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Role of professional societies in the global battle against gynecologic cancers

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Introduction

In 2008, Collingridge wrote that “Cancer kills more people than tuberculosis, malaria and AIDs combined and over two-thirds of all deaths occur in low to middle income countries where resources are scant or nonexistent… most of these deaths are needless; if the knowledge and options available today were exploited to their maximum effect, most cancer could be avoided or cured [1]”. Among the various opportunities for working together against gynecologic cancer, the role of professional societies has been underdeveloped and under-utilized.

Societies usually begin as a loose network of like-minded professionals from a similar geographic region. Over time their activities become more focused and their way of interacting with each other becomes more structured. Eventually these societies develop linkages with other regional, national or international organizations. Given that the members of the societies have both a knowledge and emotional investment in this field, they should be the leaders in their area of interest.

In the domain of gynecologic cancers, cervix cancer is by far the most pressing issue. It is the second most common cause of cancer world wide with 500,000 new cases each year and 270,000 deaths. This disease usually occurs where screening is non-existent or poorly managed. Below are some of the domains in which societies that are focused on gynecologic cancer can make an impact [2].

Role for professional societies in low and middle resource settings

Continuing education for its membership

Usually groups of like-minded individuals coalesce around an educational opportunity. Whether it is the Mongolian Obstetrics and Gynecology Society or the Society of Gynecologic Oncologists in the United States, education which emphasizes the best practice based on scientific evidence rallies the membership together. Whether it is a lecture during an evening meal, or a formal congress, presentation of the evidence is the starting point for professionals to meet together, discuss the information and determine if there is an opportunity to incorporate new information within their setting.

Societies can also link with other organizations to provide training material or formal courses to enhance skills. These courses have usually been developed from educational principles with pre and post course evaluation, incorporating different learning styles, and provide visual as well as didactic material. In the field of cervical cancer there are courses like Visual Inspection with Acetic Acid (VIA), training developed by JHIPIEGO [3] and the palliative care manual by PATH which can be accessed through the Alliance for Cervical Cancer Prevention (ACCP) website [4].

Promotion of self assessment and audits

Once the vision for a new technique is captured, individuals or groups implement the new technique. As a society develops, there are opportunities to present audits of practice. At the recent 2009 National Congress on “Improving Practice by Exchange”, experience in cryotherapy at the National Maternal Child Hospital showed how a technique previously not available in Mongolia could be adapted. One barrier that was overcome involved how to obtain access to CO2. Such national meetings provide opportunities to share information but also provide an opportunity for feedback.

Opportunities may also arise to present information internationally, i.e., the Asia-Oceania Research Organization in Genital Infection and Neoplasia (AOGIN). This is a forum not only to present results but interact with health care providers from other like resourced countries [5]. These interactions allow venues for problem solving issues with healthcare providers from other regions. It allows interaction with agencies that potentially provide equipment, financial resources, and intellectual resources. Another benefit is to receive constructive critical assessment of completed projects in an environment which fosters the tenets of evidence based medicine. The epitome of this interaction is when
the collaboration results in a well designed study. One such success story has been the work by PATH with Indian and Chinese healthcare providers during the development and testing of the careHPV test [6]. Here the sharing of leadership skills and resources were vital to building, implementing, monitoring and evaluating the careHPV test. Such interactions will ensure greater ownership and sustainability at the country level.

One example of a high level audit program is the cancer control work promoted by the World Health Organization (WHO) through the International Agency for Research in Cancer (IARC). Here information on specific indicators of cancer (i.e., incidence and mortality) are reported and help in identifying successes and barriers to care (i.e., Cancer-MONDIAL) [7]. On a national level, a society could be the leader in initiating an assessment on regional variations in practice and outcomes.

In a different domain, societies can promote self-assessment programs, allow physicians to document their continuing medical education and demonstrate its impact on their own practice patterns. The Royal College of Physicians and Surgeons of Canada has such a self-assessment program which is now mandatory for all specialists to maintain their certification with the Royal College [8].

**Standard setting**

Inadequate care fails the individual patient in front of you, her family unit, and ultimately erodes public confidence in the ‘healthcare system’. Around a decade ago when I first began visiting the National Oncology Hospital in Mongolia, many people indicated that they did not want to go to the hospital because it was a place that you go to die. Often if healthcare professionals are not seen to be meeting a need and the patient is distressed, the families will move away from health professionals and seek out traditional healers. However, if a vision of cancer care can be expanded to include prevention of cancer then one can turn a negative impression into an energized mandate. Cervical cancer is a wonderful example where screening strategies can prevent cancer or at least down-stage it such that survival is more possible with available surgical or radiation treatments.

Quality of care can also be improved through the promotion of a standard setting, practice guidelines or development of care paths. If a carepath defines that women with endstage disease are given palliative care in her community but women with potentially curable disease are sent to tertiary care centres for radical therapy, this allows resources to be available for those who will most benefit. By highlighting areas that need improvement and addressing high case fatality rates in certain regions or facilities, energy can be focused on resolving the problem.

**Awareness raising**

Professional societies have an incredible role in providing accurate information to the public concerning women’s cancers. In addition to public broadcasts, access to health information can include educational level appropriate booklets, videos and websites. Formal interactions between scientists and healthcare providers can generate an effective outreach into the community.

**Teamwork**

Extending the realm of influence from gynecologic cancer surgeons or obstetricians and gynecologists to include nurses, midwives and other oncologists (radiation oncologist, medical oncologists), educators, and basic research scientists to name a few, helps capitalize on the strengths of each discipline. One of the greatest success stories for cervical cancer is how the Alliance against Cervical Cancer and partners like EngenderHealth, the International Agency for Research on Cancer (IARC), JHPIEGO, and the Pan American Health Organization (PAHO) incorporated midwives as a point of care in assessment of the cervix using VIA and treatment using cryotherapy [9].

**Political lobbying**

Many professional healthcare workers have a broad sphere of influence. In part this is related to their level of education, their opportunity for learning other languages, their financial status and their interaction with patients and families from all strata of society. Thus if the goal is to draw attention to the unacceptable rate of cancer deaths healthcare professionals and their societies play a pivotal role by undertaking needs assessments that can clarify the specific barriers to care. They can educate those charged with health resource allocation like the Ministry of Health concerning the disease and the service requirements. They can point to specific needs to strengthen and scale up the health system to enable them to provide effective low-cost interventions. They can work toward national policies, strategies and action plans related to cancer prevention and treatment. They can suggest government policy and vigorously endorse it. They can encourage proactive legislation that protects and cares for women’s basic human rights and encourages access to quality healthcare for women [10].
Discussion

I have attempted to show the origins, development and potential of healthcare professional societies. The mandates a cancer-focused society from a low or middle resource setting can have on peer and public education, audits and research, standard setting and agenda or policy setting have been shown. Another educational opportunity that societies could take on revolves around resident and medical student education. Societies can advocate for a standard setting in licensure requirements, examinations and duration of training. The Society of Gynecologic Oncologists in Canada were pivotal players in advocating for initially the two and three year fellowship training programs over a decade ago. More recently they have been successful in launching the exit examination through the Royal College of Physicians and Surgeons of Canada.

In the domain of medical student and resident education, training could be organized so that personnel from low or middle resource countries come to a high resource centre for certain rotations. Jointly, the Department of Obstetrics and Gynecology at McMaster University, Hamilton and St. Joseph’s Hospital, Hamilton have such a program supported by the Sisters of St. Joseph Hospital. This program runs in conjunction with the Faculty of Medicine of Haiti at the University of the State of Haiti to provide their Obstetrics and Gynecology residents training in cervical cancer prevention which is a system currently not available in the country. It allows practice with new techniques under the supervision of Canadian gynecologists. It also provides the visiting residents with a vision for cervical cancer prevention that could be taken back to their country.

This exchange program can involve personnel from a high resource country going to a low resource setting which provides an opportunity for transfer of information and experience provided it is appropriately supervised with specific goals, monitoring, assessment and feedback. Currently the Society of Gynecologic Oncology (SGO) in conjunction with the International Gynecologic Cancer Society (IGCS) has a Visiting Professor Program in Guatemala and Panama with the aim of improving the training of residents in gynecologic oncology in their country [11]. Medicine for Humanity is a nonprofit charitable organization which was co-founded by Dr. Leo Lagasse, a retired gynecologic oncologist from Cedar-Sinai and Kaiser Hospitals in Los Angeles [12, 13]. Volunteers have gone with this organization to the Philippines, Africa, Pakistan, Mexico, Malawi, Uzbekistan, Poland, Croatia, Costa Rica, Panama, Bangladesh, and Nepal to work with local physicians to provide a comprehensive approach to cancer screening, prevention and treatment. Obstetrics and gynecology residents from the California programs can spend time under the supervision of American staff personnel in the low to middle resource country both providing a service, gaining experience and transferring information and practice with their peers.

What are the responsibilities and potentials for professional healthcare associations from high resource countries to assist their colleagues in low resource countries? One would postulate that there is an ethical obligation for health professional associations to promote women’s health within their country and to eventually assist other associations in their effort to also do so. This assistance can come in various forms: used equipment adaptation and donation, financial support of societies’ projects, professionals’ time and energy to build the human resources in low/middle income countries, and development of leadership. Donating professional time toward working in the low or middle resource country has the potential for modeling evidence-based healthcare decision making at the bedside as a way of life. It informs the visiting professional of the resource constraints and allows them to problem solve with their peers who live in that environment. For those professionals who partner with policy makers either at the level of leadership in a cancer centre or ministry of health, modeling decision-making based on cost-effectiveness information is incredibly valuable [14].

Currently many of the linkages between Obstetrics and Gynecology societies in low and middle resource countries with those in high resource settings are ad hoc based on convenience rather than any systematic planning. They seem to be based on historic relationships with countries i.e., the relationship of the National College of Obstetrics and Gynecology of France (CNGOF) with hospitals and universities in former colonies (i.e., Vietnam) or with countries that speak the same language (i.e., CNGOF with Africa). Eventually linkages may occur with the Federation of Gynecologic Obstetrics (FIGO) or the International Gynecologic Cancer Society. FIGO has the potential of providing medical and clinical expertise, social credibility, advocacy and lobbying. FIGO includes all major obstetric and gynecologic organizations worldwide but their mandate is broad including cancer, and infertility. The difficulty has been their limited ability to devote much attention to subspecialties. One society more focused on gynecologic cancers is the International Gynecologic Cancer Society (IGCS) which was formed in the 1980s. It is a forum for meetings and discussion among international physicians and surgeons in the specialty of gynecologic cancers [15]. The IGCS is structured to offer individual memberships rather than country representation. The IGCS is a relatively young society and formalized international programs are in their infancy. Currently, the ability of professional societies from high resource countries to influence change is by taking an initiating and collaborative role.

The success stories within and between societies have arisen where there is a vision, dedicated personnel, ability to obtain resources (specifically financial) and an accountability structure. Our future will depend on, as Franklin Roosevelt said, “The test of our progress is not whether we add to the abundance of those who have much. It is whether we provide enough to those who have little” [16].
References


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Significance of serum CA125 and TPS antigen levels for determination of overall survival after three chemotherapy courses in ovarian cancer patients during long-term follow-up

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Introduction

Tumor markers have been demonstrated to be useful in ovarian cancer for prediction of prognosis, monitoring treatment response and for early detection of recurrences. Many studies have been designed to identify prognostic factors for determination of suitable treatment and also when to change treatment [1, 2]. The most important prognostic factors in ovarian cancer patients include age, FIGO stage, performance status, histological type, absence of ascites and type of primary cytoreductive surgery [3].

CA125 is by far the most documented and the best performing single biomarker for ovarian cancer and is the only marker approved for monitoring of ovarian cancer progression and for treatment response [4-6]. The sensitivity and specificity of CA125 is limited in patients with early-stage disease. However, CA125 measurements are performed almost routinely during the course of chemotherapy; and where CA125 levels after two or three courses of chemotherapy have been demonstrated to be an independent prognostic factor [1, 7, 8].

The time at which normalization of CA125 levels should be achieved, as well as the apparent half-life of CA125 have been proposed to be valuable prognostic indicators.
factors [9-13]. However, the prognostic information derived from CA125 measurements has failed to make a major impact on patient management, as the majority of patients with epithelial ovarian cancer will relapse [14]. The level of CA125 has been shown to correlate with overall survival, and in particular CA125 levels around 100 U/l after treatment indicate poor prognosis [15, 16]. The established prognostic factor CA125 half-life and CA125 nadir level were shown to be independent prognostic factors for overall survival in advanced ovarian cancer patients during induction therapy [13, 17, 18]. Another interesting prognostic approach was to apply the baseline CA125 levels before starting maintenance chemotherapy after primary chemotherapy [19]. Patients with a pre-maintenance CA125 level below 10 U/l have a superior progression-free survival compared to higher CA125 levels. The CA125 level at the end of primary therapy was shown to be a predictor of overall survival, as well as for progression-free survival in ovarian cancer patients with surgically defined disease status, using optimized cut-off levels within the normal range [20]. A number of biomarkers have been proposed to be of additive value to CA125 during follow-up of ovarian cancer to obtain better patient management, e.g. TPA (tissue polypeptide antigen), CA72-4, LDH, TATI (tumor-associated trypsin inhibitor), CASA (cancer associated serum antigen) and TPS (tissue polypeptide specific antigen) [21-24]. Some of these markers might be particularly valuable in mucinous type tumors, where CA125 shows a relatively low sensitivity. TPS measures a specific epitope structure of soluble cytokeratin 18 fragments and reflects the activity of the tumor growth [24, 25]. A number of reports describing the use of TPS in the follow-up of ovarian cancer patients have been published [26-30]. Recently we demonstrated the prognostic significance of the combination of CA125 and TPS in ovarian cancer patients (all FIGO stages) after three chemotherapy courses [29, 31]. In particular, patients with advanced disease and elevated CA125 levels had a poor prognosis, but importantly the prognostic accuracy could be even further increased by the addition of TPS. Multivariate analysis showed that staging laparotomy, CA125 and TPS elevated were independent factors for prediction of 2-year overall survival. The purpose with this study was to analyze the clinical outcome of these patients during long-term, 10-year clinical follow-up. It is important to find out whether the elevated marker combination CA125 and TPS in ovarian cancer patients (FIGO Stage I-IV), after three chemotherapy courses, may add further information on long-term overall survival than seen after two years.

Material and Methods

Patients

The patient collective has been described before in the 2-year overall survival study [29]. In short, a total of 213 patients (mean age 56 years, SD 11 years) FIGO Stage I-IV, receiving chemotherapy were prospectively recruited between 1994 and 1997 to the multicenter study. The patients entered the study independent of CA 125 value. Of these 213 patients (all FIGO stages, all ovarian cancer histological types and all grades of differentiation were represented), originally included in the 2-year overall survival study, 212 patients could also be included in the 10-year clinical follow-up overall survival study. All patients had received at least three courses of combined chemotherapy (cisplatin or carboplatin, in combination with cyclophosphamide). Patients included in the study were classified accordingly: FIGO Stage I (n = 34), Stage II (n = 30), Stage III (n = 113) and Stage IV (n = 36).

Surgery was carried out in all hospitals according to the FIGO recommendations from 1994-1997. In patients with FIGO Stages I-II, lymphadenectomy was performed. In advanced stages, FIGO Stage III-IV optimal debulking was performed whenever feasible. Residual tumor size smaller than 2 cm was taken as optimal debulking. Further treatment was conducted according to the protocol used in the individual hospital. Detailed patient characteristics have been presented in the 2-year overall survival study [29].

Serum tumour markers

Serum CA125 antigen concentrations were determined in each hospital using CA125 (II) assays. Serum TPS antigen levels were measured by an IRMA or ELISA assay from IDL Biotech AB (Bromma, Sweden). In the earlier 2-year follow-up study, optimal discrimination levels (optimal cut-off levels) of CA125 and TPS assays were determined by receiver operating curve (ROC) analysis as 25 kU/l and 100 U/l, respectively [29].

Statistics

Statistical analysis was performed using the StatView package. Patients were grouped on the basis of FIGO Stages I, II, III, and IV and the combined Stages FIGO I + II and FIGO III + IV. Overall survival was defined as the elapsed time between operation and death. Actuarial overall survival curves were calculated using the method of Kaplan-Meier and compared by log-rank test. Variables found to be significant (p < 0.05) on univariate analysis were entered into the multivariate Cox proportional hazards model.

Results

Patients in FIGO Stage I and II

In FIGO Stage I (n = 34), the 10-year overall survival was 64.7% (22 out of 34), with a median (50%) survival > 10 years. In FIGO Stage II, the 10-year overall survival was 43.3% (13 out of 30), with a median survival of 48 months. The difference in overall survival between Stage I and II patients was not statistically significant. In the seven FIGO Stage II patients where partial debulking was performed, all had died within ten years. No significant difference in survival was observed between Stage I and II patients undergoing radical surgery (64.7% vs 56.5%). Further characteristics of the possible prognostic factors for FIGO Stage I and II (histological type, tumor grade, and tumor marker levels after three chemotherapy courses) are presented in Table 1. This Table indicates that grade 3 and the histological types of clear cell-undifferentiated carcinoma were significant predictors of low survival. In multivariate analysis these two factors were not independent prognostic factors.
Patients in FIGO Stage III and IV

A total of 148 patients were included in the combined group FIGO Stage III+IV. In FIGO Stage III (n = 112), the 10-year overall survival was 15.2% (17/112), with a median survival of 25 months. In FIGO Stage IV (n = 36), the 10-year overall survival was 5.6% (2/36), with a median survival of 15 months. The difference in survival between Stage III and IV patients was significant (p < 0.005).

In the combined Stage III+IV group, survival was highly dependent on the type of operation that could be performed. In staging laparotomy (n = 21), the overall survival was 4.8% (1/21), with a median survival of 12 months. If partial debulking could be performed (n = 67), optimal debulking resulted in an overall survival of 23.3% (14/60) and a median survival of 41 months. These survival curves are presented in Figure 1. Further characteristics of the prognostic factors for 10-year overall survival for FIGO Stage III and IV are presented in Table 2.

Further, because of the high impact on survival indicated by operation possibility and tumor marker levels above the discrimination level, these factors were also analyzed in the total patient group collective (n = 148), then excluding the grade of the tumor (Table 4).

Figure 1. — Ten-year overall survival curves in FIGO Stage III+IV ovarian cancer patients depending on operability (optimal debulking n = 60; partial debulking n = 67 and staging laparotomy n = 21; p < 0.0001).
FIGO Stage IV, partial debulking + staging laparotomy, the CA 125 level above 25 kU/l and the TPS level above 100 U/l were all predictors of low survival.

Survival curves are presented for patients with CA125 levels either above or below 25 kU/l (Figure 2), and TPS levels above or below 100 U/l (Figure 3), respectively. In Figure 4, survival curves are presented for patients with both tumor markers elevated (n = 30; survival 0%), one tumor marker elevated (n = 58; survival 3.4%) and both markers below the discrimination level (n = 60; survival 28.3%). In the group of patients with one marker elevated, where two out of 58 patients survived (3.4%), one patient showed elevated CA 125 and normal TPS, whereas it was the opposite in the other patient. The difference in survival between patients with CA 125 levels above (n = 47) and below 25 kU/l (n = 11) was not significant. This was also observed in patients with TPS levels above (n = 11) or below (n = 47) 100 U/l.
In relation to surgery, only patients with optimal debulking showed a reasonable 10-year overall survival (23.3%). Because the tumor marker levels were independent prognostic variables, we also analyzed the significance of these levels in the group of patients with optimal debulking (n = 60). Survival of only 4.8% (1/21) was registered in patients with a CA 125 level above 25 kU/l after three chemotherapy courses, versus 33.3% when CA 125 was below 25 kU/l (median survival 18 and 50 months, respectively; p < 0.0001). In patients with a TPS level above 100 U/l, comparative data were 9.1% (1/11) versus 26.5% (13/49), respectively, with a median survival of 11 and 49 months, respectively (p < 0.005).

In multivariate analysis, CA 125 and TPS were independent prognostic variables in this group of 60 patients with optimal debulking. Therefore if both markers were elevated survival was 0% (0/6), if one marker was elevated the survival was 10% (2/20), and when both markers were below the optimal cut-off level, survival was 35.3% (12/34). The survival curves are given in Figure 5.

Discussion

Patients in FIGO Stage I and II

The 10-year overall survival of patients in FIGO Stage II and partial debulking was 0% (0 out of 7); where four of the seven (4/7) patients died already within the first two years after operation, and the remaining three patients died in the third year after operation. This does not necessarily mean that these patients have been understaged, because also in patients with Stage III and partial debulking the survival was only 4.8% (1 out of 21) and furthermore all these patients had died within four years after operation.

No difference in survival was observed between patients in Stage I and II with radical surgery, which can be explained by the fact that only high-risk Stage I patients were included in the study. All patients received three chemotherapy courses (combined platinum-based chemotherapy), however the treatment after that depended on the individual hospital.

Significant differences in survival were observed between patients with histological type clear cell or undifferentiated carcinoma (these histological types with a poor prognosis were combined because of low numbers) and the other histological types (serous or mucinous). The median overall survival in patients with serous or mucinous carcinoma exceeded ten years, while the poor histological group only showed 29 months median survival.

In contrast to the survival results of our patients after only two years [29], also the histological grade of the tumor was a significant factor after ten years. Here, a 10-year overall survival of 89% is seen in grade 1, versus 35% in grade 3 tumors.

The difference in survival of patients with a CA 125 level above or below 25 kU/l did not reach statistical significance, but may well do so once the > 10 year median overall survival is known. The difference in survival of patients with a TPS level above or below 100 U/l was not significant. Therefore, tumor marker levels of CA 125 and of TPS after three chemotherapy courses did not contribute to assessing response to treatment in patients with ovarian cancer FIGO Stage I and II.

Patients in FIGO Stage III and IV

There is hardly any debate that survival of ovarian cancer patients is dependent on operability and stage of disease. Whereas radical surgery is an option in Stage I and II, optimal debulking is the maximum operability which can be achieved in Stage III and IV [3]. Many patients in Stage III and IV will have only partial debulking or even a staging laparotomy. The combined Stage III+IV patient group showed a 10-year survival of 12.8%, as compared to the 45.9% recorded in the already published 2-year survival study [29].

When divided by stage, the 10-year overall survival in Stage III (n = 112) was 15.2%, with a median overall survival of 25 months as compared with a 2-year overall survival of 52%. For patients with Stage IV disease, 94.4%, or 34 out of 36, did not survive the first 3.5 years after operation.

Significant differences in survival were not only observed between Stage III and IV, but also between the type of operation, grade of the tumor and whether the marker levels of CA 125 or TPS were above or below the optimal discrimination level after three chemotherapy courses. These results were parallel with our 2-year overall survival findings. In multivariate analysis, independent adverse factors of survival were grades 2 and 3, partial debulking + staging laparotomy, CA 125 > 25 kU/l and TPS > 100 U/l. In 2-year overall survival, grade was not an independent prognostic factor. In multivariate analysis of the 10-year overall survival (excluding grade because grading was not available in all patients) Stage IV became an independent prognostic factor in addition to the already mentioned adverse prognostic factors (Table 4).

Patients with both markers elevated after three chemotherapy courses had a worse prognosis than with only one marker elevated (Figure 4). Nearly all patients (86 out of 88) with marker elevations died within a period of five years. Only when both marker levels were normalized a survival of 28.3% was observed. In contrast, in 60 patients where both markers were normal after three cycles of chemotherapy, a survival of 28% was observed. As already mentioned, patients with staging laparotomy or partial debulking showed a poor survival of 5.7% (5 out of 88) within four years after surgery. In patients with optimal debulking a survival of 23.3% was observed (60 patients). These 60 patients differ from the 60 patients with normalized levels of both markers (independent prognostic factors in multivariate analysis). Therefore we have analyzed the 10-year overall survival of the 60 patients with optimal debulking using the tumor markers CA 125 and TPS as variables. From Figure 5 it could be
concluded that only patients with normal levels of both markers after three cycles showed a reasonable survival (10-year overall survival of 35%).

To our knowledge CA 125 levels have not been studied separately or in combination with TPS by others in this specific group of patients with a reasonable prognosis. In a recent overview about the clinical utility of cytotkeratins as tumor markers the authors concluded that TPS of all the cytotkeratins was most successfully combined with CA 125 to manage ovarian carcinoma [32]. The European Group on Tumor Markers (EGTM) recently published the contribution of CA 125 and other markers for clinical use in ovarian cancer [6]. In assessing response the authors reviewed several methods using CA 125 levels during and after chemotherapy. They concluded that the described studies were consistent in showing that measurement of CA 125 levels during initial chemotherapy could yield prognostic information, but that these studies had a number of limitations. In respect to the level of evidence it was concluded that all the reports described should be regarded as level 3 evidence studies or lower [6]. The EGTM working group also described other markers that could complement CA 125 in the detection of ovarian cancer. Less information is available for the contribution of other markers in assessing response [6, 33].

It is remarkable in our study that patients in FIGO Stage III+IV are doing rather well when both levels of CA 125 and TPS are below the optimal cut-off level. A 10-year overall survival of 28% demonstrates that this patient group has a much better prognosis than would be expected for FIGO Stages III and IV epithelial ovarian cancer. Therefore, as early as after three chemotherapy courses, we have a way of giving both short and long-term prognoses. Low levels of both markers CA 125 and TPS at that time not only indicate a short-term favorable prognosis, but also long term. From the survival curves it is concluded that in the groups of patients with an unfavorable prognosis nearly all patients had died within the first five years, and in the unfavorable operation groups already 3.5 years. In the group of patients with a favorable prognosis (i.e., patients with optimal debulking) 2-year overall survival was 67% and 10-year overall survival was 23%. The predictive value of 10-year overall survival could be increased by another relative 50%, by determining in the group of patients with optimal debulking and the patients with normal levels of both CA 125 and TPS (10-year overall survival 35%) (Figure 5).

Our 10-year survival study could be regarded as a level 2 study [6, 33]. The optimal reference values were determined in an earlier 2-year overall survival study and not further adapted. The study was prospective and multicentric and a reasonable number of patients were included. We do agree that these data should be reproduced in other centers. This could be of utmost importance for ovarian cancer patients with optimal debulking because an unfavorable patient group could be selected (nearly all patients died within 5 years after operation) by elevated levels of CA 125 and TPS. It could be questioned if these patients deserve an even more aggressive chemotherapy.

References

Significance of serum CA125 and TPS antigen levels for determination of overall survival after three chemotherapy courses in etc.


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Modulation of microRNA associated with ovarian cancer cells by genistein


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Summary

Purpose: Role of microRNAs in malignancies is well established due their regulatory role in cellular differentiation, proliferation and cell cycle control. Our purpose was to determine miRNA profiles of serially established ovarian cancer cell lines and the effect of genistein treatment. Methods: Cell lines (UL-3A, UL-3B) were established from one patient during progression of disease. miRNA profiling was performed in untreated and genistein-treated cells. Estrogen receptors (ER) were studied with real-time polymerase chain reaction (RT-PCR) and Western immunoblotting. In vitro migration and invasion assays were utilized. Results: While 108 miRNAs were expressed equally in both cell lines and their genistein-treated counterparts, an additional 53 miRNAs were differentially expressed. Genistein resulted in induction of ERα and ERβ in ovarian cancer cells. A significant reduction in migration and invasion of UL-3A and UL-3B was demonstrated in genistein-treated cells. Conclusion: Common and unique miRNA profiles were demonstrated between the two cell lines, some of which were altered by genistein.

Key words: Ovarian cancer; miRNA; Estrogen receptor; Genistein.

Introduction

Despite optimal primary treatment of ovarian cancer, overall prognosis is poor due to recurrences [1, 2]. Ovarian carcinoma (OC) is the most lethal of all gynecological malignancies, resulting in over 16,000 deaths annually in the United States. Because the symptoms of ovarian cancer are non-specific, the majority of patients do not present until their disease has metastasized [1, 2]. The first-line treatment for ovarian cancer consists of surgical cytoreduction followed by chemotherapy. Numerous studies have been directed at the understanding of ovarian cancer, however the cause of ovarian cancer is currently unknown. In order to aid in the understanding of the pathogenesis, diagnosis and management of ovarian cancer, expression profiling technologies have identified new biomarkers [3]. One such biomarker group is a class of small noncoding RNAs, termed microRNAs. They are small non coding RNAs, 22-25 nucleotides in length, and suppress the translation of the target mRNAs by binding to their 3' untranslated region [4, 5]. Post-transcriptional silencing of target genes by micro (miRNA) RNA can occur either by cleavage of homologous mRNA or specific inhibition of protein synthesis [4, 5]. It is now recognized that miRNAs are frequently deregulated in malignancy. Under-expressed miRNAs such as let-7 in lung cancer and mirs-15/16 in leukemia, are tumor suppressor genes, suppressing Ras and BCL-2, respectively. Over-expressed miRNAs such as miR-21 and the cluster mir-17-92 are oncogenes, targeting tumor suppressors PTEN and E2F1 in solid and hematologic malignancies, respectively [6].

MiRNAs have been shown to be aberrantly expressed in human ovarian cancer [7]. The overall miRNA expression can be used to separate normal versus malignant ovarian tissues. The most significantly over-expressed miRNAs were miR-200a, miR-141, miR-200c and miR-200b, whereas miR-199a, miR-140, miR-145 and miR-125b1 were among the most down-regulated miRNAs. The levels of miR-21, miR-203 and miR-205, up-modulated in ovarian carcinomas compared with normal tissues, were significantly increased after 5-aza-2’-deoxycytidite demethylating treatment of OVCAR-3 cells, suggesting that DNA hypomethylation may be involved [8]. These results indicate that miRNAs might play a role in the pathogenesis of epithelial ovarian cancer and epigenetic regulation of miRNAs may result in their aberrant expression adding to their critical role in cancer [8]. Thus, understanding of the role of miRNAs and their regulation should enable better understanding of disease and identification of disease monitoring and potential treatment targets.

Genistein has been reported to have estrogenic properties and antineoplastic activity in multiple tumor types [9-11]. Genistein possesses weak estrogenic properties and has weak affinity for the estrogen receptor. Genistein’s structural similarity to endogenous estrogen, its ability to bind to both ERα and ERβ, with preferential binding to ERβ, has been well documented [12-14]. Genistein was found to have epigenetic effects in various systems. Enzymatic assays showed genistein and 5-aza-C decreased DNMTase (DNA methyltransferase), MBD2 activity and increased HAT activity [15, 16]. Thus its antitumor activity may be at least partially mediated by epigenetic-based pathways.

There is no previous information regarding genistein’s role in miRNA regulation. The main purpose of this study was to determine if genistein results in the differential regulation of miRNAs in ovarian cancer cells.
Materials and Methods

Cell lines: Cell lines were obtained from the ascitic fluid of a patient with Stage IIIIC grade 1 papillary serous adenocarcinoma of the ovary under a University Human Studies Committee-approved protocol as described previously [17]. Cells were collected at progressive stages of disease: UL-3A, at presentation, and UL-3B at recurrence six months post-treatment and established in culture using RPMI media supplemented with 10% fetal calf serum. Cells utilized in this experiment had undergone approximately 40 passages. Genistein (5 M) treatment was for 48 hours.

Real-time polymerase chain reaction (real-time PCR): RNA was isolated with Trizol (Invitrogen Life Technologies) and resuspended in RNase-free water. Two micrograms of RNA were used for cDNA synthesis, adding dNTPs, DNaseI and RNase inhibitor and incubated at 75°C for 5 min. After adding reverse transcriptase, the mix was incubated at 42°C for 60 min followed by 5 min at 94°C. For real-time PCR 2 μl of cDNA were used with the appropriate primers and 0.5 μl of fluorescent probes. The amplification reactions were performed in LightCycler (Roche). Standard curves for quantification were generated by the human beta-microglobin transcription of various concentrations of known amounts of human DNA.

Western blotting: Cells were lysed in 2% SDS, 10% glycerol, and 50 mM Tris (pH 6.8). Equal amounts of protein (30 μg), determined by Biorad assay, were separated on 8-16% polyacrylamide gels. Following transfer to nitrocellulose the blots were blocked with 5% non-fat dry milk and incubated with primary antibodies (1-2 g/ml). Antibodies to ER and ER were rabbit polyclonals (Abcam and Zymed). Secondary antibody (peroxidase-conjugated anti-rabbit IgG) was incubated for 45 minutes. Enhanced chemiluminescence (ECL) was used to visualize the specific binding of the antibodies. Densitometric analysis was performed for quantitation.

Invasion assay: Genistein-treated and control cells were analyzed in migration and invasion assays. Five thousand cells were placed in chambers following 48 hours of treatment with genistein or control media. Migration assay consisted of chambers with 8 μm pore size with media containing 10% fetal calf serum. Cells utilized in this experiment had undergone approximately 40 passages. Genistein (5 M) treatment was for 48 hours.

Table 1. — MiRNA profile of UL-3A and UL-3B cells with and without treatment with genistein.

<table>
<thead>
<tr>
<th>MiRNA</th>
<th>UL-3A treated</th>
<th>UL-3A untreated</th>
<th>UL-3B treated</th>
<th>UL-3B untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>let-7a</td>
<td>mir-17-5p</td>
<td>mir-222</td>
<td>mir-424</td>
<td></td>
</tr>
<tr>
<td>let-7b</td>
<td>mir-181a</td>
<td>mir-23a</td>
<td>mir-425-5p</td>
<td></td>
</tr>
<tr>
<td>let-7c</td>
<td>mir-181b</td>
<td>mir-23b</td>
<td>mir-429</td>
<td></td>
</tr>
<tr>
<td>let-7d</td>
<td>mir-181d</td>
<td>mir-24</td>
<td>mir-454-5p</td>
<td></td>
</tr>
<tr>
<td>let-7e</td>
<td>mir-182</td>
<td>mir-25</td>
<td>mir-484</td>
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</tr>
<tr>
<td>let-7f</td>
<td>mir-183</td>
<td>mir-26a</td>
<td>mir-487b</td>
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</tr>
<tr>
<td>let-7g</td>
<td>mir-185</td>
<td>mir-26b</td>
<td>mir-512-3p</td>
<td></td>
</tr>
<tr>
<td>let-7i</td>
<td>mir-18a</td>
<td>mir-27a</td>
<td>mir-550</td>
<td></td>
</tr>
<tr>
<td>mir-103</td>
<td>mir-18b</td>
<td>mir-27b</td>
<td>mir-574</td>
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</tr>
<tr>
<td>mir-106a</td>
<td>mir-191</td>
<td>mir-28</td>
<td>mir-593</td>
<td></td>
</tr>
<tr>
<td>mir-106b</td>
<td>mir-192</td>
<td>mir-29a</td>
<td>mir-594</td>
<td></td>
</tr>
<tr>
<td>mir-107</td>
<td>mir-193b</td>
<td>mir-29b</td>
<td>mir-629</td>
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<tr>
<td>mir-10a</td>
<td>mir-194</td>
<td>mir-301</td>
<td>mir-638</td>
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<td>mir-10b</td>
<td>mir-195</td>
<td>mir-30b</td>
<td>mir-652</td>
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</tr>
<tr>
<td>mir-125a</td>
<td>mir-197</td>
<td>mir-30c</td>
<td>mir-660</td>
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</tr>
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<td>mir-128a</td>
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<td>mir-30d</td>
<td>mir-768-3p</td>
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<td>mir-30e-3p</td>
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<td>mir-200a</td>
<td>mir-31</td>
<td>mir-770-5p</td>
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<td>mir-200b</td>
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<td>mir-324-3p</td>
<td>mir-92b</td>
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<td>mir-20a</td>
<td>mir-339</td>
<td>mir-93</td>
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<td>mir-20b</td>
<td>mir-34a</td>
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<td>mir-210</td>
<td>mir-361</td>
<td>mir-99b</td>
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<tr>
<td>mir-15a</td>
<td>mir-212</td>
<td>mir-421</td>
<td>U46HS</td>
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<tr>
<td>mir-15b</td>
<td>mir-214</td>
<td>mir-422a</td>
<td>U47HS</td>
<td></td>
</tr>
<tr>
<td>mir-16</td>
<td>mir-220</td>
<td>mir-422b</td>
<td>U49HS</td>
<td></td>
</tr>
<tr>
<td>mir-17-3p</td>
<td>mir-221</td>
<td>mir-423</td>
<td>U50HS</td>
<td></td>
</tr>
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</table>
Table 2. — MiRNAs that were differentially regulated in UL-3A and UL-3B cells in response to genistein (GEN).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>mRNA</th>
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<tr>
<td>UL-3A untreated controls</td>
<td>miR-370, miR-371, miR-663</td>
</tr>
<tr>
<td>UL-3B untreated controls</td>
<td>miR-452</td>
</tr>
<tr>
<td>UL-3A and UL-3B controls</td>
<td>miR-148a</td>
</tr>
<tr>
<td>UL-3A controls and GEN treatment</td>
<td>miR-100, miR-196b, miR-503, miR-595</td>
</tr>
<tr>
<td>UL-3B controls and GEN treatment</td>
<td>miR-141, miR-335, miR-362, miR-585</td>
</tr>
<tr>
<td>UL-3A GEN treatment only</td>
<td>miR-122a, miR-137, miR-196a, miR-204, miR-206, miR-217, miR-331, miR-449b, miR-454, miR-501, miR-515, miR-578</td>
</tr>
<tr>
<td>UL-3B GEN treatment only</td>
<td>miR-517c, HasmiR-7</td>
</tr>
<tr>
<td>Higher expression in UL-3A than</td>
<td>miR-125b, miR-126, miR-152, miR-22, miR-30a-3p, miR-30a-5p, miR-342, miR-584, miR-625</td>
</tr>
<tr>
<td>control or GEN-treated UL-3B</td>
<td>miR-205, miR-532, miR-565</td>
</tr>
<tr>
<td>Higher expression in UL-3B than</td>
<td>miR-135, miR-765</td>
</tr>
<tr>
<td>control or GEN-treated UL-3A</td>
<td>miR-135b, miR-136, miR-766</td>
</tr>
<tr>
<td>Expression only by GEN treatment</td>
<td>miR-148b, miR-149, miR-328, miR-500</td>
</tr>
<tr>
<td>of UL-3A and UL-3B</td>
<td>miR-190, miR-296, miR-500</td>
</tr>
<tr>
<td>Expression only in control UL-3A</td>
<td>miR-497, miR-647</td>
</tr>
<tr>
<td>and control and GEN-treated UL-3B</td>
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</tbody>
</table>

Figure 1. — Analysis of ERα and ERβ in UL-3A and UL-3B cells. A. Western immunoblotting; B. RT-PCR. Cont: untreated controls; GEN: genistein-treated for 48 hours with 5 μm.

3B cells only, and miR-148a was detected in both cell lines. MiR-100, miR-196b, miR-503 and miR-595 were detected in treated and untreated UL-3A cells. MiR-141, miR-335, miR-362 and miR-585 were demonstrated in treated and untreated UL-3B cells. Twelve miRNA species (miR-122a, miR-137, miR-196a, miR-204, miR-206, miR-217, miR-331, miR-449b, miR-454, miR-501, miR-515, miR-578) were induced by genistein in UL-3A cells. UL-3B genistein-treated cells expressed miR-517c, and miR-7. MiR-135 and miR-765 were upregulated with genistein (GEN) in both UL-3A and UL-3B cells. Nine miRNAs were expressed at higher levels in UL-3A cells than control or GEN-treated UL-3B cells (miR-125b, miR-126, miR-152, miR-22, miR-30a-3p, miR-30a-5p, miR-342, miR-342 and miR-625). Similarly, miR205, miR-532 and miR-565 were expressed in higher levels in UL-3B cells than untreated or GEN-treated UL-3A cells. MiR-135 and miR-765 were expressed only in GEN-treated UL-3A and UL-3B cells. Twelve additional miRNAs were differentially expressed by UL-3A and UL-3B cells in untreated and genistein-treated cells.

The effect of GEN on the expression of ERα and ERβ was studied in ovarian cancer cells by Western immunoblotting (Figure 1A). Densitometric analysis of these gels demonstrated that UL-3A cells had a 1.2-fold increase, while UL-3B cells had a 1.4-fold increase of ER expression. Similar analysis of ERβ resulted in the demonstration of a 1.2-fold increase in UL-3A cells, and 2.6-fold in UL-3B cells. Further analysis of the effect of genistein on estrogen receptors was studied by real-time PCR (Figure 1B). Transcriptional analysis demonstrates the induction of ERα in all cell lines. Induction of ERα was 2.1-fold in UL-3A cells and 3.4-fold in UL-3B cells. Analysis of ERβ also demonstrated induction in all cell lines tested with most induction associated with UL-3B cells.

