority of gynecologist (78.2%) intend to recommend HPV vaccines before the beginning of sexually active life and 64% of them believe that the best age for a universal immunization program is 14 years or younger. However, an important minority (21.8%) preferred immunization at an older age. This is in line with the results of other surveys of gynecologists, pediatricians, and family physicians in Western countries [16-22]. In order to obtain the best protection against HPV diseases; immunization is required before the beginning of sexual activity. This finding might be explained through socio-cultural perspective; in Jordan it is not accepted and unusual for females to have sexual activity before marriage.

The present findings show that HPV vaccines will be reasonably accepted (67.1%) for use by gynecologists. In general, respondents did not strongly believe that HPV vaccines will be accepted by parents, adolescents, and young adults. This might be explained by the clinicians’ concern about negative parental reaction to a discussion of STIs with their daughter or their hesitancy to discuss issues related to sexuality with preadolescent girls.

The majority of the respondents intend to prescribe HPV vaccines either for protection against both cervical cancer and anogenital warts or for protection against cervical cancer only. It indicates that the gynecologists’ knowledge and their theoretical attitude toward HPV vaccines were congruent. This confirmed that respondents’ practice was a reflection of their belief and knowledge. It is therefore suggested
that improving gynecologists’ attitudes toward HPV vaccine would be achieved by enhancing their knowledge.

Most of the gynecologist (79.6%) intend to recommend the HPV vaccine if is publicly funded and only 48.4% of them intend to prescribe the vaccine if the patients have to pay for it. This is an expected finding because, in Jordan, HPV vaccine is not part of the government-funded, school-based immunization program, and the three-dose series of the Merck quadrivalent vaccine will cost an estimated $360. It is assumed that the cost of HPV vaccines is the biggest barrier of population vaccination in the developing world.

The authors believe that this study reflects the opinion and practice of Jordanian gynecologists, and is valuable in assessing the status of knowledge HPV infection and attitude toward its vaccine in the country and could also serve as a foundation for improvement. Obstetricians and gynecologists need to be targeted because of their pivotal role in any planned future vaccination programs against HPV.

Strengths of this study are that it had a good response rate 72.5%, the respondents were anonymous, and were all obstetricians and gynecologists.

The limitations of this study are: it only assessed the knowledge and attitudes of gynecologists, although other specialists such as pediatricians and general practitioners and family physicians also play important roles in national HPV vaccination program. Secondly, although this study was designed to be representative of Jordanian Society of Obstetricians and Gynecologists, it is possible that the knowledge and attitudes of the enrolled participants are different from Jordanian gynecologists in general.

This study clearly shows that, although most of the Gynecologists in Jordan have the intention to recommend HPV vaccine, it is essential that the deficit in their knowledge of HPV infection and vaccine must be corrected to assure an adequate degree of acceptability of the HPV vaccines by the adolescents’ patients and their parents.

References


Curcumin induces human SKOV3 cell apoptosis via the activation of Rho-kinase

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Summary

Objective: Curcumin has been showed anti-inflammation and anti-cancer effect in various cancer cells such as lung cancer, breast cancer, and so on. However the pro-apoptosis effect and the mechanism of curcumin in ovarian cell is still not very clear. In this study, the authors demonstrated that curcumin induced human SKOV3 cell apoptosis and explored the underlying mechanism concerning Rho A/Rho-kinase pathway. Materials and Methods: Human SKOV3 cell was performed with MTT assay to measure the cell viability with curcumin. The cell was treatment with 15 μM or 30 μM curcumin and flow cytometry. Cell apoptosis analysis was performed to measure the cell apoptosis level. In order to explore the mechanism concerning pro-apoptosis activity of curcumin, the cells were pre-treatment with Y-27632, a specific Rho-kinase inhibitor, before curcumin was added. Then the expression of activated caspase-3 and Rho A, Rho-kinase was detected by western blot. Results: Treatment with 15 μM or 30 μM curcumin significantly promoted the apoptosis of SKOV3 cell (p < 0.05) and the apoptosis rate is dose-dependent. Curcumin also activated the expression of Rho A and Rho-kinase in a dose-dependent effect. When pre-treatment with Y-27632, the expression of activated caspase-3 was significantly decreased compared to the group without Y-27632 pre-treatment (p < 0.05). Conclusions: Curcumin induced human SKOV3 cell apoptosis in a dose-dependent effect. The pro-apoptosis effect of curcumin is partly mediated via the activation of Rho A/Rho-kinase signal pathway. This may help to further clarify the mechanisms of curcumin in ovarian cancer therapy.

Key words: Ovarian cancer; Curcumin; Apoptosis; Rho-kinase.
membrane. The membrane was blocked with 5% non-fat milk in tris buffer saline-tween (TBST) solution and incubated at 4°C overnight with primary antibody in blocking solution. After washing with TBST, the membrane was incubated at 37°C for one hour with horse radish peroxidase–conjugated secondary antibody diluted in TBST. Proteins were detected by enhanced chemiluminescence plus detection reagents. The relative expression level of protein was reflected by the band density of target proteins relative to β-actin.

**Statistical analysis**

Data are presented as mean ± standard deviation (SD). Statistical differences among different groups were assessed with one-way analysis of variance (ANOVA) and followed independent samples T-test. All Statistical analysis was performed with SPSS 18.0. Statistical significance was considered as $p < 0.05$.

**Results**

**Curcumin inhibited the survival of SKOV3 cell**

SKOV3 cells were added with curcumin at concentrations of 15, 30, or 60 μM, incubated for 12, 24, 48, and 72 hours, respectively. The control group was without any treatment. Generally speaking, the impact of curcumin on the viability of SKOV3 cells is both time and concentration dependent. High-dose curcumin significantly inhibited the viability of SKOV3 cell, and the survival rate in all time points of the high-dose group were lower than 30%. At 12 hours, the survival rate in the 15 μM and 30 μM groups were 77.23 ± 4.38 and 56.24 ± 1.97, both were significantly higher than the other time points ($p < 0.01$, Figure 1). As a result, the cell was treated with 15μM or 30μM curcumin for 12 hours in the following study.

**Curcumin increased SKOV3 cell apoptosis in a dose-dependence effect**

SKOV3 cell was treated with 15μM, 30μM curcumin or DMSO for 12 hours, the apoptosis rate of SKOV3 cell was detected by flow cytometry. There was only a low apoptosis rate observed in the DMSO group (7.56 ± 1.25). The apoptosis rate in both the higher concentration (19.88 ± 2.32) and lower concentration (33.96 ±2.57) of curcumin treatment were significantly higher compared to the DMSO group ($p < 0.01$). Meanwhile, the apoptosis rate in the 30 μM curcumin group was much higher than in the 15 μM curcumin group ($p < 0.01$). The results showed that curcumin increased SKOV3 cell apoptosis in a dose-dependent effect (Figure 2).

**Rho-kinase mediates the activation effects of curcumin on cell apoptosis**

Next, the authors explored the role of Rho A/Rho-kinase pathway in the activation effects of curcumin on the cell apoptosis. SKOV3 cells were treated with 10μM Y-27632 for 30 minutes [13], which is a specific Rho-kinase inhibitor, prior to treatment with curcumin (15 or 30 μM, 12 hours). The control group was defined as none curcumin or Y-27632 added. The expression of activated caspase-3
Curcumin induces human SKOV3 cell apoptosis via the activation of Rho-kinase

(a specific marker of cell apoptosis), Rho-kinase and Rho A in the groups was detected by western blot.

The expression of activated caspase-3 was significantly increased both in the 15 or 30 μM curcumin group compared to the control group (p < 0.05), but when pre-treated with Y-27632, the expression of activated caspase-3 was significantly decreased compared to the same concentration of curcumin group (p < 0.05). The expression in the 30 μM curcumin group was still higher than in the 15 μM group with or without Y-27632 pre-treatment (p < 0.05, Figure 3).

The expression of Rho A in the 30μM curcumin group was also higher than the 15 μM group (p < 0.01), and both groups were significantly higher than the control group (p < 0.05). When pre-treatment with Y-27632, the expression of Rho-kinase was significantly decreased (p < 0.05) and no significantly difference was observed compared to the control group (p > 0.05, Figure 5).

Discussion

The present study demonstrated that curcumin induced apoptosis in human ovarian cancer cells. The induction is mediated through the activation of Rho/Rho-kinase pathway. Many new anti-inflammatory and anti-cancer drugs have been derived from chemical scaffolds engineered from natural products. Curcumin is a polyphenolic deriv-
Curcumin is a naturally occurring compound isolated from Curcuma longa. It has long been used as a food, coloring agent, and traditional medicine. It is safe and non-toxic and has demonstrable antitumor, anti-inflammatory, apoptotic, and antioxidant properties [14]. Several reports have demonstrated that curcumin inhibits animal and human cancers, suggesting that it may serve as a chemopreventive agent.

The chemotherapeutic potential of curcumin against cancer is due to its ability to induce cancer cell apoptosis, and to inhibit cancer growth and angiogenesis [15, 16]. In-vitro and in-vivo studies showed that curcumin induces proliferation arrest and apoptotic and necrotic death in a variety of tumor cells [17, 18]. The mechanism underlying pro-apoptosis roles of curcumin in cancer cell including down-regulation of the expression of NF-κB [19], STAT3 [20], bcl-2 and p53 [21], or induces apoptosis through inhibition of the expression of reactive oxygen species [22]. Curcumin has also been shown to suppress the expression of various NFκB-regulated genes, including Bcl-2, COX-2, cyclin D1 and adhesion molecules [23]. However, little is known about its anti-tumor mechanism in ovarian cancer. In this study, the authors found that curcumin treatment increased human SKOV3 cell apoptosis in a dose-dependence effect. This conclusion may provide new evidence to the therapeutic roles of curcumin on ovarian cancer.

Rho A belongs to the Rho protein family, and Rho-kinase is one of the main downstream effector of Rho A [24]. Rho-kinase proteins are Rho-GTPase activated serine/threonine kinases that function as regulators of actin-myosin cytoskeletal dynamics via activation of LIM kinase, MLC phosphatase, and so on [25].

Rho GTPases are essential for many cell functions, including membrane trafficking [26], transcriptional activation, apoptosis [27], cell cycle progression, cell polarity, adhesion, and migration. In particular, Rho GTPases are crucial regulators of cancer progression through modulation of cell proliferation, apoptosis, invasion, and metastasis formation [28]. RhoA signalling activates the ROCK family of kinases, promoting formation of actin stress fibres and generation of the actomyosin contractile force that is required for retraction of the cell rear in mesenchymal-type movement [29]. Rho-kinase expression positively correlates with the cell migration and invasion ability. Treatment with Y-27632, a chemical inhibitor of ROCK, could decrease the invasiveness of SW620 cells [30]. Therefore, Rho GTPases appear to be appealing targets for cancer therapy.

In this study the authors found that Rho A and Rho-kinase was activated when curcumin was added to the SKOV3 cell, which means curcumin promote the activity of Rho A/Rho-kinase signal pathway. The apoptosis activity of curcumin was inhibited when Y-27632 was pre-treatment to the cells. It manifests that the pro-apoptosis roles of curcumin partly mediates through activation of Rho-kinase. The expression of caspase-3 in the higher concentration curcumin group was still higher than the lower concentration of curcumin group when Y-27632 was pre-treatment. It signifies that there are other pathways concerning the pro-apoptosis role of curcumin which needs to be further explored in the next study.

**Conclusion**

This paper confirms that curcumin induces human SKOV3 cell apoptosis in a dose-dependent effect. More importantly, the authors found that the pro-apoptosis effect of curcumin is Rho-kinase mediated.
References


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Is HE4 a useful endometrioma marker?

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Summary

Purpose of investigation: By the comparison between most used tumor marker trend (cancer antigen 125: CA 125 and human epididymal secretory protein E4: HE4) before and after laparoscopic surgery, the aim of the present study was to assess HE4 usefulness in ovarian benign cyst and endometrioma diagnosis. Materials and Methods: Thirty-eight patients were enrolled in this prospective study: 25 women underwent unilateral endometriosis ovarian cyst excision, 13 underwent benign ovarian cyst incision, and 26 were healthy controls. CA 125 and HE4 serum levels were estimated before surgery (in the early proliferative phase of the cycle) and one month after surgery. Results: A statistically significant decrease of CA 125 serum level was found after an endometrioma surgical excision but no decreases in HE4 serum level. Conclusion: In patients with endometrioma, no alteration was found in HE4 serum levels before and after surgery, while CA125 serum levels decreased after surgery. HE4 may better distinguish a malign cyst from benign one, but it is not useful in the diagnosis of low risk endometrioma.

Key words: HE4; Ca125; Ovarian cyst; Endometrioma.

Introduction

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterus and it affects 3% to 10% fertile women [1]. Thirty-five percent of benign cysts needing surgery are endometriomas [2]. The gold standard for their diagnosis is laparoscopic inspection and histological confirmation [3]. Even if endometriosis cannot seem a premalignant condition, epidemiologic, histopathological and molecular data suggest that an endometriosis environment is associated to certain subtypes of ovarian cancer, such as endometrioid and clear-cell carcinomas [4, 5]. Several molecules, especially cancer antigen 125 (CA 125), have been studied for diagnosis of endometriosis, but no specific serum marker is available yet [6].

Elevated serum levels of CA 125 may be associated with a high false positive rate to benign gynaecological condition, such as endometriosis [7], and it distinguishes with a low sensitivity benign gynaecological condition from early-stage ovarian cancer [8].

Human epididymal secretory protein E4 (HE4) is a new tumor marker, more sensitive than CA 125 in the diagnosis of early ovarian cancer, especially in young women [9]. There are no evidences in literature regarding the use of HE4 in benign pelvic mass diagnosis but it could distinguish early stages of malignant ovarian cancer from endometriomas [10] and other benign adnexal masses.

The aim of the present study was to assess if tumor marker levels trend (HE4 and CA 125) varies in different forms of ovarian cysts (and among them, especially endometrioma) by their evaluation before and after laparoscopic removal.

Materials and Methods

This prospective study was conducted in the period between October 2010 and June 2012 in the Center of Mini-Invasive Pelvic Surgery, Department of Health of Woman and Child, University of Padua.

Patients were divided in three groups: the first one, composed of women affected by symptomatic unilateral endometriomas, the second one, of those affected by unilateral ovarian cyst (cystic teratomas, serous, and mucinous cystoadenomas) undergoing surgical laparoscopic treatment, and the third (controls, young healthy women women) that did not receive surgery. Clinical symptoms such as dysmenorrhea, dyspareunia, chronic pelvic pain, and infertility clinical history were recorded and pelvic examination was preoperatively performed.

