european journal of gynaecological oncology
an International Journal

Founding Editor
A. Onnis
Montréal (Canada)

Editors-in-Chief
M. Marchetti
Montréal (Canada)
P. Bősze
Budapest (Hungary)

Associate Editor
T. Maggino
Padua (Italy)

Assistant Editor
A. Sinopoli
Toronto (Canada)

Editorial Board

Allen H.H., London (UK)
Ayhan A., Ankara (Turkey)
Balat O., Graziantep (Turkey)
Balega J., Birmingham (UK)
Basta A., Krakow (Poland)
Bender H.C., Dusseldorf (Germany)
Charkviani T., Tbilisi (Georgia)
Chiarelli S., Padua (Italy)
De Oliveira C.F., Coimbra (Portugal)
Dexux S. Jr., Barcelona (Spain)
Di Saia P., Orange, CA (USA)
Elit L., Hamilton (Canada)
Friedrich M., Krefeld (Germany)
Gadducci A., Pisa (Italy)
Geisler H.E., Indianapolis, IN (USA)
Heintz A.P.M., Utrecht (The Netherlands)
Ioannidou-Mouzaka L., Athens (Greece)
Klastersky J., Bruxelles (Belgium)
Kubista E., Vienna (Austria)
Lee Y.S., Daegu (South Korea)
Markowska J., Poznan (Poland)
Mariani A., Rochester, MN (USA)
Marth C., Innsbruck (Austria)
Massuger Leon F.A.G., Nijmegen (The Netherlands)
Menczer J., Savyon (Israel)
Monsonego J., Paris (France)
Morice P., Villejuif (France)
Pálfalvi L., Budapest (Hungary)
Piura B., Beer Sheva (Israel)
Piver S.M., Buffalo, NY (USA)
Rakar S., Ljubljana (Slovenia)
Sartori G., Brescia (Italy)
Shepherd J.H., London (UK)
Siklós P., Budapest (Hungary)
Stankusova H., Prague (Czech Republic)
Smit B.J., Tjgerberg (South Africa)
Stelmachów J., Warsaw (Poland)
Syrijänen K., Turku (Finland)
Tjama W., Antwerpen (Belgium)
Ungár L., Budapest (Hungary)
Vermorken J.B., Edegem (Belgium)
Wang P.H., Taipei (Taiwan)
Winter R., Graz (Austria)
Yokoyama Y., Hiroasaki (Japan)
Zola P., Torino (Italy)

Publishing Organization (M. Beaucage):
7847050 CANADA, Inc. - 4900 Côte St-Luc - Apt # 212 - Montréal, Québec H3W 2H3 (Canada)
Tel. +1-514-4893242 - Fax +1-514-4854513 - E-mail: canlux@mgroup-online.com
Website: www.irog.net

Editorial Office (M. Critelli):
Galleria Storione, 2/A - 35123 Padua (Italy) - Tel. +39-049-8756900 - Fax +39-049-8752018
E-mail: irog.canada@gmail.com

EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY (ISSN 0392-2936) publishes original peer reviewed works in the fields of female genital cancers and related subjects and also proceedings of gynecologic oncology society meetings all over the world. The Journal is covered by CURRENT CONTENTS, SCISEARCH, RESEARCH ALERT, INDEX MEDICUS, MEDLINE, EMBASE/Excerpta Medica, CURRENT ADVANCES IN CANCER RESEARCH, BIOSIS.
The immune response in malignant ovarian neoplasms

A. Martins Filho, M.P. Jammal, R.S. Nomelini, E.F.C. Murta - Uberaba, BRAZIL

The role of cytokines, nitric oxide, and lymphocytes in immune response against ovarian cancer is reviewed.

The role of levonorgestrel-releasing intrauterine system for endometrial protection in women with breast cancer taking tamoxifen

Qingquan Shi, Jinhong Li, Ming Li, Jing Wu, Qiang Yao, Aiyun Xing - Chengdu, CHINA

The endometrium of women treated with tamoxifen for breast cancer is protected by levonorgestrel-releasing intrauterine system.

Treatment results for Stage Ib cervical cancer after stage subdivision by MRI evaluation

H. Yamashita, Y. Niibe, K. Okuma, M. Omori, Y. Inoue, T. Onda, K. Nakagawa, K. Hayakawa - Sagamihara, JAPAN

A different prognosis was proved for Stage Ib1 and Ib2 cervical cancer, diagnosed according to MRI information.

Microtubule-associated protein tau correlates with estrogen receptor status but not with in vitro paclitaxel sensitivity in primary breast cancer


Estrogen receptor expression predicts tau mRNA expression, suggesting that estrogen receptor expression may be associated with tau protein expression.

The progress of ALDH-1 in gynecologic oncology

L. Liu, J. Yi, D. Zhang - Guangzhou, CHINA

The progress of ALDH-1 in cervical, endometrial, and ovarian cancer is reviewed.

Preoperative prediction of poor prognostic parameters and adjuvant treatment in women with pure endometrioid type endometrial cancer: what is the significance of tumor markers?

E. Baser, T. Gungor, C. Togrul, O. Turkoglu, S. Celen - Ankara, TURKEY

CA-125, CA 15-3, and CA 19-9 are evaluable tumor markers for adjuvant treatment prediction in endometrial cancer.

Expression of hexokinase 2 in epithelial ovarian tumours and its clinical significance in serous ovarian cancer

Z. Jin, J. Gu, X. Xin, Y. Li, H. Wang - Wuhan, CHINA

Hexokinase 2 is highly expressed in epithelial ovarian tumours, especially in serous group, and is related to stage and grading.

Ductal carcinoma in situ: analysis of 250 cases

J. Böhm, M. Zikán - Hildburghausen, GERMANY

With mammography, screening the incidence of non-invasive breast lesions is increased, particularly for ductal carcinoma in situ.
## Contents

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>485</td>
</tr>
<tr>
<td>530</td>
</tr>
<tr>
<td>535</td>
</tr>
<tr>
<td>539</td>
</tr>
<tr>
<td>544</td>
</tr>
<tr>
<td>548</td>
</tr>
<tr>
<td>554</td>
</tr>
<tr>
<td>557</td>
</tr>
<tr>
<td>562</td>
</tr>
<tr>
<td>566</td>
</tr>
<tr>
<td>571</td>
</tr>
<tr>
<td>576</td>
</tr>
</tbody>
</table>

**Breast conserving surgery in multicentric breast cancer, preliminary data of our experience**  
Breast conserving surgery in comparison with total mastectomy in a multicentric breast cancer study.

**Cytoreductive surgery for isolated para-aortic lymph node recurrence of endometrial cancer: report of four cases and a review of the literature**  
H. Nakamura, K. Takehara, O. Samura, T. Mizunoe - *Kure City, JAPAN*  
Surgery, as elective therapy for isolated para-aortic lymph node metastasis, was performed, improving the prognosis of the patients.

**Comparison of the histopathological diagnoses of preoperative dilatation and curettage and Pipelle biopsy**  
Pipelle endometrial biopsy is more economical, less invasive, and easily performed.

**Report on incidence and mortality trends of cervical cancer in northern Sardinia, Italy**  
A. Cossu, G. Capobianco, M. Budroni, D. Surico, R. Cesaraccio, F. Tanda, M. Dessole, S. Dessole, G. Palmieri - *Sassari, ITALY*  

**Improving ductoscopy with duct lavage and duct brushing**  
S. Zervoudis, Y. Tamer, G. Iatrakis, A. Bothou, X. Tokou, A. Avgoulea, V. Aranitis, X. Spanopoulos, E. Tomara, X. Patralexis - *Athens, GREECE*  
Duct brushing with duct lavage has a higher accuracy with respect to duct lavage alone in cases of pathological nipple discharge.

**HPV and HPV vaccination: knowledge and consciousness of young women**  
E. Coşar, M. Gencer, S.O. Hacivelioğlu, A.Ç. Güngör, A. Uysal - *Çanakkale, TURKEY*  
The low sociodemographic levels influence the knowledge and the use of HPV vaccination, and maintain a high risk of cervical cancer.

**The immune function differences and high-risk human papillomavirus infection in the progress of cervical cancer**  
Changdong Li, Cui Ma, Weiyuan Zhang, Jiandong Wang - *Beijing, CHINA*  
To investigate immune function differences in the progress of cervical cancer, several immune cells in cervical tissues were determined.

**Protection of ovarian function during chemotherapy for ovarian cancer**  
X. Tianmin, C. Weiqin, W. Shuying, L. Yang, C. Manhua - *Changchun City, CHINA*  
The influencing factors of ovarian function and protective measure during chemotherapy are discussed.

**The surgical outcomes of abdominal radical trachelectomy: does transrectal ultrasonography determine the cervical incision site during surgery?**  
Abdominal radical trachelectomy may be the therapy for early stage cervical cancer, especially in fertile age: transrectal sonography assists in the diagnosis of the length of cervix.

**The role of ureaplasma urealyticum infection in cervical intraepithelial neoplasia and cervical cancer**  
C. Xiaolei, H. Tao, S. Zongli, Y. Hongying - *Luoyang, CHINA*  
Positive rate of ureaplasma urealyticum may be related to the genesis of cervical cancer.

**Expression of heat shock protein 20 inversely correlated with tumor progression in patients with ovarian cancer**  
Naian Qiao, Yanhui Zhu, Haiying Li, Zhongyu Qu, Zhichun Xiao - *Jihan, CHINA*  
The relationship between heat shock protein 20 expression levels and tumor progression in a patient with ovarian cancer is examined.
### CASE REPORTS

**Malignant female adnexal tumor of Wolffian origin (FATWO) positive for CD56: a possible diagnostic role for the biomarker**  
CD56-positivity may be a diagnostic biomarker to differentiate malignant female adnexal tumor of Wolffian origin from benign lesion.

**Primary ovarian malignant mixed mesodermal tumor: report of four cases**  
E.Y. Kim, J.S. Park, J.B. Mun, S.Y. Hur - Seoul, Korea  
Diagnosis, treatment, and postoperative management of four cases of ovarian mixed mesodermal tumor are discussed.

**Isolated sacral metastases as the initial presentation from an endometroid ovarian carcinoma: a case report**  
G. Xin, J. Du, Y. Xu - Jinan, China  
A rare case of sacral metastases from a ovarian carcinoma is reported.

**Paraneoplastic limbic encephalitis – neurologic paraneoplastic syndrome associated with ovarian malignancy – the importance of clinical recognition**  
I. Pestana, A. Costa, R. Mota, R. Gorgal, V. Paiva - Porto, Portugal  
Early diagnosis and treatment, also with immunotherapies, explain the favorable neurological outcome in a case of paraneoplastic limbic encephalitis.

**Primary fallopian tube carcinoma: a case report and mini-review of the literature**  
E. Kalampokas, C. Sofoudis, I. Boutas, T. Kalampokas, I. Tourountous - Athens, Greece  
A case of primary carcinoma of the fallopian tube is reported.

**Normal-sized ovary carcinoma syndrome (NOCS) detected with FDG-PET/CT**  
M. Kanda, A. Sonoyama, N. Ohara - Sanda, Japan  
FDG-PET is determinant for the diagnosis of normal-sized ovarian carcinoma, especially when CT and MRI are inconclusive.

**Multifocal microinvasive squamous cell carcinoma with extensive spread of squamous cell carcinoma in situ (CIS) into the uterine corpus, vagina, and left salpinx diagnosed five years after conization of cervical CIS**  
 Extensive spread of multifocal squamous cell carcinoma in situ in a patient treated with conization five years prior.

**Primary choriocarcinoma of the fallopian tube: a case report and literature review**  
J. Wan, X.M. Li, J. Gu - Guangzhou, China  
Diagnosis, treatment, and outcome of a choriocarcinoma of the fallopian tube are discussed.
Malignant ovarian neoplasia

Despite a vast quantity of clinical and laboratory research, improvements in surgical techniques, and an increasing number of powerful chemotherapy agents, ovarian cancer remains the most lethal gynecological malignancy for women, and is one of the main causes of morbidity [1]. The incidence of ovarian cancer peaks at the start of the sixth decade of life, and two-thirds of patients have developed an advanced stage of disease by the time of diagnosis [2]. In addition, approximately 5–10% of tumors that appear to be in an initial stage of disease, are accompanied by metastases in the aortic or pelvic lymph nodes [3]. Although nearly 90% of ovarian cancer cases are sporadic, approximately 10% have a genetic or familial component [4].

The sensitivity and specificity of gynecological exams for monitoring ovarian neoplasias remains questionable [5]. However, ultrasound imaging has been found to facilitate the diagnosis of an ovarian tumor, and can differentiate between neoplastic and non-neoplastic tumors. For example, ultrasound imaging provides important tumor information, such as size and content, and this can be used to differentiate between malignant and benign tumors [6]. Transvaginal ultrasound with color Doppler imaging can also evaluate the vascular condition and blood flow of tumors in some cases [7]. However, the early diagnosis of ovarian cancer remains challenging due to the low prevalence of ovarian cancer in the general population. In addition, detection of tumors in their initial stages is less reliable, and this makes tracking of them difficult.

Currently, the five-year survival rate for ovarian cancer worldwide is 50%, with a 95% survival rate for patients with a neoplasia restricted to the ovaries (Stage I), a 79% survival rate reported for patients with neoplasia that have infiltrated tissue adjacent to the ovaries (Stage II), and a 28% survival rate for patients with an advanced stage of disease (Stage IV) [8].

In patients with malignant epithelial tumors, tumor markers such as CA-15.3, CA-125, and CA-19.9 have been detected, as well as alpha-fetoprotein and CEA in both the blood and intracystic fluid [9, 10]. When transvaginal ultrasound is used in combination with the detection of tumor markers, the sensitivity and specificity of these techniques for predicting malignancy in ovarian neoplasia is improved [5].

Ovarian cancer does not have a defined, pre-invasive lesion identified for it, and this makes it difficult to establish preventative strategies for it. In addition, although the hypothesis that ovarian epithelial cancer originates in the epithelium of the ovarian surface is widely accepted, it is based on relatively weak histological arguments. Moreover, recent morphological and molecular genetics studies have provided valuable insight into the carcinogenesis and histogenesis of non-uterine pelvic serous carcinomas. For example, pelvic serous carcinomas that have traditionally been considered to have an ovarian origin, may originate from the distal portion of the uterine tube. Thus, it is being investigated whether clonal expansion of tubal secretory cells represent the origin of these carcinomas [11].

Dissemination of ovarian cancer can occur in various ways. For example, the tumor can invade the ovarian capsule, and based on contiguity, can compromise adjacent organs. In addition, tumor cells can reach the lymphatic system, leading to metastases in the lymph nodes of the external iliac chain, common iliac, hypogastrium, lateral and
aortic sacral, and possibly the involvement of the inguinal lymph nodes. Hematogenic dissemination is less frequent, and occurs mainly in the liver parenchyma, cerebrum, and lungs. Finally, cells that detach from the surface of the tumor can be carried by the mechanism of intestinal peristalsis and gastrointestinal reflux, and may implant on the serous surface of the omentum, peritoneum, diaphragm, liver, or other abdominal visceras [12]. Ovarian cells may also pass through the lymph canals of the diaphragm and reach the pleural cavity.

The immune system and ovarian cancer

Prognostic factors identified for ovarian cancer include tumor stage, residual disease after initial surgery, histological type, and tumor grade. However, for cases of ovarian cancer that are often diagnosed in the late stages of disease, these prognostic factors have limited value. Therefore, methods to improve disease prognosis and the selection of treatment are important [13].

Recent studies have demonstrated the prognostic significance of certain elements of the immune response for ovarian cancers. For example, in various tumor tissues, a strong infiltration of leukocytes into a tumor, and into tissues adjacent to a tumor, have been observed. The presence of these cells is related to pre-cancerous inflammatory processes, or substances produced by the tumor cells themselves [14]. Furthermore, the presence of tumor-infiltrating leukocytes, or tumor-associated leukocytes, have been found to correlate with a better disease prognosis, while other leukocytes have been associated with a poor prognosis [15, 16]. The presence of infiltrating immune cells in a tumor also provides a better prediction of clinical outcome compared with other histopathological parameters. In addition, the immunological profile obtained can be useful in selecting alternative forms of treatment, such as immunotherapy [17]. Taken together, these results indicate that leukocytes can have a protective role, and at the same time, can promote the anti-tumor effects of the immune system.

Cytokines

Cytokines are peptides or glycoproteins of low molecular weight (e.g., < 80 kDa) that communicate between each other to regulate biological processes such as: cell growth, differentiation, programmed cell death, chemotaxis, inflammation, immunity, tissue repair, fibrosis, and morphogenesis. Correspondingly, cytokines are expressed by all cells in the body, except erythrocytes, and can be secreted from the cell membrane and/or expressed and stored in the extracellular matrix [18]. In particular, cytokines have been found to have key roles in stimulation and suppression events of the immune system, including the initiation and coordination of an inflammatory response [18]. These roles contribute to regulation of cell function, growth, and differentiation by the immune system, which represent key functions in the protection of a host [19].

Cytokines are also referred to as: lymphokines (since the majority of cytokines associated with specific immunity are produced by activated T lymphocytes), monokines (because they are produced mainly by mononuclear phagocytes in natural immunity), immunotransmitters, immunocytokines (since they are mainly produced by the immune system), chemokines (because certain cytokines share the ability to stimulate leukocyte movement (chemokinesis) and directed movement (chemotaxis)), or interleukins. The latter was the result of an important hypothesis generated in the 1970s that postulated that cytokines were mainly synthesized by leukocytes, and therefore, should be called interleukins. Regardless of the term used, cytokines have been shown to play an important role in the induction of an immune response to a variety of diseases, including cancer [20].

An immune response can be evaluated according to the type of cytokines secreted by CD4+ lymphocytes. For example, a TH1 immune response involves cytokines IL-2, IL-8, TNF-α, and IFN-γ, while a TH2 response involves the secretion of IL-3, IL-4, IL-5, IL-6, and IL-10. In addition, TH1 cells participate in mediating cytotoxicity and inflammation, and also defend a host from viral, microbial, and neoplastic diseases. In contrast, TH2 cells mainly contribute to a humoral immune response by producing antibodies in atopical reactions and defend a host from parasitic diseases [21].

In the tumor stroma and in neoplastic effusions, a large quantity of leukocytes, mainly macrophage and lymphocytes, have been observed [19, 22]. Both cell types are capable of producing TH1 and TH2 cytokines. However, during tumor progression, these macrophage and lymphocytes have been found to progressively deviate from the production of TH1 and TH2 cytokine profiles, which reduces the immune response to the tumor [22]. These results indicate that cytokines are important for understanding tumor-host interactions, yet can mediate pro- or anti-tumor actions [23].

Recently, scientists have demonstrated that cytokines can stimulate cell growth and affect metastasis. For example, the actions of cytokines, either independently or in combination, have been shown to mediate immunomodulatory activities in response to neoplasias. For these processes, signaling between inflammatory cells and invasive neoplastic tissue is involved, and this signaling may or may not affect malignant cell growth [23, 24]. Furthermore, tumor microenvironments can differ according to the quantity of cytokines expressed by benign, malignant, or non-neoplastic tumors, and this suggests that cytokines play a significant role in tumor progression [10]. Moreover, if particular cytokines were constitutively secreted at detectable levels, they could represent markers
The immune response in malignant ovarian neoplasms

CD3+ T lymphocytes

These cells have been referred to as tumor associated lymphocytes (TALs). For patients diagnosed with Stage III or IV ovarian cancer, the presence of infiltrating lymphocytes is associated with a higher rate of survival compared with those patients who do not have such lymphocytes: the presence of these lymphocytes is related to a better prognosis of the clinical response ovarian cancer [25].

Compared to benign ovarian epithelial lesions, carcinomas may have significantly higher levels of CD3+ and CD8+ T cells, suggesting that the presence of these lymphocytes is related to an improved prognosis for cases of ovarian cancer [26]. However, the presence of CD3+/CD4+/CD25+ T lymphocytes has been found to correlate with a poor prognosis in ovarian cancer.

T regulator (Treg) cells play an important role in maintaining immunological tolerance to self antigens by suppressing the activation and effector function of autoreactive T lymphocytes mature; the high rate of Treg cells has been identified as a predictor of risk of death and reduced survival in patients with ovarian cancer in all stages [27].

Nitric oxide – general aspects

Nitric oxide (NO) is a free radical that acts as a gaseous messenger and affects various biological functions. For example, at low concentrations, it acts as a signal transducer for many physiological processes, including regulation of blood flow, iron homeostasis, platelet reactivity, neurotransmission, and modulating the immune response mediated by cells. In contrast, at higher concentrations, NO acts as a defense against cytotoxic pathogens and tumors [28].

Previous studies have shown that NO and radicals derived from metabolizing oxygen, including superoxides, are key molecules in the pathogenesis of various infectious diseases [30]. In addition, accumulating evidence suggests that chronically elevated levels of NO are involved in the pathogenesis of certain human pathological conditions, such as inflammatory intestinal disease [31], neurodegenerative diseases [32], and cancer [33].

It has been hypothesized that NO can initiate carcinogenesis, and in combination with other factors, can lead to dysregulated cytostasis and cell differentiation. The effects of NO appear to be related to various factors, including the concentrations of NO generated, the varying sensitivity of different cell types to NO, and the duration of NO production [34]. Moreover, the effects of NO can be contradictory. For example, high concentrations of NO have been shown to induce apoptosis, while low concentrations can stimulate

<table>
<thead>
<tr>
<th>Components of immune system</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+ T lymphocytes</td>
<td>The presence of infiltrating lymphocytes is associated with a higher rate of survival in patients diagnosed with ovarian cancer Stage III and IV compared with those patients who do not have such lymphocytes: the presence of these lymphocytes is related to a better prognosis of the clinical response ovarian cancer.</td>
<td>Zhang et al., 2003 [25]</td>
</tr>
<tr>
<td>T CD3+ CD8+ lymphocytes</td>
<td>The presence of these lymphocytes is related to a better prognosis for the clinical response of ovarian cancer.</td>
<td>Helal et al., 2004 [26]</td>
</tr>
<tr>
<td>T CD3+ CD4+ CD25+ lymphocytes</td>
<td>These cells have been related to poor prognosis of ovarian cancer. They are called regulatory T cells (Treg) and play an important role in maintaining immune tolerance to self-antigens, through the suppression of activation and effector function of autoreactive T lymphocytes mature; the high rate of Treg cells has been identified as a predictor of risk of death and reduced survival in patients with ovarian cancer in all stages.</td>
<td>Benost and Mathis, 2013 [27]</td>
</tr>
<tr>
<td>Cluster of differentiation</td>
<td>Levels of expression of CD antigens appear to reflect the biology of carcinoma, both in distinguishing the lines of some cancer cell types. Accordingly, these molecules can be used to study the function of cells and cell differentiation.</td>
<td>Liu and True, 2002 [39]</td>
</tr>
<tr>
<td>Nitric Oxide</td>
<td>High concentrations of NO induce apoptosis but low concentrations stimulate tumor growth by inducing angiogenesis, causing a complexity of NO present purposes, sometimes contribute to tumor regression herein for its progression.</td>
<td>Moncada, Palmer, and Higgs, 1991 [34]</td>
</tr>
<tr>
<td></td>
<td>Eroded a predictor of a reduced survival rate for patients with any stage of ovarian cancer [28].</td>
<td>Hao et al., 2001 [35]</td>
</tr>
</tbody>
</table>
tumor growth by inducing angiogenesis [35]. It has also been observed that tissue synthesis induced by NO enzymes, and the production of intracystic NO metabolites, may be higher in malignant ovarian neoplasias than in benign and non-neoplastic tumors. Furthermore, these observations may correlate with prognostic factors such as tumor staging and the degree of tumor differentiation [36]. Therefore, NO is an attractive target for the development of new strategies for the prognosis, diagnosis, and treatment of cancer.

Cluster of differentiation (CD)

A cluster of differentiation (generally abbreviated CD) is a set of marker molecules present on a cell surface that can be used to differentiate various kinds of cells. CD molecules are generally receptors or molecules that activate receptors, and signaling cascades derived from CDs typically change a cell’s behavior. However, there are some CD proteins that have no role in signaling, and they perform other functions, such as mediating cell adhesion [37].

Recent advances in studies of antigen expression have facilitated the identification of diagnostic clusters of differentiation. This is consistent with the observation that clusters of differentiation are related to the presence of certain infections or a neoplasia [38]. Moreover, CD antigen expression levels seem to reflect both the biology of a carcinoma present, and the distinct carcinogenic cell types involved. Therefore, CD molecules may be applicable to studies of cell function and cell differentiation [39].

Table 1 summarizes the main functions of cells of the immune system, cytokines, and NO related to cancer.

Conclusion

The objective of this study was to review the immune response in the presence of malignant ovarian neoplasia, particularly in regards to the role of cytokines and NO as markers of the tumor microenvironment. However, additional studies are needed to fully characterize the roles of these molecules.

The presence of tumor-infiltrating leukocytes, or tumor-associated leukocytes (such as CD3+ and CD8+ T cells), has been found to correlate with a better disease prognosis. These results indicate that certain leukocyte subsets can have a protective role, yet at the same time, can also promote the immune system in targeting a cancer that is present. Thus, certain cytokines (such as IL-8 and IL-10) are attractive targets for the development of new diagnostic and prognostic strategies, as well as for the development of therapeutic methods for the treatment of ovarian cancer.

Acknowledgments

The authors would like to thank the Studies and Projects Funding Body (Financiadora de Estudos e Projetos, FINEP), the Foundation for Research Assistance of the State of Minas Gerais (Fundação de Amparo à Pesquisa do Estado de Minas Gerais, FAPEMIG), and the National Council for Scientific and Technical Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq).

References

The immune response in malignant ovarian neoplasms


Address reprint requests to:
E.F.C. MURTA, M.D.
Oncology (IPON)/Discipline of Gynecology and Obstetrics, Federal University of Triângulo Mineiro, Av. Getúlio Guaritá, s/n, 38025-440, Bairro, Uberaba, MG (Brazil)
e-mail: eddiemurta@mednet.com.br
The role of levonorgestrel-releasing intrauterine system for endometrial protection in women with breast cancer taking tamoxifen

Qingquan Shi1, Jinhong Li2, Ming Li1, Jing Wu1, Qiang Yao1, Aiyun Xing1

1 Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu
2 Department of Urology, West China Hospital, Sichuan University, Chengdu (China)

Summary

Purpose of Investigation: To review the evidence concerning the efficacy of levonorgestrel-releasing intrauterine system (LNG-IUS) in preventing endometrial pathology in women treated with tamoxifen. Materials and Methods: Randomized controlled trials (RCTs) of women with breast cancer on tamoxifen that compared endometrial surveillance or placebo alone vs. the LNG-IUS were reviewed. The eligible trials were identified from the following electronic databases: Cochrane CENTRAL, Medline, and EMBASE. The authors extracted data on all reported outcomes and conducted meta-analyses on the endometrial polyps, endometrial hyperplasia, proliferative endometrium, and endometrium thickness. Results: According to the subgroup analysis, a significant reduction of endometrial polyps was obtained (OR=0.22, 95% CI 0.13-0.37, p < 0.00001). The use of LNG-IUS reduced the incidence of endometrial hyperplasia (OR=0.13, 95% CI 0.03-0.58, p = 0.007). Increased abnormal vaginal bleeding for LNG-IUS users may be an adverse aspect of LNG-IUS. Conclusion: This meta-analysis confirms that endometrial hyperplasia is also reduced as well as endometrial polyp formation reduced after long-term follow-up.

Key words: Levonorgestrel-releasing intrauterine system (LNG-IUS); Endometrial neoplasm; Endometrial hyperplasia; Endometrial polyps.

Introduction

Tamoxifen acting as a classical therapy for women suffering breast cancer has been proven to improve survival for those who are hormone receptor positive [1]. Five years of treatment of tamoxifen lowers the annual breast cancer death rate by 31% in women with estrogen receptor positive tumor, compared to use for one to two years [1, 2]. Its main action is as an anti-estrogen, by blocking the estrogen receptor on the breast cancer cells, thus reducing proliferation [3]. However, corresponding to the anti-estrogen tumor-suppressive action in the breast, tamoxifen has an estrogen-agonist effect and thereby different actions in different organs [4, 5]. It acts as a pure estrogen in the skeleton and endometrium but as an anti-estrogen in the vagina and bladder [3]. Although tamoxifen also reduced the risk of osteoporotic fractures, it increased the risk of endometrial cancer, and other undesirable side effects [6]. Recently, tamoxifen exposure was found to be associated with an overexpression of b-catenin oncoprotein, which may play a major role in the pathogenesis of endometrial adenocarcinoma [7]. As a result of this, the effect of tamoxifen on endometrium challenges its safety [8]. The most common endometrial changes include endometrial polyps and hyperplasia in untreated women. The risk for the endometrial carcinoma was 1.3–7.5-fold risk compared to untreated women [9]. Compared with tamoxifen, the third-generation aromatase inhibitors bringing fewer adverse effects are now part of the standard adjuvant treatment for postmenopausal women with breast cancer [10-13]. However, tamoxifen remains part of the standard adjuvant endocrine therapy for premenopausal and postmenopausal breast cancer patients, owing to the new uncertain adverse effects and the cost.

The only levonorgestrel-releasing intrauterine system (LNG-IUS) approved for general public use is a T-shaped plastic intrauterine device that releases levonorgestrel (20 μg per day) directly into the uterine cavity. It produces a very high local concentration in the endometrial tissues with low plasma concentrations gained systemically [14], lowering the potential systemic adverse effects [15]. LNG-IUS is approved for endometrial protection in women who are receiving estrogen replacement therapy [16], and it was shown to induce regression of endometrial hyperplasia [17]. In the UK in 2005, the LNG-IUS was licensed for endometrial protection for women using estrogen replacement therapy (ERT) who retained their uterus, although it is not licensed for this indication in the USA or Canada [18]. Additionally, because LNG-IUS causes gland atrophy as well as abundant impediment and decidualisation of the endometrium, it has been suggested that LNG-IUS may be effective in keeping from proliferative endometrial pathology in tamoxifen users [19]. However, the effects and safety of LNG-IUS for this are not clearly known, especially for a long-term (> one year) use.

The purpose of the present study and systematic review was therefore to assess the efficacy of the LNG-IUS in pre-
venting the development of endometrial hyperplasia, polyps, and carcinoma in pre- and postmenopausal women taking tamoxifen.

This systematic review analyzes the current evidence for the use of the LNG-IUS in women using tamoxifen. To determine whether long-term LNG-IUS use prevents development of endometrial pathology like hyperplasia, polyps, and endometrial adenocarcinoma in those taking adjuvant tamoxifen.

Materials and Methods

A comprehensive database search was carried out independently by Q. Shi and J. Li. The authors searched the following database: Cochrane Central Register of Controlled Trials (CENTRAL), Medline (via OVID), and EMBASE. Each Randomized controlled trial (RCT) was scored for quality to assess validity using the Jadad scoring system, which is used to evaluate studies based on randomization, blinding, and description of withdrawals and dropouts. If the Jadad score of a study was more than 3, it was considered a high-quality study [20-22]. There was no restriction on the language of the publication. The following search terms were used to identify any relevant studies: “levonorgestrel or intrauterine device or IUD or intrauterine system” and “breast neoplasm or breast cancer” and “endometrium neoplasm or endometrium hyperplasia” and “tamoxifen” and “randomized controlled trial”. Two investigators evaluated all the potentially eligible studies independently without prior consideration of the result and assess the methodological quality separately.

The following criteria were used for study selection: (1) the study was a randomized controlled trial (RCT); (2) the patient was diagnosed women with breast cancer on adjuvant tamoxifen; (3) the treatment intervention was LNG-IUS vs. endometrial surveillance or placebo alone; (4) objective and/or subject outcome measures were clearly defined. Studies were excluded if: (1) the studies were not RCTs; (2) studies that examined the use of LNG-IUS in the treatment of early invasive endometrial cancers; (3) studies comparing different doses of intrauterine levonorgestrel in reduction of endometrial cancer risk without a control group.

The primary outcome measure was incidence of endometrial pathology including polyps, hyperplasia, and endometrial adenocarcinoma in those taking adjuvant tamoxifen.

Results

The present search identified 147 reports, of which 134 were excluded on the basis of title or abstract due to irrelevant to the topic and eight were excluded from the remaining 13 literatures after we finished the reading of full
The role of levonorgestrel-releasing intrauterine system for endometrial protection in women with breast cancer taking tamoxifen

494

Therefore, data from a total of five studies were included in this systematic review. Table 1 shows the characteristics of the included studies. Overall, 393 women were randomized to insert LNG-IUS (n = 198) or endometrial surveillance alone (n = 195).

The combined search strategies identified five studies that met the inclusion criteria. However, the five included papers actually are three trials, two of which were five-year study and the two studies both reported 12-month and 60-month outcome, respectively [24-27]. The three randomized controlled trials, 393 women included, investigated the use of the LNG-IUS in women using tamoxifen 20 mg per 24 hours compared to surveillance alone. Chan et al. [25] compared endometrial surveillance alone vs. prophylactic LNG-IUS insertion before tamoxifen administration in pre- and postmenopausal women. These women suffering breast cancer required adjuvant tamoxifen after the completion of postoperative radiotherapy and chemotherapy. Wong et al. [27] report the final 60-month results of described Chan’s trial [25]. Gardnet et al. studies [24, 26] and Kesiml et al. study [28] also compared endometrial surveillance alone versus endometrial surveillance before and after insertion of LNG-IUS for 12, 60, and 36 months in postmenopausal women taking adjuvant tamoxifen treatment for at least one year, respectively. Among the above studies, no endometrium cancers or cases of hyperplasia, fewer polyps were seen in the LNG-IUS groups at both 12-month and long-term follow-up.

In this meta-analysis, two studies reported the 12-month follow-up after LNG-IUS insertion in the breast cancer patients taking adjuvant tamoxifen; heterogeneity among them is not applicable, as no endometrial hyperplasia was observed in Chan et al. study [25]. The fixed effects model was used, the result showed that there was no significant difference between the LNG-IUS insertion group and the surveillance alone group (OR = 0.12, 95% CI: 0.00, 6.18; p = 0.29, Figure 2). Additionally, compared to control group, 12-month use of LNG-IUS reduced the incidence of endometrial polyps (OR = 0.21, 95% CI: 0.07, 0.58; p = 0.003, Figure 3) and endometrial proliferation (OR = 0.12, 95% CI: 0.04, 0.35; p < 0.0001, Figure 4).

Three studies reported the long-term follow-up (>36 months) after LNG-IUS insertion in the breast cancer patients taking adjuvant tamoxifen, no heterogeneity was found among them (p = 1.0, I2 = 0%), the fixed effects model was used, the result suggested a significant reduction in the incidence of endometrial hyperplasia in the LNG-IUS users group compared to endometrial surveillance alone (OR = 0.13, 95% CI: 0.03, 0.66; p = 0.01) (Figure 2). Moreover, a significant reduction in the incidence of endometrial polyps (OR = 0.23, 95% CI: 0.13, 0.41; p < 0.00001, Figure 3) and proliferative endometrium (OR = 0.15, 95% CI: 0.08, 0.30; p < 0.00001, Figure 4) in the LNG-IUS users group compared to endometrial surveillance alone. According to the subgroup analysis, the use of LNG-IUS reduced the incidence of endometrial hyperplasia (OR=0.13, 95% CI 0.03-0.58; p = 0.007, Figure 2), endometrial polyps (OR=0.22, 95% CI 0.13-0.37; p < 0.00001, Figure 3) and endometrial proliferation (OR=0.14; 95% CI 0.08 – 0.25; p < 0.00001, Figure 4).

There was no statistical significance between sonographic endometrial thickness in the treatment and control groups during both short-term (MD = -0.88 95% CI: -1.85, 0.09; p = 0.08, Figure 5) and long-term follow-up in the selected studies (MD = -0.15 95% CI:-0.84 0.54; p = 0.67, Figure 5). Overall, a majority of women with LNG-IUS complained of abnormal vaginal bleeding or spotting at six months compared with the control groups. However, by 12 months, this difference did not reach significance in any of the studies. After 60-month follow-up, Wong et al. [27]
Figure 2. — Forest plot of pooled odds ratios in endometrial hyperplasia in women using the levonorgestrel-releasing intrauterine system (LNG-IUS) and tamoxifen compared to tamoxifen alone.

Figure 3. — Forest plot of pooled odds ratios in endometrial polyps formation in women using the levonorgestrel-releasing intrauterine system (LNG-IUS) and tamoxifen compared to tamoxifen alone.

Figure 4. — Forest plot of pooled odds ratios in endometrial proliferation in women using the levonorgestrel-releasing intrauterine system (LNG-IUS) and tamoxifen compared to tamoxifen alone.

Figure 5. — Forest plot of pooled mean difference of endometrium thickness in women using the levonorgestrel-releasing intrauterine system (LNG-IUS) and tamoxifen compared to tamoxifen alone.
identified a statistically significant increase in abnormal vaginal bleeding for LNG-IUS users \((p < 0.001)\), which may be an adverse aspect of LNG-IUS. Chan et al. [25] reported other adverse effect of LNG-IUS such as breast tenderness and acne: four women in the treatment group and two in the control group reported breast tenderness. Only one woman in the treatment group complained of mild acne during the study.

**Discussion**

Breast cancer is the most frequent cancer among women with a lifetime risk of up to 12% and a lifetime risk of death of up to 5% [29], while tamoxifen administration for adjuvant treatment made a significant decline of incidence of the estrogen receptor–positive breast cancer, resulting for its wide use to treat breast cancer [8]. A meta-analysis summary of 28 randomized clinical studies indicated a significant reduction (16%) in mortality [30]. Regardless of its proven effectiveness, tamoxifen is still in connection with series of adverse effects: increased incidence of abnormal bleeding, polyps, hyperplasia, and cancer, the most serious risk [31]. To those women taking tamoxifen, the longer time one uses it, the higher risk of endometrial carcinoma she suffers. As reported, the risk doubled after two years of treatment, and quadrupled after five years of use. [32-34]. However, for breast cancer patients, the benefit of tamoxifen still outweighs its risk [27].

The LNG-IUS, which delivers the progestin levonorgestrel (LNG) into the uterine cavity at a steady rate of 20 µg per day, leads to abundant regression and decidualisation of the endometrium with atrophic gland [14]. As a result of its abundant anti-proliferative effect, the LNG-IUS is deemed to lower the risk of endometrial carcinoma and perhaps be efficacious in treating hyperplasia.

In the present review, three randomized controlled trials explored endometrial protection of LNG-IUS for breast cancer women taking tamoxifen. All studies address that LNG-IUS users got a statistically significant reduction in endometrial polyps. Nevertheless, only one trial concluded that LNG-IUS could lower the risk of endometrial hyperplasia for tamoxifen users. The rest two trials considered that protection of endometrium uncertain. Also, the trials differ in inclusion criteria, design, and the outcome measures. Gardner et al. [24, 26] inserted LNG-IUS in postmenopausal breast cancer women who had already been taking tamoxifen for one year and then had a five-year follow-up. Wong et al. and Chan et al. [25, 27] implanted LNG-IUS in mastocarcinoma patients before using tamoxifen, and also had 60-month following. Both studies [24-27] had 12-month results and 60-month conclusion. Kesiml et al. [28] put LNG-IUS in postmenopausal mastocarcinoma women who had taken tamoxifen at least for one year and then had a 36-month follow-up. All studies used hysterectomy and endometrial biopsy to diagnose endometrial lesion.

The primary outcome for Gardner et al. [24] and Chan et al. [25] study was endometrial pathology at first year and for Gardner et al. [26], Wong et al. [27] studies were endometrial pathology at fifth year, and for Kesiml et al. [28] study was endometrial pathology at 36th month. All trials excluded the patients diagnosed endometrial lesion before randomization. Any endometrial pathology detected at baseline was treated. Subsequent hysterectomy and endometrial biopsy was performed at 12, 24, 36, 48, and 60 months.

In Gardner et al. trial, the authors analyzed the data by received therapy, making a significant reduction in endometrial polyps in LNG-IUS users. In the entry baseline of participants, there was one case of complex hyperplasia without atypia in the control group. However, the authors fail to achieve any difference in endometrial hyperplasia between experimental group and control group. The study reported vaginal bleeding, the only significant adverse effect of LNG-IUS, which seems to be more common in those women inserted LNG-IUS after six months and this bleeding seems to be due to decidualised endometrium. In addition, there was no significant difference in endometrium thickness. The second trial randomized those participants to LNG-IUS insertion or surveillance alone before the commencement of tamoxifen. Chan et al. and Wong et al. made a statistically significant reduction concerning polyps in treatment group at 12th month \((p = 0.002)\) and at 60th month \((p < 0.001)\) [25, 27]. No endometrial hyperplasia or carcinoma was found in either group. Unscheduled vaginal bleeding was not significantly different from the control group at one year. However, at fifth year, Wong et al. found a significant increase in abnormal bleeding for LNG-IUS users \((p < 0.001)\) [27]. Other adverse effects like breast tenderness and mild acne were uncommonly reported, suggesting its good acceptance. Similarly, the trial did not find a significant difference in endometrial thickness between LNG-IUS users and control group. Furthermore, the authors showed an uncertainty of LNG-IUS, the risk of breast cancer recurrence as a result of mammary cell proliferation. The third trial [28] randomized participants to the LNG-IUS or no treatment after taking tamoxifen for at least one year. There was also no significant difference between those taking or not taking tamoxifen, as local progestogen affected the stroma. The incidences of endometrial polyps (19%) and endometrial hyperplasia (5.5%) in the control group after 36 months compared to only 5.7% and 0%, respectively, in the LNG-IUS group [28]. Spotting, the most common form of vaginal bleeding, in LNG-IUS users was only observed at first year. Headache and mastalgia were observed both in control (7.8%) and treatment group (12%). This review extends the findings of the recent Cochrane systematic review investigating the efficacy of LNG-IUS in women taking ta-
moxifen [20] by including one further randomized controlled trial [28] and the long-term follow-up data from the RCT by Gardner et al. [26] and Wong et al. [27]. This meta-analysis confirms that endometrial hyperplasia is also reduced as well as endometrial polyp formation reduced after long-term follow-up. Additionally, compared to Wan and Holland [17], the present authors add one latest study [27] which was a 60-month follow-up for inserting LNG-IUS prophylactically in breast cancer patients before taking tamoxifen. However, meta-analysis regarding development of hyperplasia was limited because of the relative rarity of the event and thus the odds ratio calculation was weighted heavily towards a single study. In the trials reviewed here, in the Cochrane review [19] and in Wan and Holland review [17], the risk of bias is low but none of the trials were adequately powered to make a significant difference in the occurrence of cancer. The effect of LNG-IUS in reducing the incidence of cancer in these women is therefore unknown.

All of the included studies were RCTs and the qualities of these RCTs were assessed by Jadad scoring system. Despite a thorough search strategy, unpublished studies may have been missing leading to publication bias. Funnel plots examining the existence of bias are not meaningful due to the small number of studies in subgroup analysis. In this review, the three trials of women using LNG-IUS in conjunction with tamoxifen showed no statistical evidence of significant heterogeneity in the endometrial outcomes.

**Conclusion**

Based on the data available, the meta-analysis confirms that not only is endometrial polyp formation reduced at long-term follow-up but endometrial hyperplasia is also reduced. This is clinically significant, as endometrial hyperplasia of varying degrees confers increased risk of cancer. Whether LNG-IUS protects against or reduces the risk of endometrial cancer for women on tamoxifen following breast cancer remains uncertain. Because of its uncertainty of breast cancer recurrence, larger studies are still necessary.

**References**


The role of levonorgestrel-releasing intrauterine system for endometrial protection in women with breast cancer taking tamoxifen


Address reprint requests to:
AIYUN XING, M.D.
Department of Obstetrics and Gynecology
West China Second University Hospital,
Sichuan University, 20# Renminnan Road,
Chengdu, Sichuan 610041 (China)
e-mail: aiyunx2013@163.com
Treatment results for Stage Ib cervical cancer after stage subdivision by MRI evaluation

H. Yamashita1, Y. Niibe2, K. Okuma1, M. Omori1, Y. Inoue2, T. Onda3, K. Nakagawa1, K. Hayakawa2

1Department of Radiology, University of Tokyo Hospital, Tokyo; 2Department of Radiology and 3Gynecology School of Medicine, Kitasato University, Kitasato, Minami-ku, Sagamihara (Japan)

Summary
Purpose of investigation: The authors analyzed treatment results for cervical cancer after subdividing Stage Ib into Stages Ib1 and Ib2 according to magnetic resonance imaging (MRI) information. Materials and Methods: The subjects comprised 40 cases of Stage Ib cervical cancer treated by definitive radiotherapy in Kitasato University hospital and Tokyo University hospital from January 2000 to December 2008. The patients’ ages ranged from 28 to 85 years (median: 68 years). The maximum tumor diameter measured with MRI ranged from undetectable to 60 mm (median: 25 mm). The authors classified tumors with the greatest dimension less than 40 mm as Stage Ib1 (29 cases) and those with the greatest dimension more than 40 mm as Ib2 (11 cases). All cases were treated with a combination of external beam irradiation and high-dose-rate intra-cavitary brachytherapy. Chemotherapy was combined with radiotherapy in 11 cases. Results: The follow-up time was from four to 109 months (median: 53 months). At the time of last observation, 37 cases survived, local recurrence was seen in none, and two cases showed distant metastasis. The two- and five-year overall survival rates of all cases were 97.5% and 89.5%, respectively. When a stage was subdivided and examined, the five-year overall survival rate of Stage Ib1 was 100% and that of Stage Ib2 was 50.5% \( (p = 0.001) \). Conclusion: The authors suggest that the subdivision of stages using image information reflects the prognosis of Stage Ib cervical cancer.