Since one of the key events in ovarian cancer outcome is metastasis, effect of genistein on in vitro parameters associated with metastatic ability was determined. Both migration and invasive characteristics of UL-3A and UL-3B cells were studied following genistein treatment. UL-3B cells had significantly more migratory ability than UL-3A cells while the invasive ability of untreated cells was similar. Significant inhibition of migration was observed in UL-3A and UL-3B cells (Figure 2A). With regard to invasive ability, significantly reduced invasion of matrigel was demonstrated in both cell lines with UL-3B cells demonstrating the most inhibition (Figure 2B).

Discussion

MiRNA profiles associated with cell lines isolated from the same patient at initial diagnosis and following recurrence were studied. To our knowledge, this is the first description of miRNA profiles of cell lines isolated before therapy and after treatment failure. Our analysis demonstrated the presence of 108 miRNAs with both cell lines. We detected miR-200a and miR-200b in all samples of the four most significantly over-expressed miRNAs in ovarian cancer [7]. We had previously demonstrated eight miRNAs (miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR, miR-203, miR-205 and
miR-214) in exosomes isolated from serum specimens of women with benign disease and various stages of ovarian cancer [18]. The levels of the eight specific microRNAs were similar between cellular and exosomal microRNAs. In this study, miR-200a, miR-200b, miR-21 and miR-214 were demonstrated in both cell lines with and without treatment. MiR-141 and miR-205 were only seen in UL-3B cells and we did not detect miR-200c and miR-203. MiR-214, which was shown to induce cell survival and cisplatin resistance primarily through targeting the PTEN/Akt pathway, was detected in both cell lines [19]. In a similar fashion, six miRNAs (let7e, 30c, 125b, 130a and 335) were always diversely expressed in all resistant cell ovarian cell lines [20]. Previous studies demonstrated the utility of serum miRNA as biomarker of ovarian cancer with miRNAs miR-21, miR-92, miR-93, miR-126, miR-29a being over-expressed; and miRNAs-155, miR-127 and miR-99b were under-expressed. In our study, we demonstrated miR-21, miR-92, miR-93, miR-29a and miR-99b in all cells, but did not detect miR-126, miR-155 and miR-127. Of interest, a few of the miRNAs we detected (miR-141, miR-149 and miR-135b) have also been shown in the placenta. The most abundant placental miRNAs (miR-141, miR-149, miR-299-5p and miR-135b) were detected in maternal plasma during pregnancy and showed reduced detection rates in post-delivery plasma. The plasma concentration of miR-141, however, increased as pregnancy progressed into the third trimester [21].

Recent reports have shown the association of a selective group of miRNAs with serous ovarian cancer [22]. When compared to normal tissues miR-21, miR-125a, miR-125b, miR-100, miR-145, miR-16 and miR-99a were expressed in malignant tissues. Of these we detected miR-21, miR-125a, and miR-16 in all, miR-125b and miR-100 in UL-3A, and neither miR-145 or miR-99a in any of the cultures. Increased expression of 200,141, 18a, 93 and 429 and lower expression of let-7b and 199a were correlated with poor prognosis. Of this group, miR-18a, miR-93 and miR-429 were present in all cultures.

Functional analysis of miRNAs indicates that let-7a-2, let-7a-3, let-7b play a role in RAS regulation while miR-10b is associated with metastatic potential and 206 (seen in genistein-treated UL-3A cells), represses ER-alpha in breast cancer lines [23]. Other studies identified 27 miRNAs associated with ovarian cancer cell in vitro response to cytotoxic agents in 16 cell lines [24]. When compared to results presented by these investigators, we detected let-7e, miR-106a, miR-132, miR-181b, miR-185, miR-21, miR-23b, miR-339 and miR-99b equally in all cells while miR-371(UL-3A only), miR-331(UL-3A gen only) and miR-126 were higher in A than both treated and untreated B.

When cells were treated with genistein, UL-3A cells were associated with 19 up-regulated miRNAs and eight were induced in UL-3B cells. We also demonstrated that genistein results in a significant reduction of the invasive ability of ovarian cancer cells. Epidemiological evidence suggests that consumption of soy products is negatively correlated with the incidence of chronic diseases, such as coronary heart disease, osteoporosis, atherosclerosis and certain type of cancers, including colon, prostate and breast [25]. Modulation of nuclear receptors is believed to be an important intracellular mechanism through which soy components exert their impact on physiological functions. Isoflavones, the major soy phytoestrogens, are structurally similar to endogenous estrogens of humans.

Figure 2. — In vitro migration and invasion assays. UL-3A and UL-3B cells were studies with and without treatment with genistein (GEN).
and animals and have both estrogenic and antiestrogenic activities. Isoflavones especially genistein have been shown to modify gene expression of sex hormone receptors, including ER, PR and AR in different tissues. Phytoestrogens bind to both types of estrogen receptors but with higher affinity to ERβ than steroidal estrogens [26].

Abundant presence of not only the classic ERα and PR but also ERβ has been demonstrated in normal ovaries and in ovarian tumors [27]. The ERα/ERβ ratio is markedly increased in ovarian cancer. Thus ERβ might play a protective role against ERα mitogenic activity or ERβ as a marker of cell dedifferentiation [28, 29]. Thus, molecules specifically activating ER or inducing ER re-expression in neoplastic cells may be beneficial for tumor proliferation or invasion. Accumulated data from protein expression in neoplastic cells may be beneficial for tumor molecules specifically activating ER or inducing ERβ re-expression [30, 31]. ERβ with higher affinity to ERβ than steroidal estrogens bind to both types of estrogen receptors but tors, including ER, PR and AR in different tissues. Phytoestrogens play a protective role against ERα-mediated responses in transfected breast cancer cells [30]. Genistein can effectively inhibit beta-mediated responses in breast cancer cells [30]. Genistein and synthetic derivatives of isoflavone in cancer prevention and therapy”. Mini Rev. Med. Chem., 2006, 6, 401.

Whether the observed effects of genistein in our study are mediated via the regulation of estrogen receptors or epigenetic modulation is not known. This intriguing question requires further study to understand regulation of miRNAs in ovarian cancer and potentially in other hormone-sensitive cancers. Since the cells we studied were all obtained from the same patient, our results also indicate that heterogeneity with respect to receptor expression and sensitivity exists.

Conclusion

Common and unique miRNA profiles were demonstrated in the two cell lines isolated during progression of disease in ovarian cancer. Genistein results in the differential regulation of selected miRNAs.

References


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Sentinel lymph node biopsy in vulvar cancer: a pilot study

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Introduction
Vulvar cancer is an uncommon but devastating disease [1]. Up to now the gold standard for early lesions has been radical vulvectomy with complete bilateral inguinal-femoral lymphadenectomy [2]. Patients frequently experience complications such as lymphedema, cellulite or pain [1-4].

There is increasing interest among gynaecologic oncologists to implement the sentinel lymph node biopsy (SLNB) procedure in vulvar cancer patients in clinical practice [6].

SLNB leads to decreased short-term and long-term morbidity [7-9]. Depending on the method used the detection rate varies between 68.8% and 100% [5, 10-15].

The aim of this pilot study was to evaluate the SLNB in early vulvar cancer.

Materials and Methods
Seventeen patients were treated between February 2003 and March 2007 with a combined procedure of (99 m) Technetium labelled nano-colloid and blue dye. Both Technetium and blue dye were injected intradermally at four sites around the tumour. The SLNB was first performed on both sides using a handheld gamma detecting probe and the sentinel nodes were separately sent to the pathologist. Frozen sections were performed. During this period a third incision was made to take out the vulva specimen. Completeinguinal-femoral dissection was then performed. At minimum a laparoscopic pelvic lymph node dissection is necessary.

Statistical analyses were performed using SPSS V.16.0 for Windows.

Summary
Background: The aim of the pilot study was to assess the feasibility, efficacy, and accuracy of the sentinel lymph node biopsy (SLNB) procedure in vulvar cancer. Patients and Methods: From February 2003 to March 2007, 17 patients with vulvar cancer, clinical Stages I and II, underwent SLN (sentinel lymph node) detection, followed by a complete inguinal-femoral lymphadenectomy. Demographic, surgical, and pathologic data on all patients were reviewed. Results: 17 patients underwent the SLNB procedure. Sixteen had vulvar carcinoma and one patient suffered from melanoma of the vulva. Midline localisation was done in 11 patients (64.7%). A total of 371 lymph nodes were resected. The median number of removed lymph nodes was 15 (range 2 to 81). Nineteen lymph nodes were positive with a maximum of six in one patient. Overall the detection rate for the sentinel lymph node was 88.2% (15 out of 17). One of the two patients with a non detectable sentinel node had positive lymph nodes. Eighty lymph nodes were detected as the sentinel node. The median number of sentinel nodes was five (range 0 to 11). Seventeen sentinel nodes were involved. The sentinel node was negative in nine patients; one of these had involved lymph nodes. Conclusions: SLNB is feasible and safe to perform in vulvar cancer. Further evaluation is needed until new guidelines allow the use in early-stage vulvar cancer.

Key words: Lymphadenectomy; Sentinel lymph node biopsy; Vulvar cancer.

Results
Table 1 shows the patient characteristics. The median age was 75 (range 37 to 83). Eight patients had node involvement. In five of these laparoscopic assisted pelvic lymphadenectomy was performed. A total of 371 lymph nodes were removed on the 17 patients. Overall 19 lymph nodes were involved in seven patients (Figure 1). The resected number of lymph nodes were 254 inguinal-femoral and 117 in the pelvis. Eighty sentinel lymph nodes were detected in 15 patients with a median of five sentinel nodes (range 0-11). Seventeen sentinel lymph nodes were involved with a maximum of six sentinel nodes in one patient. Eleven involved sentinel nodes were found on the right and six on the left. There were 31 blue-stained sentinel nodes and 49 radioactive nodes. Eighteen blue-stained sentinel nodes were found on the left and nine on the right. Fourteen lymph nodes had both radioactive and blue staining. Overall the detection rate was 88.2% (15 out of 17). Blue staining occurred in 52.9% (9 out of 17) and radioactive in 76.5% (13 out of 17) (Figure 2). Out of 15 patients with detected sentinel lymph nodes seven had positive nodes. Eight patients had no sentinel involvement. None of these had further lymph node involvement. There were no false-negative findings.

Discussion
The incidence of lymph node metastases for all clinical Stage 1 patients is less than 10% [16]. Despite this low incidence complete groin dissection is established as the standard operative procedure in the treatment of vulvar cancer. This leads in general to significant morbidity. Van der Zee et al. [7] published a multicentre observational
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study on 403 patients. Two hundred and fifty-nine patients were in the sentinel lymph node group. After a median follow-up of 35 months the recurrence rate was 2.3% and three-year survival 97%. Short-term and long-term morbidity were significantly better in the sentinel lymph node group. Hampl et al. treated 127 women between 2003 and 2006 in seven centres in Germany with T1 to T3 vulvar cancer. The false-negative rate was 7.7%. The authors concluded that the sentinel procedure is safe if limited to T1-tumours [17]. As standard lymphadenectomy leads to a similar recurrence rate as the sentinel procedure Martinez-Palones et al. suggested the SLNB as an alternative to inguinal-femoral lymphadenectomy [18]. On the other hand in a study by de Hullu et al. the majority of patients preferred complete inguinal-femoral lymphadenectomy to a 5% false-negative rate of the SLNB procedure. This was not age or side-effect related [3]. The detection rate is still high [10, 14, 19, 20]. Our detection rate was 88.2% as also found by Levenback et al. [15]. They used only blue dye, and we used the combination of (99 m) technetium labelled nano-colloid and blue dye. In agreement with other authors we found no false-negative results [11, 12, 15, 21]. Any remaining positive sentinel lymph node is detrimental for the patient. Complete inguinal-femoral lymphadenectomy sometimes causes quality of life complications. The real goal is to introduce the sentinel procedure with carefully selected patients. These results are suitable for further evaluation and training until new guidelines are implemented.

Table 1. — Patient characteristics (n = 17).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number</th>
<th>Percent</th>
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<tr>
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<tr>
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Nodal status

<table>
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<td>52.9</td>
</tr>
<tr>
<td>N1</td>
<td>8</td>
<td>47.1</td>
</tr>
</tbody>
</table>

Histology

cornificated squamous epithelium | 14 | 82.4 |
uncornificated squamous epithelium | 2 | 11.8 |
vulvar melanoma | 1 | 5.9 |

Treatment

radical vulvectomy | 8 | 47.1 |
hemivulvectomy | 5 | 29.4 |
radiical vulvectomy + flap reconstruction | 4 | 23.5 |

References


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Emerging concepts in epithelial ovarian cancer: highlights of
the 4th Canadian Conference on Ovarian Cancer Research

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Key words: Ovarian cancer; Risk factors; Etiology; Treatment; Canadian Conference.

Introduction

Epithelial ovarian cancer (EOC) is the most lethal of the gynecological cancers and is the fifth leading cause of cancer-related death in Canadian women. The mortality rate of EOC, expressed as the ratio of deaths to newly diagnosed cases, is estimated at 70%, a statistic that has not improved significantly over the past 20 years. While early-stage disease can be cured with surgical approaches, the lack of early diagnostic markers combined with the diffuse and easily overlooked symptoms of early-stage disease lead most patients to present with metastatic disease. Unfortunately, while most metastatic ovarian cancers initially respond to taxol and platinum-based chemotherapy, the disease almost inevitably recurs in a resistant form. Thus, there is a tremendous effort in identifying molecular markers and novel therapeutic targets for diagnosing and managing EOC. EOC includes four major histological subtypes (serous, endometrioid, clear cell and mucinous) which resemble the epithelia of the fallopian tube, uterine endometrium and endocervix. Recent and emerging studies indicate that epithelial ovarian cancer is not a single disease but rather a complex collection of cancers with differing etiologies, risk factors, and altered molecular signaling pathways.

It has become increasingly clear that a multidisciplinary approach to broaden our understanding of this disease and to define and address the most pressing questions will facilitate advances in ovarian cancer management. To provide a forum for exchange of emerging ideas, promising research and clinical findings related to the predisposition, detection, diagnosis and management of ovarian cancer, B. Vanderhyden, the Corinne Boyer Chair for Ovarian Cancer Research, initiated a biannual national conference in ovarian cancer research. This effort was strongly supported by the Canadian Institutes of Health Research, Ovarian Cancer Canada (formerly the National Ovarian Cancer Association) and the Society of Gynecologic Oncologists of Canada. The initial conference, held in Ottawa in 2002, brought together principal investigators and academic clinicians from across Canada. Subsequent national conferences welcomed trainees, and this year’s meeting was the first to incorporate a preceeding trainee symposium that provided a broad overview of the pathophysiology and major issues of ovarian cancer. The purpose of this review is to highlight emerging concepts featured at the 4th Canadian Conference on Ovarian Cancer Research, which was organized by A.-M. Mes-Masson and D. Provencher and held on May 4-7, 2008 in Montreal, Quebec.

Risk Factors and Etiology

Risk factors

S. Hankinson of the Department of Epidemiology at Harvard University provided a broad review of risk factors associated with ovarian cancer and a discussion of recent trends in the field of ovarian cancer epidemiology. In her opening keynote address, Dr. Hankinson first summarized confirmatory findings by the Collaborative Group on Epidemiological Studies of Ovarian Cancer on the protective effect of oral contraceptives. Data from 45 epidemiological studies comprising over 23,000 women with ovarian cancer and over 85,000 controls were analyzed. A protective effect provided by oral contraceptives was greater with longer duration of use and appears to persist for several decades; however, this protection decreases over time. With the exception of mucinous tumors, the protective effect was present for all histotypes of ovarian cancer.

Other potential risk factors discussed included height, weight, postmenopausal hormone use, talc use, physical activity, inactivity (not merely the converse of physical activity), diet, and vitamin D exposure. A surprising finding was the greater incidence of ovarian cancer in women over 170 cm in height as compared to women less than 160 cm; this may indicate an impact of genetics and early development on a woman’s later risk. Somewhat consistent with this idea, obesity in early life but not at menopause was weakly associated with ovarian cancer. Also, while physical activity was not found to reduce risk as observed for colon cancer, inactivity seemed to increase risk.
The exploration of the impact of diet on ovarian cancer risk is emerging as a new area of investigation but has not yet provided clear insights. Thus far, increased lactose intake has been the only dietary factor to show a consistent, albeit modest, association with risk. Other dietary factors explored include fruits and vegetables, flavonoids, alcohol, and caffeine. One large controlled cohort study reported a 0.5 relative risk in the top versus the bottom quartile of coffee intake. Ttwoger et al. found an inverse relationship between caffeine intake and ovarian cancer among women who have never used oral contraceptives and in postmenopausal women [1]. Oral contraceptive use might influence caffeine metabolism, such that caffeine may more effectively decrease risk in a lower estrogen background. Finally, there has been considerable recent interest in vitamin D and cancer, particularly for sun-starved Canadians. While the relationship with ovarian cancer is not entirely clear, women with adequate levels of vitamin D tend to have decreased incidence. In addition, vitamin D levels are linked to lactose intake.

An important concept indicated by Hankinson that was emphasized throughout the meeting is an increased understanding that EOC is not a single disease, but rather consists of subtypes characterized by distinct molecular abnormalities, stage at presentation, response to standard chemotherapy, survival and predisposing risk factors reflecting various etiologies. In fact, the Trans-Canadian Ovarian Cancer Task Force was launched to develop a standardized, reproducible system for the accurate diagnosis of ovarian cancer subtypes. This task force is a collaborative effort by sites in Vancouver, Edmonton, Calgary, Winnipeg, Halifax, St. John’s and Toronto. B. Gilks, a driving member of this task force, presented the development of a six-part classification system (consisting of clear cell, endometrioid, mucinous, high-grade serous carcinoma, low-grade serous carcinoma and carcinoma not otherwise specified) based on a diagnostic panel of immunohistochemical markers and careful pathological review [2, 3]. Refinement of the markers used may further enhance the accuracy of these classifications.

An accurate histological classification is also important since the prognostic value of biomarkers may differ among EOC histotypes. This point was illustrated by D. Huntsman, who examined the expression of tissue-based biomarkers in a population-based cohort of 500 ovarian carcinomas. Of a total of 21 biomarkers examined, 19 were differentially expressed between subtypes and not by FIGO stage. For instance, the proliferation marker Ki67 showed differential staining between subtypes and was an unfavorable prognostic marker in the entire cohort, but was not prognostic within any specific subtype. Some prognostic associations were inverse within the entire cohort vs a particular subtype. For example, whereas WT-1 was an unfavorable prognostic marker within the entire cohort, it was a predictor of favorable prognosis when high-grade serous cancers alone were considered. These findings stress the point that failure to stratify by subtype may greatly confound survival analysis.

The difference between subtypes, as well as the need for accurate diagnosis, was further highlighted by a retrospective study by L. Gien comparing endometrioid and serous ovarian cancers. Endometrioid carcinomas were found to occur at a younger age, with an earlier stage at presentation and lower grade disease. In addition, a smaller proportion of patients with endometrioid carcinoma recurred following standard chemotherapy. Endometrioid histology was a significant independent predictor of favorable overall and disease-free survival after adjusting for age, grade, primary cytoreductive surgery, year of diagnosis and adjuvant treatment. However, after adjusting for disease stage, endometrioid histology was no longer an independent prognostic factor, suggesting that the increased survival of patients with endometrioid cancer largely reflects earlier presentation and intervention. The talks by Gilks, Hunstman and Gien clearly indicated that ovarian cancer histotypes are distinct entities, hence any epidemiologic or molecular studies must take subtype into account.

Etiology

A main contributing factor to the poor prognosis of ovarian cancer is the failure to detect disease at an early stage in the majority of cases. Currently, there is no reliable method of early detection and no reproducible precursor lesion predictive of cancer development. In order to improve our ability to detect ovarian cancer in its earliest stages, we must understand the etiology of the distinct ovarian malignancies. The most enduring theory for the development of ovarian cancer is the ‘incessant ovulation hypothesis’ which states that ovarian cancer occurs through repeated cycles of ovulation-induced trauma and repair of the ovarian surface epithelium (OSE), based on the observation that ovarian cancer risk is proportional to the number of lifetime ovulations [4]. A study presented by L. Turchet put forth the hypothesis that the OSE contains stem cells that provide the rapidly proliferating cells that close the ovulatory wounds, and that repeated ovulatory cycles may cause these stem cells to become dysregulated, eventually giving rise to cancer. Using flow cytometry, they isolated a side population of putative mouse OSE stem cells, based on the ability to exclude Hoechst dye. These cells expressed higher mRNA levels of stem cell markers (Sca1, c-Kit and Nanog). Furthermore, Sca1 mouse OSE cells had a greater spheroid generating efficiency compared to Sca1 cells, suggesting enhanced self-renewal capability.

An emerging question in serous ovarian cancer is whether the etiology of low-grade and high-grade carcinomas is distinct. Low-grade serous carcinomas (LGSC) are thought to arise from low malignant potential tumors (LMP), which are generally non-invasive. LMP tumors sometimes harbor histopathological micropapillary features (LMP-MP) that are associated with an increased risk of malignant transformation and disease recurrence. Careful study of these lesions may therefore identify early events in the specific development of LGSC. A study pre-
sent by T. May compared gene expression profiles of laser capture microdissected epithelial cells from these tumor types and high-grade serous carcinomas (HGSC). LGSC and LMP-MP had similar global gene expression profiles that were markedly different from both LMP and HGSC specimens. Statistical analysis revealed the differential expression of MAPK1/3 and EGFR pathway members between LMP and HGSC samples, suggesting that they may play a key role in low-grade ovarian serous carcinogenesis. Furthermore, these genes may represent novel therapeutic targets given that patients with LGSC do not generally respond well to standard combination chemotherapy. While the study of LGSC has historically been difficult due to a lack of suitable cell models, Y.Z. Wang presented the development of transplantable ovarian tumor cell lines by injection of primary human ovarian cancer tissue, including a serous LMP tumor, into the subrenal capsule of SCID mice [5]. This model system promises to provide an invaluable resource for the study of LGSC etiology.

Recent studies have called into question the OSE as a source of all HGSC. Occult HGSC and putative precursors within the fallopian tube epithelium (FTE) have been found in a large proportion of prophylactic salpingectomy specimens from confirmed BRCA1/2 mutation carriers at risk for adnexal (ovary and fallopian tube) HGSC, highlighting the FTE as a potential alternative source. A unifocal origin of ovarian and fallopian tube HGSC is supported by a study presented by S. Salvador. Fallopian tube involvement was detected in 10/12 cases of Stage III ovarian HGSC and similar copy number changes were demonstrated in three sets of ovarian and tubal HGSC by fluorescence in situ hybridization (FISH). In addition, potential precursor lesions of HGSC were found in prophylactic salpingectomy specimens, consistent with recent findings by other groups [6, 7]. The relationship between the FTE and HGSC of presumed ovarian or tubal origin was addressed in a study presented by Tone et al. [8]. Laser capture microdissection was used to obtain epithelial cells from non-malignant distal FTE of BRCA1/2 mutation carriers (FTEb, n = 12) and normal control women (FTEn, n = 12), and from tubal and ovarian HGSC (n = 13). Unsupervised cluster analysis of gene expression profiles confirmed the similarity of ovarian and tubal HGSC, supporting a common cell of origin. Cluster and statistical analysis also revealed that FTEb specimens as a group, and four individual FTEb samples obtained during the luteal phase of the ovarian cycle in particular were similar to HGSC. This suggests that non-malignant FTEb specimens have accumulated alterations involved in serous carcinogenesis, and further that factors associated with the luteal phase may contribute to predisposition to HGSC [8].

**Disease Management**

**Novel therapeutics**

E. Eisenhauer, from the Cancer Research Institute at Queen’s University, summarized new anti-cancer drugs currently being tested by the NCIC Clinical Trials Group. As she outlined in her keynote address, the ideal target should be expressed in the majority of tumor (but not normal) cells, mutated or amplified and drive the malignant program. A diverse array of potential targets have been investigated in ovarian cancer, including those involved in the EGFR and PI3K/AKT/mTOR pathways, apoptosis, DNA repair, cell cycle regulation and angiogenesis, as well as tumor antigens. Specifically, EGFR pathway members have been found to be overexpressed in 35-70% of ovarian cancers, and a phase II trial of the kinase inhibitor Erlotinib has shown small effects. Similarly, the p110 catalytic subunit of PI3K is amplified in HGSC and mutated in clear cell cancer, while mutations have been found in the p85 regulatory subunit. While insulin resistance has been observed in trials of PI3K inhibitors, mTOR inhibitors have shown some efficacy.

Manipulation of the intrinsic and extrinsic apoptotic pathways has also shown promise in ovarian cancer. Apoptosis is controlled by the balance of pro- and anti-apoptotic family members, with an overexpression of inhibitors of apoptosis observed in tumor cells. Hence, an effective means of therapy may be to increase pro-apoptotic signals (via agonist humanized antibodies) and/or decrease anti-apoptotic signals (via neutralizing antibodies or anti-sense oligonucleotides). Examples of treatments under investigation include the use of recombinant TNF-related apoptosis-inducing ligand (TRAIL) to target the TRAIL death receptor 4/5, as well as inhibitors of XIAP, clusterin and Bcl2.

Several studies are investigating the manipulation of DNA repair proteins as a means of enhancing tumor cell death. For instance, cells with mutations in the double strand break repair proteins BRCA1 and BRCA2 are extremely sensitive to inhibitors of the single strand break repair protein PARP, which may have implications for hereditary ovarian cancer patients. Similarly, J. Weber-pals presented data showing that inhibition of histone deacetylase (HDAC) increased the sensitivity of ovarian cancer cell lines to cisplatin/carboplatin treatment, coincident with a significant decrease in BRCA1 and ERCC1 expression, suggesting that the use of HDAC inhibitors in BRCA-deficient cells may enhance response to platinum-based chemotherapy.

Subsequent presentations discussed emerging strategies targeting cell cycle regulation and angiogenesis. G. Tremblay discussed the use of cell cycle checkpoint inhibitors in combination with DNA damaging agents as a potential way of improving chemoresponse. Cell cycle checkpoints ensure the orderly progression of the cell cycle and maintain genomic stability in normal cells. An intact G2 checkpoint contributes to resistance of cancer cells to select chemotherapeutics and radiotherapy through repair of introduced DNA lesions, leading to decreased treatment-induced cell death even in the absence of a functional p53-dependent G1 checkpoint. Agents designed to target the G2 checkpoint could prevent this repair, thereby acting as chemosensitizers. Co-treatment of ovarian cancer cells with the Chk1
inhibitor isogranulatimide and a topoisomerase inhibitor (topotecan) resulted in enhanced cell death as compared to either treatment alone. This synergistic effect on reducing tumor growth was also observed in nude mice harboring human ovarian tumor xenografts whereas combined treatments had very little effect on non-cancerous cells.

As outlined by Eisenhauer, there are 25 ongoing phase II/III clinical trials targeting vascular endothelial growth factor (VEGF) to inhibit angiogenesis associated with tumor progression. A large proportion of ovarian tumors show overexpression of the angiogenic factor VEGF, associated with a poor prognosis. H. Hirte summarized a phase II clinical research trial of the VEGF receptor inhibitor cediranib in patients with recurrent or persistent ovarian (n = 49), peritoneal (n = 8) or fallopian tube (n = 3) cancer. This orally administered agent was well-tolerated and showed promising activity in both platinum-sensitive (n = 26) and platinum-resistant (n = 34) disease, with an overall response rate of 15%.

Targeting of the tumor vasculature was also explored using a novel orthotopic syngeneic mouse model of HGSC. Briefly, spontaneously transformed mouse OSE (ID8 cells) [9] were injected into the ovarian bursa of C57BL/6 mice and allowed to colonize. By 90 days post-injection, these cells formed large primary ovarian tumors of serous histology, with numerous secondary lesions throughout the peritoneal cavity and abundant ascites similar to that observed in ovarian cancer patients [10]. The ability of an anti-angiogenic thrombospondin 1 (TSP-1) mimetic peptide (ABT-510) to oppose the effects of VEGF in this ID8 model was tested. As described by N. Solinger, injected cells were allowed to grow for 30 days, followed by 60 daily intraperitoneal injections of ABT-510. Treatment with this peptide led to a ‘normalization’ of the tumor vasculature as observed by a reduction in the number of large tortuous vessels, an increased proportion of smaller vessels and reduced tissue hypoxia. The authors proposed that this finding could be applied to clinical practice, as the increased tumor perfusion upon ABT-510 treatment would allow an increased delivery of anti-cancer agents to the core of the tumor, leading to an enhanced response. In addition, ABT-510 treatment led to decreased tumor cell proliferation and reduced expression of pro-angiogenic factors. When ABT-510 therapy was combined with the HDAC inhibitor valproic acid (VPA), a significant reduction in microvessel density and tumor expression of VEGF and basic fibroblast growth factor (bFGF) was evident. The combination treatment resulted in a greater reduction of primary ovarian tumor size relative to either treatment alone. In addition, both individual and combination treatments greatly reduced the number of secondary lesions and ascites formation.

Eisenhauer emphasized in her closing statements that future work needs to incorporate new agents and prioritize targets for further investigation, as well as bridge scientific discoveries of novel molecular targets to clinical trials. In addition, Gilks stressed that accurate subtype diagnosis is particularly important for those women diagnosed with the less common subtypes, as these women are currently receiving ineffective standard treatment and missing out on promising subtype-specific therapies.

**Mechanisms of Chemoresistance**

One of the major contributors to the poor prognosis of ovarian cancer is the recurrence of disease following initial response to combination chemotherapy. Understanding the mechanisms of chemoresistance may lead to the development of more effective treatments. M. Abedini highlighted the role of the caspase inhibitor flice-like inhibitory protein (FLIP) in determining resistance of ovarian cancer cells to cisplatin. Cisplatin decreases FLIP content through protein ubiquitination and proteasomal degradation in chemosensitive, but not chemoresistant cells. This degradation is achieved by facilitating the Akt-modulated interaction of FLIP with p53 and IκB [11].

Akt was found to be one of the main contributing factors to the intrinsic resistance to TRAIL-induced apoptosis in ovarian cancer cell lines and primary ovarian cancer samples in a separate study presented by N. Goncharenko-Khaider. Activation of Akt in TRAIL-sensitive ovarian cancer cells enhances resistance to TRAIL, while inhibition of Akt in TRAIL-resistant cells enhances sensitivity, strongly implicating Akt as a potential therapeutic target [12, 13].

The possibility that drug resistance may result from acquired chromosomal aberrations during progression of EOC is being examined by J. Squire. An advanced molecular cytogenetic analysis by spectral karyotyping (SKY) and FISH was first used to determine the sequence of chromosomal events leading to genomic instability in sporadic ovarian cancer. The extent of numerical alterations in each tumor was found to increase as a function of overall genomic content, with diploid tumors exhibiting lower instability indices compared to tetraploid and triploid tumors. Tetraploid tumors additionally showed a trend of greater percentage of cells with abnormal centrosomes. In contrast, tetraploid tumors exhibited less evident structural chromosomal aberrations compared to diploid tumors. This suggests that two distinct processes governing genome stability are disrupted in ovarian cancer, specifically those leading to changes in numerical segregation and ploidy of chromosomes and those affecting DNA repair leading to structural aberrations [14]. The impact of structural cytogenetic aberrations on gene expression changes associated with cisplatin resistance was then determined by parallel microarray and SKY analysis. This analysis revealed a cryptic homozygous deletion adjacent to the intracellular trafficking gene sorting nexin 7 (SNX7) and an insertional transposition from 13q12.12 into chromosome 22 leading to an overexpression of four contiguous genes [15]. Understanding the chromosomal aberrations contributing to the failure of current chemotherapeutic approaches could help guide management options for advanced disease when used in a prognostic manner, and will facilitate the identification of key genes involved.
Promising New Directions

Alternative splicing

While a majority of studies have used traditional expression profiling to identify novel genes and pathways with potential relevance to ovarian cancer, R. Klinck presented promising data using high-throughput analysis of alternative splicing events as a signature of ovarian cancer. Alternative pre-mRNA splicing is a post-transcriptional process occurring in the majority of genes, leading to an increased number of transcript variants generated from a single gene. These variants typically display altered function as compared to the full length transcripts. This study used LISA (Layer and Integrated system for Splicing Annotation) to monitor splicing of 600 known cancer-associated genes in 25 normal and 21 serous ovarian cancer tissues (www.Igfus.ca/Rnomics@Igfus.ca). A classifier set of 48 alternative splicing events was found to be significantly associated with serous ovarian cancer, and was then used to successfully distinguish normal tissues from cancer in a blind set of 39 ovarian cancers [16]. Emerging platforms now enable the genome-wide screening of potential splicing variants based on representation of all exons of a given gene in the array with multiple probe sets. Thus, the increasing implementation of such platforms should expand our understanding of the contribution of alternative splicing to the clinical course of the distinct ovarian cancer subtypes.

Immunoregulation in ovarian cancer

The study of the immune system in ovarian cancer is an important area of research for the investigation of mechanisms of chemoresistance and the development of novel therapies. Many studies have established the relevance of the immune system in determining ovarian cancer outcome. For instance, patients whose tumors contain high numbers of intraepithelial CD8+ cytotoxic T cells and low numbers of CD25+FoxP3+ regulatory T cells have increased disease-free and overall survival following standard treatment. Increased tumor expression of MHC class I/II molecules is a favorable prognostic factor in serous carcinoma. In addition, tumor-specific autoantibodies have been found in many cancer patients, although the association with patient prognosis is unknown [17]. B. Nelson of the IROC (Immune Response Ovarian Cancer) group studied the prognostic significance of autoantibodies to the common tumor antigen NY-ESO-1. Matched serum and tumor tissue were obtained from Stage III/IV HGSC patients (n = 36), and it was determined that 25% of these patients had developed autoantibodies to the NY-ESO-1 antigen. The presence of these autoantibodies positively correlated with expression of the antigen by tumor cells and with infiltration of tumor stroma by CD8+, FoxP3+ and CD4+ cells. Despite this, patients with serum anti-NY-ESO-1 antibodies showed a significantly shorter overall survival, suggesting an ineffective T cell response due to stromal location and/or a suppressive phenotype. Nelson and his group proposed a potential T cell therapy aimed at improving the effectiveness of the anti-tumor response and enhancing patient survival. Specifically, T cells could be harvested from the blood or tumor of a patient, and tumor-reactive T cells expanded in culture and infused back into that patient. This promising approach has been successful in the ID8 mouse model, where adoptively transferred CD8+ cells led to complete tumor regression [18].

A study presented by J. Webb examined the complexity of immune cell populations in fresh malignant ascites from patients with advanced serous ovarian cancer using flow cytometry. Hematopoietic cells (CD45+) represented the dominant cell type (up to 90%) in all but one of the ascites samples studied. Patient samples showed variable lymphocyte populations, with T cells (CD3+) representing between 10% and 90% of the cellular component. The CD4 to CD8 ratio was variable, with several patients with high T cell infiltration exhibiting a dramatic excess of CD8+ over CD4+ cells. Ascites-derived T cells were comprised of a polyclonal population suggesting the presence of many tumor antigens. In addition, the T cell receptor repertoire was distinct between ascites and the corresponding solid tumor, which may reflect phenotypic differences between the primary and metastatic tumor.

Round table discussions

Round table discussions were held to collect the views of conference delegates on highly relevant issues for the progress of ovarian cancer research in Canada. Priorities should remain the identification of markers of early stage disease, standardized tumor subclassification, mechanisms of chemoresistance and selection of optimal and individualized treatment. We should exploit our strengths in familial disease, which will facilitate the discovery of biomarkers of predisposition. An agreement on standard operating procedures for clinical data collection and tissue banking of tumors, ascites, urine and serum samples from ovarian cancer patients and appropriate controls is imperative and will facilitate sharing of these resources across the country. A national database of ongoing clinical trials and links to tissue samples and clinical data would provide an invaluable resource. An identified barrier frequently encountered is access to accurate patient follow-up data. Creation of a national gynecologic oncology registry would empower outcome analysis and benefit translational research.

The value of bringing clinicians and scientists together was evident throughout the meeting and it was felt that clinician-researcher teams should be promoted at both the local and national levels. Proposed means to achieve this included providing forums to bring researchers and clinicians together. Clinicians should be included on relevant graduate supervisory committees, research project planning and grant writing, while scientists should participate in the planning of clinical trials and attend tumor boards. Funding opportunities favoring multidisciplinary teams
provide incentives for translational research. Travel awards, such as those provided by Ovarian Cancer Canada allowing trainees to spend a few weeks in a host laboratory, enhance the training experience and promote current and future collaborations.

Concluding Remarks

The 4th Canadian Conference on Ovarian Cancer Research highlighted the importance of a multi-disciplinary approach to address the complex issues inherent in ovarian cancer biology. The need to integrate histological subtypes of EOC into all aspects of research was evident. This will enhance our understanding of this multifactorial disease, and lead to improved clinical trials, more meaningful biomarker discovery and relevant pathway analysis. An emphasis on understanding why our current treatment approaches often fail and how we can enhance the efficacy of these approaches will immediately impact clinical outcomes. Collectively, we need to identify novel strategies to exploit the emerging molecular differences associated with histological subtypes of EOC that should lead to more effective disease management.

Acknowledgements

The 4th Canadian Conference on Ovarian Cancer Research was sponsored by CIHR Institute of Cancer Research, NCIC & The Terry Fox Foundation, The Society of Gynecologic Oncologists of Canada, Institut du cancer de Montréal, Ovarian Cancer Canada, OvCaRe British Columbia, Schering-Plough, Toronto Ovarian Cancer Research Network, Amgen, Service de Gynécologie oncologique, Centre hospitalier de l’Université de Montréal, Montreal Ovarian Initiative, Pfizer Oncology and Réseau de recherche sur le cancer du Fonds de la recherche en santé. We thank the meeting organizers and the Planning Committee for the invitation to summarize the meeting highlights and B. Vanderhyden for providing a summary of the round table discussions. We also thank A-M. Mes-Masson for critical comments of the manuscript.

References


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Breast cancer patients with micrometastases in sentinel lymph nodes: differences considering additional metastatic lymph nodes

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Summary

Aims: Characterization of breast cancer patients with micrometastases in sentinel lymph node (SLN) and establish differences between micrometastatic breast cancers with additional metastatic lymph nodes (LNS) versus no other lymph node invasion.

Methods: Analysis of 30 breast cancers, N1mi or pN0(i+), diagnosed and treated in our department from July 2000 to July 2008.

Results: Micrometastases in SLNs were found in 30 patients. Complete axillary dissection revealed other metastatic LNs in 24%. Concerning breast cancers with additional LN invasion versus no other LN invasion, tumors located in the superior-external quadrant were more frequent in the former group. Other characteristics as clinical presentation, histological subtype, focality, cytonuclear grade, hormone receptors and Her2 expression were not significantly different in either group. Regarding SLN invasion, the presence of at least two micrometastatic foci were significantly more relevant in patients with other metastatic LN invasion (p < 0.01). Micrometastases diagnosed only after immunohistochemistry (IHC) were exclusively found in patients without other LN invasion, reaching statistical significance (p < 0.05).

Conclusions: Complete axillary dissection revealed additional LN invasion in 24% of patients with micrometastases in the SLN. Tumors with additional LN invasion were more frequently found in the superior external quadrant and SLNs harbored at least two micrometastatic foci. Micrometastases diagnosed exclusively by IHC techniques were more relevant in cases without additional lymph node invasion.

Key words: Micrometastases; Sentinel lymph node.

Introduction

Lymph node (LN) metastases are considered the most important prognostic factor in breast cancer patients [1, 2]. Axillary LN status is determined after histological examination of at least ten LNs are surgically removed [3]. Axillary dissection was first considered a therapeutic intervention to eliminate disease from regional LNs [4]. It later became the only surgical staging procedure available [5]. With the advancement of sentinel lymph node (SLN) biopsy in early breast cancers (T0-T2, N0), this same information can be obtained by removal of only a few nodes, avoiding much of the morbidity associated with axillary dissection [6]. Histopathologic examination of SLNs is more detailed than traditional examination of axillary nodes [1, 2]. Further axillary surgery depends on SLN status. Serial sectioning of SLNs and use of immunohistochemistry (IHC) with cytokeratin have led to increased detection of minimal lymph node involvement, classified as micrometastases (0.2-2 mm; pN1 (mi)) and isolated tumor cells (ITCs) (<0.2 mm; pN0(i+)) [1, 7].