The inclusion criteria of this study were the following: 1) age > 18 and < 38 years; 2) normal ovulatory cycles with a duration between 26 and 35 days; 3) unilateral ovarian cyst with size more than three cm; 4) absence of endocrine disease (thyroid disease, diabetes mellitus, hyperprolactinemia, polycystic ovarian syndrome); 5) no prior ovarian surgery; 6) consent to participate in the study.

The exclusion criteria were: 1) malignant cysts; 2) E/P in place or in the six month prior to surgery; 3) therapy or a history of GnRH; 4) history of chemotherapy or radiotherapy; 5) pelvic inflammatory disease.

Informed consent was obtained from all patients and the study was approved by the present department’s ethical committee (n.2735P).

Preoperative workup consisted in Pap smear, urinary and blood analysis including haemachrome, PT, PTT, and electrocardiography. Blood samples of surgical patients were collected in two periods: one month before surgery, in the early proliferative phase of the cycle, and one month after surgery (during the first menstrual cycle). Controls blood samples in the early proliferative phase of the cycle were also gained. Blood was picked up within 15-30 minutes in citrated vacationers and stored at 4°C. Serum samples aliquots were centrifuged with the speed of 4,000 rpm/sec at the temperature of 4°C with the aim to separate debris and cellular contents. They were frozen at -80°C.
CA 125 and HE4 serum levels were measured with a method of chemiluminescence, the so-called Architect CA 125, HE4. The range concentration for CA 125 was 0-35 KU/L while for HE4 in premenopausal women it was < 70 pmol/L, according to manufacturer’s instructions. All assays were tested in duplicates.

Surgical procedure

The procedure were executed under general anaesthesia by the same surgeon in day hospital as described below: laparoscopic pneumoperitoneum was obtained by CO2 insufflation with an umbilical ten-mm trocar. Two five-mm ancillary trocars were introduced under direct laparoscopic observation. The first step was the exploration of the abdominal cavity to exclude endometriosis located out from the ovary. The removal of the cystic wall from the ovarian cortex was performed using scissors and grasping forceps. After identification of the cleavage plane, the stripping of the cyst was performed using two non-traumatic grasping forceps by cautious traction. All the procedures were performed without the spillage of the cyst’s content. An intracortical suture was performed with a PDS 2.0 monofilament wire, approaching the ovary edge and not damaging the ovarian tissue. Bipolar forceps were never used to minimize the possible injury to the ovarian tissue. The ovarian cysts were removed using a disposable endobag. The procedure were executed under general anaesthesia by the same surgeon in day hospital as described below: laparoscopic pneumoperitoneum was obtained by CO2 insufflation with an umbilical ten-mm trocar. Two five-mm ancillary trocars were introduced under direct laparoscopic observation. The first step was the exploration of the abdominal cavity to exclude endometriosis located out from the ovary. The removal of the cystic wall from the ovarian cortex was performed using scissors and grasping forceps. After identification of the cleavage plane, the stripping of the cyst was performed using two non-traumatic grasping forceps by cautious traction. All the procedures were performed without the spillage of the cyst’s content. An intracortical suture was performed with a PDS 2.0 monofilament wire, approaching the ovary edge and not damaging the ovarian tissue. Bipolar forceps were never used to minimize the possible injury to the ovarian tissue. The ovarian cysts were removed using a disposable endobag.

Stated that elective laparoscopic surgery was performed for benign gynecologic conditions, antibiotic prophylaxis was not administered [11]. Every specimen was examined and the nature of the lesion was histologically confirmed. The patients were discharged on the same day or the day after surgery.

Statistical analysis was performed on the collected data using Statistics Package for Social Sciences software (SPSS 19.0) and p < 0.05 was accepted as significance level. Pre- and post-operative CA 125 and HE4 serum levels were compared using Kruskal-Wallis One Way Analysis of Variance on Ranks and were reported as median values (range). Dunn’s method for multiple comparison procedures was utilized.

Results

Thirty-eight patients fulfilled the inclusion criteria of the study: 25 of them underwent surgery for endometrioma, 13 of them had surgically removed a benign ovarian mass (cystic teratomas, serous, and mucinous cystadenomas) and 26 women were recruited as controls. Their clinical characteristics are described in Table 1. The mean age of the patients was 31.9 ± 5.7, mean BMI was 22.1 ± 4.8 kg/m², mean cyst size was 5.0 ± 1.7 cm.

Table 1. — Demographic characteristics of patients.

<table>
<thead>
<tr>
<th>Cysts</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (n)</td>
<td>38</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.9 ± 5.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 4.8</td>
</tr>
<tr>
<td>Parity (n)</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Size of ovarian cyst (cm)</td>
<td>5.0 ± 1.7</td>
</tr>
<tr>
<td>Cyst volume (cm³)</td>
<td>92.0 ± 120.0</td>
</tr>
<tr>
<td>Cyst site (n)</td>
<td>right (44%)</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>66.0 ± 32.8</td>
</tr>
<tr>
<td>Intraoperative complications (n)</td>
<td>0</td>
</tr>
<tr>
<td>Blood loss (cc)</td>
<td>24 ± 22</td>
</tr>
</tbody>
</table>

No intraoperative complications were reported. Mean blood loss was 24 ± 22 cc.

In endometriosis group, preoperative CA 125 median serum level was 40.5 KU/L (interquartile range 25% 25 - 75% 112.2), postoperative one was 32.7 KU/L (interquartile range 25% 17.87-75% 65.1).

Preoperative HE4 median serum level was 42.8 pmol/L. (interquartile range 25% 37.9-75% 46.27), postoperative one was 40 pmol/L (interquartile range 25% 36.1-75% 47.2).

In non-endometriosis group, preoperative CA 125 median serum level was 17.6 KU/L (interquartile range 25% 14.27 75% 29.27), postoperative one was 25.4 KU/L (interquartile range 25% 18.57-75% 36.07). Preoperative HE4 median serum level was 44.1 pmol/L. (interquartile range 25% 37.8-75% 49.52), postoperative one was 41.7 pmol/L (interquartile range 25% 38.57-75% 48.72). In the control group, CA 125 median serum level was 14.6 KU/L (interquartile range 25% 11.5 75% 22.2), while HE4 median serum level was 36.6 pmol/L (interquartile range 25% 29.9-75% 40.5).

In patients with endometriosis, benign pelvic mass, and healthy controls, HE4 median serum level was below the 70 pmol/L limit. In the endometriosis group, CA 125 median serum level was significantly higher than in the control group and it decreased one month after surgery. In non-endometriosis group, CA 125 median serum level increased significantly after surgery.

The results were analyzed with Dunn’s Method for multiple comparison procedures: comparing the endometriosis and non-endometriosis group, pre- and post-surgery HE4 serum levels did not statistically differ. Comparing these two groups, preoperative CA125 serum levels showed a statistically significant difference, while no difference was found in the postoperative period. The values are shown in Table 2. None of the patients required laparotomic procedure and none of the patients had diagnosis of malignancy at the histopathological specimen analysis.

Table 2. — Median CA125 HE4 levels in different groups

<table>
<thead>
<tr>
<th>Ovarian benign cyst (n=13)</th>
<th>Endometriosis (n=25)</th>
<th>Controls (n=26)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 125 (KU/L) Pre-operatory</td>
<td>17.6</td>
<td>40.5</td>
<td>14.6</td>
</tr>
<tr>
<td>CA 125 (KU/L) 1 month post-operatory</td>
<td>25.4</td>
<td>32.7</td>
<td>14.6</td>
</tr>
<tr>
<td>HE4 (pmol/L) Pre-operatory</td>
<td>44.1</td>
<td>42.8</td>
<td>36.6</td>
</tr>
<tr>
<td>HE4 (pmol/L) 1 month post-operatory</td>
<td>41.7</td>
<td>40.7</td>
<td>36.6</td>
</tr>
</tbody>
</table>

* CA 125 endometriosis vs controls p < 0.05; endometriosis vs benign cyst p < 0.05; benign cyst vs controls p > 0.05.

^ CA 125 endometriosis vs controls p < 0.05; endometriosis vs benign cyst p > 0.05; benign cyst vs controls p > 0.05.

# HE4 benign cyst vs control p < 0.05; benign cyst vs endometriosis p > 0.05; endometriosis vs controls p < 0.05.
Discussion

Ovarian cysts in fertile women are a quite common findings: in most cases requiring surgery, ovarian masses are endometriomas (35% of cases) [2]. When an endometrioma was found at ultrasound investigation, an accurate examination of the pelvis by means of multisite ultrasonographic examinations is mandatory to rule out other endometriotic nodules [12-14].

Endometriosis genesis is still matter of concern and several theories were proposed to explain it [15, 16]. Inflammation plays a key role in tissue uptake of endometriosis cells: the involvement of the phosphoinositide-specific phospholipase C (PI-PLC) enzymes, corroborate the hypothesis that phosphoinositide signal (PI) may be involved in the pathogenesis of endometriosis [17].

Tissue architecture of ectopic lesions shows a significant heterogeneity and abnormalities, compared to normal endometrium features [18]. In endometriosis, eutopic endometrium, biochemical and ultrastructural features may also present a significant difference than those of normal endometrium. They are particularly marked in the mild-secretory part of the cycle: these findings may explain the association between endometriosis and infertility [19].

Epidemiologic, histopathologic, and molecular data suggest that endometriosis has a malignant potential [4]. The most frequently used marker for ovarian cancer is CA 125: it is a high weight molecular glycoprotein, also present in the epithelium of fallopian tubes, endometrium, endocervix, peritoneum, pleura, and pericardium [20].

Elevated serum levels of CA 125 are not only associated to endometriosis, but also to many conditions, such as pelvic inflammatory disease, ovarian hyperstimulation, fibroids, pregnancy [15], tuberculosis, cirrhosis, and other carcinomas. It could help to distinguish, with a high false positive and a low sensitivity, early – stage of ovarian cancer from benign gynaecological conditions [7,8]. CA 125 concentration varies on the phases of menstrual cycle [21] and it decreases during treatment with danazol and leuporelin acetate [22].

HE4 is a new tumor marker: its gene expression is amplified in ovarian carcinomas than in normal tissue [23].

A total eradication of the lesion should be performed, minimizing the damage to the ovarian reserve, and avoiding consequent disease relapse due to incomplete treatment hence [24].

Several studies comparing CA 125 and other markers show the necessity of a really sensitive blood test marker for endometriosis. Tokmak et al. [6] compared Urocorbin and CA 125: they did not find Urocorbin to be effective as or more than CA 125 in distinguish endometrioma from other benign cysts. Mohamed et al. [25] compared CA 125 to VEGF-A and concluded that VEGF-A is better than CA 125 for the diagnosis and the follow-up of advanced endometriosis after conservative laparoscopic surgery.

Since HE4 has been identified as the molecular candidate for early stages of ovarian cancer [26], it is less frequently raised in patients with non malignant ovary disease [23]. HE4 may have a relative low specificity: in fact, some pulmonary, endometrial, and breast adenocarcinomas can express it [27].

Even if elevated serum CA 125 concentrations are associated with the presence of moderate to severe endometriosis [28], its utilization in the detection of endometriosis has shown a specificity of 85% and a sensitivity between 20% and 50% [29].

In the present series, comparing HE4 and CA125, the authors did not observe differences of HE4 serum levels in the three study group: before and after surgery its value remained under the cutoff. CA 125 was slightly higher in the endometriosis group before surgery and it decreased one month after surgery. This result was related to the effectiveness of the surgical approach. In the non-endometriosis group, the authors recorded an unexpected increase of CA 125 after surgery: it might be due to the inflammatory reactions which may alter the peritoneal permeability.

In the present study, all the patients subjected blood samples in the early proliferative phase of the cycle in order to minimize CA125 fluctuations: in menstrual period CA125 mean concentrations, could be significantly higher in endometriosis patients than in control group [30]. On the other hand, HE4 serum levels measurements can be performed at any phase of the menstrual cycle [31].

It could help to better discriminate patients with ovarian malignancies from those with endometriomas, as reported by Huhtien et al. [10]. As previously asserted in literature [32], the present authors confirmed HE4 and CA 125 combination could differentiate ovarian endometriosis from ovarian malignant masses but in the pre-surgery work up of low risk ovarian cysts, HE4 is not useful.

Given that pre- and post-surgery CA125 serum assay can help to detect the recurrence of endometrioma, while HE4 normal levels are strongly suggestive of a benign condition [33], in the authors’ opinion, its dosage is unnecessary in patients with adnexal pathology without suspect of malignancy and would increase healthcare spending.

References


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Introduction

Ovarian cancer has the highest mortality rate among gynecologic cancers. Combination paclitaxel and carboplatin (TC) therapy is a standard regimen that has improved the response and survival rates of ovarian cancer patients up to 20-30% and 60-70%, respectively [1]. However, more than 50% of patients who initially respond to treatment eventually experience disease recurrence [2]. Such patients must receive long-term treatment following recurrence. While progress in chemotherapy has extended the average lifespan of cancer patients, the complications associated with long-term use of these agents can be quite serious. In particular, secondary malignancy, notably therapy-related acute myeloid leukemia (t-AML), has become an issue in this patient population [3, 4].

t-AML accounts for 10-30% of all AML cases. Although alkylating agents and topoisomerase II inhibitors have historically been the primary agents associated with secondary t-AML, the incidence of t-AML in conjunction with paclitaxel treatment has increased in recent years [5]. In this manuscript, the authors review a case of treatment-related bone marrow dysplasia syndrome (t-MDS) progress to t-AML after TC therapy and radiation therapy.

Case Report

The patient was a 65-year-old woman with a medical history significant for primary Stage IIIc grade ovarian cancer ten years prior. At that time, she received initial surgery (total abdominal hysterectomy [TAH] / bilateral salpingo-oophorectomy [BSO] + omentectomy + appendectomy) followed by six courses of cyclophosphamide/doxorubicin/cisplatin (CAP) therapy. Beginning in February 2005, the patient achieved multiple remissions due to sternal metastasis. Chemotherapy, including paclitaxel and carboplatin (TC), was administered intermittently and was combined with radiation therapy to a sternal metastatic lesion. Pancytopenia was observed in December 2008, and she was diagnosed with t-MDS (WHO subtype, refractory cytopenias with multilineage dysplasia [RCMD]); the time from first chemotherapy to t-MDS onset was 106 months. Without evidence of blast crisis, the recurrent lesions continued to grow and caused multiple cerebral infarctions, from which she eventually died. The cumulative doses of paclitaxel and carboplatin administered to this patient were 1,968 mg and 6,480 mg, respectively.