Key words: Stage Ib; Cervical cancer; Stage subdivision; MRI.

Introduction
According to the International Federation of Gynecology and Obstetrics (FIGO) staging of cervical carcinoma, Stage Ib is a clinical lesion confined to the cervix or a preclinical lesion greater than Stage Ia, and all gross lesions, even those with superficial invasion, are Stage Ib cancers. In 1995, the FIGO staging system was revised and, using 40 mm as a cut-off value, the Stage Ib tumor was re-classified as Stage Ib1 (≤ 40 mm) and Ib2 (> 40 mm) [1]. Even if there is no stromal invasion, few reports have been published on radiation therapy of Stage Ib1 and Ib2 tumors in order to evaluate how the size influences prognosis.

Diagnostic work-up for carcinoma of the uterine cervix includes physical examination (including bimanual pelvic and rectal examinations), inspection, colposcopy, conization, dilatation and curettage, punch biopsies, cystoscopy, rectosigmoidoscopy, intravenous pyelography (last three examinations are not mandatory), chest and bone radiography. Computed tomography (CT) and magnetic resonance imaging (MRI) evaluations were increased from the revision of April 2012, when “the use of diagnostic imaging techniques to assess the size of the primary tumor is encouraged but is not mandatory”. Conventionally, it was decided, “the test results by CT or MRI may be used for treatment plan decision, but staging must not be influenced by these results.”

MRI is not officially incorporated in the FIGO staging system, but is already widely accepted as the most reliable imaging technique for the diagnosis, staging, treatment planning, and follow-up of both endometrial and cervical cancer. The role of MRI in gynecologic oncology has evolved during the past two decades. There is now a substantial body of evidence that MRI is useful in evaluating malignant conditions of the pelvis [2]. MRI has been shown to be superior to CT in staging of cervical carcinoma and has been shown to be superior to CT in measuring tumor volume. The advantages of MRI are (a) space resolving power is high in the MRI, (b) the correlation between the high signal region of the T2-weighted MRI image and the tumor diameter is high [3], (c) MRI can also be used in a treatment plan for the image-guided intra-cavitary irradiation. Wagenaar et al. [4] demonstrated that tumor volume measurement with MRI was correlated with clinical stage and that inter-observer consistency was high.

The authors analyzed treatment results for Stage Ib cervical cancer after using MRI information to subdivide it into Stages Ib1 and Ib2.

Materials and Methods
This investigation examined retrospectively MRI images that were obtained before treatment as of April 2012. The images were correlated with stages of the disease based on a new evaluation standard introduced from April 2012.
The study comprised 40 cases with Stage Ib cervical cancer treated by definitive radiotherapy in Kitasato University Hospital and Tokyo University Hospital from January 2000 to December 2008. The ages ranged from 28 to 85 years (median: 68) (Table 1). The maximal tumor diameter measured with MRI ranged from undetectable to 60 mm (median: 25 mm). The authors classified tumors with the greatest diameter dimension less than 40 mm as Stage Ib1 (29 cases) and those with the largest dimension more than 40 mm as Ib2 (11 cases) (Table 1). All cases were treated with a combination of external beam irradiation and high-dose-rate intra-cavitary brachytherapy. The chemotherapy was combined with radiotherapy in 11 cases (Table 1). Most of Stage Ib2 cases were combined with chemotherapy.

### Results

#### Patients

The follow-up time ranged from four to 109 months (median: 53). At the time of last observation, 37 cases survived, local recurrence was seen in none, and distant metastasis was seen in two cases. The two-and five-year overall survival rates of all patients were 97.5% and 89.5%, respectively (Figure 1). When the authors subdivided the stage and examined the survival, the five-year overall survival rate of Stage Ib1 was 100% and that of Stage Ib2 was 50.5% ($p = 0.001$, Figure 2). The survival curves by histopathological types are shown in Figure 3.

### Table 1. Patient and tumor characteristics.

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>No.</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ib1</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Ib2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Age Histopathology</td>
<td>SqCC</td>
<td>36</td>
</tr>
<tr>
<td>AC</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Tumor greatest dimension by MRI</td>
<td>25 mm (undetectable - 60 mm)</td>
<td></td>
</tr>
<tr>
<td>PLN swelling</td>
<td>With</td>
<td>1</td>
</tr>
<tr>
<td>Without</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Dose fraction of EBRT</td>
<td>1.8 Gy (1.8 - 2.0)</td>
<td></td>
</tr>
<tr>
<td>Total dose EBRT</td>
<td>50 Gy (45 - 50.4)</td>
<td></td>
</tr>
<tr>
<td>Dose without MB</td>
<td>20 Gy (0 - 37.8)</td>
<td></td>
</tr>
<tr>
<td>Dose fraction of ICRT</td>
<td>6 Gy (5 - 6)</td>
<td></td>
</tr>
<tr>
<td>Total dose of ICRT</td>
<td>24.5 Gy (24 - 35)</td>
<td></td>
</tr>
<tr>
<td>Combined chemotherapy</td>
<td>With</td>
<td>11</td>
</tr>
<tr>
<td>Without</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

FIGO: the International Federation of Gynecology and Obstetrics; SqCC: squamous cell carcinoma; AC: adenocarcinoma; PLN: pelvic lymph node; EBRT: external beam radiation therapy; MB: midline block; ICRT: intra-cavitary radiation therapy; MRI: magnetic resonance imaging.
types showed that the five-year overall survival rate of squamous cell carcinoma (SqCC) was 91.1% and that of adenocarcinoma was 75.0% ($p = 0.13$, Figure 3). The prognosis of Stage Ib1 was significantly better than that of Stage Ib2. The local control rate was 100%. Only two patients had disease recurrence outside the pelvis; one had lung metastases and the other pleural dissemination.

According to Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0, all non-blood toxicity was below Grade 3. Grade 3 hematological toxicity occurred in three cases, but none of them needed discontinuation of the treatment.

According to European Organization for Research and Treatment of Cancer (EORTC) / Radiation Therapy Oncology Group (RTOG) Late Radiation Morbidity Scoring Schema, late adverse events higher than Grade 3, small intestine toxicity (ileus) was recognized in only one case.

Discussion

In this study, the tumor diameter (Ib1 vs. Ib2) affected prognosis more significantly than previously thought. The relationship between tumor size and survival is not clear. Some studies have demonstrated a statistically significant relationship between tumor size and survival in univariate analysis [5-6], while others did not [7]. However, the most valuable analysis is a multivariate analysis, which determines independent prognostic factors. Some studies have demonstrated that tumor size is an independent prognostic factor [8], while others have not [9]. Rutledge et al. [5] (Ib1 vs. Ib2, median follow-up: 35 months) found that survival rate decreased from 92.5% in Stage Ib1 to 74.3% in Stage Ib2 ($p = 0.004$), but in a multivariate analysis, this decrease was not statistically significant. Horn et al. [8] (Stages Ila-Ilb, median follow-up: 54 months) found that tumors larger than 40 mm affected the five-year survival rate significantly in an invariant analysis (49.5% vs. 67.4%, $p = 0.0015$), and tumor size was an independent prognostic factor. Kamelle et al. [6] (Stage Ib2, median follow-up: 25 months) found that two-year DFS was 86% when tumor size was 40-49 mm, while it was 72% when tumor size was 50 mm ($p = 0.29$). Lee et al. [7] (Stage Ib1, median follow-up: 51 months), showed that 40 mm did not have a significant effect on disease-free survival and overall survival ($p = 0.06, p = 0.29$ respectively). The present study, which showed that the five-year overall survival rate of Stage Ib1 and Stage Ib2 were 100% and 50.5%, respectively, did not have inferiority to these previous reports.

In this study, Stage Ib2 comprised many adenocarcinoma groups. Adenocarcinoma has a very poor prognosis in cervical cancer. According to Niibe et al. [10], the five-year overall survival rate of Stage IIIb adenocarcinoma of the uterine cervix treated with high-dose-rate intracavitary brachytherapy combined with external beam radiation therapy in Japan was 20.2%, and adenocarcinoma has a generally poor prognosis.

In this study, all the recurrences were distant metastasis. The method of local radiation therapy will not have to be improved because there were few side effects. For Stage Ib2 cases, more intensive systemic therapy such as consolidation chemotherapy after concurrent chemoradiation may be necessary [11-12]. Abe et al. [11] concluded that patients with para-aortic lymphadenopathy who received concurrent chemoradiation therapy (CCRT) and adjuvant chemotherapy had a more favorable overall and disease-free survival than did those treated with CCRT alone. The same thing may be said of patients in Stage Ib2.

Conclusion

The authors suggest that the subdivision of stages using MRI information reflects prognosis of Stage Ib cervical cancer. It may be necessary to consider other regimens of chemoradiation for Stage Ib2.

References


Address reprint requests to:

H. YAMASHITA, MD, PhD,
Department of Radiology,
University of Tokyo Hospital, 7-3-1, Hongo,
Bunkyo-ku, Tokyo, 113-8655 (Japan)
e-mail: yamachan07291973@yahoo.co.jp
Microtubule-associated protein tau correlates with estrogen receptor status but not with in vitro paclitaxel sensitivity in primary breast cancer

A. Honig1, M. Gehrmann, P. Kranke2, D. Keller3, J.B. Engel1, J. Hengstler4, M. Schmidt5
1Department of Obstetrics and Gynecology, University of Wuerzburg, Bayer GmbH, Leverkusen; 2Department of Anaesthesiology, University of Wuerzburg, Wuerzburg; 3Department of Bioinformatics, Biocenter, University of Wuerzburg, Wuerzburg; 4Leibniz Research Centre, Technical University of Dortmund, Dortmund; 5Department of Obstetrics and Gynecology, Medical School, University of Mainz, Mainz (Germany)

Summary

Background: Factors or signatures predicting response to chemotherapeutic agents are of great interest for breast cancer patient care. There is conflicting data regarding microtubule-associated protein tau as a predictive marker of paclitaxel sensitivity. Paclitaxel plays an important role in the adjuvant and metastatic therapy of breast cancer. However, a substantial proportion of patients treated with paclitaxel do not derive benefit from this therapy. Therefore, evaluating potential predictive factors is increasingly important. The authors attempted to validate these findings in vitro utilizing the ATP tumorchemosensitivity assay (ATP-TCA). Materials and Methods: The in vitro drug sensitivity to paclitaxel was evaluated in 48 fresh primary breast cancer specimens using the ATP-TCA. ATP-TCA results were analysed using the area under the curve (AUC) of growth inhibition. These results were correlated with the expression of tau mRNA measured by quantitative reverse transcriptase–polymerase chain reaction (RT–PCR). Tau was also compared between patients with progesterone receptor (PgR) positive and negative and estrogen receptor (ER) positive and negative breast cancer, respectively. Results: The correlation of tau with the AUC for paclitaxel was weak, Spearman Rho was -0.267 with a p-value of 0.064. As described before, multiple regression analysis confirmed T-stage (p = 0.01) and PR status (p = 0.01) as independent predictors of paclitaxel chemosensitivity. Using multiple regression analysis and defining tau mRNA expression as a dependent variable, estrogen receptor status as measured by immunohistochemistry was a highly significant predictor for tau mRNA expression (p < 0.001). Grade (p = 0.002) as well as PgR expression (p < 0.001) were also found to be predictors of tau mRNA expression. Conclusions: In the present data set the authors were not able to show that MAP-tau mRNA could predict benefit from the addition of a taxane to adjuvant chemotherapy. They found that ER expression is associated with tau protein expression. Estrogen gene transcription is reported to carry weak predictive significance for endocrine sensitivity, therefore it might be worth pursuing whether, tau mRNA could possibly be a predictor for endocrine therapy response.

Key words: Protein tau; Paclitaxel; Adjuvant therapy; Estrogen receptor.

Introduction

Taxanes have been added to adjuvant breast cancer treatment in recent years. Studies are in progress attempting to establish a role for taxanes not only for nodal positive but also for nodal negative high risk patients. Paclitaxel and docetaxel are the two taxanes used in most of the conducted trials in the adjuvant breast cancer setting. Paclitaxel plays an important role in the adjuvant therapy of primary breast cancer [1].

Despite the well-documented antitumor efficacy of paclitaxel, many tumours exhibit intrinsic resistance to paclitaxel. A substantial proportion of patients treated with paclitaxel after or before an anthracycline-based adjuvant chemotherapy do not derive benefit from adding this therapy. Identifying these patients could not only spare them an ineffective treatment, but gives the opportunity to establish more efficient regimens for the subgroup of paclitaxel-resistant patients.

In the adjuvant setting, Henderson et al. have shown that especially patients whose tumours were estrogen receptor (ER) negative derived most of the benefit from adding paclitaxel to an anthracycline-based regimen [1]. It is yet not clear whether in this setting the prognostic impact of the hormone receptor status and the impact of adjuvant treatment with tamoxifen might thus offset a potential predictive effect of the hormone receptor status for paclitaxel chemosensitivity. A large number of trials have been performed to identify predictive markers for chemosensitivity and/or prognosis of breast cancer [2-5].

Resistance to paclitaxel can be induced by decreased expression of the spindle assembly checkpoint genes Mad2 and BubR1 [6]. On the other hand, low expression of the microtubule-associated protein tau was associated with high sensitivity to paclitaxel [7]. These findings lead to an interest in protein tau and generated results which give an insight into factors that affect tau interesting. Recently more evidence occurred that the expression of the estrogen receptor is correlated with tau protein expression [8]. Ikeda et al. found a correlation between paclitaxel sensitivity and
tau expression as well as a relation between the expression of the protein and ER expression.

Genes involved in spindle assembly have a high probability to be involved in taxane especially paclitaxel resistance. This corresponds to the mechanism of action, as paclitaxel stabilises microtubules. Resistance to paclitaxel [9] and docetaxel [10] has been reported to be associated with specific patterns of gene expression. Chemosensitivity of primary tumour cells could be increased by inhibition of P-glycoprotein [11]. These examples demonstrate that many factors might predict paclitaxel chemosensitivity. If validated, such a factor could help to reduce unnecessary treatment for patients with breast cancer or help to identify sensitive subpopulations.

In order to test whether tau mRNA expression can predict paclitaxel response, the authors performed a prospective study in 48 consecutive breast cancer patients using an in vitro chemosensitivity assay (ATP-TCA) [12,13] with primary tumour cells isolated from resected breast cancer tissue. The present analysis also allows to correlate clinicopathological factors with tau mRNA expression in order to gain information on the underlying tumorbiology.

Materials and Methods

Statistical analysis

The Software PASW Statistics 18 was used for statistical analysis. Differences between two normally distributed groups were evaluated using T-Tests. Differences between two not normally distributed ones were investigated using the non-parametric Mann-Whitney U test. Gaussian distribution of groups was evaluated using a Normal-Probability plot. Significance was considered as two-sided \( p < 0.05 \). Multiple regression analysis with backward selection was used to model dependence of tau on multiple covariates. Spearman correlation coefficient was calculated to quantify the correlation between tau and area under the curve (AUC).

Ethics

The study was approved by the ethical review board of the medical association of Rhineland-Palatinate.

Patients

The authors used fresh breast cancer tissue specimens of consecutive patients who underwent surgery for primary breast cancer at the Department of Obstetrics & Gynecology, University of Mainz, Medical School during October 2002–September 2003. In this period of time, 155 patients had surgery for primary breast cancer. In 48 of these patients, a sufficient amount of fresh breast cancer tissue (at least 0.5 cm³) allowed for in vitro chemosensitivity. In 48 of these patients, the ATP concentration was measured. An ATP standard curve was included into all assays. Three independent incubations with primary tumour cells of each patient were performed to calculate the AUC. From a total of 52 tested breast cancer specimens, three showed a too low viability of the isolated tumour cells. Therefore, the overall evaluability rate was 94%.

Table 1. — Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Number evaluated</th>
<th>%</th>
<th>Not evaluable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59 +/- 12.5a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>20</td>
<td>41.7</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>41.7</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>3</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>N-stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>20</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>20</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>2</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>5</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>7</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>12</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>30</td>
<td>61.2</td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>41</td>
<td>85.4</td>
<td></td>
</tr>
<tr>
<td>Other types</td>
<td>7</td>
<td>14.6</td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor*</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
<td>71.4</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>Progesterone receptor*</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>55.1</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>22</td>
<td>44.9</td>
<td></td>
</tr>
</tbody>
</table>

* Immunohistochemically determined.

In vitro chemosensitivity assay

The chemosensitivity test was performed with primary tumour cells that have been isolated from tumour tissue immediately after resection. A commercially available kit was used to assess chemosensitivity according to the manufacturer’s instructions. Briefly, tumour specimens were dispersed using sterile scalpels. Subsequently, small tissue fragments were enzymatically disso-
Microtubule-associated protein tau correlates with estrogen receptor status but not with in vitro paclitaxel sensitivity in primary breast cancer

Quantitative RT–PCR for TAU mRNA

Total RNA was isolated from five mM sections of formalin-fixed, paraffin-embedded tumour tissues after histopathological confirmation of a tumour cell content of at least 70%. Reverse transcriptase–polymerase chain reaction (RT–PCR) was performed as described before [14]. Forward and reverse primers were located in exon 14 and in exon 15 respectively. The sequence of the forward primer is 5’ TCAAGGACAGAGTCCAGTCGAA 3’ and the sequence of the reverse primer is 5’ GCGTTCTCGCGGAAGGT 3’ respectively. The size of the PCR product was 109 base pairs. The position of the amplicon lies between nucleotides 1185 and 1293 (1185 tcaagg acagagt ccggaagtaaatt gggtccctgg acaatatcac ccacgtc cct ggcggaggaa ataaaaagat tgaaacccac aagctgacct tccgcgagaa cgc 1293, Homo sapiens microtubule-associated protein tau (MAPT), transcript variant 3, mRNA length=5637, GENE ID: 4137 MAPT).

To standardise the amount of sample RNA, GAPDH was selected as a reference gene. Primer and probes were obtained and the sequences are shown above.

Immunohistochemistry

Serial sections of formalin-fixed slices were stained with either monoclonal ER antibodies (clone 1D5) or monoclonal PR antibodies (clone PgR 636), as described [4]. The immunohistochemical evaluation was performed by one of the authors (M.S.) trained in histological and immunohistochemical diagnostics, unaware of the ATP-TCA data.

Results

Analysis of data groups

Using normal-probability plots for each data group the authors found values for paclitaxel sensitivity were distributed following a Gaussian distribution curve and values for mRNA of tau protein were not. Therefore T-tests were performed for the former data set and Mann-Whitney U tests for the latter data set in order to analyze differences between data groups.

Evaluation of paclitaxel chemosensitivity

For evaluation of paclitaxel chemosensitivity primary tumour cells isolated from 49 breast cancer patients were analyzed (baseline characteristics: Table 1). The area under the dose–response curve (AUC) was used as a measure for chemosensitivity.

Correlation of paclitaxel sensitivity to tau mRNA expression

The authors calculated the Spearman-Rho correlation coefficient in order to measure the extent and significance of the correlation. Spearman-Rho was -0.267 with a p-value of 0.064 suggesting a trend but still being statistically non significant. They concluded that there was no statistically significant correlation between tau mRNA expression and paclitaxel sensitivity in the present data set with a limited sample size (Figure 1).

Multiple regression analysis confirms T-stage and PR status as independent predictors of Paclitaxel chemosensitivity

To analyse which of the individual parameters were independent predictors of paclitaxel chemosensitivity, the authors performed a multiple regression analysis. Using a step down approach, initially the PR status, T-stage, N-stage, grading, estrogen, and tau mRNA expression were included. PR status and T-stage remain as independent factors (p = 0.01).

In the following the authors investigated the influence of these two factors separately for paclitaxel chemosensitivity. As published before in a slightly larger study population PR status (positive vs negative, p = 0.008) proved to be a predictor of paclitaxel sensitivity. T-stage also remained a statistically significant predictor of paclitaxel sensitivity (Stages 1 and 2 vs. 3 and 4, p = 0.013) but it has to be mentioned that the number of patients with tumors greater than T2 were small.

Multiple regression analysis identifies ER status as independent predictor of Tau mRNA expression

Defining tau mRNA expression as the dependent variable the authors analyzed the individual parameters with regards to their influence on tau mRNA expression. Performing multiple regression analysis the authors found that estrogen (receptor status as measured by immunohistochemistry) was highly significantly correlated with for tau mRNA expression with a p-value of < 0.001.

The authors also found that grade (p = 0.002) as well as progesterone receptor expression (p < 0.001) were correlated with tau mRNA expression.

Discussion

The molecular rationale for investigating tau expression in samples of breast cancer patients is that studies suggested that high levels of tau partially protect microtubules from pa-
clitaxel binding and that low levels of the protein leave microtubules more accessible and vulnerable to the drug [7]. Rouzier et al. performed in vitro experiments, in which they downregulated tau mRNA expression using small interfering RNA technique. This downregulation increased the sensitivity to paclitaxel but not to epirubicin [7]. The same investigators showed in tubulin polymerization assays that tau seems to modulate binding of paclitaxel to tubulin. They preincubated tubulin with tau which resulted in decreased paclitaxel binding and reduced paclitaxel induced microtubule polymerization. These data suggested that low tau expression renders microtubules more vulnerable to paclitaxel and putatively renders breast cancer cells sensitive to this drug.

This suggested that patients with low tau expression would benefit most from paclitaxel chemotherapy whereas patients with high tau protein expression could be spared from unnecessary toxicity.

One putative reason the present authors might not have found that tau could function as indicator of paclitaxel chemosensitivity is that they measured mRNA levels and not protein. It cannot be ruled out that a measurement of protein could lead to different results than ours.

In support of this notion Ikeda et al. recently found that mRNA expression of MAPT (MAPT = microtubule associated protein tau copy number) did not correlate with sensitivity to taxanes. However, expression of MAPT protein isoforms of less than 70 kDa were correlated with a low sensitivity to taxanes [8].

On the other hand there are reports that state that tau mRNA expression has utility to predict taxane chemosensitivity. For example Andre et al. described tau mRNA expression as a useful marker to predict paclitaxel chemosensitivity in oestrogen-receptor-positive breast cancer patients [15].

Andre et al. conducted a trial investigating 82 patients to assess the predictive value of tau mRNA analysis [15]. Paclitaxel sensitivity with respect to tau mRNA expression was analyzed in the preoperative neoadjuvant setting. The investigators concluded that high tau mRNA expression in ER-positive breast cancer indicates an endocrine-sensitive but chemotherapy-resistant disease. Furthermore Andre et al. stated that low tau expression identified a subset of ER-positive cancers that have poor prognosis with tamoxifen alone and might benefit from taxane-containing chemotherapy [15].

The same investigators explored whether this correlation would hold up in predicting sensitivity to paclitaxel in the adjuvant setting. Tau protein expressions in primary breast cancer specimens from 1,942 patients in the NSABP-B28 clinical trial were analyzed.

The patients were treated with four courses of doxorubicin/cyclophosphamide (AC) or AC followed by four courses of paclitaxel. All hormone receptor-positive patients in the trial also received adjuvant endocrine therapy. The hypothesis was that patients whose tumors expressed low levels of tau would preferentially benefit from the addition of paclitaxel to their adjuvant regimen. In terms of evaluating the prognostic value, univariate and multivariate analyses found that both high tau expression and ER-positive status were associated with better disease-free and overall survival. However the researchers found no significant correlation between tau expression and benefit from paclitaxel no matter whether they investigated the total study population or patients with ER-positive or ER-negative breast cancers [16]. The latter results confirm the present results that tau protein expression might not be suitable candidate for the prediction of paclitaxel sensitivity.

Docetaxel is the other most frequently used taxane administered for breast cancer treatment. Dumontent et al. investigated whether tau protein expression was correlated to docetaxel sensitivity in the patients of the adjuvant BCIRG 001 adjuvant breast cancer trial [17]. In this cohort of node positive patients, tau protein expression was not predictive of docetaxel response.

Conclusions

The authors found that ER expression predicts tau mRNA expression suggesting that ER expression is associated with tau protein expression. Although they were not able to show that MAP-tau mRNA could predict benefit from the addition of a taxane to adjuvant chemotherapy, tau mRNA could possibly be a predictor for endocrine therapy response given that estrogen gene transcription alone carries weak predictive significance for endocrine sensitivity or for outcome [8]. Other groups have shown the predictive significance of MAP tau gene expression for patient outcome [18]. Therefore it seems worthwhile to further investigate this ER inducible tubulin modulator with respect to its utility for individualising cancer therapy.

Acknowledgements

This study was supported by the German Research Foundation (DFG).

References


Microtubule-associated protein tau correlates with estrogen receptor status but not with in vitro paclitaxel sensitivity in primary breast cancer


Address reprint requests to:
A. HONIG, M.D.
Department of Obstetrics and Gynecology,
KKM Mainz
An der Goldgrube, 11
55131 Mainz (Germany)
e-mail: arnd_hoenig@hotmail.com
The progress of ALDH-1 in gynecologic oncology

L. Liu*, J. Yi*, D. Zhang*

1Department of Gynecology, Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou
2Department of Dermatology, Foshan Maternal and Child Health Hospital, Foshan (China)

Summary
The authors present a review of the literature to illustrate the progress of ALDH-1 in gynecological malignancies.

Key words: ALDH-1; Cervical cancer; Endometrial cancer; Ovarian cancer.

Introduction
ALDH-1 was called aldehyde dehydrogenase 1, and was also known as ALDH1A1, which is located in cytosol and are responsible for oxidizing intracellular aldehydes to acetic acid, and to protect cells from the damage of acetaldehyde peroxide. Studies have shown that ALDH-1 is the cancer stem cells marker of many tumors, such as head and neck squamous cell carcinoma [1], liver cancer [2], breast cancer [3], colon cancer [4], and lung cancer [5], and ALDH-1 positive expression was correlated with poor clinical outcome in many cancers, such as breast [3, 6], pancreatic [7], lung [8], and bladder cancer [9], prostate gland carcinoma [10] and liver cancers [11].

ALDH-1 has been more and more researched in the recent years. It is expressed in normal stem cells and is a specific marker in surface of the normal stem cell. Studies have found that ALDH-1 is a tumor stem cell marker in many cancers, such as head and neck squamous cell carcinoma [1], hepatocellular carcinoma [2], breast cancer [3], colon cancer [4], lung cancer [5], breast cancer [3, 6], pancreatic cancer [7], lung cancer [8], bladder cancer [9], prostate cancer [10], liver cancer [11] et al. Tumor which was positive of ALDH-1 correlated with poor clinical outcomes. Although studies between ALDH-1 and gynecologic tumors stem cell (such as cervical cancer, endometrial cancer, ovarian cancer) has not yet been reported, it has been observed that it is correlated with clinical prognosis in gynecologic cancers.

The overview of ALDH-1
ALDH-1 was also called ALDH1A1 (Genebank), named acetaldehyde dehydrogenase -1. It is a cytosolic isoenzyme that are responsible for oxidizing intracellular aldehydes and contributing to the oxidation of retinol to retinoic acid (RA) in early stem cell differentiation, and to protect normal cells from the damage of aldehyde peroxide. It is expressed in cytoplasm, and the genome is located at chromosome 9 q21, containing 13 exons, encoding 501 amino acid. The ALDH-1 (aldehyde dehydrogenase -1) superfamily includes many types, such as ALDH1A1, ALDH1A2, ALDH1A3, ALDH1B1, and ALDH1L1 [12]. The gene sequences of ALDH1A2, ALDH1A3, ALDH1B1, and ALDH1L1 were unknown. Recently more and more research focus on ALDH-1. ALDH-1 is expressed in normal stem cells, and is a specific marker in surface of the normal stem cell. The ALDH-1 play a part in RA biosynthesis in human cells. It contributed to the oxidation of retinol to retinoic acid in the early differentiation of stem cells, and the metabolism of retinaldehyde to RA may play a role in the biology of stem cells. More and more reports [13] have shown that RA act through RA receptors and function in differentiation, reduced cell proliferation, tissue homeostasis and apoptosis in various cell type, and in the intestine RA from dendritic cells imprints T and B cell homing, induces Treg cell differentiation, and induces tolerance. This suggests that ALDH-1 and its product RA could influence tumor growth either through regulation of immune cells or by direct effects on tumor cell growth. The high activity of ALDH-1 are found in the liver and neural stem cells and progenitor cells of rat and human, multiple myeloma, and acute myeloid leukemia stem cell populations. Moreover clinical studies have found that breast cancer cells also have a small number of cells expressing ALDH-1; the patients with ALDH-1 positive had poor prognosis, which was closely related to tumor grade, the state of estrogen receptor (ER) and progesterone receptor (PR), the overexpression of ERBB2, and the formation of cytokeratin. Several studies have found that ALDH-1 is the tumor stem cell markers of the head and neck squamous cell carcinoma [1], liver cancer [2], breast cancer [3], colon cancer [4], and lung cancer [5], and ALDH-1 positive expression was correlated with poor clinical outcome in many cancers, such as breast cancer [3, 6], pancreatic cancer [7], lung cancer [8], bladder cancer [9], prostate gland carcinoma [10], and liver cancers [11]. Different tumors due to
the different expression of ALDH-1 resulted to a different response to treatment. Using a reagent for separating breast cancer ALDH-1 positive cells, the characteristics of tumor stem cells have been demonstrated in vitro experiments. In the transplantation model of nude mouse, \(5 \times 10^2\) ALDH-1 positive cells can be transplanted to form solid tumors, while \(5 \times 10^5\) ALDH-1 negative cells cannot [3], which showed that ALDH-1 positive breast epithelial cancer cells had stem cell activity, and ALDH-1 positive cell was related to breast cancer metastasis and drug resistance. Recent research has also found that ALDH-1 positive of head and neck squamous cancer cells showed high tumorigenicity, radiation tolerance, high expression of stem cell marker (Bmi-1/Oct-4/Nanog), and epithelial mesenchymal transformation (epithelial-mesenchymal transition, EMT) related genes [14].

### Research between ALDH-1 and gynecologic cancer

**First: cervical cancer**

Cervical cancer is the most common malignancy of the female lower genital tract, which seriously threatens women's health. There are about 130,000 new annual cases in China, which account for one-fifth of the world's total cases. Although the clinical effects and prognosis of cervical cancer have improved significantly, however the five-year survival rate of advanced and recurrent cervical cancer is only 3.2%-13%. The reason why the survival rate is low is that patients who have recurrence and metastasis, are not sensitive to the traditional treatment (such as radiotherapy, chemotherapy, and other treatment) and current anticancer treatments do not completely kill tumor cells, probably because these methods aim primarily at the vast majority the differentiate tumor cells, but do not affect the tumor cells (including tumor stem cells) which are resistant to drugs. Tumor stem cells are a few tumor cells which have the ability of self-renewal and multiple differentiation potential, which are the root of tumor proliferation, metastasis, and recurrence. Moreover, they show a high resistance because of their natural anti-apoptosis to chemotherapy and radiotherapy.

ALDH-1 is expressed in normal tissue stem cells, playing a critical role for oxidizing intracellular aldehydes to acetic acid in the early differentiation of stem cells. ALDH-1 is the specific cancer stem cell marker. Reports between ALDH-1 and cervical cancer stem cell have not been reported. Yao et al. [15] reported that 89 cases of cervical squamous cell carcinoma and 20 cases of adenocarcinoma of the cervix were detected for the expression of ALDH-1 with immunohistochemistry, and found that only 23 cases of squamous cell carcinoma and four cases of adenocarcinoma express ALDH-1 (expression rate: 24.77%). Moreover, the reagent kits, qRT-PCR, and Western blot have confirmed the existence of a small populations of ALDH-1 positive cells. This suggests that the tumor stem cells count for a small portion in tumor cells.

Yao et al. [16] reported that 198 patients with cervical cancer was performed with immunohistochemistry to analyse the associations between aldehyde dehydrogenase-1 (ALDH-1) tumour immunopositivity and disease-free survival in cervical carcinoma. Patients were diagnosed with invasive squamous carcinoma (n=155) or adenocarcinoma (n=43). A total of 112 patients had undergone no preoperative radiotherapy or chemotherapy, four had undergone preoperative radiotherapy, and 82 had received preoperative chemotheraphy. Their results showed that patients with ALDH-1-positive tumours (n=31) had significantly shorter disease-free survival than those whose tumours were ALDH-1-negative (n=167; 41.99 ± 0.90 vs 53.64 ± 2.67 months; \(p < 0.05\)). Lymph node metastasis (RR 5.445; 95% CI 2.014, 14.722) and ALDH-1 positivity (RR 2.727; 95% CI 1.253, 5.914) were associated with poor prognosis. Thus they concluded that ALDH-1 positivity is associated with poor prognosis in cervical carcinoma and may be an independent predictor of prognosis. Moreover, they found that the expression of ALDH-1 was related with different pathological grading, deep myometrial invasion, as well as lymph node metastasis, but not with patients’ age, clinical stages, pathological type or vascular invasion. With radiotherapy/chemotherapy in preoperation, the positive expression of ALDH-1 was found in 74 cases, of which 61 cases were low expression, 13 cases were high expression, and the positive expression of ALDH-1 was related with different clinical stage, pathological grade, depth of myometrial invasion, and lymph node metastasis, but not with patient age, histologic type, and vascular invasion. The prognostic analysis results of multivariate COX proportional hazards model showed that clinical stage, pathological grade, lymph node metastasis, and the positive expression of ALDH-1 were the main risk prognosis factors of cervical cancer. The ALDH-1 protein was related with parametrial invasion. The expression of ALDH-1 which was greater than 1/2 layers of myometrial invasion was higher than that of less than 1/2 layers. So they concluded that ALDH-1 positive cells were “stem cells” or had the potential community characters of stem cells. ALDH-1 positive cells may promote tumor invasion and metastasis of cervical carcinoma.

Rao et al. [17] transfected the siRNA of ALDH-1 with lentivirus vector to SiHa cells, and found that cell proliferation, microsphere formation rate, tumorigenesis, and migration rate of SiHa-ALDH1-interfering group were significantly lower than SiHa-ALDH1-positive cells. While higher ALDH-1 expression correlated with significantly higher rates of cell proliferation, microsphere formation, tumorigenesis, and migration. So they suggested that ALDH-1 may be a marker of cervical cancer stem cells. Thus the present authors hypothesized that ALDH-1 in cervical cancer was similar as breast cancer, lung cancer, liver cancer, head and neck squamous cell carcinoma, and other tumors. ALDH-1 positive cervical cancer patients have a
worse clinical prognosis, and higher risk of metastasis and recurrence. ALDH-1 may be a specific cervical cancer stem cell marker.

**Second: endometrial cancer**

Endometrial cancer is the most common malignant tumors of the female genital tract. In recent years, the trend of incidence is rising: close to or even more than the cervical cancer. Although the examination and treatment are promising, the prognosis of patients with endometrial cancer is still not optimistic. Kato et al. [18] confirmed that the side population of endometrial adenocarcinoma contained original tumor cells (cancer-initiating cells, CIC) and ALDH-1 was expressed in stem / progenitor cells, which contain CIC of different tissues and organs. CIC showed high expression of ALDH-1 activity, and the higher of which has stronger tumor-derived and chemotherapy resistance in colon, breast, lung, pancreatic, bladder, and prostate cancers.

Rahadiani et al. [19] detected the expression of ALDH-1 of endometrial adenocarcinoma in patients with immunohistochemistry. ALDH-1 high expression in endometrial adenocarcinoma cells has stronger tumor-derived, anticancer drug resistance and invasion than low expression of cells. High expression of ALDH-1 activity showed that endometrial cancer cells like all other cancers had the CIC characteristics. In addition, they found that high expression of ALDH-1 tumor cells from clinical specimens were mostly negative of CD9, ER, and PR, which indicated that the expression of ALDH-1 cells was in an immature state. It is also similar as the immaturity characteristics of the CIC. Moreover they found that higher expression of ALDH-1 was related with tumor stage, lymph vessel invasion, chemotherapy resistance, recurrence, and prognosis. The prognosis of patients with higher expression of ALDH-1 was worse than lower expression of ALDH-1, and the expression of ALDH-1 were independent factors. This was consistent with other tumor types (such as colon, breast, lung, pancreatic, bladder, prostate cancers et al.), and they showed that higher expression of ALDH-1 cells were much stronger resistant to cisplatin in vitro experiments. Matrigel invasive experiments showed that high ALDH-1 expression of endometrial carcinoma cells contained more invasive cells, and this indicated that high expression of ALDH-1 cells had more invasive ability. The clone formation experiments showed that high expression of ALDH-1 in endometrial cancer cells had more clone numbers. All of these experiments confirmed that high expression of ALDH-1 cells had stronger resistance to cisplatin, stronger invasion, and reproduction ability. Hence the present authors conclude that the role of ALDH-1 in endometrial carcinoma was similar as breast cancer, lung cancer, liver cancer, head and neck squamous cell carcinoma, and other tumors and the patients of endometrial cancer with ALDH-1 positive had worse clinical prognosis, higher metastasis, and more risk of recurrence. The expression of ALDH-1 positive cells in endometrial carcinoma had higher growth and reproduction ability, invasive ability, and stronger resistance to chemotherapeutic drugs. The ALDH-1 may be a CIC marker, but it still requires larger sample clinical studies and cell experiments to verify this in the future.

**Third: ovarian cancer**

The five-year survival rate of ovarian cancer is still about 25%~ 30%, which is the highest mortality of gynecologic tumors. The relapse and drug resistance are the major difficulties in the treatment of ovarian cancer; the reason for these was the presence of tumor stem cells. Compared with other tissues, the ALDH-1 expression content of the normal human ovary is the highest.

Edassery et al. [20] found that ALDH1A1 (ALDH-1) was a new antigen which was associated with ovarian autoimmunity with unexplained infertility and early menopause, and they also found the patients with ovarian cancer had antibodies to ALDH-1, and the expression of retinol binding protein which was related with RA metabolism in ovarian carcinoma was low.

Rae et al. [21] found the expression level of ALDH-1 of ovarian cells decreased through inflammatory stimulation. In addition, the ALDH-1 was high in early ovarian tumor and may correlate with clinical prognosis.

Deng et al. [22] detected the ALDH-1 expression of 439 patients with serous ovarian cancer and found the prognosis of high expression of ALDH-1 positive patients with ovarian tumors was worse than low expression, and found that increased expression of ALDH-1 was related with chemotherapy resistance. They also detected the activity of ALDH-1 with reagent kits, and the results showed that the activity of ALDH-1 in ovarian carcinoma cell lines was stronger than the primary cells, but there was no significant difference, while the activity of ALDH-1 in normal ovarian epithelial cells was stronger than ovarian cancer cell lines and there was significant difference. These results indicated that ALDH-1 may play a key role in ovarian tumors and the response to drug therapy.

Wang et al. [23] found that high expression of ALDH-1 cells had more stem cell properties (such as resistance to chemotherapy, cloning, and nude mice tumor formation ability) in ovarian cancer ES-2, TOV-21G and CP70 cells compared with low expression of ALDH-1 cells. Immunohistochemical staining results showed high expression of ALDH-1 activity in patients with epithelial ovarian carcinoma that had lower overall survival. These results showed that high expression of ALDH-1 in ovarian cancer had chemotherapy resistance characteristics and related with poor prognosis. However, in contrast with the Deng et al. [22] and Wang et al. [23] studies, Chang et al. [24] reported high expression of ALDH-1 in ovarian tumor cells of patients was a good prognosis factor. They collected 442 cases of patients with primary ovarian cancer, and found
that patients with high expression of ALDH-1 (>20%) accounted for 19%. High expression of ALDH-1 in patients was related with ovarian endometrioid adenocarcinoma, early stage, complete response to chemotherapy, low level of CA125, as well as good prognosis survival rate. They found that patients with high expression of ALDH-1 had a longer overall survival. This indicated that ALDH-1 may be a potential independent prognostic factor in ovarian cancer patients.

Consistent with Chang et al. [24] and Penumatsa et al. [13] found that expression of ALDH-1 in malignant ovarian tumor was lower than that in normal ovarian and benign ovarian tumors, and the level of ALDH-1 in ovarian tumor was lower than normal ovaries, but there was no statistically significance. This was inconsistent with breast cancer, lung cancer, colon cancer, and so on. At the same time, they found that ALDH-1 was mainly localized in the surface epithelial cells and stroma of normal ovarian cortex and medulla, and it is not obvious in the follicular and vascular endothelial cells. The high expression of ALDH-1 in normal ovaries was consistent with the physiology function of it in normal ovarian. In addition, according with the research of Deng et al [22], they also found ALDH-1 positive cells using reagent kits were less than immunostaining, which suggested that not all of ALDH-1 positive cells had activity. They also found that staining of ALDH-1 in tumor cells with poorly differentiated (high grade) was weak and the response to chemotherapy of patients with poor differentiation was weaker than well-differentiated ones, so the prognosis was worse. Thus they assumed that low-grade of patients with high expression of ALDH-1 had faster metabolism to chemotherapy drug, therefore the effect of chemotherapy was better. Whereas the low expression of ALDH-1 in patients with ovarian cancer was weaker to chemotherapy; however the number of samples was less, so it still requires further assessment whether the ALDH-1 expression in ovarian cancer can predict the prognosis of patients and the response to chemotherapy from tissue pathology.

In summary, the present authors concluded that ALDH-1 in ovarian cancer may be different with other types of tumors such as breast, lung, liver, prostate, bladder, colon cancers, and so on. Although they cannot confirm that the ALDH-1 existed in ovarian cancer stem cells, they can still conclude that it may not be a stem cell marker of ovarian cancer through previous research.

**Conclusion**

In summary, ALDH-1 was related with poor clinical prognosis of patients with cervical cancer and endometrial cancer, whose role in cervical and endometrial cancer was similar as breast, lung, and liver cancer, head and neck squamous cell carcinoma, and other tumors. Patients of cervical cancer and endometrial cancer with ALDH-1 positive had worse clinical prognosis, higher risk of metastasis, and recurrence. ALDH-1 may be a stem cell marker of cervical and endometrial cancer. In contrast, it is a good prognosis factor in ovarian cancer; however it still requires larger sample clinical research and cell experiments to verify. We are now faced with difficulties including: whether ALDH-1 can be used as the specific stem cell marker in cervical cancer or endometrial cancer? How to screen and identify? What is the difference in the mechanism between ovarian cancer and cervical cancer or endometrial cancer? Whether ALDH-1 is related with the clinical characteristics, recurrence or metastasis of patients with cervical cancer or endometrial cancer? It still requires larger sample clinical prospective studies to confirm whether ALDH-1 correlates with cervical, endometrial, ovarian, and other gynecological cancers, and to further confirm whether ALDH-1 is related with the prognosis of the patients with gynecologic cancers, and whether the regulation of ALDH-1 will result in biological characteristic changes of gynecological cancer cells (such as resistance to radiotherapy and chemotherapy, invasion, et al). So as to find the specific tumor stem cell targets, targeted killing the tumor stem cells is performed without harming normal cells. Thus better and more effective treatment schemes can be provided for patients with gynecologic oncology.

**Acknowledgements**

Research was supported by the National Natural Science Foundation of China (#81101979), the Guangdong Province Natural Scientific Grant (#S2011040004639), and by the Guangdong Province Medical Science Technology Grant (#B2011088).

**References**


The progress of ALDH-1 in gynecologic oncology


Address reprint requests to:
D. ZHANG, M.D.
Department of Gynecology
Sixth Affiliated Hospital
Sun Yat-sen University
Guangzhou 510655 (China)
e-mail: diopen@21cn.com
Preoperative prediction of poor prognostic parameters and adjuvant treatment in women with pure endometrioid type endometrial cancer: what is the significance of tumor markers?

E. Baser, T. Gungor, C. Togrul, O. Turkoglu, S. Celen

Department of Gynecologic Oncology, Zekai Tahir Burak Women’s Health Education and Research Hospital, Altindag, Ankara (Turkey)

Summary

Purpose of the study: The study was conducted to determine whether preoperative serum levels of cancer antigen (CA) 125, CA15-3, CA19-9, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) are associated clinicopathologically with poor prognostic parameters and adjuvant treatment requirements in women with pure endometrioid endometrial cancer (EEC). Materials and Methods: The authors performed a retrospective review of EEC cases that were treated between January 2008 and January 2011. The association between preoperative tumor markers and prognostic parameters, recurrence risk, and adjuvant treatment requirements were investigated. Following univariate analyses, receiver-operating characteristic (ROC) curves were constructed for each marker to assess their capacity to predict prognostic parameters and need for adjuvant treatment. Results: A total of 166 EEC cases were identified. Mean CA125, CA15-3, and CA19-9 levels were higher in cases that required adjuvant treatment (p < 0.05). CA125 had significant power for prediction of extraterine disease, tumor size > two cm, lymphovascular space invasion (LVSI), deep myometral invasion, cervical involvement, adnexital involvement, positive cytology, lymph node metastasis, and adjuvant treatment requirement. CA15-3 was a significant marker for adjuvant treatment prediction. CA19-9 could predict deep myometral invasion, cervical involvement, and adjuvant treatment requirement. However, CEA and AFP did not have adequate capacity to predict any of the poor prognostic parameters and adjuvant treatment requirements. Conclusions: CA125 is currently one of the most important preoperative markers for identifying EEC cases that exhibit postoperatively poor prognostic pathologic findings and a consequent need for adjuvant treatment. CA15-3 and CA19-9 were also significant markers with limited capacity in detecting prognostic parameters.