After detection of micrometastases that the SLN has incorporated into its staging system, many breast tumors become upstaged [8]. The significance of micrometastases remains controversial. Value of complete axillary dissection after finding micrometastases is still not uniformly defined. It would be of great value to identify a guide for estimating risk of non SLN involvement.

The aims of this study were to identify parameters more associated with other LN involvement in breast cancer patients with micrometastases in SLN. We also intended to characterize breast cancers with micrometastases in SLN and prevalence of additional metastatic invasion after axillary dissection.

Methods

Analysis of 30 breast cancer patients with micrometastases in SLNs diagnosed in our department from July 2000 to July 2008 was carried out.

In breast cancers (T0-T2) with clinical and imaging negative axillary nodes, SLN biopsy is performed using a blue dye technique.

Twenty-five patients with micrometastatic SLNs were submitted to axillary dissection. Clinical and histological parameters were compared in cases with other metastatic LNs versus no other metastatic LNs besides micrometastatic SLN.

The analytic data included patient characteristics (age, hormonal status) and clinical characteristics (presentation, location). Histopathological data studied breast tumor histological type, size, cytonuclear (Bloom Richardson) grade, focality and hormone (estrogen and progesterone) receptors. SLN was histologically analyzed after serial section (100 μm thickness) using classical staining – hematoxylin and eosin (H&E) and IHC (MNF116) when classic staining was negative or inconclusive. Lymph nodes obtained from later axillary dissection were studied to identify additional invasion.

Descriptive statistics are reported as frequencies, percentages, means and standard deviations. Distribution of categorical
variables were compared using the chi-square test. Parametric variables were tested using Student’s t-test. Statistical analysis was performed using SPSS version 15.0.

Results

Micrometastases in SLNs were found in 30 patients. Mean age was 55.2 ± 12.0 (40-81) and 50% post-menopausal with a mean menopausal age of 50.7 ± 3.1 (45-56). Tumors presented clinically as palpable lesions in 22 cases (74%). The remaining patients showed image (mammography or mammary ultrasound) alterations (n = 8). Lesions were described in the following quadrants: superior-external in 60% (n = 18), superior-internal in 20% (n = 6), inferior-external in 10% (n = 3), inferior external in 3% (n = 1) and retroareolar in 7% (n = 2). Histology revealed mainly ductal invasive carcinoma (n = 26) but also two invasive lobular carcinomas and another two mucinous invasive carcinomas were reported. Multifocal lesions were found in 21%. Tumors were pT1 in 25 cases (83%) and pT2 in five cases (17%). Concerning cytocuclear grade, G2 tumors were found in 73% (n = 22) and G1 (n = 4) and G3 (n = 4) tumors in 13% each. Lymphovascular invasion was present in four tumors (13%). Estrogen receptors (ERs) were positive in 97% (n = 29). Progesterone receptors (PRs) were positive in 68% (n = 17) and negative in 32% (n = 8). Cerb2 was positive in 21% (n = 6) and negative in 89% (n = 22).

Micrometastases in SLN measured between 0.2-2 mm in 27 patients. In the remaining three cases ITCs were found (< 0.2 mm). Considering the focality of micrometastases in SLNs, 25 were related only to one focus and in the other five all two foci were noted. Diagnosis of micrometastases was performed using H&E in 23 cases and IHC in the seven cases with negative standard staining.

Completion of axillary dissection was performed in 25 patients after SLN analysis. On average 13.9 LNs were removed. Six patients (24%) had additional metastatic invasion revealed by axillary dissection, including two (8%) macrometastases (≥ 2 mm) and four (16%) micrometastases (≤ 2 mm). Additional micrometastatic LNs harbored one focus in two cases (8%) and two foci in another two cases (8%). Table 1 summarizes the comparison between tumors with SLN micrometastases with additional metastases versus without other metastatic LNs reported in axillary dissection. Significant differences were found considering the presence of tumors in the superior-external quadrant. Table 2 correlates the number of micrometastatic foci and additional LN invasion. The presence of at least two micrometastatic foci in SLN was more frequent in patients with other metastatic LNs. Table 3 compares the method of detecting micrometastases and implications considering other LN invasion. Diagnosis of micrometastases by IHC (negative H&E) was more associated in patients without other metastatic LNs, reaching statistical significance (p < 0.05).

Table 1. — Comparison between micrometastatic tumors without additional LN invasion vs with additional LN invasion. (LN: lymph node; SLN: sentinel lymph node; n.s.: non-significant; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; IMC: invasive mucinous carcinoma; DCIS: ductal carcinoma in-situ; ER: estrogens receptor; PR: progesterone receptor).

<table>
<thead>
<tr>
<th>Micrometastases in SLN</th>
<th>Other LN invasion</th>
<th>Without LN invasion</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 19</td>
<td></td>
</tr>
<tr>
<td>Clinical presentation</td>
<td>50%/50%</td>
<td>42%/58%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Palpable lump/imaging alteration</td>
<td>83%/17%</td>
<td>84%/16%</td>
<td></td>
</tr>
<tr>
<td>Location (quadrant):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior external/other</td>
<td>6</td>
<td>8/11</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>IDC/ILC/IMC</td>
<td>100%/0%</td>
<td>42%/58%</td>
<td>n.s.</td>
</tr>
<tr>
<td>Multifocal</td>
<td>0</td>
<td>4</td>
<td>n.s.</td>
</tr>
<tr>
<td>DCIS associated</td>
<td>5</td>
<td>14</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>83%</td>
<td>74%</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>2 (33%)</td>
<td>1 (11%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>G2</td>
<td>4 (67%)</td>
<td>14 (74%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>3 (15%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>pT1/pT2</td>
<td>5/1</td>
<td>0/12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>2</td>
<td>18</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>33%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>ER+/−</td>
<td>6</td>
<td>18/1</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>95%/5%</td>
<td>n.s.</td>
</tr>
<tr>
<td>PR+/−</td>
<td>5/1</td>
<td>10/4</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>83%/17%</td>
<td>71%/29%</td>
<td></td>
</tr>
<tr>
<td>Cerb2+/−</td>
<td>0/6</td>
<td>5/12</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>0/100%</td>
<td>30%/70%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. — Number of micrometastatic foci with LN invasion. (LN: lymph node; SLN: sentinel lymph node).

<table>
<thead>
<tr>
<th>Micrometastases in SLN</th>
<th>Other LN invasion</th>
<th>Without LN invasion</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 19</td>
<td></td>
</tr>
<tr>
<td>Micrometastatic focus in SLN</td>
<td>2</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33%</td>
<td>95%</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>≥ 2</td>
<td>67%</td>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — Method of detecting micrometastases in SLNs and correlation with presence of additional LN invasion. (SLN: sentinel lymph node; LN: lymph node; H&E: Hematoxylin and Eosin; IHC: immunohistochemistry).

<table>
<thead>
<tr>
<th>Micrometastases in SLN</th>
<th>Other LN invasion</th>
<th>Without LN invasion</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 19</td>
<td></td>
</tr>
<tr>
<td>H&amp;E+</td>
<td>6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100%</td>
<td>74%</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>H&amp;E−</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>IHQ (MNF16)+</td>
<td>0%</td>
<td>26%</td>
<td></td>
</tr>
</tbody>
</table>
Breast cancer patients with micrometastases in sentinel lymph nodes: differences considering additional metastatic lymph nodes

Discussion

SLN biopsy aims to allow a correct staging procedure, avoiding the need of complete axillary dissection in cases without SLN invasion. A meticulous pathologic study is performed in SLNs, detecting the presence of metastatic disease as small as 0.2 mm.

In this series, in face of micrometastases in SLNs, we found an incidence of 24% additional LN invasion. Several studies suggested that presence of micrometases in SLNs correlates with absence of metastases in non-sentinel LNs [8, 9]. Langet al. reported in a prospective trial that SLN micrometastases do not harbor risk of axillary recurrence or distant disease [11]. This emphasizes the idea of avoiding further axillary dissections. In contrast, other investigators suggested a relevant percentage of non-SLN involvement in micrometastatic SLNs [12, 13], reaching 26% [14]. A recent meta-analysis reported the risk of non-SLN metastases associated with micrometastatic disease in SLNs of around 10-15%, depending on the method of detecting SLNs [15].

The greatest aim with a micrometastatic SLN is to identify patients at risk of additional LN invasion. Considering patients submitted to complete axillary dissection (n = 25), we compared clinical and histological parameters between tumors with other LN invasion (n = 6) and those without other LN invasion (n = 19). We evaluated, tumor characteristics, hormonal stage, clinical presentation, location (quadrant), histological subtype, focality, DCIS associated, cytonuclear grade, stage, hormonal receptors (ER, PR) and Her2 expression. Significant differences between breast cancers with other metastatic invasion besides the SLN were related to primary tumor location, with the superior-external quadrant being significantly more associated with additional invasion. Other parameters were not significantly different (Table 1).

Some previous studies have emphasized the role of some tumor aspects, as tumor size and lymphovascular invasion, as predictive factors for the presence of metastatic LNs [8, 16]. In a multicentric study predictive factors were pT stage, menopausal status, grade, lymphovascular invasion and histological type [17]. One study reported micrometastases from invasive lobular carcinoma was related with other LN involvement; on the contrary all invasive ductal carcinomas had negative axillary dissection [18].

Our results emphasize the location of the primary tumor as the only predictive factor, considering primary tumor characteristics for additional LN invasion. Lymph flows unidirectionally from the superficial to deep plexus and from the deep subareolar plexus through the lymphatic vessels of the lactiferous ducts to the peribulbar and deep subcutaneous plexus [19]. Intramammary lymphatic vessels move centrifugally toward axillary and internal mammary lymph nodes [19]. With this drainage system in mind, it seems logical that tumors located in the superior external quadrant originate more frequently from small metastatic foci in the axillary lymph nodes. In consonance with the centrifugal movement in lymphatic vessels, metastases from tumors with this topographic location flow a shorter way toward the axilla.

Focusing on SLN analysis, the presence of at least two micrometastatic foci was significantly more associated with additional axillary invasion. It has been reported that risk of non-SLN metastasis is related to the size of disease in SLNs [1, 20], being the greatest for macrometastases, intermediate for micrometastases, and the least for ITCs (14.8%), more frequently detected by IHC [21]. Besides classical H&E, the advent of IHC directed to cytoqueratin can identify up to 34% initially negative SLNs [22, 23]. With IHC we could identify more (7/30) micrometastatic SLNs. In our study, micrometastases detected by IHC were not associated with additional metastatic invasion besides the SLN, reaching statistical significance. These results emphasize the idea that SLN metastases diagnosed by routine H&E have a higher risk of non-SLN metastases than metastases detected by IHC, reflecting a larger metastatic size in the former group [24]. Studies confirm the increase incidence in the detection of micrometastases by IHC [1, 17, 21]. The prospective use of IHC by Rydén et al. only showed stage migration in 3/132 cases [1]. All these reports supports the idea that patients with micrometastases detected by IHC have a low risk of additional LN invasion.

Controversy exists surrounding the best management of patients with SLN micrometastases. In approximately 80% of patients with SLN micrometastases, the SLN is the only LN involved [20]. These particular patients would not benefit from further axillary surgery. Recent studies report that selected patients with micrometastases without further axillary dissection will not suffer from a higher incidence of regional recurrence [25, 26].

Various studies suggest that the prognosis of breast cancer patients with micrometastases should not be considered the same as that of truly node-negative patients (pN0), with a poorer survival for micrometastatic patients [27, 28]. This means that minimal LN invasion cannot be safely overlooked, particularly when tumors are located in the superior-external quadrant and SLN harbors at least two micrometastatic foci, as we proved in our results. This is the only possible way to solve this problem - the identification of predictive factors associated with LN invasion besides SLN micrometastases. The real clinical impact of micrometastases will only be assured after results of on-going randomized controlled trials are known [29, 30].

Conclusions

In this study the incidence of additional LN invasion in face of micrometastatic SLNs was 24%. Primary tumor characteristics predictive of other metastatic involvement besides SLNs was the superior-external quadrant location, reaching statistical significance. Other parameters like hormone stage, clinical presentation, histological type, focality, cytonuclear grade, hormone receptors and Her2 expression were not significantly different. Consid-
erating analyzes of SLN, the presence of at least two metastatic SLN foci was significantly more frequent in patients with additional LN invasion. Finally, micrometastases detected by IHC with negative H&E were exclusively reported in patients without other LN invasion, also statistically significant.

References


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Proteomics in mammary cancer research

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²Clinical Department of Pathology, Division of Gynecopathology, Medical University of Vienna, Vienna
³Department of Obstetrics and Gynecology, Provincial Hospital of Villach, Villach (Austria)

Summary

During the past few years, the intensified detection of small (mammary) carcinomas causes an increase in the number of mammary cancers. Cancer of the mammary tissues has an almost individually unpredictable behavior and aggressiveness. Therefore, a better insight in the molecular biological defects, which are responsible for initiation and progressive aggressiveness of mammary cancer, is necessary. Proteomics are an alternative to identify proteins which initiate carcinogenesis and can be useful to predict cancer prognosis. Today, the most commonly used technique for large-scale protein identification in clinical samples is two-dimensional electrophoresis (2-DE) in combination with image analysis and MS. Using these techniques, qualitative and quantitative information can be achieved regarding protein forms and post-translational modifications. In the following article we review proteomic techniques that are now commonly used in order to elucidate the role of proteins in breast cancer.

Key words: 2D gel electrophoresis; MALDI/ESI/TOF; Protein identification; Proteomics; Mammary cancer.

Introduction

Two-dimensional gel electrophoresis (2-DE)

This technique separates proteins according to their molecular weight (MW) and electric charge (pI). Protein expression levels can be analyzed by measuring the spot intensity. Protein patterns in gels can be compared with patterns of other gels. For identification of proteins it is often necessary to generate separate gels in which proteins are labeled with staining methods that do not interfere with MS. The spots of interest are selected and excised from the gels for protein identification by MS (Figure 1).

In 1996, Wilkins et al. [1] estimated the number of different proteins in a selected cell type to be 5,000 to 10,000 – more than the number of protein-encoding genes. In 2001, Venter and his study group [2] described one of the first analyses of the complete DNA sequence of the working draft human genome (International Human Proteome Organisation - HUPO), which was completed in 2003. Hanash [3], a pioneer in cancer proteomics, pointed out the tremendous interest in the potential of proteomics for biomedicine. Although numerous data have been reported, information has not been consistently compiled. Identification of proteins has lagged behind because of inadequacy of the mass spectrometry technique (MS). Therefore, the Proteomics Standard Initiative (PSI) was established to make information more available and comparable [4].

To obtain the best resolution for 2-DE, fresh tissue immediately immersed on ice is used. Several techniques exist to purify epithelial cells from tumor tissues [tissue microdissection (LCM)] [5]. To extract as many proteins as possible representative tumor cell material is essential, because tumors of mammary carcinomas can be heterogeneous and small tumor sizes are often difficult for sampling. Before proteins are separated in 2-D gels they have to be denatured, disaggregated and solubilized.

In the first dimension, proteins are separated by isoelectric focusing (IEF) using immobilized non-linear pH gradient (IPG) gel strips [6, 7], with a common pH gradient from 4 to 7, but narrow-range IPG strips (pH 3-10 NL; Immobiline dry strips) are used to obtain a better separation in the medium MW range. The number of distinct detectable protein spots in a standard 2-DE system varies between 1000 to 2000 spots in one gel [8] and their MW-size between 15 to 150 kD. Precast IPG strips offer advantages, such as reduced preparation time or improvement of the reproducibility of the pH gradient. Samples can also be applied to the gel strips during gel rehydration [9].

The NuPAGE system (Invitrogen Cooperation/NO- VEX, Carlsbad, CA, USA) uses a neutral pH system, consisting of Bis-TRIS precast gels for proteins with small and medium-size MW, or, TRIS-acetate gels for larger proteins with specially optimized buffers [10], offering significant advantages compared to other gel systems. Bio-Rad Laboratories (Hercules, CA, USA) have provided the ready IPG strips, which simplify first-dimension separation by immobilizing the pH gradient on an easy-to-handle support strip [7]. The strip length fit for vertical electrophoresis cells and gels and they are available in a wide selection of pH gradients. The reproducibility of 2-DE has been improved by the mini tube gel cell (Bio-Rad), where the tube gels are cast in glass tubes and attached to molded sample reservoirs for the IEF run. Following first-dimension IEF, the gels are removed by a tube gel ejector and placed between the plates of the slab gels for the second dimension run.
discovery of low-abundance protein spots is a limiting factor. At the moment, the accurate identification increase the recovery of tryptic peptides for MS-based proteins. It is also important to generate methods to technologies will be required to identify such modified diseases. Further development of 2-DE and MS-based al modification in biologic processes and in pathology of very important to understand the role of post-translation-Probes, Inc. Eugene, OR, USA) fluorescent dyes. It is detect a) phosphorylated or b) glycosylated proteins by a) in contrast to genomic analyses. Now, it is possible to detect data analyzing, after spots are picked, matched and normalized (variations can be caused by differences in sample preparation, loading, staining and imaging between gels). All software programs generate statistical reports (e.g., means, standard deviation, variation) and a variety of statistics, e.g., Mann-Whitney and Student’s t-test, can be carried out on the data. Once information about the protein spots on the gel has been generated, it can be added to the gel image or database using the annotation feature available in each of the programs. Annotations are organized in categories such as accession number, landmark, pI and MW. Some programs also allow annotations to be linked to specific data files, 2-D (including on-line) databases, or any other type of file. Master gels (fully annotated gels) can be used to copy data into other gels to identify protein spots by gel comparison. It should be kept in mind that any candidate biomarker would need further validation using, e.g., antibody base technologies. Disadvantages of the 2-DE technology, because of its identification of a considerable number of proteins, are: a) very small and very large proteins (undetectable), b) very hydrophobic ones (under-represented), c) pH range (most acid or basic proteins remain undetected), d) trypsin digestion, e) gel elution of peptides and f) identification by MS (needs a relatively large amount of protein). Low amounts of proteins and also minor genetic alterations, e.g., point mutations, are often insufficient to
Proteomics in mammary cancer research

Proteome analysis (2-DE) can generate separate protein spots. Oncogene products as well as tumor suppressor genes cannot easily be identified by 2-DE and MS. For these analyses, the non-2-DE technique is used. Finally, editing and matching of 2-D gels is labor-intensive and requires large data sets for analyzing prognostic markers.

**Mass spectrometry (MS)**

Matrix-assisted laser desorption-ionization (MALDI) is important for protein research [17]. Electro spray ionization (ESI), has a high sensitivity, allows ionization of peptides and proteins directly from the sample solution and supports protein identification [18]. For MALDI-MS, sample proteins are digested with specific proteases (e.g., trypsin) and MS determines ionized peptide fragment masses. For MALDI-MS analysis of proteins and peptides, samples are crystallized with the organic matrix that absorbs a specific wavelength (usually, UV 337 nm). Typically, the matrix of choice for large proteins is sinapinic acid (SA) and for peptide mapping hydroxy cinnamic acid (HCCA). The samples are exposed to short laser pulses, the analytes are protonated and desorbed into the gas phase, resulting in vaporization of the matrix and acceleration of the ions. The mass-to-charge (m/z) ratios of the ions are determined in a time-over-flight (TOF) mass analyzer collected by a detector, where the ion flux is converted to a proportional electrical current. Electrical signals are recorded as a function of mass-to-charge and converted to mass spectra. The mass spectra generated by MALDI-MS provide masses of analyzed peptides. The list of the masses of the tryptic fragments (i.e., peptides) is then matched to theoretical masses in databases. Mass spectrometry using MALDI-MS can produce pseudomolecular ions with little or no fragmentation. Therefore, some peptides in a sample may have a poor ionization property, which may result in a low score and ambiguous identification. Ionization, e.g., by ESI followed by tandem mass spectrometry (MS/MS), can induce fragmentation and separation to obtain peptide structural information. To identify a specific protein, several database search software programs can be used.

### Table 1. — Comparison of proteomics technologies and their contribution to biomarker discovery and early detection.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Multi-dimensional protein identification technology (MudPIT)</th>
<th>Protein identification technology</th>
<th>ELISA microarrays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>High</td>
<td>Medium sensitivity</td>
<td>Highest</td>
</tr>
<tr>
<td>Overall low, particularly for less-abundant proteins; sensitivity limited by detection method; LCM can improve specificity via isolation of selected cells out of a tissue section</td>
<td>with diminishing yield at higher molecular weights; will improve with new MS instrumentation</td>
<td>Medium/high</td>
<td></td>
</tr>
<tr>
<td><strong>Direct identification of markers</strong></td>
<td>Yes</td>
<td>No, newer MS technologies might make this possible</td>
<td>Possible when coupled with MS technologies</td>
</tr>
<tr>
<td><strong>Use</strong></td>
<td>Detection and identification of potential biomarkers</td>
<td>Diagnostic pattern analysis in body fluids and tissues; potential biomarker identification</td>
<td>Multiparametric analysis of many analytes simultaneously</td>
</tr>
<tr>
<td><strong>Advantages/drawbacks</strong></td>
<td>Significantly higher sensitivity than 2D-PAGE (much larger coverage of the proteome for biomarker discovery)</td>
<td>Protein identifications not necessary for diagnostic pattern analysis; reproducibility issues need to be addressed; need for validation; coupling to adaptive informatics tools might revolutionize the field of clinical chemistry</td>
<td>Format is flexible: can be used to assay for multiple analytes in a single specimen or a single analyte in a large number of specimens; requires prior knowledge of analyte being measured; limited by antibody sensitivity and specificity; requires use of an amplified tag detection system</td>
</tr>
</tbody>
</table>

2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; ID, identification; LCM capture microdissection; MS, mass spectrometry.
Apart from MASCOT, the “Protein Prospector” (http://www.prospectors.ucsf.edu) can be used for any MS/MS data. The peptide sequence software Protein Lynx™! Global SERVER (PLGS, http://www.waters.com; http://www.micromass.co.uk) allows, together with the NCBInr engine, to search for ESI-derived data. The software program SEQUEST (http://fields.scripps.edu/seqest) detects all peptides in the database that match the input ion masses and then theoretically calculates and matches the expected fragment ion masses against the observed MS/MS data.

Usually, databases use different MS/MS-based algorithms as an attempt to correlate uninterpreted CID (collision induced dissociation of peptide ions) spectra to theoretical spectra of peptides and scoring models to estimate the likelihood of an accurate match [21, 22]. The success of these algorithms relies on the completeness of databases and the availability of a good scoring mechanism. Correct usage and a better understanding of these search engines will also lead to higher-quality search results and less false-positive identification. Increased automation and less interactive assessment of results require quality control, especially in the developing methods to estimate the reliability of protein identification.

Non-2-DE techniques

Because the time consuming and expensive 2-DE technology requires quite a large tissue sample, several alternative techniques have been developed. In a Multidimensional Protein Identification Technology (MudPIT) peptide suspension is separated by chromatography and identified by MS. Large numbers of proteins can be identified, but no quantitative information can be obtained [23]. The method of Isotope-coded Affinity Tags (ICAT) separates very large or hydrophobic proteins and determines relative protein quantities. All proteins containing cysteine residues are isotopically labeled with ICAT probes [24]. Surface enhanced laser desorption/ionization – time-of-flight (SELDI-TOF), a protein chip technology, extracts and quantifies proteins with defined characteristic properties [25]. Unfortunately, this technology has difficulties in terms of protein identification and data validation [26].

Array-based technologies have been developed to estimate and validate potential cancer biomarkers. In the forward phase protein array (FPA), immobilized antibodies are attached on a surface to capture specific analytes from a cellular lysate or serum sample [27]. In the reverse phase protein array (RPA), cellular lysates of patient samples are immobilized on a solid phase and a single analyte specific antibody is used to detect a defined protein [28]. In summary, non-2-DE methods require smaller sample sizes and some techniques are more automated than 2-DE and suitable for analysis of a large number of samples. However these methods are restricted because most of them can only detect a limited set of proteins in each assay.

Difference gel electrophoresis (DIGE)

This highly reproducible technique allows detection of subtle changes in protein expressions and analyses of small tumor samples or LCM-collected cells. DIGE has the major advantage that both the control and experimental sample are run in the same gel. Therefore, it is less time consuming than 2-DE, uses fewer gels and simplifies correlation of protein expression patterns [29]. A recently developed DIGE system may be of potential use when studying interacting proteins [30].

Conclusion

Proteomics is “the study of all the protein forms expressed within an organism as a function of time, age, state, external factors, etc.” [31] Compared to the study of DNA or RNA expression patterns, analysis of protein expression in tissues, serum, and other biologic samples (called proteomics) may provide a more accurate understanding of the molecular complexities of human tumors [32].

In other words, proteomics characterizes the behavior of the system rather than the behavior of any single component. And the central questions asked in proteomics are: What proteins are present in which cells? How do these proteins work together in signaling pathways? What are the changes in protein expression and activity that drive the development, repair, breakdown, and death of an organism? In contrast to other levels of information, proteomics may be applied to components of the cell, such as nucleus, membrane, and organelles, which may deserve specific attention or be more convenient to study in some cases.

In relation to the clinical application the result of a proteomics analysis is the correlation of changes in protein expression with a given phenotype. Once a protein or biomarker “signature profile” has been identified, results must be validated in independent, large study sets. This requires high throughput technology, permitting the analysis of hundreds of cancer tissue samples at a time.

The identified proteins may represent important biomarkers for early diagnosis, yield valuable information about the disease process and thus identify targets for novel therapeutic intervention, or provide surrogate markers for therapeutic efficacy or treatment-related toxicity. Despite the promise of proteomic technologies in clinical cancer research, there are limitations that need to be overcome to increase sensitivity and enhance the information capture (Table 1).

References


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Expression of cyclooxygenase-2 in ovarian serous carcinoma: correlation with angiogenesis, nm23 expression and survival

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Summary

Objective: To evaluate the expression of cyclooxygenase (COX)-2 in ovarian serous carcinomas (OSC) and its correlation with microvessel density (MVD), nm23 expression, clinicopathologic prognostic factors and survival. Methods: Specimens from 44 patients with OSC were evaluated by immunohistochemistry for COX-2 and nm23 expression. Tumor MVD was assessed with CD34 immunostaining. The survival data of the patients were found from data files. Results: 40 specimens (90.1%) showed positive COX-2 staining. Patients with high COX-2-expressed tumors had shorter overall survival, but it was not statistically significant. No correlation was found between COX-2 expression and clinicopathologic variables. There was no significant correlation between COX-2 and nm23 expression or MVD. Conclusions: COX-2 is frequently expressed in OSC. Although we could not confirm the prognostic significance of COX-2 expression in the present cohort of OSC patients, the p value for overall survival was just slightly greater than alpha, and this result can be referred as almost significant. We considered that the limited number of cases in our study might affect the statistical analysis of our results. Further studies involving a larger number of patients are needed to clarify the prognostic significance of COX-2 expression in ovarian carcinomas.

Key words: Cyclooxygenase-2; COX-2; Angiogenesis; nm23; CD34; Ovarian serous carcinoma; Immunohistochemistry; Prognosis.

Introduction

Prostaglandin (PG) endoperoxidase synthase, also known as cyclo-oxgenase (COX), is an enzyme responsible for the production of prostaglandins from arachidonic acid [1]. They have different expression patterns as well as different functional characteristics [2]. COX-1 is constitutively expressed in most tissues and is thought to be involved in maintaining cellular homeostasis. In contrast, COX-2 is frequently undetectable at baseline in normal tissues, but can undergo rapid induction in response to a variety of stimuli, including mitogens, hormones, cytokines, and growth factors [3-6]. Over-expression of COX-2 was detected in various solid malignancies including ovarian cancer, and was thought to be involved in the critical steps in carcinogenesis [2, 7-9].

One of the mechanisms by which COX-2 enzyme contributes to the process of carcinogenesis is through angiogenesis. Studies on a number of human tumors have revealed strong associations between COX-2 expression and tumor vascularization [10-12]. Recent evidence suggests that COX-2 contributes to neovascularization and may support vascular-dependent solid tumor growth and metastasis [11, 13, 14]. A higher microvessel density (MVD) is associated with poor prognosis and the overexpression of COX-2 in colorectal and gastric carcinomas [12, 15]. A recent study also found a correlation between COX-2 overexpression and vascular endothelial growth factor expression in epithelial ovarian neoplasms [16].

Gene nm23 was initially cloned as a metastasis suppressor gene. In vitro experiments using cancer cells transfected with nm23 have also established that nm23 expression alters the motility, invasiveness, and colonization of cancer cells and reduces the metastatic potential of these cells [17, 18]. The relationship between nm23 and metastatic potential may vary between different tumor types. Most studies found nm23 overexpression associated with a more aggressive tumor phenotype [19, 20], and some others indicated that nm23 overexpression might have a favorable prognostic role in ovarian cancer [21]. The relationship between expressions of COX-2 and nm23 has been investigated in gastric cancer but, to our knowledge, has not been reported in ovarian serous carcinoma.

It has been shown that COX-2 overexpression was associated with higher stages, poorer survival rates, and poorer responses to therapy. Also, numerous studies revealed increased COX-2 expression to be associated with invasiveness, decreased survival and aggressive clinical behavior in a wide variety of different malignancies [22]. Recently, increased COX-2 levels have also been shown in epithelial ovarian carcinomas [23].

The objectives of the current study were 1) to evaluate the expression of COX-2 protein in serous ovarian carcinomas and its correlation with microvascular proliferation and nm23 expression; 2) to investigate the association of COX-2, nm3 expression and MVD with known clinicopathological prognostic parameters, survival and outcome.
Expression of cyclooxygenase-2 in ovarian serous carcinoma: correlation with angiogenesis, nm23 expression and survival

Materials and Methods

Tissues and patients: Approval to conduct the study was obtained from the Uludag University Faculty of Medicine-Ethics Board. Between 1997 and 2007, 44 patients were included in the study. Availability of adequate tissue material was the only criterion for selection. The tumors were graded according to the FIGO (International Federation of Gynecology and Obstetrics) grading system. The medical records of the patients were reviewed. Survival data of the patients were found from data files. All patients were treated by total abdominal hysterectomy, bilateral salpingo-oophorectomy, and 39 patients also had omentectomy. All patients received postoperative platinum-based chemotherapy. No patient received neoadjuvant chemotherapy. In all patients, formalin-fixed, paraffin-embedded tumor tissue was available. For each carcinoma, one paraffin block containing representative tumor was used for immunohistochemistry.

Immunohistochemistry: Immunohistochemical staining of COX-2 (clone SP21, rabbit monoclonal antibody prediluted in 0.05mol/l, Neomarkers) nm23 (NDP Kinase/nm23 Ab-1, rabbit polyclonal antibody prediluted form, Neomarkers), and CD34 (clone QBEnd/10 monoclonal antibody prediluted in 0.05mol/l, Neomarkers) was performed by applying the streptavidin-biotin complex method. Briefly, 5-μm tumor sections were deparaffinized and hydrated through graded alcohols to water. The slides were immersed in 10% citrate buffer, pH 6.0, for 15 min in a microwave oven for antigen retrieval and then allowed to cool for 20 min. Following endogenous peroxide blocking by immersion of the slides in 3% H2O2, and a serum-blocking step (LabVision Protein Block), the slides were incubated with primary antibodies at room temperature for 1 h. The sections were then washed and incubated with linking reagent for 15 min, followed by incubation with labeling reagent streptavidin-conjugated horseradish peroxidase for 15 min. Staining was visualized using diaminobenzidine. Mayer’s hematoxylin was used as a counterstain. Known positive controls, colonic adenocarcinoma tissue for COX-2, breast carcinoma tissue for nm23, and tissue samples of tonsillectomy for CD34 were used. For negative controls, sections were treated similarly with the exception of the primary antibody. Immunostaining was independently evaluated by two pathologists unaware of the prognosis factor and clinical outcome, and any differences were resolved by consensus.

Assessment of COX-2 and nm23: For COX-2 assessment, both staining intensity and the percentage of cells stained were analyzed. Staining intensity was scored as 0 (negative), 1+ (weak), 2+ (medium), and 3+ (strong). A combined score based on the intensity and percentage was used as the final score. Low expression was defined as intensity 1, 2, or 3 and < 10% and/or intensity 1 and < 50%. High expression was defined as intensity 2 or 3 and > 10% and/or intensity 1, 2, or 3 and > 50%. and expression of COX-2 [2, 24]. Immunoreactivity to nm23 was invariably cytoplasmatic and perinuclear. Moderately and strongly expressed staining results were considered positive [25].

Quantification of angiogenesis: MVD was quantified as previously described [26]. Briefly, sections stained with CD34 were scanned at low magnification (x 40) on an Olympus BX51 microscope (Olympus, Japan) to identify the most vascular areas (hot-spots). Stained individual endothelial cells or endothelial cell clusters that were clearly separate from adjacent microvessels, tumor cells and other connective tissue elements, were regarded as as a single countable microvessel, even in the absence of lumen. Large vessels with thick muscular walls and lumina greater than approximately eight blood cells were excluded from the count. Vessels only in the area of carcinoma were analyzed. In each case, capillaries in the hot-spots were counted per field of view at x 400 magnification (1.1 mm2) in five consecutive fields and mean counts were recorded as MVD [27]. For statistical analysis, the patients were split into two groups based on the median vessel counts (< 29 or > 29).

Statistics: Clinicopathologic features of the patients were compared with the expression of COX-2, in tumor cells and checked by 2 tests. The 2 tests were also used to analyze the association between COX-2 expression and expression of CD34 and nm23. Survival curves were estimated using the Kaplan-Meier method. The distribution of survival was compared using the log rank test. For statistical analyses, the SPSS system for personal computer (version 13.0 for windows; SPSS Inc., Chicago, IL) was used and p < 0.05 was regarded as statistically significant. Overall survival (OS)-time between date of first operation and death, or time to study closure.

Results

Patient characteristics: The mean age of the patients was 54.2 years (range, 34-75 years). The age of 13 (29.5%) women was higher than 60 years. According to the FIGO grading system, four of 44 carcinomas were grade 1, 27 were grade 2, and 13 were grade 3. We combined grade 1 and 2 tumors as a low-grade group, so 70.5% of the tumors were low grade and 29.5% were high grade. Two patients were in Stage I, two in Stage II, 34 in Stage III, and six in Stage IV. We considered Stage I and II tumors as early stage (9%) and Stage III and IV tumors as advanced stage (91%). Thirty-four (77.2%) patients had optimal debulking of gross neoplastic masses. Ten (22.8%) patients had residual disease (> 2 cm) after surgery and 25 (56.8%) patients had omental involvement at primary diagnosis.

Immunohistochemistry: Forty specimens (90.1%) showed positive COX-2 immunostaining. COX-2 expression was low in 23 (57.5%) and high in 17 (42.5%) of the cases (Figure 1). No significant COX-2 staining was observed in the stromal compartment. Immunohistochemical staining for nm23 was observed in the cytoplasm of tumor cells in 35 of 44 cases (79.5%) (Figure 2). Of these positive cases, 17 (48.6%) showed moderate and 18 (51.4%) showed strong staining intensity. The number of microvessels for x 400 microscopic fields ranged from 18 to 116 (mean MVD: 35 microvessels/high power field; median MVD: 29). The 35 cases (79.5%) presented a MVD > 35 microvessels/high power field and nine cases (20.5%) presented a MVD < 35 microvessels/high power field (Figure 3).

Clinicopathological correlations: No significant correlations were observed between COX-2 expression and patient age, FIGO stage, histological grade, residual tumor size and omental metastasis at diagnosis. Similarly, expression of nm23 and MVD evaluated by CD34 expression was not associated with clinicopathological parameters.

No correlation was observed between the COX-2 expression and MVD or nm23 expression. The association of cyclooxygenase-2 (COX-2) expression with
MVD, nm23 expression and clinicopathological parameters is shown in Table 1.

Analysis of survival: Survival data were available for 38 patients. Six patients who were lost to follow-up were excluded from the survival analysis. The median survival time was 40 months, with a range of 5-93 months. Twenty-seven of 38 patients died during the follow-up period. The OS curves in relation to COX-2 staining intensity status is shown in Figure 4. Although the difference was not statistically significant, there was a trend toward longer OS in patients with strong COX-2 staining ($p = 0.05$). The mean survival in patients with tumors that had low COX-2 expression was 68.4 (± 5.6) months, compared with 49.1 (± 6.2) months in patients who had high COX-2-expressed tumors. Nm23 expression and MVD evaluated by CD34 expression did not correlate with survival.

Discussion

COX-2 is a cytokine-inducible enzyme that is upregulated in a wide variety of human tumors. COX-2 seems
Expression of cyclooxygenase-2 in ovarian serous carcinoma: correlation with angiogenesis, nm23 expression and survival

Conflicting results reported by different authors. Different rates between histologic subtypes may account for the serous type, and the possible differences in expression were mined to be higher when compared with previous studies. The carcinomas in our study were limited to those of ovarian serous carcinomas. The expression of COX-2 in 40 (90.9%) of 44 cases of ovarian serous carcinomas. The ratio of COX-2-positive cases in our series was determined to be higher when compared with previous studies. Klimp and co-authors [33] found an expression of COX-2 in 79% of 28 ovarian carcinomas. In this study, we showed that COX-2 was expressed in 40 (28 carcinomas, 21 borderline tumors, and 8 cystadenomas) and normal ovaries. Immunohistochemistry staining against COX-2, CD34, and vascular endothelial growth factor (VEGF) showed significantly higher COX-2 expression in ovarian carcinomas (78.6%) compared with benign cystadenomas (50%). There was a significant correlation between MVD (based on CD34 immunostaining) and VEGF expression; however, no correlation was observed between COX-2 expression and MVD. In our study, though statistically not significant, tumors with higher COX-2 expression had slightly increased mean MVD compared to tumors with low COX-2 expression (36 vs 34). The smaller number of patients included in the study may have limited its statistical power.

The ovarian carcinomas demonstrated variable levels of expression of the nm23 gene product. The ratio of nm23-positive cases (79.5%) in our series was similar to that in other studies on ovarian cancer [35-37]. Researchers have been trying to assess the real prognostic significance of nm23 overexpression in ovarian cancer, but results of the studies have been controversial. For instance, some studies found that nm23 overexpression shows a significant association with more advanced tumor stages, according to them it also seems to exert a suppressive action on the development of lymph node metastasis. This apparently contradictory finding is still devoid of a plausible explanation. The mechanism of ovarian tumor invasion may be very complex, involving several biological or time- and patient-dependent factors that could drastically affect the metastatic diffusion process and partially mask the nm23-HI effects. It may be that a high nm23-HI mRNA expression exerts an inhibitory effect on the lymphatic dissemination, and this inhibition may become ineffective when nm23-HI drops below a threshold level. In our series, nm23 expression did not correlate with common prog-

### Table 1. — Association of cyclooxygenase-2 (COX-2) expression with clinicopathological parameters, MVD and nm23 expression.

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of patients (n = 44)</th>
<th>No. of patients (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>&lt; 60</td>
<td>31</td>
<td>18 (58.0)</td>
</tr>
<tr>
<td></td>
<td>&gt; 60</td>
<td>13</td>
<td>9 (69.2)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td>Early (I/II)</td>
<td>4</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td></td>
<td>Advanced (III/IV)</td>
<td>40</td>
<td>25 (62.5)</td>
</tr>
<tr>
<td>Grade</td>
<td>Low (1/2)</td>
<td>31</td>
<td>18 (58.0)</td>
</tr>
<tr>
<td></td>
<td>High (3)</td>
<td>13</td>
<td>9 (69.2)</td>
</tr>
<tr>
<td>Residual tumor size</td>
<td>&lt; 2 cm</td>
<td>34</td>
<td>20 (58.8)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 cm</td>
<td>10</td>
<td>7 (70.0)</td>
</tr>
<tr>
<td>Lymph node status</td>
<td>Positive</td>
<td>6</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>38</td>
<td>24 (63.1)</td>
</tr>
<tr>
<td>Omental involvement</td>
<td>Positive</td>
<td>25</td>
<td>16 (64.0)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13</td>
<td>8 (61.5)</td>
</tr>
<tr>
<td>Survival***</td>
<td>Alive</td>
<td>11</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td>27</td>
<td>18 (66.7)</td>
</tr>
<tr>
<td>MVD (CD34)</td>
<td>&lt; 29***</td>
<td>22</td>
<td>11 (50.0)</td>
</tr>
<tr>
<td></td>
<td>&gt; 29</td>
<td>22</td>
<td>16 (72.8)</td>
</tr>
<tr>
<td>Nm23 expression</td>
<td>Positive</td>
<td>35</td>
<td>20 (57.1)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>9</td>
<td>7 (77.8)</td>
</tr>
</tbody>
</table>

* Non significant, ** Appendectomy was performed in 38 patients, *** 6 patients were lost to follow-up, *** Median MVD.