Key words: Ovarian carcinoma; Myelodysplasia; Secondary leukemia; Paclitaxel; Radiation treatment.
Table 1. — Clinical course of the patient.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Total dose of each chemotherapy agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial treatment</td>
<td></td>
</tr>
<tr>
<td>6 courses of CAP therapy</td>
<td></td>
</tr>
<tr>
<td>12 courses of TC therapy</td>
<td>Paclitaxel 135-155 mg/150-168 mg/body</td>
</tr>
<tr>
<td></td>
<td>1,968 mg Carboplatin AUC=3.5-4 2,580 mg</td>
</tr>
<tr>
<td>6 courses of irinotecan/carboplatin therapy</td>
<td>60 mg/×2 720 mg Carboplatin AUC=5 1,800 mg</td>
</tr>
<tr>
<td>Radiation therapy to sternum (combination with cisplatin)</td>
<td>Cisplatin 30 mg/30 mg/body 30 mg</td>
</tr>
<tr>
<td>Treatment for recurrent disease</td>
<td></td>
</tr>
<tr>
<td>6 courses of irinotecan/carboplatin therapy</td>
<td>60 mg/×2 720 mg Carboplatin AUC=5 1,200 mg</td>
</tr>
<tr>
<td>6 courses of irinotecan/carboplatin therapy</td>
<td>60 mg/×2 720 mg Carboplatin AUC=5 900 mg</td>
</tr>
<tr>
<td>Onset of MDS</td>
<td></td>
</tr>
<tr>
<td>1 course of GD therapy</td>
<td>Docetaxel 80 mg/×60% 50 mg 900 mg/×60%×2 1,200 mg</td>
</tr>
<tr>
<td></td>
<td>50 mg/body 600 mg/body×2</td>
</tr>
</tbody>
</table>

CAP = cyclophosphamide/doxorubicin/cisplatin; TC = paclitaxel/carboplatin; GD = gemcitabine/docetaxel
Therapy-related myelodysplastic syndrome and acute myeloid leukemia following chemotherapy (paclitaxel and carboplatin) etc.

Pancytopenia was observed when the patient was hospitalized for a recurrence to the adrenal gland in December 2008. Hematologic values were: hemoglobin (Hb), 7.7 mg/dl; white blood cell (WBC) count, 3.0 x 10^9/l; and platelet count, 51 x 10^9/l. Moreover, bone marrow examination showed dysplastic changes in all blood cell types. Based on these results, the patient was diagnosed as having myelodysplastic syndrome (MDS), subtype refractory cytopenias with multilineage dysplasia (RCMD), characterized by bone marrow blasts, 2.6%; severe chromosomal aberrations; IPI score, Int-1; and therapy-related s/o) (Figure 3).

Karyotype analysis revealed that 15 out of 20 cells harbored aberrations within chromosomes 6, 13, 17, and 21 (Figure 4). These findings were compatible with t-MDS. At this point, 106 months had passed since initial treatment, and her cumulative doses of paclitaxel and carboplatin were 1,968 mg and 6,480 mg, respectively.

Because the patient and her family hoped to continue treatment, one course of GD therapy (gemcitabine 600 mg/body [900 mg/m^2 × 60%] x 2 + docetaxel 50 mg/body [80 mg/m^2 × 60%]) was administered. However, following this treatment, further chemotherapy was cancelled due to severe pancytopenia (Hb, 6.3 g/dl; WBC count, 1.5 x 10^9/l; and platelets, 1.3 x 10^9/l). While supportive therapy (e.g., pain management) and blood transfusion were continued, the recurrent tumors continued to grow (liver metastasis, 22 mm; right adrenal gland metastasis, 62 mm). Finally, the patient died of multiple cerebral infarctions caused by a tumor embolism 15 months later (Figure 5).
Platinum-based chemotherapy has remarkably improved the survival of ovarian cancer patients in the past 20 years [2]. However, chemotherapy with platinum agents and taxanes has also increased the risk of developing t-AML [6]. Combination paclitaxel and carboplatin (TC) has been considered to be the gold standard for ovarian cancer treatment. These agents exhibit synergistic efficacy, enabling response rates as high as 75%. Although moderate bone marrow suppression is usually observed, TC therapy is associated with relatively few serious side effects [7]. The dose-limiting toxicities of TC therapy include neurotoxicity, nephrotoxicity, and bone marrow toxicity [8, 9]. In most cases, these toxicities are both well tolerated and controllable. However, as the survival period of ovarian cancer patients has lengthened, concern about side effects associated with chronic chemotherapy administration, such as t-MDS, has increased.

Table 2. — Classification of MDS

<table>
<thead>
<tr>
<th>Type</th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducer</td>
<td>Alkylating agents, platinum agents, radiation therapy</td>
<td>Topoisomerase inhibitor II</td>
</tr>
<tr>
<td>Time to onset</td>
<td>4-7 years</td>
<td>6 months to 5 years</td>
</tr>
<tr>
<td>Type of disease</td>
<td>MDS, AML, M1, M2</td>
<td>AML, M4, M5</td>
</tr>
<tr>
<td>Chromosomal abnormality</td>
<td>Deletions 5q-, -7</td>
<td>Translocation type 11q23</td>
</tr>
<tr>
<td>Sensitivity to chemotherapy</td>
<td>Poor</td>
<td>Relatively good</td>
</tr>
</tbody>
</table>

t-MDS is divided into two subtypes according to certain characteristics, including causal drugs, clinical features, and chromosomal aberrations (Table 2). Recently, paclitaxel-induced t-MDS and t-AML have been reported to develop relatively soon after initial administration of paclitaxel and to harbor several chromosomal aberrations. Clinically, these conditions develop as leukemias without passing through a state of dysplasia, and are associated with a serious clinical course similar to that of type 2 MDS [10].

Several reports about paclitaxel-induced t-AML have been published in recent years [1, 5, 7, 11-16]; these reports, as well as those describing t-MDS and the current case, are summarized in Table 3 [5, 13-16, 17-19]. Paclitaxel has also been shown to promote mutations in cultured cells in vitro [13]. However, the precise mechanisms that cause t-AML remain unknown. Carboplatin is also a leukemogenic drug. Thus, it has been hypothesized that the addition of carboplatin to paclitaxel therapy may potentially increase the risk of developing leukemia [11,12].

The treatment history of the present patient included radiation therapy, an alkylating agent, and > six g of carboplatin. She developed t-MDS 106 months (~8.8 years) after initial treatment, and harbored chromosome 5 and 7 deletions in many cells. Based on these findings, she was diagnosed with type 1 MDS. The risk of developing type 1 MDS has been reported to depend on the total dosage of anticancer agents administered [20]. Therefore, patients receiving long-term treatment must be carefully followed-up. In addition, chemotherapy combined with radiation therapy may increase the risk of developing t-MDS, since the present patient underwent radiation therapy for her sternal...
Table 3. — Literature review of t-AML cases following exposure to paclitaxel or radiation therapy

<table>
<thead>
<tr>
<th>Author</th>
<th>Age (years)</th>
<th>Primary tumor</th>
<th>Initial chemotherapy until onset of MDS</th>
<th>Period from initial treatment to onset of MDS</th>
<th>Preceding MDS</th>
<th>Prognostic group (prognosis of MDS or AML and cause of death)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seymour et al.(13)</td>
<td>57</td>
<td>Ovarian carcinoma</td>
<td>PTX 1.800 DOC 0 CBDDA 3.600 CBDCA 840 CPA 8.400 EPI 0 CPT-11 0 DXR 0 GEM 0</td>
<td>22</td>
<td>M4</td>
<td>inv(16)(p13q22) 13M Poor (recurrence)</td>
</tr>
<tr>
<td>Seymour et al.(13)</td>
<td>53</td>
<td>Ovarian carcinoma</td>
<td>PTX 450 DOC 0 CBDCA 750 CBDCA 2.400 CPA 0 CPT-11 0 DXR 0 GEM 0</td>
<td>17</td>
<td>M4</td>
<td>inv(16)(p13q22) 10M Poor (t-AML)</td>
</tr>
<tr>
<td>See et al.(14)</td>
<td>52</td>
<td>Ovarian carcinoma</td>
<td>CPA+CBDCA (6 months), RT (50 Gy)</td>
<td>14</td>
<td>No</td>
<td>45XX,t(1,5)(q25;q35)del(4)q21q26,ins(7)(p15:?),del(12)(p12),-13,-17,-17,add(19)(p13.3),add 22 (q13) 23M Poor (t-AML)</td>
</tr>
<tr>
<td>Tasaka et al.(15)</td>
<td>43</td>
<td>Ovarian carcinoma</td>
<td>PTX (8 months)</td>
<td>33</td>
<td>NS</td>
<td>46,XX,inv(16)(p13q22)[15] Favorable</td>
</tr>
<tr>
<td>Dissing et al.(16)</td>
<td>51</td>
<td>Leiomyosarcoma</td>
<td>PTX (3 months)</td>
<td>33</td>
<td>M4</td>
<td>46,XX,inv(16)(p13q22) Favorable</td>
</tr>
<tr>
<td>Sajiyo et al.(21)</td>
<td>71</td>
<td>Ovarian carcinoma</td>
<td>CPA+CBDCA (6 months)</td>
<td>61</td>
<td>NS</td>
<td>46,XX,inv(16)(p13q22) Favorable</td>
</tr>
<tr>
<td>Abe et al.(22)</td>
<td>70</td>
<td>Ovarian carcinoma</td>
<td>CPA+CBDCA (6 months), RT (50 Gy)</td>
<td>61</td>
<td>NS</td>
<td>46,XX,inv(16)(p13q22)[23];46,XX[11] Favorable</td>
</tr>
<tr>
<td>Abe et al.(22)</td>
<td>58</td>
<td>Ovarian carcinoma</td>
<td>PTX (8 months)</td>
<td>33</td>
<td>M4</td>
<td>46,XX,inv(16)(p13q22) Favorable</td>
</tr>
<tr>
<td>Sakate et al.(10)</td>
<td>56</td>
<td>Ovarian carcinoma</td>
<td>CPA+CBDCA (6 months)</td>
<td>61</td>
<td>NS</td>
<td>46,XX,inv(16)(p13q22) Favorable</td>
</tr>
<tr>
<td>Murakami et al.(23)</td>
<td>59</td>
<td>Ovarian carcinoma</td>
<td>CPA+CBDCA (6 months)</td>
<td>61</td>
<td>NS</td>
<td>46,XX,inv(16)(p13q22) Favorable</td>
</tr>
<tr>
<td>Yeasmin et al.(5)</td>
<td>73</td>
<td>Ovarian carcinoma</td>
<td>CPA+CBDCA (6 months)</td>
<td>61</td>
<td>NS</td>
<td>46,XX,inv(16)(p13q22) Favorable</td>
</tr>
</tbody>
</table>

ND = Not described; PTX = paclitaxel; DOC = docetaxel; CBDDA = carboplatin; CBDCA = cisplatin; EPI = epirubicin; CPT-11 = irinotecan; DXR = doxorubicin; GEM = gemcitabine

AUTHOR = Much of the data need units, e.g., are columns F-N doses? Are columns O and S months? Also, the See et al. data are missin, and the meaning of
metastasis. Concurrent radiation therapy/chemotherapy is generally performed for cervical cancer. Recently, concomitant radiation therapy/chemotherapy has been reported to also be highly effective for endometrial cancer [21]. However, combination radiation therapy/chemotherapy may increase the risk of t-AML and t-MDS [22]. The risk of t-AML and t-MDS also depends on the patient’s age at initial therapy [23]. Thus, any treatment plan should consider patient age.

Consistent with the current case, risk factors for secondary leukemia include using agents with high accumulation, long-term chemotherapy, patient age at initial treatment, and combination radiation therapy/chemotherapy [24]. Since paclitaxel is an effective agent for gynecologic malignancies and is used in most patients, gynecologists must consider the risk of t-MDS and t-AML when making treatment plans for their cancer patients. Proper usage of paclitaxel may prevent cancer patients from developing t-MDS and t-AML [3].

References

Rectus abdominis muscle resection and fascial reconstruction for the treatment of uterine leiomyosarcoma invading the abdominal wall: a case report

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Department of Obstetrics and Gynecology, CHA Gangnam Medical Center, CHA University, Seoul (Korea)

Summary
The authors present a case of intra-abdominal recurrent leiomyosarcoma invading a large area of the abdominal wall. The patient underwent cytoreductive surgery, including resection of the rectus abdominis muscle, followed by reconstruction of the defect using synthetic mesh. The tumor was surgically removed by en bloc resection, including most of the rectus abdominis muscle and ileum. The abdominal wall defect was repaired using synthetic mesh. The patient underwent radiotherapy and chemotherapy after the surgery and was healthy one year later.

Key words: Leiomyosarcoma; Abdominal wall; Mesh repair; Recurrence; Reconstruction.

Introduction
Uterine leiomyosarcoma is highly aggressive and more than half of patients develop a recurrence of the tumor. The most common sites of recurrence are the lung, abdomen, and pelvis [1]. Surgery is the mainstay of the treatment when the recurrent lesion is localized, and its complete removal is associated with a complete cure or prolonged survival [2].

When a large defect in the abdominal wall occurs following the radical excision of tumors, abdominal wall reconstruction is required. For fascial reconstruction, recently synthetic mesh has been applied [3], but some complications following surgery to repair an abdominal wall defect with synthetic mesh have been reported [4, 5]. If bowel or genitourinary tract reconstruction is also required, the risk of postoperative mesh infection is slightly increased due to bacterial contamination [6]. However, defect reconstruction associated with intra-abdominal malignancy that invades the abdominal wall has been reported only rarely [7-9], and there are no reports of cases with leiomyosarcoma.

Herein the authors present the case of a patient with intra-abdominal recurrent leiomyosarcoma invading a large area of the abdominal wall. The patient underwent cytoreductive surgery including resection of the rectus abdominis muscle and defect reconstruction using synthetic mesh successfully.