Key words: Adjuvant treatment; Endometrial cancer; Endometrioid type; Tumor marker.

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in women living in developed countries, and the second most common in those living in developing countries [1, 2]. About 90% of EC consists of the endometrioid histological subtype, which is generally diagnosed at early stages (International Federation of Gynecology and Obstetrics, FIGO, Stages I–II) [3]. Surgery is the mainstay of treatment and is generally the only intervention needed in many cases of endometrioid EC (EEC). Following surgical treatment, risk of recurrence is determined by various pathological risk parameters such as disease stage, tumor size and grade, myometral invasion, cervical involvement, lymphovascular space invasion (LVSI), status of lymph nodes, and peritoneal cytology [4]. Patients who carry significant risk for recurrence receive adjuvant treatment in the form of radiotherapy (RTx) and/or chemotherapy (CTx). The value of tumor markers in the preoperative prediction of prognosis in EEC is not well established. Most studies on this subject have been conducted using limited sample sizes that included various histological types of EC [5, 6].

In this study, the authors investigated whether preoperative serum levels of carbohydrate/cancer antigen (CA) 125, CA15-3, CA19-9, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) are useful for predicting poor prognostic parameters and adjuvant treatment requirements in women with EEC.

Materials and Methods

After gaining approval from the institutional review board, the authors retrospectively reviewed their cancer registry database for EC cases that were treated at the present gynecologic oncology department between January 2008 and January 2011. Cases with endometrioid type histology only were included in the study. Clinical data including age, gravidity, parity, and menopausal status were obtained from patient files. Preoperative serum levels of CA125, CA15-3, CA19-9, AFP, and CEA were acquired from a laboratory database. The serum markers were measured using electrochemoluminescence immunoassays.

All cases underwent a complete surgical staging procedure including peritoneal washings for cytology, total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), bilateral pelvic and para-aortic lymph node dissection, and infracolic omentectomy. Modified radical hysterectomy instead of a TAH/BSO was performed in cases with gross cervical involvement. To retain uniformity in staging, pathological reports were re-analyzed and cases were staged according to the FIGO 2009 staging system for EC [7]. Prognostic data were recorded for each case, including tumor size, tumor grade, LVSI, myometrial invasion depth, cervical involvement, adnexal involvement, peritoneal cytology, and pelvic para-aortic lymph node metastasis.
Patients were categorized according to risk of recurrence after surgical staging in the following groups: low risk, low-intermediate risk (LIR), high-intermediate risk (HIR), and high risk [8]. Low risk cases were defined as grade 1 or 2 tumors that were confined to the endometrium. Intermediate risk cases were defined as Stage I or II tumors with myometrial invasion. Within the intermediate risk group, HIR cases were defined as: 1) Age < 50 years with a) FIGO Stage I-II with grade 2 or 3 tumors, b) LVSI positivity, and c) outer one-third myometrial invasion; 2) Age 50-69 years with any two risk factors above; 3) Age ≥ 70 years with any risk factor above [8]. Intermediate risk cases without these criteria were placed in the LIR group. High risk cases were defined as: Stage III or IV disease (regardless of grade) or cases with gross involvement of the cervix. Because this study included only endometrioid type cancers, high-risk histological subtypes such as serous papillary and clear cell tumors were not included in the risk group definitions. Assigned risk group and adjuvant treatments were recorded for each case.

Statistical analyses were performed using the SPSS software Version 15. Numeric variables were expressed as means ± standard deviation (SD) or median (range). Categorical variables were expressed as numbers and percentages. To determine associations between variables, the Chi-square, Fisher’s exact, Student’s t, Mann Whitney-U, one-way ANOVA and Kruskal-Wallis tests were used, where appropriate. The capacity of serum tumor marker levels in predicting poor prognostic parameters and adjuvant treatment requirements were analyzed using receiver operating characteristics (ROC) curve analysis. When a significant cut-off value was observed for a variable, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were presented. Relative risks of poor prognostic parameters were calculated for the defined cut-offs. A 5% type I error level and p values < 0.05 were considered statistically significant.

Results

Among the 201 cases of EC that were treated at the present institution within the study period, 166 patients with EEC were identified and included in the study. Mean patient age was 57.3 ± 9.1 years (range, 35–84). Mean gravidity and parity were 2.5 and 1.3, respectively. Nineteen patients (11.4%) were premenopausal and 147 (88.6%) were postmenopausal at the time of surgery. None of the patients had an accompanying malignancy other than EC. Nine patients (5.4%) with gross cervical involvement underwent modified radical hysterectomy and 157 (94.6%) underwent TAH/BSO during surgical staging. Of the study patients, 107 (64.5%) underwent surgery only, whereas 59 (35.5%) required additional adjuvant treatment.

According to the postoperative risk stratification criteria, 14 cases (8.4%) had low risk, 93 cases (56%) had LIR, 34 cases (20.5%) had HIR, and 25 had high-risk for recurrence. Close follow-up without further treatment was planned for cases in low risk and LIR groups. All of the cases in the HIR group received vaginal brachytherapy (RTx). Of the 25 cases in the high-risk group, 16 (64%) received concurrent CTx, and nine (36%) received only CTx.

Mean serum levels of CA125, CA15-3, CA19-9, CEA and AFP were 30.3 ± 61.3 U/ml, 20.3 ± 27.2 U/ml, 48 ± 103.6 U/ml, 1.03 ± 1.3 ng/ml, and 2.8 ± 2.3 U/ml, respectively. Associations of serum tumor markers with clinicopathological parameters, risk groups and adjuvant treatment are presented in Table 1. Mean serum levels of CA125, CA15-3, and CA19-9 were significantly higher in the group of cases that required adjuvant treatment (p < 0.05) (Table 1). Adjuvant treatment status was significantly associated with tumor size > two cm, grade, LVSI, deep myometrial invasion, and lymph node metastasis (p < 0.001).

ROC curve analyses were performed for CA125, CA15-3, CA19-9, CEA, and AFP for their ability to predict poor prognostic parameters and adjuvant treatment requirement (Table 2). CA125 had significant capacity to predict extraperitoneal disease, tumor size > two cm, LVSI, deep myometrial invasion, cervical involvement, adnexal involvement, positive peritoneal cytology, lymph node metastasis, and adjuvant treatment requirement. CA15-3 could predict adjuvant treatment requirement. CA19-9 could predict deep myometrial invasion, cervical involvement, and adjuvant treatment requirement. However, CEA and AFP did not have adequate capacity to predict any of the poor prognostic parameters and adjuvant treatment requirements (Table 2). Relative risks above the defined cut-offs for prognostic clinicopathological parameters are presented in Table 3.

Discussion

Endometrial cancer is generally diagnosed and treated at early stages of disease. Although surgery is the cornerstone of treatment, a significant percentage of women with EC require adjuvant treatment after primary surgical intervention. Many studies have investigated optimal therapeutic modalities (CTx, RTx) for postoperative treatment of EC cases that are prone to risk of recurrence [9-13]. Preoperative evaluation and patient counseling regarding the anticipated disease stage and postsurgical treatments contribute not only to a stronger adherence to therapy but also to a more accurate determination of prognosis. To date, many different methods including imaging studies and serum markers have been evaluated for their ability to predict disease burden in EC preoperatively. However, an optimal marker that accurately reflects the prognosis of EC and the need for adjuvant therapy has yet to be defined. In this study, the authors investigated the value of preoperative CA125, CA15-3, CA19-9, CEA, and AFP levels in predicting poor prognostic parameters and adjuvant treatment requirement in EEC cases.

Preoperative prediction of disease stage and prognosis by measuring serum levels of various markers has long been an area of interest. Among these, CA125 is the most extensively studied marker, especially following immunohistochemical studies that demonstrated significant tumoral expression of this glycoprotein [14-17]. Hsieh et al. investigated the role of preoperative CA125 in determining the extent of endometrial cancer preoperatively [15]. In this
Table 1. — Associations of serum tumor markers with clinicopathological parameters, risk groups, and adjuvant treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n (% of all patients)</th>
<th>Mean CA125 (U/ml)</th>
<th>Mean CA15-3 (U/ml)</th>
<th>Mean CA19-9 (U/ml)</th>
<th>Mean CEA (ng/ml)</th>
<th>Mean AFP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60</td>
<td>106 (63.9)</td>
<td>35.9</td>
<td>20.7</td>
<td>46.6</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>60 (36.1)</td>
<td>20.4</td>
<td>19.8</td>
<td>50.6</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>FIGO Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>129 (77.7)</td>
<td>23.0</td>
<td>17.9</td>
<td>35.4</td>
<td>1.1</td>
<td>2.7</td>
</tr>
<tr>
<td>II</td>
<td>20 (12)</td>
<td>28.9</td>
<td>28.4</td>
<td>64.1</td>
<td>0.9</td>
<td>3.5</td>
</tr>
<tr>
<td>III</td>
<td>17 (10.2)</td>
<td>87.3</td>
<td>29.1</td>
<td>124.7</td>
<td>0.9</td>
<td>2.9</td>
</tr>
<tr>
<td>IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>0.006</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2 cm</td>
<td>62 (37.3)</td>
<td>31.7</td>
<td>24.6</td>
<td>41.6</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>&gt; 2 cm</td>
<td>104 (62.7)</td>
<td>29.4</td>
<td>17.8</td>
<td>51.8</td>
<td>1.0</td>
<td>2.8</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>0.006</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Tumor grade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99 (59.6)</td>
<td>24.8</td>
<td>16.5</td>
<td>43.4</td>
<td>0.8</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>56 (33.7)</td>
<td>42.3</td>
<td>27.7</td>
<td>61.9</td>
<td>1.3</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>11 (6.6)</td>
<td>18.3</td>
<td>17.3</td>
<td>18.4</td>
<td>1.2</td>
<td>3.0</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>LVSI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>136 (81.9)</td>
<td>27.1</td>
<td>19.7</td>
<td>43.4</td>
<td>1.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Positive</td>
<td>30 (18.1)</td>
<td>44.4</td>
<td>23.2</td>
<td>69.1</td>
<td>1.1</td>
<td>3.0</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>0.002</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Myometrial invasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>14 (8.4)</td>
<td>9.9</td>
<td>13.1</td>
<td>17.8</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>≤1/2</td>
<td>100 (60.2)</td>
<td>29.3</td>
<td>22.5</td>
<td>45.2</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>&gt;1/2</td>
<td>52 (31.3)</td>
<td>37.6</td>
<td>18.1</td>
<td>61.7</td>
<td>1.3</td>
<td>3.2</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>0.03</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Cervical involvement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>135 (81.3)</td>
<td>26.2</td>
<td>19.2</td>
<td>37.0</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Positive</td>
<td>31 (18.7)</td>
<td>48.0</td>
<td>25.5</td>
<td>96.0</td>
<td>0.8</td>
<td>3.6</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>0.005</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Adnexal involvement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>162 (97.6)</td>
<td>29.0</td>
<td>19.4</td>
<td>46.7</td>
<td>0.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Positive</td>
<td>4 (2.4)</td>
<td>83.2</td>
<td>59.0</td>
<td>107.1</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>0.003</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Cytology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>158 (95.2)</td>
<td>28.8</td>
<td>19.4</td>
<td>47.6</td>
<td>0.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (4.8)</td>
<td>60.0</td>
<td>39.0</td>
<td>566.2</td>
<td>2.1</td>
<td>3.2</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Pelvic or Paraaortic node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>148 (89.2)</td>
<td>23.8</td>
<td>19.4</td>
<td>39.5</td>
<td>1.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Positive</td>
<td>18 (10.8)</td>
<td>83.2</td>
<td>28.0</td>
<td>117.8</td>
<td>0.9</td>
<td>3.0</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Risk group for recurrence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>14 (8.4)</td>
<td>9.9</td>
<td>13.1</td>
<td>17.8</td>
<td>0.7</td>
<td>1.9</td>
</tr>
<tr>
<td>LIR</td>
<td>93 (56)</td>
<td>24.8</td>
<td>19.7</td>
<td>39.3</td>
<td>0.9</td>
<td>2.8</td>
</tr>
<tr>
<td>HIR</td>
<td>34 (20.5)</td>
<td>20.8</td>
<td>18.5</td>
<td>44.2</td>
<td>1.3</td>
<td>3.5</td>
</tr>
<tr>
<td>High</td>
<td>25 (15.1)</td>
<td>74.9</td>
<td>29.3</td>
<td>102.7</td>
<td>1.2</td>
<td>2.5</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td><strong>Adjuvant therapy requirement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>107 (64.5)</td>
<td>22.9</td>
<td>18.9</td>
<td>36.5</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Yes</td>
<td>59 (35.5)</td>
<td>43.8</td>
<td>23.0</td>
<td>69.0</td>
<td>1.2</td>
<td>3.1</td>
</tr>
<tr>
<td><em>p</em></td>
<td></td>
<td>&lt; 0.001</td>
<td>0.02</td>
<td>0.01</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2. — Diagnostic values of preoperative serum tumor marker levels for predicting poor prognostic factors and adjuvant treatment in endometrioid endometrial cancer.

<table>
<thead>
<tr>
<th></th>
<th>Cut-off value</th>
<th>AUC(^a)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV(^b) (%)</th>
<th>NPV(^c) (%)</th>
<th>Accuracy (%)</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CA 125</strong> U/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exaularterine disease (Stage III-IV)</td>
<td>18</td>
<td>0.81</td>
<td>82.4</td>
<td>64.4</td>
<td>20.9</td>
<td>97.0</td>
<td>66.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td>15</td>
<td>0.62</td>
<td>58.6</td>
<td>64.5</td>
<td>73.5</td>
<td>48.1</td>
<td>60.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td></td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td>18</td>
<td>0.68</td>
<td>63.3</td>
<td>64.7</td>
<td>28.3</td>
<td>88.9</td>
<td>64.4</td>
<td>0.002</td>
</tr>
<tr>
<td>&gt; 1/2 myometrial invasion</td>
<td>15</td>
<td>0.68</td>
<td>71.1</td>
<td>59.6</td>
<td>44.5</td>
<td>81.9</td>
<td>63.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td>18</td>
<td>0.72</td>
<td>67.7</td>
<td>65.9</td>
<td>31.3</td>
<td>89.9</td>
<td>66.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td>40</td>
<td>0.93</td>
<td>75</td>
<td>88.9</td>
<td>14.2</td>
<td>99.3</td>
<td>88.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Positive peritoneal cytology</td>
<td>28</td>
<td>0.86</td>
<td>75</td>
<td>79.1</td>
<td>15.3</td>
<td>98.4</td>
<td>78.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>18</td>
<td>0.79</td>
<td>77.8</td>
<td>64.2</td>
<td>21</td>
<td>95.9</td>
<td>65.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adjuvant treatment requirement</td>
<td>15</td>
<td>0.71</td>
<td>69.5</td>
<td>60.7</td>
<td>49.4</td>
<td>78.3</td>
<td>63.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>CA 15-3</strong> U/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exaularterine disease (Stage III-IV)</td>
<td></td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td></td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td></td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td></td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>&gt; 1/2 myometrial invasion</td>
<td></td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td></td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td></td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Positive peritoneal cytology</td>
<td></td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>Adjuvant treatment requirement</td>
<td>15</td>
<td>0.61</td>
<td>61</td>
<td>55.1</td>
<td>42.8</td>
<td>71.9</td>
<td>57.2</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>CA 19-9</strong> U/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exaularterine disease (Stage III-IV)</td>
<td></td>
<td>0.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td></td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.60</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td></td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.48</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td></td>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td>&gt; 1/2 myometrial invasion</td>
<td>17</td>
<td>0.62</td>
<td>61.5</td>
<td>52.6</td>
<td>37.2</td>
<td>75</td>
<td>55.4</td>
<td>0.009</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td>17</td>
<td>0.66</td>
<td>74.1</td>
<td>53.3</td>
<td>26.7</td>
<td>90</td>
<td>57.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td></td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>Positive peritoneal cytology</td>
<td></td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>Adjuvant treatment requirement</td>
<td>17</td>
<td>0.61</td>
<td>61</td>
<td>53.2</td>
<td>41.8</td>
<td>71.2</td>
<td>56</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>CEA</strong> ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exaularterine disease (Stage III-IV)</td>
<td></td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td></td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td></td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.91</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td></td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>&gt; 1/2 myometrial invasion</td>
<td></td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.52</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td></td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td></td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>Positive peritoneal cytology</td>
<td></td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Adjuvant treatment requirement</td>
<td></td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.37</td>
</tr>
<tr>
<td><strong>AFP</strong> ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exaularterine disease (Stage III-IV)</td>
<td></td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td></td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td></td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td></td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>&gt; 1/2 myometrial invasion</td>
<td></td>
<td>0.56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td>Cervical involvement</td>
<td></td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>Adnexal involvement</td>
<td></td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>Positive peritoneal cytology</td>
<td></td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.57</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>Adjuvant treatment requirement</td>
<td></td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.56</td>
</tr>
</tbody>
</table>

\(^a\) area under the curve; \(^b\) positive predictive value; \(^c\) negative predictive value.
study, elevated CA125 levels were significantly correlated with advanced stage disease, larger tumor size, increasing depth of the myometrial invasion, cervical involvement, positive peritoneal cytology, and lymph node metastases. An optimal cut-off level of 40 U/ml was determined to predict lymph node metastases with a sensitivity and specificity of 77.8 and 81.0%, respectively [15]. In their prospective study, Dotters et al. investigated the clinical utility of preoperative CA125 measurement in determining the need for lymph node dissection in EC cases [16]. The authors reported that preoperative CA125 levels higher than 20 U/ml correctly identified patients at risk for lymph node metastasis with 75% sensitivity [16]. Subsequent reports concluded that CA125 had significant value in preoperative determination of extrauterine disease spread, lymph node metastasis, and prognosis in EC [18-20]. These studies included various histological subtypes of EC, in which expression of CA125 may have significant variation. A recent study analyzed the association of CA125 with important prognostic parameters in 413 cases of endometrioid type EC [21]. The authors found that the optimal cut-off levels for determining HIR and high-risk disease were 17.3 U/ml and 21.9 U/ml, respectively. However, CA125 had low predictive value in determining whether adjuvant treatment was required in endometrioid EC cases [21]. Nonetheless, a strong association was demonstrated between preoperative CA125 level > 80 U/ml and adnexal involvement, which led the authors to conclude that CA125 levels > 80 U/ml may be useful for preoperative counseling of premenopausal women who may require adnexectomy [21]. In the present study, the authors found that CA125 was a significant predictor for many poor prognostic parameters such as tumor size, LVSI, deep myometrial invasion, cervical and adnexal involvement, positive cytology, and lymph node metastasis (Table 2). In contrast with the study by Kim et al. [21], the present authors noted that CA125 levels > 15 U/ml significantly predicted adjuvant treatment requirement with nearly 70% sensitivity and 60% specificity. The optimal CA125 threshold for lymph node metastasis prediction in this study was 18 U/ml, which was very close to the cut-off defined by Dotters et al. [16], and was lower than the 40 U/ml reported by Hsieh et al. [15]. The differences between the reported serum CA125 cut-off levels in these studies may reflect the individual expression pattern of CA125 in various histological types.

Despite being a marker primarily of breast cancer, the significance of CA15-3 has been previously investigated in EC [5]. Lo et al. compared serum CA125, CA15-3, CA19-9, CEA, and tissue polypeptide antigen in 97 cases with EC, 47 cases with benign gynecological diseases, and 100 controls [5]. The authors reported that elevations in CA125 and CA15-3 levels were significantly associated with poor prognostic factors. They also found that increased CA125, CA15-3, and CA19-9 were associated with shorter survival. In the present study, serum CA15-3 levels were significantly higher in cases that required adjuvant treatment (Table 1). A cut-off level of 15 U/ml significantly predicted adjuvant treatment with 61% sensitivity and 55% specificity.

CA19-9 is a tumor marker that is used primarily in monitoring patient response to therapy or in detecting recurrence of several cancer types including gastric, pancreatic, gallbladder, cholangiocarcinoma, and adenocarcinoma of the ampulla of Vater. Occasionally, it may exhibit elevated levels in women with ovarian cancer [22]. A previous study reported significantly elevated levels of CA19-9 in cases with EC [23]. Another study showed that increased CA19-9 levels in EC cases were associated with decreased survival [5]. In the present study, mean CA19-9 levels were significantly higher in EEC cases with extrauterine disease (i.e., Stage III), deep myometrial invasion, cervical involvement, and adjuvant treatment requirement (Table 1). The authors also found that a CA 19-9 cut-off level of 17 U/ml significantly predicted deep myometrial invasion, cervical involvement, and adjuvant treatment (Table 2).

CEA is a glycoprotein normally found in embryonic or fetal tissues. After birth, the serum level of this marker decreases significantly. Although described initially as a tumor-specific marker of colon cancer, subsequent studies reported elevated CEA levels in many malignant and benign conditions [24]. CEA levels may be increased, especially in mucinous tumors of the gastrointestinal tract or ovary. Previous studies demonstrated that EC tissues express limited amounts of CEA, and the role of this marker as a prognostic parameter is unclear [25]. This study demonstrated that mean CEA levels were not significantly different among poor prognostic parameter groups, nor did they predict the need for adjuvant treatment in EEC cases.

Similar to CEA, AFP is produced mainly in the developing fetus. Its role as a tumor marker is predominantly recognized in hepatocellular carcinoma and germ cell tumors.

### Table 3. — Relative risks of clinicopathological variables above the defined cut-off values for significant tumor markers.

<table>
<thead>
<tr>
<th>Cut-Off</th>
<th>Clinicopathological parameters</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 125</td>
<td>Extrauterine disease (Stage III-IV)</td>
<td>2,315</td>
</tr>
<tr>
<td></td>
<td>LVSI</td>
<td>2,552</td>
</tr>
<tr>
<td></td>
<td>Cervical involvement</td>
<td>3,103</td>
</tr>
<tr>
<td></td>
<td>Pelvic or paraaortic node metastasis</td>
<td>5,172</td>
</tr>
<tr>
<td></td>
<td>Tumor size</td>
<td>1,419</td>
</tr>
<tr>
<td></td>
<td>Myometrial invasion</td>
<td>2,099</td>
</tr>
<tr>
<td></td>
<td>Adjuvant therapy requirement</td>
<td>2,278</td>
</tr>
<tr>
<td></td>
<td>Cytology</td>
<td>9,769</td>
</tr>
<tr>
<td></td>
<td>Adnexal involvement</td>
<td>20,714</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>Myometrial invasion</td>
<td>1,437</td>
</tr>
<tr>
<td></td>
<td>Cervical involvement</td>
<td>2,674</td>
</tr>
<tr>
<td></td>
<td>Adjuvant therapy requirement</td>
<td>1,456</td>
</tr>
<tr>
<td>CA 15-3</td>
<td>Adjuvant therapy requirement</td>
<td>1,528</td>
</tr>
</tbody>
</table>
of the female and male genital tract [26-28]. In a previous report, mean AFP was lower in EC cases when compared with healthy controls [23]. However, the present authors did not observe a significant difference in terms of a decrease or elevation among cases that carry various risk factors and adjuvant requirements.

In summary, according to the present findings and evidence from the literature, the authors conclude that CA125 is currently one of the most effective markers in identifying EEC cases that may subsequently exhibit poor prognosis and require adjuvant treatment. This study also demonstrated that CA15-3 and CA19-9 were significant markers with limited capacity in predicting adjuvant treatment. Although CEA and AFP were not associated with the parameters studied in EEC, increased levels of these markers may indicate coexisting malignancies such as breast, colon, or hepatic cancers. Further investigation of novel markers and their predictive value in prognostic determination is warranted in cases with EEC.

References


Expression of hexokinase 2 in epithelial ovarian tumors and its clinical significance in serous ovarian cancer

Z. Jin¹, J. Gu², X. Xin¹, Y. Li¹, H. Wang¹

¹Department of Obstetrics and Gynecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan
²Department of Obstetrics and Gynecology, Beijing Aerospace General Hospital, Beijing (China)

Summary

“Warburg effect” emphasizes that malignant cells exhibit active glycolysis even under aerobic conditions. Hexokinase 2 (HK2) is a key glycolytic enzyme that helps to exhibit a “Warburg effect”. In the present study, the main aim was to detect the expression of HK2 in epithelial ovarian tumor tissues. Immunohistochemistry and qRT-PCR were used to examine the expression of HK2 in different epithelial ovarian tissues. The expression of HK2 in ovarian cancer tissues was significantly higher than that in normal ovarian, benign, and borderline tumors both in protein ($p < 0.001$) and mRNA ($p < 0.05$) levels. HK2 expression was significantly higher in Stage III/IV compared to Stage I/II ($p < 0.001$). Expression of HK2 in poorly-differentiated carcinoma was higher than that in well-differentiated carcinoma ($p = 0.008$). The level of HK2 was higher in serous groups than in non-serous groups in both protein ($p = 0.008$) and mRNA ($p < 0.05$) level. Collectively, HK2 is highly expressed in epithelial ovarian cancer, especially in serous groups. Its expression is related with clinical stage and histological differentiation.

Key words: Ovarian cancer; Hexokinase 2; Serous ovarian cancer; Immunohistochemistry; Glycolysis.

Introduction

Ovarian cancer is one of the most common three gynecological malignancies and is the leading cause of gynecological cancer death [1]. The malignant epithelial tumor accounts for more than 90% of all ovarian cancers. There have been major advances in cellular and molecular biology referred to ovarian cancer. However, there has been little change in the survival of women with epithelial ovarian cancer since platinum-based anticancer drugs were introduced over three decades ago [2]. Therefore, new therapeutic targets are urgently needed for novel approaches to deal with ovarian cancer.

Warburg first emphasized that tumor cells exhibit active glycolysis even under conditions where oxygen is sufficient [3]. To date, encouraging evidence indicated that high glycolytic rate plays an important role in rapidly proliferating cancers, not only to provide energy but also to supply with precursors for nucleotide and lipid synthesis [4]. Hexokinase as the first rate-controlling enzymes in the glycolysis pathway catalyzes the process of glucose converting to glucose-6-phosphate (G-6-G). There are four hexokinase isozymes (I, II, III, IV) to our knowledge, and with HK2 in particular, the isoform’s activity is markedly higher in many tumors cells as compared to normal differentiated cells and it can bind to mitochondria to inhibit apoptosis [5]. However HK2 expression in gynecologic malignancies is rarely reported in the literature.

Here, for the fist time, to the authors’ knowledge, they detected HK2 expression in normal ovarian tissue and ovarian epithelial tumors (benign, borderline, and malignant) by immunohistochemistry and qRT-PCR and analyzed the correlation of HK2 expression with clinicopathologic parameters to explore the glycolysis condition in ovarian epithelial tissues and evaluate whether HK2 is involved in the development of ovarian cancer.

Materials and Methods

Clinical samples for immunohistochemistry

Tissue samples were selected from the Department of Pathology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan. The selected cases received surgical treatment with epithelial ovarian cancer which was confirmed by pathological examination, from November 2004 to March 2007. The samples included five normal ovarian specimens, five benign ovarian tumors (two serous cystadenomas and three mucinous cystadenomas), five borderline ovarian tumors (three borderline serous cystadenomas and two borderline mucinous cystadenomas), and 31 malignant epithelial ovarian tumors (16 serous cystadenocarcinomas, five mucinous cystadenocarcinomas, four clear cell carcinomas, three endometrioid carcinomas, and three undifferentiated adenocarcinomas). The patients were aged from 17 to 71 years (median 47.6) and underwent no prior radiotherapy or chemotherapy.

Tissues collection for quantitative reverse transcription polymerase chain reaction

Tissue samples from patients who were suspected with ovarian neoplasms were collected at initial surgery, from August 2008 to October 2009. Samples were stored initially in liquid nitrogen upon collection and then transferred to -80°C refrigerator for long-term storage. Clinical and pathological data,
such as histology, FIGO stage (2000), grade, and differentiation state were collected afterwards and recorded for future reference.

The present study was approved by the Ethics Committee of Tongji Medical College.

**Immunohistochemistry**

Immunohistochemical staining for HK2 was performed using the SABC kit according to the manufacturer’s instructions. Rabbit polyclonal antibody to HK2 (1:200, AB3279) was incubated overnight at 4°C. Positive cells were defined as having brown-yellow granules distributed in cytoplasm, with stain intensity higher than the unspecific background. HK2 expression was evaluated by a digital image system. Five images of representative fields were captured at a magnification of ×400 and saved as TIFF files. Images were analyzed with Image-Pro Plus 6.0 software. The area and the integrated optical density (IOD) of positive staining of HK2 were measured, and the mean density was calculated as IOD/area in each image. The average of mean densities of five images from each slide was used to represent an individual sample [6].

**Quantitative reverse transcription polymerase chain reaction (qRT-PCR)**

Approximately 100 mg of tissue samples were harvested in one ml Trizol reagent to extract total RNA. Complementary DNA was synthesized using MMLV transcriptase according to the manufacturer’s recommendations. Complementary DNA was amplified by polymerase chain reaction (PCR). All reactions were performed on an ABI 7700 Real-time PCR system. HKII primers amplify a fragment of 154bp, sense strand: 5’-TG-GAGCCACCACTCACCCTAC-3’, antisense strand: 5’-GAGCCATTTGTCCGTTACTTTC-3’. GAPDH was applied as the internal housekeeping gene control. The GAPDH primers amplify a fragment of 220 bp, sense strand: 5’-GTCCATTGTCCGTTACTTTC-3’, antisense strand: 5’-CAGCATCG-CCCACCTTGATTTTG-3’. The cycling conditions were as follows: initial denaturation at 94°C for three minutes, followed by 45 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, with a final incubation at 72°C for five minutes. Relative gene expression was calculated using the $2^{-\Delta\Delta C_T}$ method. Each measurement was performed in triplicate.
**Expression of hexokinase 2 in epithelial ovarian tumors and its clinical significance in serous ovarian cancer**

**Statistical analysis**

Results were expressed as mean ± standard deviation. Non-parametric statistical tests (Mann-Whitney Test, Kruskal-Wallis Test) were carried out for analyzing relationships between variables. All statistical analyses were performed with SPSS statistical software 13.0, and p values less than 0.05 were considered statistically significant.

**Results**

**HK2 expression in different epithelial ovarian tissues**

The levels of HK2 staining detected by immunohistochemistry were observed in normal, benign, borderline, and malignant epithelial ovarian tissues. All of the malignant tissues were HK2 positive while the others were negative. So the conclusion can be made that HK2 is specifically expressed in malignant ovarian tissues. HK2 was mainly located in the cytoplasm (Figure 1).

**Table 1. — The relationship between HK-2 expression and clinical pathologic characteristics.**

<table>
<thead>
<tr>
<th>Clinicopathologic Parameters</th>
<th>No. of Patients</th>
<th>IOD/Area (Mean ± SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FIGO stage (2000)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>17</td>
<td>0.1723±0.0346</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>III/IV</td>
<td>14</td>
<td>0.2694±0.0537</td>
<td></td>
</tr>
<tr>
<td><strong>Histological grade</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1+G2</td>
<td>11</td>
<td>0.1758±0.0462</td>
<td>0.008*</td>
</tr>
<tr>
<td>G3</td>
<td>20</td>
<td>0.2383±0.0649</td>
<td></td>
</tr>
<tr>
<td><strong>Histotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>16</td>
<td>0.2485±0.0706</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>5</td>
<td>0.1679±0.0320</td>
<td>0.065†</td>
</tr>
<tr>
<td>Clear cell</td>
<td>4</td>
<td>0.1670±0.0225</td>
<td>0.008§</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>3</td>
<td>0.2089±0.0679</td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>3</td>
<td>0.1970±0.0083</td>
<td></td>
</tr>
</tbody>
</table>

* Mann-Whitney Test; † Kruskal-Wallis Test; § Serous vs. other histotypes together using Mann-Whitney Test.

Figure 2. — IOD/area of HK2 protein expression in malignant epithelial ovarian tissues was presented together with clinicopathological features. A) HK2 expression levels in Stage I/II and III/IV ovarian epithelial carcinomas. There is a significant difference between the two groups (p < 0.001). B) HK2 expression levels in grade 1/2 and grade 3 ovarian epithelial carcinomas. The expression levels considerably differed between grade 1/2 and grade 3 (p = 0.008). C) HK2 expression levels in histological subtypes of ovarian epithelial carcinomas. No significant differences were observed among tumors (p = 0.065). D) HK2 expression levels in serous epithelial carcinomas was compared with that in other epithelial histologies. Significant difference was observed between the two groups (p = 0.008).
HK2 expression in malignant epithelial ovarian tissues

According to the World Health Organization (WHO) grading system, the ovarian cancer groups were divided into three subgroups: G1 group, well-differentiated (n=2); G2 group, moderately differentiated (n=9); G3 group, poorly differentiated (n=20). Surgical pathology staging was carried out for all the cancer patients according to FIGO 2000 criteria. There were nine cases in Stage I, eight cases in Stage II, 13 cases in Stage III, and one case in Stage IV. The correlation between HK2 expression by immunohistochemistry and clinicopathologic parameters in epithelial ovarian cancer patients is analyzed (Table 1). HK2 overexpression was significantly related with advanced FIGO stage and poorly differentiated state. In more detail, the HK2 level was higher in serous epithelial carcinomas compared with that in other histotype carcinomas together (Figure 2).

HK2 mRNA expression in different ovarian tissues

The authors detected HK2 mRNA expression in normal (n=4), benign (n=6), borderline (n=3), and malignant (n=15) ovarian tissues by using quantitative Real-time PCR. HK2 mRNA expression was significantly higher in malignant ovarian tissues than in normal, benign, and borderline ovarian tissues. There was no significant difference among the normal, benign, and borderline ovarian tissues in the HK2 mRNA level. HK2 mRNA level was considerably higher in Stage III/IV than that in Stage I/II of the ovarian epithelial carcinomas. They then compared the HK2 mRNA expression in serous epithelial carcinomas with that in other epithelial histotypes. There was considerably difference between the two groups (p < 0.05). It was concordant with the immunohistochemical expression profiles (Figure 3).

Discussion

This study reports an HK2 expression in both protein and mRNA levels in ovarian tissues. We found that the expression of HK2 was cytoplasmic. Previous studies indicated that HK2 is bound to mitochondria, consistent with our findings. The authors have analyzed for the first time that HK2 expression among different histological types of ovarian epithelial tissues. An interesting finding was that malignant epithelial ovarian tissues were specifically HK2 over expression compared with normal, benign and borderline epithelial ovarian tissues. Further more, malignant serous epithelial ovarian tissues were characterized by an abnormally high HK2 expression both in protein and mRNA level in all the malignant epithelial ovarian tissues. We concluded that HK2 over expression may participate in the development of ovarian cancer, especially in serous epithelial ovarian cancer. Further in vitro studies should be carried out to confirm it.

Warburg pointed out that even under aerobic conditions cancer cells can also provide energy largely dependent on the glycolytic pathway [3]. Active glycolysis is a specific metabolic characteristic in malignant cells. In clinic, based on the difference in glucose metabolism between malignant
and normal cells, [18F]-fluorodeoxyglucose positron emission tomography (FDG-PET) was applied to diagnose and monitor malignant tumors. FDG-PET has a large advantage in the detection of early stage, recurrence, and metastasis in malignant ovarian epithelial tumors [7]. It conversely confirmed a high glycolytic rate in ovarian cancer.

Hexokinase catalyzes the first step of the glycolytic pathway. Previous studies have shown that the overexpression of HK2 in malignant tumors, including liver [8], brain [9], stomach [10], and pancreas [11] cancers, plays a pivotal role in glucose metabolism and was correlated with malignancy and prognosis. In this study, the authors found that HK2 expression was significantly higher in malignant ovarian tissues than in normal ovary, benign, and borderline ovarian neoplasm tissues. It provides the evidence that malignant ovarian tumors is in an active glycolysis state and the molecular elucidation for the FDG-PET application in ovarian cancer patients. However, studies in malignant melanoma [12] and breast cancer [13] reported that HK2 played no role in FDG uptake. They suggested that tumor heterogeneity may contribute to the different relationships between HK2 expression and FDG uptake and that other rate-limiting step of cellular FDG uptake like GLUT-1 may dedicate to it [12]. In ovarian tumors no research has been done in the relationship between the HK2 activity and FDG uptake.

Bustamante and Pedersen [14] first found that HK2 was one of the fundamental protein components which enhanced the malignant liver cells glycolysis. Later studies confirmed that HK2 is strategically harbored on the outer mitochondrial membrane protein voltage-dependent anion channel (VDAC) [5]. In this way, HK2 gains preferential access to mitochondrial generated ATP and escapes from inhibition by its product G-6-P. Overproduced HK2 in liver cancers brings about glycolysis and biosynthetic metabolic pathways providing optimal support for uncontrolled tumor proliferation. In addition, HK2-VDAC appeared to help cancer cells survival from apoptosis. One elucidation is that HK2 binding to VDAC suppresses the release of mitochondrial intermembrane space proteins and inhibits apoptosis, but the precise mechanism remains unknown. On the other hand, the lactic acid produced by the tumors contributes tumor acidic microenvironment, which helps tumor progression either by suppressing attacks by the immune system, facilitating tumor local invasion and metastasis, or both [15].

In current study, the association between HK2 expression, cancer stage, differentiation grade, and histological type might therefore suggest that abnormal glycolysis was an outstanding feature in ovarian epithelial cancers, especially in serous cancers. HK2 expression differed significantly in advanced and poorly differentiated when compared with early and well/moderate differentiated ovarian epithelial cancers. These results may indicate that the advanced and poorly differentiated cancer cells lead higher glycolysis rate.

The important role of HK2 in both glycolysis and apoptosis makes HK2 an attractive therapeutic target. Inhibitors of HK2, such as 2-deoxy-D-glucose (2-DG) and 3-bromopyruvate (3-BrPA), have already appeared in preclinical trials. Two previous studies have already indicated that the HK2 expression condition was not well known in ovarian caners. 2-DG, a glucose analog that is phosphorylated by hexokinase and its phosphorylated molecule (2-DG-6P) cannot be metabolized, appeared as a new therapeutic agent against ovarian cancer cell lines in vitro [16]. To date, no in vivo clinical trials for 2-DG were found in ovarian cancers. In another recent study, cisplatin induced ovarian tumor-initiating cells (TICs) which were resistance to chemotherapy were sensitive to combination treatment with cisplatin and 3-BrPA, which dissociates HK2 from mitochondria binding site [17]. The present study provides the HK2 expression evidence in ovarian epithelial cancers to further elucidate the molecular basis. However, more studies are needed to explore HK2 targeted therapies in ovarian cancers. Studies in other tumors indicated that agents that could detach HK2 from mitochondria would have a very small impact on normal cells but would sensitize malignant cells to a second hit from conventional chemotherapeutic agents, thus placing malignant cells on the verge of death [18-20].

Conclusion

This study confirmed that HK2 is specifically overexpressed in epithelial ovarian caners, especially in progressive serous ovarian cancers. Therefore targeting mitochondrial-HK2 to induce tumor cells apoptosis may represent a promising strategy to overcome chemo-insensitivity and chemo-resistance in ovarian epithelial cancers. HK2 targeted drug may enhance therapeutic efficacy or combat drug resistance when combined with current conventional chemotherapy drugs in serous epithelial ovarian cancer. Further studies would focus on the possibility that HK2 expression as a specific molecular marker for the HK2 inhibitor combined chemotherapy regimen in serious epithelial ovarian cancer.

Acknowledgement

The authors thank Dr. Bangxing Huang from Pathology Department for her immunohistochemistry assistance.

References


Address reprint requests to:
H. WANG, M.D.
Department of Obstetrics and Gynecology,
Union Hospital, Tongji Medical College,
Huazhong University of Science and Technology,
Wuhan 430022 (China)
e-mail: drwanghongbo@gmail.com
Ductal carcinoma in situ: analysis of 250 cases

J. Böhm¹, M. Zikán²

¹Department of Obstetrics and Gynaecology, Henneberg Kliniken, Hildburghausen (Germany)
²Oncogynaecologic Center, Department of Obstetrics and Gynaecology, Charles University in Prague,
First Faculty of Medicine and General University Hospital, Prague (Czech Republic)

Summary
Background: In the mammography screening era, we experience increasing incidence of non-invasive lesions of the breast, particularly the ductal carcinoma in situ (DCIS). It is the authors’ goal to better understand this disorder in order to be able to tailor therapy individually for each patient and, most of all, to avoid overtreatment. Materials and Methods: The authors analyzed all cases of DCIS diagnosed within one mammography screening unit between 2007 and 2013. Medical reports as well as a detailed case conference protocol were used to gain all required data. Results: In a seven-year period, DCIS has been diagnosed 250 times in 249 women. Mostly the authors were able to obtain relevant information regarding tumor size, grade, biological characteristics, as well as surgery. This data was compared with current literature. Discussion: Participating women who screen positive constitute a large patient sample. Thus, we have a large amount of clinical and histological information available for planning and conducting studies regarding DCIS as well as invasive breast cancer.

Key words: Mammography; Screening; DCIS.

Introduction
According to the decision of Bundestag from year 2002, a national mammography screening program has been established in Germany [1]. The purpose of this program is the early breast cancer detection and thus the risk reduction of dying from this most frequent female malignancy. By the participating women, not only invasive breast cancer (IBC) but also premalignant (in situ) lesions are increasingly diagnosed. As a result, ductal carcinoma in situ (DCIS) represents the second most common tissue alteration being diagnosed within the screening program. In Germany as well as in other countries with established mammography screening, the incidence of DCIS among women who screen positive is as high as 20 per cent [1].

Our current understanding of DCIS is limited. Most of all we search for objective criteria to differentiate between an aggressive lesion and the one without risk of recurrence. The lack of knowledge about this disorder is reflected in the current guidelines of the German Working Committee of Gynaecologic Oncology (Arbeitsgemeinschaft Gynäkologische Onkologie - AGO) [2]. Regardless of the size or the grade of the lesion, negative surgical margins should be obtained for DCIS patients, followed by radiotherapy by women treated with breast conserving surgery (BCS). This “one-size-fits-all” principle is not optimal, yet we still lack prognostic factors for determining a certain group of patient who do not need any adjuvant radiotherapy or even better, any treatment at all except intensive observation. Additional prognostic or predictive factors should be explored in order to individualize the management of DCIS and, most of all, to avoid overtreatment.

Materials and Methods
The aim of the present analysis was to evaluate clinical and histological features of DCIS. The authors decided to make use of mammography screening database, since this provides a large cohort of patient diagnosed with DCIS. All procedures within organized mammography screening are clearly defined. [3] In case of suspect X-ray and ultrasound imaging followed by a positive biopsy, patients are referred to a cooperating hospital to undergo a surgery. After completing surgical therapy, every case is a subject of discussion within a case conference. Together with a medical report, a detailed protocol from this case conference was used in the present retrospective study to obtain all required data.

In the present study, all patients with diagnosed pure DCIS between May 2007 and January 2013 were included. IBC and DCIS in one tissue sample were excluded. DCIS with microinvasion were excluded from this analysis as well, because of their biological potential and management similar to IBC. Pure DCIS was included regardless its X-ray features, clinical appearance, size, focality, and laterality or grade.

Results
Between May 2007 and January 2013, all together 297 thousand women underwent an X-ray examination of their breast in this particular mammography screening unit. Within this cohort, IBC has been diagnosed 1,465-times. DCIS with microinvasion has been diagnosed in 19 cases. Pure DCIS without any invasive component has been diagnosed 250 times in 249 patients (one bilateral lesion). This constitutes 14.41% of all breast malignancies diagnosed within this screening unit.

Out of the medical record and the case conference protocol, the authors were able to obtain and evaluate following information:
Age at diagnosis

Mammography screening program in Germany is generally designed for women between 50 – 69 years of age. In the present study, the mean age of patients diagnosed with DCIS was 61 years (average 60.52). Although it was possible to discover the age at diagnosis for each patient, there was generally no information in the medical record about the menopausal status whatsoever. Considering the age, though, the authors assumed that most patients were postmenopausal.

Surgery for DCIS

All 249 patients with DCIS in biopsy underwent a breast surgery. Of these, 84.8% (n=212) were treated with breast conserving surgery (BCS), 5.6% (n=14) received a primary skin (+/- nipple) sparing mastectomy, and in 9.6% (n=24) patients a mastectomy was required.