Many studies showed a correlation between COX-2 expression and tumor vasculization [11]. Fehmi and co-authors figured out a significant correlation between COX-2 expression and MVD in their study of 125 ovarian carcinomas [2]. In recent studies, it has been shown that COX-2 either directly affects angiogenesis by increasing the proangiogenic growth factor or indirectly regulates angiogenesis with increased prostaglandin (PG) synthesis [14]. Prostaglandins, in turn, can stimulate the angiogenic process by endothelial cell migration and tube formation. Recently, Fehmi and co-authors have reported that high COX-2 expression correlated significantly with high MVD in patients with advanced stage [2]. Nonetheless, one recent study found no correlation between COX-2 expression and tumor MVD in ovarian carcinoma [16]. That study included 57 epithelial ovarian neoplasms (28 carcinomas, 21 borderline tumors, and 8 cystadenomas) and normal ovaries. Immunohistochemistry staining for COX-2, CD34, and vascular endothelial growth factor (VEGF) showed significantly higher COX-2 expression in ovarian carcinomas (78.6%) compared with benign cystadenomas (50%). There was a significant correlation between MVD (based on CD34 immunostaining) and VEGF expression; however, no correlation was observed between COX-2 expression and MVD. In our study, though statistically not significant, tumors with higher COX-2 expression had slightly increased mean MVD compared to tumors with low COX-2 expression (36 vs 34). The smaller number of patients included in the study may have limited its statistical power.

Researchers have been trying to assess the real prognostic significance of nm23 overexpression in ovarian cancer, but results of the studies have been controversial. For instance, some studies found that nm23 overexpression shows a significant association with more advanced tumor stages, according to them it also seems to exert a suppressive action on the development of lymph node metastasis. This apparently contradictory finding is still devoid of a plausible explanation. The mechanism of ovarian tumor invasion may be very complex, involving several biological or time- and patient-dependent factors that could drastically affect the metastatic diffusion process and partially mask the nm23-HI effects. It may be that a high nm23-HI mRNA expression exerts an inhibitory effect on the lymphatic dissemination, and this inhibition may become ineffective when nm23-HI drops below a threshold level. In our series, nm23 expression did not correlate with common prog-
nostic parameters, lymph node metastasis and survival. Various studies also have failed to show any correlation between clinicopathological parameters including lymph node metastasis or survival and nm23 immunostaining [19, 36].

There are only few in vitro studies on the relation of nm23 and COX-2 proteins. Recently Kaul and co-authors [42] showed that nm23-H1 can upregulate the COX-2 promoter element transcription in micro-assays. Yu et al. [43] observed that the effects of COX-2 inhibitors may be related to nm23 upregulation in breast cancer cell line. In our study we did not find a correlation between COX-2 and nm23 expression. A relation between COX-2 and nm23 expression does not seem to have been explored in any previous study on ovarian carcinomas and needs to be clarified.

In ovarian epithelial tumors, conflicting results exist on the correlation between COX-2 expression and tumor progression [29]. Many studies about COX-2 expression in ovarian carcinomas and correlation with clinicopathologic variables revealed that COX-2 expression affected patient prognosis [29, 44]. Some studies have found that COX-2 expression increases significantly according to the stage and grade of ovarian epithelial tumors [16, 30, 31].

Seo et al. [30] found that expression of COX-2 was significantly associated with FIGO stage, histologic type, tumor grade, and status of residual disease by univariate analysis. Denkert and co-authors [31] reported recently that COX-2 did not correlate with any of the clinicopathological variables, including histological grade in 86 invasive ovarian cancer patients of various histological types but that elevated COX-2 expression was a marker for poor prognosis. Moreover, elevated COX-2 expression was identified as an independent prognostic factor. The exact cellular mechanisms underlying the poor prognosis associated with COX-2 expression need to be delineated further. In contrast, Shigemasa et al. [34] and Matsumoto and co-authors [16] did not find any correlation between COX-2 expression and survival; their conflicting results may have been due to the fact that their patient populations were heterogeneous and included patients who had ovarian epithelial tumors of various histologic types and grades.

In conclusion we could not find any relationship between clinicopathological prognostic factors or survival and COX-2 expression. Although the difference was not statistically significant, we found that COX-2 may be a favorable prognostic factor in ovarian serous carcinoma. There was a trend toward increased overall survival (p = 0.05). It should be pointed out that because of the small number of patients in our study the statistical power of the analysis may be insufficient to detect prognostic factors. Large-scale prospective and retrospective studies are needed to establish whether COX-2 expression is indeed of practical utility as a prognostic predictor. In addition to the prognostic significance, a better understanding of the biologic mechanism of these molecular changes may help to identify new targets for ovarian cancer therapy.

Aknowledgement

The study was sponsored by the Uludag University Research Fund Project T-2006/15.

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Expression of cyclooxygenase-2 in ovarian serous carcinoma: correlation with angiogenesis, nm23 expression and survival


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Plasma concentration of angiopoietin-1, angiopoietin-2 and Tie-2 in cervical cancer

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Summary

**Purpose of investigation:** Angiogenesis is important in the promotion and progression of malignancy. The formation of new blood vessels is coordinated by many factors, angiopoietins among others. Overexpression of angiopoietins has been observed in various tumors. The aim of the study was to evaluate plasma concentration of Ang-1, Ang-2 and Tie-2 in cervical cancer. **Methods:** The study group consisted of 34 patients with cervical cancer and the control group of 20 healthy volunteers. Plasma concentrations of Ang-1, Ang-2 and Tie-2 were evaluated by ELISA. **Results:** Plasma concentrations of Ang-1, Ang-2, Tie-2 and Ang-1/Ang-2 ratios were significantly higher in cervical cancer patients than in controls. Although there was no correlation between concentration of Ang-1, Ang-2, Tie-2 and clinical stage (FIGO), the Ang-1/Ang-2 ratio was higher in Stage I than in II-III. Ang-1 correlated positively with Ang-2 and Tie-2 in a subgroup with Stage II-III and Ang-2 with Tie-2 in a subgroup with Stage I. **Conclusion:** Plasma concentrations of Ang-1, Ang-2 and Tie-2 may be useful additional tumor markers in cervical cancer.

Key words: Angiopoietins; Cervical cancer.

Introduction

Angiogenesis is important in the promotion and progression of malignancy. New blood vessels are formed in a few stages with the participation of many factors, angiopoietin-1 and angiopoietin-2 (Ang-1 and Ang-2), among others. Ang-2 binding to the Tie-2 receptor on endothelial cell (EC) surfaces causes removal of pericytes from the endothelium, vessel wall weakness, and finally their destabilization. After pericyte loss, proliferation mediated by VEGF, FGF, EGF and migration, followed by adhesion influenced by VEGF, FGF, integrin αVβ3 of endothelial cells become possible. The next stage of angiogenesis is a tube formation of a new blood vessel (FGF, PDGF, TNF-alpha, ephrin 2A) which is settled by mesenchymal cells (PDGF, Ang-1). TGF-alpha participates in the differentiation of these cells into mature pericytes. The last stage of the process is stabilization of vessels with participation of survival factors for ECs (VEGF and Ang-1) [1].

Ang-1, as an agonist of the Tie-2 receptor, has a stabilizing effect on blood vessels, acts as an anti-inflammatory, and as a sealing-up factor. Instead, Ang-2, depending on cell type and microenvironmental conditions, could act as an antagonist or agonist. If it is expressed at sites of blood vessel remodelling, it promotes destabilization of the vessel. When it acts mostly solely, it causes vessel regression, whereas in conjunction with VEGF it may form vessels and cause their sprouting [2-4].

Tie-2 and Ang-2 expression in endothelial cells is induced by hypoxia and pro-inflammatory cytokines as they prevail in cancer tissues. However, these factors decrease Ang-1 expression. Disturbance of balance between both angiopoietins, due to a relatively higher expression of Ang-2 compared to Ang-1, causes induction of tumor angiogenesis (“angiogenic switch on”). In the angiogenic phase Ang-1, Ang-2 and Tie-2 expression is increased. The last stage of this process is a maturing phase, in which the Ang-1/Ang-2 ratio is increased (“angiogenic switch off”) [5-8].

The aim of this study was to answer two questions: 1) whether the above-mentioned alterations in angiopoietins expression in cancer tissue are reflected by changes in the concentration in the peripheral blood of cervical cancer patients, 2) whether plasma angiopoietin concentration may be of diagnostic use in this condition.

Materials and Methods

**Patients**

The study group consisted of 34 patients with cervical cancer ranging in age from 30 to 84 years (mean age 50.7 ± 14.0). By means of clinical staging, 22 patients were classified as Stage I and 12 patients as Stage II-III. All patients had squamous cell carcinoma. The most frequent histological grade was G2 (moderately differentiated). All patients were treated with brachytherapy, and surgical treatment was applied to 17 patients.

Ang-1, Ang-2 and Tie-2 concentrations were evaluated upon ascertainment of cancer, before treatment.

The subjects were patients of the University Hospital Department of Oncology and Brachytherapy Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun.

The control group consisted of 20 women aged from 24 to 58 years (mean age 36.2 ± 11.7). These women were introduced to current normal cervical cytology, which excluded cervical cancer. Other tumors were not ascertained in these women during enquiry.
Material

Ang-1, Ang-2, and Tie-2 concentrations were evaluated in plasma. Blood (2 ml) was taken from the elbow vein. EDTA was used as an anticoagulant. Within 30 min of collection, the blood samples were centrifuged at 2-8°C for 15 min at 1000 x g. For complete platelet removal, an additional centrifuge at 10,000 x g for 10 min was applied. The plasma was stored at –70°C.

Methods of determination

Ang-1, Ang-2 and Tie-2 concentrations were assayed by commercially available sandwich enzyme-linked immunosorbent assay kits from R&D Systems (Quantikine Human Ang-1, Ang-2, Tie-2 Immunoassay, R&D Systems Inc., Minneapolis, MN, USA). Kits are designed to measure human Ang-1, Ang-2 and Tie-2 in cell culture supernates, serum, plasma, and other biological fluids. Plasma samples for detection of these three factors were diluted 15-, 5- and 10-fold respectively.

Statistical analysis

Statistical analysis was done using the nonparametric Mann-Whitney test and Pearson’s linear correlation. The results were considered statistically significant at \( p < 0.05 \).

Results

Table 1 shows the comparison of Ang-1, Ang-2, Tie-2 concentrations and the Ang-1/Ang-2 ratio between the study and control groups. The medians of all parameters were significantly higher in the study group than the controls.

The correlation between Ang-1, Ang-2, and Tie-2 concentrations and clinical stage of cervical cancer (FIGO classification) are given in Figure 1 and the correlation between the Ang-1/Ang-2 ratio and clinical stage in Figure 2.

The concentrations of Ang-1, Ang-2, and Tie-2 did not differ statistically in the two subgroups: in the subgroup with cancer restricted to the uterine cervix (Stage I) and with cancer infiltrating sites beyond the uterine cervix (Stage II-III).

Although the median of Ang-1 concentration did not differ in the subgroups of patients with tumor Stages I and II-III, it should be noted that: in 27.3% patients with Stage I the Ang-1 concentration exceeded 10,000 ng/ml, however such high Ang-1 was observed in only 17.0% of patients with Stage II-III (Figure 1). Moreover, the Ang-1 concentration was above the reference interval, which means above 1278 pg/ml (upper quartile, 75th percentile), more frequently in patients with Stage II-III than patients with Stage I (91.7 vs 63.6%).

The Ang-1/Ang-2 ratio was higher in subjects with Stage I than II-III (average 3.41 vs 1.77) (Figure 2).

In this study the estimation of factors correlation was carried out. Weak correlations between Ang-1, Ang-2,
and Tie-2 were identified in the study group (Ang-1 vs Ang-2: r = –0.06; Ang-1 vs Tie-2: r = 0.08; Ang-2 vs Tie-2: r = 0.17). However, these correlations were stronger in the subgroup with Stage II-III (Ang-1 vs Ang-2: r = 0.36; Ang-1 vs Tie-2: r = 0.33) and in the subgroup with Stage I (Ang-2 vs Tie-2: r = 0.28) (Table 2).

**Discussion**

Undoubtedly, changes of angiopoietins and their receptor expression have been observed in cancer. Results of investigations related to angiopoietin-2 expression in various tumors are more unequivocal. This expression was usually increased [9-15]. Instead, on the subject of Ang-1 opinions are varied. For example, the overexpression of Ang-1 has been observed in breast cancer [16] and colorectal adenocarcinoma [9]. Not only the angiopoietins but also their receptor Tie-2 was overexpressed in tumors [9].

In many studies, the correlation between angiopoietin expression and tumor progression was observed [17-22]. The Ang-1/Ang-2 ratio is more important than angiopoietin expression. Hypoxia, inflammation, and gene mutations disturb the balance between angiogenesis inductors and inhibitors. Increased expression of the Ang-2 and Ang-2/Ang-1 ratio affects angiogenesis induction (plastic phase). In the angiogenic phase expression of Ang-1, Ang-2, and Tie-2 is increased. In the maturing phase Ang-1/Ang-2 ratio is increased. The rate of Ang-1 to Ang-2 expression was negatively correlated with density of blood vessels and clinicopathological features in hepatocellular carcinoma [23] and ovarian cancer [24].

After analysis of angiopoietin expression in tumors, the question arises whether the changes in angiopoietin concentration in peripheral blood appear. Increased plasma (serum) concentration of Ang-1 and Ang-2 was observed in breast and prostate cancer [25], and Ang-2 in lung cancer [26]. An increased concentration of Tie-2 was indicated in colorectal cancer [27] and also in relation to metastasis [28].

In our study, plasma concentrations of Ang-1, Ang-2 and Tie-2 were significantly higher in cervical cancer patients than in controls. These results suggest that angiogenesis was increased in the study group. Also the Ang-1/Ang-2 ratio was significantly higher in cancer patients than in healthy subjects. The question appears whether the increased Ang-1/Ang-2 ratio resulted from the angiogenic cycle phase, or if it was caused by different release of both angiopoietins. Ang-1 is secreted by periendothelial cells and is incorporated into the extracellular matrix (ECM), however Ang-2 is stored in Weibel-Palade bodies in the cytoplasm of endothelial cells and after secretion is not associated with the extracellular matrix [29].

A correlation of angiopoietins or Tie-2 concentrations with the stage of cervical cancer was not observed. The Ang-1/Ang-2 ratio was higher in patients with Stage I than in those in Stage II-III. It may be suggested that in
the Stage I the maturing phase prevails, after which the quiescent phase follows. However, it may be suggested that in more advanced stages the angiogenic phase should occur.

The correlation between studied factors in the whole group was weak, while in the subgroups with different stages – a little stronger. These observations may confirm earlier ascertainment that in each clinical stage of tumor, angiogenesis may be in different phases.

Conclusion

Ang-1, Ang-2 and Tie-2 plasma concentrations may be useful additional tumor markers in cervical cancer.

Acknowledgement

Supported by a grant from Nicolaus Copernicus University in Torun, no. 29/2007.

References

Do women with glandular abnormalities require colposcopy follow-up?

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Summary

Purpose: To determine whether cervical glandular abnormalities can be safely followed up by community cytology. Methods: A retrospective database review was conducted identifying women with a histological report of cervical glandular abnormalities over a three-year period. Results: Fifty women were found to have a glandular abnormality diagnosed histologically. Twenty were initially referred for colposcopy with cytological glandular abnormalities. Thirty women with cytological squamous abnormalities were later found to have cervical glandular intraepithelial neoplasia (CGIN) at histological assessment. Two women had invasive adenocarcinoma and all others with endocervical dyskaryosis or CGIN were treated using large loop excision of the transformation zone (LLETZ). At follow-up 43 women had negative cytology, one woman defaulted further appointments, one had moved out of the area, seven were successfully treated by a repeat LLETZ following incomplete excision of CGIN at the endocervix, and five had negative cytology in the community. Three women continue to have cytological/histological abnormalities with one subsequently having a hysterectomy. Conclusion: Women with endocervical dyskaryosis or CGIN should be treated by LLETZ. Provided LLETZ is repeated in cases of incomplete endocervical CGIN excision cytological follow-up can take place in the community.

Key words: Endocervical dyskaryosis; Cervical glandular intraepithelial neoplasia; Large loop excision of the transformation zone; Cytology.

Introduction

The current National Health Service Cervical Screening Programme (NHSCSP) guidelines ‘Colposcopy and Programme Management [1] suggest that follow-up for women treated for glandular abnormalities should be ‘cytology alone’ with samples ‘taken by appropriately trained staff’ in treatment centres. This is seen as the best practice. The guidance then states that an alternative arrangement could include follow-up cytology ‘undertaken in the primary care sector’.

In Newcastle upon Tyne the colposcopy service sees all women post treatment for glandular abnormalities, undertaking a colposcopic examination and cervical smear. However we wished to consider community cytological follow-up as it was felt to be a safe option particularly since a recent local audit suggested that liquid-based cytology (LBC) when compared to conventional cytology led to a substantial increase in the positive predictive value of cytology to predict invasive and pre-invasive disease of the cervix (unpublished data).

Newcastle was part of the pilot project assessing LBC in three cytology screening laboratories across England [2]. The results of this study led the National Institute for Health and Clinical Effectiveness in 2003 to recommend the roll out of LBC across England and Wales as the primary method for collecting and preparing cervical cytological specimens for the cervical screening programme [3]. Since that time Newcastle continues to use the Surepath LBC system. Its colposcopy service, based at the Royal Victoria Infirmary, sees almost 1,300 new patients a year of which approximately 0.9% are found to have glandular abnormalities. The aim of this audit was to confirm that women with successfully treated cervical glandular abnormalities can be followed-up by community-based cytology.

Methods

The Colposcopy Department of the Royal Victoria Infirmary, Newcastle upon Tyne has a fully computerised record database holding clinical information on all women attending the service including details of their referral cervical cytology and subsequent histological/cytological reports. A retrospective database review was conducted interrogating data from 1 January 2004 to 31 December 2006. Women with a histological and/or a cytological report suggesting a cervical glandular abnormality were identified.

Results

During the three-year investigation period 50 women were identified as having either a cervical cytology result and/or histological report indicating that a glandular abnormality had been detected. A total of 20 women (0.5%) had endocervical dyskaryosis out of 4,037 women referred with a cytological abnormality to the department during this three-year period. A further 30 women were found to have glandular intraepithelial neoplasia (CGIN) on histological sampling. None of these women were later found to have any endometrial pathology.

Seventeen of the 20 women with endocervical dyskaryosis at referral were treated by LLETZ at their...
first colposcopy appointment. Six women were found to have CGIN, eight had cervical intraepithelial neoplasia grade three (CIN 3) and one woman had epithelial changes of uncertain significance (ECUS). Subsequent cytological smears for 13 women showed no abnormality. One defaulted follow up. Two women were found to have invasive adenocarcinoma and were referred to the Northern Gynaecology Oncology Centre at Gateshead.

Three of the 20 women with endocervical dyskaryosis initially had cervical punch biopsies however all had subsequent LLETZ with two demonstrating CGIN and one viral change. Follow-up cervical cytology was negative in all three.

Five of the 20 women had a second LLETZ because of incomplete CGIN excision of the endocervical margin in three, CGIN incomplete at the ectocervical margin in one and recurrence of CIN 2 in the final woman. Again follow-up cytology in all five was negative.

A total of 30 women referred with cytological squamous abnormalities were found to have CGIN on histology. Twelve had been referred with severe squamous dyskaryosis, one severe squamous dyskaryosis - query invasion, nine women moderate squamous dyskaryosis, one mild squamous dyskaryosis, four borderline nuclear changes and three endocervical atypia. Twenty-one of these women had a LLETZ at their first colposcopy visit and all showed CGIN histologically. Follow-up cytology was normal in 18 women, one is still under colposcopic review and one had a hysterectomy. One defaulted further clinic appointments.

Nine women initially had punch biopsies which revealed CGIN. All had subsequent LLETZ with seven demonstrating CGIN, one CIN 3 and one CIN 1. Follow-up cytology was negative in eight women and one is still under review.

Of these 30 women referred with cytological squamous abnormalities but were later found to have CGIN four had a repeat LLETZ and one underwent a hysterectomy for incomplete excision of CGIN at the endocervical margin. All had subsequent negative cervical smears.

Discussion

This audit shows that the colposcopy service in Newcastle follows the standards set by the National Health Service Cervical Screening Programme (NHSCSP) [4]. All women referred with cytology showing endocervical dyskaryosis underwent LLETZ because punch biopsy is known to be unreliable in the management of high-grade cytological glandular abnormality [4]. However a further LLETZ was only performed if CGIN remained at the endocervical margin. Colposcopic and cytological follow-up suggests that this is a successful approach and avoids unnecessary repeat cervical treatments which have a known morbidity [5].

There was a high correlation between cytological referral showing endocervical dyskaryosis and the finding of high-grade glandular and high-grade squamous changes. In these 20 women eight had CGIN, eight had CIN 3, two women had invasive adenocarcinoma of the cervix, one had CIN 1 and another woman had ECUS. These findings are very similar to previously published work highlighting the importance of adequate investigation and treatment for women found to have glandular abnormalities [5].

Many women find repeated visits to a colposcopy unit stressful with almost one-third of women still having a significant fear of cancer two years after their initial referral following an ‘abnormal smear’ [7]. Being able to be followed-up by their family doctor or nurse for cervical cytology may help elevate these concerns and decrease those who default review appointments. From this audit community cytological surveillance appears safe as long as women are informed about the nature of the glandular abnormality detected and the need for repeated follow-up cytology testing.

Conclusion

The NHSCSP guidelines state that a further LLETZ is required if there is incomplete excision of CGIN at the ectocervical and/or endocervical margins. This audit demonstrates that women with endocervical dyskaryosis or CGIN should be treated by LLETZ. As long as the NHSCSP guidelines are followed cytological follow-up in the community is safe. The general practitioner or nurse practitioner should ensure that endocervical cells are sampled when the cervical smear is performed.

References


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Sexual and psychological functioning in women after pelvic surgery for gynaecological cancer

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Introduction

Over the last decade, modifications in surgical and adjuvant treatment modalities have resulted in an improved outcome of gynecological cancer treatment. Consequently, there is an increased interest for quality of life issues in both clinical practice and research of patients with gynecological cancer. Although sexuality is regarded as an important contributor to quality of life, it is only scantily studied in the context of gynecological cancer.

Recent studies have demonstrated that treatment of cervical cancer by radical hysterectomy result in changes in vaginal anatomy and function, resulting in changes in sexual functioning [1-5]. Jensen et al. [3] and Lindau [5] demonstrated that 63% of women treated by radical hysterectomy for cervical cancer experience long-term sexual problems and that such problems were significantly more prevalent compared with a population-based control group. A higher degree of sexual dysfunction is also reported by women following vulvectomy for vulvar cancer [6]. The importance of discussing sexual function with patients suffering from genital tract cancer has recently been shown by Lindau et al. [5] who demonstrated that women who report that no such discussion had taken place had a much higher risk of complex sexual morbidity.

These studies [1-6] suggest that surgical treatment of women with gynaecological cancer may result in anatomical and physiological changes that interfere with sexual function (due to e.g., damage to pelvic nerves, damage to small vessels, fibrosis or hormonal deficiencies), sexual experience (due to e.g., the experience of disfigurement, perception of vaginal shortness and of decreased vaginal elasticity) and may give rise to sexual dysfunction.

Considering these findings, this study aimed to: (1) examine the presence of sexual problems after pelvic surgery in women with endometrial, vulvar and cervical cancer, (2) describe the specific relation between psychological variables and sexual dysfunction in this group of patients, and (3) examine the effect of surgery for gynecological cancer on the partner relationship.

Materials and Methods

Setting and sample

All patients who underwent pelvic surgery for vulvar cancer Stage I-III, cervical cancer Stage I-IIb or endometrial cancer Stage I-III in a gynecological oncology unit of the University Hospitals Gasthuisberg in Leuven between January 2002 and August 2003 were invited to participate in the study. The minimal interval between surgery and administration of the questionnaires was six months. The maximum interval was 18 months. Exclusion criteria were: patients with advanced stage disease and metastatic disease, patients with ovarian cancer, patients who had received chemotherapy or radiotherapy and patients confronted with a relapse of the disease or poor general condition.

Summary

Pelvic surgery for gynecological cancer can affect sexuality through a number of anatomical, physiological and psychological mechanisms. We aimed to examine the prevalence of sexual dysfunction and psychological functioning in women who underwent pelvic surgery for gynaecological cancer. Fifty women who underwent pelvic surgery for gynaecological cancer. Fifty women who underwent pelvic surgery for vulvar, cervical or endometrial cancer in a gynecological oncology unit completed questionnaires evaluating marital satisfaction (DAS), depression (BDI-II) and sexual functioning (SSFS and an in-house Specific Sexual Problems Questionnaire). Medical records were used to obtain disease-specific data. The control group consisted of 39 healthy age-matched control women attending an outpatient screening clinic. Significantly more women with gynaecological cancer than controls reported sexual problems (83 vs 20%), including decreased desire (76 vs 14%) and impaired vaginal lubrication (42 vs 9%). Pelvic surgery was specifically related to changed intensity of orgasm (43%), reduced vaginal sensitivity (38%), vaginal elasticity (30%), superficial dyspareunia (27%), vaginal narrowing (26%) and shortening (22%). Although no significant differences were found between either group for depression (17% vs 13%) or total quality of the partner relationship, women with a history of gynecological cancer reported significant lower marital cohesion. These results indicate that although the psychological adjustment of women who underwent pelvic surgery seems to be satisfactory, they seem to be at risk for sexual dysfunctions.

Key words: Oncology; Surgery; Quality of life; Sexual dysfunction.
Controls were recruited from women visiting an outpatient gynecology clinic for routine screening reasons (n = 180). Women were eligible for inclusion in the control group if they were 18 years or older, had a stable heterosexual partner relation for at least one year, and had no somatic diseases.

**Methods**

First, patients who underwent surgery for vulvar cancer Stage I-III, cervical cancer Stage I-IIb or endometrial cancer Stage I-III were called to ask whether they would be interested in participating in the study. Patients were assured that participation was voluntary and refusal would not jeopardize their treatment in any way. After subjects gave written informed consent questionnaires were sent to their home address with an accompanying letter. One reminder letter was sent after six weeks. Established self-report questionnaires were used to assess psychological adjustment, marital satisfaction, depression and relevant aspects of sexual functioning. Medical records were used to obtain data on current medication, the presence of other illnesses and type of operation. The study protocol was approved by the local ethics committee.

**Questionnaires**

The 21-item Beck Depression Inventory (BDI) was used to assess current self-reported symptoms of depression [7]. Each item measures the presence and severity of a symptom of depression and by summing up the item scores a total depression score is determined. A cut-off score of 17 on the BDI was used as an indication for the presence of clinical depression. Higher scores indicate a higher number of depressive symptoms. Reliability analysis of the BDI has shown a Cronbach’s α of 0.94, and good one-week test-retest reliability of 0.93, and good content and construct validity [7].

The Dyadic Adjustment Scale (DAS) was used to assess the quality of marital relation [8]. The DAS consists of 32 items with higher scores indicating better marital quality. The DAS has shown good reliability (Cronbach’s α = 0.94) and construct validity as reflected in high correlations with the Locke-Wallace Marital Adjustment Test [8].

The ‘Short Sexual Functioning Scale (SSFS)’ is a self-constructed questionnaire to assess the presence, severity and burden of sexual dysfunctions. The scale contains items related to sexual desire, sexual arousal and orgasm. Each item is rated on a 4-point scale (range 0 to 3). Higher scores indicate a higher severity of the problem. Sexual dysfunctions are conservatively defined: only moderate (score 2) and severe (score 3) problems are taken into account. Apart from presence and severity of the problem, this questionnaire also measures the burden the problem presents to the patient, partner and relationship. Reliability analysis has shown a Cronbach’s α of 0.96.

The “Questionnaire on Female Sexuality” is a self-constructed instrument to assess the subjectively perceived influence of surgery on sexual functioning. The questions request a comparison of the best sexual situation ever compared to the current sexual situation. Visual analogue scales (VAS-scale) aim to assess the influence of surgery on desire, arousal, orgasm and overall sexual satisfaction. Reliability analysis showed a Cronbach’s α of 0.83.

The ‘Specific Sexual Problems Questionnaire’ is a self-constructed questionnaire to assess presence, severity and burden of specific sexual problems such as vaginal narrowing, vaginal shortening, decreased elasticity, reduced swelling of the labia, superficial and deep dyspareunia, and intensity of orgasm. Symptom selection was based on a literature review and on clinical experience. Each symptom is rated on a 4-point scale (ranging from 0 to 3) with higher scores indicating increasing severity. A specific symptom is conservatively defined: only moderate (score 2) and severe (score 3) problems are taken into account. Apart from presence and severity, this questionnaire also measures the burden of these symptoms on the patient, partner and relationship.

**Data analysis**

Analyses were performed using SPSS (version 14.0, Chicago, Inc., IL). Scores are presented as mean ± SD. Student’s t-test and Mann-Whitney-U test were used to calculate differences between groups. Chi-square tests and – if applicable – Fisher’s exact test were used to study associations between variables. Based on a Bonferroni correction the initial level of significance, p < .05, was changed to p < 0.005.

**Results**

**Patient population**

Of 60 questionnaires sent by mail we received 50 questionnaires back resulting in a response rate (RR) of 83%. Three of the non-responders stated they were not interested in the topic of the study and seven did not respond for unknown reasons. Mean age of subjects at the time of treatment was 52.0 ± 13.4 years (range: 30-76 years) and questionnaires were completed 0.5 to 1.5 years postoperatively. The majority (48%) of subjects had endometrial carcinoma, and the remainder of the group had either vulvar (26%) or cervical carcinoma (26%). Surgical procedures included hemi-vulvectomy (20%), total vulvectomy (6%), Wertheim-Meigs procedure with (20%) or without bilateral salpingo-oophorectomy (BSO) (6%) and total hysterectomy with (44%) and without BSO (4%).

To compose an age-matched control group, 39 out of 135 controls (RR: 80.5%) were randomly selected with a mean age of 48.1 ± 6.9 (range: 36-62 years).

**Prevalence and type of sexual dysfunction**

Significantly more patients with a history of surgically treated gynecological cancer reported sexual dysfunctions (20% vs 83%; p < .001) compared with healthy controls. While 39% of the cancer patients reported one, 28% two and 17% three sexual problems, only 5.7%, 8.6% and 5.7% of controls reported these problems respectively (p < .001).

Table 1 shows that significantly more women with a history of gynecological cancer reported a decrease in sexual desire (76.3 vs 14.3%, p < .001). Measurement of the burden of the decreased desire in subjects showed that this was a moderate or severe problem for the patient herself in 44.8%, a problem for the partner in 50% and a problem for the partnership in 40%. Combining the data on severity (score ≥ 2; n = 29) and burden (score ≥ 5; n = 11) 38% of women from the study group met the severe criteria for a decreased sexual desire disorder.
Table 1. — Sexual dysfunctions in the group of patients after surgical treatment for gynecological cancer compared to the group of age-matched control women from the general population.

<table>
<thead>
<tr>
<th>Sexual dysfunction</th>
<th>Patient n (%)</th>
<th>Control n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased sexual desire</td>
<td>29/38 (76.3)</td>
<td>5/35 (14.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Decreased vaginal lubrication</td>
<td>16/38 (42.1)</td>
<td>3/35 (8.6)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Problems with orgasm</td>
<td>12/40 (30.0)</td>
<td>6/34 (17.6)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s. = not significant.

Table 2. — Postoperative sexual complaints of the pelvic gynecological cancer group.

<table>
<thead>
<tr>
<th>Sexual complaint</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changed intensity of orgasm</td>
<td>15/35</td>
<td>42.8</td>
</tr>
<tr>
<td>Reduced vaginal sensitivity</td>
<td>14/37</td>
<td>37.8</td>
</tr>
<tr>
<td>Reduced vaginal elasticity</td>
<td>11/37</td>
<td>29.7</td>
</tr>
<tr>
<td>Superficial dyspareunia</td>
<td>10/37</td>
<td>27.0</td>
</tr>
<tr>
<td>Vaginal narrowing</td>
<td>10/39</td>
<td>25.6</td>
</tr>
<tr>
<td>Vaginal shortening</td>
<td>8/37</td>
<td>21.6</td>
</tr>
<tr>
<td>Reduced swelling of labia</td>
<td>6/37</td>
<td>16.2</td>
</tr>
<tr>
<td>Deep dyspareunia</td>
<td>5/36</td>
<td>13.9</td>
</tr>
</tbody>
</table>

Compared to controls, subjects reported significantly poorer vaginal lubrication (42.1 vs 8.6%; \( p < 0.005 \)). Measurement of the burden of the sexual dysfunction in subjects showed that this decrease in vaginal lubrication was moderately or severely affecting the patient herself in 57.1%, the partner in 46.2% and the relationship in 29.6%. Combining the data on severity (score \( \geq 2; n = 16 \)) and burden (score \( \geq 5; n = 12 \)) 32% of women from the study met the severe criteria for a decreased sexual arousal disorder.

No significant differences were found between the treatment and control group regarding the ability to reach orgasm (30 vs 17.6%). The measurement of the burden of sexual dysfunction in subjects showed that an inability to reach orgasm was moderately or severely affecting the patient herself in 53.3%, the partner in 41.4% and the relationship in 34.4%. Combining the data on severity (score \( \geq 2; n = 12 \)) and burden (score \( \geq 5; n = 10 \)) 25% of women from the treatment group met the severe criteria for an orgasmic dysfunction.

The results of the ‘Specific Sexual Problems Questionnaire’, as listed in Table 2, show that pelvic surgery was associated with several changes in sexual functioning. In decreasing order of prevalence patients with a history of surgery for gynecological cancer listed moderate to severe changes in intensity of orgasm (42.8%), vaginal sensitivity (37.8%) and vaginal elasticity (29.7%), apart from problems with superficial dyspareunia (27.0%), vaginal narrowing (25.6%), vaginal shortening (21.6%), reduced swelling of the labia (16.2%) and deep dyspareunia (13.9%).

**Psychosocial variables**

There was no significant difference in the mean BDI-depression score of women from the study group (9.7 ± 8.7) compared to controls (7.8 ± 6.5). Based on a cut-off score of \( \geq 17 \) on the BDI, 17% of subjects and 12.8% of the controls were depressed. No significant differences were found between either group for total quality of relationship with the partner (108 vs 110), including expression of emotions (8.5 vs 8.6), consensus in the relationship (47.0 vs 48.4) and marital satisfaction (37.3 vs 38.4). Nevertheless, women from the study group had significantly lower scores for marital cohesion (11.5 vs 14.7, \( p < 0.005 \)).

**Sexual dysfunctions and psychosocial variables**

Women from the study group who reported sexual dysfunctions were younger than those not reporting sexual dysfunctions (44.1 vs 52.1 years, \( p < 0.05 \)). There were no differences between these two groups with respect to depression scores (12.2 vs 8.1) or any aspect of the quality of the relationship: marital consensus, marital satisfaction, expression of emotions, marital cohesion or total marital quality. There were however obvious differences in the way women with a history of cancer, regardless of presence of sexual dysfunctions, rated VAS-scales on the impact of surgery on certain aspects of sexual functioning. Subjects were more likely to report an impact on frequency of sexual activity (76 vs 39, \( p < 0.005 \)), on vaginal lubrication (79 vs. 44, \( p < 0.005 \)) and on the ability to achieve an orgasm (84 vs 40, \( p < 0.001 \)). Although there was a similar trend for impact on sexual desire (69 vs 34, \( p < 0.005 \)) and overall satisfaction of sexual life (19 vs 45, \( p < 0.05 \)) these did not reach significance.

**Discussion**

This study examined sexual dysfunctions in women following pelvic surgery for endometrial, vulvar or cervical cancer. The study describes the specific relation between psychological variables and sexual dysfunction. To date this is the first study not only focussing on sexual dysfunctions after pelvic surgery for gynecological cancer. The study describes the specific relation between psychological variables and sexual dysfunction. In decreasing order of prevalence patients with a history of surgery for gynecological cancer listed moderate to severe changes in intensity of orgasm (42.8%), vaginal sensitivity (37.8%) and vaginal elasticity (29.7%), apart from problems with superficial dyspareunia (27.0%), vaginal narrowing (25.6%), vaginal shortening (21.6%), reduced swelling of the labia (16.2%) and deep dyspareunia (13.9%).
tion, while no impact on orgasm could be found in this study. Isolated sexual problems do not seem to occur since almost half of the women that underwent pelvic surgery reported two or even three sexual dysfunctions.

Our results about the impact on sexual desire are in line with the literature. While we found a postoperative decrease of sexual desire in 38% of our sample, other authors reported a decrease in 25 to 54% of surgically treated women with gynecological cancer (Pieterse et al. [2, Bergmark et al. [1]). Our finding that 32% of women with a history of gynecological cancer have vaginal lubrication problems is higher than previously reported. While Bergmark et al. [1] found that 26% of the patients had insufficient vaginal lubrication after surgery, Gruman et al. [9] only noticed a slight downward trend postoperatively. Jensen et al. [3] and Pieterse et al. [2] reported a persistent lack of lubrication during 24 months after treatment. Although, 25% of the patients in our sample reported a problem with reaching orgasm, this result did not reach significance when compared with the control group suggesting that there is no impact of pelvic surgery on the ability to experience orgasm. This is in contrast to Pieterse et al. [2] who found that 33% of patients had no orgasm after surgery compared to 20% preoperatively. Bergmark et al. [1] even reported that 9% of the women had no or little orgasmic pleasure after treatment. Jensen et al. [3] noted severe orgasmic problems in the first six months after surgery. Our findings on concurrent sexual problems are in accord with Lindau et al. [5] who reported that 50% of survivors of vaginal or cervical cancer, versus 15% of an age-matched control group, suffered concurrent sexual problems.

The present study not only focused on the classic sexual dysfunctions but also systematically assessed more uncommon complaints that could be induced by pelvic surgery and affect sexual functioning. In particular, our study shows that pelvic surgery is specifically related to changes in intensity of orgasm, reduced vaginal sensitivity, vaginal elasticity, superficial dyspareunia, vaginal narrowing and - shortening. These results are in line with previously published data [1-6, 9, 10] confirming the presence of a real risk to the sexuality of women undergoing pelvic surgery.

In the current study we further evaluated the association of pelvic surgery for gynecological cancer with depression and the quality of the relationship. There were no significant differences in the prevalence of depression in the study group compared with the control group. These findings are similar to Grumann et al’s observation in a group of women that underwent radical hysterectomy [9]. This finding suggests that patients who survived pelvic gynecological cancer seem to cope well psychologically.

Similarly, our results showed that evaluation of the relationship of women with a history of surgery for gynecological cancer did not differ from that of controls. This in is in line with Gruman et al’s [9] observation that there was no decrease in postoperative satisfaction with the partner relationship. Corney et al. [10] found in their study that 16% of the patients felt that their marriage had worsened after radical hysterectomy or vulvectomy. Strikingly, women from the study group reported, however, significantly lower marital cohesion. One possible explanation could be that the confrontation with the diagnosis and treatment of cancer is so intense that it may lead to a kind of detachment as preparation for the final separation of death [11]. This situation can potentially drive partners apart and result in partners beginning to live more emotionally separate lives. It is important to reassure couples that such a reaction is initially natural but that they should attempt to redefine the balance between separateness and togetherness in the long run [11].

The present study also has limitations mostly due to its size of only 50 women with a history of different surgical treatments for vulvar cancer I-III, cervical cancer I-IIb or endometrial cancer I-III. This small number and the heterogeneity of the subjects restrict our conclusions on the specific influence of different surgical techniques for a range of cancers. The negative impact of the relative small number of participants is partially compensated by the high response-rate of 83%, taking into account the sensitivity of the subject and the personal character of the questions. Furthermore, the inclusion of an age-matched control group allowed comparisons with age-matched women not affected by treatment for cancer. The size of the study is comparable to other recently reported studies [1-6, 9, 10].