Case Report
A 52-year-old female patient (gravid 1, para 1) was treated at the present gynecological oncology clinic in January 2011. She complained of a large palpable abdominal mass and lower abdominal pain for three days. Her body mass index (BMI) was 25.2 kg/m², and she had no medical history. On pelvic examination, a hard stony mass near the umbilicus was palpated. Transabdominal sonography revealed 15.0´8.7- and 11.7´10.1-cm masses in the uterus with mixed and irregular cystic changes. Pelvic magnetic resonance imaging (MRI) revealed a 22.0´16.0´9.5-cm mass with two cystic change compartments (Figure 1A). No metastasis was found during the preoperative evaluation. A laparotomy was performed through an extended midline incision. Operative findings showed an enlarged uterus including two large interconnected masses measuring ~21 cm in total with an intact serosa (Figure 1B). The frozen sections revealed the leiomyosarcoma, and thus, a total abdominal hysterectomy (TAH) with bilateral salpingo-oophorectomy (BSO), omentectomy, bilateral pelvic lymph node dissection, and para-aortic lymph node dissection were performed. On final pathologic examination, the mass comprising the leiomyosarcoma included most of the thickness of the myometrium with geographic ischemic necrosis, marked atypia, and frequent bizarre mitoses. A metastasis was present in one pelvic lymph node of total 47 resected para-aortic and pelvic lymph nodes. Her final diagnosis was leiomyosarcoma of the uterus, of Stage IIIc. The patient underwent postoperative adjuvant chemotherapy including six cycles of platinum-based multiagent chemotherapy: paclitaxel 175 mg/m² on day 1, cisplatin 75 mg/m² on day 2, and ifosfamide five g/m² over 24 hours with mesna five g/m² on day 2, and three g/m² on day 3, with continued hydration.

On follow-up examination three months after the completion of chemotherapy, positron emission tomography-computed tomography (PET-CT) showed a newly developed hypermetabolic lesion in lungs, suggesting metastasis. She underwent a multifocal wedge resection of the lung. The pathology was determined to be leiomyosarcoma metastatic from the uterus. She was followed-up four months after the lung surgery. She revisited the clinic in January 2012, complaining of a palpable large mass in her abdomen with intestinal obstruction. CT revealed a new heterogeneous enhancing mass in the lower abdominal cavity measuring 15´11´9 cm with abdominal wall invasion (Figure 2A). PET-CT also showed a new large cystic and solid mass with increased fluorodeoxyglucose (FDG) uptake of the solid portion (SUV = 16.5) suggestive of a recurrent malignancy, without other sites of recurrence (Figure 2B).

She underwent an exploratory laparotomy through the previous midline skin incision. The mass invaded the entire depth of the rec-
B.S. Yoon, S.J. Seong, T. Song, M.L. Kim, M.K. Kim

450
tus muscle of about 10·10 cm horizontally (Figure 2C). After the incision of the subcutaneous tissue at the midline, dissection along the anterior rectus sheath was performed until the linea semilunaris was encountered laterally. At the linea semilunaris, the fascia and the peritoneum were opened bilaterally. The upper margin of the mass was not cleared, because the mass adhered to the ileum and the anterior abdominal wall with aggregation. The fascia of the distal part of the rectus abdominis muscle that was connected to the symphysis pubis was free of any tumor mass, so it was ligated and cut at this site. The posterior wall of the mass was not severely adhered, so the mass was lifted up together with an adhered part of the ileum of ~1 m in length. Ileal resection with the margin free and reanastomosis were performed. Finally, the rectus muscle and fascia were ligated and cut at a site one cm distal from the upper margin of the tumor. A closed drain was inserted into the pelvic cavity. Biodegradable adhesion barrier film was applied over the bowel underneath the abdominal wall to prevent further adhesion. The defective rectus abdominis muscle, fascia, and peritoneum were replaced with a synthetic mesh (monofilament polypropylene). Mesh fixation was performed with separate 2/0 prolene sutures on the fascial margin. An open silastic drain was inserted over the mesh before the abdomen was closed. The final pathology was determined to be recurrent leiomyosarcoma and the patient underwent sequential chemo-radiotherapy postoperatively; three cycles of cisplatin 50 mg/m² and adriamycin 60 mg/m² on one day, continuing whole-pelvis irradiation 5,040 cGy, followed by three cycles of chemotherapy with the same regimens. She is well with no complications one year after secondary cytodebulking surgery.

**Discussion**

Surgical resection is important for the treatment of locally recurrent leiomyosarcoma, as *en bloc* resection of this tumor is associated with prolonged survival [2]. However, abdominal wall defect is inevitable due to *en bloc* resection of the tumor, when the size of the intra-abdominal tumor increases and the anterior abdominal wall is invaded. If the abdominal wall defect is small, primary closure is possible due to the sufficient tensile strength. When the defect is large, primary closure is impossible, and if the wall is approximated by

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**Figure 1.** — (A) The first MRI of the uterine leiomyosarcoma conducted prior to the staging laparotomy. (B) Photograph of the resected uterus, including the huge mass with intact serosa.

**Figure 2.** — (A) CT of the abdominal mass recurrence invading the rectus abdominis muscle (arrows-margin of mass). (B) PET-CT showing the recurrent mass with increased FDG uptake of the solid portion. (C) Photograph of the resected recurrent mass attached to the ileum and invading the abdominal wall structure.
force, the chance of a postoperative hernia is increased. In the past, resorbable mesh or autologous fascia flaps were used to repair large defects [3]. Due to development of materials, synthetic mesh is now commonly used, inducing intense fibrovascular infiltration that is incorporated into the surrounding myofascial tissue to provide a strong repair. Although there is a risk of complications, such as hernia, wound complication, and fistula, after use of synthetic mesh, the incidence is low [4, 5].

In many instances, cancer patients have undergone radiotherapy and/or chemotherapy prior to the surgery. These conditions may suppress the immune system, decrease vascularization, and impair wound healing. In addition, advanced malignancy is commonly accompanied by bowel or genitourinary tract surgery, promoting intra-abdominal bacterial contamination and impairing wound healing. To overcome these drawbacks, an acellular dermal matrix (ADM) biologic mesh was used as a substitute for a synthetic mesh in high-risk patients with prior radiation therapy or bacterial contamination, reducing the rate of wound complications. This patient also had a weak immune system due to previous chemotherapy and bowel resection. Nevertheless, abdominal wall reconstruction was successful and no complications related to the synthetic mesh occurred.

The reports of abdominal wall reconstruction for en bloc resection after the invasion of the abdominal wall with advanced intra-abdominal malignancy are rare [7-11]. Luna–Perez et al. reported treatment of 17 patients with colon cancer that invaded the abdominal wall with abdominal wall reconstruction using a synthetic mesh. Wound complications occurred in 5.8% of patients; no postoperative hernias occurred [7]. Yezhelyev et al. reported that in patients with tumors originating from the gastrointestinal tract, 6.7% and 13.3% showed wound infections and postoperative hernias, respectively, after repair using synthetic and/or biological mesh [9].

Herein, the authors presented a case of advanced leiomyosarcoma that invaded the abdominal wall. En bloc resection and abdominal reconstruction, with the aid of synthetic mesh, were performed with no complications, although the patient had previously undergone chemotherapy and bowel surgery.

References


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A case of accessory mammary cancer in a male patient and a literature review

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Summary
A 68-year-old Chinese male patient was referred to the present hospital because of a right axillary lump in May 2011. Physical examination showed a rigid movable mass measuring 35 mm in diameter in the right axilla. No mass was palpable in either breast. Mamograms were normal. Physical and imaging examination of the head and neck region, lung, and upper and lower gastrointestinal tract also revealed no evidence of a primary tumor. Ultrasonography and resonance imaging (MRI) revealed no evidence of tumors in the bilateral mammary glands. Fine needle histological biopsy for suspected malignancy was performed, and the patient underwent tumor resection with axillary lymph node dissection on Jun 23, 2011. Moderately differentiated adenocarcinoma in ectopic breast tissue was diagnosed based on the pathologic result, the tumor was immunohistochemically positive for ER, PR, and HER-2.

Key words: Accessory breast; Breast neoplasms; Axilla; Male.

Introduction
It was reported that cancer originating from accessory or aberrant breast tissue with an incidence of 0.3-0.6% of all breast cancers, 70-80% of these cases originated from the axillary region [1-3]. Breast cancer in men accounts for <1% of all breast cancer cases in the world and ~0.1% of cancer mortality in men [4, 5]. Therefore the occurrence of accessory breast cancer in male is even rarer. Here the authors report an axillary malignant tumor in a male, which was diagnosed pathologically as breast carcinoma. According to their search, there are only five cases of axillary accessory breast malignant tumor in male up to today since the first case was reported in Russia [6].

Case Report
A 68-year-old male patient first noticed a nodule in the right axilla in 2002 at age of 60. He consulted a local dermatologist and was diagnosed as a benign skin nodule. He was advised to have no treatment and to just follow up conservatively. However, In May 2010, after follow-up for eight years, the mass began to enlarge at a discernible rate with no presence of tenderness. The patient denied any nipple discharge, retraction, or skin ulceration. There was no medical and surgical history of fever, cough, weight loss, appetite loss, or melena. The patient denied any known risk factors for the development of breast cancer, including a history of breast trauma, irradiation exposure, or known family history.

The patient was referred to the Breast Ward of Department of General Surgery in the present hospital in May 2011 for further therapy. At the first visit, a physical examination revealed an inelastic hard movable mass with an irregular surface, measuring 35×30 mm, in the right central axilla. Part of the covering skin was purplish pallor but no tenderness. The size of the resected tumor was 3.5×2.5×2.5 cm, and the final histopathologic examination of the resected sample demonstrated moderately differentiated adenocarcinoma originate from accessory breast gland with cleaning cutting edge, and none of eleven axillary lymph nodes were invaded by carcinoma. The immunohistochemical staining showed (Table 1) that 80% estrogen receptor (ER) and 70% progesterin receptor (PR) were positive, human epidermal receptor (HER-2) were (+) (Figures 1-4). Immunohistochemical staining yielded other following results: negativity for P63 and a literature review.
protein and smooth muscle actin (SMA) and positivity for cytokeratin (CK), carinoembryonic antigen (CEA), and focal positivity for gross cystic disease fluid protein–15 (GCDFP15); Ki-67 index (Figures 5, 6) was 15-20%. These features strongly suggested a moderately differentiated invasive ductal carcinoma. Based on the pathologic results, the authors diagnosed the tumor as breast cancer originating from the accessory mammary gland in the right axilla rather than cutaneous adnexal carcinoma.

Table 1. — *The pathological and immunohistological analyses.*

<table>
<thead>
<tr>
<th>Location</th>
<th>Pathologic types</th>
<th>Estrogen receptor (ER)</th>
<th>Progestin receptor (PR)</th>
<th>HER-2-neu/ C-erbB2</th>
<th>Ki-67 index</th>
<th>Cytokeratin (CK)</th>
<th>Carinoembryonic antigen (CEA)</th>
<th>P63</th>
<th>GCDFP-15</th>
<th>Smooth muscle actin (SMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right axilla (80)</td>
<td>adenocarcinoma</td>
<td>80% (+)</td>
<td>70% (+)</td>
<td>positive(+)</td>
<td>15-20%</td>
<td>positive</td>
<td>negative</td>
<td>focal positive</td>
<td>negative</td>
<td>negative</td>
</tr>
</tbody>
</table>

Table 2. — *Summary of the articles reviewed about the previous reported cases of male accessory breast cancer.*

<table>
<thead>
<tr>
<th>Author and year [Reference]</th>
<th>Age (yrs)</th>
<th>Location (Largest Dimension, mm)</th>
<th>Pathologic types</th>
<th>Estrogen receptor (ER)</th>
<th>Progestin receptor (PR)</th>
<th>HER-2-neu/ C-erbB2</th>
<th>Ki-67 index</th>
<th>P53</th>
<th>GCDFP-15</th>
<th>Treatment</th>
<th>Prognosis</th>
<th>Clinical follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takeyama et al., 2010 [7]</td>
<td>58</td>
<td>Right axilla (80)</td>
<td>Adenocarcinoma</td>
<td>40% (+)</td>
<td>30% (+)</td>
<td>Negative</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>Two cycles of neoadjuvant chemotherapy + right axillary lymph node dissection + endocrine therapy</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

Schneider et al., 2009 [8]  | 82        | Right axilla (70)                | Metaplastic carcinoma with prominent squamous differentiation | Positive | Positive | Weakly positive | Positive | Positive | NC | Modified radical mastectomy | NC | NC       |

Lin et al., 2011 [9]       | 65        | Right axilla (55)                | Moderately differentiated adenocarcinoma of unknown origin | Positive | Positive | negative | 5% positive | Positive | Radical mastectomy | No residual or 18 months | metastasis | |

Figure 1. — Postoperative H&E staining demonstrates solid nests and cord-like arrangements of cancer cells with tubule and alveoli formation (HE stains original magnification ×100).

Figure 2. — IHC stain of 80% ER was positive. Showing positive expression of ER in carcinoma cell cytoplasm (SP ×100).

**Discussion**

Ectopic breast tissue has been reported to occur up to 6% of the population, more frequently in women, and in the axilla. The first case of male accessory breast cancer was reported in 1957 in Russia [6]. To the present authors’ knowledge, this report is the fifth case of male breast cancer originated from an accessory mammary gland reported in the literatures [7-9]. Literature related to male accessory
A case of accessory mammary cancer in a male patient and a literature review

breast cancer were searched using PubMed and three reports were listed in Table 2. Therefore a primary breast cancer arising in an accessory mammary gland in male is extremely rare disease.

For this patient with adenocarcinoma arising from a unilateral axilla, the first step of the diagnosis was to identify whether the tumor was a primary lesion or a metastatic epithelial neoplasm from the breast, gastrointestinal tract, lung, or prostate. Histopathologically, no lymph node tissue or lymphovascular infiltration was identified in the specimen. There was also no evidence suggesting axillary node metastasis, such as intraductal spread, necrosis, or nodular lesions associated with a well-circumscribed tumor. The presence of isolated regions of non-cancerous breast tissue adjacent to the malignant tissue suggested that the carcinoma originated from accessory breast tissue. Moreover, a precise imaging assessment of the bilateral mammary gland excluded the possibility of an occult primary breast cancer with unilateral axillary metastasis. Therefore the authors believed that this tumor was a primary breast cancer originating from the accessory mammary tissue in the axilla.