In 4.8% of all specimen (n=12) the pathologist could not detect any further malignant tissue. Therefore, it was assumed that there was complete removal of a small DCIS already through the biopsy. Nevertheless, there was no reliable information regarding the width of surgical margin in these cases.

In 23.5% (n=58), the authors did not find any information what so ever in the medical record regarding the width of surgical margin, even though negative margins were confirmed by the pathologist. In three women treated with BCS as the definite surgery, positive margins were confirmed (R1). Thus, there were 177 cases all together with negative and clearly defined surgical margin width eligible for the authors' further analysis.

Tumor size

Mostly, the size of the DCIS was clearly defined in the histopathological report. As already mentioned above, in 12 cases, however, the pathologist could not detect any malignant tissue within the excised surgical specimen. Therefore, complete removal of a small DCIS through the biopsy was assumed. In these 12 patients, mammography images had to be consulted in order to estimate the extent of the lesion.

In 8% of all DCIS (n=20), there was a multicentre growth pattern of DCIS detected.

In the present analysis, the DCIS size ranged from two mm to 100 mm in diameter. There was no DCIS smaller than two mm – these are generally not described as DCIS but per definition as atypical ductal hyperplasia (ADH). The mean size of DCIS in this study was 14.5mm (average 20.5 mm). Following the VNPI classification (see below), the authors present the distribution of DCIS size divided in three groups (≤ 15 mm, 16-40 mm, and ≥ 41 mm) (Figure 1).

Grading

In the present analysis, the authors could assess the grading for each DCIS. All samples were classified as low-grade (G1, or non-high-grade without necrosis), intermediate-grade (G2, or non-high-grade with necrosis) or high-grade (G3, or high-grade with/without necrosis). There were 45 low-grade DCIS, 98 intermediate-grade DCIS, and 107 high-grade DCIS (Figure 2).

Immunohistochemical analysis

An immunohistochemical (IHC) staining was carried out if there was enough DCIS tissue available in the excised specimen (n=229). In all of these samples, estrogen (ER) and progesterone receptors (PgR) detection was carried out. Almost two-thirds of all DCIS (n=154) were both ER and PgR positive. Both ER and PgR negativity were found in 43 cases. The results of hormone receptor analysis and correlation between grading and hormone receptor status is shown in (Figures 3 A-D).

Her2/neu tested positively 26 times. Negative result was seen in 53 cases. Triple-negative DCIS (ER and PgR and HER2 negative) was found only once.

The Ki-67 protein known as a marker of cellular proliferation [5] was tested in as much as 83 cases. This was surprising, because there was still a lacking routine clinical consequence of the results. Lower indices in low-grade DCIS and higher indices in high-grade DCIS were seen. There was a medium correlation between grade and Ki-67 value (r = 0.43) The mean proliferation index was 27.4% (Figure 4).
Van Nuys Prognostic Index

In order to simplify the decision when treating DCIS, Silverstein et al. presented the Van Nuys Prognostic Index (VNPI) in 1996. [6] The combination of three predictors of recurrence was supposed to help by deciding for the most appropriate treatment. Each lesion was assigned with a score from 1 to 3 for each of three factors: tumor size, margin width, and grading. By using VNPI, an attempt was made to identify subgroups of patients with different risk of recurrence and thus to individualize the therapy. A decade ago, modification of VNPI by adding the age of the patient was proposed. [7]

This so called University of Southern California/Van Nuys prognostic index (USC/VNPI) is frequently used nowadays, although in general the attempt failed to clearly identify through this scoring system patients who do not require irradiation after BCS, as well as patients whose risk of recurrence rate is so high that mastectomy is preferable.

As mentioned above, after evaluating medical records of every 250 DCIS in the present group, the authors were able to obtain reliable information regarding age at diagnosis, tumor size, margin width, and grading for 180 patients (72%). After analysing these prognostic factors and assigning 1 to 3 points for each of them, the recurrence risk for each patient was calculated (Figure 5); 40.6% (n=73) patients had USC/VNPI score 4 to 6, which would make them eligible
for excision only. The largest was the intermediate-risk group (score 7-9) with 58.8% (n=106) women. These should receive BCS followed by radiotherapy. In only one case, the score was as high as 10 and thus indicating mastectomy.

Axillary staging
Axillary staging via sentinel lymph node biopsy (SLNB) should be routinely performed in all patients with DCIS, who present with an extensive lesion or where mastectomy is required. [2]. Complete axillary lymphadenectomy, on the other hand, has become an obsolete procedure, mostly due to its high morbidity and poor risk-benefit ratio. [8]

In the present study, the authors focused also on the frequency and method of axillary staging. In 76.8 % (n=192) there was no axillary surgery performed at all. In one case, a complete axillary dissection was done; 22.8 % (n=57) of all patients received a sentinel node biopsy. Out of these 57 SLNBs, only in half (n=29), though, the indication was correct according to the current German guidelines [2]. In the remaining (n=28), a SLNB was executed even though there was neither a mastectomy required nor was the extension of DCIS greater than 40 mm.

On the other hand, the authors observed undertreatment in 25 women. In 14 of them (5.6%) there was a mastectomy executed without simultaneous SLNB, and in 11 cases (4.4%), SLNB was omitted even though the extension of DCIS was greater than 40 mm (Figure 6). In all subjects, axillary lymph nodes were free from metastases.

Discussion
In this paper the authors presented an analysis of characteristics of DCIS. They selected all cases with pure DCIS diagnosed within one mammography screening unit between May 2007 and January 2013. All available morphological and biological features were studied. After analyzing this patient sample, the acquired data with information available in the literature was compared.

As for the age at diagnosis, there was a relatively homogenous distribution of patients with DCIS within the range of 50-69 years (Figure 7). This signified that in women aged 50-59 years, DCIS was diagnosed as frequent as in women aged 60-69 years. This is in contradiction to studies which prove, that younger women are more often affected with in situ malignancy of the breast than older women, who, on the contrary, are more likely to develop invasive breast cancer. [9]

Several prior studies have examined the correlation between age and histological characteristics of DCIS [10, 11]. As for the size of DCIS, the results mostly suggested that DCIS in younger women is more extensive than in older

Figure 6. — Axillary staging in DCIS.

Figure 7. — Age at diagnosis.

Figure 8. — Correlation between age and tumor size (mm).
women. In the present analysis, the authors could not confirm this theory (Figure 8).

The results of studies examining correlation between age and grade are not that coherent. While Goldstein et al. found that DCIS in younger women was more likely to exhibit high nuclear grade compared to DCIS in older women [10], a number of other studies did not find any difference in grading across the different age groups [12]. In the present DCIS sample, the distribution of tumor grade in relationship to patient age was equal. (low grade - mean age 60.5 years, intermediate grade - mean age 61 years, high grade – mean age 61 years)

In the present analysis, the authors could confirm results which showed that ER and PgR expression range from 60% to 78% in DCIS [9, 11] (Figure 3). So far, no studies have found correlation between expression of ER, PR, or HER2 in relation to age [12].

Overexpression of HER-2/neu has been observed in 50-65% DCIS [9, 11]. In the present sample, only 32.9% of DCIS displayed HER-2/neu overexpression. This difference can be due to small number of studied cases. HER-2/neu analysis has been conducted only in 79 specimens out of 250 (31.6%).

Regardless of the type of surgery procedure performed (sentinel node vs. axillary lymph node dissection), the frequency of axillary lymph node metastasis range from 1% to almost 7% [9, 14]. In the present study, out of 58 patients who received an axillary staging, none presented with lymph node metastasis. The present findings indicated that axillary metastases in pure DCIS are very rare.

Analysis of the adjuvant therapy of diagnosed malignancies was not the primary goal of this paper. Nevertheless, one issue concerning the adjuvant radiotherapy should be mentioned. When studying the case conference protocols the authors noticed that there has been a routine recommendation expressed for adjuvant radiotherapy after BCS, regardless tumor extension, grade, margin width or overall USC/VNPI score. This reflects also the recommendation of the latest German guidelines [2].

In the future, more prospective studies in larger patient samples should be carried out in order to investigate novel prognostic and predictive factors in regards of surgery as well as adjuvant therapy of breast malignancies. Population-based mammography screening can provide us with the necessary large population sample.

Conclusions

This article describes some clinical and histopathological characteristics of DCIS diagnosed within German mammography screening. The results are similar to those found in literature and suggest, that positive screen patient provide a large patient sample, which can be used by planning and running trials concerning DCIS.

Acknowledgement

This work was supported by the Charles University in Prague UNCE No. 204024.

References

[9] Zhang W., Gao E.L., Zhou Y.L., Zhai Q., Zou Z.Y., Guo G.L.: “Overexpression of HER-2/neu has been observed in 50%

Address reprint requests to:
J. BÖHM, M.D.
Department of Obstetrics and Gynaecology,
Henneberg Kliniken, Schleusinger Strasse 17
98646 Hildburghausen (Germany)
e-mail: bazy001@atlas.cz
Breast conserving surgery in multicentric breast cancer, preliminary data of our experience


1TEI Technological University of Athens, Athens; ‘Rea and Leto Hospital, Athens
2University of Ioannina, Department Obstetrics & Gynaecology, Ioannina
3University of Montpellier-Nimes, Department of Obstetrics & Gynecology & Biostatistics, Montpellier-Nimes (France)
4University of Athens, Attikon Hospital, 3rd Department of Obstetrics & Gynaecology, Athens (Greece)
5University of Montpellier-Nimes, Department of Obstetrics & Gynecology & Biostatistics, Montpellier-Nimes (France)

Summary

Introduction: It is widely supported that multicentric disease of the breast (MCDB) is a contraindication of breast conservative surgery (BCS). Materials and Methods: This is a multicentric study (two breast cancer units from Greece, one from France) involving patients with at least two primary tumors in separate quadrants of the breast and no diffuse suspicious microcalcifications on mammography. Sixty-one patients were included in the study, but 49 were followed up to the end. Patients were randomly assigned in total mastectomy (TM) and BCS groups. End point of the study was disease-free survival rates three and five years after initial operation. Results: Three years after BCS, local recurrence (LR) was observed in two patients (7%) and one after five years (total recurrence rate: 11%). A TM was performed in these patients, and in two there was no LR or distant metastasis (DM) five years after. The third patient was disease free two-years later. Three years after TM, eight patients (36.4%) had DM and 14 (63.6%) did not (p = 0.004). Five years after TM, eight patients (36.4%) had DM and 14 patients (63.6%) did not (p = 0.03). Conclusion: The results showed that conservative surgery was an alternative surgical option in multicentric breast cancer with good results regarding disease-free survival and recurrence.

Key words: Multicentric breast cancer; Conserving surgery; Total mastectomy.

Introduction

Multicentric disease of the breast (MCDB) is characterized by two or more primary tumors in separate quadrants of the breast. It was suggested that this kind of cancer is a contraindication of breast conservative surgery (BCS) [1]. However, there are no convincing data comparing BCS (wide excision of primary tumors) to total mastectomy (TM) in relation to survival. Furthermore, most existing research is retrospective [2, 3] or related to local disease control [3]. On the other hand, a possible impact on survival was shown in patients with ductal carcinoma in situ (DCIS) and multicentric (and contralateral) invasive tumors identified with preoperative magnetic resonance imaging (MRI) [4].

The purposes of the present study were: 1) to list patients characteristics with MCDB and 2) to prospectively compare the outcome of patients who underwent BCS with those who underwent TM in relation to disease-free survival (distant metastasis and local/regional recurrence).

Materials and Methods

This was a multicentric study involving patients from three breast cancer units (two from Greece and one from France). Patients included in the study had the following characteristics: 1) (at least) two primary tumors in separate quadrants of the breast. These patients, classically, could be considered “ideal” candidates for TM; 2) there were no diffuse suspicious microcalcifications on their mammography.

Clinical information regarding age, tumor location, operation type, tumor size, lymph node involvement, stage, histologic grade, histopathology, necrosis, hormone receptor status, HER2/neu status, and disease-free survival were obtained from patient charts. Taking into account that tumor size and axillary lymph node involvement (Stage), hormone receptor status, and histologic grade have all been reported as important prognostic factors in breast cancer, these data were further analyzed. In total, 49 cases included in the study divided in two groups. In the first group (TM group), 22 cases (aged 23 to 83 years, mean = 57.5) were treated with TM and in the second one (BCS group), 27 cases (aged 29 to 75 years, mean = 58.1) were treated with BCS. Most patients were randomly divided in the above groups. However, three patients of the TM group ultimately refused TM and they were included in the BCS group. In the latter group, the following techniques were used, mainly depended on the size and the location of the tumors: 1) For seven patients, the round block (RB) technique (donut technique) was performed (Figure 1). This technique, originally described by Benelli in 1990 [5], allows a larger surgical field, securing a negative surgical margin of at least one cm around the lumps, and giving a good cosmetic result [6]; 2) for 20 cases, lumpectomy-tumorectomy (T), quadrantectomy (Q) (Figure 2), zonectomy (Z), and periareolar lumpectomy (periareolar tumorectomy [PT]) (Figure 3), were performed in nine, eight, two and one patients, respectively. In the present series, other oncoplastic techniques (vertical or lateral segmentectomy and batwing mastopexy) were not performed for the above cases. The procedures were explained to the patients who gave informed consent. Patients in the above groups had a histologically proven breast carcinoma and were staged both with the diameter of the...
largest tumor (LT) and the sum of the diameter of all tumors (ST). In this way, a patient with apparent earlier-stage with LT method, could be upstaged with ST method. However, in multiple foci of microinvasion (extension of cancer cells beyond the basement membrane within ≤ one mm in greatest dimension), the size of only the largest focus was used. Dimpling of the skin, nipple retraction, or any similar skin changes were not recorded in data analysis because they do not change staging. None of the present patients had received multiple core biopsies, because the original tumor size should be reconstructed based on a combination of imaging and histologic findings. None of the present patients belonged to TX or NX classification, as these patients could not have a stage assigned to their disease, making them ineligible for inclusion in the study. In eight of the Q-T-Z-PT patients (BCS group), largest tumor was ≥ two cm (2.1 - five cm) and resection of the tumors was achieved with a negative surgical margin both in frozen section and final pathology report. On the contrary, a patient of TM group, with a large tumor (five cm), had positive surgical margins in the final pathology report. Nevertheless, a positive surgical margin was discovered in two cases of small tumors (< 2 cm and < 1 cm) in the final pathology report of the RB-Q-T-PT-Z patients.

The purpose of the present study was to compare free survival rates between TM and BCS patients three and five years after the initial operation. Initially, 61 patients were included in the study, but, in the final results, 12 patients were excluded because their surveillance period was less than three years and/or due to incomplete information. Data of some of the above patients will be included in a further study after the completion of at least three years after the initial operation. A negative clinical history and negative examination were used to designate a case as M0 (no metastasis). Chi-square analysis was applied in group comparisons (Mac Chi Square software). In cases of invalid results, due to cells with expected values less than 5, a Fisher-exact-test was used to measure the association between two variables in 2 x 2 contingency tables. In 2 x 3 contingency tables, the Freeman-Halton extension of Fisher’s exact test was used to measure the association between two variables in 2 x 2 contingency tables. In 2 x 3 contingency tables, the Freeman-Halton extension of Fisher’s exact test was applied. In the above tests, p values were 2-sided and p values ≤ 0.05 were considered significant. A further study is planned with more patients and further analysis of the data (using Kaplan-Meier curves and log-rank test).
Breast conserving surgery in multicentric breast cancer, preliminary data of our experience

Although better cosmetic results was the main reason that the present patients selected BCS, it must be emphasized that this factor was not examined after the operations and it was not included in the study (Figure 1).

### Results

Most tumor characteristics were roughly similar between TM and BCS groups. However, percentages of positive (+) and negative (-) estrogen receptors (ER) and progesterone receptors (PR) differed in tumors of TM and BCS patients. In particular, in TM group, 13 (59%) patients were both ER+ and PR+, 6 (27%) patients were both ER- and PR-, two (9%) patients were ER+ and PR- and one (4.5%) patient was ER- and PR+. In BCS group, 24 (89%) patients were both ER+ and PR+ and three (11%) patients were both ER- and PR- ($p = 0.02$). Even so, in both groups, most patients had ER+ and/or PR+ (16 patients [73%] of the TM group and 23 [89%] of the BCS group) ($p = 0.3$).

Rates of HER2+ were similar between groups with four patients in each group (18% and 15% for TM and BCS respectively) ($p = 1$). However, in BCS group, three HER2+ patients had both ER+ and PR+ and one HER2+ patient had ER- and PR-. In TM group, HER2+ patients had ER and PR in different combinations. Rates of grade 1, 2, and 3 tumors were similar in TM and BCS patients ($p = 0.11$) although a greater percentage of BCS patients had grade 1 tumors (five patients [23%] of the TM group and 13 patients [48%] of the BCS group). Interestingly, in TM group, grade 1 and/or grade 2 tumors had both ER+/PR+ in greater percentage (11 of 14 [79%]) compared with grade 3 tumors (three of eight [56%]), although this difference was not significant ($p = 0.08$). In TM group, 15 patients had ductal carcinoma, four patients had lobular carcinoma, and three patients had other histologic types or a histologic combination. In BCS group, 25 patients had ductal carcinoma and two patients had lobular carcinoma (Table 1).

Staging of both groups is shown in Tables 2 and 3. As expected, taking into account sum of tumors’ diameter upstaged some patients (six in TM group and four in BCS group). Different staging (Table 3 instead of Table 2) altered distribution of TM patients in Stages I and IIA ($p < 0.05$). Distribution of TM patients in Stages IIB and IIIA remained actually unchanged. In BCS patients, different staging did not alter significantly distribution of patients within stages.

Three years after BCS, local recurrence was observed in two patients (7%). Five years after BCS, local recurrence was observed in one more patient (total percentage of recurrences was 11%). A successful TM was performed in all three patients as second surgery procedure. In the first two patients, five years after TM, there was no new local/regional recurrence (LR) or distant metastasis (DM). Similarly, in the third patient, two years after TM, there was no new LR or DM. Therefore, none of the present patients had LR or DM, after TM as a second surgery procedure, in the follow-up period.

Three years after BCS, 26 (96.3%) patients had ductal carcinoma and two patients had lobular carcinoma (Table 1). As expected, taking into account sum of tumors’ diameter, upstaged some patients (six in TM group and four in BCS group). Different staging (Table 3 instead of Table 2) altered distribution of TM patients in Stages I and IIA ($p < 0.05$). Distribution of TM patients in Stages IIB and IIIA remained actually unchanged. In BCS patients, different staging did not alter significantly distribution of patients within stages.

### Table 1.

Patients of both groups (total mastectomy [TM] and breast conservative surgery [BCS]) distributed according to positive (+) and negative (-) estrogen receptors (ER)/progesterone receptors (PR), HER2+/HER2-, grade (G) and histologic type (ductal carcinoma [DC], lobular carcinoma [LC] or other).

<table>
<thead>
<tr>
<th>Receptors</th>
<th>HER2/neu</th>
<th>Grade</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+PR+</td>
<td>HER2+</td>
<td>G1</td>
<td>DC/LC</td>
</tr>
<tr>
<td>ER-PR-</td>
<td>HER2-</td>
<td>G2</td>
<td>DC</td>
</tr>
<tr>
<td>ER+PR-</td>
<td>HER2+</td>
<td>G3</td>
<td>DC/LC</td>
</tr>
<tr>
<td>ER-PR+</td>
<td></td>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>ER+PR+</th>
<th>ER-PR-</th>
<th>ER+PR-</th>
<th>ER-PR+</th>
<th>HER2+</th>
<th>HER2-</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>DC</th>
<th>LC</th>
<th>DC/LC</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>18</td>
<td>5</td>
<td>9</td>
<td>8</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>BCS</td>
<td>24</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>23</td>
<td>13</td>
<td>10</td>
<td>4</td>
<td>25</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>$p$</td>
<td>0.02</td>
<td>1.00</td>
<td>0.11</td>
<td>0.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2.

Patients of both groups (total mastectomy [TM] and breast conservative surgery [BCS]) distributed according to Stage (I to IIA) taking into account greatest diameter of tumor.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Group</th>
<th>I</th>
<th>IIA</th>
<th>IIB</th>
<th>IIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td></td>
<td>9</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>BCS</td>
<td></td>
<td>14</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 3.

Patients of both groups (total mastectomy [TM] and breast conservative surgery [BCS]) distributed according to Stage (I to IIA) taking into account sum of tumors’ diameter.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Group</th>
<th>I</th>
<th>IIA</th>
<th>IIB</th>
<th>IIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td></td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>BCS</td>
<td></td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
Tables 1 and 2: “On the contrary”, five years after TM, 14 (63.6%) patients had no DM and eight (36.4%) patients had DM ($p = 0.03$). Again, “as expected”, the greatest percentage: six out of eight patients (75%) in staging of Table 1 and seven out of eight (87.5%) patients in staging of Table 2 had Stage ≥ 2A. DM comparisons between groups reached significance, however, small numbers could influence related results.

**Discussion**

Multicentric breast tumor (BT) could be defined as BT presenting as a separate focus outside the index quadrant and multifocal BT as BT present in two or more foci separated by 5 mm in the same breast quadrant [7]. However, they are different definitions of multicentric/multifocal (M/M) tumors in several studies. Taking into account that there is no distinct border between the quadrants of the breasts and evaluating the actual borders radiologically between the tumor foci is difficult, other authors considered M/M as one entity and defined M/M tumors as more than one tumor foci in the same breast histopathologically [8].

The reported incidence (RI) of multicentricity of breast cancer may depend on the extension of the pathological review and therefore varies in different references. As an example the RI for DCIS varies widely from 18% to 60%, raising questions about the importance of multicentricity. However, DCIS multicentricity is more likely to be around 30% to 40% [7]. Furthermore, pathological examination may incorrectly interpret contiguous intraductal spread as multicentricity. Similarly, >95% of local recurrences after treatment of DCIS occur in the same quadrant as the index lesion. This finding could be interpreted as residual untreated disease rather than multicentricity [7].

Surgical management options for breast cancer include mastectomy or BCS (surgical removal of the tumor, with negative surgical margins, followed by moderate-dose radiation therapy to eradicate any residual disease) [9]. Although it was suggested that the latter approach is not suitable for MCDB, more than ten years ago, the first suggestions appeared for BCS in MCDB [10]. To analyze the present data, patients staged initially with the diameter of the largest tumor. However, it could be suggested that taking the combined diameters of the multicentric tumors, and not just the diameter of the largest tumor, may give a clearer indication of the actual breast cancer stage, an approach that could alter the final decision related to the planned procedure. The mode by which the diameter of the tumor is measured may be extremely important because it was shown that tumor size (before any tissue is removed for special studies, such as hormone receptors or HER2/neu status) is an important prognostic factor for multicentric and multifocal breast cancer. Recently, it was found that the diameter of the largest deposit provides a better fit in a multivariate model for overall survival than aggregate diameter (and aggregate volume) [11]. Therefore, it is suggested that tumor size in multicentric and multifocal breast cancer should (continue to) be measured using the diameter of the largest deposit [11]. Furthermore, some physicians could be hesitant to assign a classification of M0, feeling that there is always a remote possibility that occult metastatic disease may exist. However, the present authors used a negative clinical history and a negative examination to designate a case as M0 according to related staging guidelines [12]. The present data dispute that MCDB is not suitable for BCS. On the contrary, it seems that conservative surgery, at least, does not adversely influence disease-free survival (DFS). In case that the present data will be confirmed in further studies, it could be hypothesized that BCS actually improves DFS. Operation method could be added to well known factors predicting DFS as the positivity of the ER and PR, histologic grade, the presence of necrosis, pT stage, pN stage, and the presence of inflammatory breast cancer [8]. On the contrary, the presence of adjuvant chemotherapy and radiotherapy and chemotherapy type such as taxanes or antracycline-based regimens were not related with DFS in multicentric tumors [8].

In accordance with the present findings, in the aforementioned retrospective trials [2,3], it was suggested that in selected patients with MCDB, wide conservative surgery is a safe therapy [2] that is not associated with poor local disease control and can be considered whenever acceptable cosmetic results can be achieved [3]. The “wide” negative surgical margins of tumor resections must be emphasized in BCS procedures. These findings should not be confused with the fact that multicentric (and multifocal) disease of the breast imparts an unfavorable prognosis on the disease-free survival of breast cancer patients in comparison to unifocal tumors and the presence of multicentric (and multifocal) tumors is associated with advanced pT and pN stages (pathologic classification [8]).

**Conclusion**

The present study showed that conservative surgery is an alternative surgical option in multicentric surgery with good results regarding the “disease-free survival” and the percentage of “recurrence”. Moreover the oncoplastic techniques performed give very good cosmetic results with high rate of satisfaction in women who avoid the damaging mastectomy. Further studies with more cases should confirm the present experience.

**References**


Breast conserving surgery in multicentric breast cancer: preliminary data of our experience


Address reprint requests to:
S. ZERVoudis, M.D., PhD
REA Hospital, Department of Mastology,
Syggrou Avenue 383, Athens 17564 (Greece)
e-mail: szervoud@otenet.gr
Cytoreductive surgery for isolated para-aortic lymph node recurrence of endometrial cancer: report of four cases and a review of the literature

H. Nakamura¹, K. Takehara¹,², O. Samura¹,³, T. Mizunoe¹

¹Department of Obstetrics and Gynecology, National Hospital Organization, Kure Medical Center and Chugoku Cancer Center, Kure City, Hiroshima; ²Department of Gynecologic Oncology, National Hospital Organization, Shikoku Cancer Center, Matsuyama; ³Department of Gynecologic Oncology, Onomichi General Hospital, Onomichi, Hiroshima (Japan)

Summary
Isolated para-aortic lymph node recurrence of endometrial cancer occurs occasionally, but management of such patients has been controversial. The authors performed cytoreductive surgery in four patients with isolated para-aortic lymph node metastasis of recurrent endometrial cancer. They resected metastatic foci by laparoscopic method for three cases and by laparotomy for one case. After the surgery, three cases underwent radiation therapy and one case was given chemotherapy as adjuvant therapy. After the treatment for recurrence, progression-free interval was from 64 to 127 months and all cases had no evidence of disease. Cytoreductive surgery may improve prognosis of isolated para-aortic lymph node metastasis of recurrent endometrial cancer. As laparoscopic surgery is superior to laparotomy in terms of less invasiveness, further examinations will reveal that it is feasible for such an isolated lymph node recurrence situation.

Key words: Isolated para-aortic lymph node recurrence; Endometrial cancer; Surgery.

Introduction
The morbidity rate of endometrial cancer is steadily increasing in Japan. According to the statistics of the National Cancer Center of Japan, it was estimated that 5,600 new uterine endometrial cancer cases would be diagnosed in 2000, with 1,139 deaths resulting from this disease. These numbers have increased four-fold in 20 years [1]. Fung-Kee-Fung et al. reported a recurrence rate of endometrial cancer at about 13% [2]. With the frequency of recurrent endometrial cancer increasing, it is expected that treatment opportunities will increase.

Although the endometrial cancer mortality rate is lower than that of ovarian and cervical cancer, patients who develop recurrences have a very poor outcome. When a recurrence of endometrial cancer is diagnosed, it should be evaluated and identified as a local or regional recurrence, or systemic, disseminated metastases. Isolated vaginal metastases are the most amenable to therapy with curative intent. Patients with a vaginal recurrence require a thorough investigation to detect any associated metastatic foci. If no other metastatic foci are detected, patients who have had prior pelvic radiation may undergo an exploratory laparotomy with a view to some type of pelvic exenteration. According to the National Comprehensive Cancer Network Practice Guidelines (version 1.2011), if patients with regional recurrence have not been treated with radiation therapy before diagnosis, radiation therapy or cytoreductive surgery should be considered. For patients with isolated pelvic recurrence, some authors report pelvic exenteration improves survival [3-6]. Similarly, for patients with isolated pulmonary metastases, tumor resection can improve survival [7, 8]. In these situations, the management of patients with isolated para-aortic lymph node recurrence is still controversial.

The authors report four cases that underwent surgical resection for isolated para-aortic lymph node recurrence of endometrial cancer.

Materials and Methods
The authors treated four cases of isolated para-aortic lymph node recurrence of endometrial cancer by surgery at the National Hospital Organization Kure Medical Center and Chugoku Cancer Center from 2001 to 2008. All cases were diagnosed as isolated para-aortic lymph node recurrence using imaging, computed tomography (CT), or positron emission computed-tomography (PET-CT), which demonstrated enlarged para-aortic lymph nodes. Surgical resection was performed in all cases.

Results
The range of ages at the initial therapy was from 40 to 56 years. At the diagnosis of endometrial cancer, they underwent surgery as primary therapy. They underwent surgery with total hysterectomy, bilateral salpingo- oophorectomy and pelvic lymphadenectomy. For case no.2, and case no.3, para-aortic lymphadenectomy was additionally executed. The stage classification was FIGO IIIc in all cases. The
pathological diagnosis for all cases was endometrioid adenocarcinoma, and histologic grade 1 (G1) in cases no.1, G2 in cases no. 2 and 4, and G3 was diagnosed for case no. 3. Adjuvant chemotherapy was applied in case no. 1, 2, and 3, and regimens of cases no.1 and 2 were CAP therapy (cyclophosphamide, doxorubicin, and cisplatin), case no. 3 was TC therapy (paclitaxel and carboplatin). Whole pelvic irradiation was carried out for case no. 4 (Table 1).

Three cases (cases no. 1, 2, and 3) were diagnosed as recurrent by scheduled imaging methods. In case no. 1, a CT study revealed a 20 × 20 mm para-aortic lymph node swelling. In case no. 2, a CT study revealed a 25 × 22 mm para-aortic lymph node. In case no. 3, a 19 × 15 mm lymph node enlargement was indicated by PET-CT (Figure 1). In case no. 4, the patient complained of lower-back pain and the PET-CT study revealed a 30 × 30 mm para-aortic lymph node swelling (Figure 2). A tumor marker was found elevated in only case no.1, CA19-9 was measured at 91 u/ml. The period from the first treatment to recurrence ranged from seven months to 6.5 years. The performance status of all patients was 0 at the recurrence date.

Table 1. — Patients characteristics.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>PS</th>
<th>Operation</th>
<th>Stage</th>
<th>Histology</th>
<th>Adjuvant Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>0</td>
<td>mRH+BSO+PLA+OMTx+APDx</td>
<td>IIc</td>
<td>Em G1</td>
<td>chemotherapy</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>0</td>
<td>TAH+BSO+PLA+PALA(b2)</td>
<td>IIc</td>
<td>Em G2</td>
<td>chemotherapy</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>0</td>
<td>mRH+BSO+PLA+PALA(b2)+OMTx+APDx</td>
<td>IIc</td>
<td>Em G3</td>
<td>chemotherapy</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>0</td>
<td>mRH+BSO+PLA+PABx</td>
<td>IIc</td>
<td>Em G2</td>
<td>radiation therapy</td>
</tr>
</tbody>
</table>

mRH: modified radical hysterectomy; BSO: bilateral salpingo-oophorectomy; OMTx: omentectomy; APDx: appendectomy; TAH: total abdominal hysterectomy; PABx: para-aortal lymph node biopsy; PALA: para-aortic lymphadenectomy; PS: performance status; Em: endometrioid adenocarcinoma

Table 2. — Clinical findings of recurrence.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age*</th>
<th>Period from the first treatment recurrence</th>
<th>Diagnosis of recurrence</th>
<th>Size of recurrent tumor (mm)</th>
<th>Operation</th>
<th>Operation time (minutes)</th>
<th>Total blood loss</th>
<th>Adjuvant therapy</th>
<th>PFS (months)</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>6y6m</td>
<td>CT</td>
<td>20×20</td>
<td>RLT</td>
<td>166</td>
<td>small</td>
<td>radiation therapy</td>
<td>70</td>
<td>NED</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>1y1m</td>
<td>CT</td>
<td>25×22</td>
<td>tumor resection OMTx</td>
<td>130</td>
<td>170g</td>
<td>radiation therapy</td>
<td>127</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>0y7m</td>
<td>PET-CT</td>
<td>19×15</td>
<td>RLT</td>
<td>79</td>
<td>small</td>
<td>radiation therapy</td>
<td>69</td>
<td>NED</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>6y6m</td>
<td>PET-CT</td>
<td>30×30</td>
<td>RLT</td>
<td>137</td>
<td>small</td>
<td>chemotherapy</td>
<td>64</td>
<td>NED</td>
</tr>
</tbody>
</table>

*Age at recurrence; PFS: progression-free survival (after recurrent treatment); RLT: retroperitoneal laparoscopic tumor resection; CT: computed tomography; PET-CT: positron emission computed-tomography; NED: no evidence of disease
The authors performed operations of retroperitoneal laparoscopic tumor resection (RLT) in cases no.1, 3, and 4, and tumor resection and omentectomy at laparotomy in case no. 2. Total blood loss was small in all RLT cases, and 170 ml in laparotomy. In the RLT, operative time ranged from 79 min to 166 min, with an average time of 127 min. In laparotomy, the procedure took 130 min. For the RLT, all of the patients walked and took a general diet on the next day (Table 2).

Histopathological diagnosis of all cases revealed lymph node metastases of endometrial cancer. As adjuvant therapy, three patients were treated by radiation therapy for the para-aortic lymph node area, and a patient was treated with chemotherapy of TC. In case no.1, additional radiation therapy was given on the recurrent site because the authors could not exclude the possibility of residual tumor due to heavy adhesion around the lymph node metastasis. In case no. 3, radiation therapy was selected. Case no. 3 had a short duration from the initial therapy to recurrence, so the authors considered it to be chemo-resistant. All patients are still alive without disease. The progression-free interval from the recurrence is from 64 to 127 months (Table 2).

Discussion

At the relapse of endometrial cancer, there are various patterns of recurrence. Many of these patients have extensive recurrences. Abu-Rustum et al. reported that recurrent sites for 154 patients included the pelvis, 52 (34%), abdomen, 51 (33%), distant, 41 (27%), and 10 (6%) isolated para-aortic [9].

Recently, Niibe and Hayakawa proposed the new notion of oligo-recurrence. Oligo-recurrence is the state in which patient shows distant relapse in only a limited number of regions, and the primary site of the cancer is controlled. In the state of oligo-recurrence, all gross tumors could be treated with local therapy, considered as a curative treatment [10].

For treatment of recurrent endometrial cancer, understanding the recurrent pattern is important. Extensive recurrences or unresectable isolated metastases cannot be a candidate for surgery. In such cases, chemotherapy with or without RT is considered. Surgical excision is considered if tumors are resectable and localized. Pulmonary resection is also reported to be useful for isolated and smaller than four cm pulmonary metastases [7, 8]. For recurrent ovarian carcinoma, there are many studies for second line chemotherapy, secondary debulking surgery (SDS), molecular target treatment, and so on. However, for recurrent endometrial cancer, there are not as many clinical studies except for chemotherapy. Surgical therapy of recurrent endometrial cancer has not been well-established.

Some authors [11-14] have reported on cytoreductive surgery of recurrent endometrial cancer where its optimal rate was 56-74.7%, and the median survival period of optimal cases was 11.8 to 53 months, and median survival period of suboptimal cases was four to 13.5 months. The authors have different definitions of optimal surgery, Scarabelli et al. and Bristow et al. defined the optimal surgery as no gross tumor [11, 14], Campagnutta et al. defined optimal surgery as less than one cm residual tumor [6], Awtrey et al. defined it as less than two cm residual tumor [7]. In all reports, multiple classification analysis for the median survival period showed optimal surgery to be the only factor of a good prognosis. Furthermore, Campagnutta et al. reported that solitary recurrence is only the factor for optimal surgery. All of the present four cases were able to avoid gross tumors and there has been no evidence of recurrence.

On the other hand, regarding the SDS of recurrent ovarian cancer, some authors [15-18] reported that optimal rates were 52% to 85%, median survival periods of optimal cases were 20 to 56 months, and median survival periods of suboptimal cases were 20 to 27 months. Although the optimal rate and median survival period of optimal cases are almost same results in comparing recurrent ovarian cancer and recurrent endometrial cancer, the median survival period of suboptimal cases of recurrent endometrial cancer is clearly shorter than that of ovarian cancer. This suggests that for the recurrent endometrial cancer, optimal surgery, especially no gross tumor, is important to obtain good prognosis. It is possible that solitary recurrence may be able to get a good prognosis by surgery, because solitary recurrence is one of the successful factors for optimal surgery. Scarabelli et al. reported a median survival period based on an optimal 20 cases is 11.8 months. On the other hand, the present four cases’ progression-free interval from the surgery of para-aortic lymph node recurrence is from 64 to 127 months. Therefore they have survived longer than Scarabelli’s patients [11].

There are no reports of cytoreductive surgery for solitary para-aortic lymph node recurrence, hence additional examinations will be needed in the future. There are some questions to be solved. The efficacy of therapies after surgical cytoreduction should be discussed, and the best treatment method (for example chemotherapy or radiation therapy) should be considered.

Operative procedures have to be reviewed. In general, if cure rate is equivalent, the less-invasive surgery should be chosen. The authors excised metastatic para-aortic lymph nodes surgically of four of the recurrent endometrial cancer cases: case no.1, 3, and 4 by RLT and case no.2 by laparotomy. RLT is less-invasive than laparotomy in that adjuvant therapy could be commenced earlier by RLT than by laparotomy. RLT may be a feasible operative procedure because the patient’s prognosis and quality of life (QOL) should be considered concurrently in recurrent cases. The indication should also be carefully considered before RLT, because after the first surgery and radiation therapy, some cases are difficult to expand in the retroperitoneal space.
Conclusion

The authors reported four cases of cytoreduction for isolated para-aortic lymph node recurrence after surgical treatment of endometrial cancer. The isolated para-aortic lymph node recurrence of endometrial cancer has a high possibility of indicating no gross tumor, and that contributes to improved prognosis. RLT is less-invasive and able to keep a good patient QOL. Regarding adjuvant therapy, further investigations will be necessary.

Acknowledgement

The authors wish to thank Dr. Masanobu Shigeta, Department of Urology, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, for instructing them on the RLT methodology.

References


Address reprint requests to:

H. NAKAMURA, M.D.
Department of Obstetrics and Gynecology
National hospital Organization
Kure Medical Center and Chugoku Cancer Center
3-1 Aoyama-cho, Kure City
Hiroshima 737-0023 (Japan)
e-mail: hirokon@kure-nh.go.jp
Comparison of the histopathological diagnoses of preoperative dilatation and curettage and Pipelle biopsy

K. Gungorduk1, O. Asicioglu2, I.E. Ertas1, I.A. Ozdemir1, M.M. Ulker3, G. Yildirim3, G. Ataser1, M. Sanci1

1Department of Gynecologic Oncology, Tepecik Training and Research Hospital, Izmir
2Department of Obstetrics and Gynecology, Sisli Etfal Training and Research Hospital, Istanbul
3Department of Obstetrics and Gynecology, Istanbul Training and Research Hospital, Istanbul (Turkey)

Summary
Purpose: To evaluate the accuracy of dilatation and curettage (D&C) and Pipelle biopsy for the diagnosis of endometrial pathologies and determine whether the amount of endometrial tissue obtained using these techniques is sufficient for further histopathology of hysterectomy specimens. Materials and Methods: Patients undergoing hysterectomy for various indications were evaluated via Pipelle endometrial biopsy or D&C from 2009–2011. A total of 267 women were included with 78 women enrolled in the Pipelle group and 189 in the D&C group. Uterine findings were grouped as normal, hyperplasia, focal lesion, atypia, and atrophy. Histological sections from the Pipelle biopsy or D&C specimens were compared to each other and hysterectomy specimens. Results: The concordance rate between Pipelle biopsy and hysterectomy was 62% and between D&C and hysterectomy was 67%. The sensitivity of Pipelle biopsy and D&C for detecting hyperplasia was 41.7% and 45%, respectively, and for detecting atypia was 71.4% for both techniques. The sensitivity of detecting atrophic endometrial tissue was significantly higher in the D&C group at 80% compared to 37.3% in the Pipelle biopsy group (p = 0.030). All other parameters were similar in both groups. Conclusion: Pipelle biopsy and D&C were equally successful for diagnosing endometrial pathologies. Neither Pipelle biopsy nor D&C was adequate for detecting focal endometrial pathologies and endometrial hyperplasia. In contrast, both techniques were sufficient for the diagnosis of atypia. The Pipelle biopsy technique is a reasonable pre-hysterectomy procedure that is more economical, less invasive, and can easily be performed in multiple clinics.

Key words: Pipelle biopsy; Dilatation and curettage; Endometrial pathologies.

Introduction
Gynecologists routinely sample the endometrium before a hysterectomy to detect unsuspected or asymptomatic endometrial pathologies as part of the preoperative workup regardless of the indication for hysterectomy [1]. Several endometrial sampling techniques are used to diagnose endometrial abnormalities in patients with or without abnormal uterine bleeding, including dilatation and curettage (D&C), aspiration techniques (Pipelle biopsy), and hysteroscopy [2].

D&C is the method of choice for obtaining an endometrial sample [3]. However, patients must undergo general anesthesia and are at risk for complications such as infections, bleeding, and uterine perforation, which collectively cause physicians to question the suitability of the procedure [4, 5]. In contrast, hysteroscopy is an effective procedure, although more expensive than D&C. Hysteroscopy also requires general anesthesia with similar complications to D&C. Thus, there is a need for an accurate, less invasive, more economical, and easily applicable method for early histological diagnosis of premalignant and malignant pathologies. Pipelle is a flexible polypropylene endometrial biopsy cannula that does not require a syringe or pump. A Pipelle biopsy can be performed during an office visit without general anesthesia or cervical dilatation and is less invasive [6].

This study examined the accuracy of D&C and Pipelle biopsy in pre-hysterectomy endometrial sampling for the diagnosis of endometrial pathologies and determined whether the amount of endometrial tissue obtained with the techniques is sufficient for further histopathology of hysterectomy specimens.

Materials and Methods
The authors retrospectively analyzed the charts of all patients who underwent a hysterectomy for various indications at the Departments of Gynecology and Obstetrics, Istanbul Teaching Hospital and Şişli Etfal Teaching Hospital from 2009 to 2011. Patients were excluded from the study if their medical records were incomplete or if the endometrium was sampled more than 30 days before hysterectomy. A total of 267 patients were enrolled with these criteria. This study was approved by the local ethics committee and informed consent of all of the patients was obtained before the procedure by informing patients about the implementation details of the diagnostic methods to be used and possible complications before the procedures.

Detailed gynecological histories of all of the cases were collected, and following physical and gynecological examinations, blood was collected for laboratory tests. β-human chorionic gonadotropin was measured to rule out pregnancy in patients that had not entered menopause. Transvaginal ultrasound examination prior to endometrial biopsy was performed in all patients using an ultrasound eight-MHz transvaginal probe. Endometrial thick-
ness was measured in the sagittal plane. The authors chose a cut-off level of ten mm because they defined the top ten mm as thick endometrium.

To perform a D&C, the patient was placed on the table in the lithotomy position and general anesthesia administered as necessary. After a careful pelvic examination to locate the position of the uterine body, the vagina, and perineum were cleaned. The cervix was dilated with small Hegar dilators as a preliminary step to curettage of the uterine cavity.

The authors implemented the Pipelle device in the dorsal lithotomy position. If necessary, the cervix was held with tenaculum forceps during Pipelle insertion into the cervical canal. After reaching the fundus, the pistol was pulled back to provide negative pressure and endometrial tissue was aspirated. The procedure was attempted twice and samples were preserved in formalin.

All samples were evaluated in the pathology department of two institutions (Istanbul Teaching Hospital and Şişli Etfal Teaching Hospital pathology department). Histopathological findings were categorized into six groups: normal, hyperplasia, focal lesions, atypia, atrophy, and insufficient material. Proliferative and secretory endometrium were included in the normal group; polyps and submucous myomas were included in focal lesion group; simple and complex hyperplasia without atypia were included in hyperplasia group; atypical hyperplasia and carcinoma were included in the atypia group; and atrophic endometrium was included in the atrophy group. The pre- and postoperative histopathological findings were evaluated for each case and the histopathological diagnosis of the endometrial sample was compared to the histopathological findings for the hysterectomy specimen. The sensitivity and specificity of Pipelle and D&C were calculated by comparison with the final pathological diagnosis.

SPSS 17 for Windows was used for statistical analysis. The data are presented as means ± standard deviation (SD) or percentage according to the variables. Chi-squared tests were used to analyze categorical variables; the Student’s t-test and Mann–Whitney U-test were used for continuous variables. Relative risk (RR) with a 95% confidence interval (CI) was calculated. Statistical significance was considered to be at \( p < 0.05 \).

### Results

A total of 267 women were included in the study, with 78 in the Pipelle group and 189 in the D&C group. Maternal demographic characteristics are shown in Table 1. No differences were observed between the Pipelle group and the D&C group regarding mean maternal age (49.8 ± 6.1 vs. 48.2 ± 6.5 years) or gravidity (4.7 ± 2.4 vs. 4.5 ± 2.4 years). Furthermore, the rate of premenstrual status (61.5% vs. 54.5%), endometrial thickness measures (42.3% vs. 47.6%), and the patients who subsequently underwent abdominal hysterectomy (80.8% vs. 81.5%) were similar between groups.