In conclusion, our results suggest that the changes in vulvar anatomy and vaginal function following pelvic surgery for gynecological cancer have deleterious effects on sexual functioning, experience and satisfaction of women. In the field of medical illness, however, there is a tendency to overestimate the importance of illness-related factors and deny the impact of other factors in the causation of sexual problems. Therefore, more longitudinal research is needed to rule out the specific impact of pelvic surgery on sexuality compared to psychological and social factors. In view of the fact that women who underwent pelvic surgery for gynecological cancer are at risk for severe sexual problems, gynecologists should offer patients and their partners realistic information before and after surgical treatment. Lack of information and support for both partners can lead to misunderstandings and conflicts in the relationship, that can however be prevented by good clinical practice that includes routinely questioning overall well-being, sexuality and the marital relation.

Acknowledgement

This research was supported through a grant from “Kom op tegen kanker”.

References


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13th Biennial Meeting of the International Gynecologic Cancer Society (IGCS 2010)

Prague, Czech Republic, European Union
October 23-26, 2010

mailto: IGCS_2010@mail.vresp.com
Intraoperative assessment of epithelial and non-epithelial ovarian tumors: a 7-year review

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Summary

Objective: To determine the accuracy of frozen section diagnosis of ovarian tumors and to discuss discrepant diagnostic cases.

Methods: 932 ovarian tumors were submitted for frozen section examination. Cases with a significant diagnostic discrepancy between the intraoperative and the final histological diagnosis were reviewed. Results: The sensitivity of frozen section diagnosis for benign, borderline and malignant epithelial tumors was 98.82%, 98.97% and 87.66% and the specificity 98.01%, 97.06% and 100%, respectively. There were 27 cases with diagnostic discrepancy. All non-teratomatous sex cord/stromal and germ cell tumors were correctly diagnosed while a diagnostic discrepancy was observed in teratomatous tumors. Conclusion: Frozen section diagnosis is a reliable method for the surgical management of an ovarian mass. Nevertheless, care should be taken for large tumors measuring > 20 cm in diameter, particularly when the intraoperative diagnosis reveals an epithelial borderline tumor or a teratomatous tumor with an extensive neural component.

Key words: Ovary; Frozen section; Intraoperative assessment.

Introduction

Accurate intraoperative diagnosis of an ovarian mass is of great importance since it defines the appropriate surgical management. Over-diagnosis might lead to unnecessary pelvic clearance and staging procedures, which would be disastrous especially when performed in young patients who want to preserve fertility. On the other hand, under-diagnosis would lead the patient, most of the time, to a second laparotomy. Unfortunately, preoperative imaging studies or serum levels of tumor markers have limited value for the recognition of ovarian cancer [1-3]. Limitations also exist when using existing diagnostic models that are used to distinguish malignant from benign masses [4].

The aim of the present study was to determine the agreement between frozen and final paraffin section diagnosis of ovarian masses in our department and to discuss the discrepant diagnostic cases.

Materials and Methods

The pathology reports of 932 ovarian lesions evaluated according to frozen and paraffin section diagnosis at the Pathology Department of “IASO” Hospital between January 2000 and October 2006 were reviewed. Age of the patients, major diameter of the tumor and tumor uni- or bilaterality was noted. Moreover, the existence of intraoperative clinical information in the patient’s medical record was indicated. Both pathologists who reported the frozen and paraffin section diagnoses were experienced in gynecological pathology. Depending on the gross and histological appearance of the lesion, one to three 4 μm sections were cut in a cryostat and stained with hematoxylin and eosin. Diagnoses of tumors were made according to the guidelines defined by Scully [5].

In all discrepant diagnostic cases, all frozen and paraffin-section pathology slides were reviewed to ascertain whether the error was the result of a misinterpretation of the pathologist, a sampling error, or an error due to other causes such as technical or lack of communication with the surgeon. Failure to specify the precise histological subtyping of a carcinoma was not considered a significant error. For the tumors with equivocal intraoperative assessment, the higher grade diagnosis was used for correlation.

Frozen section diagnoses were compared with the final paraffin section diagnoses in terms of whether it was a nonneoplastic lesion, a benign, borderline or malignant tumor, or a tumor of uncertain malignant potential.

Statistical analysis

For the purpose of the study, the final histological diagnosis was assumed to be correct. Overall accuracy was defined as the total number of agreements between the frozen section and the final diagnosis, divided by the total number of tests performed. Sensitivity, specificity and positive predictive value (PPV) and negative predictive value (NPV) of frozen section for the diagnosis of various categories of neoplasms were calculated using the standard 2 x 2 method [6]. Overall frozen section (FS) accuracy was calculated as the ratio of the total successful FS divided by the total FS performed.

Results

During the study period, 932 frozen sections were performed on ovarian or related masses. The mean age of the patients was 47.3 (range 19 to 75). On paraffin block examination, 594 of these 932 cases were benign epithe-
lial tumors or nonneoplastic lesions, while 97 were borderlined and 154 malignant primary epithelial tumors. There were also 16 tumors with uncertain biological behavior, namely granulosa cell tumors, three dysgerminomas, two yolk sac tumors, seven metastatic carcinomas and 59 teratomatous germ cell tumors.

As shown in Table 1, FS was capable of diagnosing benign and malignant primary epithelial lesions, since PPV and NPV for benign lesions was 99.15% and 97.23% and for malignant lesions 100% and 97.32%, respectively. However, in assessing borderline tumors, FS has not been proven such a powerful tool. Out of 118 FS that had been diagnosed as borderline tumors (Table 2), only 96 proved true. Fifteen cases of borderline tumors were underestimated and proved malignant. These cases were responsible for the rather unsatisfactory PPV (81.36%) of the FS regarding borderline tumors and the rather reduced but acceptable FS sensitivity regarding malignant tumors (87.66%). Furthermore, seven out of the 118 cases that were diagnosed on FS as borderline were overestimated, since on histological examination they proved to be benign tumors. These cases were expected to affect FS sensitivity regarding benign tumors. Nevertheless, when compared to the larger sample of the correctly diagnosed benign lesions, the current seven cases were a negligible portion exerting a rather insignificant effect.

Table 1. — Presentation of frozen sections of epithelial tumor cases compared to histology results.

<table>
<thead>
<tr>
<th>Frozen sections</th>
<th>Benign</th>
<th>Borderline</th>
<th>Malignant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>587</td>
<td>1</td>
<td>4</td>
<td>592</td>
</tr>
<tr>
<td>Borderline</td>
<td>7</td>
<td>96</td>
<td>15</td>
<td>118</td>
</tr>
<tr>
<td>Malignant</td>
<td>0</td>
<td>0</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>594</td>
<td>97</td>
<td>154</td>
<td>845</td>
</tr>
</tbody>
</table>

Table 2. — Frozen section of epithelial tumor cases: features analyzed by original diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>98.82</td>
<td>98.01</td>
<td>99.15</td>
<td>97.23</td>
</tr>
<tr>
<td>Borderline</td>
<td>98.97</td>
<td>97.06</td>
<td>81.36</td>
<td>99.86</td>
</tr>
<tr>
<td>Malignant</td>
<td>87.66</td>
<td>100</td>
<td>100</td>
<td>97.32</td>
</tr>
<tr>
<td>Overall</td>
<td>92.03</td>
<td>98.82</td>
<td>97.05</td>
<td>96.70</td>
</tr>
</tbody>
</table>

Comparison of FS diagnosis with final paraffin diagnosis of the 27 ovarian epithelial tumors in which there was a disagreement, revealed the following: 15 cases with an intraoperative diagnosis of borderline tumor were classified on paraffin sections as mucinous (9 cases), serous (4 cases) and endometrioid (2 cases) carcinomas. Moreover, seven more cases were later classified as mucinous (2 cases) and serous (5 cases) cystadenomas. In five cases the intraoperative diagnosis of benign tumor turned out to be a mucinous borderline tumor (1 case) and a mucinous carcinoma (4 cases). In most of the discrepant cases, the incorrect diagnosis was due to sampling error. Yet, in two cases of mucinous carcinomas that were initially diagnosed as borderline tumors, the error was due to the pathologist's underestimation. In both cases, the tumor was well differentiated with an "expansile" growth pattern. In two other cases, one serous and one endometrioid carcinoma, the error was due to the surgeon, as only a small part of the tumor was sent for FS.

Out of the seven metastatic carcinomas, two were incorrectly diagnosed on FS as primary carcinomas and were proven to be metastatic colorectal carcinomas only after the paraffin and immunohistochemical examination with the antibodies CK7 and CK20 was performed. In both cases the surgeon was aware of the existence of the primary tumor but had provided no clinical information to the pathologist. In three out of the remaining five cases, only after the frozen and paraffin examination were given as metastatic, did the clinical examination reveal the existence of two primary gastric carcinomas and one primary infiltrating lobular breast carcinoma.

All non teratomatous sex-cord/stromal and germ cell tumors were correctly diagnosed on FS, yet with an uncertainty sometimes on the exact classification of the tumor.

Out of the 59 teratomatous germ cell tumors that were sent for FS examination, there were six high-grade immature teratomas and three low-grade immature teratomas – two were harboring a follicular thyroid carcinoma, two with a carcinoid (one of which was a mucinous carcinoid), one with a tumor of epidermal appendages that recurred two years later and one with a squamous cell carcinoma. Of these tumors, only five out of the six high-grade immature teratomas and the case with the squamous cell carcinoma were correctly diagnosed on FS examination. Both the mucinous carcinoid and a mucinous tumor with mucinous ascites that rose in relation to mature cystic teratomas had been considered as mucinous borderline tumors on FS examination. All the other teratomatous germ cell tumors had been diagnosed as mature teratomas.

Benign cases comprised 149 serous cystadenomas, 72 mucinous cystadenomas, 92 endometriotic cysts, 59 mature teratomas, 38 corpus luteum cysts, 62 mesosalpingial cysts, 40 stromal tumors, 20 Brenner tumors, 10 hydrosalpinx and 40 miscellaneous cases.

Discussion

The optimal surgical performance in the treatment of an ovarian mass could be related to the accurate pre- and intraoperative evaluation. Several studies have used preoperative diagnostic models combining ultrasound findings, color Doppler tests, CA-125 measurement, age, and/or menopausal status [1-3, 7], but their diagnostic performance did not prove to be of great value.

On the other hand, intraoperative frozen section diagnosis has been established as a reliable diagnostic method with acceptable sensitivity and almost perfect specificity [8-11]. Yet, in all studies, the majority of diagnostic discrepancies were the result of over- or under-diagnosing borderline epithelial tumors.
The results of the present study, which was performed on the material from a general gynecological center with no oncological orientation, are in agreement with those of the former investigators. As shown in Table 2, the sensitivity of frozen section diagnosis for benign, borderline and malignant epithelial tumors was 98.82%, 98.97% and 87.66% and the specificity 98.01%, 97.06% and 100%, respectively. Diagnostic problems occurred mostly in mucinous and borderline epithelial tumors.

Mucinous tumors accounted for 16 out of the 27 primary epithelial tumors that were incorrectly diagnosed on frozen section. Of these, nine cases with an intraoperative diagnosis of borderline tumor were classified on paraffin examination as mucinous carcinomas and two cases as mucinous cystadenomas while in five cases the intraoperative diagnosis of a benign mucinous tumor turned out to be a mucinous borderline tumor (1 case) and mucinous carcinoma (4 cases). Moreover, it has been shown that non-epithelial tumors or metastatic carcinomas can masquerade on frozen section as primary mucinous tumors [12, 13]. In the present study, two teratomatous germ cell tumors, specifically one mucinous carcinoid and one enteric type mucinous tumor with mucinous ascites that arose on a mature cystic teratoma, were erroneously diagnosed on frozen section as mucinous borderline tumors and two metastatic colon carcinomas as primary mucinous ovarian carcinomas. Due to the high rate of discrepant diagnostic cases one has to be aware, when dealing with frozen section of a mucinous tumor, of the multiplicity of tumors with a mucinous epithelial component that can be encountered in the ovary. Moreover, it is known that nearly 80% of ovarian mucinous tumors can simultaneously have features of benign, borderline and infiltrating mucinous tumors [14]. This renders necessary the detailed gross inspection and performance of more than one frozen section.

In our study, serous (4 cases) and endometrioid (2 cases) carcinomas were underestimated on frozen section and diagnosed as borderline tumors, while five cases of serous cystadenomas were over-diagnosed on frozen section as serous borderline tumors. Under-diagnosis was due to sampling error or to a misinterpretation of the pathologist. Specifically, two of the serous carcinoma cases were of the micropapillary type and the infiltrating component occupied an area of 12 mm² and 15 mm², while one case was a clear cell carcinoma and one a serous carcinoma that was misinterpreted as borderline seromucinous tumor. In both endometrioid tumors the frozen section diagnosis was that of a borderline endometrioid adenofibroma. The misinterpretation of the pathologist was due to the presence of a dense fibrous stroma and the absence of a “confluent” growth pattern or of grade 3 cytological atypia. Since borderline endometrioid tumors are quite rare, one should be very cautious before rendering such a diagnosis. Over-diagnosis was due to tangential cutting of the frozen section or to the fact that the section for intraoperative evaluation was taken from the papillary part of the cystic tumor which ended up representing less than 10% of the whole surface of the tumor.

All mucinous, serous and endometrioid tumors that were under-diagnosed on frozen section were bulky tumors measuring more than 20 cm in diameter. This observation is in agreement with the study of Geomini et al. [15] who stated that the accuracy of frozen section diagnosis is dependent on tumor size, considering that a benign result in tumors ≥ 10 cm is less reliable than in tumors < 10 cm. Moreover, Puls et al. [16] evaluated the effect ovarian weight has on the accuracy of frozen section diagnosis. They suggested that for patients with large ovarian tumors, particularly mucinous, consideration should be given to performing staging at the time of the initial laparotomy even with a benign frozen section.

In our series, all non teratomatous sex-cord/stromal and germ cell tumors were correctly diagnosed on FS, yet with an uncertainty sometimes on the exact classification of the tumor. On the contrary, all low-grade immature teratomas and one high-grade immature teratoma were incorrectly diagnosed. On frozen section all four cases had an extensive neural component and were also bulky tumors of more than 25 cm in diameter. One would suggest the utility of multiplying the number of frozen sections when dealing with large tumors harboring an important neural component. However, the number of our cases was not large enough to allow us to make definite statements. Nevertheless, this is the first study evaluating separately the impact of frozen section diagnosis on teratomas.

The incidental finding on paraffin-section examination of a small focus of a malignant tumor arising in a mature cystic teratoma, that had not been found on frozen section, as in our cases, would not have changed the surgical procedure and as such does not seem to be of major importance.

The communication between pathologists and gynecologists is of great importance when dealing with frozen section of an ovarian tumor that might be metastatic to the ovary. Yet sometimes, the surgeon is unaware of the existence of the primary tumor. For such cases a simple rule that has been suggested by Seidman et al. [17] particularly for mucinous tumors, would be of great help. This rule classifies all bilateral mucinous carcinomas and unilateral mucinous carcinomas < 10 cm as metastatic and unilateral mucinous carcinomas ≥ 10 cm as primary and leads to the correct diagnosis in 90% of the cases. Going back to our files, all seven metastatic carcinomas that were included in our series fit the rule.

In conclusion, the results of our large series of cases confirm, as shown elsewhere, that in most cases of ovarian tumors, performing a frozen section is an accurate test. Care should be taken for large tumors, measuring > 20 cm in diameter, particularly when the intraoperative diagnosis reveals an epithelial borderline tumor or a teratomatous tumor with an extensive neural component.
References


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Incidence of multiple primary malignancies in women diagnosed with breast cancer

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Introduction

Breast cancer is the leading cause of cancer incidence and mortality in women in Italy and in many other countries [1, 2]. In Italy, an estimated 37,302 new cases of invasive breast cancer were expected in females in 2005. The number of incident cases probably increased in recent years because of the spread of the organized screening programs in many areas of Italy. Since then the effectiveness of prevention and advances in medical treatments [3, 4], has reduced the number of deaths to about 8,500 [5].

Umbria, a Region of central Italy, had a population of 457,404 women on 31-12-2007. The incidence of female breast cancer was quite low compared with data from the pool of Italian cancer registries. In Umbria the number of newly diagnosed cases every year was about 600, with 165 deaths, making female breast cancer first among the cancer causes of incidence and mortality [6]. The estimated number of prevalent cases was about 416,000 in Italy and about 7,000 in Umbria [5].

Several studies reported risks of second tumors in women with breast cancer which may be due to shared environmental, lifestyle, or genetic factors or treatment effect [7-9]. In the past more aggressive radiotherapy regimens increased the risk of the radiotherapy-associated cancers [10] and nowadays treatment with adjuvant tamoxifen seems to cause an excess of endometrial cancers [11, 12]. On the other hand there appears to be a low risk of some cancers, e.g., gastrointestinal, in women who have had breast cancer [13, 14].

This present study used incidence data from the Umbrian Population Cancer Registry (RTUP) from 1994 to 2006 to test the hypothesis that direct or inverse relationships link several later cancer sites in women who have had breast cancer.

Materials and Methods

Between January 1, 1994 and December 31, 2006, 7,840 female breast cancer (C50 ICD10) [15] patients were collected from the Umbrian Population Cancer Registry as incident cases. Twenty-four DCO cases were excluded. Over the same time-period, 332 subsequent multiple cancers in 320 patients were recorded. Subsequent breast cancers were included only if they presented a different morphology. One hundred and forty metachronous contralateral breast cancers, with the same histology, were recorded. Cases were collected, coded, stored and analyzed in accordance with the standard methods recommended for cancer registries [16]. Contralateral breast cancers with the same histology, were excluded from the calculation of incidence rates. Bladder cancers were considered malignant if not reported as non-infiltrating. One hundred and fifty-two patients presented synchronous bilateral breast cancers.

In this cohort of 7,816 breast cancer patients multiple cancers that were recorded in the RTUP archives over the same time-period were selected. Only sites that were observed in more than five cases were included in the analysis. The 45,195 person-years were calculated from the date of first breast cancer diagnosis up to date of death or follow-up on 31/12/2007. The expected number of cases was obtained from indirect standardization with regional incidence rates of several sites relative to the overall period. The significance of the observed/expected ratios (SIR) and the corresponding 95% confidence intervals were based on the Poisson distribution [17].
Results

Table 1 reports distribution by site of observed and expected multiple cancers and corresponding SIR.

Table 1. — Distribution by site of observed (Obs) and expected (Exp) multiple primary cancers, corresponding standardized incidence ratio (SIR), and 95% confidence interval.

<table>
<thead>
<tr>
<th>ICD 10</th>
<th>Site</th>
<th>Obs</th>
<th>Exp</th>
<th>SIR</th>
<th>C.I. 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C44</td>
<td>Skin non melanoma</td>
<td>68</td>
<td>66.5</td>
<td>1.02</td>
<td>0.78-1.27</td>
</tr>
<tr>
<td>C18-C21</td>
<td>Colon-rectum</td>
<td>52</td>
<td>71.5</td>
<td>0.73</td>
<td>0.53-0.92</td>
</tr>
<tr>
<td>C54</td>
<td>Uterine corpus</td>
<td>28</td>
<td>22.4</td>
<td>1.25</td>
<td>0.79-1.71</td>
</tr>
<tr>
<td>C50</td>
<td>Breast</td>
<td>20</td>
<td>105.5</td>
<td>0.19</td>
<td>0.11-0.27</td>
</tr>
<tr>
<td>C50</td>
<td>Breast + contralateral</td>
<td>160</td>
<td>105.5</td>
<td>1.52</td>
<td>1.28-1.78</td>
</tr>
<tr>
<td>C16</td>
<td>Stomach</td>
<td>16</td>
<td>32.0</td>
<td>0.50</td>
<td>0.26-0.75</td>
</tr>
<tr>
<td>C34</td>
<td>Lung</td>
<td>16</td>
<td>20.8</td>
<td>0.77</td>
<td>0.39-1.14</td>
</tr>
<tr>
<td>C43</td>
<td>Melanoma</td>
<td>16</td>
<td>7.1</td>
<td>2.24</td>
<td>1.14-3.34</td>
</tr>
<tr>
<td>C56</td>
<td>Ovary</td>
<td>16</td>
<td>15.4</td>
<td>1.04</td>
<td>0.53-1.55</td>
</tr>
<tr>
<td>C67</td>
<td>Bladder</td>
<td>15</td>
<td>15.0</td>
<td>1.00</td>
<td>0.49-1.51</td>
</tr>
<tr>
<td>C82-C85</td>
<td>n-H lymphoma</td>
<td>13</td>
<td>14.5</td>
<td>0.90</td>
<td>0.41-1.38</td>
</tr>
<tr>
<td>C64-C66</td>
<td>Kidney, ureter, urethra</td>
<td>13</td>
<td>10.4</td>
<td>1.25</td>
<td>0.57-1.93</td>
</tr>
<tr>
<td>C91-C95</td>
<td>Leukaemias</td>
<td>12</td>
<td>12.0</td>
<td>1.00</td>
<td>0.44-1.57</td>
</tr>
<tr>
<td>C71</td>
<td>Brain</td>
<td>6</td>
<td>6.5</td>
<td>0.92</td>
<td>0.18-1.66</td>
</tr>
<tr>
<td>C25</td>
<td>Pancreas</td>
<td>6</td>
<td>12.8</td>
<td>0.47</td>
<td>0.09-0.84</td>
</tr>
<tr>
<td>C73</td>
<td>Thyroid</td>
<td>6</td>
<td>7.3</td>
<td>0.82</td>
<td>0.16-1.48</td>
</tr>
<tr>
<td>All but contralateral</td>
<td>332</td>
<td>479.0</td>
<td>0.69</td>
<td>0.62-0.77</td>
<td></td>
</tr>
<tr>
<td>All but C44 &amp; contra</td>
<td>264</td>
<td>413.0</td>
<td>0.64</td>
<td>0.56-0.72</td>
<td></td>
</tr>
<tr>
<td>All with contralateral</td>
<td>472</td>
<td>466.0</td>
<td>1.01</td>
<td>0.92-1.10</td>
<td></td>
</tr>
<tr>
<td>All with contra but C44</td>
<td>404</td>
<td>413.0</td>
<td>0.98</td>
<td>0.88-1.07</td>
<td></td>
</tr>
</tbody>
</table>

Contra: contralateral.

The increase in skin melanomas (SIR = 2.24) in female survivors of breast cancer has been reported by several authors, who discussed the relationship between these cancers and BRCA2 mutations as well as CDKN2A mutation-positive patients [8, 18-21]. The excess risk due to the CDKN2A mutation should also involve pancreatic cancer [18] which, on the contrary, presented a significantly lower SIR (0.47) in our study.

Primary breast cancer with different histologies, presented a significantly low SIR (0.19) but, if we consider contralateral malignancies, the SIR rose significantly to 1.52. The risk of developing a second primary breast cancer decreases over time since the first diagnosis of breast cancer and by age at first diagnosis [22]. Our cohort presented a mean age at diagnosis of 67.6 years and the mean relative survival at five years was 87% [5]. This very low SIR can be explained and is very similar to what was observed in female patients who presented a second primary breast tumor 10-19 years after the first one (significant SIR = 0.4 in females aged 60-69 and SIR = 0.20 in females aged 70+) [22]. On the other hand, the high risk of developing contralateral cancer has been emphasized by many authors [22-24].

Our study shows a significantly reduced risk for second tumor incidence in the colon-rectum (SIR = 0.73), stomach (SIR = 0.50) or pancreas (SIR = 0.47). Pappo et al. [13] recently demonstrated that the rate of a second primary gastrointestinal malignancy in breast cancer patients was lower than in matched patients without breast cancer. Trentham-Dietz et al. [14] reported a reduced risk of colorectal cancer after breast cancer in relation to several patient characteristics. On the other hand, some authors showed a different picture, with no significant [21, 24] or significantly increased SIR [8, 9, 20, 22] for several gastrointestinal cancers.

In our study the SIR for second genitourinary cancers was very close to 1. Soerjomataram et al. [20, 26] reported an increased risk of a second ovarian cancer, but not other genital and urinary organs. In particular the results of many studies are contradictory on the incidence of ovary and endometrium second cancers [8, 9, 14, 25].

Potentially radiotherapy-associated cancer sites [9], in the region of Umbria, showed a non-significant SIR. The lower SIR related to gastrointestinal cancers (colorectal, gastric and pancreatic) and to a second breast cancer with a different histology, produced significantly low SIR for all sites except for contralateral breast cancer and skin carcinomas.

Discussion

The results of this study seem to confirm some hypotheses advanced by different authors.

One major result is the significant excess of melanoma and total second breast cancers, including contralateral.

Considering all second cancers, including metachronous contralateral breast cancer, the SIR was non-significant and very close to 1. Excluding these cases, SIRs were significantly lower than 1, whether with (SIR = 0.69, 332 obs. vs 479 exp.) or without (SIR = 0.64, 264 obs. vs 413 exp.) second carcinoma skin cancers. The SIR of all second cancers including the metachronous contralateral but excluding skin carcinomas was non-significant at 0.98.

Significantly lower risks were shown by SIR for the colorectum (SIR = 0.73, 52 vs 72), stomach (SIR = 0.50, 16 vs 32), pancreas (SIR = 0.47, 6 vs 13) and metachronous breast cancer with different histologies (SIR = 0.19, 20 vs 106). Only melanoma presented an excess risk with a significant SIR of 2.24 (16 obs. vs 7 exp.). Non-melanoma skin cancer, ovary and bladder cancer, non-Hodgkin’s lymphomas, leukemias and brain cancer presented a non-significant SIR of 1 ± 0.10; the uterus corpus and kidney, ureter and urethra cancers had non-significant SIRs of 1.25, and lung and thyroid cancers had SIRs below 1.

Conclusion

In conclusion the relationship between breast cancer and several subsequent primary cancers is still contradictory and controversial. The results of the present study seem to confirm the protective effect on gastrointestinal cancers and an excess of skin melanomas.
Acknowledgement

This work was supported by the Department of Health, Regional Government of Umbria, Italy.

References

Endometrial stromal sarcoma: experience from a district hospital and literature review

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Summary

Purpose: Endometrial stromal sarcoma (ESS) is a rare malignancy of the uterus. Most cases are incidentally diagnosed on histological examination of hysterectomy specimens. Evidence on the management is accrued mainly from retrospective studies. This study aims to review the clinical experience of a district hospital in the diagnosis and management of this rare tumour over a 12-year period.

Methods: Information on cases of ESS from 1995 to 2007 were retrieved from the histological database. All case files of identified patients were analysed and data extrapolated. A literature review was performed.

Results: There were seven cases identified over this time period highlighting the rarity of ESS. Most cases were low-grade ESS and diagnosed retrospectively following surgery for presumed benign pathology. Cases with high-grade ESS and advanced stage low-grade ESS received adjuvant therapy.

Conclusion: The primary treatment of ESS is surgery. The role of adjuvant therapy remains debatable, but generally involves radiotherapy, chemotherapy and hormonal therapy. Optimal treatment protocols may be achieved by the conduct of randomised controlled clinical trials.

Key words: Endometrial stromal sarcoma.

Introduction

Endometrial stromal sarcoma (ESS) is a rare disease with an estimated incidence of one to two per million women. It constitutes 0.2% of all gynaecological malignancies and is among the uterine sarcomas, a group of rare tumours of mesodermal origin with aggressive features and poor prognosis [1]. It is commonly staged using the 1988 modified International Federation of Gynecology and Obstetrics FIGO staging system for endometrial adenocarcinoma. Tumour grade and stage are known prognostic factors. It is a heterogeneous condition with largely unknown genetic or familial predispositions. An observational study in a cohort of 100 patients showed younger aged patients (average age 42), long-term hormonal treatment and a familial history of hormone dependent cancer as risk factors [2].

The relative rarity of this condition has resulted in limited clinical experience in identifying prognostic factors and designing appropriate therapy protocols. Its similarity in clinical presentation with benign disorders of the female genital tract may impede early diagnosis.

The clinical experience of a district hospital in the diagnosis and management of this rare tumour over a 12-year period is reviewed. In addition a literature review on its management was carried out.

Materials and Methods

Data was obtained from the histological database in our unit from 1995 to 2007. All case files of identified patients were analysed and data extrapolated. Information retrieved included age at diagnosis, prior hormonal therapy, parity, family history of cancer, clinical presentation, investigation, management, and histological description as well as postoperative therapy.

Results

During the study period seven patients were identified with ESS. Most of the patients were in their forties at diagnosis. Ages ranged from 28 to 52 years with a median age of 45 years. Three patients had a history of prior hormonal therapy (hormone replacement therapy, combined oral contraceptive pills and tamoxifen). Three women were nulliparous while four were multiparous with parity ranging from one to three. There were solitary family histories of cancer in three patients (gastric, bowel and breast cancer). Follow-up ranged from 10-153 months with a mean and median of 74 and 80 months, respectively. Clinical presentation was universally with heavy persistent vaginal bleeding in all seven cases. One of the cases had heavy postmenopausal bleeding having attained an early menopause, while another presented with acute urinary retention secondary to a pelvic mass.

In five cases uterine fibroids were diagnosed by ultrasound (US), whereas two had clinically diagnosed fibroids 16 to 20 weeks’ gestation in size. Three patients had a pre primary surgical diagnosis of ESS (one by myomectomy and two by hysteroscopy and endometrial biopsy). In the other four, ESS was diagnosed at the time of histological examination of the hysterectomy speci-
men following surgery for a presumed fibroid. To date in this series there has been 100% overall survival.

Treatment, histological and immunohistochemical outcomes and postoperative therapy and follow-up are detailed in Table 1.

### Discussion

ESS is subdivided into two separate clinical entities: low-grade ESS and high-grade ESS with a poorer clinical outcome. ESS typically presents as low-grade disease which is hormone sensitive. In this review five out of the seven patients had low-grade ESS. Reported cases suggest a progression from low-grade ESS to high-grade ESS over time (up to 25 years) [3]. Transition from low to high grade is associated with loss of hormone sensitivity, increased cellular atypia, absence of spiral arterioles and increased mitotic index [3].

The disease may present with vaginal bleeding or a pelvic mass. However, diagnosis is often not made until histological examination following hysterectomy for a presumed benign condition (fibroid, menorrhagia). This was noted in four out of the seven patients in this series in whom a postoperative diagnosis of ESS was made. Hysteroscopic findings are non specific; in this series they ranged from suspicious thickened endometrium, bulky multifibroid uterine cavity to normal appearance. Histological diagnosis can be made upon examination of specimens obtained at hysteroscopy [4, 5]. Inadvertent diagnosis has been reported following hysteroscopic resection for presumed benign pathology [6, 7]. The solitary case that had an endometrial ablative procedure had a preoperative negative endometrial biopsy. The rarity of the disease compounds the ability to make preoperative diagnosis. Various diagnostic modalities have been discussed in the literature. Gupta et al. recently described fine needle aspiration cytology of low-grade ESS [8]. Suggested preoperative US findings of a short series of ESS include a polypoid mass, an intramural mass, and an ill-defined central cavity mass or diffuse myometrial thickening [9]. These findings are non specific and may be seen in benign pathology. Comparative magnetic resonance imaging (MRI) findings of endometrial carcinoma (EC) and ESS have been described [10]. They showed irregular margins, nodular lesions at the margin, intramyometrial nodular extensions and multi-nodular mass formations in more frequent occurrence with ESS. These findings suggest that MRI may have a diagnostic role as well as an ability to differentiate ESS from EC.

Chromosomal aberrations are heterogeneous and do not clearly correlate with the histological grade. The commonest cytogenetic alteration observed in low-grade ESS is the t (7; 17) (p15; q21) translocation resulting in the fusion of the JAZF1 and JJAZ1 genes [11]. Spindle shaped cells with scant to moderate cytoplasm, round to ovoid nuclei, fine chromatin and rare mitotic figures are cytomorphological features of low-grade ESS [12]. High-grade ESS shows more cytological atypia and will to a variable degree lose the typical morphological features and may even become completely undifferentiated. Immunohistochemical studies are routinely performed to aid diagnosis [13]. ESS may show variable labelling for desmin and smooth muscle tumours by characteristic labelling for CD10. There is often no correlation between tumour grade and antigen expression.

There are limited randomised controlled trials designed to investigate the optimal treatment option for ESS. Most data are accrued from retrospective studies. Current stan-

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### Table 1. — Surgical treatment, histological and immunohistochemical outcomes, postoperative treatment and outcome.

<table>
<thead>
<tr>
<th>Surgical treatment</th>
<th>Histology</th>
<th>Immunohistochemistry</th>
<th>Postoperative therapy</th>
<th>Follow-up/outcome</th>
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<tbody>
<tr>
<td>Total abdominal hysterectomy &amp; bilateral salpingo-oophorectomy</td>
<td>High-grade ESS Stage 3C</td>
<td>Positive for vimentin, CD10, smooth muscle actin, negative for cytokeratin (MNF116), desmin, oestrogen and progesterone receptors</td>
<td>Chemo/therapy</td>
<td>10 months no recurrence</td>
</tr>
<tr>
<td>Total abdominal hysterectomy &amp; right salpingo-oophorectomy</td>
<td>Low-grade ESS Stage 1C</td>
<td>Positive for CD10 and oestrogen receptor, negative for CD34, desmin and smooth muscle actin</td>
<td>Adjuvant hormonal therapy (Zoladex)</td>
<td>14 months no recurrence</td>
</tr>
<tr>
<td>Mirena IUCD, Thermal Balloon Ablation, Vaginal hysterectomy</td>
<td>Low-grade ESS Stage 1B</td>
<td>Variable stain with desmin, smooth muscle actin, CD10, oestrogen and progesterone receptors</td>
<td>No adjuvant therapy</td>
<td>109 months no recurrence</td>
</tr>
<tr>
<td>Myomectomy, Wertheims hysterectomy</td>
<td>Low-grade ESS Stage 1C</td>
<td>Not reported</td>
<td>No adjuvant therapy</td>
<td>80 months no recurrence</td>
</tr>
<tr>
<td>Laparoscopic assisted vaginal hysterectomy &amp; bilateral salpingo-oophorectomy</td>
<td>High-grade ESS (within focus of adenomyosis) Stage 1C</td>
<td>Positive for vimentin and progesterone receptors</td>
<td>No adjuvant therapy</td>
<td>14 months recurrence, intraabdominal metastases, solitary lung metastases</td>
</tr>
<tr>
<td>Total abdominal hysterectomy</td>
<td>Low-grade ESS Stage 3</td>
<td>Not reported</td>
<td>Hormonal therapy/ radical pelvic radiotherapy</td>
<td>125 months no recurrence</td>
</tr>
</tbody>
</table>
dard treatment of ESS involves total abdominal hysterectomy and bilateral salpingo-oophorectomy. However, in Stage 1 low-grade ESS bilateral salpingo-oophorectomy does not appear to affect overall survival or recurrence interval [14]. Preservation of the ovaries may thus be considered for premenopausal women with low-grade ESS. However proponents for removal of the ovaries would suggest the benefits of removal of a potential site for metastases and a source of continued stimulatory oestrogen. The role of lymphadenectomy for low-grade disease remains uncertain. Lymphadenectomy does not appear to affect overall outcome with different studies showing varied outcomes. Riepol et al. demonstrated a 33% lymph node involvement in low-grade ESS suggesting the benefit of lymph node clearance [15]. However, a more recent retrospective study showed that leaving the lymph nodes in situ in low-grade ESS, does not alter clinical outcome [16]. Management should therefore be discussed on an individual basis within a multidisciplinary context. This would enable planning of appropriate surgery to improve outcome.

Aggressive cytoreduction with minimal residual disease is associated with improved survival in high-grade ESS [17]. Postoperative adjuvant treatment is debatable and has been evaluated by various investigators. ESS has been known to be more radiosensitive among the uterine sarcomas. Adjuvant radiotherapy following surgery has been shown to be effective due to good local disease control in all stages and good overall survival in early stages [18]. However, this benefit can not be translated to advanced disease, with the majority of recurrences and failures having a distant component [19].

There are no available data from randomised trials demonstrating improved survival following chemotherapy in ESS. Outcome following chemotherapy remains varied in the literature. Adjuvant chemotherapy may lengthen overall survival in advance-stage disease, with less impact on early-stage disease. Various regimens used traditionally include CYVADIC, DECAV,IFO, VAC and VAD [20-23].

ESS expresses steroid receptors and is thus considered to be hormone sensitive. Progestins, aromatase inhibitors and gonadotrophin releasing hormone analogues are effective hormonal alternatives to chemotherapy and radiotherapy in low-grade stromal sarcoma patients as first and second line treatment [24, 25]. As progestins are associated with side-effects, aromatase inhibitors are becoming the preferred option. The appropriate duration of hormonal treatment has not been clearly defined. Recurrence and higher risk has been identified in women receiving oestrogen replacement therapy (ERT) or tamoxifen. Thus withdrawal of ERT and tamoxifen is strongly advised in women with a history of low-grade ESS.

The follow-up period in this series ranged from 10-153 months. There was a single case of recurrence in a patient with low-grade ESS. Following adjuvant hormonal therapy there has been no further recurrence and to date there has been no recorded mortality. A lengthened overall survival is associated with early tumour stage, low myometrial invasion and low mitotic count [26]. Age, race, free resection margins at primary surgery, malignancy grade, tumour diameter and menopausal status are important prognostic factors [27, 28].

We have presented the experience of endometrial stromal sarcoma in a district hospital setting. Due to its rarity, experience in its management continues to be acquired from individual and multi-institutional studies. Primary treatment remains surgery, with or without adjuvant therapy as indicated by the pathology. Randomised controlled clinical trials would be necessary to determine optimal treatment for this rare cancer. Gynaecologists should be aware of this potential diagnosis in patients having surgery for presumed benign pathology.

References


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Serum YKL-40 levels in patients with ovarian cancer and women with BRCA1 gene mutation - comparison to CA 125 antigen


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Summary

**Purpose of investigation:** Our work was undertaken to determine the usefulness of YKL-40 as a tumor marker in patients with ovarian cancer and women with BRCA1 gene mutations. **Methods:** Our study population consisted of 111 patients. They were divided into five study groups: I - newly diagnosed ovarian cancer, II - recurrence of ovarian cancer, III - complete remission, IV - benign epithelial tumors and V - patients with BRCA1 gene mutations. YKL-40 and CA 125 were determined in patient sera. **Results:** YKL-40 in newly diagnosed ovarian cancer patients was significantly higher (181.17 n/ml) than in patients with BRCA1 gene mutation (97.74 ng/ml, \( p < 0.01 \)), women with benign epithelial cancer (57.19 ng/ml, \( p < 0.005 \)) and patients with ovarian cancer at the time of complete remission (58.12 ng/ml, \( p < 0.005 \)). Taking 124 ng/ml as a cut-off value for YKL-40 (95th percentile for healthy women) we observed higher levels in 50% of patients from group I and in 38% from group II. **Conclusions:** YKL-40 appears to demonstrate no advantage over CA 125 as a biomarker of ovarian cancer, particularly in women with early-stage tumors. More research is needed on carriers of the BRCA1 gene mutation in view of the elevated YKL-40 concentrations in this group.

**Key words:** Ovarian cancer; Biomarkers; YKL-40; CA 125; BRCA1 gene mutation.

Introduction

Ovarian cancer is notable for its highest mortality among female cancers [1] and for the fact that only 20% of cases are disclosed during its early stages (Stage I and II according to FIGO). The 5-year survival rate in women with advanced ovarian cancer is 20-25%. As many as 80-90% of patients respond to initial chemotherapy but permanent clinical remission is achieved in just 10-15% [2].

Currently, no effective screening procedure for ovarian cancer is known. Screening is undertaken in high-risk women but the sensitivity of the tests is low [3]. The CA 125 antigen attracts most interest while the search for other markers continues with the hope of improving survival through more effective screening and early detection of recurrence. In the case of ovarian cancer, recurrence reduces the chances for total recovery to almost zero [4].

Researchers working in the field of oncologic diagnostics have recently turned their attention to the YKL-40 protein belonging to the mammalian chitinase protein family. This protein, discovered in vitro more than ten years ago, is produced in significant quantities by osteosarcoma cell lines [5]. Other cell lines producing YKL-40 in vitro include glioblastoma, melanoma, colorectal, ovarian, and prostate cancers [5-7]. Immunohistochemistry disclosed the expression of this protein in biopsies of glioblastoma, breast cancer, and colorectal cancer [6, 8-10]. There are suggestions that under pathologic conditions YKL-40 participates in the degradation of the extracellular matrix and/or in angiogenesis [11]. This work was undertaken to determine the usefulness of YKL-40 as a tumor marker. We decided to measure serum concentrations of this protein in patients with ovarian cancer at three occasions: diagnosis, recurrence, and clinical remission. Our control group comprised healthy women carrying the BRCA1 gene mutation who underwent prophylactic adnexectomy done routinely at our center since many years. Postoperative histopathology was unrevealing in all carriers [12]. Our long-time interest in the BRCA1 gene is in connection with one of the largest databases in the world on carriers of the BRCA1 mutation collected at the International Hereditary Cancer Center of the Pomeranian Medical University. We have previously reported on some types of the BRCA1 mutation [12, 13] and on patterns of serum levels of several proteins in patients with breast and ovarian cancer [14-16].