When facing the cases manifestation of the axillary tumor in clinic, we should discriminate accessory mammary cancer from the occult breast cancer [10]. Among malignant neoplasm of the axilla, malignant lymphoma is the most common cases. Some types of adenocarcinoma also metastasize to axillary lymph nodes, the possible primary sites including the mammary gland, lung, thyroid, stomach etc. They usually present lymph node metastasis of axilla, supraclavicular or infraclavicular fossa as the primary manifestation [11]. In case of seeing similar patients in clinic in the future, the present authors better suggest some relative examination such CT
and MRI to find the key of diagnosis and avoid misdiagnosis. In this case, the patient presented axillary tumor as the first sign. The final diagnosis on this patient was made after many pathologists’ consultation and a series of examinations for confirmative diagnosis. Hereby, the histological examination and IHC stain of the pathological samples is very important in the diagnosis of the accessory breast cancer.

In this case, it was showed that 80% ER, 70% PR, and HER-2 were positive in the immunohistochemical staining. The gross cystic disease fluid proteins (GCDFP-15) were first identified in the fluid of breast cysts and in the serum of patients with mammary carcinomas. It is considered a marker for both apocrine and breast differentiation. It is more specific for diagnosing breast carcinoma than mammaglobin which is reportedly produced from mature differentiated mammary gland tissue [9, 12]. P63 is expressed in both eccrine and apocrine cutaneous carcinoma in more than 25% of cells, but it is not expressed in most types of breast cancer.

Breasts are skin appendages that arise from mammary lines extending from the forelimb to the hind limb in the embryo. A search of the literature showed that 73% of female patients with carcinoma of ectopic breast tissue occurred in the axilla worldwide before 2000. Neoplasm that arise in ectopic breast tissue may be associated with remnants of normal mammary structures; however this is not necessary for the diagnosis for ectopic mammary cancer, especially in men, in whom there is no female hormone stimulation [13]. There is no convincing evidence showing that this tissue is more prone to become malignant than normal breast tissue [1]. Since the axillary region has plenty of sweat glands and sebaceous glands, the diagnoses of cutaneous adnexal malignancies with ductal differentiation should be differentiated. Patients with apocrine adenocarcinoma generally present with a solitary cutaneous or subcutaneous nodule of long duration (7.3 years on average) and no symptoms [14]. Although features including transition from normal to neoplastic apocrine epithelium and the context of other benign adnexal tumors favor the diagnosis of this lesion, the majority of apocrine adenocarcinoma develops de novo [15]. Histologic examination of cutaneous apocrine adenocarcinoma reveals characteristics common to the apocrine glands from which they arise. It is still difficult to determine whether the original site is mammary gland or sweat gland on the basis of H&E staining alone in cases of poor differentiation. In this case, the solid tubular invasive growth pattern lacking apocrine and the immunohistochemical results of ER, PR, and p63 supported the diagnosis of primary breast cancer originated from vestigial accessory mammary glands.

The standard treatment for male accessory breast cancer has not yet been well established. It is believed that wide resection of the axillary region while lymph node dissection is needed if a preoperative diagnosis can be established and additional mastectomy is required if a preoperative diagnosis cannot be identified. In the present case, the male accessory breast cancer was unclearly diagnosed before surgery and the patient refused the modified radical mastectomy. Hence the authors performed axillary lymph node dissection. Because there is no standard treatment for male accessory breast cancer, they referred to the strategy recommended by the National Comprehensive Cancer Network (NCCN) for women with early-stage breast cancer [16]. The chemotherapy was performed followed by the endocrine therapy. The treatment effect of this patient needs to be followed-up.

References


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Successful treatment of a large symptomatic lymphocyst with percutaneous drainage and repeated iodopovidone sclerotherapy

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Gdynia Oncology Center. Gynecological Oncology Department, Gdynia (Poland)

Summary
The objective of the case report was to present an easy and safe method for treatment of a large, persistent lymphocyst, through a procedure performed in an ambulatory setting. The patient diagnosed with large (1,800 ml), symptomatic (pains, renal insufficiency) lymphocyst after lymphadenectomy for cervical cancer, was successfully treated with percutaneous drainage (using vascular drains) and five sessions of sclerotherapy with 10% iodopovidone, performed in ambulatory settings. The method was minimally invasive, safe, and effective in management of symptomatic lymphocyst.

Key words: Lymphocyst; Lymphadenectomy; Iodopovidone; Percutaneous; Drainage.

Introduction
Symptomatic lymphocyst (LC) can be a complication following pelvic and para-aortic lymphadenectomy for gynecologic cancers. Treatment options include observation, drainage, sclerotherapy, and surgery [1]. Here the authors describe a case with large, bilateral LC causing pain and ureters obstruction with renal insufficiency, treated in ambulatory setting with repeated iodopovidone sclerotherapy.

Case Report
A 59-year-old women diagnosed with cervical cancer, Stage IB1 (International Federation of Obstetricians and Gynaecologists (FIGO) staging system 2009), planoepithelial carcinoma G2 from biopsy, was admitted for radical hysterectomy. There were no signs of disease spread noted in preoperative abdominal and pelvis computer tomography and chest X-ray. Laboratory tests were within normal ranges. The patient had well-controlled hypertension, and had undergone type C viral hepatitis in the past. Her body mass index (BMI) was 21.4 kg/m². She had had no previous surgery.

She underwent a radical hysterectomy type C1 (Querleu and Morrow classification) [2] combined with pelvic (type III) [3] and para-aortic (up to IMA) lymphadenectomy (LN) (January 27th, 2012). Peritoneum was left open. The surgery took 3.5 hours. Two drains were inserted in the pelvis to control bleeding and these drained 100 ml/day serous fluid on the right side (removed on the third day), and 40 ml/day on the left side (removed on the first day). The bladder catheter was removed on the fifth day, and a spontaneous micturition was observed with 50 ml urine retention. An urography performed on day 7 showed correct renal function and no obstruction in urine passing.

Histopathology revealed planoepithelial carcinoma G2 of the cervix with an infiltration depth to eight mm, involving the external os and lower part of the cervical canal. There was no disease spread neither in the uterus specimen nor in the lymph nodes (0/42). There were no significant laboratory test abnormalities in postoperative course.

Differential diagnosis, investigations, and treatment
During follow-up examinations, the patient did not manifest any complications until June 29th, 2012 (five months after surgery), when she was referred from the hospital emergency unit with suspicion of ascites, renal insufficiency [creatinine 4.1 mg/dl (range: 0.5 - 0.9), urea 187 mg/dl (range: 15 - 50), and anemia (hemoglobin 10.1 g/dl (range: 10.8 - 14.2)]). Sonography revealed retroperitoneal LC with an estimated volume of >1,300 ml on the right side and 500 ml on the left side with bilateral hydronephrosis. Percutaneous vascular drains were inserted in local anesthesia, under ultrasonography control – 1,800 ml and 550 ml were drained from the right and left side, respectively. The clear serous fluid was negative for cancer cells and infection. During the next two days, the patient passed 3,200 ml urine a day. In the following four days, active drainage was performed with 1,000 ml and 100 ml a day from the right and left side, respectively. In sonography no lymphocyst space was observed. In the next 12 days normal creatinine levels were noted, with no signs of hydronephrosis on the right side, and slight enlargement of the pelvocalyceal system on the left side (10 mm). The urinary tract infection with Esherichia coli was diagnosed and antibiotic treatment was required.

One month after the drains insertion there were 900-1,400 ml/day of lymph fluid on the right side and 50-100 ml/day on the left side. During sonography a heterogenic structure 103 x 67 mm in diameter was noted, with no lumen on the right side. The option of surgical laparoscopic treatment was discussed but the patient refused this approach. After complete lymphocyst draining, 20 ml of 10% iodopovidone was inserted and the drains closed on both sides. The patient changed position for two hours, and the drains were opened again.

On the 39th day, there were 1,000 ml/day drainage on the right side and no fluid on the left side. The right kidney showed a normal diameter of the pyelocalyceal system, while a diameter of 15 mm was registered on the left side. A second iodopovidone application to the right LC was performed with the same procedure as before. The drain was removed from the left side. There was no drainage for the next three days after the procedure, but then 600-1,100 ml/day drainage on the right side was noted again. In every two weeks next iodopovidone applications were performed, with drainage of a 600-800 ml/day after third, a 400-500 ml/day after forth, and 0 ml/day after fifth infusion, respectively.

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**Outcome and follow-up**

On the 81st day, no drainage was noted. Sonography did not reveal any lymphocyst nor any heterogenic structures. Drain was patent. The pyelocalyceal system diameter was 20 mm on the left side, and no enlargement was noted on the right side. Laboratory test showed normal renal function. The drain was removed. During follow-up examinations in the next six months, no lymphocyst recurrence nor renal dysfunction was noted.

**Discussion**

The authors describe a successful large lymphocyst percutaneous drainage with multiple (five times) sclerotherapy sessions using 10% iopodovide in a patient with symptomatic, persistent lymphocyst, following lymphadenectomy, and complicated with bilateral hydroureteroscopy and renal insufficiency.

Lymphocyst can be detected in 12-32% of cases after radical pelvic lymphadenectomy, but symptomatic LC are seen in 0.5-10% [1]. Diagnosis is based on anamnesis, symptoms (not always), imaging where ultrasonography plays a major role. Most lymphocysts appear in period of three weeks to few months after LN. Treatment should be suggested to those with symptoms, or when there is a risk of complications, or no optimal adjuvant cancer treatment can be performed [1].

Fine needle aspiration should be used as a diagnostic modality only, because the recurrence after complete LC emptying is seen in 50-100% of cases. The effectiveness of prolonged drain placement alone is estimated at 57-72% [1]. In the case the drainage alone for one month eliminated ureters obstruction but did not stop lymph leakage. Sclerotherapy is considered to be a feasible and effective method of LC treatment. The regimen infused to the LC lumen causes local inflammation and fibrosis that obliterate lymph vessels [4]. Iopodovide, ethanol, doxycycline, bleomycine, and polidokanol were described as sclerotherapy regimes with an effectiveness of 80-100% [1]. In general, one sclerotherapy procedure should be effective when LC drainage is less than 150 ml/day, and a few procedures are often required when the volume is larger [5]. The most often suggested schemas described one to three infusions [1]. The risk factors for sclerotherapy failure are: large LC volume before treatment and the need to repeat the procedure more than four times [6]. The proposed procedure involved LC emptying followed by 10% iopodovide infusion, and the drains were opened after two hours [6]. Complications of sclerotherapy may occur in 12% of cases. These involve subcutaneous tissue inflammation, peritonitis, and elevated creatinine serum levels [6]. Surgical treatment involves laparoscopy and LC fenestration to the peritoneal cavity, and is considered very effective and safe [7-9]. The most recent microsurgery method called lymphaticovenular Anastomosis is a minimally invasive procedure, considered effective in the treatment of pelvic lymphocysts [10].

The only lymphocyst prevention procedure after pelvic lymphadenectomy is leaving the peritoneum open. The drain placement is associated with a higher risk of short and long-term symptomatic lymphocyst formation [11, 12]. In the case, after surgery with lymphadenectomy, according to guidelines, drains were put in the pelvis, not retroperitoneal space, to control bleeding only. Despite this lymphocysts appeared and were of major clinical significance.

Percutaneous lymphocele drainage with multiple sclerotherapy sessions using 10% iopodovide appears to be a safe, acceptable, and effective method for symptomatic, large, and persistent lymphocyst treatment possible to perform in ambulatory settings. Further studies are needed to standardize the procedure (timing, volume etc).

**References**


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Heterologous type of malignant mixed Müllerian tumor of the uterus presenting as a vulvar mass

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Summary
Carcinosarcoma is a rare, extremely aggressive tumor of the uterus with a poor prognosis. The authors describe a case of a 78-year-old woman who presented with a giant mass protruding through the cervix, vagina, and vulva. A total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. The histopathological examination of the surgical specimen revealed a malignant mixed Müllerian tumor. The clinical and pathological features, molecular data, and prognosis of this aggressive neoplasm are discussed. Although uterine carcinosarcomas are extremely rare, when a postmenopausal woman with a vulvar mass is admitted to the gynecology clinic, the physician should consider that the mass may be a carcinosarcoma.

Key words: Carcinosarcoma; Malignant mixed Müllerian tumor; Vulvar mass.

Introduction
Carcinosarcoma of the uterus, also called malignant mixed Müllerian tumor, is a rare, extremely aggressive biphasic neoplasm. The term carcinosarcoma reflects the origin of these mixed tumors, characterized by a combination of epithelial and mesenchymal (stromal) elements, traditionally divided into homologous and heterologous subtypes. Carcinosarcoma is quite uncommon, with an incidence of fewer than two per 100,000 women per year. It has an extremely poor prognosis, with a five-year survival rate of 33% to 39% [1].

Case Report
The authors present the case of a 78-year-old woman diagnosed with carcinosarcoma of the uterus. She came to the hospital because of a large tumoral mass protruding through her vagina. She was not able to specify the time she first observed this mass. The patient (weight, 64 kg; body mass index, 25) had no pathological antecedents. Clinical examination revealed a large tumoral mass with areas of necrosis and hemorrhage; the origin of this mass was the uterus (Figure 1). An initial biopsy revealed only necrosis. The authors performed a total abdominal hysterectomy with bilateral salpingo-oophorectomy. Because of the patient’s critical condition and the high anesthesiologic risk, a bilateral pelvic lymphadenectomy and aortic lymphadenectomy were not performed.

The histopathological analysis showed a heterologous malignant mixed Müllerian tumor (Figure 2). The epithelial differentiation was endometrioid and poorly differentiated papillary serous carcinoma, and the mesenchymal component was rhabdomyosarcoma (Figure 3). After surgery, the patient was sent to the oncology unit.

Discussion
Carcinosarcoma is a rare, extremely aggressive tumor of the uterus with a poor prognosis. It is predominantly identified in postmenopausal woman (median age 65 years), but it can also be found in young women or children [2, 3].

Carcinosarcomas and carcinomas of the uterus have similar risk factor profiles. Their incidence increases in association with marked obesity, which generates increased exposure to estrogen hormones, nulliparity, use of exogenous estrogen, treatment with tamoxifen (for breast cancer), and pelvic radiation. The incidence decreases in association with oral contraceptive use [4, 5]. None of the risk or protective factors was found in the present patient.