The authors compared the results of Pipelle endometrial sampling and the endometrial histopathology obtained from hysterectomy in 78 cases (Table 2). The highest histopathological compliance between Pipelle and hysterectomy was seen in patients with normal endometrial tissue and atypia. Among 49 patients with normal endometrial

---

### Table 1. — Demographic characteristics of patients.

<table>
<thead>
<tr>
<th></th>
<th>Pipelle group (n = 78)</th>
<th>D&amp;C group (n = 189)</th>
<th>( p ) value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ± SD (year)</td>
<td>49.8 ± 6.1</td>
<td>48.2 ± 6.5</td>
<td>0.066</td>
<td>—</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal, n (%)</td>
<td>48 (61.5)</td>
<td>103 (54.5)</td>
<td>0.291</td>
<td>1.3 (0.7–2.2)</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>30 (38.5)</td>
<td>86 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 10 mm, n (%)</td>
<td>33 (42.3)</td>
<td>90 (47.6)</td>
<td>0.428</td>
<td>0.8 (0.4–1.3)</td>
</tr>
<tr>
<td>&lt; 10 mm, n (%)</td>
<td>45 (57.7)</td>
<td>99 (52.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of hysterectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal, n (%)</td>
<td>63 (80.8)</td>
<td>154 (81.5)</td>
<td>0.892</td>
<td>0.9 (0.4–1.8)</td>
</tr>
<tr>
<td>Vaginal, n (%)</td>
<td>15 (19.2)</td>
<td>35 (18.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity ± SD</td>
<td>4.7 ± 2.4</td>
<td>4.5 ± 2.4</td>
<td>0.550</td>
<td></td>
</tr>
<tr>
<td>Tobacco use, n (%)</td>
<td>11 (14.4)</td>
<td>28 (14.8)</td>
<td>0.881</td>
<td>0.9 (0.4–2.0)</td>
</tr>
</tbody>
</table>

CI: confidence interval; D&C: dilatation and curettage; OR: odds ratio; SD: standard deviation.

### Table 2. — Clinical outcomes of patients who underwent Pipelle biopsy and hysterectomy (n = 78).

<table>
<thead>
<tr>
<th></th>
<th>Pipelle</th>
<th>Hysterectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (43) Hyperplasia (12) Focal lesion (8)</td>
<td>Atypia (7) Atrophy (8) Insufficient (0)</td>
</tr>
<tr>
<td>Pipelle</td>
<td>Normal (48) 32 6 5 1 4 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hyperplasia (6) 1 5 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Focal lesion (6) 2 0 3 1 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atypia (5) 0 0 5 0 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atrophy (9) 6 0 0 3 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient (4) 2 1 0 0 1 0</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as the number of patients (n) in each category.
Comparison of the histopathological diagnoses of preoperative dilatation and curettage and Pipelle biopsy

541

six of 49 lesions (12%) were diagnosed as hyperplasia based on the final pathology results obtained by hysterectomy. Furthermore, five of 49 lesions (10%) were diagnosed as focal lesions with the final pathology results. Two normal endometrial tissues obtained from Pipelle biopsy were upgraded to atypia upon final histopathological analysis.

D&C histopathology findings were compared to those of the subsequent hysterectomy specimen in 189 cases (Table 3). The highest histopathological compliance rate between D&C and hysterectomy was seen in patients with normal, atypia, and atrophy endometrial tissue. In contrast, the lowest histopathological compliance rate between D&C and hysterectomy was seen in patients with hyperplasia, which was similar to the Pipelle group. All insufficient tissue samples (7/7) obtained from D&C were upgraded to normal endometrial tissue upon final histopathological analysis.

D&C histopathology findings were compared to those of the subsequent hysterectomy specimen in 189 cases (Table 3). The highest histopathological compliance rate between D&C and hysterectomy was seen in patients with normal, atypia, and atrophy endometrial tissue. In contrast, the lowest histopathological compliance rate between D&C and hysterectomy was seen in patients with hyperplasia, which was similar to the Pipelle group. All insufficient tissue samples (7/7) obtained from D&C were upgraded to normal endometrial tissue upon final histopathological analysis.

The authors also compared the sensitivity and specificity to the two different techniques (Table 4). The sensitivity was similar between the groups for detection of normal endometrial tissue, 74.4% vs. 71.2% ($p = 0.687$); hyperplasia, 41.7% vs. 45.0% ($p = 0.854$); and focal lesions, 37.5% vs. 35.1% ($p = 0.899$). Furthermore, the rate of atypia was the same between the groups (71.4% vs. 71.4%). Only the sensitivity of detecting atrophic endometrial tissue was significantly different with 37.5% in the Pipelle group vs. 80.0% in the D&C group ($p = 0.030$). The highest sensitivity was normal endometrial tissue at 74.4% for the Pipelle group and atrophy at 80.0% for the D&C group. The specificities of histopathological findings were high in both groups; only normal endometrial tissue specificity was lower than 90% in both groups, with 51.4% in the Pipelle group vs. 62.7% in the D&C group ($p = 0.257$). The rate of insufficient tissue sampling was also similar between the groups with 5.1% in the Pipelle group vs. 3.7% in the D&C group ($p = 0.594$). The four cases of insufficient tissue obtained in the Pipelle group were from patients with an endometrial thickness < ten mm.

Discussion

Endometrial sampling is a frequently performed gynecological procedure that is an important step during the pre-hysterectomy workup. Various methods of endometrial sampling are used in practice; D&C is accepted as the traditional method but a Pipelle biopsy is a minimally invasive, more economical, and less time-consuming outpatient procedure. The authors evaluated the accuracy of Pipelle biopsy and D&C for the diagnosis of endometrial

<table>
<thead>
<tr>
<th>Table 3. — Clinical outcomes of patients who underwent D&amp;C and hysterectomy (n = 189).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (111)</td>
</tr>
<tr>
<td>D&amp;C</td>
</tr>
<tr>
<td>Pipelle</td>
</tr>
<tr>
<td>Hysterectomy</td>
</tr>
<tr>
<td>Data are expressed as the number of patients (n) in each category. D&amp;C: dilatation and curettage</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4. — Comparison of sensitivity and specificity between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong>&lt;br&gt;N: Normal tissue (%)</td>
</tr>
<tr>
<td>Pipelle</td>
</tr>
<tr>
<td>D&amp;C</td>
</tr>
<tr>
<td><strong>Specificity</strong>&lt;br&gt;N: Normal tissue (%)</td>
</tr>
<tr>
<td>Pipelle</td>
</tr>
<tr>
<td>D&amp;C</td>
</tr>
<tr>
<td>Data are expressed as the number of patients (n) in each category. D&amp;C: dilatation and curettage; OR: odds ratio; SD: standard deviation. *statistically significant.</td>
</tr>
</tbody>
</table>
pathologies and determined whether the amount of tissue obtained is sufficient for further histopathological analysis. The results demonstrated that both techniques resulted in an equally accurate diagnosis of endometrial pathologies.

Fothergill et al. [7] was the first to compare the diagnostic accuracy of Pipelle biopsy and D&C and found 84% concordance between the two methods for 187 cases. Goldschmit et al. [8] performed Pipelle endometrial biopsy prior to D&C in 176 consecutive patients and reported that Pipelle biopsy resulted in a 39% rate of false-negative results for endometrial polyps and hyperplasia in premenopausal patients. The authors suggested that the low sensitivity of Pipelle sampling may be correlated with the focal location of hyperplasia. In the present study, a lower sensitivity rate was seen in focal lesions and hyperplasia in both the Pipelle and D&C groups, confirming the findings of Goldschmit et al. [8]. Thus, hysteroscopy would be superior to Pipelle sampling and D&C for detecting hyperplasia in high-risk patients, such as diabetics, the obese, and low parity-postmenopausal women, because the entire uterine cavity can be observed and the area in question curated [9, 10].

The authors performed a Pipelle biopsy prior to hysterectomy in 78 patients to evaluate the diagnostic accuracy of Pipelle for endometrial pathology. The histopathologic results on the specimen were 62% (48/78) concordant with the Pipelle biopsy. Remarkably, hyperplasia, atrophy, and focal lesion sensitivity were very low but specificity was very high. However, the sensitivity for atypia was reasonable with only two cases missed by Pipelle biopsy, which is an important indicator because atypia is a life-threatening pathology. In 2000, Dijkhuizen et al. [11] published a meta-analysis that reported 25–100% sensitivity and 93–100% specificity of Pipelle biopsy for endometrial carcinoma. They concluded that endometrial biopsy with the Pipelle is superior to other endometrial techniques for detecting endometrial carcinoma and atypical hyperplasia. In contrast, the present authors found that Pipelle was not superior to traditional techniques. In another study, one in three cases of adenocarcinoma of the endometrium could not be detected by Pipelle [12], which was similar to the present results that two in seven cases of atypia of the endometrium could not be detected by Pipelle. Guido et al. [13] performed Pipelle biopsy prior to hysterectomy in 65 cases diagnosed previously as endometrial carcinoma. Malignancy was detected in 54 patients, a sensitivity of 83 ± 5%. Of the 11 patients with false-negative results, five had tumors present in only an endometrial polyp, and three had disease localized to <5% of the surface area of the endometrium. The authors concluded that the Pipelle endometrial suction curette is an effective device for evaluating patients at risk of endometrial cancer; however, tumors localized to a polyp or small area of endometrium may go undetected. In the present study, the rate of atypia sensitivity was high at 71.4%, but a lower sensitivity rate was seen in focal lesions at 37.5%, similar to that reported by Guido et al. [13].

The present authors found a concordance rate of 67% (128/189) between D&C and hysterectomy, higher than that between Pipelle biopsy and hysterectomy. Epstein et al. [14] reported that D&C missed 58% (25/43) of polyps, 50% (5/10) of hyperplasias, 60% (3/5) of complex atypical hyperplasias, and 11% (2/19) of endometrial cancers. Here, the present authors report similar results in that D&C failed to diagnose focal lesions in 11/24 cases and hyperplasia in 11/20 cases. Bettocchi et al. [15] confirmed the inadequacy of D&C as a diagnostic tool for all uterine disorders because major intrauterine diseases (myomas, polyps, and hyperplasia) were missed in 62.5% of patients. The limited value of D&C for the diagnosis of endometrial polyps and submucous myomas has been reported [15, 16], which supports the present results.

Another important issue is the power of obtaining sufficient material for endometrial sampling techniques. The insufficient sampling rate of Pipelle biopsy and D&C was 5% and 4%, respectively. Thus, the sampling rate of the two techniques is acceptable. The rate of insufficient tissue for Pipelle biopsy in the present study was consistent with the 8% failure rate reported by Clark et al. [17]. In addition, the rate of insufficient tissue from D&C in the present study was much lower than previous reports of 22.6% by Barut et al. [18].

The present authors also compared the sensitivity and specificity of the two different techniques and found that only the sensitivity rate of atrophic endometrial tissue was statistically higher in the D&C group. They attributed this to atrophic endometrial tissue not being aspirated with negative pressure.

In conclusion, Pipelle biopsy and D&C had an approximately equal success rate for the diagnosis of endometrial pathologies. Neither Pipelle biopsy nor D&C was an adequate method for diagnosis of focal endometrial pathologies and endometrial hyperplasias. In contrast, both methods seem sufficient for diagnosing atypia. The Pipelle biopsy technique is a reasonable pre-hysterectomy procedure that is more economical, less invasive, and can be easily performed in multiple clinics.

References

Comparison of the histopathological diagnoses of preoperative dilatation and curettage and Pipelle biopsy

543


Address reprint requests to:
K. GUNGORDUK, M.D.
Department of Gynecologic Oncology,
Tepecik Training and Research Hospital,
Gaziler Street
35120 Izmir (Turkey)
e-mail: maidenkemal@yahoo.com
Introduction

Cervical cancer is one of the most common malignant neoplasias in women worldwide, with more than 500,000 new cases and more than 275,000 deaths estimated in 2008 [1]. Large part of these cases are registered in developing countries, especially in Africa and South America, while incidence and mortality rates in Western countries were reduced in the last decades, due to the improvements in the detection and treatment of cervical infections and pre-neoplastic or early neoplastic neoplasias [2].

Previous reports [3-5] investigated the epidemiological characteristics of cervical cancer in northern Sardinia, Italy from 1965 to 2000.

The aim of this study was to analyze and describe the incidence and mortality trends of cervical cancer in northern Sardinia, Italy, in the period 1992–2010.

Materials and Methods

Data were obtained from the tumor registry of Sassari province which is part of a wider registry web, coordinated today by the Italian Association for Tumor Registries.

Results:

The overall number of cervical cancer cases registered in the period under investigation was 311. The mean age of the patients was 51.8 years. The standardized incidence and mortality rates were 6.6 / 100,000 and 0.7 / 100,000, respectively. A stable trend in incidence and mortality of cervical cancer was evidenced. Relative survival at five years from diagnosis was fairly good (66.3%).

Conclusions:

The incidence and mortality trends of cervical cancer in northern Sardinia remained relatively stable in the last decades. Furthermore, survival of patients with cervical cancer is good in the area, sanctioning the adequacy of the preventive and clinical measures in use.

Key words: Cervix cancer; Adenocarcinoma; Squamous carcinoma; Screening; Pap test; Sardinia.
ports, radiological referrals, death certifications, etc) in four cases (1.3%). The mean age of the sufferers was 51.8 years. The cumulative risk of developing the disease between zero and 74 years of age was 0.52%.

Among the 307 tumors that had a histological or cytological diagnosis, 238 (77.5%) were squamous cell carcinomas, 41 (13.5%) were adenocarcinomas, 21 (6.8%) were other histotypes, while in the remaining seven (2.3%) cases the exact histologic subtype was not specified.

The crude and standardized incidence rates of cervix cancer in the period under investigation were 7.3 / 100,000 and 6.6 / 100,000 respectively.

Table 1 shows the distribution of cases in percentages in relation to age, while Table 2 shows the distribution of incidence rates per age-class. Peak incidence occurred at 45–49 years.

Figure 1 depicts the trend of incidence rates in the period 1992–2010; there was no registered substantial modification in the incidence rates oscillating between 5 / 100,000 and 7.7 / 100,000; a stable trend was registered with mortality rates oscillating between 3 / 100,000 and 9 / 100,000. In fact, from the early 1990s until 2000, the authors observed a reduction in the incidence of cervical cancer due to the wide spread of the screening campaign by Papanicolaou test in hospitals and in family counseling. Finally, relative survival at five years from diagnosis was 66.3%.

Analysis of the trend of mean age at disease onset for the same period of time did not reveal any relevant changes. Furthermore, no substantial modifications of the proportions of the histological types mentioned before were found. Table 3 shows the comparison of the incidence and mortality in the province of Sassari with those in other Italian provinces.

There were 41 deaths registered in the period under investigation. Crude overall mortality rate was 1 / 100,000, while standardized mortality rate was 0.7 / 100,000. Mean

---

**Table 1. — Age-class incidence distribution of cervical cancer in north Sardinia, 1992-2010.**

<table>
<thead>
<tr>
<th>Age class (years)</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>0</td>
</tr>
<tr>
<td>15-29</td>
<td>1.93</td>
</tr>
<tr>
<td>30-44</td>
<td>32.15</td>
</tr>
<tr>
<td>45-59</td>
<td>37.94</td>
</tr>
<tr>
<td>60-74</td>
<td>20.58</td>
</tr>
<tr>
<td>75+</td>
<td>7.40</td>
</tr>
</tbody>
</table>

**Table 2. — Age-class incidence and mortality rates of cervical cancer in North Sardinia, 1992-2010.**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Incidence/100,000</th>
<th>Mortality/100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20-24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25-29</td>
<td>1.9</td>
<td>0</td>
</tr>
<tr>
<td>30-34</td>
<td>7.3</td>
<td>0.3</td>
</tr>
<tr>
<td>35-39</td>
<td>9.3</td>
<td>1.2</td>
</tr>
<tr>
<td>40-44</td>
<td>13.9</td>
<td>0.3</td>
</tr>
<tr>
<td>45-49</td>
<td>16.7</td>
<td>1.7</td>
</tr>
<tr>
<td>50-54</td>
<td>15</td>
<td>1.8</td>
</tr>
<tr>
<td>55-59</td>
<td>10.9</td>
<td>0.4</td>
</tr>
<tr>
<td>60-64</td>
<td>11.7</td>
<td>0.8</td>
</tr>
<tr>
<td>65-69</td>
<td>7.7</td>
<td>2.3</td>
</tr>
<tr>
<td>70-74</td>
<td>10</td>
<td>3.2</td>
</tr>
<tr>
<td>75-79</td>
<td>6</td>
<td>1.3</td>
</tr>
<tr>
<td>80-84</td>
<td>5.6</td>
<td>2.8</td>
</tr>
<tr>
<td>85+</td>
<td>8.7</td>
<td>6.5</td>
</tr>
</tbody>
</table>

---

**Figure 1. — Incidence and mortality rates trends of cervical cancer in northern Sardinia, 1992-2010.**
age at death was 63.6 years. The cumulative risk of death between zero and 74 years of age was 0.06%. Table 2 shows the age-class distribution of mortality rates. There was a relevant increase in mortality rates after the sixth decade of life.

Discussion

Cervical cancer is the most incident cancer of the female reproductive apparatus. More than 530,000 new cases and more than 275,000 deaths were estimated in the world in 2008 [1]. Cervical cancer is substantially more common in developing areas of the globe; 85% of the new cases and 88% of the deaths registered in 2008 worldwide occurred in those areas, where it accounts for approximately the 13% of all female malignancies [1, 11-13]. In several countries in Eastern Africa and South-Central Asia, cervical cancer is the most frequent malignancy and cause of neoplastic death in women [1]. The causes of such a geographical variations in incidence and mortality rates of cervical cancer are linked to the high incidence of human papilloma virus (HPV) infections and to the lack of adequate facilities for surveillance, early detection, and treatment in most industrialized countries [12-15].

In Europe there were estimated more than 54,000 new cervix cancer cases in 2008 and the standardized incidence rate of the disease was 10.6 / 100,000 [16]. High risk European regions include North-Eastern countries (Russian Federation, Ukraine, Lithuania, Estonia) and the Balkans (Romania, Serbia, FYR Macedonia). More than 25,000 deaths were estimated in 2008 in Europe (standardized mortality rate: 3.9 / 100,000) [16].

In Italy it is estimated that there will be approximately 2,000 new cases of cervical cancer in 2013 [17]. This signifies that it will be one case for every 163 woman in the country. These figures make cervical cancer the fifth most frequent malignancy in women with less than 50 years of age. However, a steady decrease in incidence rates (-3.8% / year) was registered in the last decade in the country, along with a parallel decreasing of mortality rates for all uterine cancers (- 2.1 % / year) [17]. The five-year survival of patients with cervical cancer was considerably improved in Italy in the last decades, and it is currently estimated in approximately 71% [17].

Concerning Sardinia, the incidence of cervical cancer remained quite stable in the last four decades, according to previous reports. Estimated incidence rates were 10.15 / 100,000, 6.17 / 100,000, and 9.8 / 100,000 in the periods 1965-1969, 1974-1983, and 1992-2000, respectively [3-5]. The standardized incidence rate the present authors found for the period 1992-2010 was 6.6 / 100,000 confirming the steady trend of incidence previously reported. Nevertheless, this rate was considerably inferior to those of several other Italian regions, especially the northern ones (Table 3). Other reports evidenced a discrepancy in cervix cancer incidence rates between the northern, central, and southern regions of Italy; it was estimated that central and southern areas present respectively the -8% and -12% of incidence in comparison to northern regions [17]. These results could be due to the fact that in the Sardinia region, there has been an enhanced screening campaign with more extensive involvement of the population.

Concerning mortality, no relevant modifications in time trends were registered in the present region. The standardized mortality rate in northern Sardinia was 0.7 / 100,000 and it is one of the lowest in Italy; also the cumulative risk to die from the disease was extremely low (0.06%). As regards survival, the relative five-years rate estimated in the present region was 66.3%. This figure was slightly lower to that estimated for the entire country, but also in the case of survival, a certain discrepancy between rates in northern, central, and southern areas was evidenced. Five-year survival rates in the South and the Islands was estimated in approximately 65%, while the corresponding rate in the central regions of Italy was approximately 70% [17].

Conclusions

The incidence and mortality trends of cervical cancer in northern Sardinia remained relatively stable in the last decades. Mortality and cumulative risk of death from the disease were extremely low. Furthermore, survival of patients with cervical cancer was relatively good in the area, sanctioning the adequacy of the preventive and clinical measures employed in the management of the disease.

Table 3. — Comparison of incidence and mortality rates of cervical cancer in North Sardinia with those of other Italian provinces [13-14].

<table>
<thead>
<tr>
<th>Province</th>
<th>Incidence/100,000</th>
<th>Mortality/100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alto Adige</td>
<td>9.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Biella</td>
<td>10.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Ferrara</td>
<td>9.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Firenze</td>
<td>6.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Friuli V.G.</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td>Genova</td>
<td>8.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Macerata</td>
<td>6.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Modena</td>
<td>8.3</td>
<td>1</td>
</tr>
<tr>
<td>Napoli</td>
<td>6.4</td>
<td>-</td>
</tr>
<tr>
<td>Parma</td>
<td>8.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Ragusa</td>
<td>7.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Reggio Emilia</td>
<td>7.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Romagna</td>
<td>9.5</td>
<td>1</td>
</tr>
<tr>
<td>Salerno</td>
<td>7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Sassari</td>
<td>6.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Torino</td>
<td>7.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Trento</td>
<td>4.6</td>
<td>1</td>
</tr>
<tr>
<td>Umbria</td>
<td>6.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Varese</td>
<td>5</td>
<td>0.9</td>
</tr>
<tr>
<td>Veneto</td>
<td>6.3</td>
<td>1</td>
</tr>
</tbody>
</table>
References


Address reprint requests to:
G. CAPOBIANCO, M.D., Ph.D
Gynecologic and Obstetric Clinic
Department of Surgical,
Microsurgical and Medical Sciences
University of Sassari
Viale San Pietro 12, 07100 - Sassari (Italy)
e-mail: capobia@uniss.it
Improving ductoscopy with duct lavage and duct brushing

S. Zervoudis¹, Y. Tamer², G. Iatrakis³, A. Bothou¹, X. Tokou¹, A. Augoulea¹, V. Aranitis¹, X. Spanopoulos³, E. Tomara³, X. Patralexis¹

¹Rea Hospital, Athens (Greece); ²Meet Ghmmr Oncology Center, Mansoura (Egypt); ³Technological University of Athens, Athens (Greece)

Summary

Aim: To assess the combined technique of duct lavage (DL) and duct brushing (DB) performed during ductoscopy in pathological nipple discharge (PND). Materials and Methods: The study was conducted in two hospitals: Rea (Greece) and in Meet Ghmmr Oncology Center (Egypt), from January 2011 to April 2013. Sixty-four women were enrolled. A sample of cells was collected with the use of DB. Afterwards, DL was performed. For each case, liquid cytology was compared to the final histology. Results: From the 19 histological diagnosis of duct ectasia, cytology by DL plus DB (CDLDB) was correct in 17 cases (89.5%). For 28 papillomas, CDLDB was correct in 19 cases (67.9%). For breast cancer (six cases), CDLDB was correct in five cases (83.3%). Also, CDLDB found 45.5% of miscellaneous benign cases. In total, cytology performed by CDLDB was correct in 46 of 64 patients: 71.9%. Thus, the sensitivity of CDLDB ranged from 67% to 90%, depending on the histological diagnosis. Conclusion: This technique showed a high accuracy, in contrast to other studies that used only DL.

Key words: Duct lavage; Duct brushing; Nipple discharge; Breast ductoscopy.

Introduction

Recent interest has focused on the topic of intraductal approaches to the evaluation of breast disease, as it is shown by the literature and by medical conferences [1-3]. An important goal in the management of breast cancer is still the early detection of breast lesions and this involves the use of special techniques.

Mammary ductoscopy is a useful endoscopic technique, which allows direct visual access to the mammary ductal epithelium, through nipple orifice cannulation. This surgical tool is able to estimate intraductal lesions with nipple discharge [4-10].

Duct lavage (DL) is a new method of cell collection used to identify precancerous and cancerous changes within the breast ducts, especially in women who are at high risk for breast cancer [5, 11]. It should be noted that there are various opinions about the utility of DL. In contrast to DL, the data about duct brushing (DB) technique are limited. This new technique may offer the detection of breast cancer, as it could help collect more breast cells with the use of one microbrush.

Several studies were published during the last decade, related to the DL technique. However, as these methods examine only a small number of 15-20 nipple’s ducts, they might fail to detect focal abnormalities [12]. The aim of this study was to assess the sensitivity of the combined technique of DL and DB performed during ductoscopy in women with pathological nipple discharge. For this purpose, the authors compared cytology results with those of final histology, after surgical procedure which is known as microdochectomy or pyramidectomy [13].

Materials and Methods

The study was conducted at Rea hospital in Athens (Greece) and at Meet Ghmmr Oncology Center in Mansoura (Egypt), from January 2011 to April 2013 (Figures 1 and 2). Sixty-four women with pathological nipple discharge were enrolled, after obtaining an informed consent. Only 53 of these women had a complete cytological and histological control. The mean age of the patients was 47 years with a range of 31 to 62 years (Figure 3).

Initially, the patient was placed in the supine position and the skin in the nipple area was cleansed with 70% alcohol. After that, a sample of cells was collected with a microbrush (diameter of 0.5 mm) (Figure 4), which was inserted in the duct through the ductoscope (DB technique) (Figure 5). The handle of the microbrush was rotated to collect the cells. Then the microbrush was pulled out and DL technique was performed (Figure 6).

The first step of DL technique included breast massage, which was performed by the physician, in order to identify the duct where the discharge originated. Then a small catheter was inserted into the breast duct in a maximum depth of one cm. The next step was the injection of saline solution (approximately two to three ml), which was then followed by aspiration. There were no reported serious side effects during the clinical trial.

The recovered sample of cells was placed in Thinprep, according to the manufacturer’s guidelines and was sent for cytological examination (liquid cytology).

Furthermore, for each case, discharge characteristics and mammography, ultrasound, and galactography findings were related to ductoscopic appearance. The results of liquid cytology were compared to the final histology after pyramidectomy (Figure 7). Finally, it is noteworthy that the time required for the procedure of DL and DB was approximately 20-30 minutes.

Results

A comparison between the cytological and histological findings was accomplished. The histological findings were summarized in four categories, which were: duct ectasia (Figure 8 a, b), papillomas and papillomatosis (Fig-
Figure 1: Tools for DL and DB.

Figure 2: Tools for DL and DB.

Figure 3: Age distribution.

Figure 4: Microbrush.

Figure 5: DB technique.

Figure 6: DL technique.
From 19 histological diagnosis of duct ectasia, cytology by DL plus DB (CDLDB) technique was correct in 17 cases (89.5%) and was non-conclusive in two cases (10.5%).

In 28 cases of papillomas and papillomatosis, CDLDB was correct in 19 cases (67.9%). In contrast, in five cases, cytology found duct ectasia, one probable breast cancer, and was non-conclusive in three cases. Therefore, cytology was not successful in nine of 28 cases (32.1%).

Moreover, in six cases of breast cancer, cytology of DL and DB detected five cases (83.3%) and was non-conclusive in one case (16.7%).

Furthermore, from 11 miscellaneous benign cases, cytology was correct in five cases (45.5%), non-conclusive in five cases, and also revealed one duct ectasia. Thereafter, from these 11 cases, cytology was unsuccessful in six cases (54.5%). Interestingly, from these benign cases, thorough
diagnosis was: fibrocystic disease, chronic mastitis or hormonal discharge.

In total, CDLDB was reliable in 46 of 64 patients (71.9%) (Figure 11) and was non-conclusive in 11 cases (17.2%), because the specimen was insufficient for the diagnosis by cytology. Consequently, the failure detection in non-conclusive cases, was not related to DL and DB technique. Also, cytology actually failed in seven cases (10.9%).

Thus, the sensitivity of CDLDB ranged from 67% to 90%, depending on the histological diagnosis.

**Discussion**

Previous studies have attempted to determine the value of DL technique during ductoscopy in the detection of breast cancer. Also, there are many studies which estimated the rate of duct cytology’s sensitivity and specificity (Table 1) [14-21].

Badve et al. assessed the utility of mammary ductoscopy and DL and reported that a considerable number of patients (15-30%) undergoing mastectomy for breast cancer did not have an intraductal component. Furthermore, they concluded that mammary ductoscopy and DL were not effective methods for detecting most forms of breast cancer [12].

Khan et al. in 2004 investigated the relevance between DL cytologic findings and histologic findings in women with known breast cancer, who had undergone mastectomy. They demonstrated that only 37% of fluid-yielding ducts were related to the cancer. They deduced that DL should not be recommended to high risk women. Therefore, imaging modalities in early detection of breast cancer was considered as a more useful procedure [17]. Similar recommendation was documented by Fabian et al. They concluded that further developments are necessary for the evaluation of DL as a risk assessment tool [22]. Also, Khan et al. in 2009 mentioned that the utility of DL was questionable [23].

![Figure 10 a, b: Ductal carcinoma.](image)

![Figure 11: Sensitivity.](image)

**Table 1. — Results of duct cytology sensitivity and specificity in the present study and in other studies.**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>DL &amp; DB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee (2003) [14]</td>
<td>58%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Baitchev et al. (2003) [15]</td>
<td>75%</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>Pritt et al. (2004) [16]</td>
<td>85%</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>Khan et al. (2004) [17]</td>
<td>17%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Beechey-Newman et al. (2005) [18]</td>
<td>87.5%</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Lang et al. (2007) [19]</td>
<td>26.7%</td>
<td>81.1%</td>
<td></td>
</tr>
<tr>
<td>Beechey-Newman et al. (2008) [18]</td>
<td>94.2%</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Koonstra et al. (2009) [21]</td>
<td>16.7%</td>
<td>66.1%</td>
<td></td>
</tr>
<tr>
<td>Zervoudis et al. (2013)</td>
<td>71.9%</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Conversely, some studies summarized that atypia identified by DL was not related to a higher risk of developing breast cancer [24, 25]. Moreover, another study indicated that the ducts, which were found on DL had atypia, however repeat lavage in some cases failed to demonstrate atypia a second time [26].

In contrast to the studies, which showed that the usefulness of DL is limited, latest studies pointed out that the combination of visualization and DL is the most accurate predictor of diagnosis. Specifically, Vaughan et al. reviewed their experience of 89 cases of patients with pathologic nipple discharge, who had undergone ductoscopy and collection of ductal washings specimens. They showed that the highest predictive value for the diagnosis of papilloma provided by visualization and cytology examination of washings [27].

In addition, other investigators demonstrated that DL is a well-tolerated procedure for repeated evaluation to obtain material for cytology and to create a biobank for future studies [28, 29].

Although, there are restricted data about DB technique, Beechey-Newman et al. significantly improved the sensitivity of cytologic examination by using a microbrush. In this study, 50 patients participated and the results of microbrush cytology compared to those of DL. Interestingly, the sensitivity of brushing’s cytology for the diagnosis of papilloma was 87.5%, while the sensitivity of DL was 18% [18]. According to our aforementioned data, with a relative small number of cases, the sensitivity of DL plus DBs ranges, roughly from 67% to 90%, depending on the histological diagnosis.

Conclusion

Ductoscopy plays a very important role as a useful tool for the detection of breast lesions, especially in women who have pathological nipple discharge. During ductoscopy, cytology provides objective assessment of the lesions. In this study, the combination of DL and DB during breast ductoscopy for pathological nipple discharge showed increased accuracy and sensitivity. Summarizing, this technique is an enough reliable procedure and should be recommended.

References


Address reprint requests to:
S. ZERVoudIS, M.D., PhD
Rea Hospital, 383-Suggrou Avenue,
Palaio Faliro 17564 (Greece)
e-mail: szervoud@otenet.gr
Human papilloma virus (HPV) is the most common sexually transmitted disease, with a prevalence of 42.5% in women aged 14-59 years and is associated with cervical cancer [1]. Quadrivalent and bivalent HPV vaccines were licenced for use in Turkey in 2006-2007, but immunization programs targeting school-aged girls and young women that did not continue with school in the same city from January to September 2011. All the students answered the questionnaire voluntarily and independently. Results: The participants had low level of knowledge about the risk factors for cervical cancer. Smoking is the major risk factor that was known by the participants (65%). Proportion of the participants that were aware of pap smear test and HPV were 65% and 17% respectively. A small proportion of young women had knowledge regarding protection from HPV. Educational stream, educational level, family income, and family size had significant association knowledge level ($p<0.05$). Conclusion: There has not been any improvement in HPV and risk factor of cervical cancer awareness in young women. Health members of the National Cancer Control Programme and delegates of the vaccine corporations have major work in order to increase the level of knowledge so that general public can easily take preventative measures.

Key words: Cervical cancer; HPV; Knowledge level.

Introduction

Human papilloma virus (HPV) is the most common sexually transmitted disease, with a prevalence of 42.5% in women aged 14-59 years and is associated with cervical cancer [1]. Quadrivalent and bivalent HPV vaccines were licenced for use in Turkey in 2006-2007, but immunization programs targeting school-aged girls and young women that did not continue with school in the same city from January to September 2011. All the students answered the questionnaire voluntarily and independently. Permission to use the questionnaire form was obtained from the Ethics Committee of the University. The study’s objective was explained orally to the participants at the study site. First part of the questionnaire collected information on age, education level, category of the education –social, medical or engineering, place of permanent residence, family income, and family size. The other part of the questionnaire included the most prevalent cancer types among the Turkish women, usual age of occurrence of cervical cancer, risk factors, role of sexual intercourse, and contraception methods in causing the cancer, and whether aware of pap smear test, HPV, and HPV vaccine. The questions were developed based on previously established facts for cervical cancer [4-6]. In most of the questions ‘yes’, ‘no’, or appropriate multiple choices were given as answers.

The authors used SPSS version 19 to analyze the data. $T$ test and $\chi^2$ tests were used where appropriate. A level of $p<0.05$ was considered statistically significant.

Results

Table 1 describes socio-demographic characteristics of the 650 participants. Mean age was 19.7 ± 1.8 (mean ± SD) years, education of 60% of them were from science category, majority of the participants (90%) came from families with less than three members.

Knowledge level of the young women about cervical cancer and related information is presented in Table 2. The participants had low level of knowledge regarding the
risk factors for cervical cancer. Smoking was the major risk factor known by the participants (65%). Proportion of the participants who were aware about pap smear test and HPV were 65% and 17%, respectively. Few proportion of young women had the knowledge for protection from HPV.

Table 3 shows the association between knowledge level and demographic variables. Educational stream, educational level, family income, and family size had significant association knowledge level ($p < 0.05$).

**Discussion**

The present study revealed a low level of knowledge of the graduate and postgraduate students of Canakkale 18 March University which is located in the western part of Turkey and represents a photograph of all Balkan States and Anatolia. Most of the risk factors for cervical cancer were recognized by much less than 50% of the study participants. The present analysis showed that students from the science disciplines and those that had a higher education level had significantly better awareness level about cervical cancer and HPV. Those young women are more interested and research and/or read about medicine and science related subjects.

Out of all the respondents 35%, 20%, and 22%, respectively, could identify having multiple partners, early onset of sexual intercourse, and parity as risk factors of cervical cancer. In an Indian study, young women identified these risk factors below 30% [6], while in a Malaysian study, women aged 21-56 years could not recognize any of these risk factors [7]. Studies about the knowledge level in etiologic involvement of sexually transmitted disease (STDs) and HPV in cervical cancer vary in different countries.

However maximum level is 31.5% in Korean survey [8]. A strong association was established in the present study between personal hygiene and nutrition: 27% and 30%, respectively. Great interest in herbal medicine currently may be the cause of these percentages. Awareness about the carcinogenic effect of smoking as a cause had 65% of true answer in the respondents.

A large number of young women (65%) in the present study had never heard of ‘pap smear test’, but 83% of them were not aware of HPV. Surveys about awareness of HPV throughout the world differ in the range of 7.9% in Ghana to 40% in United States [9, 10]. Increased education level, family income, and age positively influence the awareness of pap smear test [11, 12]. A review from different countries reported that overall, general public has low level knowledge of HPV infection [13]. A qualitative study by Bingham et al. reported low level of knowledge on HPV and cervical cancer among children, parents, teachers, community leaders, and even health service providers of developing countries [14].

In conclusion, despite the advent of vaccines to prevent HPV, the impact of cervical cancer deaths especially in developing countries, has not shown any improvement in
HPV and risk factors of cervical cancer awareness in young women. Health members of the National Cancer Control Programme and delegates of the vaccine corporations have major work in order to increase the level of knowledge so that general public can easily take preventative measures.

References

Address reprint requests to:
E. KOŞAR, M.D.
Çanakkale 18 March University
Department of Obstetrics and Gynecology
Terzioglu Kampüsü, Çanakkale (Turkey)
e-mail: dremineay@hotmail.com
The immune function differences and high-risk human papillomavirus infection in the progress of cervical cancer

Changdong Li1*, Cui Ma2*, Weiyuan Zhang2, Jiandong Wang2
1Department of Gynecology & Family planning, Beijing; 2Department of Gynecology Oncology, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing (China)

Summary
Investigation: To study the differences immune function in normal cervix, cervical intraepithelial neoplasia (CIN) and cervical cancer tissue, and study the relationship between human papillomavirus (HPV) infection and cervical local immune function. Materials and Methods: This study determined the form, quantity, distribution, and intensity of CD4-Th cells, S-100+ dendritic cells (DCs) and CD57+NK cells in the normal cervical tissue, CIN cervical tissue, and cervical cancer by histopathological and image analyses. Results: The immune function was differences in the progress of cancer genesis. The numbers of the CD4+ Th cells, S-100+ DCs, and CD57+NK cells increased with the progress of the disease in CIN, but when cancer occurred, immune cells decreased in local cervical tissue. Conclusion: From lesion precancerous to infiltrating carcinoma, the form, quantity, and intensity of expression of immune cells changed, which may indicate that the cervical local immune function has changed. Furthermore, high-risk HPV infections are more active in local immune function.

Key words: Cervical cancer; Cervical intraepithelial neoplasia; Immune function; High-risk HPV.

Introduction
Cervical cancer is secondary in women’s most common malignant tumor around the world [1]. It is clear that high-risk human papillomavirus (HPV) infection is a necessary element to cervical cancer [2]. Cervical intraepithelial neoplasia (CIN) reflects a continuous and progressive process of cervical cancer, thus is considered as a cervical precancerous lesion [3]. After high-risk HPV virus infects the genital tract continuously, high-grade squamous intraepithelial lesions would develop and progress to invasive cervical cancer after about eight to 12 years [4]. In this process, the body’s immune function has also changed [5]. Cell-mediated immunity plays a major role in the HPV-induced immune responses, and the mainly effector cells are T cells, NK cells, macrophages, and antigen-presenting cells [6, 7]. Through histopathological and image analyses, the authors determined the form, quantity, distribution, and intensity of CD4+ Th cells, S-100+ dendritic cells (DCs) and CD57+NK cells in the normal cervical tissue, CIN tissue, and cervical cancer tissue.

Materials and Methods
Cases and collection of tissues
From January 2009 to August 2012, cervical tissues were obtained, after acquiring written informed consent under a study protocol approved by the institutional review board of Beijing Obstetrics Gynecology Hospital, from patients who had undergone therapeutic surgery. Each sample was divided into two parts, one part was paraffin blocked and the other part was quickly frozen in liquid nitrogen and stored at −80°C in a freezer until further study. All patients did not accept radiotherapy or chemotherapy before pathological examination. Twenty-four cases of normal cervical tissue were obtained from patients who accepted hysterectomy because of uterine fibroid. The cervical Thineprep cytological test (TCT) were negative, and high-risk HPV were positive or negative as tested by HC-II. There were 78 CIN cases (including HPV-positive or HPV-negative), including 28 cases of CIN I, 26 cases of CIN II, and 24 cases of CIN III. There were 48 cases of cervical squamous cancer (all of HPV positives), including 24 cases of early invasive carcinoma and 24 cases of invasive carcinoma.

Immunohistochemistry analysis
Paraffin-embedded cervical tissues specimens were sliced to three-μm thicknesses. The Th cells, DCs, and NK cells were immunohistochemistry stained using mouse-anti-human monoclonal CD4, S-100 antibodies and rabbit-anti-human polyclonal CD57 antibodies, respectively. The steps of immunohistochemistry were followed according to the manufacturer instructions. In brief, following deparaffinization, the endogenous peroxidase activity was quenched by 0.3% hydrogen peroxide for five minutes followed by washing. Primary antibodies diluted at 1/50, were incubated for 30 minutes, and then incubated with secondary antibody for 30 minutes. The revelation was done with SABC detection kit. Finally, diaminobenzidine tetrachloride (DAB) was applied for five minutes. The known positive tissues were used as positive control while replacement of the antibodies by Tris buffer was used as negative control for the procedure. Immunohistochemistry results were evaluated either as positive or negative under a light microscope. Cytomembrane (CD4, CD57) or cytoplasm (S-100) was positive for brown coloring. First, each antibody-positive cell’s shape and distribution in human cervical intraepithelia were observed. Then quantitative analysis was done. One section was taken from each case and each antibody, cells were selected from the intensive area under low magnification microscopy, then the number of positive cells of three non-consecutive high power fields was recorded.

*Contributed equally to this manuscript.

Revised manuscript accepted for publication November 13, 2013
CD4+ Th cells was not statistically significant (antibody expression was much lower. Cells located between cancer cells or around cancer nest and were various, and antibody expression decreased. In invasive tissue, CD4+ Th cells distributed in the normal cervical tissue (x¯± s). AOD of CIN II group was higher than that of other groups, which was statistically significant (p < 0.05). The AOD of CIN II group was higher than that of other groups, which was statistically significant (p < 0.05).

Using a DMLA microscope and QWIN image statistical analysis system, the average optical density (AOD), and the integral optical density (IOD) of positive cells were measured. Average optical density (AOD) demonstrates the color depth of the positive target in the tissue. IOD reflects the total tissue’s content of the positive target. It can reflect both the optical density and the area of positive target. These two indicators could be more objective and accurate on the quantitative analysis.

Statistical analysis

Dates appeared as mean ± standard deviation (x¯ ± s). The One-Way ANOVA and Multiple Comparison in ANOVA and Independent Samples T Test were used to analyze the differences between the groups, using the SPSS program (Version 11.5). A difference was considered significant when the p-value was less than 0.05.

Table 1. — Number, AOD, and IOD of CD4+ Th cells in cervical tissue (x¯ ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of positive cells /HP</th>
<th>Average optical density (AOD)</th>
<th>Integral optical density (IOD) ×10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
<td>2.57±1.04</td>
<td>170.72±15.72</td>
</tr>
<tr>
<td>CIN I</td>
<td>28</td>
<td>3.14±2.18</td>
<td>192.17±20.15</td>
</tr>
<tr>
<td>CIN II</td>
<td>26</td>
<td>3.47±2.21</td>
<td>207.73±14.03</td>
</tr>
<tr>
<td>CIN III</td>
<td>24</td>
<td>2.86±1.35</td>
<td>185.11±18.50</td>
</tr>
<tr>
<td>Early cervical</td>
<td>24</td>
<td>3.83±1.65</td>
<td>186.04±23.03</td>
</tr>
<tr>
<td>Invasive cervical</td>
<td>24</td>
<td>2.80±2.57</td>
<td>189.31±13.23</td>
</tr>
<tr>
<td>F</td>
<td>1.841</td>
<td>13.337</td>
<td>3.518</td>
</tr>
</tbody>
</table>

CD4+ Th cells distributed in the normal cervical subcutaneous stroma, little quantity, small, and nearly round. From CIN I to CIN II, then to CIN III tissue, antibody expression increased, the number raised, the cell was much bigger, and the majority distributed in the subcutaneous tissue. In early cervical cancer tissue, CD4+ Th cells were slightly more than normal and CIN groups, cell sizes were various, and antibody expression decreased. In invasive cervical carcinoma tissue, a small number of CD4+ Th cells located between cancer cells or around cancer nest and antibody expression was much lower.

One-way ANOVA among the six groups, the number of CD4+ Th cells was not statistically significant (p = 0.108 > 0.05), the AOD and the IOD were statistically significant (p < 0.05, Table 1). Multiple Comparison in ANOVA among the six groups, the number of CD4+ Th cells in early cervical cancer tissues was greater than that of normal cervical tissues, which was statistically significant (p = 0.008, <0.05). The AOD of normal group was lower than that of other groups, which was statistically significant (p < 0.05). The AOD of CIN II group was higher than that of other groups, which was statistically significant (p < 0.05). The integral optical density (IOD) of normal group was lower than that of CIN I group and early cervical cancer group, which was statistically significant (p < 0.05). Among CIN I group and invasive cervical cancer group, CIN II group, and early cancer group, there was statistical significance (p < 0.05). The other comparisons were not statistically significant (p > 0.05).