Materials and Methods

**Study population**

Our study population consisted of 149 patients treated at the Department of Gynecological Surgery and Gynecological Oncology of Adults and Adolescents, Pomeranian Medical University, Zgorzelec, between January 2006 and March 2008. Patients with any form of arthritis were excluded. The following conditions qualified for enrollment:

- Initial diagnosis of epithelial ovarian cancer based on ultrasound (US) findings and levels of the CA 125 marker;
- Clinical remission or recurrence of ovarian cancer;
- BRCA1 gene mutation (prophylactic salpingo-oophorectomy performed);
Following histopathology, our population was reduced to 111 patients. We disqualified patients with endometriosis, gonadal and germinal tumors, and one patient with the BRCA1 mutation and lymph node involvement after treatment for breast cancer. Five study groups were formed:

I - Patients with newly diagnosed ovarian cancer;
II - Patients with histopathologically confirmed recurrence of ovarian cancer;
III - Patients with histopathologically confirmed clinical remission of ovarian cancer;
IV - Patients with benign epithelial tumors;
V - Patients with BRCA1 gene mutations at high risk of ovarian cancer who underwent prophylactic salpingo-oophorectomy (control group).

Fourteen patients from group V (subgroup Va) underwent measurements of YKL-40 and CA 125 levels after 3-17 months from surgery.

Histopathology and molecular biology tests were done at the Department of Genetics and Pathomorphology, Pomeranian Medical University.

Biochemical analysis

Blood was collected preoperatively (usually 1 day) in all patients and centrifuged to obtain serum which was next stored for two to three weeks at −20°C pending receipt of histopathology results.

YKL-40 levels were determined in serum using the commercially available YKL-40 ELISA Kit from Metra Biosystems (Mountain View, CA) according to the manufacturer’s protocol. Serum CA 125 levels were determined using a CA 125 immunoassay (EIA) system according to the manufacturer’s instructions (IMX, Abbott CA125, Abbott Laboratories, Chicago, IL).

Statistics

Distribution of the results deviated from normal according to the Shapiro-Wilk W test. Consequently, means were compared using the non-parametric Mann-Whitney U-test for two variables and the level of significance was taken as p < 0.05. Spearman’s rank test was applied to correlations.

Results

Patients with newly diagnosed ovarian cancer

Concentrations of YKL-40 in group I were significantly higher (181.17 ng/ml) than in controls (97.74 ng/ml; p < 0.01). No statistically significant difference was found between group II (122.38 ng/ml) and group V. For groups I and II, CA 125 antigen levels were significantly higher than for controls. Taking 124 ng/ml (95th percentile for healthy women) as the cut-off value for YKL-40 [17], we observed higher levels in 50% of patients from group I and in 38% from group II. By reducing the cut-off level to 62 ng/ml (mean + 2 SD in healthy women) [18] we observed higher YKL-40 levels in 73% of patients from group I and in 44% from group II. CA 125 levels were elevated in 90% of patients from group I and in 55% from group II.

Statistically significant correlations between CA 125 and YKL-40 concentrations were ascertained for groups I (r = 0.404) and II (r = 0.738).

Patients with ovarian cancer during clinical remission and patients with benign epithelial tumors

No significant differences were disclosed for YKL-40 and CA 125 antigen levels in groups III and IV as compared with controls. For the cut-off value of 124 ng/ml, YKL-40 was elevated in 7% of patients in group III. No elevation was disclosed in group IV. When the cut-off value was reduced to 62 ng/ml, elevated YKL-40 levels were revealed in 35% of patients in group III and in 21% of patients in group IV. CA 125 antigen (cut-off value 35 U/ml) was elevated in 15.3% of patients in group III (peak level 53.7 U/ml) and in 21% in group IV (peak concentration was 64.67 U/ml).

The correlation between serum CA 125 and YKL-40 levels in groups III and IV was significant.

Healthy patients with BRCA1 gene mutations

The preoperative mean YKL-40 concentration in women at high risk of ovarian cancer due to the BRCA1 gene mutation was 97.74 ng/ml (n = 25). After three to seven months postoperatively, the mean decreased to 63.63 ng/ml (n = 14). The decrease was not statistically significant.

In this group, YKL-40 concentrations above the cut-off value of 124 ng/ml and 62 ng/ml were found in 12% and 40% of patients, respectively.

Patients with ovarian cancer FIGO Stage I and II

The mean YKL-40 concentration in this group (n = 14) was 104.25 ng/ml and was not significantly different from controls. Concentrations above the cut-off value of 124 ng/ml and 62 ng/ml were disclosed in 21% and 50% of patients, respectively.

Discussion

The prognosis in ovarian cancer is generally poor and this fact has stimulated research into novel tumor biomarkers. Preliminary reports on YKL-40 released by several cancer cell types [3-6] appeared promising to us in terms of more effective screening. The exact function of this glycoprotein in cancer remains unknown. It has been suggested that YKL-40 participates in processes of differentiation and proliferation of malignant tumor cells, protecting them from apoptosis and stimulating angiogenesis and fibroblast growth around the tumor [19, 20]. All these mechanisms have been studied under in vitro conditions and so far their operation in vivo awaits confirmation. Unfortunately, the number of reports on YKL-40 in patients with malignant ovarian cancers is rather small [21-24].

The aim of our work was to evaluate the diagnostic usefulness of YKL-40 in ovarian cancer and in women at high risk of this cancer type due a marker mutation of the BRCA1 gene [12, 13]. In agreement with other researchers, we disclosed elevated YKL-40 in patients with ovarian cancer regardless of the clinical stage (181.17 ng/ml) (121.8 ng/ml in the report of Dupont et al.
The mean YKL-40 level in patients with Stage I and II according to FIGO was 104.25 ng/ml (Dupont et al. [12] reported 109 ng/ml). At the time of recurrence, we observed a mean of 122.38 ng/ml (Dupont et al. [18], Dehn et al. [15] 94 ug/l). Dupont et al. [18] reported levels above 62 ng/ml in 72% of ovarian cancer cases and in 65% during the early stage of the tumor. We found levels above 62 ng/ml in 73% of all ovarian cancer patients and in 50% of patients with Stage I and II. The discrepancy between the report of Dupont et al. [18] and ours relates to the CA 125 marker. Elevated CA 125 levels were observed by Dupont et al. [18] in 46% of patients with ovarian cancer and in 35% with Stage I and II according to FIGO, whereas our study revealed 90% and 71%, respectively. Dupont et al. [18] have been the only researchers so far attempting to determine the usefulness of YKL-40 for preoperative screening for ovarian cancer, while other researchers concentrated on the prognostic role of this protein [21-24]. According to Dehn et al. [2], high levels of YKL-40 accompanying recurrence (above 160 ug/l) suggest a very poor prognosis and a markedly shorter survival time. Gronlund et al. [23] reported that high YKL-40 concentrations at recurrence are associated with a lack of response to second-line chemotherapy, while Hogdall et al. [22] observed that elevated YKL-40 levels are an independent prognostic factor.

Table 1. — YKL-40 and CA 125 levels in groups I and II, III, IV, V.

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<tr>
<td>Mean</td>
<td>181.17</td>
<td>122.38</td>
<td>181.17</td>
<td>58.12</td>
<td>181.17</td>
<td>57.19</td>
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<td>Median</td>
<td>131.85</td>
<td>83.18</td>
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<td>47.25</td>
<td>131.85</td>
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<td>95% CI</td>
<td>131.65-181.27</td>
<td>NS</td>
<td>131.65-51.03</td>
<td>&lt;0.005</td>
<td>131.65-30.13-</td>
<td>&lt;0.005</td>
<td>131.65-43.14-</td>
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<td>CA 125</td>
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<tr>
<td>Mean</td>
<td>729.58</td>
<td>579.14</td>
<td>729.58</td>
<td>16.41</td>
<td>729.58</td>
<td>24.88</td>
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<tr>
<td>Median</td>
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<td>234.4</td>
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<td>95% CI</td>
<td>362.1-125.22-</td>
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<td>362.1-7.43-</td>
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<td>362.1-13.86-</td>
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<td>1097.04</td>
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Table 2. — YKL-40 and CA 125 levels in groups II and III, IV, V.

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<td>Median</td>
<td>56.45</td>
<td>12.62</td>
<td>56.45</td>
<td>18.94</td>
<td>56.45</td>
<td>13.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>125.22-1283.5</td>
<td>7.43-25.39</td>
<td>125.22-1283.5</td>
<td>1.8-65.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — YKL-40 and CA 125 levels in groups III, IV, V.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IV</td>
<td>p</td>
<td>III</td>
<td>IV</td>
<td>p</td>
<td>V</td>
<td>IV</td>
<td>V</td>
</tr>
<tr>
<td>YKL-40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>58.12</td>
<td>57.19</td>
<td>58.12</td>
<td>97.74</td>
<td>57.19</td>
<td>97.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>51.03</td>
<td>47.25</td>
<td>51.03</td>
<td>52.89</td>
<td>47.25</td>
<td>52.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>35.63-80.62</td>
<td>30.13-84.26</td>
<td>35.63-80.62</td>
<td>43.14-152.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>16.41</td>
<td>24.88</td>
<td>16.41</td>
<td>33.58</td>
<td>24.88</td>
<td>33.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>7.43-25.39</td>
<td>13.86-35.91</td>
<td>7.43-25.39</td>
<td>1.8-65.35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — YKL-40 levels between group V and subgroup Va.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>YKL-40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>97.74</td>
<td>63.63</td>
</tr>
<tr>
<td>Median</td>
<td>52.89</td>
<td>46.65</td>
</tr>
<tr>
<td>95% CI</td>
<td>43.14-152.33</td>
<td>27.39-99.88</td>
</tr>
</tbody>
</table>
gene mutation, the mean concentration of YKL-40 was 97.74 ng/ml. This value appears to be high when compared with the mean of 43 ug/l [18] in the healthy population. The mean concentration after prophylactic salpingo-oophorectomy fell to 63.64 ng/ml but the difference in comparison with the preoperative value was not significant. Dupont et al. [18] studied women at high risk of ovarian cancer but gave no information as to the presence of any mutation in this group. The mean YKL-40 level for these patients was as low as 38 ng/ml. According to our study and to other researchers, patients at high risk for ovarian cancer demonstrate markedly higher levels of this protein than in the general population. It should be remembered that YKL-40 is not produced by every cancer type of the ovary, pointing to differences in the biology of the ovarian cancer cells. For example, we found low levels of YKL-40 in clear cell carcinoma.

In summary, YKL-40 appears to demonstrate no advantage over CA 125 as a biomarker of ovarian cancer, particularly in women with early-stage tumor. More research is needed in carriers of the BRCA1 gene mutation in view of the elevated YKL-40 concentrations in this group.

References


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Endometrial carcinoma clinical management: results of a regional survey

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Department of Gynecological Sciences and Perinatology;
“La Sapienza” University of Rome, 1st Faculty of Medicine B School of Midwifery, Rome (Italy)

Summary
In Southern regions of Italy, many women affected by oncologic pathology go to referral hospitals to be treated. However there is the impression that this does not apply to endometrial cancer, which affects older women less prone to seek care far from home. To verify this premise and to ascertain the quality of treatment these patients received, and the degree of compliance of their treatment with the International Federation of Gynecology and Obstetrics (FIGO) recommendations, a database was created collecting information concerning the clinical management of endometrial cancer in 13 different gynecological units in the Campania region. We confirmed that endometrial carcinoma, the most frequent oncologic pathology in all participating units, was treated independently from the dimensions of all of these units. The adopted diagnostic and therapeutic procedures seemed to be largely compliant with FIGO guidelines. Limited dishomogeneity only regarded the smallest units. Overall, a good quality of treatment seemed to be given to women affected by this pathology who wanted to be treated in, or close to, their town of residence.

Key words: Endometrial cancer; Clinical management.

Introduction
In Southern regions of Italy, many women affected by oncologic pathology go to referral hospitals in larger towns to be treated. However it was our impression that this does not apply to endometrial cancer which affects older women less prone to seek treatment far from home. To verify this premise and to ascertain the quality of treatment these patients received, and the degree of compliance of their treatment with the International Federation of Gynecology and Obstetrics (FIGO) recommendations [1] a database was created collecting information concerning the clinical management of endometrial cancer in 13 different gynecological units in the Campania region. The present paper analyzes the collected data.

Material and Methods
All topical information concerning the clinical management of endometrial carcinoma over one year was reported by 13 gynecological units (Table 1). Few basic data were used in order to achieve maximum questionnaire fulfillment from every participating unit.

Results and Discussion
The 13 participating units, all in the Southern Campania region of Italy, differed in size and served a variable number of people (estimated on the basis of number of assisted deliveries), as well as a widely different range of surgical activity (Table 2). Endometrial carcinoma was the most frequent oncologic pathology in all participating units, with figures ranging from 36% to 57% of all treated cases. Epidemiologically, data emerging from single units showed no relation between unit size and major surgical activity: the maximum surgical activity occurred in two relatively small units (nearly 30 beds), while larger units (more than 50 beds) did not even reach 350 major gynecological operations per year. A sort of “surgical appeal” of some units seems to exist, which is more evident if the number of oncologic cases per 100,000 of estimated assisted women is compared: the mean value for all the units was 26/100,000 with a very wide range (6-87/100,000); only 5/13 units had values close to the mean. However, as expected, this did not

Table 1. — Collected data.

<table>
<thead>
<tr>
<th>Assisted population calculated on the number of assisted deliveries</th>
<th>Major gynecological surgery</th>
<th>Gynecological oncology surgery</th>
<th>Presurgical diagnostic and staging procedures</th>
<th>Intra-surgery diagnostic facility availability (e.g., frozen section histopathology, peritoneal washing cytology)</th>
<th>Surgical approach for endometrial carcinoma</th>
<th>Distribution of FIGO stages</th>
<th>Criteria mode and local availability of adjuvant therapy</th>
<th>Follow-up program</th>
</tr>
</thead>
</table>

Table 2. — Dimensions of participating gynecologic units.

<table>
<thead>
<tr>
<th>No. of beds</th>
<th>Calculated assisted population</th>
<th>Major gynecological surgery/year</th>
<th>Oncologic gynecological surgery/year</th>
<th>Endometrial carcinoma surgery/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>426</td>
<td>1,247,000</td>
<td>3,477</td>
<td>296</td>
<td>165</td>
</tr>
<tr>
<td>33</td>
<td>95.000</td>
<td>267</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>15-55</td>
<td>35.000-150.000</td>
<td>70-420</td>
<td>7-78</td>
<td>4-31</td>
</tr>
</tbody>
</table>

Revised manuscript accepted for publication March 25, 2009

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XXX, n. 6, 2009
apply to the cases of endometrial carcinoma; the mean value for all the units was 14,100,000 (range 3-32,100,000) and 11/13 units had figures close to, or greater than, the mean.

The adopted diagnostics and presurgical staging procedures in participating units are reported in Table 3. These data show homogeneity among the participating units and substantial compliance with FIGO guidelines.

Data concerning the surgical approach are shown in Table 4. Pathological FIGO stage distribution was comparable with that reported by a very large recent review (Table 5) [2]. Some dishomogeneity was expected in the surgical approach and compliance with corresponding FIGO guidelines, as observed in a recent European review [3]. In contrast, given the figure of nearly 20% FIGO stages greater than Ib, we found 40% of hysterectomies with associated lymphadenectomy. In only 4/13 units lymphadenectomy was never performed, but we stress that these units accounted for less than ten treated endometrial carcinomas per year, self-limited to FIGO Stages Ia-b. Higher FIGO staged cases (greater than Ib or Ib/G3) were performed in the remaining nine units and were treated and staged following FIGO guidelines. Positive nodes were found in 12 out of 66 lymphadenectomies performed after gross examination of the uterus revealed Stage Ic disease during surgery, or after random node frozen section, or palpable nodes. The vaginal approach was performed in a few cases, mostly due to a high degree of obesity or medical indications, while the laparoscopic approach was carried out in only 4/13 units in a limited number of cases.

Adjuvant therapy was performed in a total of 27 cases: radiotherapy in 11, radiotherapy and chemotherapy in four, and chemotherapy in 12. This seems to follow FIGO guidelines, given the observed frequency of positive nodes or high-stage cases. A relatively low number of radiochemotherapies were noted if compared with the number of chemotherapies alone; this seems mostly due to the small number (2/13) of units having locally available radiotherapeutic facilities. A follow-up program for treated patients was activated in only six out of 13 participating units, while the remaining units only recommend follow-up be done by the patient’s gynecologist.

### Conclusions

Given the frequency of endometrial carcinoma and its diffusion among elderly women who find it difficult to seek treatment in major gynecologic units far from home, we found that the clinical management was substantially correct in most of the participating units, even those minor units located in small community hospitals of the Campania region – a self-limitation of very initial cases being observed in the smallest units (less than 10 cases per year). This is in agreement with the cited FIGO guidelines: “Low risk tumors will have positive nodes in less than 5% cases (well differentiated and < 1/2 myometrial invasion) and do not require full surgical staging. These women can generally be safely operated on by a general gynecologist”.

After evaluating the reported data, we believe there should be more homogeneity in the surgical approach, especially a standardized hysterectomy with a Piver I degree of radicality, excluding those cases better treated by vaginal hysterectomy due to high-grade obesity or medical reasons. Greater homogeneity in the adjuvant therapy approach is also needed and should not be conditioned by the level of the locally available facilities. Finally all gynecologic units treating endometrial cancer cases should provide a follow-up program on an institutional basis.

| Table 3. — Routine diagnostics and staging work-up before surgery. |
|-----------------------|-----------------------|
| Total number of units adopting the procedure |
| Hysteroscopy and biopsy | 13/13 |
| Hysteroscopy and biopsy followed by dilatation and curettage | 9/13 |
| Echography | 13/13 |
| Computerized tomography | 9/13 |
| Nuclear magnetic resonance * | 7/13 |

* NMR not available in 5/13.

<p>| Table 4. — Surgical approach in participating Gynecologic Units. |</p>
<table>
<thead>
<tr>
<th>In all units</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laparotomic hysterectomy (intrafascial)</td>
<td>42.13%</td>
</tr>
<tr>
<td>Laparotomic hysterectomy (radicality Piver I)</td>
<td>47.81%</td>
</tr>
<tr>
<td>Laparotomic hysterectomy (radicality Piver II)</td>
<td>8.69%</td>
</tr>
<tr>
<td>Associated peritoneal washing cytology</td>
<td>100%</td>
</tr>
<tr>
<td>Associated lymphadenectomy</td>
<td>40%</td>
</tr>
<tr>
<td>Indicated by gross examination of the uterus during surgery, or random node frozen section or palpable nodes</td>
<td></td>
</tr>
<tr>
<td>Vaginal hysterectomy or surgical risk</td>
<td>3.7%</td>
</tr>
<tr>
<td>Indicated by high-grade obesity</td>
<td></td>
</tr>
<tr>
<td>LAH and laparoscopic lymphadenectomy</td>
<td>1.37%</td>
</tr>
<tr>
<td>Performed in only 4/13 units</td>
<td></td>
</tr>
</tbody>
</table>

| Table 5. — Pathological FIGO stage distribution. |
| In all units |
|----------------|-------|
| Ia | 26.46% |
| Ib | 53.23% |
| Ic | 13.77% |
| II | 4.54% |
| III | 1.15% |
| IV | 0.85% |
| Positive nodes (in 12 of 66 lymphadenectomies) | 18.18% |
Acknowledgement

Gratitude is expressed to Riccardo Arienzo, Chairman of the Obstetrics and Gynecology Department at SS. Annunziata Hospital in Naples, who solicited data from the 13 participating units.

References


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Comparison of paired cervical scrape and tumor tissue samples for detection of human papillomaviruses in patients with cervical cancer

N. Jančar1, B.J. Kocjan2, M. Poljak2, E. Vrtačnik Bokal1

1Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, Ljubljana
2Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana (Slovenia)

Introduction

Cervical cancer (CC) is the seventh most common cancer overall and the second most frequent cancer in women worldwide. More than 80% of CC occurs in the developing countries. In the developed countries with well established screening programs the age-standardized rate (ASR) of CC is now generally less than 14.5/100,000 women [1]. The ASR of CC in the year 2002 in Slovenia was 16.1/100,000, which is the 5th highest in Europe [2]. After the introduction of an organized national cervical cancer screening program in Slovenia in 2003, the incidence had already fallen from 21.0/100,000 to 17.6/100,000 in the year 2005 [3].

Several epidemiological, molecular and clinical studies performed in the last decade clearly showed that sexually transmitted infection with human papillomaviruses (HPV) has an important role in the development of preinvasive cervical lesions and CC [4-7]. Persistent infection with one of the 15 high-risk HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 or 82) is a necessary although not sufficient etiological factor for CC [7-9]. HPV testing has consequently become an important part of CC screening and detection algorithms in several countries. Thus, the US Food and Drug Administration (FDA) has approved a concurrent HPV and Pap smear screening of women aged 30 or more years in 2004. Additionally, several consensus guidelines recommend HPV testing in the management and triage of women with borderline cytological findings (e.g., atypical squamous cells of undetermined significance) or as a follow-up test after therapy of high-grade cervical intraepithelial lesions [10-12].

Molecular diagnostics of HPV infection can be performed in various types of samples that contain HPV infected host cells. For screening purposes, cervical scrape specimens are widely used, since the material can easily be obtained along with the material for conventional cytological testing [10]. For detection of HPV in women with preinvasive cervical lesions and CC fresh or fixated material obtained by biopsy or surgical procedure can also be used.

The distribution of HPV genotypes in cervical scrape specimens and matching biopsies in women with or without preinvasive cervical lesions has already been studied [13-18] in order to compare the detection rate of HPV and the spectrum of HPV genotypes in paired samples. The concordance between HPV genotypes detected in paired cervical scrape specimens and biopsies was between 88% and 97.5%. However, we were unable to find any study to date comparing the distribution of HPV genotypes in paired cervical scrapes and tumor tissue samples in patients with CC.

The aim of present study was to compare the distribution of HPV genotypes in paired cervical scrape samples and tumor tissue samples in patients with CC in order to assess whether cervical scrape specimens are accurate for preoperative HPV genotype determination.

Summary

Purpose of investigation: To compare the detection and distribution of HPV genotypes in paired cervical scrape samples and tumor tissue samples in patients with cervical cancer. Methods: Forty cervical scrape samples and 40 paired archival or fresh frozen tissue samples were collected from women with cervical cancer. Polymerase chain reaction with GP5+ and GP6+ primers was performed in all samples for HPV DNA detection. All GP5+/GP6+ negative samples were additionally tested using INNO-LiPA HPV Genotyping Extra Test. Results: Overall, 39/40 (97.5%) of CC samples were HPV DNA positive. HPV 16 was found in 24/40 samples, HPV 18 in 5/40 samples. A co-infection with two different HPV genotypes was identified in one cervical scrape specimen, while in tissue samples only single infections were detected. Overall agreement between paired samples was 98.75%. Conclusion: The present study has shown that cervical scrape samples are equally useful for HPV genotype determination as tumor tissue samples in patients with cervical cancer. They can be used as accurate clinical samples for detection of HPV genotype causing cervical cancer or for epidemiological molecular studies.

Key words: Human papillomavirus; HPV genotype; Cervical cancer; Cervical scrape; Tissue sample.
Materials and Methods

The women included in the present study were recruited in the year 2006 at the Advisory Board for Gynecological Oncology, which is held weekly at one of the three tertiary referral centers in Slovenia (the Department of Obstetrics and Gynecology at the University Medical Centre Ljubljana). Cervical scrapes were taken from every consecutive woman with CC until 40 samples were collected. Cervical scrape specimens were collected in 1 ml of Digene Specimen Transport Medium (Qiagen, Gaithersburg, MD) and stored at 4°C. DNA extraction was performed within five days. Informed consent was obtained from every included woman. The study was approved by the Medical Ethics Committee at the Ministry of Health of the Republic of Slovenia.

For each woman included in the present study, a formalin-fixed paraffin-embedded (FFPE) sample or fresh tissue sample was obtained at the Pathology Unit of Department of Obstetrics and Gynecology at the University Medical Centre Ljubljana, Department of Pathology at the General Hospital Celje, or Department of Pathology at the University Medical Centre Maribor. These samples were harvested by biopsy or hysterectomy. All archival samples were cut centrally at the Pathology Unit of the Department of Obstetrics and Gynecology at the University Medical Centre Ljubljana. From each sample, 3-5 tissue sections (10 μm thick) were cut and collected into tubes. The microtome blade was changed after each use. The DNA extraction was done within one hour.

Fresh tissue samples were obtained at the Department of Obstetrics and Gynecology at the University Medical Centre Ljubljana by hysterectomy. A small representative sample was taken from the tumor tissue before fixation of the whole pathological specimen in 4% buffered formalin. Samples were then kept frozen at -70°C until analysis.

Year of birth, place of residence, patient age at the time of diagnosis, histological type with differentiation grade, FIGO stage [19], and type of diagnostic and surgical procedure (when appropriate) were retrieved from the Cancer Registry of Slovenia or from medical records for each included woman.

DNA was extracted from clinical samples using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany), following the manufacturer’s instructions. Extracted DNA was stored at -20°C until molecular analysis. DNA concentrations were estimated by spectrophotometric analysis at 260 nm using a spectrophotometer (Biophotometer, Eppedorf, Berlin, Germany). The quality of each DNA sample was verified by PCR amplification of a 268-bp fragment of beta-globin gene [20], on the real-time PCR instrument LightCycler® v1.5 (Roche Diagnostics, Mannheim, Germany) using LightCycler® FastStart DNA Master SYBR Green I kit (Roche Diagnostics) and PC04/GH20 primers. Successful and specific amplification of the beta-globin gene fragment indicated that the DNA sample was adequate for HPV DNA analysis and that no PCR inhibitors were present.

For detection of alpha-HPV, PCR amplification was performed on all samples using HotStarTaq® Plus DNA Polymerase kit (Qiagen) and consensus GP5+ and GP6+ primers [21], targeting approximately 150-bp fragments of the HPV L1 gene, as described previously [22]. Up to 200 ng of the extracted DNA was used for PCR in a 50 μl reaction volume. All GP5+/GP6+ PCR-negative samples were additionally tested using the commercially available assay INNO-LiPA HPV Genotyping Extra Test (Innogenetics, Gent, Belgium), capable of recognizing 27 different alpha-HPV genotypes, as described previously [23].

The HPV genotypes present in CC samples were determined by direct sequencing of the GP5+/GP6+ PCR products with the same primers as those used for PCR, as described previously [24]. All samples, in which more than one HPV genotype was initially suspected from GP5+/GP6+ sequencing, were tested additionally with the INNO-LiPA HPV Genotyping Extra Test.

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to test for a normal distribution of numeric variables. Analysis of variance, Pearson’s χ2 test and logistic regression analysis were used when appropriate. Differences were considered significant when p values were < 0.05.

Results

Forty cervical scrape specimens obtained from women with CC and 40 paired CC tissue samples (35 FFPE samples and 5 fresh frozen tissue samples) were included in the present study. Mean age of included women was 45.15 ± 11.31 years (range 22 to 77 years). Most carcinomas were squamous cell carcinomas (34/40; 85%), most carcinomas were moderately differentiated (20/40; 50%) and most carcinomas were FIGO Stage IB (30/40; 75%) (Table 1).

Table 1. — Sample characteristics.

<table>
<thead>
<tr>
<th>Age at diagnosis (years) (n = 40)</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 40</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>41 - 60</td>
<td>25 (62.5)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>2 (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histological diagnosis (n = 40)</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>34 (85)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>2 (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade of differentiation (n = 40)</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 - well differentiated</td>
<td>8 (20)</td>
</tr>
<tr>
<td>Grade 2 - moderately differentiated</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Grade 3 - poorly differentiated</td>
<td>9 (22.5)</td>
</tr>
<tr>
<td>Not known</td>
<td>3 (7.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FIGO stage (n = 40)</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>4 (10)</td>
</tr>
<tr>
<td>IB</td>
<td>30 (75)</td>
</tr>
<tr>
<td>IIA</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>IIB</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>III</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (2.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of pathological specimen (n = 40)</th>
<th>Number of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>10 (25)</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>30 (75)</td>
</tr>
</tbody>
</table>

The 268-bp fragment of beta-globin gene was amplified successfully from all 80 samples. HPV DNA was detected using GP5+/GP6+ primers in 78/80 samples (97.5%). Two initially HPV DNA negative samples — paired samples of adenosquamous carcinoma — were additionally tested for the presence of HPV DNA using the INNO-LiPA HPV Genotyping Extra Test, but remained HPV DNA negative.
Table 2. — HPV genotypes in paired cervical scrape and tumor tissue samples in patients with cervical cancer.

<table>
<thead>
<tr>
<th>HPV genotype</th>
<th>Cervical scrape</th>
<th>Tissue sample</th>
<th>Total percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>24</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>5</td>
<td>11.25</td>
</tr>
<tr>
<td>18+51</td>
<td>1</td>
<td>0</td>
<td>1.25</td>
</tr>
<tr>
<td>31</td>
<td>4</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>33</td>
<td>3</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>51</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>45</td>
<td>1</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>HPV negative</td>
<td>1</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Overall, 78/80 CC samples were HPV positive (97.5%). HPV 16 was a predominating HPV genotype, followed by HPV 18 (Table 2). Direct sequencing of GP5+/GP6+ PCR products revealed the presence of a single HPV genotype in 77/78 HPV DNA positive samples (98.7%). In one sample, a co-infection with at least two different HPV genotypes was detected and was verified additionally by INNO-LiPA HPV Genotyping Extra Test. The distribution of HPV genotypes in paired CC samples is shown in Table 2.

There was a 100% agreement between 38 pairs of samples where a single HPV genotype was detected. In one cervical scrape sample, HPV 51 was found along with HPV 18, which was the only HPV genotype found in paired FFPE sample (also confirmed by the INNO-LiPA HPV Genotyping Extra Test) (Table 2). Since both genotypes belong to the high-risk group, each was given a relative proportion of 0.5 in this case; overall agreement was thus 98.75%.

Discussion

The aim of the present study was to investigate whether HPV genotypes found in cervical scrape specimens of patients with CC are consistent with HPV genotypes found in tumor tissue samples from the same patients.

Studies comparing the distribution of HPV genotypes in paired cervical scrape and biopsy specimens have already been performed using in situ hybridization [13, 14], antigen detection [15] and PCR [16-18], but none of them have focused on patients with CC. Therefore, it remained unclear how well cervical scrape samples obtained from patients with CC represented the actual HPV status of the patient’s cervical region and to what extent they included HPVs from other cervical regions, if they are present. Cervical scrape samples in the present study were taken by gynecologists from visible tumor on the cervix or from the part that was suspicious; adjacent parts of the cervix might also have been scraped. Tissue samples were obtained from visible tumor tissue or from FFPE blocks in which tumor tissue was present according to the pathologist’s report. In the present study, we found a 98.75% agreement between HPV genotypes present in cervical scrape samples and paired tissue samples, which is better than in previous studies [17, 18] investigating paired samples of women with low- and high-grade cervical lesions.

It has already been reported [17, 18] that infections with multiple HPV genotypes are more frequently detected in cervical scrapes or exfoliated cell samples than in tissue samples. Similarly, in the present study, we did not find any multiple HPV infections in CC tissue samples and only one CC scrape sample contained more than one HPV genotype. We believe that the additional HPV genotype (HPV 51) found in one cervical scrape specimen represents transitional infection and is not involved in the etiology of CC, as only HPV 18 was found in a paired tumor tissue sample from the same patient. Alternatively, an additional HPV genotype in a cervical scrape specimen may stem from parts of the cervix surrounding the tumor. In previous studies, the prevalence of multiple HPV infections in women with CC ranged from 7.83% [25] to 22% [26]. The 1.3% prevalence of multiple HPV infections determined in the present study might be to some extent attributed to direct sequencing of HPV PCR products, which is known to detect mainly the predominant HPV genotype present in a particular sample [23, 27].

The HPV genotype distribution in CC on a worldwide perspective has already been published [25, 28]. HPV 16, 18, 45, 31, 33, 52, 58 and 35 were identified as eight predominant genotypes in decreasing order of frequency in the first meta-analysis [25], while HPV 16, 18, 33, 45, 31, 58, 52 and 35 were found as predominant genotypes in the most recent meta-analysis update [28]. Interestingly, even on a relatively small number of CC samples, we found a similar distribution of HPV genotypes in similar order of frequency. The only exception was HPV 51, which ranked as 12th in a previous report [25], and was in the present study found in three CC cases (7.5%). HPV 16 and HPV 18 were responsible for 71.9% of CC in the present study, which is also in accordance with the published data [25, 28].

In conclusion, the present study has shown that cervical scrape samples are equally useful in HPV genotype determination as tumor tissue samples in women with CC. Since cervical scrape samples are relatively easy to obtain, they can be used as accurate preoperative clinical samples for exact determination of HPV genotype causing cervical cancer or for epidemiological molecular studies.

Acknowledgements

The authors thank Jasna Šinkovec, Simona Šramek-Zatler and Rajko Kavalar for providing archival cervical cancer samples. The authors also thank Joža Škof and the staff of the Pathology Unit of the Department of Obstetrics and Gynecology at the University Medical Centre Ljubljana for their excellent technical assistance.

References


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Assessment of serum paraoxonase and arylesterase activity in patients with endometrial cancer

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Summary

Purpose of investigation: Serum paraoxonase (PON 1) is one of the most important enzymatic antioxidants that hydrolyzes lipid peroxidation, an indicator of carcinogenic activity. The aim of this study was to compare the serum levels of paraoxonase and arylesterase activity in patients with endometrial cancer to those of healthy controls. Methods: Serum paraoxonase and arylesterase activities, total free sulphhydryl (-SH) groups and lipid hydroperoxide (LOOH) levels were measured in patients with endometrial cancer (n = 20) and controls (n = 23). Results: Serum paraoxonase, arylesterase activities and total –SH group levels were significantly lower in patients compared to controls (p < 0.05, p < 0.05 and p < 0.001; respectively), while LOOH levels were significantly higher (p < 0.001). Among patients, serum paraoxonase and arylesterase activities were inversely correlated with LOOH levels (r = -0.680, p < 0.05; r = -0.708, p < 0.001; respectively), while these were positively correlated with the total –SH group (r = 0.526, p < 0.05; r = 0.508, p < 0.05; respectively). Conclusion: Reduced serum PON 1 activity might contribute to an impaired antioxidant defense system which plays a critical role in carcinogenesis in patients with endometrial cancer.

Key words: Arylesterase; Endometrial cancer; Lipid peroxidation; Paraoxonase.

Introduction

Endometrial cancer is one of the most common gynecologic cancers. According to the American Cancer Society, there was an estimated 40,100 new cases and 7,470 deaths due to uterine corpus cancer in 2008 [1]. Unlike the treatment advances that have been made for other cancers, similar progress has not been achieved for endometrial cancer. In fact, the death rate associated with endometrial cancer appears to be rising. Further investigation into the pathologic mechanisms at play is critical. Oxidative stress has a crucial role in the pathophysiological mechanisms of some diseases, including miscellaneous cancers, cardiovascular diseases and aging. When organisms are exposed to oxidative stress, proteins and lipids, especially low-density lipoprotein (LDL), are subject to oxidation. Nonetheless, the human organism has a strong antioxidant system with a variety of mechanisms that protect against oxidative products. The cytoprotective and antioxidant properties of high-density lipoproteins (HDL) that protect against LDL oxidation, inhibit the toxic signaling, and protect the vascular cell, are well known [2, 3]. It is commonly accepted that the antioxidant features of HDL depend on HDL-associated enzymes [4]. One of the most important HDL-associated enzymes is paraoxonase-1 (PON 1) which is located on circulating HDL. PON 1 can break down oxidized LDL into non-harmful products [5-7]. PON1 is a HDL-associated enzyme with three activities: paraoxonase, arylesterase and dyazoxonase [8]. Originally PON 1 was thought to only break down organophosphates, chemicals that are used as insecticides and nerve gases, but it is now believed to also protect against LDL oxidation [9]. Prior studies have assessed serum PON 1 activity in cardiovascular diseases [10, 11] and various other diseases [12, 13]. To date, only a few studies exist that have reported an association with cancer [14-16]. The aim of this study was to investigate paraoxonase and arylesterase levels in patients with endometrial cancer compared to healthy controls, to determine their role in pathogenesis and prognosis of disease.

Materials and Methods

Subjects

Between July 1, 2007 and March 31, 2008, a total of 20 subjects with endometrial cancer and 23 healthy controls were enrolled in the study. The study was conducted at the following institutions: Afyonkarahisar Kocatepe University Medical School, Department of Obstetrics and Gynecology, Afyonkarahisar; Harran University Medical School, Department of Obstetrics and Gynecology and Biochemistry, Sanliurfa; and Izmir Aegean Maternity and Women’s Health Hospital, Izmir, Turkey. The diagnosis of endometrial cancer was based on histopathologic findings. The FIGO surgical staging system was used for staging and histologic subtyping followed the World Health Organization classification. Study procedures were explained to each participant and written consent was obtained from all women. The study protocol conforms to the principles of the Helsinki Declaration and was approved by the Medical Ethics Committee of Harran University.
Blood sample collection

Blood samples were obtained in the morning from the cubital vein after an overnight fast preoperatively. Samples were drawn from the cubital vein into blood tubes and immediately separated from the cells by centrifugation at 3000 x g for 10 min, stored on ice at -80°C, then analyzed.

Measurements of paraoxonase and arylesterase activity

Paraoxonase and arylesterase activity was measured using commercially available kits (Relassay, Turkey). The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorption at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290 M⁻¹ cm⁻¹ [17]. Paraoxonase activity was expressed as U/l serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310 M⁻¹ cm⁻¹.

Measurement of lipid hydroperoxide (LOOH) levels and total free sulfhydryl (-SH) groups

Serum LOOH levels were measured by the ferrous ion oxidation-xylene orange (FOX-2) method. The principle of the assay depends on the oxidation of ferrous ion to ferric ion via various oxidants and the produced ferric ion is measured with xylene orange. LOOHs are reduced by triphenyl phosphate (TPP), which is a specific reductant for lipids. The difference between with and without TPP pretreatment gives LOOH levels [18]. -SH groups of serum samples were assayed according to the method of Elman as modified by Hu et al. Briefly, 1 ml of buffer containing 0.1 M tris, 10 mM EDTA, pH 8.2, and 50 μl serum was added to cuvettes, followed by 50 μl 10 mM DTNB in methanol. Blanks were run for each sample as a test, but there was no DTNB in the methanol. Following incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer. Sample and reagent blanks were subtracted. The concentration of sulfhydryl groups was calculated using reduced glutathione as free sulfhydryl group standard and the result was expressed as millimolars [19].

Measurement of other biochemical parameters

The levels of triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were determined using commercially available assay kits (Abbott®, IL, USA) with Abbott Aeroset auto-analyzer (Abbott, IL, USA).

Statistical analysis

All analyses were conducted using SPSS 11.5 (SPSS for Windows 11.5, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD). Parameter comparisons were performed using the Mann-Whitney U test. Normality of distribution was evaluated with the Kolmogorov-Smirnov test. Spearman’s correlation test was used for group comparison. A p value < 0.05 was considered significant.

Results

The demographic and biochemical data are summarized in Table 1. There were no significant differences between patients and controls in connection with age and body mass index (BMI). However, as was expected, patients with endometrial cancer had lower parity values than controls (p < 0.001).

There were no significant differences between serum LDL-C and TG levels of patients compared to controls, while TC and HDL-C levels were significantly lower in patients compared to controls (p < 0.05 and p < 0.001, respectively) (Table 1).

Seventeen out of 20 patients were categorized as FIGO Stage I and 3 were categorized as Stage II. All the patients were endometrioid adenocarcinoma.

Parafoxonase and arylesterase activities, LOOH levels and total -SH group levels in patients and controls are shown in Table 2. Serum paraoxonase and arylesterase activities and total -SH group levels were significantly lower in patients than controls (p < 0.05, p < 0.05, and p < 0.001, respectively), while LOOH levels were significantly higher (p < 0.001).