Uterine carcinosarcomas typically present with abnormal vaginal bleeding and also may present with bloody discharge, watery discharge, abdominal pain, or an abdominal mass [3]. Carcinosarcoma usually develops as a large, soft, polyloid mass, filling and distending the uterine cavity. In the present case, the tumor was very large (15 cm) and protruded outside the vagina through the cervical os. Histologic examination of standard hematoxylin and eosin-stained sections showed the two components of carcinosarcoma: malignant-appearing epithelial and stromal (mesenchymal). These two morphologies can be intimately admixed or may appear as distinct components. The epithelial malignant tissue can have the appearance of any of the malignant epithelial neoplasms encountered in the female genital tract: serous, endometrioid, clear cell, mucinous, and squamous patterns [3]. Of these patterns, the most frequent one encountered is serous [6]. The stromal component may resemble mesenchymal cell types normally present in the uterus (i.e., homologous differentiation) or

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may have heterologous elements that are not normally found in the uterus, such as rhabdomyosarcoma, chondrosarcoma, and osteosarcoma, listed in decreasing order of frequency [7]. Frequently, the histologic pattern is simply high-grade sarcoma, without appreciable specific differentiation.

Genetic and molecular data provides evidence of a monoclonal origin of most carcinosarcomas. The arguments for this monoclonal origin are the presence of similar chromosomal aberrations, concordant loss of heterozygosity, identical \( p53 \) and \( K-ras \) mutations, and matching X inactivation patterns in both histologic components of most carcinosarcoma cases studied [6-9].

Additionally, the specific patterns of genetic aberrations are more consistent with a high-grade carcinoma than a sarcoma, providing strong support for divergent differentiation within a primarily epithelial neoplasm (carcinoma) as the histogenesis. Although the theory of being monoclonal or biclonal is under investigation, the clinical implication of this fact is unknown. The carcinomatous component has been shown to have more aggressive behavior and be a better predictor of clinical outcome in carcinosarcomas. The carcinosarcoma evolution is unfavorable, even if it is identified and treated in the initial phases. The recurrence or survival do not correlate with the patient’s age and the histological type of the tumor (homologous or heterologous) [10].

**Conclusions**

Carcinosarcomas are rare, highly aggressive biphasic tumors composed of malignant epithelial and mesenchymal components. Although they can arise anywhere in the female genital tract, they are more common in the uterus. They usually appear to arise from the transformation of pluripotent stem cells capable of giving rise to cells with divergent differentiation. The authors present a rare case of a carcinosarcoma with an unusual clinical presentation as a giant tumor mass outside the vagina. Recent data have advanced understanding of the biology of this lesion, and clinical trials are underway to determine the most efficacious chemotherapeutic regimens.

**References**


Heterologous type of malignant mixed Müllerian tumor of the uterus presenting as a vulvar mass


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Uterine endometrial carcinoma with trophoblastic differentiation: a case report with literature review

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Summary
Choriocarcinoma is categorized as either gestational or nongestational depending on its origin. Nongestational choriocarcinoma originated in the trophoblastic differentiation is a rare but an aggressive tumor. This article reports a nongestational case of a uterine endometrial carcinoma with trophoblastic differentiation. A 54-year-old woman with a history of atypical genital bleeding that underwent semi-radical hysterectomy, bilateral salpingo-oophrectomy, and pelvic lymph nodes dissection. Pathological investigation showed that the tumor had endometrioid adenocarcinoma and choriocarcinomatous components. Although a series of multimodality treatments including craniotomy were performed, she died of aggressive lung and brain metastases one year after the primary surgery.

Key words: Nongestational choriocarcinoma; Endometrial carcinoma; Trophoblastic differentiation.

Introduction
Gestational trophoblastic diseases, characterized by abnormal proliferation of pregnancy-associated trophoblastic tissues, include a range of rare disorders such as hydatidiform moles, invasive moles, and choriocarcinoma. Choriocarcinoma is a highly malignant tumor and is categorized as either gestational or nongestational. Gestational choriocarcinoma can occur preceding any gestational event. A few choriocarcinomas called nongestational choriocarcinoma are independent of the gestational events and arise from the trophoblastic differentiation of germ cell tumor and carcinoma, or residual germ cell. The authors describe here a rare case of uterine endometrial carcinoma with trophoblastic differentiation, while reviewing pertinent literature.

Case Report
A 54-year-old woman (gravida2, para2) came to the present gynecologic department with postmenopausal atypical genital bleeding. Pelvic examination revealed a large uterine mass and transvaginal ultrasonography showed the enlarged uterus of 12 × 8 cm and irregular thickness in the endometrium (Figure 1a). Endometrial biopsy revealed endometrioid adenocarcinoma (grade 1). Pelvic magnetic resonance imaging (MRI) showed a solid bulky mass, filling the uterine cavity, and infiltrating the uterine wall (Figure 1b), and chest computed tomography (CT) showed multiple lung nodules suggesting lung metastases (Figure 1c). The patient underwent semi-radical hysterectomy, bilateral salpingo-oophrectomy, and pelvic lymph nodes dissection.

Pathological examination revealed a mass 14×80×55 mm in size (Figure 2a) in the uterine cavity. The tumor extended to the cervix, penetrating greater than 50% of the myometrium and spreading to the right ovary and Douglas peritoneum. Microscopically, there were two different histological types of tumor in the specimen; namely, 95% of the tumor showed poorly differentiated endometrioid adenocarcinoma and the remaining part showed extensive hemorrhage and necrosis (Figure 2b). This remaining portion consisted of multinucleated giant cells showing a sporadic/focal pattern and eosinophilic cytoplasm resembling syncytiotrophoblastic cells, and other cells showing oval nuclear and pale cytoplasm resembling cytrophoblastic cells (Figure 2c). In addition, the immunohistochemistry stain for human chorionic gonadotropin–β (hCG-β) (Figure 2d), human placenta lactogen (hPL), and inhibin-α turned out to be positive; therefore, this part is thought to exhibit the trophoblastic feature. Although the preoperative serum hCG level was not measured, the postoperative serum hCG level was elevated to 1,632 mIU/ml. Finally, the patient was diagnosed as having endometrioid adenocarcinoma with trophoblastic differentiation of uterine corpus and classified as International Federation of Gynecology and Obstetrics (FIGO) Stage IVb (pT3aN0M1).

The patient started to receive tri-weekly AP (doxorubicin, 60 mg/m²; CDDP, 50 mg/ m²) chemotherapy. Since metastatic lung tumors were enlarged (Figure 2b) with elevated serum hCG (8,318 mIU/ml) after three cycles of AP, the regimen was changed to MEA (methotrexate, 450 mg/body, day 1; etoposide, 100 mg/body, days 1–5; actinomycin D, 0.5 mg/body, days 1–5). Three cycles of MEA regimen could reduce the size of lung metastatic lesions (Figure 1d) as well as serum hCG value (291 mIU/ml). However, CT image again revealed increased number of lung metastatic lesions (Figure 1e) and new metastatic lesion appeared in the left occipital lobe of brain after five cycles of MEA. Craniotomy was performed to remove the metastatic brain tumor and pathological diagnosis of the tumor was pure choriocarcinoma. Soon after the craniotomy, another brain metastatic lesion was found and the patient died one year after primary surgery.

Discussion
Based on the information obtained from clinical observations, histopathological facts, and genetic origin, choriocarcinoma is categorized as gestational or nongestational. In
Figure 1. — Imaging studies. (a) Transvaginal ultrasound examination of enlarged uterus. (b) Pelvic magnetic resonance imaging (MRI) showing a solid bulky mass measured about 12 cm, filling the uterine cavity and infiltrating the uterine wall. (c) Chest computed tomography (CT) of lung field showing multiple lung metastasis before primary surgery. (d) Lung metastatic regions were enlarged after three cycles of AP treatment. (e) Lung metastatic regions were reduced after three cycles of MEA treatment.

Figure 2. — Pathological findings. (a) Gross picture showing yellowish and white tumor occupying the endometrial cavity. (b) Histological examination showing the tumor composed of poorly differentiated adenocarcinoma (left upper) and choriocarcinoma (right) (HE×100). (c) The choriocarcinomatous component is characterized by a sheet of both proliferative cytotrophoblasts and syncytiotrophoblasts (HE×250). (d) Syncytiotrophoblasts were positive for hCG-β immunostaining (×250).
particular, nongestational choriocarcinoma is defined based primarily on the patient’s reproductive history. Recently, DNA polymorphism analysis has been successfully utilized to identify the genetic origin of choriocarcinoma [1-2]. Although DNA analysis was not performed in the case in this report, the authors diagnosed it as nongestational choriocarcinoma because she also had endometrioid adenocarcinoma. Gestational choriocarcinoma is chemosensitive and the prognosis is good even in advanced stages. On the other hand, nongestational choriocarcinoma is less sensitive to chemotherapy and has a poor prognosis. Therefore, it is clinically important to determine the exact type of choriocarcinoma for identifying the appropriate treatment strategy.

Although three causes of nongestational trophoblastic tumor have been proposed, the pathogenesis of nongestational choriocarcinoma is still uncertain [3]. Thirdly, nongestational choriocarcinoma rarely has its origin in residual germ cells that could not migrate to the gonads without other neoplastic components [2].

Trophoblastic differentiation is histologically found in several types of tumors including stomach, lung, colon, esophagus, bladder, breast, and gynecologic cancers. Uterine endometrial carcinoma with trophoblastic differentiation is rare, and only a small number of cases have been reported so far [3].

### Table 1. Cases of uterine endometrial carcinoma with trophoblastic differentiation.

<table>
<thead>
<tr>
<th>Author</th>
<th>Age</th>
<th>G/P</th>
<th>Area of choriocarcinoma component</th>
<th>Coexisting tumor</th>
<th>Histology at metastasis or recurrence</th>
<th>Chemotherapy regimen</th>
<th>Metastasis region</th>
<th>Follow up (month)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Civantos et al.</td>
<td>87</td>
<td>3/2</td>
<td>ND</td>
<td>SA</td>
<td>ND</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Savage et al.</td>
<td>70</td>
<td>1/1</td>
<td>Small area</td>
<td>Well EA</td>
<td>Choriocarcinoma</td>
<td>MPA, 5-FU+ DXR+megestrol acetate</td>
<td>brain, lung, kidney, liver</td>
<td>14</td>
<td>DOD</td>
</tr>
<tr>
<td>Pesce et al.</td>
<td>78</td>
<td>ND/0</td>
<td>ND</td>
<td>Poor AC</td>
<td>ND</td>
<td>CDDP+BLM+VCR</td>
<td>pelvic lymph node</td>
<td>1.5</td>
<td>DOD</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>ND/0</td>
<td>ND</td>
<td>Poor AC</td>
<td>ND</td>
<td>MTX, BEP</td>
<td>lung</td>
<td>2</td>
<td>AWD</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>ND</td>
<td>AC</td>
<td>Same as primary</td>
<td>-</td>
<td></td>
<td>lung, liver, peritoneum</td>
<td>14</td>
<td>DOD</td>
</tr>
<tr>
<td>Kalir et al.</td>
<td>83</td>
<td>0/0</td>
<td>ND</td>
<td>Mod EA</td>
<td>ND</td>
<td>CDDP, etoposide</td>
<td>lung</td>
<td>1</td>
<td>AWD</td>
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<tr>
<td>Black et al.</td>
<td>88</td>
<td>ND/1</td>
<td>ND</td>
<td>CCC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>NED</td>
</tr>
<tr>
<td>Bradley et al.</td>
<td>68</td>
<td>4/4</td>
<td>Focal area</td>
<td>Mixed SA</td>
<td>SA</td>
<td>PTX, CBDDCA</td>
<td>pelvic lymph node</td>
<td>24</td>
<td>NED</td>
</tr>
<tr>
<td>Tunc et al.</td>
<td>54</td>
<td>6/6</td>
<td>10-20%</td>
<td>Mod EA</td>
<td>AC</td>
<td>MEA, CPA+folic acid+ etoposide</td>
<td>retroperitoneum</td>
<td>24</td>
<td>DOD</td>
</tr>
<tr>
<td>Nguyen et al.</td>
<td>34</td>
<td>0/0</td>
<td>Major area</td>
<td>MMMT</td>
<td>Choriocarcinoma</td>
<td>BEP, EMACO</td>
<td>brain, lung</td>
<td>4</td>
<td>AWD</td>
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<tr>
<td>Khue et al.</td>
<td>71</td>
<td>0/0</td>
<td>ND</td>
<td>Carcinosarcoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
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<tr>
<td>Horn et al.</td>
<td>61</td>
<td>3/3</td>
<td>30%</td>
<td>SA</td>
<td>ND</td>
<td>MEA, EMACO</td>
<td>lung</td>
<td>3</td>
<td>DOD</td>
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<tr>
<td>Akbulut et al.</td>
<td>42</td>
<td>ND</td>
<td>Small area</td>
<td>Mod EA</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>NED</td>
<td></td>
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<tr>
<td>Yamada et al.</td>
<td>58</td>
<td>1/1</td>
<td>50%</td>
<td>Well EA</td>
<td>Choriocarcinoma</td>
<td>CBDDCA, therarubicin, CPA, EMACO</td>
<td>vagina</td>
<td>45</td>
<td>NED</td>
</tr>
<tr>
<td>Olson et al.</td>
<td>68</td>
<td>6/4</td>
<td>Focal area</td>
<td>EA</td>
<td>Choriocarcoma</td>
<td>-</td>
<td>axillary lymph node</td>
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<td>ND</td>
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<tr>
<td>Present case</td>
<td>54</td>
<td>2/2</td>
<td>Poor EA</td>
<td>Choriocarcoma</td>
<td>-</td>
<td>DXR+CDDP, MEA</td>
<td>lung, brain</td>
<td>12</td>
<td>DOD</td>
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</table>

G/P: gravid/para; MP: multiparous; SA: serous adenocarcinoma; EA: endometrioid adenocarcinoma; AC: adenocarcinoma; CCC: clear cell adenocarcinoma; MMMT: malignant mixed mesodermal tumor; Mod: moderately differentiated; Poor, poorly differentiated; MPA: medroxyprogesterone; 5-FU: 5-fluorouracil; DXR: doxorubicin; CDDP: cisplatin; BLM: bleomycin; VCR: vincristine; MTX: methotrexate; PTX: paclitaxel; CBDDCA: carboplatin; CPA: cyclophosphamide; MEA: methotrexate+etoposide+actinomycin D; EMACO: etoposide+methotrexate+actinomycin D+cyclophosphamide+vincristin; NED: no evidence of the disease; AWD: alive with the disease; DOD: dead of the disease; ND: not described.
necarcinoma, two poorly differentiated adenocarcinoma, and remaining cases included clear cell carcinoma and carcinosarcoma. Metastatic lesions varied; eight cases had lung metastasis and four cases had brain metastasis. Histology of metastatic lesions was recorded in 11 cases and five of them were pure choriocarcinoma.