Among the HPV(+) and HPV(-) cases in the normal group, the number of CD4+ Th cells was not statistically significant (p = 0.864 > 0.05). Among the HPV(+) and HPV(-) cases in the CIN and cancer group, the number of CD4+ Th cells in HPV(+) was less than that of HPV(-), which was statistically significant (p = 0.017 < 0.05).

Among the HPV(+) and HPV(-) cases in the normal group (p = 0.419 > 0.05) and in the CIN group (p = 0.776 > 0.05), the AOD of CD4+ Th cells was not statistically significant, as was the IOD.

Statistical analysis

One-Way ANOVA among the six groups, the number of CD4+ Th cells was not statistically significant (p = 0.108 > 0.05), the AOD and the IOD were statistically significant (p < 0.05, Table 1). Multiple Comparison in ANOVA among the six groups, the number of CD4+ Th cells in early cervical cancer tissues was greater than that of normal cervical tissues, which was statistically significant (p = 0.008, <0.05). The AOD of normal group was lower than that of other groups, which was statistically significant (p < 0.05). The AOD of CIN II group was higher than that of other groups, which was statistically significant (p < 0.05). The integral optical density (IOD) of normal group was lower than that of CIN I group and early cervical cancer group, which was statistically significant (p < 0.05). Among CIN I group and invasive cervical cancer group, CIN II group, and early cancer group, there was statistical significance (p < 0.05). The other comparisons were not statistically significant (p > 0.05).

Using a DMLA microscope and QWIN image statistical analysis system, the average optical density (AOD), and the integral optical density (IOD) of positive cells were measured. Average optical density (AOD) demonstrates the color depth of the positive target in the tissue. IOD reflects the total tissue’s content of the positive target. It can reflect both the optical density and the area of positive target. These two indicators could be more objective and accurate on the quantitative analysis.

Statistical analysis

Dates appeared as mean ± standard deviation (x¯ ± s). The One-Way ANOVA and Multiple Comparison in ANOVA and Independent Samples T Test were used to analyze the differences between the groups, using the SPSS program (Version 11.5). A difference was considered significant when the p-value was less than 0.05.

Table 2. — Number, AOD and IOD of S-100+ DC cells in human cervical tissue (x¯ ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of positive cells /HP</th>
<th>Average optical density (AOD)</th>
<th>Integral optical density (IOD) ×10⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
<td>6.47±5.93</td>
<td>143.94±12.25</td>
</tr>
<tr>
<td>CIN I</td>
<td>28</td>
<td>7.95±4.72</td>
<td>159.32±12.78</td>
</tr>
<tr>
<td>CIN II</td>
<td>26</td>
<td>7.77±4.14</td>
<td>162.62±14.82</td>
</tr>
<tr>
<td>CIN III</td>
<td>24</td>
<td>12.92±5.41</td>
<td>154.76±9.25</td>
</tr>
<tr>
<td>Early cervical</td>
<td>24</td>
<td>20.97±7.50</td>
<td>145.22±9.66</td>
</tr>
<tr>
<td>Invasive cervical</td>
<td>24</td>
<td>19.61±7.71</td>
<td>146.58±11.70</td>
</tr>
<tr>
<td>F</td>
<td>35.869</td>
<td>16.690</td>
<td>20.977</td>
</tr>
</tbody>
</table>

Using a DMLA microscope and QWIN image statistical analysis system, the average optical density (AOD), and the integral optical density (IOD) of positive cells were measured. Average optical density (AOD) demonstrates the color depth of the positive target in the tissue. IOD reflects the total tissue’s content of the positive target. It can reflect both the optical density and the area of positive target. These two indicators could be more objective and accurate on the quantitative analysis.

Statistical analysis

Dates appeared as mean ± standard deviation (x¯ ± s). The One-Way ANOVA and Multiple Comparison in ANOVA and Independent Samples T Test were used to analyze the differences between the groups, using the SPSS program (Version 11.5). A difference was considered significant when the p-value was less than 0.05.

S-100 immunohistochemical staining results

S-100 expressed in the cytoplasm, showing brown. In normal cervical tissue, most of the S-100+ DC cells were similarly round, and some were strip-type with branches, which distributed in the subcutaneous tissue near the epithelia. In CIN I and CIN II, S-100+ DC cells were strip-type mostly with branches. The number was similar to the normal group, and mostly distributed around the lesion; antibody expression increased. In CIN III, S-100+ DC cells still had branches, and the branches enlarged; the number of S-100+ DC cells had a slight increase and antibody expression showed little change. In the early cervical cancer tissue, almost all of the S-100+ DC cells were deformed, which appeared like earthworms and their distribution was concentrated. In the invasive cervical cancer tissue, deformation of the S-100+ DC cells decreased, were almost round, with fewer branches, and the number and antibody expression were almost the same as the early cancer.

One-way ANOVA among the six groups, the number of S-100+ DC, the AOD, and the IOD were statistically sig-
Table 3. — Number, AOD and IOD of CD57+ NK cells in human cervical tissue (x ± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>The number of positive cells /HPF</th>
<th>Average optical density (AOD)</th>
<th>Integral optical density (IOD) ×10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>24</td>
<td>9.06±3.14</td>
<td>159.63±4.69</td>
<td>1.12±0.64</td>
</tr>
<tr>
<td>CIN I</td>
<td>28</td>
<td>20.83±12.45</td>
<td>149.42±7.88</td>
<td>4.12±3.25</td>
</tr>
<tr>
<td>CIN II</td>
<td>26</td>
<td>23.08±9.35</td>
<td>151.01±8.77</td>
<td>4.38±1.35</td>
</tr>
<tr>
<td>CIN III</td>
<td>24</td>
<td>42.58±16.94</td>
<td>155.60±7.05</td>
<td>8.24±4.09</td>
</tr>
<tr>
<td>Early cervical cancer</td>
<td>24</td>
<td>3.40±1.77</td>
<td>157.76±9.77</td>
<td>0.52±0.31</td>
</tr>
<tr>
<td>Invasive cervical cancer</td>
<td>24</td>
<td>27.89±10.75</td>
<td>157.52±7.84</td>
<td>6.08±3.56</td>
</tr>
<tr>
<td>F</td>
<td>61.169</td>
<td>9.433</td>
<td>41.283</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

The immune function differences and high-risk human papillomavirus infection in the progress of cervical cancer

Significant (p < 0.05, Table 2). Multiple comparison in ANOVA among the six groups, the number of S-100+ DC cells of normal group, CIN I group, and CIN II group were not statistically significant (p > 0.05). The same result was seen between the early cervical cancer group and the invasive cancer group. The AOD of normal group was lower than that of CIN groups, which was statistically significant (p < 0.05). There were no statistically significant (p > 0.05) between normal group and cancer group. The IOD of normal group, CIN I group, and CIN II group were not statistically significant (p > 0.05). The IOD of normal group was lower than that of CIN III group and cancer groups, which were statistically significant (p < 0.05).

Among the HPV(+) and HPV(-) cases of the normal group, the number of S-100+ DC cells in HPV(+) was less than that of HPV(-), which was statistically significant (p = 0.022 < 0.05). Among the HPV(+) and HPV(-) cases of the CIN and cancer group, the number of S-100+ DC cells in HPV(+) was more than that of HPV(-), which was statistically significant (p = 0.006 < 0.05).

Among the HPV(+) and HPV(-) cases of the normal group (p = 0.419 > 0.05) and the CIN group (p = 0.950 > 0.05), the AOD of S-100+ DC cells was not statistically significant. Among the HPV(+) and HPV(-) cases, the IOD of S-100+ DC cells was not statistically significant (p = 0.206 > 0.05) in the normal tissue, but was statistically significant (p = 0.046 < 0.05) in the CIN tissue.

**CD57 immunohistochemical staining results**

CD57 expressed on the NK cell membrane, showing brown. In normal cervical tissue, CD57+ NK cells were distributed in the subcutaneous tissue. In CIN I and CIN II tissue, the number of CD57+ NK cells obviously increased, most CD57+ NK cells distributed in the subcutaneous tissue near the epithelia basilar part, clustered, and occasionally infiltrated into the epithelial tissue. In CIN III tissue, much more CD57+ NK cells aggregated. In the early cancer tissue, the number of positive cells significantly reduced, antibody expression slightly changed. In the invasive carcinoma tissue, the number of CD57+ NK cells increased significantly than in the early cancer tissue, but decreased compared to CIN III tissue, and most cells distributed around the cancer nest.

One-way ANOVA among the six groups, the number of CD57+ NK cells, the AOD and the IOD were statistically significant (p < 0.05, Table 3). Multiple comparison in ANOVA among the six groups, the number of CD57+ NK cells of normal group was less than that of CIN groups and cancer groups, which was statistically significant (p < 0.05). The number of CD57+ NK cells of CIN I group and CIN II group was similar, which was not statistically significant (p > 0.05). The AOD of normal group was higher than that of CIN groups, which was statistically significant (p < 0.05), but was not statistically significant (p > 0.05) compared to the cancer groups. The IOD of normal group and the early cervical cancer group was not statistically significant (p > 0.05), and the same result was seen between CIN I group and CIN II group. The other two-two comparisons were statistically significant (p < 0.05).

Among the HPV(+) and HPV(-) cases of the normal group (p = 0.925 > 0.05) and the CIN group (p = 0.065 > 0.05), the number of CD57+ NK cells was not statistically significant.

Among the HPV(+) and HPV(-) cases of the normal group (p = 0.642 > 0.05) and the CIN group (p = 0.141 > 0.05), the AOD of CD57+ NK cells was not statistically significant, as was the IOD.

**Discussion**

Cell mediated immunity is the main anti-tumor immunity. T cells play a critical role in the immune function [8]. Soluble tumor antigens were in taken and processed into short peptides by antigen-presenting cells (APC), and then by the MHC-II type of antigen presenting and activated CD4+ T cells, by secreting cytokines to promote the special cytotoxic effect of CD8+ T cells [9]. In many cases, CD4+ Th cells are essential for inducing anti-tumor immune responses, as well as for the maintenance of immunological memory. CD4+ Th cells can also directly kill tumor cells [10,11]. This study found that a small number of CD4+ Th cells existed in cervical tissue and the number of positive cells among the groups was not statistically significant, as observed with the naked eye; however, the optical density difference mechanically detected was statistically significant, which showed that the AOD and the IOD of positive cells are more objective and accurate for the quantitative analysis. In CIN tissue, CD4+ Th cells gradually increased as the illness worsened. The CD4+ Th cells of the early cancer group were more than the invasive cancer group, the reason of which may be related to, or the tumor growth process, especially in advanced tumor. However after removing the tumor by surgery or giving another effective treatment with consequent remission, the patient’s
immune function can recover on various levels [12]. At present, tumor immune suppression is mainly related to the abnormal inhibitory cells and lymphokines, immune inhibitory factor, and effect of cell dysfunction, and so on [13]. In addition, this study found in CIN group, that the CD4+ Th cells of the cases infected with HPV increased in number, which showed that HPV infection gathered the CD4+ Th cells in the lesion area to play a role in immune function. Moreover, the higher HPV testing value was, the more CD4+ Th cells were generally found.

DCs are not only full-time APC, but also currently known as the most powerful antigen-presenting cells. Less of them exist in the body, about less than 1% of the total number of peripheral blood mononuclear cells. They can intake various types of antigens and express rich major histocompatibility complex (MHC) I, II molecules, co-stimulatory molecules, and adhesion molecules. They can also stimulate the original T cell proliferation in vitro and vivo and induce specific cytotoxic T lymphocyte (CTL) generation. Accordingly they regulate the body’s immune response [14-15]. Therefore detection of the DC’s quantity, appearance, distribution, and expression in cervical tissue can reflect the immune function state in local cervical tissue. This study found that many S-100+ DC were in the lesion tissue, and mostly deformed with dendritic branches which confirmed their active function. While in normal tissue they were almost nearly round and less in number, which meant that they were not active. In the invasive cervical cancer tissue, a larger number of S-100+ DC had few changes in morphology and immunosuppressive phenomena existed. Therefore, S-100+ DC is closely related to the occurrence and development of cervical precancerous lesions and cervical squamous cell carcinoma [16]. The present authors also found that in normal and CIN tissue S-100+ DC of HPV (+) cases were obviously more than that of HPV (-) cases. It was shown that HPV infection could gather S-100+ DC and then S-100+ DC could uptaken antigen to acquire information.

NK cells are a kind of lymphocytes. When they kill target cells, they do not require specific antibodies to participate in and do not require antigen presentation. They can be quickly activated, then suppress and destruct all kinds of tumor cells. IL-2, IFN-γ and IFN-α can enhance the NK cells’ activity, especially IL-2. NK cells contain a variety of anti-factors. Recently it was found that NK cells are one of the precursors of the IL-2 activated killer cells (LAK). Bearing cancer patients’ suppressor T-cells, suppressor monocytes, suppressor macrophages, and tumor cells can produce PGE-2, which can inhibit the activity of NK cells. Therefore, the NK cells activity of most cancer patients are decreased. It indicated that, the immune defense system will not have serious consequences, but persistent infection can cause tumor cell immune competence decreased, which may be due to the depletion of the immune system or function decline, or immune escape of tumor cells and tumor micro-environment depressant effect to immune cells [22]. In addition, although there is substantial evidence that HPV infection and HPV-related tumors can cause humoral immunity and cell immunity, it is not yet clear whether these immune responses are the consequences of disease or the key process to remove the disease. Most of the normal immune persons can effectively clear the HPV infection and will not have serious consequences, but persistent infection is likely to evolve into cervical cancer.

As the above mentioned study regarding Th cells, dendritic cells, and NK cells surface marker molecules suggested that human cervical precancerous local lesions had more active immune function compared to the cancer tissue. Therefore, it is necessary to give cell immunity therapy to the patients with precancerous lesions and early cervical cancer, in order to enhance their antigen-presenting cells’ and immune effector cells’ function, which will not develop into cervical cancer. Currently, DC vaccine used in cancer immune therapy has had preliminary results in animal experiments and some clinical trials, however, it is far from the clinical treatment requirements. This study also suggests that the study of NK cell vaccine has broad prospects. It is believed that cervical cancer will be the first malignancy that people prevent and eradicate by a variety of methods.
Acknowledgements

This study was supported by a grant “High-level Talents Project” (2013-3-029) and by a grant “Hundred Level of Health Talents”, from Beijing Municipal Health Bureau.

References


Address reprint requests to:
WEIYUAN ZHANG, M.D.
Beijing Obstetrics and Gynecology Hospital,
Capital Medical University,
No.251 Yao Jiayuan Road, Chaoyang District,
Beijing, 100026 (PR China)
e-mail: zhangwy9921@hotmail.com
Protection of ovarian function during chemotherapy for ovarian cancer

X. Tianmin, C. Weiqin, W. Shuying, L. Yang, C. Manhua
Department of Obstetrics and Gynecology, The Second Hospital of Jilin University, Changchun City (China)

Summary
The protection of ovarian function during chemotherapy is an urgent issue to be resolved after the fertility preserving surgery on patients with ovarian cancer. The paper summarizes and analyzes the research progress on the protective measures in the aspects of gonadotropin releasing hormone analogue (GnRHa), cell protecting agents, and traditional Chinese medical science and drugs.

Key words: Ovarian cancer; Protection; Ovarian function.

Introduction
Ovarian cancer is a malignant tumor that seriously jeopardizing women’s health. Surgical-pathological staging, cytoreductive surgery for patients in advanced stage, and platinum-based chemotherapy are among the milestone progresses in the diagnosis and treatment of ovarian cancer. However, the trend of a younger average age of patients with ovarian cancer is obvious [1], and more and more patients are afflicted with this disease during child-bearing period, even younger girls and during puberty; fertility preserving surgery is often required in such patients. The chemotherapy is an indispensable important adjuvant therapeutic measure after most ovarian cancer surgeries. Apart from myelosuppression, toxic and side effects on the digestive tract, urinary system, heart, nervous system, and respiratory system, chemotherapy will also cause a negative influence to various degrees on ovarian function, impeding the achievement of true target of preserving fertility [2]. Therefore, the protection of ovarian function during chemotherapy is an issue acquiring much attention and must be resolved urgently.

The main influences on ovarian function by chemotherapy are the lowering of ovarian reserve and premature ovarian failure [3]. The manifestations include less menstrual volume, menopausal syndrome, temporary or permanent amenorrhea, and sterility.

The protective measures for ovarian function can be taken before and during surgery, such as freezing preservation of ovum or fertilized ovum, frozen section and transplantation of ovarian cortex, in vitro maturation of ovarian cortex, ovary transplantation, etc. However, these techniques may cause reimplantation of tumor cells, ethical issues from coupling embryo ovary transplantation, or potential technical problems [4], and cannot be applied as “regular” therapeutic measures. The current focus is the protection of ovarian function during postoperative chemotherapy in ovarian cancer patients [5]. This paper will discuss the influencing factors of ovarian function and protective measures during chemotherapy, with a view to minimize the adverse effects that chemotherapy has on ovarian function by achieving the true significance of fertility preservation [6].

Influencing factors of ovarian function by chemotherapy

Age
As women become older, the number of ovarian follicles gradually decrease: it is about two million at birth, down to 300-500 thousands during puberty, and in a lifetime, only about 400-500 of them mature and are released during ovulation, and all others degenerate automatically; therefore, the older the patient is undertaking chemotherapy, the less the remaining number of ovarian follicles will be, the poorer the ovarian reserve will be, and the higher the probability of premature ovarian failure after chemotherapy will be. It is reported that the probability of amenorrhea caused by same chemotherapeutic program on patients less than 36 years of age is 35%-40%, and 90%-100% in those above 36 years of age [7].

Drug type
Among five types of common chemotherapeutic drugs (alkylating agent, platinum-based, antimetabolite, antibiotic, alkaloid), the alkylating agent has the obvious gonad toxicity; some researches show that compared to control group without administrating drugs, the incidence of premature ovarian failure caused by alkylating agent increases by 4.52 times, and 1.77 and 1.22 by platinum-based and alkaloid, respectively [8]. The gonad toxicities of antimetabolite and antibiotic are lower. The alkylating agent not only interrupts the maturation of ovarian follicles, causing temporary amenorrhea, but also may destroy the primordial follicles, resulting in permanent amenorrhea and premature ovarian failure [9].
Protection of ovarian function during chemotherapy

The damage to ovarian function and premature ovarian failure are major long-term side effects of chemotherapy. During chemotherapy after ovarian cancer surgery, the protective measures for ovarian function mainly include gonadotropin releasing hormone analogue (GnRHa), cell protecting agents, traditional Chinese medical science and drugs, and ovarian protection [10].

The chemotherapeutic drugs mainly act upon the cells with active proliferation; the oocytes have a strong cell division and proliferation potential, therefore, they are very likely to be infringed by the chemotherapeutic drugs. The chemotherapeutic drugs may destroy a large amount of developing ovarian follicles, causing an extreme exhaustion of following primordial follicles. Therefore, the purpose of drug treatment is to maintain the ovary static, to stop the development of follicles [11], to prevent the follicles from entering the chemotherapeutic sensitive stage, so as to protect more primordial follicles.

GnRHa

GnRHa is a kind of synthetic polypeptide substance, capable of competitively combining with luteinizing hormone-releasing hormone (LHRH) receptor of the hypophysis, to suppress the secretion of endogenous luteinizing hormone (LH) and follicle-stimulating hormone (FSH), resulting in gonadotropic function suppression; it can also act on the ovary directly, blocking the synthesis of estrogens and progestogens. GnRHa’s ability to protect the ovary is related to the suppression of secretion of endogenous gonadotropin, capable of arresting the development and maturation of primordial follicles, and making the ovary relatively static, hence reducing damage to mature follicles by chemotherapeutic drugs [12]. It can also reduce ovarian blood circulation, and subsequently reduce the local concentration of chemotherapeutic drugs in the ovary, and afterwards reduce their damage to ovarian function. Recchia et al. reported a clinical stage II research including 64 patients with early-stage breast carcinoma before menopause (at 27-50 years of age) were selected. At three weeks after surgery, they were administered long-acting GnRHα before and during chemotherapy, one injection every 28 days, lasting one year in total. The results from average 55 months of follow-up show that 86% patients recovered their normal menstruation, 84% patients were cured, and 94% patients survived, indicating that the GnRHa as adjuvant chemotherapy, not only do not affect the curative effect, but also protect the ovarian function [13]. Besides cyclophosphamide (CTX) chemotherapy on rhesus monkeys, Ataya et al. also administered GnRHa to them; when compared to the control group without GnRHa, it was proved that GnRHa can reduce the damage to primordial follicles caused by CTX [14]. Tanyi et al. also administered GnRH antagonists during CTX chemotherapy on rats; when compared to the control group without administration, they found that GnRH antagonists can protect the ovary during chemotherapy; the protection mechanism may be by strengthening BCL-2 apoptosis resistance and suppression of caspases-3 apoptosis path [15]. Blumenfeld performed clinical experiments in 60 cases and 60 control cases of 14-40 years-old patients, in order to evaluate whether GnRHa can protect the ovary under combined chemotherapy, and the result was positive [16].

Cell protecting agents

With regards to the signalling pathways of oocyte apoptosis caused by chemotherapy, the sphingomyelin pathway is considered important. Its main metabolites are ceramide, sphingosine, and sphingosine-1-phosphate (SIP), which can transform each other. SIP is the active metabolite of ceramide, capable of causing cell proliferation and suppressing apoptosis [17]. Radiochemotherapy will cause the generation of ceramide in egg cells to increase, and induce apoptosis, and SIP can block the induction action of ceramide. Doyle et al. [18] explored the prevention of chemotherapy-induced ovarian damage by sphingosine-1-phosphate in rat model, as well as its mechanism; the results show that SIP can prevent the damage to ovarian function of rat caused by CTX, and its apoptosis resistant action may be achieved through adjustment of expression of Bcl-2 family members [19]. Amifostine is a kind of protecting agent, capable of significantly alleviating the toxicity caused by chemotherapeutic drugs to kidney, marrow, heart, ear, and nervous system, without compromising their effect, as well as protecting the normal cells [20]. It fulfills this protective effect by stabilizing DNA molecules in normal tissues and eliminating the free radicals generated by chemotherapy [21].

Traditional Chinese medical science and drugs and ovarian protection

Modern medical researches indicate that the traditional Chinese medical measures such as traditional Chinese medical science and drugs, and acupuncture [22], can activate the dopamine system in brain, and adjust the natural functions of brain-hypophysis-ovary, assisting the genital endocrine system recover its normal physiological dynamic activities [23]. Traditional Chinese drugs are characterized by a multi-system and multi-link adjusting effect; they are not hormones themselves, but possess obvious capability of adjusting the endocrine system. In recent years, as experimental studies on traditional Chinese medical science and drugs make progress [24], the researchers have found that the kidney invigorating Chinese drugs can improve the reactivity of hypophysis, and possess certain adjusting effect upon every link of gonadal axle; the kidney invigor-
ating Chinese drugs can prevent damage to gonads by chemotherapeutic drugs. The commonly used Chinese drugs include rehmannia, fructus corni, pachyma cocos, Chinese yam [25], asiatic plantain seed, fructus ligustri lucidi, mulberry white, and fructus licii [26].

Others

The selection of a chemotherapeutic regimen should be well-regulated and reasonable, considering not only the pathophysiological mechanism of tumor [27], sensitivity to chemotherapy, mechanism of drug action (such as cell cycle specific agents, but also toxic and side effects, and compatibility of drugs [28]. The chemotherapeutic regimen for ovarian cancer should be as simple as possible, include two preferably to three drugs, and the number of courses is usually three to six [16].

In summary, the patients receiving fertility preserving surgery against ovarian cancer should be confronted with chemotherapy, during which, the protection of ovarian function, as well as prevention from premature ovarian failure, should be attended, in order to ensure normal function and complete child-bearing, fulfilling the true significance of fertility preservation, and this is also the ultimate objective of the present authors.

Acknowledgments

The authors are thankful for the financial support received from the National Natural Science Foundation of China (81272875, 81302242), Ministry of Education for Young Teacher Foundation of China (20110061120084) and Jilin Science and Technology Funds (20110755 and 20130102094JC), Basic Scientific Research of Jilin University Funds.

References


Address reprint requests to:
C. MANHUA, M.D.
Department of Obstetrics and Gynecology,
The Second Hospital of Jilin University,
218 Ziqiang Street, Nanguan district
Changchun City, 130041 (China)
e-mail: manhuacui@126.com
The surgical outcomes of abdominal radical trachelectomy: does transrectal ultrasonography determine the cervical incision site during surgery?

F. Demirkiran1, T. Bese1, E. Meseci1, C. Onculoglu2, H. Erenel3, V. Sal1, M. Arvas4

1Department of Obstetrics and Gynecology, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul
2Department of Obstetrics and Gynecology, Acibadem Kozyatağı Hospital, Istanbul
3Department of Obstetrics and Gynecology, Sisli Etfal Training and Research Hospital, Istanbul (Turkey)

Summary

Purpose: To evaluate the surgical outcomes of abdominal radical trachelectomy (ART) and the efficacy of transrectal ultrasonography in determining the upper end of cervical incision during this operation. Materials and Methods: ART was performed in five patients with early-stage cervical cancer in the present clinic. In the first three patients, uterine corpus was transacted blindly at a level of approximately five mm below the internal os. In the last two patients, the authors performed transrectal ultrasonography before vaginal incision to evaluate the distance between upper margin of tumoral mass and internal os of cervical canal. Results: Mean follow-up was 21 months. During this period, menstrual abnormality occurred in three patients. The two patients in which transrectal ultrasonographies were taken intraoperatively had 9- and 12-mm postoperative cervical canal length and both of them were asymptomatic postoperatively. Conclusions: ART is usually associated with menstrual abnormality at late postoperative period and transrectal ultrasonograph during this procedure may decrease postoperative morbidity.

Key words: Abdominal radical trachelectomy; Cervical cancer; Transrectal ultrasonography; Cervical incision.

Introduction

Nearly 15% of all cervical cancers are diagnosed in women under the age of 40 years [1]. At the same time, the number of women wishing to have their first child between 35–39 years of age has increased [2]. Radical trachelectomy has been the most popular fertility-sparing surgery for patients with early-stage cervical cancer since the first published study in 1994 by Dargent et al. [3]. Although vaginal radical trachelectomy (VRT) with laparoscopic pelvic lymphadenectomy is considered the standard technique; recently abdominal radical trachelectomy (ART), which can be performed easily and safely, has been the acknowledged surgical technique for fertility preservation in cervical cancer [4-6]. Obstetrical outcomes are encouraging and the literature revealed a 67% pregnancy rate after radical trachelectomy [7]. Unfortunately, the rate of abortion and preterm delivery is more than 30% [8]. Additionally, there have been problems related to reproductive outcomes at postoperative period after radical trachelectomy. The crucial factor affecting obstetrical and reproductive outcomes is the amount of remaining cervical tissue after the surgery. The desirable cervical remnant is minimum one cm of cervical stroma [8-10]. Obviously, successful term pregnancy rates will increase as the cervical remnant is extended.

The present authors have been practicing ART since 2007 as a fertility-sparing surgery for women who would otherwise be treated with radical hysterectomy or pelvic radiation therapy. The use of transrectal ultrasound during ART has not been previously described. The aim of this study was to evaluate the surgical outcomes of ART and the efficacy of transrectal ultrasonography in determining the upper end of cervical incision during this operation.
F. Demirkiran, T. Bese, E. Meseci, C. Onculoglu, H. Erenel, V. Sal, M. Arvas

567

to the lymph nodes before the commencement of ART. If positive lymph nodes were identified, procedure was abandoned. Except two cases, round ligaments were kept intact in all subjects. The paravesical and pararectal spaces were developed just before the lymphadenectomy. Uterine artery was severed at its origin from the internal iliac artery. Complete ureter dissection was performed from beginning of internal iliac artery to bladder. After vesicouterine ligaments were dissected, bladder was mobilized inferiorly to the upper vagina and then rectovaginal space was opened. Uterosacral ligament, parametrium, and paracolpium were severed similar to standard abdominal radical hysterectomy. Following all these procedures, vaginal cuff was transected two cm below the fornices in the first three patients. The uterine corpus was transected at a level of approximately five mm below the internal os and cervix was removed out. In the last two patients, before vaginal incision was made, the authors performed transrectal ultrasonography to evaluate the distance between upper margin of tumoral mass and internal os of cervical canal (Figure 1a, 1b). In these patients, the fundus was divided from cervix at ten mm upper margin of cervical tumor by using a needle guide (Figure 2). A shaved margin was sent as a frozen section from the remaining upper endocervix and distal vaginal cuff. Also, the authors performed endocervical curettage to the remaining endocervix for frozen section evaluation. A re-anastomosis of the fundus, attached by the utero-ovarian ligaments, to the vagina was then performed using interrupted 2-0 vicryl sutures. In the first three patients, a cerclage using one prolene suture was applied to the neo-cervix. After drain placement abdominal wall was closed. All patients received prophylactic low molecular weight heparin and standard antibiotic prophylaxis. Urinary catheter was removed on postoperative 7th day.

Ultrasonography

In the last two patients, transrectal ultrasonography was performed using a machine with a 5-9 MHz probe.

Results

A total of five patients underwent radical abdominal trachelectomy with pelvic lymphadenectomy. Clinico-pathologic characteristics of patients are shown in Table 1. The mean age was 31.6 years with a range of 26 to 38 years. All patients had squamous cell carcinoma, except one. One patient had Stage Ia2 disease with lymphovascular space invasion and the remaining four patients had Stage Ib1 cervical cancer. Maximum tumor diameter was less than 24 mm in all patients and all were nulligravida. Diagnosis was established by cervical conization in two patients, loop electrosurgical excision procedure (LEEP) excision in one patient, and punch biopsy in two patients.

The mean surgical time was 213 minutes with a range of 160 to 265 minutes. The results of frozen-section evaluation performed during operation were negative in all of the patients. There were no intraoperative and early postoperative complications. Mean number of nodes removed during pelvic lymphadenectomy was 25 (range, 19-36) and the mean length of hospital stay was 4.7 days (range, 4 to 7). On final pathology examination, the vaginal, endocervical, and parametrial resection margins were tumor-free in all
The surgical outcomes of abdominal radical trachelectomy: does transrectal ultrasonography determine the cervical incision site during surgery?

568 patients and no patient had lymph node involvement. There was no residual tumor at the final pathology report in the patient diagnosed by conization (Stage Ia2). The length of clear surgical margins were 14, 17, and 22 mm at pathology report and the length of remaining cervical canal measured by transvaginal ultrasonography at postoperative 4th month were two, three, and five mm respectively in the same patients operated classically. Ten and 12 mm cervical canal were measured by sonography four months postoperatively in the two patients in whom the authors performed transrectal ultrasonography for upper margin evaluation during operation. These patients had Stage Ib1 disease and clear surgical margins were nine and 12 mm at the final pathology. None of the patients was subjected to any adjuvant therapy.

Mean follow-up was 21 months (range, 7-52 months) and no recurrence has been seen so far. During this follow-up period, menstrual abnormality occurred in three patients (Table 2). All of them had clinically notable stenosis. Three patients complained of newly developed severe dysmenorrhoea requiring analgesics. On transvaginal ultrasonography, haematometra associated with cervical stenosis was detected in two of these patients. One of these three patients was amenorrheic at 8th month after the operation, and hysterectomy was performed because of patient desire. One patient did not require any intervention. The last patient of this group had severe stenosis and underwent dilation of her neocervix three times. This patient presented with tubo-ovarian abscess at postoperative 14th month and she was re-operated. Three months after the re-operation, T-IUD was installed into the uterine cavity and removed after eight months (patient 1). The patient finally recovered following this procedure. Two patients attempted to conceive for six and four months respectively, but were not successful.

Discussion

Early-stage cervical cancer patients are potential candidates for fertility-sparing surgery since the vast majority of patients are in reproductive period. A number of studies have previously evaluated the oncologic, reproductive, and obstetrical results associated with radical trachelectomy [11, 12]. Many side effects have been reported for this type of procedure. Complications are seen in about 10–15% of cases of abdominal or vaginal radical trachelectomy with laparoscopic lymphadenectomy. Intraoperative and early postoperative complications include bladder or bowel trauma, pelvic hematomata, pelvic abscess, and transient neuropathy requiring long-term catheterization [13]. Postoperative complications may also occur and include dysmenorrhoea, dysplastic Pap smears, irregular or intermenstrual bleeding, excessive vaginal discharge, isthmic stenosis, and amenorrhoea [14]. Also some authors have reported a high rate of first- and second-trimester miscarriage (16% - 20% and 8% - 10%, respectively) and preterm delivery (20% - 30%) [12, 15].

Most of these unfavorable experiences such as isthmic-cervical stenosis, menstrual abnormality (hypo-menorrhoea, oligo-menorrhoea, and amenorrhoea), miscarriage, and preterm delivery may be related to uterine hypo-perfusion and shortening or disappearing of cervical canal and/or cervical tissue during radical trachelectomy.

The main uterine arterial supply is from uterine artery, a branch of internal iliac artery. Tubal branches of ovarian artery and arterial branches on the round ligament also provide arterial perfusion of uterus. The uterine artery is usually sacrificed in ART and many surgeons sever the round ligaments during operation. Actually, it is not necessary technically to sacrifice these ligaments. Hypo- and amenorrhoea emerged at late postoperative period in two of the present patients whose

Table 1. — Clinical and pathologic characteristics of patients.

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age</th>
<th>Histology</th>
<th>Stage</th>
<th>Tumor diameter</th>
<th>Op time</th>
<th>Nod number</th>
<th>Clear margin</th>
<th>Post op CL (‡)</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(†)</td>
<td>28</td>
<td>Squamous ca</td>
<td>Ib1</td>
<td>19 mm</td>
<td>265 min</td>
<td>26</td>
<td>14 mm</td>
<td>3 mm</td>
<td>52 months</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>Adenoca</td>
<td>Ib1</td>
<td>15 mm</td>
<td>180 min</td>
<td>36</td>
<td>17 mm</td>
<td>2 mm</td>
<td>8 months</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>Squamous ca</td>
<td>Ia2</td>
<td>–</td>
<td>160 min</td>
<td>19</td>
<td>22 mm</td>
<td>5 mm</td>
<td>24 months</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>Squamous ca</td>
<td>Ib1</td>
<td>24 mm</td>
<td>200 min</td>
<td>24</td>
<td>9 mm</td>
<td>12 mm</td>
<td>14 months</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>Squamous ca</td>
<td>Ib1</td>
<td>19 mm</td>
<td>260 min</td>
<td>22</td>
<td>12 mm</td>
<td>10 mm</td>
<td>7 months</td>
</tr>
</tbody>
</table>

(†) re-operated for pelvic abscess and intrauterine device installed postoperatively; (‡): postoperative remaining cervical length measured by ultrasonography

Table 2. — Surgical and clinical characteristics of patients

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Lig. rotundum</th>
<th>Cerclage</th>
<th>Transrectal USG</th>
<th>Cervical stenosis</th>
<th>Menstrual abnormality</th>
<th>Final result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>not severed</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>dysmenorrhoea</td>
<td>asymptomatic</td>
</tr>
<tr>
<td>2</td>
<td>severed</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>dysmenorrhoea</td>
<td>hysterectomy</td>
</tr>
<tr>
<td>3</td>
<td>severed</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>dysmenorrhoea</td>
<td>hypo-menorrhoea</td>
</tr>
<tr>
<td>4</td>
<td>not severed</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>asymptomatic</td>
</tr>
<tr>
<td>5</td>
<td>not severed</td>
<td>no</td>
<td>yes</td>
<td>no</td>
<td>no</td>
<td>asymptomatic</td>
</tr>
</tbody>
</table>

Lig: ligamentum; USG: ultrasonography
round ligaments were severed and cerclage was applied. Not preserving this ligament may have caused the menstrual abnormality with or without the cervical cerclage in these two patients. However, it is not possible to reach a conclusion on the effect of preserving round ligament on menstrual cycle with these two cases, and additionally there is no consensus on this point; while some surgeons favor preserving the ligament while others favor severing it. The present authors’ suggestion is to preserve the round ligament.

Many authors have performed permanent cervico-isthmic cerclage as a routine procedure of radical trachelectomy [11, 16-18]. The main problem related to cerclage is cervical stenosis, which may cause dysmenorrhea and unfavorable reproductive outcomes. Cervical stenosis has been reported in 11-15 % of patients who had cerclage performed in VRT [17, 19]. On the other hand, Carter et al. reported that 33% of the patients had clinically notable stenosis not requiring neo-cervical dilation while 40% had stenosis requiring a neo-cervical dilation procedure in their series [20]. In a study published by Li et al. [18] in 2011, cerclage was performed and a failed T-IUD was routinely installed during ART in all patients, and the rate of stenosis was reported to be 8%. Based on the present authors’ experience, cervical stenosis is the major and embarrassing unique postoperative complication for radical abdominal trachelectomy. IUD placement during ART may prevent cervical stenosis, but this practice is associated with increased infectious morbidity and chronic discharge. In the present last cases, the authors’ preference was not to perform cervical cerclage and to keep an intact cervical canal and/or cervical tissue as long as possible during the surgery.

Decision on the length of the cervix to be excised is the critical point of operation, since the length of remaining cervical canal affects both the rate of cervical stenosis and obstetrical outcomes. Also, it clearly affects prognosis of cancer. There is no standard for an adequate negative endocervical margin for oncological outcomes in radical trachelectomy. Some authors believe that a five-mm negative margin is sufficient, while others prefer a ten-mm negative endocervical margin [5, 21, 22]. Also it is still unclear how long the remaining cervical canal is acceptable for good obstetrical and reproductive results. Generally, it has been preferred to divide the cervix at the level of the internal os to obtain the maximum margin; however, this may have a significant impact on reproductive morbidity. Inherently, while the length of remaining cervical canal decreases, the rate of first- and second-trimester miscarriage and preterm delivery increases. Transrectal ultrasonography can be easily used to identify the upper margin of cervical cancer and internal os of cervical canal at ART, so decision on the length of the cervix to be excised can be made. The present authors perform transrectal ultrasonography using a vaginal probe and they also use two-dimensional ultrasound imaging. Scanning is begun just before cervical incision. Cervix is examined using sagittal plane as well, by turning the probe plane 90 degrees. This makes the endocervical canal and tumor tissue to be the most prominent structure on screen. With these manipulations, the authors identify the correct incision plane using the needle guide that is embedded in the cervix during ultrasonographic evaluation. They have used transrectal ultrasonographic evaluation as a part of ART in two patients with Stage Ib1 cervical cancer. This is the first report addressing the intraoperative use of ultrasound in ART.

Conclusion

In conclusion, there are numerous outstanding problems, such as cervical stenosis, dysmenorrhea, and menstrual abnormalities, related to reproductive outcomes following radical trachelectomy. Most of these may be based on the length and volume of cervical remnant after the surgery. Consequently, one of the most crucial steps in ART is to make an accurate decision on the length of the cervix to be excised. For this purpose, transrectal ultrasonography guidance can be used efficiently during the operation.

References

The surgical outcomes of abdominal radical trachelectomy: does transrectal ultrasonography determine the cervical incision site during surgery?


Address reprint requests to:
F. DEMIRKIRAN, M.D.
Department of Obstetrics and Gynecology, Istanbul University Cerrahpasa, Faculty of Medicine Cerrahpasa St. 181
34098 Istanbul (Turkey)
e-mail: fuatdemirkiran@hotmail.com
The role of ureaplasma urealyticum infection in cervical intraepithelial neoplasia and cervical cancer

C. Xiaolei¹, H. Tao¹, S. Zongli², Y. Hongying³

¹Department of Gynecology, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang, Henan Province
²Department of Central Lab, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang, Henan Province
³Department of Pathogeny, Medical College, Henan University of Science and Technology, Luoyang, Henan Province (China)

Summary

Aim: To investigate the role of ureaplasma urealyticum (UU) infection in cervical intraepithelial neoplasia (CIN) and cervical cancer and to study the correlation between UU and HPV infection in CIN/cervical cancer. Materials and Methods: A total of 233 research subjects were divided into the case group and the control group. UU and pathogenic load UU were detected in the case group and the control group by fluorescence quantitative polymerase chain reaction (PCR) method, human papillomavirus (HPV) in case group by PCR + membrane hybridization method. Results: There was statistically significant difference in the case group and control group with respect to the positive rate and pathogenic load of UU (p < 0.05). The positive rate of UU among CIN II group, CIN III group, and the cervical cancer group were not statistically significant difference (p > 0.05). There may be statistically significant difference in the result of testing UU coinfection with HPV (p = 0.002). Conclusion: Positive rate and the pathogenic load of UU infection may be related to the genesis of cervical cancer. Significant combined effect could strengthen the process of the disease and lead to the pathogenesis of cervical cancer between infection of HPV and UU.

Key words: UU; HPV; PCR; Cervical cancer; CIN.

Introduction

Cervical cancer is the third malignant tumor and the main cause of women’s deaths, which has been shown that approximately 275,000 women died of the disease in 2008 [1]. Cervical intraepithelial neoplasia (CIN) is cervical pre-cancerous lesions, reflecting continuous process in the development of cervical cancer. Cervical squamous intraepithelial lesions are divided into low-grade squamous intraepithelial lesions (LSIL; including CIN I) and highly squamous intraepithelial neoplasia (HSIL; including CIN II, CIN III), according to TBS2001 classification from International Society of Gynecological Pathology.

Now it is believed that high-risk HPV (HR-HPV) infection is a major risk factor for cervical cancer, but only a few of which lead to cervical cancer, so it is inevitable that there are other synergistic human papillomavirus (HPV) factors [2] or other factors leading to cervical cancer.

Some researches show that there were correlation between mycoplasma and tumor: mycoplasma infection exists in human tumor tissues [3-5]; mycoplasma infection can cause malignant transformation of the cell [6-8]. Ureaplasma urealyticum (UU) belongs to the ureaplasma of mycoplasma. Genital tract is the important site for UU infection. Is there some correlation between the infection of UU and cervical cancer? Do UU and HPV infections produce any synergistic effect on cervical cancer and its development? In the present study, UU DNA expression of cervical secretions from patients with CIN or cervical cancer were investigated by means of fluorescence quantitative polymerase polymerase chain reaction (FQ-PCR) and detected HPV DNA by PCR + membrane hybridization method, and then explored the correlation of UU, HPV, and CIN or cervical cancer.

Material and Methods

Patients and specimens

Randomly selected from The First Affiliated Hospital of Henan University of Science and Technology from December 2011 to June 2012, the groups cervical secretions were taken from patients with cervical cancer, CIN, or via physical examination. Thirty-nine cases who were diagnosed with cervical cancer were women aged from 24 to 67 years with an average age of 44.21. They were confirmed to be cervical squamous carcinoma patients by pathological diagnosis. Seventy-four cases with CIN included 24 with CIN I, 27 with CIN II, and 23 with CIN III who were confirmed by pathological diagnosis or thinprep cytologic test, and aged from 20 to 65 years with an average age of 37.43, which were matched with the case group regarding age distribution frequency, and were chosen to be the control group. Their vaginal mucosa was pink and the quantity of vaginal discharge was little and coloured white or light yellow. The cervical surfaces were smooth and without neoplastic vegetations.

Exclusion criteria

Patients with a history of treatment including radiotherapy, chemotherapy, surgery, those taking any antibiotics within seven days, those using vaginal drugs within three days or washing the vagina, those having sexual activity within 24 hours, those suffering from Neisseria gonorrhoeae, candida infection, and bacterial vaginosis disease before taking cervical specimens were excluded from the study.

Revised manuscript accepted for publication October 21, 2013
**Ethics**

Before the study, the patients were voluntarily asked to sign an informed consent form approved by the Institutional Ethics Committee of The First Affiliated Hospital of Henan University of Science and Technology including name, age, purposes of the research, possible risks, accessible benefits, and the assurance that all the information would be completely confidential, and so on.

**UU test**

Sterile cotton swab was inserted in the cervix canals, twisted after five seconds to collect cervical secretions, placed in sterile glass tube, and immediately stored in a refrigerator with a temperature of -20 °C. The UU test was taken with FQ-PCR diagnostic kit for UU DNA. Negative and positive quality control and 233 cases of specimens were analyzed with DNA extract (NaOH, Tirs - HCl, TritonX - 100, the NP - 40, CheleX - 100, EDTA) for DNA extraction. UU positive quantitative reference for product were centrifuged at the speed of 8,000 rpms / few seconds, and placed aside. The processed samples were then placed in PCR reaction tube. PCR program as recommended by manufacturer was as follows: ten cycles of two minutes predegenerated in 93°C followed by five seconds in 95°C and 55°C for 66 seconds, 30 cycles of 30 seconds in 93°C followed by 55°C for 45 seconds. The system automatically saved the test data file after the reaction. Then the fluorometer value was adjusted to F1 / F2 and r value between 1.0 ~ 0.91. It was ensured that there was no value in the CT value of negative quality control. The calculated val-

---

![Amplification Plot](image1)

Figure 1. — The standard and sample curve of FQ-PCR.

![Standard Curve](image2)

Figure 2. — Standard curve used to calculate the minimum detection limit for UU DNA in unknown samples.
The role of *Ureaplasma urealyticum* infection in cervical intraepithelial neoplasia and cervical cancer

Uues of the unknown specimen were then automatically analyzed by a recording instrument. There were some curves of UU FQ-PCR (Figures 1-4).