Among patients, serum paraoxonase and arylesterase activities were inversely correlated with LOOH levels (r = -0.680, p < 0.05; r = -0.708, p < 0.001, respectively), while these were positively correlated with total -SH group (r = 0.526, p < 0.05; r = 0.508, p < 0.05, respectively). In addition serum paraoxonase and arylesterase activities were inversely correlated with LDL-C (r = -0.502, p < 0.05; r = -0.527, p < 0.05, respectively), while these were positively correlated with HDL-C (r = 0.626, p < 0.05; r = 0.744, p < 0.001, respectively). There were no correlation found between serum TG levels and serum paraoxonase and arylesterase activities (Table 3).

Discussion

Oxygen-free radicals, also known as reactive oxygen species (ROS), are chemical species containing two unpaired electrons. They are well known for both their

| Table 1. — Demographic and biochemical data in patients and controls. |
|-----------------------------|-----------------------------|-----------------------------|
| Parameters                  | Patients (n = 20)           | Controls (n = 23)            | p value    |
| Age (yrs)                   | 54.5 ± 7.4                  | 51.4 ± 7.7                  | 0.176      |
| BMI (kg/m²)                 | 29.5 ± 5.8                  | 30.6 ± 5.4                  | 0.715      |
| Parity                      | 3.9 ± 2.2                   | 6.7 ± 1.9                   | < 0.001    |
| TC (mg/dl)                  | 170 ± 26                    | 201 ± 39                    | 0.012      |
| LDL-C (mg/dl)               | 107 ± 30                    | 117 ± 33                    | 0.201      |
| HDL-C (mg/dl)               | 35.1 ± 7.9                  | 54.1 ± 13.2                 | < 0.001    |
| TG (mg/dl)                  | 141 ± 62                    | 149 ± 81                    | 0.855      |
| Parity                      | 3.9 ± 2.2                   | 6.7 ± 1.9                   | < 0.001    |
| TC (mg/dl)                  | 170 ± 26                    | 201 ± 39                    | 0.012      |
| LDL-C (mg/dl)               | 107 ± 30                    | 117 ± 33                    | 0.201      |
| HDL-C (mg/dl)               | 35.1 ± 7.9                  | 54.1 ± 13.2                 | < 0.001    |
| TG (mg/dl)                  | 141 ± 62                    | 149 ± 81                    | 0.855      |

Table 2. — Parafoxonase, arylesterase, lipid hydroperoxide and total free sulphhydryl group activity in patients and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients (n = 20)</th>
<th>Controls (n = 23)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>Patients (n = 20)</td>
<td>Controls (n = 23)</td>
<td>p value</td>
</tr>
<tr>
<td>Paraoxonase (U/l)</td>
<td>169 ± 85</td>
<td>235 ± 88</td>
<td>0.024</td>
</tr>
<tr>
<td>Arylesterase (kU/l)</td>
<td>152 ± 33</td>
<td>182 ± 35</td>
<td>0.006</td>
</tr>
<tr>
<td>LOOH (μmol H₂O₂ Eqv/l)</td>
<td>10.3 ± 2.3</td>
<td>6.5 ± 1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>-SH (mmol/l)</td>
<td>0.22 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

LOOH: Lipid hydroperoxide, SH: Total free sulphhydryl groups. Values are mean ± SD.
deleterious and beneficial effects. In healthy individuals, ROS are formed continuously at low concentrations as a result of internal reactions as well as external factors, and some ROS have important physiological functions [20]. Antioxidant defense systems can provide protection from the harmful effects of ROS. However, if these systems are insufficient for protection, there can be severe metabolic malfunctions and oxidative damage to DNA, which experimental studies in animals and in vitro have suggested are an important factor in carcinogenesis [21, 22].

Impaired enzymic antioxidant activities have long been associated with the development of cancer. Multiple studies have shown significantly higher levels of various antioxidant molecules with certain cancers, which may indicate the activation of oxidant-antioxidant systems [23, 24]. On the other hand, studies that investigated lipid peroxidation and the antioxidant status of cancers have found significantly increased lipid peroxidation and significantly decreased antioxidant enzyme activity (i.e., superoxide dismutase, catalase, glutathione peroxidase) in the erythrocytes and tissue of endometrial or cervical cancer patients [25-27]. It has been suggested that the lessened activity of antioxidant enzymes in gynecological cancers could be a result of disturbed redox status, while elevated lipid peroxidation seems to be a consequence of the disease rather than its cause [27]. Similarly, in our study, we observed that one lipid peroxidation end product, LOOH, was elevated in patients. Serum PON 1 is one of the most important enzymatic antioxidants which hydrolyzes lipid peroxidation, indicating carcinogenic activity. However, serum PON 1 activity is highly variable, and its regulation is complex. In fact, most studies report reduced PON 1 activity in several groups of patients, including those with atherosclerosis or other medical conditions [10-13]. A small number of studies have assessed PON 1 activity in cancer patients. Akcay et al. found that patients with pancreatic and gastric cancer had lower PON and HDL levels compared to those of healthy individuals [14, 15]. Similarly, Elkiran et al. found that serum PON 1 activity was significantly lower in patients with lung cancer, irrespective of metastatic status and cigarette smoking [16]. In our recent study, we found significantly lower paraoxonase and arylesterase activities in patients with epithelial ovarian cancer [28]. Similarly, in the present study, we found that patients with endometrial cancer had significantly lower paraoxonase and arylesterase activities and total-SH group levels, but significantly higher LOOH levels than healthy controls. To the best of our knowledge this is the first study that has assessed paraoxonase and arylesterase activity in patients with endometrial cancer and can therefore contribute greatly to the knowledge of the activity of these enzymes in cancer patients.

In conclusion, despite the fact that the small study population of patients makes it difficult to reach a definite conclusion, these results suggest that lowered activity of paraoxonase and arylesterase activity could be related to an impaired antioxidant defense system, and thus the physiopathology of endometrial cancer. However, larger prospective studies are needed in this area to better elucidate the relationship between PON 1 and endometrial cancer.

Table 3. — Relationships among paraoxonase and arylesterase activity, lipid hydroperoxide and total free sulphhydryl groups, HDL-C, LDL-C and TG levels in patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>SH</th>
<th>LOOH</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.510</td>
<td>0.526</td>
<td>-0.680</td>
<td>0.626</td>
<td>-0.502</td>
</tr>
<tr>
<td>p</td>
<td>0.022</td>
<td>0.017</td>
<td>0.001</td>
<td>0.003</td>
<td>0.024</td>
</tr>
<tr>
<td>Arylesterase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.508</td>
<td>-0.708</td>
<td>0.744</td>
<td>-0.527</td>
<td>-0.011</td>
</tr>
<tr>
<td>p</td>
<td>0.022</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.017</td>
<td>0.962</td>
</tr>
<tr>
<td>-SH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.594</td>
<td>0.466</td>
<td>-0.249</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.006</td>
<td>0.038</td>
<td>0.290</td>
<td>0.037</td>
<td></td>
</tr>
<tr>
<td>LOOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.638</td>
<td>0.371</td>
<td>-0.055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.002</td>
<td>0.017</td>
<td>0.107</td>
<td>0.518</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.399</td>
<td>-0.044</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.081</td>
<td>0.054</td>
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<tr>
<td>LDL-C</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>r</td>
<td>-0.303</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.195</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

-SH: total free sulphhydryl groups, LOOH: lipid hydroperoxide, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, TG: triglyceride.
Brain metastases from epithelial ovarian cancer - report of cases

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Summary
Epithelial ovarian cancer is one of the gynecological malignancies most commonly diagnosed late and one of the principal causes of mortality among women. The majority of women present with advanced disease. However, 5-year survival of patients with ovarian cancer has improved in recent years. Brain metastases from epithelial ovarian cancer are rare but in the last few years the incidence of brain complications seems to be increasing. Among all patients registered as having epithelial ovarian cancer at the Department of Oncology, Division of Gynecological Oncology, Poznan University of Medical Sciences, Poland between August 1998 and March 2008, four patients (4/669) who developed central nervous system (CNS) metastases were identified. Patients with symptoms of the CNS were evaluated by a neurologist, with a CT scan of the brain. The most common symptom of brain metastases are headaches which occur in 40-50% of patients. Because of the rarity of these patients, the optimal treatment for brain metastases is ill-defined. Brain metastasis usually appears with a poor prognosis, however early diagnosis and aggressive multimodal treatment can improve the quality of life in patients.

Key words: Ovarian cancer; Central nervous system metastasis; Chemotherapy; Radiotherapy.

Introduction
Epithelial ovarian cancer is one of the gynecological malignancies most commonly diagnosed late and one of the principal causes of mortality among women [1]. In 2004 in Poland epithelial ovarian cancer was the sixth most common malignancy among women and the fourth most common cause of death in women with gynecological malignancies. In 2004 there were 3,264 morbidity and 2,273 mortalities reported. The incidence of morbidity was estimated to be 10.93 per 100,000 women [2]. The majority of women present with advanced stage (FIGO III to IV) disease. However, 5-year survival of patients with ovarian cancer has improved in recent years. This increased survival is due to better public awareness, better detection of the tumor, cytoreductive surgery, and taxanes and platinum-based chemotherapy.

Brain metastases from epithelial ovarian cancer are rare. Before 1968 central nervous system (CNS) metastases from ovarian cancer were not reported in the medical literature. In recent years the incidence of brain complications seem to be increasing [3].

Materials and Methods
A retrospective review of all patients with ovarian carcinoma between August 1999 and March 2008 was performed by the Department of Oncology, Division of Gynecological Oncology of Poznan University of Medical Sciences, Poland. Four cases of brain metastases (0.6%) out of the 669 patients treated for epithelial ovarian carcinoma in our centre were noted.

Cytoreductive surgery
All four patients had to undergo staging laparotomy and tumor reduction surgery. Three of the patients had to undergo a total hysterectomy, bilateral salpingo-oophorectomy and omentectomy. There was no residual disease. During the operation two of the three patients had abdominal fluid of more than 1000 ml (two patients with FIGO IC and one patient with FIGO IIIC). The fourth patient had to undergo bilateral salpingo-oophorectomy and omentectomy and had residual disease above 5 cm. This patient had 6000 ml of abdominal fluid during the operation (FIGO IV - metastases to the lungs).

Histopathology of the primary tumor was adenocarcinoma serosum G3.

Adjuvant chemotherapy regimen
Three patients received paclitaxel and carboplatin/cisplatin-based chemotherapy (six courses) before developing CNS metastases. One of the patient received six courses of PC (cisplatin and cyclophosphamid).

Three patients who underwent radical cytoreductive surgery after chemotherapy were found to have pathologic complete response. The fourth patient underwent second-look surgery and was found to be in partial remission.

Diagnosis of cerebral metastasis
Patients with symptoms relating to disorders of the CNS were evaluated by a neurologist, with a computed tomography (CT) scan of the brain. The most common symptoms associated with CNS metastases were dizziness, headaches, motor weakness of the upper limbs, vomiting, visual disturbances (double vision), and dry eye syndrome.

The cerebellum was the most common site of metastasis (three patients), followed by the frontal hemisphere (one patient). Median time between the initial diagnosis of ovarian carcinoma and brain metastases varied from one year (1 patient), to three years (2 patients), to 12 years (1 patient).

Revised manuscript accepted for publication January 7, 2009
Three of the patients received palliative radiotherapy alone 60Co (30Gy/T), while the patient with frontal lobe metastasis was treated by craniotomy, which was not followed by whole brain radiotherapy.

The patient who had a partial remission after the operation and first-line chemotherapy died 45 months after the primary diagnosis of ovarian carcinoma. The other patients treated by complete surgical resection followed by chemotherapy are still alive, receiving further chemotherapy.

Discussion

Among all patients registered as having epithelial ovarian cancer at our Department between August 1998 and March 2008, four patients (4/669) who developed CNS metastases were identified. The incidence in our series was 0.6% and in the literature the incidence of brain metastases in patients with ovarian cancer varies from 0.29-6% [1, 4-6]. The cerebral hemisphere is the most common site of metastasis (the parietal lobe, the frontal lobe and the temporal lobe) followed by the cerebellum. The falk cerebri and spinal cord have less frequent occurrences of metastasis.

Brain metastases can be demonstrated with specific or generalized symptoms. The most common symptom are headaches which occur in 40-50% of patients with brain metastases. Headaches are most common in patients with multiple metastases or local metastases in the posterior fossa. Headaches are probably caused by increased intracranial pressure. It may also be connected with visual disturbances, vomiting, nausea, and the episodes of syncope and epilepsy [1].

In the literature brain metastases in ovarian carcinoma are uncommon. Due to this fact it is difficult to find a correlation between the probability of development of brain metastasis and prognostic factors of ovarian carcinoma [7]. An increased incidence of CNS metastasis from ovarian carcinoma is probably associated with prolonged survival achieved by use of cytoreductive surgery and aggressive chemotherapy. It is also connected to better accessibility of high-resolution imaging technology [1, 7].

Ovarian cancer usually spreads by lymphatic dissemination. It has been suggested that probability of developing of brain metastasis is increased in patients with disseminated disease (beyond the peritoneum) [7, 8]. Chemotherapy used in ovarian cancer may cross the blood-brain barrier poorly so we suggest that it increases the probability for brain metastases [1].

Two of our four patients had FIGO Stage III and IV cancers at the initial diagnosis. In the literature 75% of all brain metastases are connected with disseminated disease [1, 6]. LeRoux et al. [9] noted that the interval between the initial diagnosis of ovarian cancer and the diagnosis of brain metastasis was five times shorter in FIGO Stages III and IV than in FIGO Stages I and II. Brain metastasis in FIGO Stage I are uncommon as ovarian cancer is usually diagnosed in the disseminated stage. However, in our group two of the four patients (50%) had brain metastases diagnosed in FIGO Stage IC.

Kolomainen et al. [4] noted that in 50% of all patients the CNS was the only site of disease and brain metastasis of ovarian cancer.

According to the literature the diagnosis of brain metastasis is routinely made on high-resolution imaging technology such as CT scan and MRI. In our department CT scans were used to diagnose brain metastases.

Tay and Rajesh [6] in their literature review reported that brain metastases were single in 46% and multiple in 54%. In their series 75% of metastases were multiple. This is contrary to our observation as all our patients were diagnosed with single (5-45 mm) brain metastasis.

Brain metastasis usually appears with a poor prognosis, however early diagnosis and aggressive multimodal treatment can improve the quality of life in patients [1, 7].

Because of the rarity of these patients, the optimal treatment for brain metastases is ill-defined. The therapeutic options take into account the number and location of the metastases, the presence or absence of extracranial disease, previous treatment, and performance status [1]. Therapeutic options for brain metastasis include: surgery, radiotherapy and chemotherapy. Radiotherapy remains the most common treatment for brain metastasis. Some authors [1, 10] reported that it may be possible to use carboplatin, particularly in patients who have had a previously documented response to platinum-based chemotherapy. Melichar et al. showed that cisplatin and gemcitabine are the most effective in patients with brain metastasis [11]. Watanabe et al. [12] showed complete remission after treatment with docetaxel and carboplatin. In the literature there are no reports of using paclitaxel for the treatment of brain metastases [1].

Rodriguez et al. [3] noted that patients who were treated with systemic chemotherapy and radiotherapy or radiotherapy and surgery or all three methods had improved survival compared with patients who were treated with whole-brain radiotherapy alone. People who underwent surgical resection of brain metastases followed by whole-brain radiotherapy usually had better quality of life and survival rates above 12 months [1, 3, 13]. Multiple brain metastases cannot be treated with surgery but several recent studies showed that treatment with a gamma knife can be an alternative treatment for patients with multiple metastases. According to Anupol et al. [14] patients who underwent surgical resection with gamma knife had a survival rate of around 27 months. Stereotactic radiosurgery can also be an alternative approach in which high doses of focused radiation are delivered to the brain metastases [1].

In our group, three of the patients received palliative radiotherapy and two of those patients are still alive and receiving chemotherapy. One of the four patients underwent a surgical procedure followed by chemotherapy.

The prognosis of patients with brain tumors is not influenced by histopathologic grade or FIGO stage or type of tumor [11, 15-17]. From the literature, no clear relationship between size of the tumor before surgery, the residual disease after primary surgery or the response to chemotherapy and development of brain metastases was found [15, 17]. In our group all the patients were diag-
nosed with a serous type of ovarian cancer (G3). As this type of ovarian cancer is the most common it is difficult to confirm any correlation. The most significant prognostic factor in brain metastases is the concomitant systemic metastasis of the tumor [14].

Conclusion

The longer survival of ovarian cancer patients is connected with using aggressive methods of treatment. Better imaging techniques allow a better diagnosis of brain metastases. It is a new challenge for patients and oncologists [6].

Treatment and extent of the disease are the only factors significantly affecting survival [7]. Statistical conclusions are difficult to formulate from such a small number of cases of brain metastases in patients with ovarian cancer.

References


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Adenoid cystic carcinoma of the Bartholin’s gland in a young patient: Eight-year follow-up

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Summary

Adenoid cystic carcinoma (ACC) of the Bartholin’s gland is one of the rarest neoplasms of the female genital tract. Including this report there are 65 cases mentioned in the literature. We report a case of a 36-year-old woman who presented at our hospital after excision of the right Bartholin gland elsewhere which proved to be ACC. The therapy of this rare tumor has many controversial questions and dilemmas, especially in young patients. Our patient underwent surgical treatment only (hemivulvectomy and lymph node dissection) without radiotherapy and is free of disease eight years after.

Key words: Adenoid cystic carcinoma; Bartholin’s gland; Hemivulvectomy.

Introduction

Klob [1], described primary carcinoma of Bartholin’s gland in 1864 and Billroth first documented ACC of the salivary glands in 1895 [2]. The initial diagnostic criteria were established in 1897 by Homan and reported by Masterson and Goss in 1955 [3]. The new criteria were established by the Institute of Pathology of Armed Forces (AFIP) concretely by Chamlian and Taylor in 1971 [4]: (1) areas of apparent transition from normal to neoplastic elements must be present on histologic study; (2) the tumor involving the area of Bartholin’s gland should be histologically compatible with origin from Bartholin’s gland; (3) there is no evidence of tumor elsewhere.

Case Report

A 36-year-old woman presented at our hospital after excision of the right Bartholin’s gland elsewhere. The patient had received antibiotic treatment due to Bartholin’s swelling three months before with no improvement and she finally underwent excision of the gland. The histological examination reported growth of various sized islands of rounded uniform cells with a small amount of protoplasm developed mainly with a cribriform pattern and PAS positivity in the pseusotubules (Figure 1). In certain sites these arrangements appeared to evolve to the epithelium of the remaining tubules of the gland. Immunohistochemically the neoplastic cells were positive in S-100, vimentin and focally in actin. Further infiltration of nerves and muscles was noted and the excised margins were positive. The specimen showed increased expression of MIB-1 antigen in less than 10% of the neoplastic cells and rare mitoses.

On clinical examination, the inguinal and femoral lymph nodes were not palpable. Laboratory tests were normal and chest X-ray, abdominal and pelvic computed tomography (CT) exams were negative. After informed consent, the patient underwent wide local excision but exhibited positive margins (Figure 2). The histological examination confirmed foci of ACC of Bartholin’s gland with a maximum diameter of 0.9 and 0.5 cm in the inferior border of the specimen corresponding to the posterior fourchette. She further underwent right hemivulvectomy with ipsilateral inguinofemoral lymph node dissection and left hemivulvectomy. The excised specimens and the 14 lymph nodes showed negative histology. Eight years follow-up revealed no recurrence of disease.

Discussion

ACC of the Bartholin’s gland constitutes about 10% of all carcinomas arising in this gland and approximately 0.1-0.5% of all vulvar malignancies. It is often found in the salivary glands, nasopharynx, breast and skin, while it can also be found in the cervical glands and ovaries [2, 5]. It has been reported as malignant cylindroma, adenomyoepithelioma and pseudoglandular carcinoma [6]. The more frequent histologic type of ACC of the Bartholin’s gland is the cribriform or mixed type (with small cylindrical hearths or dense regions) [5].

The etiology of this neoplasm remains unknown. It seems to be antigenically distinct from both squamous and adenocarcinomas and is believed to originate from the myoepithelium. It also seems unrelated to HPV infection in contrast with tumors derived from the transition zone of the epithelium [7, 8].

ACC is a slow growing tumor that often exhibits local relapse [9]. The primary lesion usually extends between 0.5-4 cm [10]. A high percentage of incidents are usually diagnosed during pregnancy [11]. Local relapse can be expected even in cases of free surgical margins, while distant metastases could present 16 years after initial diagnosis [5].

Although obtaining clear surgical margins for ACC of the salivary gland is associated with lower recurrence [12], for the Bartholin gland there is still considerable...
bias. The positivity of resection margins is reported to be 48% for simple excision and 30% for radical vulvectomy and the importance of clear surgical margins has been pointed out [9, 13]. Anaf et al. [14] suggested radical vulvectomy in order to achieve tumor clearance, lower recurrence and avoid re-intervention, while Rosenberg et al. [2] advocated wide local excision and unilateral lymph node dissection. Lelle et al. [10] noticed that patients with negative margins will most probably develop local recurrence and therefore radical operation with considerable morbidity could not be justified, while in some patients the disease-free interval will be longer than the natural life expectancy. Nevertheless, because the number of patients was still small, they concluded that the necessity of obtaining free margins should not be dismissed.

Metastatic spread of this neoplasm appears to be hematogenic and geographic. The first pathway could result in lung, bone and less often in liver, kidney and brain metastases, while the second could be responsible for frequent local recurrence with extension along the neural sheath [10]. Adjuvant radiotherapy is recommended when margins are positive or when local perineural invasion is observed [6]. Postoperative external beam radiation is reported to give good control in patients with recurrent disease [2, 11], while the role of chemotherapy can not be established due to limited data. Several treatment options such as cyclophosphamide [10], cyclophosphamide and Adriamycin [15] and cyclophosphamide, Adriamycin and cisplatin have been reported [13], but with no actual benefits from this additional therapy.

In our case, we initially treated the patient surgically but as conservatively as possible. Due to positive margins, bilateral hemivulvectomy with clear margins and ipsilateral lymph node dissection were performed. This therapy has offered up to now an 8-year free-interval of disease and good quality of life. Rosenberg et al., [2] reported two similar cases. Initially, the patients were treated non-radically, but due to positive margins the first underwent hemivulvectomy and radiotherapy and the second radical vulvectomy and radiotherapy. It has also been suggested that a 10-15-year survival rate is a better indicator of treatment effectiveness than 5-year survival, since this malignancy is characterized by the appearance of late hematogenic metastases [2]. Nevertheless, 5-year survival still remains the “gold” standard in following-up oncology patients. Furthermore, the dilemma of radical vulvectomy versus wide excision or hemivulvectomy, especially in young patients, is still present. Young women are more sexually active and psychologically sensitive. Radical operations and radiotherapy increase the risk of complications and necessitate conspicuous changes in body image, the loss of sexual and ovarian function, and psychiatric disorders [14].

This is the only case in the literature that describes therapy of a young patient (less than 40 years old) with ACC of the Bartholin’s gland by surgical treatment only. In agreement with other authors [6, 13], we contemplate that every clinical situation is unique for each patient. Although radical vulvectomy is more likely to give clear surgical margins, higher operative and especially higher psychological complications may occur in young patients, and it seems best to tailor the surgery to the patient. Encouraged by our report we could postulate that in younger patients initial treatment should be as conservative as possible considering that tumor size is small and surgical margins are clear.

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A successful pregnancy and delivery after resectoscopic surgery for early invasive endometrial cancer

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Summary

Among young women, the incidence of uterine corpus cancer is increasing. Most young women cannot preserve fertility because simple total hysterectomy with bilateral salpingo-oophorectomy is the standard method for early endometrial cancer so far. We present a case of early endometrial adenocarcinoma which succeeded in pregnancy and delivery after resectoscopic surgery. Following a circumferential resection of the lesion including the mucosa and muscle layer under resectoscopic guidance, the patient became pregnant by means of in vitro fertilization-embryo transfer with hormone replenishment. She underwent cesarean section at 33 weeks and five days of gestation and had a healthy baby. Resectoscopic surgery can help to preserve fertility among young women who have early invasive endometrial cancer.

Key words: Resectoscopic surgery; Endometrial cancer; Pregnancy.

Introduction

Uterine cancer is the most common type of gynecologic malignancy, and among young women, the incidence of the disease is increasing [1]. For early corpus cancer, physicians try to preserve fertility with hormone therapy and total endometrial curettage resulting in recurrence.

Case Report

In June 2001, a 28-year-old nulliparous woman, who had been married almost five years, visited an obstetrics and gynecology clinic for infertility consultation. The patient was examined, and the resulting diagnosis was polycystic ovary syndrome. Hysterosalpingography revealed endometrial thickening. After total endometrial curettage, the surgical specimen revealed well-differentiated (grade 1) endometrial adenocarcinoma. As the patient had a strong desire to have children, we selected gestagen therapy (progestin using medroxyprogesterone acetate; MPA), and prescribed MPA (200-400 mg/day) and low dose aspirin as an anti-coagulant agent. Six months later, we performed another total endometrial curettage. Cancer tissue was detected in part of the curetted tissue. On magnetic resonance imaging (MRI) two months later, the junctional zone was unclear, suggesting muscle layer invasion by endometrial carcinoma.

Before selecting simple total hysterectomy, the patient consented to receiving resection of the lesion under resectoscopic guidance. A full circumferential resection was performed, focusing on the lesion and extending to the upper two-thirds of the corpus without resecting the lesion-free lower corpus. The tissue resection depth was 3-4 mm (including the mucosa and myometrium), when measured after formalin fixation. No cancer tissue was histopathologically detected in the resected tissue. Five months after surgery, the intrauterine device that had been inserted during the resection procedure was removed, and menstruation resumed with Kauffmann therapy (estrogen-progesterone treatment). Without hormone replenishment, the endometrium did not thicken to a level equivalent to that of the luteal phase. In October 2003, one year after confirming the junctional zone preservation on MRI and the absence of cancer by cytology and histopathological examination, the patient became pregnant by means of in vitro fertilization-embryo transfer (IVF-ET). In January 2004, a spontaneous abortion occurred at 19 weeks of gestation. No cancer was detected in the placenta or decidua. Thereafter, no abnormalities were found on cytology, histopathological examination, or MRI until January 2006 when the patient became pregnant a second time by means of IVF-ET. Because the previous abortion seemed to be attributable to cervical incompetence, we applied cervical cerclage (Shirodkar’s operation) at 15 weeks of gestation. Her pregnancy followed a favorable course thereafter (Figure 1). At 33 weeks and five days of gestation, she underwent cesarean section. A girl weighing 1926 g with an APGAR score of 9 at 5 min was delivered. During the cesarean section, endometrial biopsies were performed and the results were negative. The child developed normally after birth.

Figure 1. — Ultrasonography showing a well-demarcated posterior wall of the uterine corpus at 16 weeks of gestation. No placenta previa was observed and the placenta occupied the anterior wall to the fundus of the uterus at 34 weeks of gestation.
Discussion

Cancer of the uterine corpus is a gynecologic tumor whose incidence has been increasing worldwide. Young patients with this cancer often want to preserve fertility. In the past, no valid operative procedure for this cancer, while preserving the uterus, was available. Hormone therapy and total endometrial curettage are often employed for patients with this cancer who want to preserve fertility. However, these therapies do not allow adequate treatment of uterine corpus cancer, and the post-treatment recurrence rate is high [1].

Among others, Stage 1a uterine corpus cancer complicated by myometrial invasion is not indicated for these therapies and is often treated by simple total hysterectomy. Mazzon et al. [2] reported a case of hysteroscopic resection and birth by cesarean section but the lesion was too small to eradicate wider lesions of Stage 1a endometrial cancer. Furthermore, it is particularly difficult to make a pathological distinction between atypical endometrial hyperplasia complex and Stage 1a uterine corpus cancer complicated by stromal invasion [3].

Our experience with the present case suggests that resection of intrauterine lesions with a resectoscope allows adequate treatment, at least of Stage 1a or less advanced Stage 1b uterine corpus cancer, while preserving a woman’s fertility postoperatively. However, no prior investigator has reported that extensive resection of endometrial tissue together with the muscle layer, using a resectoscope, resulted in regeneration of endometrial tissue or successful pregnancy and delivery.

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Pure vulvar Langerhans cell histiocytosis: 
a case report and literature review

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Summary
Langerhans cell histiocytosis (LCH) of the female genital tract is a very rare disease. Only 16 cases of primary vulvar LCH without subsequent systemic spread of disease have previously been published in the literature. We describe an additional case of LCH in which the lesion was confined to the vulva. A 52-year-old Caucasian woman presented for further investigation with a 6-month history of vulvar pruritus. Physical examination revealed multiple fine red papules on both labia minor. A metastatic workup did not reveal any evidence of disease beyond the vulva. The lesion was biopsied, and histological findings were characteristic of LCH. The patient was treated by local extirpation of both labia minor. Ten months after surgery, the patient has no signs of local recurrence or systemic spread. It is necessary to perform a biopsy of the lesions when a woman presents atypical chronic lesions on the vulva. Although different treatment has been proposed, complete surgical excision is fundamental in “pure” genital Langerhans cell histiocytosis as initial therapy.

Key words: Langerhans cell histiocytosis; Vulva; Treatment.

Introduction
Langerhans cell histiocytosis (LCH), also known as Langerhans cell granulomatosis or histiocytosis is a rare systemic disorder of unknown etiology, characterized by an abnormal monoclonal proliferation of cells of dendritic cell lineage (bone marrow origin), which phenotypically resemble normal activated Langerhans cells [1, 2].

Paul Langerhans was the first to describe this disorder in 1868. Langerhans cells (LCs) are normally found in the skin, cervix, vagina, stomach, and esophagus [4, 5]. LCH was initially categorized into eosinophil granuloma (when disease affects only one organ), Hand-Schuller-Christian disease (a chronic progressive disease), and Letterer-Siwe disease (a fulminant systemic illness) depending on the site and degree of spread. Later these were found to be different manifestations of a single disease named histiocytosis X by Lichtenstein [6, 7].

Recently this classification has changed to LCH, in recognition of the primary cell involved.

LCH is generally considered to be a disease of childhood, mainly in young males, while adults are rarely affected. Approximately one in 200,000 children and one in 560,000 adults are affected [8-10].

The clinical presentation varies greatly, ranging from mild to life-threatening [11, 12]. The estimated total mortality of LCH is approximately 3% in adults and 15% in children [13, 14]. LCH of the female genital tract is very rare. The disease may involve the vulva, cervix, endometrium and ovary [14, 15]. LCH of the lower female genital tract was first reported in a six-year-old child by Lane and Smith in 1939 [16, 17]. Only 16 cases of primary vulvar LCH have been published in the medical literature. In this report we present an additional case of isolated LCH of the vulva.

Case Report
A 52-year-old Caucasian woman, initially visited her local dermatologist in April 2007 complaining of vulvar pruritus. The clinical manifestation had first been diagnosed as simple fungal dermatitis. Therefore, she was treated locally by corticoids and antifungal ointments. She had not experienced any improvement and was admitted to our department six months later for further investigation. The physical examination revealed multiple fine red papules on both labia minor. A metastatic workup did not reveal any evidence of disease beyond the vulva. The lesion was biopsied, and histological findings were characteristic of LCH.

The patient was treated by local extirpation of both labia minor. Ten months after surgery, the patient has no signs of local recurrence or systemic spread. It is necessary to perform a biopsy of the lesions when a woman presents atypical chronic lesions on the vulva. Although different treatment has been proposed, complete surgical excision is fundamental in “pure” genital Langerhans cell histiocytosis as initial therapy.

Key words: Langerhans cell histiocytosis; Vulva; Treatment.

Revised manuscript accepted for publication February 14, 2009
LCH is a rare disease which can occur at any age, with a peak between one and three years [2]. There is no significant sex bias [18], although recent studies have suggested a slight female predominance in patients above 15 years of age [19]. This disorder is often misdiagnosed because of the wide range of symptoms that can be local or systemic [10]. The clinical presentation and course range widely from indolent to aggressive and from spontaneous remission to rapid death due to “risk” of organ system involvement and subsequent failure. “Risk” organs are the liver, spleen, bone marrow and lungs [20].

Localized LCH lesions are unusual and may be found on the skin or mucosa [21]. There is no correlation between histological findings and the outcome of the genital lesions. According to the literature 33% of isolated vulvar LCH are subsequently disseminated.

There are four patterns of LCH involvement in the female genital tract: 1) pure genital LCH, in which the disease is limited to the genital tract only, 2) genital tract LCH with subsequent multi-organ involvement, 3) oral or cutaneous LCH with subsequent genital and multi-organ involvement and 4) diabetes insipidus with organ involvement [15].

In this study LCH was isolated in the vulva, which is a very rare disorder and should be differentiated from other dermatologic diseases, such as diaper rash eczema, seborrheic dermatitis, tuberculosis, as well as sexually transmitted disorders (syphilis, herpes and granuloma inguinale) or trauma. Neoplastic processes should also be included in the differential diagnosis: squamous cell carcinoma, sarcoma, malignant melanoma and Paget’s disease of the vulva. It is important to exclude systemic disease in any patient suspected of having vulvar LCH. The differential diagnosis of LCH includes immunodeficiency syndromes with graft-versus-host disease such as leukemia or lymphoma, reticuloendothelial storage disease, congenital infections, Erdheim-Chester disease and popular xanthomas. Therefore a biopsy of the lesion is mandatory. The diagnosis of LCH is based on hematological and histological criteria established by the International Histocyte Society in 1987 [22]. Definitive diagnosis requires the finding of Birbeck granules in lesional cells by electron microscopy or CD1a+ positivity on the surface of lesional cells [2, 5]. It has to be pointed out that LCs are predominantly involved in antigen presentation (S-100 protein+, CD1a+, CD45+, CD21-, and CD35-).

The routine evaluation should include measuring serum electrolytes and performing a chest X-ray, CT of the abdomen, thorax, pelvis, brain and a bone scan.

During the last decade, by utilizing modern molecular biology techniques, a greater understanding of this rare but interesting disease has been acquired. Present knowledge has altered the prior conception of LCH as a hyperplastic disorder to that of neoplasia of LCs [2], but whether it is malignant or not is still not clear. It results from proliferation and accumulation of distinct tissue histiocytes with the phenotype of LCs. Moreover, high expression of a large panel of cytokines such as TNF-a, IL-1α, GM-CSF, IL-10 and IFN-γ has been demonstrated in these lesions. T-cells and LCs have proved to be the predominant sources of this “cytokine storm”. Associated pathogenic effects of these cytokines include chemotaxis of additional inflammatory cells, over-expression of adhesion molecules, fibrosis, necrosis and osteolysis [20, 23]. The presence of an increased amount of GM-CSF receptor and TNF-a in infiltrating LCs are crucial factors for the proliferation of CD34+ precursors and for LC differentiation and survival [24-26] (Figure 1).

Since the very first case of primary vulvar LCH described in 1939 by Lane et al. [16], a total of 16 cases have been published in the literature. Due to the rarity of this disease, treatment of genital LCH is still very diverse without any randomized clinical studies. Generally, localized genital tract or cutaneous disease is treated with resection, radiation therapy, topical nitrogen mustard, corticosteroids, trimethoprim-sulfamethoxazole, phototherapy (PUVA), thalidomide, as well as systemic chemotherapy and 2CDA [22, 27, 28].

Radiotherapy for mucosal or cutaneous LCH is questionable because of its controversial efficacy and because of the natural history of the disease. Isolated LCH as genital lesions have a tendency to spontaneous regression and radiotherapy should not be a front-line treatment mainly for adolescents.

Complete surgical excision has been advocated as the recommended initial therapy for LCH of the genital tract. Wide excision is necessary for complete removal of genital disease, but unfortunately this does not eliminate the risk of recurrence. Axiotis et al. [22] suggest treating genital lesions initially by complete excision, but 50% of the patients with genital LCH relapsed after surgery [29]. If LCH indeed has its origins in the bone marrow, then it is hardly surprising that such a high relapse rate exists.

Chemotherapy for multisystem disease is beneficial [30], but there are few data referring to the use of chemotherapy for localized disease. Systemic treatment with vincristine did not clear up the lesions of the patient described by Solano et al. [21].
The successful use of thalidomide for genital LCH was first reported by Gnassia et al. [31]. The efficacy of thalidomide in treating several inflammatory skin diseases suggests that the mechanism of action is related to immune modulation, cytokine inhibition and/or antiangiogenesis [32]. The reason for its use to treat LCH is that thalidomide is an inhibitor of tumor necrosis factor, which plays a prime role in generating LCs for their CD34+ bone marrow precursors. However there are some significant side-effects of using thalidomide. In addition to the teratogenic effect of thalidomide its prolonged use may lead to substantial neuropathy and sedation. The likelihood of developing neurological symptoms does not seem to correlate with either the daily dose of thalidomide or the duration of treatment. Based on retrospective data, women and older patients seem to be at greater risk of developing neuropathy. Trimethoprim-sulfamethoxazole in the setting of localized cutaneous LCH has also been used with temporary remission but, like thalidomide, patients frequently relapse upon discontinuation of therapy [33]. Although several investigators found thalidomide to be effective in treating cutaneous and genital LCH [7], the response in some cases was temporary remission but, like thalidomide or the duration of treatment. Based on retrospective data, women and older patients seem to be at greater risk of developing neuropathy. Trimethoprim-sulfamethoxazole, interferons, retinooids or other immunomodulatory agents as initial first-line therapies of genital LCH, rather than surgical excision or radiotherapy.

2-Chlorodeoxyadenosine (2-CDA), a purine analog with activity in indolent lymphoproliferative disorders, has also been used and durable remissions as well as partial responses were reported [35]. According to Rodriguez-Galindo [36] and Mottl [14] 2-CDA is a good option for treatment of recurrent LCH because of early local improvement and achievement of complete response with good toleration.

In the presented case local excision seemed to be effective since the patient did not have systemic involvement. The fact that the surgical margins were free of disease with a safety margin of at least 10 mm could be crucial.

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Sclerosing peritonitis associated with bilateral luteinized thecoma, linked to anticonvulsant therapy

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Summary

Objective: To present a new case of sclerosing peritonitis associated with bilateral luteinized thecoma of the ovaries, linked to anticonvulsant therapy. Case: A 22-year-old patient, receiving carbamazepine for seizures and anxiety attacks presented with shortness of breath, abdominal pain, nausea and vomiting. Clinical and imaging examinations revealed bilateral ovarian masses with massive ascites. At emergency surgery, bilateral ovarian luteinized thecoma with sclerosing peritonitis was found. Due to recurrent, postoperative episodes of small bowel obstruction she was treated with nasogastric suction, intravenous fluids and electrolyte replacement. Total parenteral nutrition was introduced. Since only partial improvement was achieved tamoxifen was administered for downregulation of TGF-β production should be considered as a treatment modality, as it proved to be very helpful in the presented patient.

Key words: Sclerosing peritonitis; Luteinized thecoma; Carbamazepine; Tamoxifen.

Introduction

In 1994, Clement and colleagues [1] were the first to describe sclerosing peritonitis associated with luteinized thecoma of the ovary in a series of six patients. Since then, 12 additional cases have been reported, most of them as isolated cases [2-11]. The combined disease is characterized by extensive peritoneal reactive fibrosis often requiring repeated surgery to repair bowel obstruction; the ovarian masses may be unilateral or bilateral.

The present paper presents a new case of sclerosing peritonitis and luteinized ovarian thecoma in a patient receiving anticonvulsant therapy.

Case Report

A 22-year-old patient, married + 2 (G2P2) presented at our emergency unit with abdominal pain, nausea and vomiting of two weeks duration. Her periods were normal and regular. For the last five months she was treated with carbamazepine 200 mg bid and fluoxetine 20 mg daily for recurrent seizures and anxiety attacks.

On admission the patient appeared pale and distressed with shortness of breath and abdominal swelling and distention. On gynecological examination, the adnexae could not be palpated and the abdomen was severely distended with signs of massive ascites. Abdominal and vaginal sonography showed bilateral solid ovarian masses measuring 15-20 cm each. Ascites occupied the entire abdominal cavity. The tentative diagnosis was bilateral ovarian tumors, and the patient was admitted to our division of gynecological oncology for evaluation. Blood test results were all within normal limits, but her hormone profile showed elevated levels of estradiol 3910 pmol/l (normal mid-cycle peak: 440-1400); total testosterone 4.3 nmol/l (0.22-2.9); free testosterone 1.08 nmol/l (0.1-0.5); and a low level of dehydroepiandrosterone sulfate (DHEA-S) < 8.81 nmol/l (0.94-11.6); Thyroid-stimulating hormone measured 6.880 mIU/l (0.4-4.0) on admission and 9.780 mIU/l on day 2; free thyroxine was 2.9 pmol/l (10.3-24.5), and total triiodothyronine 1.17 nmol/l (1.0-2.7). Serum tumor markers were all within normal limits. The serum carbamazepine level was low at 35 mcg/ml.