The management of choriocarcinoma is well established by the FIGO 2000 staging and risk factor scoring system, however, that of the rare uterine endometrial carcinoma with trophoblastic differentiation has not been well developed. The present authors’ review of the previously reported cases show that most patients received surgery followed by multi-agent chemotherapy such as MEA, EMACO (etoposide, methotrexate, and actinomycin D, followed by cyclophosphamide and vincristine), or BEP (bleomycin, etoposide, cisplatin) regimens. Yamada et al. in 2009 reported that recurrent vaginal tumor was completely diminished by administration of EMACO [15]. In the present case, tri-weekly AP was ineffective for lung metastasis and subsequent MEA could only temporarily reduce lung tumor volume. The fact that metastatic brain tumor showed pathologically pure choriocarcinoma supported the idea that metastatic lung tumors are also composed of choriocarcinomatous component.

Conclusion

The present review of the field has shown that there are few case reports of uterine choriocarcinoma in postmenopausal women. Furthermore, uterine endometrial carcinoma with trophoblastic differentiation is an extremely rare form of nongestational choriocarcinoma. Since prognosis of the rare tumor is thought to be worse than that of pure gestational choriocarcinoma, the early accurate diagnosis is clinically important.

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References


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Aggressive angiomyxoma of the female genital tract: report of two cases

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Summary

Aggressive angiomyxoma (AA) was identified in 1983 and 250 cases of this rare tumor have since been reported in the literature. It is characterized by a locally infiltrative and recurrent nature; however, it rarely shows distant metastasis. Surgical managements can successfully treat AA patients but may result in a significant morbidity due to the large size and infiltration of the tumor. Less radical surgeries have recently been recommended in the treatment of this tumor, but adjuvant therapies have not yet been fully established. The authors report here two AA cases that were treated at their hospital, with a brief review of the literature.

Key words: Aggressive angiomyxoma; Genital tract; Treatment.

Introduction

Aggressive angiomyxoma (AA) is a rare, locally aggressive myxoid mesenchymal neoplasm that preferentially arises in the pelvic and perineal regions of young women, most commonly during the reproductive age [1, 2]. Its peak incidence is the third to fifth decades of life [3-5]. In men, the tumor involves analogous sites, including the scrotum and inguinal area, and usually appears at older ages [1, 2, 6]. AA characteristically grows slowly and insidiously, is gelatinous in nature, presents as a painless lump, has no capsule, and is focally infiltrative [2, 7, 8]. It has a tendency towards local recurrences and rare metastases. Females are affected more commonly than males, at a ratio of slightly more than 6:1 [6, 9, 10]. The term “aggressive” was introduced to emphasize its locally aggressive behavior and high potential for local recurrences and does not imply a high probability of metastases [3]. AA was first described in 1983 by Steeper and Rosai and since then, there have been fewer than 250 AA cases reported in the English literature—mostly in the form of small case series or case reports. The authors report herein two cases of AA of vulva with a brief review of the literature.

Case Report

Case 1

A 46-year-old nulliparous woman presented with a palpable mass on the suprapubic area. She had undergone total abdominal hysterectomy due to myoma three years prior. Other gynecological and medical histories were unremarkable. The mass was first noted six months prior to her visit and gradually grew without any remarkable symptoms. Transvaginal ultrasonography exhibited that there were no abnormal findings in the pelvis. Sonography of the suprapubic mass revealed that the mass was a cystic hypeoechoic lesion without blood color Doppler. She underwent a wide local excision of the suprapubic mass. Gross examination revealed a 3.0 × 1.2-cm mass with an irregular margin and of glistering gray color (Figure 1). Microscopically, the mass had a hypocellular background that contained spindle-shaped fibroblasts infiltrating the surrounding tissue. Delicate thick-walled blood vessels were also visible (Figures 2a and 2b). These findings established a final diagnosis of aggressive angiomyxoma. The patient has been asymptomatic during the period of 29 months after surgery.

Case 2

An 18-year-old nulliparous woman was referred to the present clinic for operation. She underwent removal of a left labium minor mass and her pathologic diagnosis was aggressive angiomyxoma involving the surgical margin. Magnetic resonance image (MRI) of her pelvis showed a 2.5 × 1.8-cm edematous swelling in the left labium minor. She underwent a wide excision along with vulvar reconstruction using unilateral modified V-Y advancement flap coverage (Figures 3a and 3b). Pathologic examination revealed no evidence of tumor. The patient has been asymptomatic during the period of 31 months after her first surgery.

Discussion

The pathogenesis of AA remains to be clarified, but chromosomal abnormalities involving chromosome 12, which are associated with rearrangement of the high-mobility group protein isoform I-C (HMGIC) gene, have been reported in a series of AA cases [6]. The tumor is rare and arises predominantly in the pelvic and perineal region in reproductive women. It is misdiagnosed in more than 80% of cases and is most often mistaken for a Bartholin cyst, vulvar abscess, lipoma, Gartner duct cyst, vaginal mass or polyp, vaginal prolapsed, pelvic floor hernia, or obturator/levator hernia [1, 6, 7, 9].

There is no definitive method for preoperative diagnosis, but imaging findings may be helpful in differentiating be-
between this tumor and others. It may grow and occupy the whole pelvic region and invade paravesical and pararectal spaces, displacing pelvic structures. AA displays an unusual growth pattern of translevator extension with growth adjacent to the pelvis and perineal structures [9]. Imaging studies are important for preoperative evaluation of AA because the extent of the tumor is often underestimated by physical examination. Sonography shows a mass that is hypoechoic or appears to be a nonspecific cystic mass [11]. MRI gives more specific findings than computed tomography (CT), displaying a ‘swirled’ pattern as in angiomyxoma [1]. On T1-weighted images, the tumor may demonstrate isointense signal compared to muscle; on T2-weighted images, it may show high signal intensity and enhanced avidly following intravenous gadolinium contrast administration. These findings may be due to the high water content of angiomyxoma [1, 4, 12]. On CT scan images, the tumor has a well-defined margin and an area of attenuation less than muscle [9]. Extension from the perineum into the pelvis is common and often clinically unsuspected. Imaging studies are valuable tools for preoperative evaluation of these patients because the extent of the tumor is often underestimated by physical examination.
Macroscopically, AA often has a smooth surface, partially or completely encapsulated, and cut surfaces have a glistening, gelatinous appearance, bluish grey, with areas of hemorrhage and congestion [6]. Histologically, AA is a hypocellular mesenchymal lesion consisting of a sparse population of bland spindled and stellate cells against a background of myxoid stroma. The stroma contains collagen fibers and prominent vascular components containing large and thick-walled vessels. No evidence of coagulative tumor blood cell necrosis is observed, and there are neither cytologic atypia, atypical mitotic features, nor discernible mitotic activities [6, 9, 13, 14]. AA is difficult to histologically differentiate from myxoma, myxoid neurofibromas, myxoid liposarcomas/fibrosarcomas, myxoid leiomyosarcoma, and myxoid type of malignant fibrous histiocytoma [9, 13]. In most of these tumors vessels are usually absent and neoplastic cells resemble fibroblasts rather than myofibroblasts [9]. Immunohistochemically, AA expresses receptors for vimentin, desmin, actin, estrogen, and progesterone [9, 13].

AA tumor cells appear to be hormonally influenced. Hormonal treatments with tamoxifen, raloxifene, and gonadotropin-releasing hormone analogs ( GnRH agonist) have been attempted by many clinicians [15-18]. In particular, GnRH agonists have been used as a primary treatment for small tumors. Sereda et al. [5] reported partial responses in AA patients who were treated with GnRH agonists. They demonstrated that preoperative shrinking of tumors by GnRH analogues may decrease tumor size and increase the chances of complete excision. In contrast, Magtibay et al. [8] reported one patient in whom tamoxifen treatment failed to stop tumor growth. Surgery is the most effective method for the treatment of AA. It has been generally recognized that complete excision with a wide margin of safety is necessary to avoid recurrence [8, 13, 19]. However, a 70% local recurrence rate after complete excision has been reported [8]. Furthermore, Chan et al. [20] reported that there is no significant difference in recurrence rates in patients with negative margins compared to those with positive margins. Owing to the high incidence of AA in women of reproductive age, conservative treatments should be considered over radical surgery. In AA patients, radiotherapy and chemotherapy may not be useful adjuvants to primary surgery due to AA’s low mitotic activity. To the best of the authors’ knowledge, there have been few reports on the use of chemotherapy in the treatment of AA. It has been suggested that preoperative external beam irradiation and intraoperative electron beam radiotherapy are useful for controlling local tumors [21]. There have been two reported cases of successful control of recurrent angiomyxoma through relatively high doses of external radiotherapy [22, 23].

High local recurrence rates, one of the findings most specific for AA, have been reported to be between 9% and 72% and may occur months to years after operation (two months to 15 years) [6, 13, 24]. Recurrences generally occur in the first five years after the first operation and may correlate partially with incomplete excision [6]. Except for positive resection margins, there are no significant relation between recurrences and predictors, such as size or location [8, 13, 25]. There is no standard method of monitoring recurrences. Ahmet et al. have suggested that all AA patients should be followed up for a long time with imaging studies because this tumor may not be discernible on pelvic examination or ultrasonography.

Conclusion

AA is a rare, locally aggressive tumor that arises mainly in the pelvis and in females and has a high recurrence rate. The authors presented two cases of AA that were diagnosed histologically after operation. Due to the rarity of this tumor, a standard treatment has not yet been established. Many investigators have emphasized that patients counseling, multidisciplinary approaches, individualization for each case, and follow-up are essential for the treatment of AA patients. The present authors reported two cases of AA experienced over the last 20 years along with a brief review of the literature.

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Written informed consent was obtained from the patients for publication of this case report. The study was approved by our institutional review board (KC13ZISE0065).

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Rupture of an endometrioma with extremely high serum CA-125 level (>10,000 IU/ml) and ascites resembling ovarian cancer

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Summary
Carbohydrate antigen 125 (CA-125) is a type of cell surface glycoproteins present in more than 80% of non-mucinous epithelial ovarian carcinomas; however, benign gynecologic conditions commonly cause a smaller increase in CA-125 level. This report presents the details regarding a 44-year-old woman with extremely high serum CA-125 level and ascites. She complained of having abdominal pain and abdominal distension. Her serum CA-125 level had been markedly elevated (>10,000 IU/ml) and computed tomography (CT) revealed an ovarian tumor and massive ascites. The cytological analysis showed no evidence of malignancy, however, the positron emission CT (PET-CT) scan suggested ovarian malignancy with peritoneal carcinomatosis. Under the impression that the patient had ovarian cancer, the present surgical team carried out an explorative laparotomy and discovered the ruptured bilateral ovarian endometriomas. In this study, it is suggested that clinicians carrying out differential diagnosis of pelvic mass with high serum CA-125 level and ascites should consider not only ovarian cancer but also ruptured endometrioma.

Key words: CA-125; Ruptured endometrioma; Ascites.

Introduction
Carbohydrate antigen 125 (CA-125) is a type of cell surface antigens present in more than 80% of non-mucinous epithelial ovarian cancers that was discovered by Bast et al. in 1981[1]. CA-125 is known as a high-molecular-weight antigenic determinant expressed on the surface of the coelomic epithelium, including endometrium, pelvic peritoneum, and placental tissue [2]. Therefore, CA-125 occurs in the serum of healthy females at low concentration (< 35 IU/ml), however, the concentration appears to be moderately elevated in patients with several benign conditions such as pelvic inflammatory disease, uterine fibroids, adenomyosis, pregnancy, menstruation, and especially endometriosis [3]. Serum concentrations of CA-125 in patients with endometriosis are rarely higher than 100 IU/ml [4]. Since the serum CA-125 levels higher than 1,000 IU/ml have been rarely seen in patients with benign diseases, the index of serum CA-125 level may be used to differentiate malignant and benign ovarian masses from other diseases, through combination with other diagnostic methods [5] and endometriosis with massive ascites is an unusual combination that is not familiar to most gynecologists.

Consequently, the authors report an uncommon case of ruptured endometrioma associated with extremely high serum CA-125 level (> 10,000 IU/ml) and ascites resembling ovarian cancer.

Case Report
A 44-year-old nullipara complained of right flank pain that had lasted for a week, which led to sudden lower abdominal pain and abdominal distension. She consulted to local hospital and had abdominal computed tomography (CT) scan. It revealed an irregularly shaped solid mass, mainly a cystic mass in her pelvis, including large amount of ascites, but no distant metastasis or lymphadenopathy (Figure 1). She was referred to the present hospital under the impression of ovarian cancer.

There was nothing remarkable in her medical history until then. Her menstrual cycle was regular, but a moderate degree of dysmenorrhea was identified. She seemed to be acutely ill. A physical examination revealed her body temperature and blood pressure were 36.9°C and 144/102 mmHg respectively, including her pulse rate at 114 beats/min with a regular rhythm. The patient’s abdomen was markedly distended with tenderness, including rebound tenderness. According to the laboratory data, WBC count was 12,200 /ul, hemoglobin 11.4 g/dl, hematocrit 34.4%, platelet 31.8 x 104 /ul, HS-CRP 24.37 mg/dl, ESR 76 mm/hr, total protein 5.7 g/dl, albumin 2.9 g/dl, CA-125 > 10,000 U/ml, and CA 19-9 3,926.1 U/ml.

The positron emission CT (PET-CT) scan showed a cystic mass lesion in her pelvic cavity with moderate fluorodeoxyglucose (FDG) uptake, including large amount of ascites with mild diffuse FDG uptake, which was suspected of being peritoneal carcinomatosis (Figure 2). Through a paracentesis, the authors were able to extract 500 ml of dark-brown colored fluid, but the cytological analysis of ascites showed no evidence of malignancy. Therefore, they performed an exploratory laparotomy with suspicion of ovarian malignancy. During the operation, they found out normal uterus, but they also found a ruptured tumor on the left ovary, which was about six cm long with thick wall, including the unruptured right ovarian cyst, which was about two cm long and filled with chocolate colored fluid. Both ovaries were severely adhered to omentum and large bowel. There was a large amount of dark brown ascites and turbid fluid. The omentum, large bowel, uterus, and pelvic peritoneum were covered with dark brown
blood clot. By carrying out a frozen biopsy, the authors confirmed that the ovarian tumor was benign endometriotic cyst (Figure 3). Therefore, they performed a right salpingooophorectomy and a left ovarian cystectomy and the surgery was completed after carrying out massive peritoneal irrigation with 10,000 cc of normal saline.