**HPV testing**

HPV genotyping was tested by PCR + membrane hybridization technique diagnostic Kit.

**Results**

**UU test results**

Positive rate of UU in case groups: CIN I, CIN II, CIN III, and cervical cancer group were 33.33%, 62.96%, 65.22%, and 65.22%, respectively (Table 1). Comparing positive rates of UU between groups of CIN I and CIN II, there was statistical significance in the difference: $\chi^2 = 4.464, p = 0.035$ (Table 1). Through a comparison among the positive rates of UU in CIN II, CIN III, and cervical cancer groups, there was no statistical significance in the difference: $p > 0.05$ (Table 1). The difference between positive rates of UU in case group (63%) and control group (43.33%) was statistically significant: $\chi^2 = 8.873, p = 0.003$, OR = 2.211 (Table 1).

There was statistically significant difference between positive rates of UU in LSIL group (33.33%) and HSIL and above group (70.79%); $\chi^2 = 11.354, p = 0.001$ (Table 2). There were statistically significant differences between positive rates of UU in control group and HSIL and above group: $\chi^2 = 15.562, p = 0.000$ (Table 2).

Pathogen loading does of UU by log conversion in case group and control group were 2.754 - 4.812, 4.231 - 6.071, respectively. There was statistically significant difference between pathogenic load of UU in control group and case group: $p < 0.001$, however, there was no statistically sig-

![Amplification Plot](image-url)  
**Figure 3.** — UU negative curve of FQ - PCR.

![Amplification Plot](image-url)  
**Figure 4.** — FQ - PCR positive curve.
Table 1. — Test results of UU in case group and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Positive rate %</th>
<th>( \chi^2 )</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case group</td>
<td>113</td>
<td>71 63</td>
<td>8.873</td>
<td>0.003</td>
</tr>
<tr>
<td>CIN I</td>
<td>24</td>
<td>8 33.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN II</td>
<td>27</td>
<td>17 62.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN III</td>
<td>23</td>
<td>18 65.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>39</td>
<td>28 71.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>120</td>
<td>52 43.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Compared with control group.

Table 2. — Test results of UU in LSIL group and HSIL and cervical cancer group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Positive rate %</th>
<th>( \chi^2 )</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSIL</td>
<td>24</td>
<td>8 33.33</td>
<td>0.823</td>
<td>0.364</td>
</tr>
<tr>
<td>HSIL and above</td>
<td>89</td>
<td>63 70.79</td>
<td>15.562</td>
<td>0.000</td>
</tr>
<tr>
<td>Control group</td>
<td>120</td>
<td>52 43.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Compared with control group.

Table 3. — Interrelationship of UU and HPV.

<table>
<thead>
<tr>
<th></th>
<th>+ HPV</th>
<th>— HPV</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>58</td>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td>—</td>
<td>24</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>Total number</td>
<td>82</td>
<td>27</td>
<td>109</td>
</tr>
</tbody>
</table>

Significant differences between pathogen loading does of UU in HSIL group and cervical cancer group: \( p > 0.05 \).

UU and HPV detection results

In the HPV detection of the case group, four of the 113 cases with LR-HPV (3.54%) were excluded. Hence statistical analysis were taken in the remaining 109 patients, among which 58 cases were positive to both UU and HPV (53.2%), 17 were negative to both UU and HPV (15.6%), ten positive to UU and negative to HPV (9.2%), 24 negative to UU and positive to HPV (22.0%) (Table 3). The detection results of UU were statistically consistent with that of HPV: the coefficient Kappa = 0.287, \( p = 0.002 \) (Table 3).

Discussion

Ureaplasmas are part of the class Mollicutes, which are obligate parasites of eukaryotes, lacking a cell wall, with a non-standard genetic code, and extremely small genome size, and require cholesterol. Although there is no evidence that ureaplasmas produce toxins, they do possess several potential virulence factors. IgA protease has been considered as one of the major factors contributing to the pathogenic potential of ureaplasmas. This can provide them with an advantage in evasion of the host-defenses.

Ureaplasmas have also been reported to have phospholipase A1, A2, and C activities which could activate the synthesis of prostaglandins leading to reproductive disease. In recent years, much attention has been paid to the diseases caused by UU, especially related to female reproductive health system disease. Disease caused by UU are implicated in a variety of clinical outcomes including but not limited to non-gonococcal urethritis, pelvic inflammatory disease, infertility, chorioamnionitis [9], and bronchopulmonary dysplasia in premature infant [10]. However the relationship between UU and cervical cancer was rarely reported.

In the present study, the authors concluded that infection of UU may be related to the genesis of cervical cancer. The pathogenic load of UU was related to CIN and cervical cancer, but without relationship with the development of CIN; significant combined effect between infection of HPV and UU could strengthen the process of the disease and lead to the pathogenesis of cervical cancer.

The study by Szostek et al. [11] on microorganism infection in 44 cases of CIN patients suggested that UU dominated in women with CIN (40.5%) . Lukic et al. [12] found a high association between UU infection and the grade of cytological cervical lesion (35% for LSIL and 45% for HSIL). Nineteen percent of the control group samplings were positive for UU. The presence of a high UU level seems to be a cofactor of HPV infection, a necessary cause of precancerous lesions of the uterine cervix. In the present study, the authors found that UU infection was 58.10% in CIN, 33.33 % in LSIL, 70% in HSIL, and 43.33% in the control group. The infection rate of UU in LSIL, HSIL, and in the control group in the present study were higher than that in Lukic et al. study which tested UU by culturing method. It may be caused by different grouping methods, different experimental methods, and so on. The present authors showed that UU may be related to the genesis of cervical cancer, that significant combination between HPV and the infection of UU led to the pathogenesis of cervical cancer, and strengthen the process of the disease, so they assumed that the presence of UU may play a role both in initiating viral cellular anomalies and in viral persistence.

In a study of abnormal cervical cytology, Biernat-Sudolska et al. [13] found that the risk of HPV infection was 4.7-fold greater when combined with UU infection. Zheng et al. [14] showed that there was a significant association between the infected HPV and UU (> 10,000 CCU/ml; all \( p < 0.01 \)) which may increase the infection risk of HPV. Pisani et al. [15] considered that double and triple infections were found in groups two (50 presented signs of flogosis) and three (100 resulted positive for an abnormal transformation zone), with mycoplasmas being the most common microorganisms present in association and quite almost copresent with papillomaviruses. In the present study, the authors tested UU coinfection with HPV in 109 patients of the case group (Kappa coefficient = 0.287, \( p = 0.002 \), and found that the
positive rates of UU and HPV were parallel and there may be synergistic effect between the infection of UU and HPV in CIN/cervical cancer caused by HPV infection. The difference between Zheng et al. study and the present may be in the diverse experiment methods: Zheng et al. employed the culture method.

Verteramo et al. [16] proposed a significant association between HPV and UU but only at a high colonization rate was found, and that perhaps it was significantly related to CIN. In the present study, the authors found that the difference of pathogenic load of UU in the case group and the control group was statistically significant ($p < 0.001$), but there was no statistically significant difference in the HSIL group and the case group ($p > 0.05$). Thus, the present authors inferred that the pathogenic load of UU was related to CIN and cervical cancer, but not related to the development of CIN.

At present, it is widely accepted that UU is divided into ureaplasma Parvum (biovar 1, parvo) and UU (biovar 2, T960); the authors did not test genotyping of positive UU specimen in this experiment and is subject of future experiments. The arguments addressed in the present study need to be confirmed in a larger sample, however it can be a significant reference for further study and clinical diagnosis of the relationship between UU and cervical cancer.
Expression of heat shock protein 20 inversely correlated with tumor progression in patients with ovarian cancer

Naian Qiao1*, Yanhui Zhu2*, Haiying Li3, Zhongyu Qu4, Zhichun Xiao2

1Department of Radiotherapy, Shandong University Qilu Hospital, Jinan
2Medical Informatics Center, Peking University, Beijing
3Division of Ultrasonography, Shandong University Qilu Hospital, Jinan
4Division of Ultrasonography, Shandong Provincial Hospital, Jinan (China)

Summary
Objective: To investigate a possible correlation between expression levels of heat shock protein 20 (HSP20) and tumor progression in patients with ovarian cancer. Materials and Methods: The study included 34 patients with ovarian cancer who were to undergo surgery, seven patients with ovarian carcinoid tumors, and five patients with normal ovaries as a control group. Ovarian tissues were obtained from patients by surgical resection and then analyzed by western blot. Results: Expression levels of HSP20 were inversely correlated with the grade of malignancy. Conclusion: The present findings suggest that HSP20 may play a protective role against the progression of ovarian cancer. Thus, HSP20 may represent a new target for the prediction and treatment of ovarian cancer.

Key words: HSP20; Ovarian cancer; Tumor progression.

Introduction

Heat shock proteins (HSPs) are a subset of the molecular chaperones; they are best known for their rapid and abundant induction by stress. HSPs, classified by their molecular weight, are highly expressed in many malignant tumors, including ovarian cancer. Most HSPs seem to play a role in many aspects of tumor progression and response to therapy, probably due to their antiapoptotic properties [1, 2]. Previous studies have indicated that HSP27, in addition to its typical function as a chaperone, also plays fundamental roles in maintaining the intracellular redox potential and in stabilization of the cytoskeleton [3, 4]. High expression of HSP27, induced by chronic cellular stress, may lead to the suppression of apoptosis; thus, HSP27 may facilitate malignant transformation [5].

In previous studies, the authors showed that HSP20, a member of the small HSP family, has an antiapoptotic effect on cardiomyocytes [6,7]. Therefore, they hypothesized that HSP20 would also have an antiapoptotic effect on ovarian cancer cells. In the present study, the authors investigated the relationship between HSP20 expression levels and ovarian cancer progression by comparing HSP20 expression levels in specimens of ovarian cancer, ovarian carcinoid, and normal ovaries.

Revised manuscript accepted for publication November 25, 2013

* Contributed equally and should be considered co-first authors.

Materials and Methods

Subjects
The Ethics Committee of Shandong University Qilu Hospital approved the research protocol. Informed consent was obtained from each of the patients and control participants.

A total of 41 women who were hospitalized for a suspected ovarian tumor from February 2011 to January 2013 and who intended to undergo surgical intervention were randomly selected as study subjects. The demographics and clinical characteristics of the study population are indicated in Table 1. Of these, 34 women were diagnosed with ovarian cancer, seven were diagnosed with ovarian carcinoid tumors, and five were diagnosed with normal ovaries. Finally, samples of healthy ovarian tissue from five age-matched women who had died in traffic accidents were used for the healthy control group. All of the participants were Asian Chinese women.

Women with ovarian cancer and ovarian carcinoid were excluded if they had received hormone therapy or chemotherapy or if their condition occurred in combination with other malignancies. After screening, the ovarian cancer group included 34 patients: six (18%) International Federation of Gynecology and Obstetrics (FIGO) Stage I cases, ten (29%) FIGO Stage II cases, 12 (35%) FIGO Stage III cases, and six (18%) FIGO Stage IV cases. The cancers had different histological types, as follows: serous papillary carcinoma (n = 24), mucinous carcinoma (n = 4), clear cell carcinoma (n = 3), endometrioid carcinoma (n = 2), and mixed cystadenocarcinoma (n = 1). Another seven women with benign ovarian carcinoid were recruited for the ovarian carcinoid group. The ovarian carcinoid patients had different histological types: serous cystadenoma (n = 2), mucinous cystadenoma (n = 1), mixed cystadenoma (n = 1), and simple ovarian cyst (n = 2).

Surgical specimens
Ovarian tissues were obtained from patients by surgical resection at the Department of Obstetrics and Gynecology, Shandong University Qilu Hospital. The resected tissue was snap-frozen in liquid nitrogen and stored at −80°C until used for Western blot analysis.
Western blot analysis
Snap-frozen samples were homogenized and sonicated in lysis buffer containing 20 mmol/L Tris-HCl pH 7.4, 1% Trion X-100, 150 mmol/L sodium chloride, one mmol/L ethylenediaminetetraacetic acid (EDTA), 2.5 mmol/L sodium pyrophosphate, one mmol/L sodium fluoride, one mmol/L sodium orthovanadate, and 0.1 mmol/L phenylmethylsulfonyl fluoride. Aliquots were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene difluoride membranes and incubated with primary polyclonal anti-HSP20 antibodies at 4°C overnight. Bound antibodies were detected with a secondary antibody conjugated to horseradish peroxidase, visualized by use of an enhanced chemiluminescence kit, and exposed to X-ray film for the appropriate time [6, 7]. Protein band intensities were determined by integrating the optical density over the band area (band volume) with NIH imaging software. HSP20 levels were normalized to those of β-actin.

Statistical analysis
Data were expressed as means ± standard deviation (SD). Differences were analyzed for significance by one-way repeated-measures ANOVA and further analyzed with the Newman–Keuls test for multiple comparisons between treatment groups. The results were considered significant at \( p < 0.05 \). GraphPad Prism version 4.0 for Windows was used for the analysis.

Results
Expression of HSP20
Western blot images of HSP20 expression in six representative patients with normal ovaries; ovarian carcinoid; and ovarian cancer Stages I, II, III, and IV are shown in Figure 1. There were decreased levels of HSP20 expression in the tumor tissues, a trend toward decreased expression levels of HSP20 in tumor tissues was observed.

Comparison of HSP20 levels in different stages of ovarian cancer
HSP20 levels in tissue samples of different stages of ovarian cancer were compared to HSP20 levels in ovarian carcinoid tumors and normal ovaries. There were significant differences in HSP20 levels with respect to tumor progression \( (p < 0.05, \text{Figure 2}) \). There were also significant differences in HSP20 levels when any two ovarian cancer Stages (I, II, III, and IV) were compared \( (p < 0.05 \text{ for all comparisons}) \). The present authors observed a trend toward decreased expression levels of HSP20 in tumor tissues that were inversely correlated with increasing cancer stage.

Discussion
The present investigation of the association between HSP20 expression levels and tumor progression in patients with ovarian cancer revealed a trend toward decreased HSP20 expression levels in tumor tissues. The results suggest that levels of HSP20 in tumor tissues were attenuated in parallel with ovarian cancer progression; thus, HSP20 expression levels were inversely related to the grade of malignancy. To the best of the authors’ knowledge, this is the first report of a significant association between HSP20 levels and the progression of ovarian cancer.

The idea that HSPs may protect against disease is not unprecedented In a previous study, the present authors demonstrated that overexpression of HSP20 in rat hearts is protective against ischemia/reperfusion; the protective effect is related to a reduction in the necrosis and apoptosis of ventricular cardiomyocytes both in vitro and in vivo [6, 7]. Another previous study reported that HSP60 mRNA lev-
Expression of heat shock protein 20 inversely correlated with tumor progression in patients with ovarian cancer

Although heat shock proteins (Hsps) are a valuable prognostic factor for epithelial ovarian cancer, variations in expression levels were not due to amplification of this gene [8]. Moreover, Olejek et al. reported that the mean concentration of anti-Hsp27 antibodies in a group of patients with ovarian carcinoma was significantly higher than in the control group. Their analysis of the association between anti-Hsp27 antibodies and the stage of clinical progression revealed that the concentration of anti-Hsp27 antibodies was higher in less advanced ovarian carcinoma specimens [9].

**Conclusion**

The present results strongly suggest that expression levels of HSP20 decrease with tumor progression in ovarian cancer patients; thus, HSP20 could have a suppressive effect on the progression of ovarian cancer. The present authors are currently conducting studies to investigate the underlying mechanism for this effect and to optimize the detection of anti-HSP20 antibodies in serum for the early identification of ovarian cancer.

**References**


---

![Figure 1. Western blot showing HSP20 levels in six representative specimens: four ovarian cancer, one ovarian carcinoid, and one control. Protein extracts were analyzed with antibodies against HSP20 and β-actin.](image1)

![Figure 2. HSP20 levels in ovarian cancer and control specimens. Protein extracts from 34 ovarian cancer specimens, seven ovarian carcinoid tumor specimens, and five control specimens were analyzed with antibodies against HSP20 and β-actin. Signal intensities on X-ray film were quantified with NIH imaging software. Histograms show quantitative representations of HSP20 levels after normalization to β-actin levels. Values on the vertical axis represent the mean ± standard error of the mean of independent experiments. *p < 0.05 compared to control tissue samples, p was also < 0.05 when any two ovarian cancer Stages (I, II, III, and IV) were compared.](image2)


Address reprint requests to:
YANHUI ZHU, M.D.
Peking University Medical Informatics,
38 Xueyuan road, Beijing 100191 (China)
e-mail: gzyh@hsc.pku.edu.cn
Introduction

Female adnexal tumors of Wolffian origin (FATWOs) are exceptionally rare neoplasms. The entity was first described in 1973 by Kariminejad and Scully [1], and fewer than 100 cases have been reported to date. The tumor usually arises from the remnants of the mesonephric (Wolffian) duct [2] and is most often located in the broad ligament, but occasionally can be found in the mesosalpinx, fallopian tube, ovarian hilum, and peritoneum [3, 4]. Most FATWOs behave in a benign fashion. To the best of the present authors' knowledge, there are only 14 case reports worldwide describing malignant FATWOs [3-12], some of which detail recurrence or metastasis to the omentum, lung, and liver after initial surgery [5]. It is important to be aware that these tumors may demonstrate malignant behavior at the time of initial diagnosis.

The authors report herein a 69-year-old woman with a malignant FATWO. They evaluated the biomarkers present in her tumor for their diagnostic value and review 14 prior case reports describing malignant FATWOs. The aim of this report is to evaluate the distinctive immunohistochemical markers, in a patient with a FATWO known to be malignant at initial diagnosis, that are helpful in differentiating benign from malignant lesions.

Case Report

A 69-year-old postmenopausal Japanese woman, gravida 3 para 2, was referred to the present hospital in January 2012. Her complaint was continuous vaginal bleeding and lower abdominal pain. She had no significant past medical or family history.

Ultrasound examination and magnetic resonance imaging revealed a solid left adnexal mass, five cm in diameter. The serum cancer antigen 125 (CA-125) level was 42 U/ml (normal, < 35.0 U/ml). The serum sialyl-Tn antigen level was within normal limits.

A total abdominal hysterectomy (TAH) and bilateral salpingo-oophorectomy (BSO) were performed, with removal of the mass in the left broad ligament. The number of mitoses was somewhat high in the active areas, numbering five to seven per ten high-power fields. The tumor cells were strongly positive for glutathione S-transferase π, and positive for calretinin, vimentin, c-Kit, CD99, and CD56; neuron-specific enolase was also partially expressed. The tumor cells were negative for inhibin α, estrogen receptors, progesterone receptors, B-cell lymphoma 2, and S100. Taken together, these immunohistochemical and pathological findings gave the diagnosis of malignant FATWO. The patient experienced a recurrence one year after her initial surgery.

CD56 immunostaining was negative in two benign FATWO cases at the present institution. These findings suggest that CD56-positivity may be a diagnostic biomarker to differentiate malignant FATWOs from benign lesions.

Key words: FATWO; CD56 antigen; Malignant.
demonstrated a three-cm mass in the right lobe of the liver, and multiple tumors in the outer aspect of the left iliopsoas muscle, around the bilateral iliac arteries, and in the right side of the pelvis. The serum CA-125 level was 73 U/ml. She was started on imatinib, 400 mg daily. The authors are hopeful that this treatment will prove effective, and plan to follow her carefully over the long term.

Pathological findings

Macroscopic examination showed a tumorous mass located in the left broad ligament of the uterus, measuring approximately 55 × 50 × 20 mm. No evidence of metastasis or dissemination was observed. The ipsilateral ovary was atrophic, and the contralateral ovary and fallopian tube appeared to be normal. The mass was ash-colored, solid, and encapsulated, without apparent cystic areas, containing some hemorrhagic and necrotic regions (Figure 1A). The formalin-fixed, paraffin-embedded tissue was stained with hematoxylin and eosin and evaluated microscopically. Microscopic examination of tumor revealed many cysts of varying sizes. These cysts were lined by flattened cuboidal epithelial cells, which occasionally showed a hobnail appearance. Solid areas separated the cyst creating a sieve-like pattern. In the other areas, the dominant pattern was characterized by a closely packed tubular pattern and solid pattern with no apparent tubular formation. There was an eosinophilic secretion within the lumens of some of the cysts and tubules (Figure 1B). There were five to seven mitoses per ten high-power fields in the active areas. The diagnosis was malignant FATWO. There was no evidence of malignant cells in the resected sigmoid colon.

Immunohistochemical findings

The tumor cells were strongly positive for glutathione S-transferase (GST π) (Figure 2A). They also stained positive for calretinin, vimentin, c-Kit, GSTπ, CD99, and CD56 (Figures 2B, C, and D). Neuron-specific enolase (NSE) was partially expressed. The tumor cells were negative for inhibin α, estrogen receptors, progesterone receptors, B-cell lymphoma 2 (bcl 2), and S 100.

Discussion

FATWO is generally a benign tumor, but recurrence or metastasis despite treatment has been described in a few cases. FATWO is therefore considered to possess low-grade malignancy. The period required for recurrence or metastasis varies, with some tumors demonstrating metastasis at the time of initial diagnosis and others demonstrating recurrence several years after initial treatment. To the best of the authors’ knowledge, there are 14 existing case reports describing malignant FATWOs. Only five of 14 were diagnosed as malignant at initial diagnosis [3, 9-11]; the remaining were initially considered benign, but were later revealed to be malignant after recurrence or metastasis occurred. It is important to aware of a tumor’s benign or malignant status at the time of diagnosis, in order to prognosticate.

In the previously described cases, the median patient age was 41 years, ranging from 15 to 81 years. Four patients were of reproductive age (15 to 27 years) and desired future fertility. A total of eight patients had documentation of mitotic activity, with four demonstrating brisk mitotic activity. In the other six patients, low or absent mitotic activity was seen. Histological features such as mitotic activity may increase the rate of recurrence and metastasis [3], hence it is important to evaluate these carefully.

The majority of patients were primarily treated with surgical debulking, including TAH+BSO and resection of the tumor mass. TAH was mainly performed in patients who were not concerned with future childbearing; eight of ten patients who had completed their childbearing underwent TAH as the initial treatment. The four patients who wished to retain future fertility underwent laparotomy with resection of the tumoral region and preservation of the normal-appearing ovaries and uteri.

Owing to the rarity of this disease, the necessity of adjuvant chemotherapy or radiation therapy is unknown. Some previous reports describe the use of platinum-based regimens, including cisplatin, however this treatment was unsuccessful in most patients [4-8]. Recently, the effectiveness of imatinib in patients with c-Kit positivity

Figure 1. — (A) Gross appearance of the female adnexal tumor of Wolffian origin, with areas of hemorrhage and necrosis. (B) Uniform epithelial cells with irregularly distributed tubules and cystic structures (hematoxylin and eosin; × 100).
Malignant female adnexal tumor of Wolffian origin (FATWO) positive for CD56: a possible diagnostic role for the biomarker

has been reported. The expression of c-Kit has emerged as the most important defining feature in gastrointestinal stromal tumors (GISTs), and is considered the gold standard for diagnosis. Harada et al. described a patient with a benign FATWO, positive for c-Kit [13], and indicated that imatinib (STI 571; Gleevec) may be useful therapy, as it is effective in the treatment of GISTs. The medication selectively inhibits tyrosine kinases, such as c-Kit and break-point cluster region-Abelson, and is said to have very few side effects. The two patients with malignant FATWOs positive for c-Kit were described by Steed et al. and Syriac et al. [7, 12]. Steed et al.’s patient underwent imatinib chemotherapy for a tumor recurrence, taking 300 mg daily for five weeks. The tumor was reduced in size after only 12 weeks of therapy, and there was no evidence of disease after ten months of follow-up [7]. Syriac et al. described the successful use of imatinib chemotherapy, 400 mg daily for six months after TAH+BSO, to inhibit recurrence or metastasis [12]. These findings suggested that imatinib therapy may be effective for FATWO recurrence. The present authors’ presumed that their patient was likely to experience recurrence or metastasis after TAH+BSO, and indeed she had a recurrence one year after surgery. She was started on imatinib and is undergoing close long-term follow-up.

It is difficult to characterize malignant FATWOs because of their rarity. All reported patients with malignant FATWOs had evidence of recurrence or metastasis—some at the time of initial diagnosis, and some as late as 16 years after treatment. The authors predicted the possibility of recurrence or metastasis in their patient and therefore followed her closely. As a result, they were able to find the metastases in a timely fashion. The histologic criteria for recurrence or metastasis are not absolute. Therefore, close follow-up is recommended for patients with FATWOs, especially those with malignant disease.

The diagnosis of FATWO is based on the morphological features of neoplastic epithelial cells growing in sieve-like,
tubular, and diffuse patterns. This tumor must be distinguished from Sertoli-Leydig cell tumors, granulosa cell tumors, endometrioid carcinoma, and primitive neuroectodermal tumors (PNET). The histology of Sertoli-Leydig cell tumors is architecturally similar to the tubular patterns of the FATWO. However, the latter tend to be multi-insular or even lobulated, with cellular, featureless areas. In addition, Sertoli-Leydig cell tumors generally occur in younger patients. Granulosa cell tumors are also usually found in younger females. They are composed of typical granulosa cells, exhibiting grooved nuclei, that are not seen in FATWOs. Endometrioid carcinoma is generally positive for epithelial membrane antigen, has abnormal, hyperchromatic nuclei and high mitotic activity, and causes squamous metaplasia; these are not features of FATWOs. PNETs are small round blue cell tumors, occurring in children and young adults, with 80% found in patients between the ages of five and 20 years. They are extremely rare in patients older than 30 years of age. In the present patient, the intraoperative frozen section stained positive for GSTπ, hence the authors could not rule out the possibility of a PNET. Consequently, they added immunostaining for S100 and NSE. The negative and partially positive results, respectively, were atypical for PNET.

The reported immunohistochemistry results of primarily benign FATWOs describe positivity for vimentin (100%), pancytokeratin (AE 1/3, CK 1) (100%), anti-cytokeratin 5.2 (100%), calretinin (91%), cytokeratin 7 (88%), inhibin (68%), and epithelial membrane antigen (12%), among others [14, 15]. GSTπ may be a comparatively specific marker for a tumor originating from the Wolffian duct such as a FATWO [14]. c-Kit oncogene was found to be positive in the present patient and her tumor cells were negative for inhibin α and bcl 2, results consistent with FATWO. Immunostaining for CD56 and CD99 was carried out in an attempt to determine malignancy; the tumor cells were positive for both. To the best of the authors’ knowledge, no previous cases, benign or malignant, have demonstrated CD99-positivity [3]. Only one previous malignant case was examined for immunoreactivity to CD99, with a negative result [3]. A single prior malignant FATWO was evaluated for CD56-immunoreactivity, with weakly positive results [3]. One of co-authors’ institution had previously performed CD56-immunostaining in two patients with benign FATWOs, with negative results in both. Therefore, the authors suggest that CD56-positivity may indicate malignancy.

The authors presented a patient with a malignant FATWO arising from the leaves of the broad ligament. The tumor expressed GSTπ, calretinin, vimentin, e-Kit, CD99, CD56, and NSE. Of special interest is that the positive CD56 finding in this case may indicate the presence of a diagnostic biomarker to differentiate between benign and malignant lesions. The authors are limited by the single-case nature of this report, given the rarity of FATWOs. A larger retrospective study is needed to confirm the present findings and to more fully explore the role of CD56 staining in malignant FATWOs.

References


Primary ovarian malignant mixed mesodermal tumor: report of four cases

E.Y. Ki1, J.S. Park1, J.B. Mun2, S.Y. Hur1

1Department of Obstetrics and Gynecology, Seoul St. Mary’s Hospital, The Catholic University of Korea, Seoul
2Department of Obstetrics and Gynecology, Presbyterian Medical Center, Jeonju (Korea)

Summary

Malignant mixed mesodermal tumors (MMMTs) are highly aggressive and usually diagnosed at advanced stages. MMMT originates from either the ovary or the uterus. Because this disease is relatively rare, an optimal treatment modality has not yet been established. The authors report four cases of ovarian MMMT (one heterologous MMMT and three homologous MMMTs) during 1990-2011. The patients underwent operation immediately after histopathologically confirmation and were treated with platinum-based combination chemotherapy. The extent of operation, the outcomes of radiation therapy, and the proper chemotherapeutic regimen are still controversial. The authors report herein four cases of ovarian MMMTs along with a brief literature review.

Key words: Ovarian MMMTs; Carcinoma; Sarcoma.

Introduction

Malignant mixed mesodermal tumors (MMMTs, also termed carcinosarcomas, sarcomatoid carcinomas, or malignant mixed Müllerian tumors) are relatively rare in the female genital tract and are most commonly found in the uterus [1]. Ovarian MMMTs account for only one percent of all ovarian tumors [2].

Histologically, MMMTs are epithelial tumors that are comprised of both carcinomatous and sarcomatous components. It is subclassified as heterologous or homologous according to the absence or presence of stromal components containing mesenchymal tissue which are not normally found at the primary tumor site [1]. Heterologous elements most frequently include rhabdomyosarcoma, followed by chondrosarcoma, osteosarcoma, and liposarcoma [3].

MMMTs are highly aggressive and rapidly progressive with poor long-term prognoses when compared to epithelial ovarian cancers. The median survival ranges from six to 12 months and more than 70% of the patients died of the disease within one year [2]. Most patients present with widespread metastases at the time of surgery, making optimal tumor debulking difficult [4]. Treatments of the advanced disease include complete surgical staging and debulking along with postoperative adjuvant chemotherapy, which may have unproven benefits in patients with ovarian MMMTs [5, 6]. Prognostic variables analyzed previously included stage, histologic type, treatment method, and surgical approach. The authors report herein four cases of ovarian MMMTs along with a brief literature review.

Case Report

Case 1

A 65-year-old woman who experienced one month of dyspnea, abdominal distension, and weight loss presented at the present hospital. No fever, vaginal bleeding, or abdominal pain was noted. By pelvic examination, a large, hard, and unmovable mass was palpated in the left lower quadrant. Transvaginal sonography showed a large mixed echoic left ovarian mass. Computed tomography (CT) revealed a 16×20×25-cm multilobulated hypodense mass with multifocal enhancing irregular soft tissue components (Figure 1A). A right inguinal lymph node, approximately 2.3 cm in size, and multiple pelvic/para-aortic enlarged lymph node were noted. Small amounts of ascites and large amounts of bilateral pleural effusions were noted. Positron emission tomography (PET) exhibited a large lobulated mass in the lower abdomen and pelvic cavity with central photopenia as well as multiple lymphadenopathies in the pelvic, para-aortic, and right inguinal lesions. The serum CA 125 level was 986 U/ml and other tumor markers were within the normal range. Endoscopic studies yielded normal results, but a colonoscopic study could not be undertaken because the mass crushed her rectum. Thoracentesis was performed and pleural effusion contained many neutrophils, macrophages, and mesothelial cells, which showed no evidence of malignancies. During the laparotomy, the authors found a 20-cm multilobulated left ovarian tumor that densely adhered to the uterus, sigmoid colon, rectum, peritoneum, cul-de-sac, and omentum. Diffuse carcinomatosis was noted and multiple seeding nodules were noted beneath the diaphragm. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, both pelvic, para-aortic and right inguinal lymph node dissection, peritonectomy, and multiple biopsy. Histopathological examination demonstrated an ovarian MMMT at Stage IIIc. Following the surgery, the patient was placed on an adjuvant combination chemotherapy regimen consisting of cisplatin and ifosfamide. Chemotherapy was administered as follows: cisplatin (20 mg/m²) and ifosfamide (1.5 mg/m²). She is currently being treated with chemotherapy and remains in a stable status.
Case 2

A 48-year-old woman who experienced low abdominal pain and had a palpable mass visited the present hospital. CT showed a 12×10×11-cm solid mass in her left ovary (Figure 1B). During the laparotomy, a large mass in the left ovary was partially ruptured. In the right ovary, there was a two-cm endometrial cyst that adhered to the pelvic peritoneum. An enlarged lymph node was noted five cm over the aortic bifurcation site. The patient underwent total hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and para-aortic lymph node dissection. Histopathologic examination demonstrated MMMT of the left ovary and left tube at Stage Ib. Following the surgery, the patient received six cycles of adjuvant combination chemotherapy consisting of cisplatin (75 mg/m²) and paclitaxel (135 mg/m²). She underwent a second-look operation and her pathologic results were negative. She is still healthy during a follow-up period of two years.

Case 3

A 64-year-old woman who experienced low abdominal pain for one month visited the present hospital. CT showed large amounts of ascites and a 7×4.5×3.5-cm mass in the right ovary (Figure 1C). Laparotomy revealed about three litres of bloody ascites and a solid mass in the right ovary. Cancerous masses had infiltrated the uterus and posterior cul-de-sac. An omental cake was also noted. Enlarged lymph nodes were noted in bilateral external iliac areas, but the other internal organs did not show any abnormal findings. She underwent right adnexectomy, omentectomy, and both iliac node biopsy. Histopathologic examination demonstrated a MMMT, including heterologous component, chondrosarcoma, involving the omentum and both external iliac nodes. Following the surgery, she received six cycles of combination chemotherapy, cisplatin (20 mg/m²), ifosfamide (1.28 mg/m²), and etoposide (75 mg/m²). CT taken at 12 months

Figure 1. — CT images of ovarian MMMTs. (A) Shows a large multilobulated hypodense mass with a multifocal enhancing irregular margin. (B) Shows heterogeneously enhanced internal irregular-shaped nonenhanced low-density lesions in a mass. (C) Shows a huge heterogeneous enhancing lobulated mass in the pelvic cavity.
Figure 2. — The pathologic finding of the ovarian MMMT. (A) Low power showing adenocarcinoma with an underlying sarcomatous area; H&E, x40. (B) x100. (C) Malignant glandular epithelium is immunohistochemically positive for pancytokeratin (CK MNF116) (x100). (D) Malignant stromal component is immunohistochemically positive for vimentin (CK MNF116) (x100). (E) The tumor was composed of adenocarcinoma and homologous high grade sarcoma; H&E, x40. (F) Low power showing well-formed glands amid pleomorphic spindle cells; H&E, x100.
showed multiple recurrences in the liver and lung. She received a seventh cycle of the same regimen; she experienced pancytopenia. She died of sepsis during the chemotherapy.

Case 4

A 66-year-old woman presented with a low abdominal palpable mass. CT showed large amounts of ascites and a large mass in the right ovary. Laparotomy revealed a 17.5×15×4.5-cm solid mass in the right ovary with brownish ascites. The left adnexa showed no abnormal lesions but adhered to the peritoneum, while the right ovarian mass adhered to the posterior cul-de-sac. There were no abnormal findings in the other internal organs. She underwent a right adnexectomy and omentectomy. Histopathologic examination revealed a MMMT containing endometrioid carcinoma and sarcoma. After the operation, she received six cycles of combination chemotherapy of cisplatin (20 mg/m²) and ifosfamide (1.5 mg/m²). Twelve months later, she was checked with CT. A CT taken 12 months after chemotherapy revealed that there was a solid mass in the pelvic cavity. She underwent the secondary debulking operation. At operation, the mass was found to adhere to the small bowel and thus the authors resected the mass along with the small bowel. The pathologic report revealed that the mass was a MMMT involving the small bowel serosa. After the secondary operation, the disease progressed and the patient died of the disease.

Discussion

Primary ovarian MMMTs are very rare, accounting for approximately one percent of all ovarian malignancies [7]. This disease is most prevalent in old women, with the median age being 67.5 years [8]. As with epithelial ovarian cancer, the most common clinical symptom is abdominal distension [8]; in the present series, all patients complained of abdominal distension and discomfort. Patients are usually at an advanced stage of disease by the time of diagnosis; approximately 70% of patients present at Stage III or IV disease and die of the tumor shortly after diagnosis [9, 10]. Widespread metastases are common at the time of surgery, which makes optimal surgical debulking more difficult. Of the present cases, only one underwent an optimal surgery. It is generally known that, in ovarian carcinoma, bulk residual tumors are associated with worse prognoses but this has not been statistically proven with ovarian MMMTs [11]. Sood et al. compared overall survival between the optimal surgery and suboptimal surgery groups in 47 cases for 17 years and found that in the optimal surgery group (residual mass < two cm), median survival was 25 months, while it was eight months in the suboptimal group. Harris et al. [11] compared survival between the optimal surgery and suboptimal surgery groups and found that there was no significant difference between the two groups, while the time to tumor recurrence was longer than in the optimal surgery group. Table 1 shows the survival difference between the optimal and suboptimal groups: five studies showed that survival was prolonged in the optimal group, while two studies showed that there was no significant difference between the two groups. Surgery alone is seldom curative even in patients with optimally resectable disease [12]. As with other epithelial ovarian cancer cases, adjuvant radiation therapy was not effective in MMMT cases; furthermore, this therapy increased the incidence of side effects.

Until the mid-1990s, whole abdominal radiation therapy was performed; however, it was unsuccessful in controlling MMMTs and had many side effects. Even now the value of radiation therapy (with or without chemotherapy) is disputed [13].

Many authors agreed that combination chemotherapy is better than single agent chemotherapy. However, there are different opinions regarding the proper regimen. Until the 1980s, a combination therapy of vincristine, actinomycin D, and cytoxan (VAC) integrated with whole pelvic/abdominal radiation was used. The response rates were 31%-42% [10, 14]. Morrow et al. [10] showed an objective response rate of 10% in patients who received doxorubicin adjuvant chemotherapy. After the mid-1980s, Moore et al. [12, 14] reported on cisplatin-based chemotherapy, and thereafter it has been used in combination with cisplatin or carboplatin. Sood et al. [7] found that an objective response rates were 12% in patients with non-platinum-based adjuvant treatment and 80% in patients with platinum-based adjuvant treatment. Table 2 shows comparisons between groups which received platinum-based chemotherapy. Survival ranged from 8.2 to 53 months. In the present cases, the authors used cisplatin-based chemotherapy. The side effects of the platinum-based chemotherapy regimen, especially ifosfamide, are significant. In the present series, one patient developed pancytopenia and sepsis, after which she had to be treated at the intensive care unit. The combination of paclitaxel/carboplatin has been found by the Gynecologic Oncology Group (GOG) to have effects comparable to cisplatin/paclitaxel but less toxicity [4]. Duska et al. [2] reported that 57% of the 28 patients with MMMTs, showed a complete response to the combination of paclitaxel and carboplatin and that their mean survival was 27.1 months. However, Sit et al. [4] reported a mean survival of 19 months when the same regimen was used.

As mentioned above, MMMTs are comprised of carcinomatous and sarcomatous components. MMMT can be subclassified as heterologous or homologous according to the absence or presence of a stromal component contain-

Table 1. — Mean survival between optimal and suboptimal group.

<table>
<thead>
<tr>
<th></th>
<th>Optimal Surgery</th>
<th>Suboptimal Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sood et al. [7]</td>
<td>25 months</td>
<td>8 months</td>
</tr>
<tr>
<td>Rutledge et al. [8]</td>
<td>25 months</td>
<td>16 months</td>
</tr>
<tr>
<td>Duska et al. [2]</td>
<td>No difference between both groups</td>
<td></td>
</tr>
<tr>
<td>Brown et al. [1]</td>
<td>14.8 months</td>
<td>3.1 months</td>
</tr>
<tr>
<td>*Harris et al. [11]</td>
<td>No difference between two groups</td>
<td></td>
</tr>
<tr>
<td>Silasi et al. [5]</td>
<td>46 months</td>
<td>27 months</td>
</tr>
<tr>
<td>Muntz et al. [13]</td>
<td>24 months</td>
<td>10 months</td>
</tr>
</tbody>
</table>

*In the optimal group, the time to recurrence was longer.
ing mesenchymal tissue which is not normally found at the primary tumor sites.

Survival differences between patients with homologous and heterologous components are still controversial [12, 15]. Mok et al. [12] demonstrated that the presence of a heterologous component has no significant impact on survival. In contrast, Sood et al. [5] and other investigators showed that the presence of a heterologous component is related to poor prognoses.

Ovarian MMMTs are a rare malignancy with poor prognoses. Because of their rarity, an optimal treatment modality has not yet been established. For advanced-stage ovarian MMMTs, many authors recommend optimal cytoreduction and adjuvant chemotherapy-including platinum as part of the regimen. The present authors reported four cases of ovarian MMMTs with a brief literature review.

### Acknowledgement

Written informed consent was obtained from the patients for publication of this case report. The study was approved by our institutional review board (KC13ZISE0200).

### References


Address reprint requests to: S.Y. HUR, M.D.
Department of Obstetrics and Gynecology, Seoul St. Mary’s Hospital, 505 Banpo-Dong, Seoul 137-040 Seoul (Korea)
e-mail: hursy@catholic.ac.kr

<table>
<thead>
<tr>
<th>Patients evaluated</th>
<th>Chemotherapy regimen</th>
<th>Progression-free survival</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutledge et al. [8]</td>
<td>20 Carboplatin (AUC:6) + taxol (175 mg/m2)</td>
<td>12 months</td>
<td>21 months (55% alive at 2 years)</td>
</tr>
<tr>
<td>Mok et al. [12]</td>
<td>10 Cisplatin (75 mg/m2) + ifosfamide (1.2 mg/m2)</td>
<td>5.2 months</td>
<td>11.7 months</td>
</tr>
<tr>
<td>Thiggen et al. [16]</td>
<td>132 Cisplatin (50 mg/m2)</td>
<td>6.4 months</td>
<td>8.2 months</td>
</tr>
<tr>
<td>Brown et al. [1]</td>
<td>65 Platinum-based chemotherapy</td>
<td>8.7 months</td>
<td></td>
</tr>
<tr>
<td>Harris et al. [11]</td>
<td>40 Platinum-based chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duska et al. [2]</td>
<td>28 Carboplatin (AUC 5-7.5) + taxol (175 mg/m2)</td>
<td>9 months</td>
<td>27.1 months</td>
</tr>
<tr>
<td>Sit et al. [4]</td>
<td>13 Carboplatin (AUC 5) + taxol (175 mg/m2)</td>
<td>10 months</td>
<td>Carboplatin + taxol: 19 months</td>
</tr>
<tr>
<td>Silasi et al. [5]</td>
<td>22 Cisplatin (40 mg/m2) + ifosfamide (1.2 g/m2)</td>
<td>13 months</td>
<td>Carboplatin + ifosfamide: 23 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 months</td>
<td>Carboplatin-taxol: 38 months</td>
</tr>
</tbody>
</table>
Isolated sacral metastases as the initial presentation from an endometrioid ovarian carcinoma: a case report

G. Xin¹, J. Du², Y. Xu¹

¹Department of Gynecology and Obstetrics, The Second Hospital of Shandong University, Jinan
²Department of Gynecology and Obstetrics, Women and Children’s Hospital of Jinan, Jinan (China)

Summary
Bone metastases are rarely in ovarian carcinoma. It usually occurs only when the cancer is advanced or recurrent. A case of endometrioid carcinoma in right ovary with intact capsule is reported. The isolated sacral metastasis was found as the initial presentation, and no distant metastases were reported.

Key words: Ovarian cancer; Bone metastases.

Introduction
Ovarian cancer can spread by intraperitoneal seeding, direct invasion or through the lymphatic and vascular circulation. The bone metastases of ovarian cancer are rare with an incidence of less than four percent in previous literatures [1-4]. Bone metastases usually occur at the advanced or recurrent stage of ovarian carcinoma. In this case, the isolated bone metastasis was reported at the early stage of ovarian carcinoma.

Case Report
A 41-year-old woman complained of left buttock pain for one and a half months. Her sacrococcygeal X-ray showed no abnormalities in local hospital. Oral painkillers did not relieve her pain. The patient visited local hospital again in November 2011 and her sacral computed tomography (CT) showed a soft tissue mass at the posterior superior space of bladder in pelvic cavity in localized bone injured area on the left sacrum (Figure 1). The pelvic CT showed a mixed pelvic mass (diameter: 12 cm). A cystic and solid mass was found at right posterior space of uterus by the pelvic ultrasound. She was then submitted to the department of gynecology in the present hospital because of the sacral metastasis of ovarian carcinoma. The enhanced pelvic CT scan showed a heterogeneous soft tissue mass in pelvic cavity (Figure 2). There was a soft tissue mass behind the sacrum and the sign of sacral destruction. Her CT findings revealed the malignant tumor and bone metastasis in pelvis. Tumor marker results were as follows: CEA 48.53 ng/ml, CA125 30.29 U/ml, and CA199 34.24 U/ml. The routine abdominal ultrasound was unremarkable. The urinary system ultrasound reported a slight right hydronephrosis without ureterectasia on both sides. The upper and lower gastrointestinal Barium X-rays confirmed gastritis. The chest CT scan showed no abnormal findings. Colonoscopy was normal. The result of radioactive nucleus elements 99m Tc systemic bone scanning showed no abnormalities. After consultation with orthopedic specialists, she was primarily diagnosed to have ovarian carcinoma with sacral metastases. She was scheduled to undergo surgical exploration in pelvis and then the resection of the sacral tumor.