Abdominal paracentesis removed 4.8 l of clear fluid, which was negative for malignant cells. Intravenous fluids and a nasogastric suction tube were introduced. Computed tomography (CT) (Figure 1) showed both ovaries to be exceedingly enlarged, with peritoneal and mesenteric thickening, omental caking, massive ascites, and bilateral pleural effusion. The patient continued to complain of severe pain and shortness of breath. Abdominal examination revealed diffuse tenderness with new formation of ascites, confirmed by sonography. A second paracentesis yielded 6.21 l of bloody ascitic fluid. Diffuse abdominal guarding and signs of peritoneal irritation developed, and the hemoglobin level dropped to 8.0 g%. Under urgent exploratory laparatomy, an additional 4 l of bloody fluid were removed and two huge (~20 cm in length each), infarcted, smooth, dark red ovarian masses were noted occupying the entire abdominal cavity and extending to the rib cage (Figure 2). Bowel loops were indurated, convoluted and dilated, consistent with obstructive ileus. The omentum appeared edematous, granular, and indurated. Bilateral, enlarged lymph nodes were palpated in the external iliac area. The rest of the abdomen appeared normal. Complete removal of the right adnexa and a partial left salpingo-oophorectomy were performed, leaving only a small segment of the left ovarian tissue. Massive ovarian edema was diagnosed by frozen section. Due to the abnormal findings in the cavity, partial omentectomy and pelvic lymph node sampling of the palpated enlarged nodes was also carried out.
Two days postoperatively, hormonal levels were as follows: estradiol 139 pmol/l, progesterone 1.10 nmol/l, DHEA-S < 0.81 mc mol/l, total testosterone < 0.1 nmol/l, and free testosterone 0.02 nmol/l.

Small bowel obstruction was diagnosed on the third postoperative day, which was treated with nasogastric suction, intravenous fluids and electrolyte replacement. Repeat CT scan revealed severe edema of the small bowel loops and sigmoid colon, with thickening of omental and gastric fat (Figure 3). Total parenteral nutrition through the left subclavian vein was introduced. With only a partial improvement, tamoxifen 40 mg bid was added on day 21, with gradual resolution of the obstructive symptoms.

After 32 days of hospitalization, bowel movement resumed. The patient was discharged two days later to the outpatient follow-up clinic. At present, 72 months after surgery, the patient is doing very well with normal and regular bowel movements. Serum LH and FSH, measured because of complaints of amenorrhea, showed menopausal levels, suggesting nonfunctioning of the left remnant ovarian tissue. The patient is now receiving tamoxifen 20 mg daily and carbamazepine 200 mg tid.

**Pathology**

The right ovary weighed 1,770 grams and measured 23 x 19 x 8 cm, and the left ovary weighed 950 grams and measured 19 x 13 x 5 cm. The external surface of both ovaries was smooth, glistening and reddish-blue. The cut surface revealed greyish tissue, which was partially solid and partially gelatinous. Congested and hemorrhagic areas were also noted. The portion of omentum studied consisted of slightly firm, yellow-grey tissue.

Microscopic examination of the ovaries revealed a tumorous process of fascicles of spindle and oval-shaped cells. The tumor was of variable cellularity, with solid areas interspersed with edematous areas, imparting a loose appearance. The cells contained mildly pleomorphic spindle to oval nuclei and a low to moderate amount of cytoplasm, which was occasionally vacuolated, compatible with luteinization. Mitotic activity was focally high, reaching 10 mitoses per 10 high power fields. The tumor was highly vascular, with areas showing a hemangiopericytomatosus pattern, and contained widespread hemorrhage (Figures 4 and 5). Immunohistochemical stains directed against estrogen and progesterone receptors (clone 6F11, Ventana, AZ, USA; clone 16, Novocastra, UK) demonstrated nuclear positivity of mild to moderate intensity in most of the tumor cells (over 90%) for the former and in about one-third of the tumor cells for the latter. In addition, the tumor cells were positive for vimentin (clone V9, DAKO, Denmark) and negative for keratin (polyclonal, DAKO, CA, USA) and desmin (clone D33, DAKO, Denmark). Microscopic examination of the omentum revealed a fibroscopic process composed of plump spindle-shaped cells embedded within a collagenous stroma and accompanied by mild lymphocytic infiltrates (Figure 6). The spindle-shaped cells in this process showed positivity for vimentin and smooth muscle actin (clone 1A4, DAKO, Denmark), indicating that these cells were myofibroblasts. Immunostains for estrogen and progesterone receptors were negative.

The distinctive combination of features noted in the ovaries and omentum were considered typical of bilateral luteinized thecomas with sclerosing peritonitis. Eight lymph nodes showed normal lymphatic tissue without any evidence of malignant cells.

**Discussion**

Ovarian thecomas are usually divided into typical and luteinized forms. Luteinized thecoma is a relatively rare tumor occurring in patients younger than those with typical thecomas, and accounts for less than 1% of all ovarian neoplasms. It is usually unilateral. In one series of 50 cases, about 50% of the luteinized thecomas were estrogenic and 11% were androgenic [12]. Histologically, luteinized thecomas include a background of ovarian thecoma/fibroma with well-defined foci of luteinized cells.

Sclerosing peritonitis is a rare entity, previously described in patients undergoing chronic ambulatory peritoneal dialysis (CAPD) possibly related to chlorhexidine in the dialysate [13], and following prolonged treatment with β-adrenergic blocking agents (mainly practolol) [14].

The main features of sclerosing peritonitis are thick fibrous depositions on the peritoneal surface, especially covering the small bowel loops, which occasionally lead to bowel obstruction. Histologically, the depotsions are characterized by the proliferation of fibroblasts and myofibroblasts separated by collagen, fibrin, and inflammatory cells.

Patients usually present with abdominal pain, nausea and vomiting. Physical examination reveals abdominal distention, occasionally with ascites and a palpable mass. These findings may develop even a long time after the patient is transferred to hemodialysis [13]. Sclerosing peritonitis in patients on CAPD is treated by cessation of the peritoneal route and conservative management of the bowel obstruction followed eventually by surgery. The surgical procedure is often very difficult and sometimes associated with severe complications. The mortality rate is high (40-60%), especially in patients with complications [15].

Luteinized thecomas associated with sclerosing peritonitis can occur in one or both ovaries [1]. Ovarian size may range from normal to slightly enlarged and up to a considerable size; the largest mass described was 31 cm in diameter [1]. The histological picture consists of a proliferation of spindle cells with focal differentiation into smaller lutein cells than in luteinized thecoma alone. Brisk mitotic activity may be found, especially in the spindle cells, despite the lack of evidence of metastatic potential of the tumor [16]. Our careful review of the case descriptions in the literature yielded a total of 18 cases besides ours [1-11] (Table 1).

Treatment of this rare entity consists mostly of conservative management of the obstructive symptoms - intravenous fluids, nasogastric suction, and parenteral nutrition. Some authors administered intravenous hydrocortisone [10, 17] to inhibit cytokine-induced fibroblast proliferation, and others used tamoxifen to inhibit tumor growth factor beta 1 (TGFβ1) gene expression on peritoneal mesothelial cells [18].

Surgical intervention consists of removal of the tumor/s, with or without the uterus, possibly with omentectomy, multiple peritoneal biopsies, adhesiolysis and, in severe cases of bowel obstruction, partial bowel resection. Several patients required repeated surgical procedures [8].
Sclerosing peritonitis associated with bilateral luteinized thecoma, linked to anticonvulsant therapy

Figure 1. — Preoperative CT scan of the abdomen showing huge, bilateral solid ovarian masses with ascites.

Figure 2. — Bilateral ovarian masses at operation. Note smooth, glistening, partially lacerated capsule.

Figure 3. — Postoperative CT scan showing small bowel distention with wall thickening (white arrow) and peritoneal thickening (black arrow).

Figure 4. — Ovarian tumor composed of fascicle of spindle and oval-shaped cells with variable cellularity and edematous foci (H & E, original magnification x 100).

Figure 5. — Higher magnification of the ovarian tumor demonstrating mildly pleomorphic nuclei and eosinophilic to foamy cytoplasm compatible with luteinization (H & E, original magnification x 400).

Figure 6. — Omentum with fibrosing process containing scattered lymphocytes (H & E, original magnification x 100).
Table 1. — Sclerosing peritonitis associated with bilateral luteinized thecoma - review of the literature.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yrs)</th>
<th>Presentation</th>
<th>Intraoperative findings</th>
<th>Surgery</th>
<th>Follow-up</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>Abd. swelling, nausea, vomiting,</td>
<td>Ascites-bloody-5 l, large bil. ov. tu, 12, 15 cm, omental thickening</td>
<td>BSO, partial omentectomy, peritoneal biopsies, appendectomy</td>
<td>Chemotherapy, 2nd look 3.5 mo, Chemotherapy, 3rd look NED 6 y.</td>
<td>Ethosuximide - 6 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bil. pelvic mass</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>Vomiting, diarrhea, obstruction</td>
<td>Slight bil. ov. enlargement, 4.3, 5.5 cm, ascites, fibrotic SB serosa</td>
<td>TAH+BSO, partial SB resection, omentectomy, peritoneal biopsies</td>
<td>Two laparotomies for SB obstruction</td>
<td></td>
</tr>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>3</td>
<td>14</td>
<td>Abd. swelling, bil. pleural effusion, pelvic mass</td>
<td>Ascites-9 l, large lt. ov. - 31 cm, tumor</td>
<td>LSO, omentectomy, biopsy-peritoneum &amp; rt. ovary</td>
<td>NED 6 y</td>
<td>2nd lap. - SB fibrous thickening, NED 2.2 y</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>Abd. pain, bil. ov. enlargement</td>
<td>Ascites-1 l, large bil. ovarian tumors - 16, 14 cm</td>
<td>RSO, partial LO, partial omentectomy</td>
<td>2nd lap. - 3 w - omentectomy, peritoneal biopsies, NED 9 mo</td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>76</td>
<td>Ascites, 4 mo.</td>
<td>Slight bil. ov. enlargement, - 4.3, 3 cm mesenteric fibrosis</td>
<td>BSO, omentectomy, serosa biopsies</td>
<td>SB obstruction 1 mo. Died 2 mo - pul. emboli, peritoneal adhesions</td>
<td>Ethosuximide - 6 mo, elevated estradiol &amp; testosterone</td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>Abd. distention &amp; pain, SB obstruction, ascites</td>
<td>Slight bil. ov. enlargement, 5.5 cm SB thickening, omental &amp; mesenteric nodularity</td>
<td>Wedge biopsies of ovaries, partial SB resection, partial omentectomy</td>
<td>NED - mo.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>Abd. distention, anorexia, leg swelling, ascites - 0.5 l, pelvic effusion</td>
<td>Ascites-bloody 1.6 l, bil. solid ovaries - 13 cm each, omental nodules</td>
<td>BSO, peritoneal &amp; omental biopsies</td>
<td>NED - mo.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>Abd. distention,</td>
<td>Bil. ov. enlargement, ascites 14 x 9 x 6 cm - 4 cm</td>
<td>TAH + BSO</td>
<td>2nd lap. - 16 d, small bowel edema-mult bxs, 3rd lap. 74 d, peri. space obliterated, gastrostomy, bxs.</td>
<td>2nd lap. - 16 d, small bowel edema-mult bxs, 3rd lap. 74 d, peri. space obliterated, gastrostomy, bxs.</td>
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<tr>
<td>9</td>
<td>28</td>
<td>Acuteabd. rt. adnexal mass</td>
<td>Ascites-bloody, rt. ov.-collapsed cyst, lt. ov. - 3 x 4 cm cyst</td>
<td>Bil. partial resection</td>
<td>2nd lap. - 1-m bowel resection, 3rd lap. dense fibrous pelvis+abd. Died 1.5 mo</td>
<td>Treated with steroids &amp; tamoxifen</td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>Abd. distention, nausea</td>
<td>Bil. ovarian enlargement</td>
<td>TAH + BSO</td>
<td>2nd lap. - 3 w, small bowel obstruction, fibrous small+ colon+pelvis</td>
<td>Treated with steroids &amp; tamoxifen</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>Upper + lower abd. masses</td>
<td>Bil. ov. enlargement sclerosing mesenteritis</td>
<td>Expl. lap, partial ovarian resection</td>
<td>Died - postoperative complications</td>
<td>Treated with steroids &amp; tamoxifen</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>Abd. pain, parakalimbal mass, fever</td>
<td>Bil. ov. enlargement sclerosing mesenteritis</td>
<td>Expl. lap, partial ovarian resection</td>
<td>Died - postoperative complications</td>
<td>Treated with steroids &amp; tamoxifen</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>Uremia, pelvic mass, hydrenephrosis</td>
<td>Bil. ov. enlargement retroperitoneal fibrosis</td>
<td>Expl. lap, partial ovarian resection</td>
<td>NED - 2 y</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>19</td>
<td>Acute abdomen, pelvic mass, fever</td>
<td>Lt. ov. mass 20 x 10 cm, bloody fluid</td>
<td>LSO, partial omentectomy, bxs</td>
<td>Lost to follow-up</td>
<td>Treated with intra-peritoneal cisplatinum</td>
</tr>
<tr>
<td>15</td>
<td>52</td>
<td>Abd. distention, diarrhea</td>
<td>Ascites, bil. ov. tumors bloody fluid</td>
<td>TAH + BSO, partial small bowel resection, adhesiolysis</td>
<td>7 lap. - adhesiolysis, short bowel syndrome - 7 y</td>
<td>Treated with hydrocortisone</td>
</tr>
<tr>
<td>16</td>
<td>28</td>
<td>Abd. distention, diarrhea</td>
<td>Ascites, bil. ov. tumor, colon + small bowel thickening</td>
<td>LSO, rt. ov. mass resection, partial colectomy</td>
<td>2nd lap. - adhesiolysis,</td>
<td>Treated with hydrocortisone</td>
</tr>
<tr>
<td>17</td>
<td>28</td>
<td>Abd. distention</td>
<td>Ascites-bloody, bil. ov. Tumor - 7, 6.5 cm</td>
<td>TAH + BSO, omentectomy, LNS</td>
<td>Laparoscopic bxs-2w</td>
<td>Treated with hydrocortisone</td>
</tr>
<tr>
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</tr>
<tr>
<td>18</td>
<td>43</td>
<td>Abd. distention</td>
<td>Ascites, bil. ov. masses - 12 cm each</td>
<td>TAH + BSO, omentectomy</td>
<td>3 laparotomies</td>
<td>Leuprolide, Toremifene</td>
</tr>
<tr>
<td>19</td>
<td>22</td>
<td>Abd. distention &amp; pain, nausea,</td>
<td>Ascites-bloody 4 l, bil. ov. tumors-rt. - 23 x 19 cm - 19 x 13 cm</td>
<td>LSO + partial RSO, omentectomy, lymph node sampling</td>
<td>NED - 72 mo</td>
<td>Carbamazepine - 5 mo, blood E2 - 3910 pmol/l</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(our case)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abd.: abdominal; bil.: bilateral; BSO: bilateral salpingo-oophorectomy; ov.: ovary; tu.: tumor; NED: no evidence of disease; SB: small bowel; TAH: total abdominal hysterectomy; LSO: left salpingo-oophorectomy; RSO: right salpingo-oophorectomy; Ac.: acute; expl. lap.: exploratory laparotomy; pul.: pulmonary; bxs: biopsies; LNS: lymph-node sampling.
Most of the reported patients with luteinized thecoma associated with sclerosing peritonitis had normal hormonal levels [1]. Lacson et al. [2] described an 8-year-old child with elevated levels of blood estradiol and testosterone [2]. In our patient, very high levels of estradiol together with elevated testosterone were found, and for the first time, estrogen (and progesterone) receptors were noted in the ovarian tumors.

The use of tamoxifen to treat the present patient was based on the drug’s known inhibitory action on fibrogenic reactions [18] and its downregulation of TGF-β gene expression and protein production [19]. Tamoxifen has also been shown to be effective in the treatment of other abnormal proliferative healing disorders, such as retroperitoneal fibrosis and desmoid tumors [20, 21].

The association of anticonvulsant therapy with ovarian thecoma has been described in six patients [1, 2, 22-24], all children aged three to 13 years. Phenobarbital, trimethadione, paramethadione, phenacetyleurea, ethosuximide and diphenylhydantoin were alternately administered for different periods of time. All the children presented with bilateral ovarian masses associated with ascites. The three youngest ones had neither luteinized cells nor sclerosing peritonitis, so they were excluded from our case analysis.

The first of the included cases, a 13-year-old girl described by Clement et al. [1], had been receiving ethosuximide for six months before she presented with bilateral luteinized thecomas associated with sclerosing peritonitis. The second child, an 8-year-old girl described by Lacson et al. [2], also received ethosuximide for six months and had multiple, firm, nodular masses on the omentum and retroperitoneum. The authors defined these findings as “fibroma-like proliferations infiltrated by lymphocytes and plasma cells”. The ovaries showed many clusters of lutein cells in the hilar area.

Our patient, a 22-year-old, was treated with anticonvulsant agents for five months. She represents the third case linking anticonvulsant therapy with bilateral luteinized thecomas associated with sclerosing peritonitis. She was older than the other two patients, and the only one taking carbamazepine (Table 2). The cause-effect relationship of this entity with anticonvulsant therapy is still unresolved. McIntyre et al. [24] reported an association of anticonvul-
sant therapy (especially long-term valproate) with hyperandrogenism and polycystic ovaries or reproductive endocrinologic problems. Herzog [25] suggested that the mechanism could be the valproate-induced decrease in estradiol metabolism, leading to higher estradiol levels and thereby, lower FSH levels. Although in our patient, the estradiol (and testosterone) levels were indeed very high, we do not know when the rise started. Since her period was regular and she had no history of endocrine problems, this explanation is doubtful. The fact that all hormonal levels returned to normal immediately after surgery (i.e., following removal of almost the entire ovarian tissue) with improvement in the patient’s general condition suggests that hormones were in some way responsible.

In summary, the occurrence of sclerosing peritonitis associated with luteinized thecoma may be explained by various mechanisms, including stimulation of mesothelial cells by sex hormones produced by the ovaries or by inflammatory activation of TGF-β production initiating the fibrogenic reactions. Treatment should be aimed at relief of the bowel obstruction symptoms with conservative methods if possible, and surgery in complicated cases. Tamoxifen for downregulation of TGF-β production should be considered as a treatment modality, especially given its simplicity of use and safety.

Acknowledgments

The authors wish to thank Gloria Ginzach and Marian Propp for their editorial and secretarial assistance.

References


Table 2. — Three cases associated with anticonvulsant therapy.

<table>
<thead>
<tr>
<th>Author (Ref. No.)</th>
<th>Age (yrs)</th>
<th>Presentation</th>
<th>Ascites</th>
<th>Operative findings</th>
<th>Anticonvulsive drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clement et al. [1]</td>
<td>13</td>
<td>Abd. swelling, nausea, vomiting</td>
<td>4.9 l - bloody</td>
<td>Bil. ovarian tumor - 4 cm, 15 cm, ovarian thickening</td>
<td>Ethosuximide - 6 mo</td>
</tr>
<tr>
<td>Lacson et al. [2]</td>
<td>8</td>
<td>Abd. distention, pleural effusion, anorexia, leg swelling</td>
<td>1.6 l - bloody</td>
<td>Bil. solid, 13 cm each, ovarian nodules</td>
<td>Ethosuximide - 6 mo</td>
</tr>
<tr>
<td>Levavi et al.</td>
<td>22</td>
<td>Abd. distention, pain, nausea, vomitng, shortness of breath</td>
<td>4.0 l - bloody</td>
<td>Bil. ovarian tumors, 23 x 19, 19 x 13 cm, ovarian thickening</td>
<td>Carbamazepine - 5 mo</td>
</tr>
</tbody>
</table>

Abb.: abdominal; bil.: bilateral.

Sclerosing peritonitis associated with bilateral luteinized thecoma, linked to anticonvulsant therapy


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Incidental diagnosis of atypical polypoid adenomyoma in a young infertile woman

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Summary

Atypical polypoid adenomyoma (APA) is considered to be a rare, benign form of a polypoid mass that exhibits glandular and squamous epithelial cell proliferation with varying degrees of atypia in association with cellular smooth muscle stroma. More frequently it manifests during the reproductive and premenopausal age with a reported mean age of 40 years. The published series indicates an average risk of endometrial carcinoma of 8.8% in women with a history of polypoid adenomyoma. The differential diagnosis of APA includes complex endometrial hyperplasia with atypia, invasive adenocarcinoma, adenofibroma, adenosarcoma and carcinosarcoma. The recognition of these unusual benign uterine tumors is very important, because they can easily be misdiagnosed. Treatment may vary depending on the patient's age, her desire to preserve fertility and the severity of her symptoms. We present a case of an infertile 39-year-old woman with an incidental diagnosis of APA.

Key words: Atypical polypoid adenomyoma; Menopause; Uterine tumors; Endometrial carcinoma.

Introduction

Atypical polypoid adenomyoma (APA) is considered to be a rare, benign form of a mixed epithelial and mesenchymal uterine tumor. It is a polypoid mass that exhibits glandular and squamous epithelial cell proliferation with varying degrees of atypia in association with cellular smooth muscle stroma. The differential diagnosis of APA includes complex endometrial hyperplasia with atypia, invasive adenocarcinoma, adenofibroma, adenosarcoma and carcinosarcoma. Because of complicating matters, APA sometimes is misdiagnosed as endometrial carcinoma. Differentiating it from endometrial carcinoma with myometrial invasion can be difficult, especially in a curettage specimen. Important distinguishing features of APA include: age of patients - usually premenopausal, minimal cytological atypia, and non-cribiform endometrial glands, with benign non-reactive smooth muscle stroma. This tumor should be carefully evaluated and cannot be strictly regarded as being a totally benign entity. It is important to diagnose these unusual benign uterine tumors because they can be misdiagnosed.

Medline and Embase databases in English were used to identify papers relevant to atypical polypoid adenomyoma. Although more than 100 cases of this lesion have been reported in the English literature, its true nature remains unclear.

Case Report

A 39-year-old woman presented with a history of irregular vaginal bleeding for the previous six months. She also complained of postcoital vaginal bleeding. She had been on oral treatment for iron deficiency anemia for the last three months. She was nulliparous, with a history of unexplained infertility and menarche at 13 years of age. In the past she had had five unsuccessful cycles of in vitro fertilization (IVF) treatment. Physical examination showed an enlarged uterus, normal vaginal smear, and transvaginal ultrasound (TVS) examination revealed a 2 cm endometrial echogenic mass arising from the fundus of the uterine body and also two intramural fibroids measuring 5 x 5 cm and 4 x 5 cm. Office hysteroscopy with a rigid 2.7 mm camera disclosed an exophytic, polypoid mass protruding from the posterior fundus of the uterus. Dilatation and curettage was performed and the specimen was sent to histopathology. The histologic examination revealed endometrial glands with moderate atypia mixed with benign non-reactive smooth muscle cells. There was no squamous metaplasia of the glandular epithelium (Figures 1, 2, 3). The pathologic diagnosis was atypical polypoid adenomyoma of the uterus. The findings and the pathology report were discussed with the woman and her husband and the option of conservative management and close follow-up was offered to them. The couple though was determined that a future pregnancy was no longer an option due to severe health problems of the husband, and asked for a hysterectomy. In the end, a subtotal abdominal hysterectomy with preservation of the ovaries was performed.

Discussion

APA is an uncommon tumor which occurs as a polypoid mass in the uterine cavity and may be either sessile or pedunculated. It is classified as a mixed benign tumor with epithelial and non-epithelial components. Approximately about 2% of endometrial polyps are adenomyomas [1]. Usually the mass averages 2.0 cm [1]. Typi-
cally, it presents with abnormal bleeding. More frequently, it manifests in reproductive and the premenopausal period with a reported mean age of 40 years (ages range from 21 to 73 years) [2]. Of the nearly 100 cases reported up to today, only six have been postmenopausal women [3]. TVS is increasingly being used as a diagnostic modality, and most adenomyomas are diagnosed as leiomyomas preoperatively. The histogenesis of the lesion still remains uncertain, although it is suggested that estrogen-related factors could play a key role in the pathogenesis [4]. A clinical history of infertility is common in patients with APA. It has been suggested that estrogen-related factors and ovarian dysfunction may be involved in the pathogenesis of the tumor [4, 5]. APA was found in three patients with Turner syndrome, possibly as a complication of the long-term estrogen therapy [5].

The histologic appearance of APA is that of admixture of endometrial glands having varying degrees of structural atypia and stroma consisting mainly of smooth muscle cells. Architecturally complex glands with a back to back pattern are not typically seen. Basically, the histologic features are similar to those seen in atypical endometrial hyperplasia [6]. APA has an epithelial component which consists of branching endometrial glands with cytologic atypia and a non-epithelial component which consists of smooth muscle and fibroblasts. Varying degrees of glandular atypicality have been observed and a few cases of APA have shown low-grade malignant potential or have been associated with adenocarcinoma [7, 8]. Squamous differentiation of an epithelial component is a frequent feature found in more than 90% of the cases and is often extensive [5]. The smooth muscle may show mild to moderate nuclear atypia and focal mitotic activity, usually less than 2 mitotic figures per 10 high power fields [9]. The cellularity of the smooth muscle component can be variable, ranging from hypocellular to greater cellularity than the adjacent endometrium. No APA has been reported to spread beyond the uterus and also Ki-67 immunohistochemical studies have shown the proliferative activity of the glands in APA to be fairly low [1]. In rare cases, APAs are synchronous with or appear to be the site of origin for well differentiated adenocarcinoma. In five cases up to today endometrial adenocarcinoma has most likely arisen from APA [10]. The published series indicate an average risk of endometrial carcinoma of 8.8% in women with a history of polypoid

Figure 1. — Large atypical glands surrounded by smooth muscle with foci of squamous differentiation (HE x 100).

Figure 2. — Large atypical glands surrounded by smooth muscle with foci of squamous differentiation (HE x 200).

Figure 3. — Cytologic atypia of the glands (HE x 400).
Incidental diagnosis of atypical polypoid adenomyoma in a young infertile woman

adenomyoma [3]. This risk is about ten times higher than the overall risk of endometrial polyps, which is 0.8% [11]. These observations suggest that it is extremely important to understand the histologic profile of APA by performing a careful histologic examination and employing numerous tissue specimens.

Treatment may vary depending on the patient’s age, her desire to preserve fertility and the severity of her symptoms. Because of the association between APA and endometrial adenocarcinoma, hysterectomy is considered to be the most effective treatment in perimenopausal or postmenopausal women. In case preservation of fertility is desired, careful follow-up with hysteroscopy and endometrial sampling is recommended [12].

References

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A giant ovarian mucinous cystic neoplasm weighing 8,500 grams with functional stroma.

A case report and literature review

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¹Pathology Laboratory, ²2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, Athens (Greece)

Introduction

In medical history, the largest reported female tumors are genital tract tumors and especially epithelial tumors of the ovary [1]. At present however, the development of health care systems and diagnostic procedures result in earlier diagnosis of ovarian tumors that usually present a small or medium size is (usually less than 15 cm in diameter). There is always special interest in the problems encountered in the diagnosis and surgical management of large ovarian tumors, often complicated by circulatory and cardiopulmonary problems.

Mucinous ovarian tumors are classified as neoplasms of surface epithelial-stromal elements of the ovary, with an incidence of less than 20% of all ovarian tumors [1, 2]. Seventy-five percent of them are benign, 10% are of low (borderline) malignancy and 15% are malignant [3]. Mucinous borderline tumors of the ovary usually occur in middle adult life [1] and present an excellent 10-year survival (over 90%), when diagnosed in Stage I [3]. Borderline mucinous tumors are typically multilocular with thin cystic walls, presenting focally solid areas. The cystic component is filled with mucus produced by the mucus-secreting epithelial cells lining the cysts. The abundant mucus production is the reason why such tumors may become very large.

We present such a case of a giant borderline mucinous ovarian tumor because of the special interest of the clinical manifestations, pathological findings and surgical procedure.

Summary

Large ovarian tumors weighing more than 5,000 g are rarely reported in the recent literature because of the improvement in health care systems. Such tumors present many challenges in diagnosis and in surgical approach due to severe circulatory and cardiopulmonary complications. The case of a 27-year-old unmarried patient, who complained of progressive increased abdominal girth of more than six months duration, metrorrhagia, constipation and dysuria is presented. A cystic ovarian tumor weighing 8,500 g (after preoperative fluid aspiration) proved to be a mucinous neoplasm of borderline malignancy with functional stroma. Problems in establishing a correct diagnosis are discussed together with a literature review.

Key words: Ovary; Mucinous tumours; Borderline tumors; Giant tumors.

Case Report

A 27-year-old unmarried woman was referred to the 2nd Department of Obstetrics and Gynecology of Aretaion University Hospital because of abdominal enlargement, constipation, dysuria and metrorrhagia observed during the previous nine months. She also complained of dyspnea when in the prone position. From her personal and familial history no serious diseases or cancer history were reported. No other serious clinical problems were detected.

On clinical examination, a thin girl with a remarkable abdominal enlargement was observed, the abdominal size in discordance with her general status. Her blood tests and tumor markers (CA-125, CEA and CA 19-9) were at normal levels. The pregnancy test was negative. The chest X-ray was free of neoplastic lesions but showed a high displacement of the diaphragm.

Transvaginal ultrasound revealed a giant (> 50 cm in diameter) cystic mass with solid elements in the abdomen. The patient was referred for abdominal computed tomography (CT) scanning which showed a normal liver, pancreas and kidneys in ectopic positions and a giant cystic mass with solid lesions, which extended from the posterior wall of the uterus up to the second lumbar vertebra showing invasion of the parametric tissues bilaterally.

Intrapelvic lymph nodes were not observed. Colonoscopy showed no findings apart from pressure phenomena of the colon. The clinical and radiological differential diagnosis included ovarian cancer and pseudomyxoma peritonei.

The patient underwent exploratory laparotomy where a giant cystic mass with solid lesions measuring more than 50 cm was found. The mass originated from the left adnexa and extended up to the diaphragm. No ascites was found.

After inspiration of part of the intracystic fluid, a copious procedure because of the viscosity of the fluid, excision of the tumor, abdominal hysterectomy with adnexa and omentectomy was performed.

There were no significant early or late postoperative complications and the patient was well 24 months after surgery.
The pathological examination showed a giant cystic neoplasm measuring 55 x 45 x 30 cm, weighing 8,500 g (Figure 1). The external surface of the tumor was smooth. On dissection, the tumor presented a multicystic surface, with cystic spaces measuring 0.5-6 cm, separated by thin septa and filled with thick mucus (Figure 2). Foci of solid whitish tissue were observed. The uterus, the right ovary and fallopian tube were grossly normal. The left fallopian tube was grossly attenuated, and presented a length of 13 cm.

The giant size of the neoplasm presented additional problems in the pathology examination. We followed the guidelines for the examination of large gynecological tumors and examined at least one section/cm of the tumor, as well as most of the solid areas. In total, 120 sections were examined and at least 240 histological levels.

The histological study showed a mucinous neoplasm of borderline malignancy, with epithelial cells mainly of endocervical type with focal development of intestinal epithelium with goblet cells (Figure 3). Areas of hyperplastic and luteinized stroma surrounding the cystic spaces were observed. No signs of infiltrative growth of the tumor or infiltration of the parametria were observed.

The right ovary showed stromal hyperplasia and a luteinized cyst, but no corpus luteum. The endometrium showed irregular proliferative glands with extensive tubal metaplasia, suggestive of estrogenic stimulation.

Histological examination of the cervix, fallopian tubes and omentum did not show any remarkable pathological changes.

The patient was well 24 months after surgery.

Discussion

Giant ovarian tumors are rarely seen in modern medicine [4]. Mucinous tumors are the largest of all ovarian tumors, the largest was reported to weigh over 150 kg [1, 4, 7-9].

Borderline mucinous tumors usually appear as large cystic multilocular masses containing sticky gelatinous fluid (mucus). Most of them are unilateral, well-differentiated and when diagnosed in Stage I present a recurrence rate of 1%. They tend to present with abdominal distention and signs of pressure or displacement of the peritoneal organs.

In certain cases, such as ours, mucinous tumors present hormonal manifestations, such as endometrial hyperplasia or endometrial polyps because of the activation of tumor stroma [3]. Ultrasound, CT and MRI scans are performed to investigate such an entity. Serum tumor markers are also involved in the preoperative investigation, although they are usually negative. The preoperative differential diagnosis includes benign or malignant conditions such as serous ovarian tumors, ascites or pseudomyxoma peritonei and is often difficult. Microscopically, borderline mucinous tumors are classified as endocervical, intestinal or mixed cell type and are characterized by significant nuclear and epithelial stratification, epithelial bridging, prominent papillary projections and nuclear atypia [5]. Argyrophilic nucleolar organizer...
regions and Ki-67 staining have been reported to be increased in borderline tumors [6].

Large ovarian tumors present technical difficulty at surgery - risk of massive hemorrhage and postoperative complications such as circulatory failure due to changes in venous return, postoperative ileus and respiratory failure associated with pulmonary edema, elevated diaphragm and disorders of the respiratory muscles [7-9]. An intensive care unit supplied in the postoperative period to prevent cardiovascular and respiratory complications might be necessary and in certain cases plastic reconstruction of the abdominal wall is necessary.

Cooperation of the gynecologist, surgeon, radiologist and oncologist is necessary for the evaluation and treatment of such patients. The surgeon should always be prepared to perform major surgery.

However, borderline mucinous tumors when of smaller size can be managed conservatively due to minimal recurrence reported in patients with Stage I disease [10].

Pathological investigation also presents problems in the method of examination in order to establish a correct diagnosis and in certain cases a differential diagnosis from metastatic colonic neoplasms must be considered.

References
Possible relationship between chronic inflammation and pyloric metaplasia in a patient with lobular endocervical glandular hyperplasia

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Summary

Background: Lobular endocervical glandular hyperplasia (LEGH) is a rare entity of a pseudoneoplastic benign condition of the uterine cervix, and its histogenesis and pathological significance including a connection with carcinogenesis of the endocervical gland has not yet been fully recognized. Case: We describe a rare case of localized LEGH, which developed adjacent to a cesarean section scar. A 53-year-old premenopausal woman presented with a recent onset of abdominal distention and menorrhagia. Magnetic resonance imaging revealed multiple uterine myomas including submucosal myoma and localized small cystic lesions in the proximal area of the anterior wall of the cervix. Total hysterectomy was performed. The cystic lesions were diagnosed as LEGH. Thread-like foreign bodies and inflammatory reaction were demonstrated around several hyperplastic lesions. Focal immunoreactivity for MIB-1 was detected only in the LEGH cells adjacent to the fibrosis and foreign body reaction. Discussion: The histological findings, in relation to the previous cesarean section suggest that the ectopic pyloric hyperplasia in the present case could represent a heteroplastic or metaplastic process due to a multidirectional differentiation of cervical glands during chronic inflammation by foreign bodies.

Key words: Lobular endocervical glandular hyperplasia; Inflammation; Pyloric metaplasia; MIB-1.

Introduction

Lobular endocervical glandular hyperplasia (LEGH) is a rare entity of a pseudoneoplastic benign condition of the uterine cervix proposed by Nucci et al. [1] in 1999 as a lesion histologically similar to minimal deviation adenocarcinoma (MDA). However, its histogenesis and pathological significance, including a connection with carcinogenesis of the endocervical gland, has not yet been fully recognized. We describe a rare case of localized LEGH, which developed adjacent to a cesarean section scar, and discuss the histogenesis of LEGH with respect to any possible relationship between chronic inflammation and pyloric metaplasia.

Case Report

A 53-year-old premenopausal woman, gravida 1, para 1, whose child had been delivered by cesarean section with a transverse lower uterine segment incision 25 years earlier, presented with a recent onset of abdominal distention and menorrhagia. The postoperative course of the cesarean section was uneventful. Findings at surgery included a markedly enlarged uterus with multiple uterine myomas. The rest of the abdomen appeared normal.

Macroscopically, no apparent tumoral mass was seen on the surface of the uterine cervix. On the cut surface of the cervix, a lesion composed of aggregations of variable sized cysts was detected. This cystic lesion was detected in the proximal area of the anterior wall of the cervix. The size of the lesion was 12.5 mm in width and 7.5 mm in depth. The lesions, microscopically recognized to be cystic, were composed of dilated glands lined with high columnar mucinous cells. These columnar cells were frequently arranged in a lobular pattern without desmoplastic stromal reactions and their nuclei were basal and uniform. These hyperplastic glandular lesions were diagnosed as LEGH. Thread-like foreign bodies and an inflammatory reaction including marked fibrosis and infiltrates of histiocytes and lymphocytes were demonstrated around several hyperplastic lesions (Figure 1). In close proximity to the fibrosis and foreign body reaction, an extensive pyloric gland, together with mild to focally moderate dysplastic changes and strong immunoreactivity for pyloric gland mucin (HIK1083) and MIB-1 in the metaplastic epithelium were observed. Cellular atypia and immunoreactivity for MIB-1 became weaker in LEGH cells remote from the inflammatory reaction, while they demonstrated strong positivity of HIK1083 (Figure 2).
K. Takeuchi, T. Tsujino, R. Yasumizu, S. Kitazawa

Discussion

Several investigators have focused on pyloric gland mucin in glandular lesions of the cervix and its connection with endocervical gland carcinogenesis [2-5]. It has been speculated that hyperplastic or metaplastic glandular lesions with gastric mucin are precursors of endocervical mucinous adenocarcinoma independent of human papillomavirus, when the correspondent pyloric immunophenotype of these lesions is considered [6]. However, the histogenesis of metaplastic changes in LEGH has not yet been fully recognized.

The present case is unique because localized LEGH lesions developed adjacent to a cesarean section scar, and cellular atypia and marked immunoreactivity for MIB-1 were detected in several hyperplastic lesions in the vicinity of thread-like foreign bodies and inflammatory reaction, while these changes were weak when remote from

Figure 1. — Histological findings with a polarizing filter. Map shows the portion of the LEGH (A). Thread-like foreign bodies (arrows) are observed adjacent to LEGH lesions (B). Inflammatory reaction including marked fibrosis and infiltrates of histiocytes and lymphocytes is seen (C). Apparent thread-like foreign bodies are demonstrated (D).
Possible relationship between chronic inflammation and pyloric metaplasia in a patient with lobular endocervical glandular etc.

Foreign body reaction. MIB-1 antibody reacts with the Ki-67 nuclear antigen that is found throughout the cell cycle (G1, S, G2, and mitosis) but not in the resting phase (G0). The proportion of cells that express Ki-67 (Ki 67 index) are an excellent and reproducible measure of cellular proliferation [7]. The histological findings, in relation to the previous cesarean section, were challenging and led us to the hypothesis that this ectopic pyloric hyperplasia could represent a heteroplastic or metaplastic process due to multidirectional differentiation of cervical glands during chronic inflammation by foreign bodies.

Pseudopyloric or pyloric gland metaplastic epithelium has been described in several other organs such as the pancreas, gallbladder, and the small and large intestine [8]. Although the pathogenesis of this metaplastic change in most organs is still under discussion, pyloric metaplastic changes in chronically inflamed epithelia are well established in the gall bladder [9]. Furthermore, the close relationship between early gall bladder cancer and pyloric metaplasia, in the setting of chronic inflammation has been emphasized several times [8, 10, 11].

With this information, and from our results, it might be speculated that chronic inflammation plays an important role in the development and progression of pyloric metaplasia-related mucinous lesions in the uterine cervix, in terms of the metaplasia-hyperplasia-dysplasia sequence.

Figure 2. — Histological (A, B) and immunohistochemical (C, D) findings of Square 1 of Figure 1, and histological (E, F) and immunohistochemical (G, H) findings of Square 2 of Figure 1.

Adjacent to foreign body reaction, extensive pyloric glands, together with mild to focally moderate dysplastic changes are seen (A, B). Strong immunoreactivity for pyloric gland mucin (HIK1083) (C) and MIB-1 (D) in the metaplastic epithelium are observed. Cellular atypia (E, F) and immunoreactivity for MIB-1 (H) become weaker in LEGH cells remote from the inflammatory reaction, while they demonstrate strong positivity of HIK1083 (G).
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