Postoperatively the patient did not express abdominal discomfort and her CA-125 and CA 19-9 concentration decreased gradually. In November 24th, 2011, after three-month follow-up, the levels of tumor markers had nearly normalized (CA-125: 30.30 U/ml and CA 19-9: 55.23U/ml).

**Discussion**

CA-125 is a 220-kDa cell surface glycoprotein which has been investigated as a specific tumor-marker of ovarian malignancy, especially for non-mucinous epithelial ovarian carcinomas[1]. Although CA-125 has been proposed as a specific marker for ovarian cancer, it may present in many other benign and malignant conditions. Kabawat et al. reported that this antigen was present in tissues related to a coelomic epithelium[2]. It occurs in
serums of healthy females at low concentrations (< 35 IU/ml) and elevations of serum CA-125 may occur in several benign conditions such as adenomyosis, uterine fibroids, pelvic inflammatory disease, early pregnancy, menstruation, or endometriosis [3]. The most common benign gynecologic condition associated with elevated serum CA-125 level is endometriosis, especially ovarian endometrioma. Serum CA-125 concentrations in patients with endometriosis are rarely higher than 100 IU/ml [4]. Since the serum CA-125 levels more than 1,000 IU/ml have been rarely seen in patients with benign gynecological diseases, the index of serum CA-125 level may be used to differentiate malignant and benign ovarian masses from other diseases, through combination with other diagnostic methods [5]. It is generally believed that the higher the CA-125 concentration, the greater probability for the ovarian mass to be malignant.

The mechanisms, by which endometriosis may elevate serum CA-125 or CA 19-9 levels, are partly understood. As already known, the human endometrium produces and secretes CA-125 and endometriotic cyst fluids contain very high concentration of CA-125 [6, 7]. Therefore, plasma CA-125 concentrations reflect the eutopic or ectopic endometrial production and the size of ovarian endometriotic cysts. As a result of endometrioma rupture, the sudden release of endometriotic cyst fluids containing very high concentrations of CA-125, which is combined with peritoneal irritation, may contribute to the extreme rise of serum CA-125 [8] and peritoneal mesothelial cells are even more potent than ovarian cancer cells in producing CA-125 [9]. CA-125 molecules leaking from the endometriotic cyst may be transferred through the peritoneum, and the associated inflammatory reaction of mesothelial cells of peritoneum is probably the very important contributor to the very high serum level of CA-125. Kurata et al. suggested that diffusion of cystic fluid on peritoneal surface and into circulation, which occurs after rupture of endometrioma, probably was a contributing factor in the rapid increase in serum CA-125 and CA 19-9 concentrations [10]. Takemori et al. reported that extensive adhesions associated with advanced stage of endometriosis reflect the chronic and repetitive inflammatory reactions which cause damage to the epithelium of chocolate cyst, possibly causing CA 19-9 leakage into the circulation, which results in abnormally high serum levels of the tumor marker [11]. An explosive rise of serum CA-125 of up to 9,300 IU/ml following the rupture of ovarian endometrioma is the highest level reported so far [12], but the authors report the new record in which the serum CA-125 level was markedly elevated up to or over 10,000 IU/ml of ruptured endometrioma with massive ascites.

Certain gynecological diseases, such as ovarian tumors, pelvic tuberculosis, ovarian hyperstimulation syndrome and Meigs Syndrome can produce ascites, but endometriosis is uncommonly encountered [13]. The simultaneous occurrence of endometriosis and ascites is still a matter of debate. Bernstein et al. reported that blood and endometrial cells shed into the peritoneal cavity, so that peritoneum is irritated and stimulated, thereby allowing ascites to occur eventually [14]. Another theory is that the rupture of endometriotic cysts results in peritoneal irritation and subsequent formation of reactive exudate [15]. Women who present with endometriosis-associated ascites usually have advanced and extensive disease, which may be accompanied by pleural effusion [16].

In conclusion, the present case emphasizes the association of high levels of CA-125 with benign gynecologic conditions, especially endometriosis. Very high CA-125 levels and ascites are not always associated with malignant diseases and there is no cut-off value of CA-125 for diagnosis of malignancy. Therefore, clinicians carrying out differential diagnosis of pelvic mass with high serum CA-125 level and ascites should consider not only ovarian cancer but also ruptured endometrioma. Therefore, the authors report ruptured endometrioma with extremely high serum CA-125 level (> 10,000 IU/ml) and ascites.

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Adenosarcoma of the uterine body initially presenting as an interstitial small tumor of the uterus: a case report

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Summary

Adenosarcoma of the uterine body is a rare mixed tumor in which a benign epithelial component is mixed with a malignant stromal element. It has been considered that this tumor originates from the endometrium and its most common finding of imaging is a polypoid tumor occupying the uterine cavity. The authors herein present a case of 37-year-old female with a complaint of abnormal vaginal bleeding. At the first visit, transvaginal ultrasound and magnetic resonance imaging (MRI) showed a round mass with a diameter of one cm in the uterine wall. No malignant pathological finding was detected. The patient visited the authors again one year later, because of continuous bleeding. At that time, they found a polypoid tumor in the uterine cavity, which turned out to be adenosarcoma with sarcomatous overgrowth. The round mass in the uterus detected at first time seems to have been incipience of adenosarcoma. Prodromal sign of adenosarcoma has not been reported previously.

Key words: Adenosarcoma; Diagnosis; Magnetic resonance imaging; Prodromal sign; Ultrasound.

Introduction

Adenosarcoma of the uterus is a rare mixed tumor in which a benign epithelial component is mixed with a malignant stromal element. This tumor has been considered to be present as a solid polypoid mass usually arising in the endometrium [1-4]. Therefore, it is sometimes misdiagnosed as a benign endometrial polyp, which can lead to a delay of the accurate diagnosis. The authors experienced a case presenting with an interstitial small tumor of the uterus which finally turned out to be adenosarcoma. This tumor seemed to be the prodromal stage of uterine adenosarcoma.

Case Report

A 37-year-old gravida 4 para 1 female visited a physician with a complaint of abnormal vaginal bleeding and hypermenorrhea for couple of years. She had had hormonal therapy of dysmenorrhea. She had not had any other past medical history, such as tamoxifen use, pelvic radiation, and so on. She had no family history of malignancy and gynecologic disease. Transvaginal ultrasound showed a hyperechoic round shadow with a diameter of one cm at uterine fundus muscle (Figure 1). To diagnose the mass and the cause of abnormal vaginal bleeding, magnetic resonance imaging (MRI) were performed. It revealed a mass with low intensity on T1-weighted images and high intensity on T2-weighted images compared to the myometrium and areas of small cysts at the lower pole. The polypoid tumor seemed to be continuous with the previously detected fundus mass (Figure 4). It was not typical as adenomyosis or endometrial polyp. FDG-PET/CT was positive for the mass in the uterine cavity (SUV max = 6.4), suggesting that the mass could be malignant. The patient’s tumor markers: LDH, CA125, CA19-9, CEA, SCC, and NSE were all within normal limits.

To diagnose the polypoid tumor, the authors performed hysteroscopy. Hysteroscopic examination showed that there was a dark red polypoid mass arising from the fundus of the endometrium. They resected this polypoid mass and a pathological examination was performed.

At that time, transvaginal ultrasound showed an irregularly-shaped mass measuring three cm in diameter in the endometrial cavity (Figure 3). MRI demonstrated this mass to be a polypoid tumor in the uterine cavity. The mass contained solid components with low intensity on T1-weighted images and high intensity on T2-weighted images compared to the myometrium and areas of small cysts at the lower pole. The polypoid tumor seemed to be continuous with the previously detected fundus mass (Figure 4).

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Microscopically, this mass had irregularly enlarged glandular epithelial components, lined by epithelium of the endometrial type. These glands were surrounded by low-grade sarcomatous stroma. The tissue was positive for MIB-1 and CD10 staining (Figure 5). These findings suggested that this polypoid mass was an adenosarcoma or endometrial stromal sarcoma. A simple total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. A small remnant of the polypoid mass was seen in the left corner of the uterine cavity (Figure 6).

Microscopically, there was adenomyosis, benign glandular epithelial elements surrounded by low-grade sarcomatous stroma, and pure sarcomatous components. The pure sarcomatous compo-
Adenosarcoma of the uterine body initially presenting as an interstitial small tumor of the uterus: a case report

The tumor comprised more than 25% of the tumor. Therefore, the authors diagnosed this tumor to be adenosarcoma of the uterine body with sarcomatous overgrowth. Tumor invasion was revealed in about one-third depth of myometrium. After surgery, adjuvant therapy was not administered. The patient has been alive with no evidence of disease for about 48 months after the surgery.

Discussion

Adenosarcoma of the uterine body is a rare mixed tumor in which a benign epithelial component is mixed with a malignant stromal element. The tumor usually affects post-menopausal patients, and its most common symptom is genital bleeding. Several papers have reported that adenosarcoma usually originates in the endometrium and grows as a polypoid mass within the endometrial cavity [1-4].

In the present case, the small round tumor in the uterine fundus muscle seemed to turn into a polypoid tumor, which was found to be adenosarcoma. There have been no previous reports of prodromal signs of adenosarcoma. As this case shows, the incipience of adenosarcoma may possibly be detected as a hyperechoic mass by ultrasound and high intensity on T2-weighted images by MRI. Takeuchi M et al. [4]
reported that the presence of small hyperintense cysts scattered within the mass on T2-weighted imaging, reflecting glandular epithelial components, may be a characteristic finding of adeno- sarcoma. Therefore, when examining a patient who complains of genital bleeding and has a uterine intramural tumor as seen in this case, adenosarcoma should be considered when making a differential diagnosis. Adenosarcomas of the uterus with sarcomatous overgrowth are aggressive tumors frequently associated with postoperative recurrence or metastases and a fatal outcome [1]. The authors will continue to follow this patient carefully.

References


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Foreword

The importance of this book is included in its very theme, as it presents gynecological cancer of the most unfavorable prognosis. In fact, despite the numerous advances in surgery, chemotherapy, and molecular therapies, the survival rates have only slightly improved. Selecting ovarian tumors as the object of study, as assessed by a multi-specialized team, can assist the gynecological oncologists, and also refine the approach to the disease and increase their professional standard.

This book, written by 32 international acknowledged experts, with rich and clear illustrations, offers an expert guide to all aspects of this neoplasia. From the epidemiology, through risk, management in early and advanced stages, pediatric neoplasia, to the quality of life, the author explores all the possible aspects of this disease and all the implications that affect the outcome.

The chapters are all written very clearly, allowing anyone from the student to the expert to fully benefit from consultation of the manual, and the in-depth information makes it easier to understand its contents.

In conclusion, I believe that the comprehensive text conveys a significant progress in understanding this complex neoplasia.

M. MARCHETTI

Contents

Chapter 1: Epidemiology of Ovarian Cancer: An Update
Jennifer Permuth-Wey, Andrea Besharat, Thomas A. Sellers

Chapter 2: Genetic Risks of Ovarian Cancer
Christopher A. Friedrich

Chapter 3: Management of Hereditary Ovarian–Breast Cancer
Andrea Tinelli, Sarah Gustapane, Antonio Malvasi, Daniele Vergara, Michele Maffia, Marilena Greco, Caterina Accettura, Marianna Giampaglia, Silvana Leo, and Vito Larusso

Chapter 4: Ovarian Cancer Screening and Early Detection
Brian M. Nolen and Anna E. Lokshin

Chapter 5: Surface Epithelial Tumors of the Ovary
Purnima Makhija and Naveena Singh

Chapter 6: Pathology of Non-Epithelial Malignancies of the Ovary
Eleni Ieremia and Naveena Singh

Chapter 7: Strategies for the Management of Ovarian Cancer
Tim Mould

Chapter 8: Ovarian Cancer in the Pediatric Population
Anne C. Fischer

Chapter 9: Management of Patients with Early-Stage Ovarian Cancer
Samir A. Farghaly

Chapter 10: Treatment of Advanced Stage Ovarian Cancer
John Butler and Alexandra Lawrence

Chapter 11: Diagnosis and Management of Epithelial Ovarian Cancer with Peritoneal Metastases
Paul H. Sugarbaker

Chapter 12: Targeted Molecular Therapy for Patients with Ovarian Cancer. Samir A. Farghaly

Chapter 13: Psychological Aspects of Hereditary and Non-Hereditary Ovarian Cancer
Kate Absolom, Elena Takeuchi, Geoff Hall, Galina Velikova

Chapter 14: Quality of Life in Patients with Ovarian Cancer
Sally E. Jensen and David Cella

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A Manual for Cervical Cancer Screening and Control: Principles, Practice and New Perspectives

This book is edited by Margherita Branco, former Director of Cervical Cancer Screening and Cytopathology Unit, National Institute of Heath, Rome (Italy) and by Adhemar Longatto-Filho, of the Laboratory Medical Investigation 14, Faculty of Medicine, Sao Paulo (Brazil).

The topic covered in this book is connected to the prevention and early detection of cervical cancer.

Although cancer of the cervix is a disease that is well-detected and almost eradicated in developed countries that have introduced individual screening programs, it still remains the second or third most common cause of death in developing countries.

The 14 chapters of this textbook thoroughly examine all the “aspects” related to prevention and early detection.

From the general information on this neoplasia, through primary prevention, HIV infection, risk factors, methods of screening, study of biomarkers, organization of training for personnel involved in screening programs, to the general instruction for prevention, this manual offers a complete contribution to improve women’s health.

Contents


Chapter 3: Human Papillomavirus (HPV) infections. M. Branca and A. Longatto-Filho.

Chapter 4: Risk factors for cervical cancer. M. Branca.


Chapter 6: Cancer prevention in developing countries. A. Longatto-Filho.

Chapter 7: Cervical cytology and alternative methods of screening. A. Longatto-Filho.

Chapter 8: Management of women with abnormal cytological results. M. Branca and A. Longatto-Filho.


Chapter 10: Basic concepts of quality and accreditation in Health Care Services. M. Branca.


Chapter 13: Instruction and training of personnel in a cervical cancer screening program. M. Branca and A. Longatto-Filho.

Chapter 14: Universal hygienic measures and precautions for infection prevention in gynecological ambulatory centers and hospitals. M. Branca.

We believe that this book also provides comprehensive coverage and expert guidance of all persons implicated in screening programmes.

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