On December 6, her surgery was performed. During surgical exploration in pelvis, it was found that her right ovarian became larger (15 × 15 × 13 cm) with a cystic and solid mass. There was no lesion on the surface of the right ovarian. No abnormal appearances of the right uterine tube, uterus, left ovarian, and uterine tube were found. There were no tumor metastases on the surface of intestine, liver, spleen, and omentum manus. Also there were no enlarged lymph nodes in pelvis. Her right uterine appendages were resected. The quick stain for frozen sections showed poorly differentiated adenocarcinoma of the ovary. The gynecological operation was performed as follows: uterus, bilateral uterine appendages, omentum majus and appendix were removed followed by lymphadenectomy of pelvic and para-aortic lymph nodes. Subsequently, orthopedic surgeons found that sacrum was damaged by her tumor at the left of the posterior median line (diameter: six cm). The mass seemed loose without an obvious border. There were necrotic tissues flowing from sacrum. The tumor tissues in spinal canal and peripheral vertebra were resected. Pathological examination showed poorly differentiated adenocarcinoma suggesting a metastatic ovarian carcinoma (Figure 3). Also, immunohistochemical examination revealed positive ER, P53, and CD10 (Figure 4). There were no cancer cells in para-aortic lymph nodes, pelvic lymph nodes, and bilateral uterine tubes. There was poorly differentiated carcinoma in spinal canal of sacrum. It was considered to be the poorly differentiated endometrioid adenocarcinoma metastases according to ovarian pathology and immunohistochemical results.

Postoperatively, systemic chemotherapy and radiotherapy on the sacrum were recommended. She refused radiotherapy and then received TP (paclitaxel-cisplatin) chemotherapy. CA125 was normal before each chemotherapy. After six cycles, her chemotherapy was stopped. Thus far, the patient went to the hospital for a review every six months and CT displays no recurrent mass in the pelvic. Serological tests displayed that CA125, CA199, and CEA are normal.

Discussion
Female bone metastases are most common in breast cancer followed by lung, kidney, and thyroid cancers. It rarely occurs in gynecologic malignancies [5]. The autopsy studies showed that it was mostly found in cervical cancer. Bone metastases of advanced ovarian carcinoma accounted...
for six to 14% of gynecologic malignancies [6-8]. The common metastases of ovarian carcinoma spread by intraperitoneal and lymphatics via 91 approaches. The usual metastasis sites included pelvic cavity, omentum majus, and gastrointestinal tract, liver surface, and para-aortic lymph nodes. The bone metastasis always occurred with advanced rather than with early carcinoma.

Several points need to be highlighted in the present case. First, the initial symptom was bone pain. Second, surgical
Isolated sacral metastases as the initial presentation from an endometroid ovarian carcinoma: a case report

exploration found a unilateral ovarian tumor with a complete capsule. No distant metastases in pelvic and peritoneal organs and pelvic lymph nodes were noted. Therefore the patient would have been at Stage Ia if she had had no sacral metastasis. Therefore, her prognosis was poor. Third, isolated sacral metastases are rare. Bone metastases are usually include multiple involvements such as vertebral body, ribs, clavicle, skull bone, and femur [6]. To the authors’ knowledge, isolated sacral metastasis was never reported before. Fourth, the serum CA125 was at a normal level which was different from the increased CA125 level in common ovarian carcinoma.

Until now, there are only two reported cases of ovarian endometrioid carcinoma with isolated sacrum metastases as initial symptoms: one by Sansom et al. [9] who reported a patient with poorly differentiated endometrioid carcinoma in left ovary. Her tumor appeared on the surface of ovary without distant or lymphatic metastases. An isolated metastasis in left acetabulum was found four weeks postoperatively; the other by Chen et al. [10], who described a patient with mid-back pain as initial presentation. The thoracic lesion was found and confirmed to be carcinoma after biopsy. A large mass of left ovary was found after further examination. It was diagnosed to be the ovarian carcinoma containing endometrioid adenocarcinoma and adenosarcoma as well as multiple lymph node metastases after surgical exploration. The present patient was similar to these previous two cases, however, in the present case, the bone metastases occurred earlier in a rare site.

In conclusion, this case suggests that the ovarian carcinoma with initial bone metastases is rare. The risk of bone metastases should be taken into account if patients complain of bone pain and distension without any obvious reasons.

References


Address reprint requests to:
Y. XU, M.D.
Department of Gynecology and Obstetric,
The Second Hospital of Shandong University,
247 Beiuyuan Road, Jinan 250033 (China)
e-mail: xgdjxrp@163.com
Introduction

Paraneoplastic limbic encephalitis (PLE) is a rare disorder characterized by personality changes, irritability, depression, seizures, memory loss, and sometimes dementia. It was first described in 1968 as a distinct clinical and pathological entity [1]. The diagnosis is challenging, as clinical markers are often lacking, and symptoms usually precede the diagnosis of cancer or mimic other complications [2]. The prevalence and incidence are not estimated, as the disease is under-reported due to the difficulty in establishing the diagnosis [3].

The pathogenesis of PLE implicates an autoimmune process involving antigens shared by tumor and neuronal cells [4]. The most frequently associated neoplasm is small-cell lung cancer, followed by germ cell tumor of the testis, breast cancer, Hodgkin’s lymphoma, thymoma, and immature teratoma of the ovary [2]. In 2004, an international panel of neurologists established diagnostic criteria for suspected paraneoplastic neurological syndrome into ‘definite’ and ‘probable’ categories, based on the presence or absence of a typical clinical picture, cancer, and specific autoantibodies [5]. According to these criteria, a patient with the typical clinical picture is considered to have a ‘definite’ PLE, when he or she has positive ‘well-characterized’ paraneoplastic antibodies and/or known cancer and other possible causes of encephalitis have been excluded [5].

Case Report

A 32-year-old Caucasian, nullipara woman was brought to the Emergency Department of the present hospital because of sleep disturbances, mental confusion, and hallucinations. She was admitted to the Psychiatric Department for diagnostic evaluation. Eight hours after admission she developed mental impairment and presented generalized tonic-clonic seizure. Intravenous valproic acid, phenytoin administration was started. Her past medical history included Hashimoto thyroiditis and a benign left ovarian cystectomy. Initial workup included blood tests (complete blood count, renal and hepatic function, and ionogram), which were within normal range, and cerebral imaging. The brain computed tomography (CT) scan was normal. The cerebrospinal fluid analysis revealed 17 cells and both glucose and protein levels were normal. The clinical suspicion of viral encephalitis sustained the antiviral empirical therapy with acyclovir. Polymerase chain reaction (PCR) tests for herpes simplex, varicella zoster, lymphocytic choriomeningitis, and enterovirus were negative. Acyclovir therapy was stopped at the second day. Electroencephalogram recordings did not reveal epileptic features. Magnetic resonance imaging was normal. The patient remained in an uncontrolled state of psychomotor agitation and rapidly evolved to coma. In order to exclude a paraneoplastic syndrome, an abdominopelvic CT was performed. The images revealed a 119 x 132 mm right adnexal mass, which was multiloculated and contained focal areas of fatty and calcified tissue - aspects suggestive of ovarian teratoma with no other relevant remarks in the abdominopelvic cavity. At this point, the most probable neurologic diagnosis was paraneoplastic limbic encephalitis secondary to a primary ovarian tumor. In accordance to this hypothesis, the presence of specific antineuronal antibodies (anti-NMDA) were investigated and were positive. Tumor markers: CA125 and alpha-fetoprotein were elevated (five times above the upper limit of normal range) and other remained within the normal range (CEA, CA 19.9,
diagnosis of these syndromes is essential, as they can be difficult to remove because of the unstable clinical condition. The patient initiated oral corticosteroids and human intravenous immunoglobulin. Definite histopathologic examination confirmed a germ cell malignant tumor with no ovarian surface or tubal involvement. Peritoneal washing, lymphatic nodes, and omentum were free from disease. The diagnosis of immature ovarian teratoma surgical Stage FIGO IA R0 with paraneoplastic limbic encephalitis was confirmed. The patient received three cycles of adjuvant systemic cytotoxic chemotherapy with bleomycin, etoposid, and cisplatin (BEP). She was discharged one month after surgery without any neurologic deficit and remains three years later in oncological remission.

Discussion

Approximately one percent of all malignant tumors are associated with paraneoplastic neurological syndromes (PNS). The etiopathogenesis is based on an autoimmune cross-reaction. The immune system recognizes antigens expressed on cancer cells and similar epitopes found on cells of the nervous system. This immunological phenomenon can suppress the growth of the cancer, but when activated lymphocytes and onconeural antibodies cross the blood–brain or blood–nerve barriers, it can lead to an autoimmune-driven damage to cells of the nervous system and thus to a PNS [6]. These onconeural antibodies can be detected in the serum and cerebrospinal fluid of patients with underlying cancer and therefore they represent an important analytical marker for this PNS. PNS are not frequently associated with gynecologic malignancies. The paraneoplastic syndromes associated to gynecologic tumors are endocrine, neurological, musculoskeletal, hematological, and skin. As part of the diagnosis interdisciplinary cooperation involving the participation of not only oncogynecologist, but also other clinicians according to the current state and the dominant issue patients is necessary. PNS are associated with ovarian, endometrial, and breast cancer [7]. Limbic encephalitis is a result of an inflammatory process mediated by onconeural antibodies, which is confined to structures of the limbic system. Patients present mood and sleep disturbances, seizures, hallucinations, and short-term memory loss that can progress to dementia. The presence of antibodies against N-methyl-D-aspartate (NMDA) receptors are predictive of an underlying tumor, usually a cystic ovarian teratoma in about 65%. The teratoma can be mature or immature, in some cases difficult to demonstrate, or, if found, difficult to remove because of the unstable clinical condition. The diagnosis of these syndromes is essential, as they can be occasionally life-threatening or result in long-term neurological sequelae including recurrent seizures, anterograde amnesia, cognitive impairments, and chronic progressive encephalopathy with associated cerebral atrophy, particularly involving the mesial temporal and limbic structures [8].

Treatment of paraneoplastic syndromes such as limbic encephalitis is still unsatisfactory and further pathophysiology research is clearly needed. The timely implementation of combination of immunotherapy and tumour eradication is key to the successful treatment. The immune treatment is based on high-dose corticosteroids or IVIG [2,10]. Plasma exchange, rituximab or cyclophosphamide are preferred as second-line therapy [10]. Antiepileptic drugs also play a critical role in patients who manifest seizures as well as cognitive symptoms. Individual factors that need to be considered when formulating a program of maintenance treatment include disease severity, antibody specificity and proclivity for disease relapse [10]. Azathioprine and mycophenolate mofetil are frequently used for the purpose of remission maintenance, and should permit gradual withdrawal of steroids, IVIG or more toxic immunosuppressants. The duration of maintenance therapy is uncertain [10]. However, growth stimulation of the tumour by immunosuppression remains a theoretically risk. Prognosis is poor, if immune therapy is administered without concomitant treatment of the underlying malignancy.

Conclusion

This case report illustrates the complex diagnostic and treatment decisions that clinicians will need to make in a relatively short period of time. The delay in treatment could potentially lead to devastating neurological outcomes. In the setting of known cancer, the decision to treat patients for paraneoplastic neurological syndrome should be empirically considered. Early diagnosis and treatment of the underlying malignancy and prompt intervention with immune therapies in this patient at the onset of presentation will probably explain the favorable neurological outcome.

References


Address reprint requests to:
I. PESTANA, M.D.
Gynecological and Obstetrical Department,
Centro Hospitalar São João, Al. Prof Hernani Monteiro,
4200-Porto (Portugal)
e-mail: minespestana@gmail.com
Primary fallopian tube carcinoma: a case report and mini-review of the literature

E. Kalampokas¹, C. Sofoudis¹, I. Boutas¹, T. Kalampokas¹, I. Tourountous²

¹ Second Department of Obstetrics and Gynecology, “Aretaion” Hospital, University of Athens Medical School, Athens
² Department of Obstetrics and Gynecology, Thriasio Hospital, Eleusis (Greece)

Summary
Primary fallopian tube carcinoma (PFTC) is an uncommon gynecologic tumor, responsible for 0.14% to 1.8% of genital malignancies, with a mean incidence of 3.6 per million women per annum. The factors that contribute to its appearance are not well-known. Overall survival percentages for patients with PFTC are generally low. Although the preoperative diagnosis rarely occurs and it is usually first confirmed by the pathologist, an earlier diagnosis occurs with early clinical manifestation and prompt investigation leading to better prognosis. Both PFTC and epithelial ovarian cancer (EOC) are treated with similar surgical and chemotherapy methods. The authors report a case of a patient with bilateral high grade serous carcinoma of the fallopian tube, whose initial presentation was bilateral cystic adnexal masses and serosanguinous discharge, with no other pelvic involvement. This article also reviews in brief and presents updates of this rare gynecological malignancy.

Key words: Fallopian tube carcinoma; High-grade serous carcinoma; Risk factors; Treatment.

Introduction
Primary fallopian tube carcinoma (PFTC) is an uncommon gynecologic malignancy which constitutes 0.14% to 1.8% of genital malignancies [1-3], first described by Reynaud in 1847 [4]. It most frequently occurs in women aged between 18-88 years, with the most common age of occurrence being between 40-65, with a mean age of 55 years [5].

Population studies show that the mean incidence of PFTC is 3.6 per million women per annum [5]. The true incidence of PFTC is perhaps underestimated [1, 5] because some of the cases have been wrongly diagnosed as ovarian tumors during initial surgery and/or microscopic examination due to the indistinguishable histological appearance of these neoplasms [1]. PFTC is associated with chronic tubal inflammation, infertility, tuberculosalpingitis, and tubal endometriosis [6]. High parity seems to have a protective effect [2]. Overall survival percentages for patients with PFTC are generally low and mostly ranging between 22% to 57% [7, 8]. The authors report a case of a patient with bilateral high grade serous carcinoma of the fallopian tube, whose initial presentation was serosanguinous discharge and abnormal vaginal bleeding.

Case Report
A 44-year-old, gravida 3, para 2, premenopausal woman with referred two to three months duration episodes of abnormal menstrual cycle/vaginal bleeding was admitted to the department of Obstetrics and Gynecology in Thriasio Hospital, Eleusis, Greece. There was no significant past medical and gynecological history apart from two previous dilatations and curettages (D+C), for treating cervical no significant past medical and gynecological history apart from

The patient was staged according to International Federation of Obstetrics and Gynecology (FIGO) staging for fallopian tube carcinoma, and allocated as high grade serous carcinoma Ic.
Discussion

PFTC is a rare but aggressive gynecological malignancy accounting for <1% of all genital cancers. Serous carcinomas originating in the fallopian tube are asymptomatic in their early stages until their spread to other pelvic sites [9]. Although it shares common characteristics with epithelial ovarian cancer (EOC) (such as surgical FIGO staging and surgical management, epidemiological characteristics, correlation residual tumor size with prognosis, better response with platinum-based chemotherapy, there also seems to be a great difference between them: PFTC is diagnosed at an earlier stage, despite the fact that in many cases, diagnosis may occur only postoperatively by the pathologist [10]. That may be a result of abdominal pain, resulting from tubal dilatation, and/or bloody-watery vaginal discharge [10].

Preoperative tumor markers, such as CA-125, vary. Other authors report high CA-125 levels [11], while others state that CA-125 is always elevated in advanced disease but not in earlier stages of the disease [12].

The first line of therapy for PFTC consists of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, bilateral pelvic lymphadenectomy, and adjuvant chemotherapy [13].

Peritoneal washings should be taken at the time of surgery because positive washings are an adverse prognostic indicator suggesting extratubal spread and are associated with an increased risk of lymph node metastasis [14]; these washings have been found to contain malignant cells, in up to 20% of cases [14].

In most previous studies, PFTC has been graded subjectively and not according to the more objective Silverberg criteria. Rosen et al. [14] found no correlation between tumor grade and patient outcome; a correlation found by Hellstrom et al., even with marginal statistical significance [15].

The survival rates of patients with PFTC are reported to be poor. Moreover, it seems to be worse than that of patients with equivalent stages of EOC; survival is found to be worse than other early-stage gynecological malignancies [14, 15].

Recently, it has become apparent that PFTC is a multietiological disease with different clinical and morphological aspects. It seems to have an increased incidence and its accurate diagnosis and differentiation from EOC is important for better prognosis and management. Improvements in treatment and, therefore, outcome can only materialize if preceded by an early and accurate diagnosis.

References


Address reprint requests to: 
T. KALAMPOKAS, M.D. 
18 Estias street 
115 26 Athens (Greece) 
e-mail: kalamp@yahoo.com
Normal-sized ovary carcinoma syndrome (NOCS) detected with FDG-PET/CT

M. Kanda, A. Sonoyama, N. Ohara

Department of Obstetrics and Gynecology, Sanda Municipal Hospital, Sanda (Japan)

Summary

Background: Normal-sized ovary carcinoma syndrome (NOCS) is an ovarian cancer with ovaries being of normal size, accompanied by diffuse metastatic disease of the peritoneal cavity. Case: A 39-year-old woman presented with lower abdominal pains. The computed tomography (CT) of the chest, esophagogastroduodenography, and colonoscopy showed no remarkable findings. A magnetic resonance imaging (MRI) displayed a slightly enlarged right ovary, thickening of the peritoneum, and massive ascites. The right ovary showed high intensity on T2 images and scattered low intensity spots on diffusion-weighted images. The cytology of ascites suspected adenocarcinoma cells. A positron emission tomography (PET) and CT using 18F-fluorodeoxyglucose (FDG) demonstrated markedly increased FDG uptake at the right ovary and peritoneum. The presumptive diagnosis of normal-sized ovary carcinoma syndrome was made. She underwent a total hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy, and partial omentectomy. The pathological examination revealed serous cystadenocarcinoma of the right ovary. Conclusion: FDG-PET/CT is useful for the detection of NOCS.

Key words: Normal-sized ovary carcinoma syndrome; FDG-PET/CT; Primary tumor detection.

Introduction

A positron emission tomography (PET) and computed tomography (CT) using 18F-fluorodeoxyglucose (FDG) has been shown to increase the diagnostic accuracy of pre-treatment stage of ovarian cancer patients in comparison with contrast enhanced CT [1] and to have a high diagnostic value in differentiating between malignant and benign ovarian tumors [2]. In addition, FDG-PET/CT is reported to be useful for predicting the diagnosis and restaging of suspected recurrent ovarian carcinoma [3-5], the detection of recurrence of ovarian cancer and predicting patients’ survival [6], and early prediction of response to neoadjuvant chemotherapy [7].

In 1989, normal-sized ovarian carcinoma syndrome (NOCS) was defined as a clinical situation in which diffuse metastatic disease of the peritoneal cavity is noted, but the ovaries are of normal size, with or without a fine granularity on their external surface [8]. The histology of NOCS was reported to be the same as common epithelial ovarian cancer with variable degrees of differentiation, but has a great tendency to spread externally [9]. The preoperative diagnosis of NOCS remains a diagnostic challenge. When the patients with peritoneal carcinomatosis with unknown origin are encountered, preoperative radiologic assessment and surgical exploration are warranted to discern the site of origin. A recent report has demonstrated that the use of FDG-PET/CT detected the site of NOCS despite the failure with magnetic resonance imaging (MRI) and CT [10]. Nonetheless, little information is available regarding the usefulness of FDG-PET/CT for the diagnosis of NOCS.

Case Report

A patient was a 39-year-old nulliparous woman with no medical history of malignancy. She presented with persistent left lower abdominal pains. Series of examinations including a CT of the chest, esophagogastroduodenography, and colonoscopy showed no remarkable findings. A MRI of the pelvis displayed the slightly enlarged right ovary, thickened peritoneum, and massive ascites. The right ovary showed the high intensity at the solid parts (Figure 1A), and the peritoneum anterior to the rectum was thickened with showing low intensity on T2 images (Figure 1A). The scattered low intensity spots were noted in the solid components of the right ovary on diffusion-weighted images (Figure 1B). A CT of the pelvis failed to detect any apparent tumors in the pelvis (Figure 1C). The cytology of ascites suspected adenocarcinoma cells. Serum CA125 levels were elevated at 248 IU/ml. She was diagnosed as having peritoneal carcinomatosis. However, the site of origin remained undetermined.

Subsequently, in order to detect a possible site of primary malignancy, she underwent FDG-PET/CT at one and two hours after intravenous injection of 2.23 MBq/kg body weight of 18F-FDG. FDG-PET/CT demonstrated markedly increased FDG uptake at the solid components of the right ovary (the maximum standard uptake value (max SUV) = 9.3) (Figure 2A, B) and the irregularly thickened peritoneum at the cul-de-sac (max SUV = 4.8) (Figure 2A, B). The left ovary had the solid components, but showed no FDG accumulation. Besides these findings, no abnormal FDG accumulation was noted in the whole body scanning.

Based on the imaging studies in addition to the cytological malignancy of ascites and elevated serum CA125 levels, a presumptive diagnosis of NOCS was made. On laparotomy, the bladder was adherent to the anterior wall of the uterus, and the sigmoid and rectum were adherent to the peritoneum of the cul-
de-sac, forming a frozen pelvis. The ascites amounted to 1,650 ml. Numerous peritoneal implants less than five mm in diameter were noted on the serosa of the intestines and omentum. A frozen section analysis of the right ovary revealed serous cystadenocarcinoma. She underwent a total hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy, and partial resection of the omentum. Macroscopically, the tumor was 20 x 30 x 20 mm in size. The microscopic examination confirmed serous cystadenocarcinoma of the right ovary. She has been treated with adjuvant chemotherapy consisting of paclitaxel and carboplatin.

**Discussion**

The authors presented a case of NOCS that was correctly diagnosed with FDG-PET/CT preoperatively. Wu et al. described that normal-sized ovarian serous surface papillary carcinomas should be kept in mind as an origin of disease in patients who have peritoneal carcinomatosis [11]. Nevertheless, the diagnosis of NOCS still remains a diagnostic challenge. In the present case, a CT of the pelvis failed to detect any pelvic mass and localize peritoneal involvement. MRI
displayed the slight swelling of the right ovary and thickened peritoneum on T2-weighted images and low intensity of the right ovary on DW images, but these findings were inconclusive for determining the site of primary malignancy. However, FDG-PET/CT clearly demonstrated markedly increased FDG accumulation in the sites corresponding to the right ovary and peritoneum, indicating the right ovarian cancer with peritoneal dissemination.

A recent report has also demonstrated that the use of FDG-PET/CT detected the site of origin in a case of ovarian serous surface papillary carcinoma with manifestation of NOCS [10]. They reported that PET/CT confirmed the intense FDG uptake in both the right and left ovaries with max SUV of 5.0 and 6.0, respectively, and abnormal FDG uptake in the nodular/irregular thickening of the mesentery and peritoneum with max SUV of 2.5 to 4.6 [10]. The origin of the peritoneal carcinomatosis was thought to be the left ovary with the right ovary being involved by tumor implant. They suggested that FDG-PET/CT seems to be advantageous in evaluating peritoneal carcinomatosis in NOCS.

With FDG-PET/CT, peritoneal implants were shown to appear as nodular soft-tissue masses, often with a variable degree of increased metabolic activity, and omental thickening and nodularity with diffuse FDG uptake were indicative of omental involvement [5]. The present patient had numerous military peritoneal implants on the intestines and omentum, but these lesions were not displayed with FDG-PET/CT. This may be due to the inability of FDG-PET/CT to depict small volume lesions. However, Sanli et al. evaluated the diagnostic value of FDG-PET/CT in comparison with MRI for the detection of recurrent ovarian cancer, and demonstrated that although PET/CT was similar to MRI for the detection of recurrent ovarian cancer, PET/CT had greater accuracy in the detection of small-to-medium-sized (< two cm) peritoneal implants compared with MRI [12]. Furthermore, Kim et al. demonstrated that in ovarian cancer patients with peritoneal carcinomatosis, the sensitivity and the specificity for the diagnosis of peritoneal carcinomatosis were 96.2% and 90% for PET/CT and 88.5% and 65% for enhanced abdominal CT and that the accuracy of PET/CT was statistically higher compared with enhanced CT [13]. They suggested that FDG-PET/CT imaging is efficient in the diagnosis of peritoneal carcinomatosis and that its performance is superior to that of enhanced CT [13].

Recent study has demonstrated that FDG-PET/CT is a useful method for the detection of cancer of unknown primary (CUP), which represents a heterogeneous group of metastatic tumors for which no primary site can be detected following a thorough medical history, careful clinical examination, and extensive diagnostic workup [14]. A meta-analysis showed that FDG-PET/CT could detect 37% of primary tumors in patients with CUP with high sensitivity and specificity of 84% [14]. In three cases (1.9%) among patients with CUP, the ovary was found to be the location of primary tumors detected with FDG-PET/CT [14]. This fact may reinforce the usefulness of FDG-PET/CT for the detection of NCOS.

Collectively, this case emphasizes that FDG-PET/CT is useful for the diagnosis of NOCS in combination with the evidence of malignant ascites and elevated serum CA125 levels, even when imaging results of CT or MRI are negative or inconclusive.

References


Address reprint requests to: M. KANDA, M.D., PhD.
Department of Obstetrics and Gynecology
Sanda Municipal Hospital
3-1-1 Keyaki-Dai, Sanda, 669-1321 (Japan)
e-mail: kanda_masatoshi@hospital.sanda.hyogo.jp
Multifocal microinvasive squamous cell carcinoma with extensive spread of squamous cell carcinoma in situ (CIS) into the uterine corpus, vagina, and left salpinx diagnosed five years after conization of cervical CIS


1 Department of Obstetrics and Gynecology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul
2 Department of Pathology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul (Korea)

Summary

Background: Multifocal microinvasive squamous cell carcinoma (SCC) with extensive spread of squamous cell carcinoma in situ (CIS) into the uterine corpus, salpinx, and vagina is extremely unusual. Case: The authors present a case of 69-year-old woman with hydrometra who was found to have multifocal microinvasive SCC in the endometrium. The CIS had spread superficially throughout the entire endometrium up to the fundus, completely replacing the epithelium. The uterine cervix, vaginal surface, and left salpingeal mucosa were involved. She had previously undergone conization due to cervical CIS five years prior. The pathologic reports showed clear resection margins at that time. Conclusion: The present case suggests that CIS in the endometrium spread back to the cervix and vagina, although the definite origin of the first CIS was not determined.

Keywords: Uterine cervix; Microinvasion squamous cell carcinoma of the cervix; Squamous cell carcinoma in situ; Superficial spreading squamous cell carcinoma; Endometrium.

Introduction

Superficial spread of squamous cell carcinoma in situ (CIS) of the uterine cervix to the endometrium, fallopian tubes, and vagina is an extremely rare phenomenon [1-8]. Although cervical squamous cell carcinoma (SCC) generally directly invades the uterine wall with or without parametrial involvement, the histological continuity of the lesion in these cases suggests a horizontal spread [5]. Microinvasive carcinoma of the uterine cervix has been designated as a minimal depth of stromal invasion of three mm or less and is believed to carry little or no risk of metastatic disease. Some cases of cervical CIS have been associated with invasive SCC in the corpus, fallopian tube, ovary or combinations thereof [1]. Pins et al. [2] suggested that such lesions arose in the cervix and extended to the corpus, tubes, and ovaries, where the tumor cells acquired invasive capability. Kushima et al. [1] demonstrated that loss of heterozygosity (LOH) analyses with a panel of microsatellite markers revealed a monoclonal process that could determine primary origin.

In this report, the authors describe a patient with multifocal microinvasive SCC with extensive spread of squamous CIS into the uterine corpus, cervix, vagina, and left salpinx. Notably, the endometrium was completely replaced by CIS. The patient had previously undergone conization five years prior due to cervical CIS, with clear resection margins. Six months after the conization, a colposcopy examination showed no recurrent disease in the cervix or vagina.

Case Report

A 69-year-old Korean housewife, gravida 6, para 3, presented with a palpable pelvic mass in January 2013. She underwent conization in December 2008 in the present hospital. The pathologic evaluation at that time showed clear resection margins. In May 2009, colposcopically directed punch biopsy was performed because of a positive human papillomavirus (HPV) DNA testing result during a routine follow-up. It was reported as chronic cervicitis. The patient had no other significant past medical or surgical history. She was thereafter lost to follow-up.

A physical examination revealed a fetal head-sized pelvic mass without tenderness and the atrophied uterine cervix was completely obliterated. A transvaginal ultrasound demonstrated two cystic pelvic masses sized 8.8 x 6.3 cm and 8.4 x 6.8 cm. Her Pap smear was negative for intraepithelial lesion or malignancy. Computed tomography (CT) of the abdomen and pelvis showed dumbbell-shaped hydrometra without a significant cervical mass (Figure 1). There was homogeneous low attenuated endometrium and no evidence of adenopathy or metastatic disease. She underwent an extraperitoneal hysterectomy with bilateral salpingo-oophorectomy and incidental upper vaginectomy.

The gross surgical specimen confirmed hydrometra. The external surface of the uterus appeared smooth. Sectioning of the specimen revealed no tumor mass, although the endometrium had a corrugated gray-white appearance. The fallopian tubes and the ovaries were grossly unremarkable. Frozen sections demonstrated that the specimen was CIS, and the authors finalized surgery.

Microscopically, multifocal microinvasive SCC (maximum depth of invasion less than one mm) with extensive spread of
CIS into the entire uterine corpus, cervix, vagina, and left salpinx was observed (Figure 2). Including the CIS, the tumor size was 11.0 (width) x 5.0 (length) cm. The CIS involved the distal resected margin and cervix. The endometrium was replaced by CIS. The tumor was initially thought to be FIGO Stage IB2 because of the total tumor size; however, the FIGO staging system has no definite guidelines for this condition. She was referred to the Radiation Oncology Department, and adjuvant radiotherapy was chosen due to the resected margin CIS and total tumor size.

Discussion

In the present report, the authors described a patient with multifocal microinvasive SCC with extensive spread of squamous CIS into the uterine corpus, cervix, vagina, and left salpinx. She underwent conization in 2008 due to cervical CIS, with clear resection margins. The recurrence of CIS completely treated by conization in the cervix with spread to the upper genital tract within five years is unlikely. Moreover, the endometrial pathological status was more advanced than those of the cervix and vagina. In the presented case, both the cervix and endometrium were initially thought to be involved simultaneously. The lesions are believed to be discontinuous. Kanbour et al. [3] concluded that the study of margins in conization specimens of cervical intraepithelial neoplasia (CIN) cannot predict the presence or absence of residual CIN in the uterine cavity or the existence of multicentric CIN. The tumors may have originated in the cervix and spread to the upper genital tract. However, the authors cannot exclude the possibility that the tumor originated in the endometrium. Nonetheless, they believe that the CIS on the upper genital tract spread superficially to the cervix and vagina after conization.

Pins et al. [2] reached no definite conclusion regarding whether these tumors are a composite of multiple independent neoplastic clones or a single clonal process arising from the cervix. Kushima et al. [1] suggested a single
clonal process for the tumors by LOH analyses. LOH analyses using a panel of microsatellite markers revealed a monoclonal process in four of the five cases. Homogeneous LOH throughout the microdissected lesions was most frequently detected on 6p and 6q (three cases), followed by 11p and 11q (two cases), which are loci known to be commonly lost in typical cervical SCC. However, one of the five cases was negative for LOH. Ishida et al. [9] suggested that increased expression of CD138, a cell-surface heparan sulfate proteoglycan, on carcinoma cells may participate in superficial spread by cell-cell interactions. Decreased expression of CD138 has been reported to correlate with tumor invasion.

Superficial spread of SCC of the cervix is a rare phenomenon, and a guideline for the management of these cases has not been established [10]. In addition, the FIGO staging system has no descriptions for such a condition. To date, too few cases of superficial spreading SCC of the cervix have been reported to establish a conclusion regarding their treatment and prognosis [10]. Gupta et al. [11] reported a case of superficial endometrial spread of CIS followed by radiotherapy. Gungor et al. [10] performed radical hysterectomy with bilateral salpingo-oophorectomy and pelvic and para-aortic lymph node removal in a case with three-cm cervical cancer spread to the endometrium, focal myometrium, bilateral tubal mucosa, fimbriae, and bilateral ovaries. Kushima et al. [1] reported that CIS cases without a diagnosed cervical squamous cell lesion underwent simple hysterectomy and with a diagnosed cervical squamous cell lesion, they performed simple hysterectomy with pelvic lymphadenectomy without adjuvant therapy.

Conclusion

In conclusion, multifocal microinvasive SCC with extensive spread of squamous CIS into the uterine corpus, cervix, vagina, and left salpinx is a rare phenomenon. Notably, the lesions in this case were not detected five years prior to this presentation despite cervical conization. It is possible that upper genital lesions can spread back to the cervix and vagina following conization. Although an LOH analysis may determine the definite origin, more data are required because of the rarity of such cases. The management of these cases remains unclear.

References


Address reprint requests to:
S.J. LEE, M.D., Ph.D.
Department of Obstetrics and Gynecology,
Konkuk University Hospital,
Konkuk University School of Medicine
4-12, Hwayang-dong,
Kwangjin-gu, Seoul (Korea)
e-mail: lsj671121@gmail.com
Primary choriocarcinoma of the fallopian tube: a case report and literature review

J. Wan, X.M. Li, J. Gu

Department of Gynecology, The 3rd Affiliated Hospital of Sun-yet Sam University, Guangzhou (China)

Summary

Choriocarcinoma is a highly malignant tumor of trophoblastic origin. Primary fallopian tube choriocarcinoma is an extremely rare occurrence, especially in women over 50 years of age. This article concerns a case of tubal choriocarcinoma developing in a 54-year-old woman, which the authors present together with a brief review of the literature. The woman presented with irregular vaginal bleeding for two months, following three months of amenorrhea. Transvaginal doppler and pelvic computed tomography (CT) scan showed an adnexal cystic-solids mass. Her serum human chorionic gonadotropin (hCG) levels were 29,1116 mIU/ml. The patient underwent hysterectomy and bisalpingo-oophorectomy. Histology was suggestive of tubal choriocarcinoma. Immunohistochemistry tests were positive for the hCG, Ki 67, CK,PLAP, and negative for CD30, supporting the diagnosis of choriocarcinoma. A combination of 5-Fu and KSM was administrated postoperatively. After four cycles of chemotherapy, her serum hCG level fell to the normal range. The patient remains disease-free 14 months after disease diagnosis.

Key words: Fallopian tube; Choriocarcinoma; Immunohistochemistry; Chemotherapy.

Introduction

Choriocarcinoma is an extremely aggressive form of gestational trophoblastic disease composed of two types of cells, syncytiotrophoblasts and cytrophoblasts. The most common site of origin is uterus. Primary fallopian tube choriocarcinomas are extremely rare. The reported incidence of ectopic tubal choriocarcinoma is approximately 1.5/1,000,000 births [1]. Most patients are in reproductive-age. Here, the authors present a case of tubal choriocarcinoma in a 54-year-old woman which was successfully treated. They summarize its histopathological and clinical features, as reported in the literature.

Case Report

A 54-year-old woman, gravid 2, para 2, presented to the present outpatient department with irregular vaginal bleeding for two months, following three months of amenorrhea. Her previous cycles were also irregular, varying from one to three months. She had diagnostic curettage in another hospital before coming to the attention of the present authors, and the pathological result showed a secretary endometrial with A-S reaction. A transvaginal ultrasound scan showed an empty uterus, a 44 x 30mm left adnexal solid mass and 44 x 35mm right adnexal solid mass, and little pelvic free fluid. Her urine pregnancy test was positive and her serum hCG levels were 29,1116 mIU/ml. The patient received four cycles of chemotherapy, following monthly serum hCG levels fell rapidly 24 hours following surgery and remained < 5 IU/l from four weeks post-surgery. Follow-up at 14 months revealed no evidence of recurrence. The patient is still being followed up at regular intervals.

Discussion

Choriocarcinoma is a highly malignant disease of trophoblastic origin. Most cases occur in woman of reproductive age, usually within one year following all kinds of pregnancies, and are classified as gestational choriocarci-
Gestational choriocarcinoma is common in the clinic. The non-gestational choriocarcinoma is rare and not associated with pregnancy. It can arise from germ cell or trophoblastic differentiation in different types of carcinoma, and it can occur at any age and in any gender, including males and children. Most cases of choriocarcinoma are intrauterine. Extrauterine choriocarcinoma has been reported to arise at a site of fallopian tube, ovary, cervix, great omentum, rectouterine pouch, lung, and brain. Ectopic tubal choriocarcinoma is extremely rare, the majority of publications refer to case reports. There are limited data available regarding the epidemiology of fallopian tube choriocarcinoma. The reported incidence is less than five percent of all choriocarcinomas [2]. The patients in the most of reported cases of recent twenty years were in reproductive-age, ranging from 24 to 38 years old. Patients over 50 is rare. A comparison of the number of case reports of tubal choriocarcinoma in the last twenty years is presented in Table 1.

The clinical presentation include amenorrhea, vaginal bleeding, abdominal pain, and adnexal mass, which are usually similar to those of an ectopic pregnancy. The patients always had a history of all kinds pregnancies several months or years before. The longest interval between last pregnancy and choriocarcinoma is reported as 38 years and after menopause [15]. In the presented case, the patient had no pregnancy history recently, her last pregnant was 21 years prior. Since her previous cycles were irregular, varying from one to three months, it is impossible to rule out entirely the possibility that this followed a recent undetermined pregnancy.

The serum β-hCG level is an important diagnostic tool before surgery. As in other gestational choriocarcinoma, it
mostly rises to abnormally high levels, from three to 100 times higher than in normal pregnancy. However there is also exception, and it did not allow the present authors to distinguish between gestational and non-gestational disease. CT, endovaginal ultrasound, and colour flow Doppler also played a role in the diagnosis of ectopic gestational choriocarcinoma. The hypervascularity found on sonography is consistent with the known hypervascular nature of choriocarcinoma. Other various serum tumor markers (AFP and CA-125) are also useful in the differential diagnosis of choriocarcinoma.

Given the rarity of the condition, the diagnosis is usually made on histological examination of a surgically resected specimen. The histological features of choriocarcinoma are columns of malignant syncytiotrophoblasts and cytotrophoblasts, commonly separated by coagulated blood, that invade the muscular tissue, but no chorionic villi are present. Immunohistochemistry analysis is useful in differential diagnosis. The tumour cells had strong diffuse b-hCG immunoreactivity. The Ki67 index was also strong stained. As trophoblastic cells are derived from epithelia, CK staining is usually positive in choriocarcinomas as cytokeratin is expressed on trophoblastic cells. There is also focal PALP staining. CD-30 is a marker of various germ cell tumors. Negative staining for both markers helps in ruling out a germ cell origin of such tumors [15].

The non-gestational choriocarcinoma is pathologically indistinguishable from gestational ones, except in patients who are sexually immature or virgin. Microsatellite polymorphism analysis by examination of restriction fragment length polymorphisms (RFLPs) using locus specific mi-
crosatellites would be a better option to distinguish gestational from non-gestational in married, reproductive-aged women. However, they do not always give conclusive results. Recently, Nakayama et al. demonstrated the usefulness of a combination of p57KIP2 immunostaining and DNA polymorphism analysis in determining the origin of extratubal choriov carcinoma (i.e. gestational or non-gestational) [5]. However, it is time-consuming and limited to clinical applications.

Due to rarity of the disease, to date, no therapeutic strategy has been established. The majority of the literature suggests that tumor resection followed by chemotherapy using single or combination drugs offer the best chance of treating. However, in some recent cases, only unilateral salpingo-oophorectomy has led to long-term survival without chemotherapy. In the present case, the authors performed total abdominal hysterectomy and both salpingo-oophorectomies, considering the age of the patient and the malignancy of the disease. After the operation, the serum hCG level decreased rapidly. Then they used multigent chemotherapy (5-FU +KSM), which is commonly used in patients with choriov carcinoma in China, and the response is positive. The serum hCG level fell to within the normal range after two cycles, and the patient was given two more cycles. The response to multiple agent chemotherapy was positive, and the prognosis was good.

Serial hCG levels after primary surgery provide a useful guide to postoperative treatment and its response. If the hCG titers fail to fall continuously or if disease recurs after cessation of chemotherapy, then the patient should be re-evaluated, and a different therapy should be initiated. Alternatively, if the hCG level reaches an undetectable level, treatment should be continued for two more courses and lifelong hCG monitoring is recommended as it is still unclear when to stop monitoring.

Primary tubal choriov carcinoma is quite rare. Most of the patients have clinical presentation mimicking ectopic pregnancy. The diagnosis is dependent upon pathological examination and special and immunohistochemistry staining. Management includes complete surgical resection with or without subsequent adjuvant chemotherapy. Although the disease is aggressive, most of the patients have a favorable prognosis.

References


Address reprint requests to:
X.M. Li, M.D.
Department of gynecology,
The 3rd Affiliated Hospital of Sun-yet Sam University,
Tianhe Road 600, 510630, Guangzhou (China)
e-mail: tigerlee777@126.com
Join us for our 2014-2015 CONGRESSES!

OVARIAN CLUB IV
Blastocysts Development and the Process of Implantation
November 15-16, 2014
Paris, France
www.comtecmed.com/oc

EUROGIN 2015
International Multidisciplinary Congress
HPV Infection and Related Cancers: Translating Research Innovations into Improved Practice
February 4-7, 2015
Sevilla, Spain
www.eurogin.com/2015

International IVI Congress
Reproductive Medicine and Beyond
April 23-25, 2015
Alicante, Spain
www.comtecmed.com/ivi

www.comtecmed.com
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

2014, XV, 270 p. 59 illus., 50 illus. in color.
ISBN 978-1-4614-8270-3
ISBN 978-1-4614-8271-0 (eBook)
DOI 10.1007/978-1-4614-8271-0
Springer New York Heidelberg Dordrecht London
Library of Congress Control Number: 2013954553
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.

Executive Board:
Pier Luigi Benedetti Panici (Italy)
Carlos F. de Oliveira (Portugal)
Giuseppe de Palo (Italy)
Santiago Dexeus (Spain)
William Dunlop (UK)
Stelios Fotiou (Greece)
Gerald Gitsch (Austria)
A. Peter M. Heintz (Netherlands)
Michael Hoeckel (Germany)
Jan Jacobs (UK)
Jacques Lansac (France)
Tiziano Maggino (Italy)
Harald Meden (Germany)
Joseph Monsonego (France)
Laszlo Palfalvi (Hungary)
Sergio Pecorelli (Italy)
Denis Quelleu (France)
Stelio Rakar (Slovenia)

Executive Board:
Piero Sismondi (Italy)
Claes Tropé (Norway)
László Ungár (Hungary)
André Van Assche (Belgium)
Raimund Winter (Austria)

International Advisory Board
Chairman: Antonio Onnis (Italy)
Hugh Allen (Canada)
Curt W. Burger (Netherlands)
Alberto Costa (Italy)
André Gorins (France)
Neville F. Hacker (Australia)
Maria Marchetti (Italy)
Stelios P. Michalas (Greece)
Maria Teresa Osorio (Portugal)
Ulf Uldsten (Sweden)
Jan B. Vermorken (Belgium)
George D. Wilbanks (USA)
Jan Zielinski (Poland)

All questions concerning the Academy may be sent to:
Peter Bosze, M.D. - P.O. Box 46 - Budapest 1301 (Hungary)
Phone: +36 1 4290317 - Fax: +36 1 2752172 - E-mail: eagc@cme.hu

www.cme.hu

Administrative Office:
1301 Budapest, P.O. Box 46 - Hungary
Fax (36 1) 4290318 - E-mail: eagc@cme.hu
CLINICAL AND EXPERIMENTAL OBSTETRICS & GYNECOLOGY

Subscription Order Card 2014

Founded in 1974 (ISSN 0390-6663) - Vol. XLI. Issued bimonthly. All subscriptions are entered on a calendar-year basis. Individual rate is not applicable if payment is made through an Institution.

Subscriptions are entered with prepayment only and are accepted per calendar year only but can be backdated depending on availability. If not cancelled by the end of October, they will be tacitly considered as renewed; cancellations will not be refunded.

Discounts: 10% to book sellers and subscription agencies.

Please enter my subscription at the rate I've checked:

<table>
<thead>
<tr>
<th>PAPER ISSUE</th>
<th>ONLINE ISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Institutional: 600 USD</td>
<td>Institutional: 450 USD</td>
</tr>
<tr>
<td>Individual: 400 USD</td>
<td>Individual: 270 USD</td>
</tr>
<tr>
<td>Single copy: 120 USD</td>
<td>Single copy: 100 USD</td>
</tr>
<tr>
<td></td>
<td>Single article: 30 USD</td>
</tr>
</tbody>
</table>

Payment: (USD ONLY)

- for PDF file: online through PayPal (all credit cards)
- for hard copy

Credit Card: [ ] Mastercard  [ ] Visa  [ ] Diners

Bank transfer: Beneficiary: 7847050 Canada Inc. - 4900 Côte St-Luc, #212 - Montréal, Québec, H3W 2H3 Canada - Account number 00001 003402-402245 SWIFT ROYCCAT2

N° ___________________________ Exp. Date ___________________________

Signature ___________________________ Date ___________________________