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Nerve sparing radical hysterectomy in early stage cervical cancer. Latest developments and review of the literature

A. Kavallaris1,2,4, D. Zygouris3, A. Dafopoulos2, I. Kalogiannidis1, E. Terzakis3

1Fourth Department of Gynecology and Obstetrics, Aristotle University of Thessaloniki, Thessaloniki (Greece)
2Department of Obstetrics and Gynecology, University of Schleswig-Holstein, Luebeck (Germany)
3Second Department of Gynecology, St. Savvas Anticancer, Oncological Hospital, Athens (Greece)
4Department of Gynecologic Oncology, St. Loukas Hospital, Thessaloniki (Greece)

Summary

Background: Cervical cancer is the second most common malignancy of the female genital tract worldwide. Radical hysterectomy with pelvic lymphadenectomy exemplifies the treatment of choice for early stage disease, whereas even if it is performed by gynaecologist-oncologist, still has the drawback of significant postoperative morbidity, especially for urinary bladder function. Nerve-sparing radical hysterectomy (NSRH) is a technique in which the neural part of the cardinal ligament which encloses the inferior hypogastric plexus, as well as the bladder branch (distal part of the plexus), remains intact. By this way, the bladder’s innervation is safe and its functional recovery is more rapid. There is sufficient data to support the feasibility of the technique via laparotomy and laparoscopy, as well as the effectiveness related to the postoperative bladder dysfunction compared to conventional radical hysterectomy. On the other hand, the evidence related to survival outcomes is weak and derives from non-randomized trials. However, the low rate of local relapses after NSRH in early stage disease (IA2-IB1) with tumor diameter less than two cm makes the procedure suitable for this group of patients.

Conclusion: According to the current evidence NSRH seems to be a suitable technique for gynaecologist-oncologist familiar with the method in early stage cervical cancer. It is a technique which improves significantly postoperative bladder recovery and the patients’ quality of life (QoL), without compromising the oncological standard.

Key words: Radical hysterectomy; Nerve-sparing; Laparoscopy.

Introduction

Cervical cancer is the most common cancer of the female genital tract in the developing world and the second most common gynaecological malignancy after endometrial cancer in developed countries. Epidemiologic report from 27 European countries showed 40,000 new cases of cervical cancer every year [1, 2]. The overall five-year survival of patients with early stage cervical cancer is almost 90% [3, 4]. The appropriate therapy of patients with cervical cancer depends on the stage of the disease. Surgical approach or chemo-irradiation are both acceptable treatments especially for the early stages [5, 6]. Fertility-sparing procedures, such as deep cold-knife cone or radical trachelectomy with additional evaluation of pelvic nodes (via abdomen or laparoscopy), are also developed in case of young patients with early stage disease, who desire to preserve their fertility, although radical hysterectomy with additional pelvic lymphadenectomy is the standard surgical approach [7, 8]. However, radical hysterectomy and pelvic lymphadenectomy are surgical procedures with significant perioperative morbidity.

Nerve-sparing radical hysterectomy (NSRH), although it already known from old reports, in the last two decades became an attractive technique because of the low rate of postoperative pelvic organs’ dysfunction (especially of the urinary bladder) and of improved patients’ quality of life (QoL), compared to conventional radical hysterectomy.

In the present review there is a presentation of the different types of radical hysterectomy, a historical overview, and description of the technique of NSRH. Bladder postoperative function and survival outcomes after NSRH of patients with cervical cancer are also analyzed according to the evidence of the current literature.

Radical hysterectomy

The extension of the radical hysterectomy was not always unequivocal and of the same acceptance. In the early 1970s, Piver et al. introduced a classification of the different types of hysterectomy [9]. According to this classification, five different types (Piver I-V) of hysterectomy were presented based on the radicality of the surgical procedure. Although, Piver’s classification was used widely in gynaecological oncology,
recently another comprehensive classification with four types of radical hysterectomies (type A-D) originated by Querleu and Morrow [10]. This new classification, does not include Piver I hysterecomy (simple hysterecomy), while lymphadenectomy is considered obligatory in any type of radical hysterectomy [10].

According to this classification: Type A, radical hysterectomy is an extrafacial hysterectomy in which direct vision or palpation is used to identify the ureters. The uterosacral and vesico-uterine ligaments are not dissected far away from the uterine body, while the paracervix (parametrium) is dissected medial to the uteri. Vaginal resection is less than ten mm from the fornix. By this technique the vascular supply of the ureters remains intact. The procedure is suitable in group of patients with micro-invasive (IA1) cervical cancer according to International Federation of Gynecology and Obstetrics (FIGO) staging, who do not desire to preserve their fertility.

Type B is a proximal radical hysterectomy in which the resection of paracervix extends up to the level of the lateral part of the ureteral tunnel. The vesico-uterine and uterosacral ligament (anterior and posterior parametrium, respectively) are partially transected, while dissection of ten mm of the vaginal wall is acceptable. The operation is separated in two subtypes (B1 & B2), depending on the extension of paracervical lymphadenectomy. Using as a landmark the obturator nerve, subtype B1 and B2 are defined according to the paracervix nodal dissection, medial or lateral to the obturator nerve, respectively. It is indispensable to mention that the causal (deep) part of the paracervix, including the neural component of paracervix, is not dissected in type B radical hysterectomy, preventing the damage of the autonomous pelvic plexus.

Type C is an extended radical hysterectomy. The resection of the uterosacral and vesico-uterine ligaments extends up to the bladder and rectal wall, respectively. The paracervix tissue is dissected laterally to the junction with the internal iliac vessels. The causal part of the paracervix remains intact in subtype C1 hysterectomy, known also as modified radical hysterectomy or NSRH. Furthermore, in C1 hysterectomy the identification of the hypogastric nerve in the lateral wall of the uterosacral ligament must be done before the dissection of the uterosacral ligament up to the level of the rectum. Vaginal dissection 20 mm from the tumor or from the cervix margin is acceptable. Subtype C2 radical hysterectomy (suitable for advance cervical cancer, IB2-IIA), includes compete dissection of the paracervix, without preservation of causal (neural) part of paracervix.

Type D is an extra-radical hysterectomy, in which the dissection of the paracervix is extended to the pelvic side walls, including the hypogastric vessels (subtype D1) or further facial and muscular structure of pelvic wall (subtype D2). Dissection of uterosacral and vesico-uterine ligaments is done as previously described (type C).

**Autonomic pelvic plexus**

The hypogastric nerves originate from superior hypogastric plexus and contain sympathetic nerves. Presacral area of the pelvis is the origin point of hypogastric nerves, while then lay in the lateral part of the uterosacral ligaments at the level of pararectal space. Additionally, sacral nerves (S2-S4) from plexus sacralis constitute the splanchic nerves, which compose a plexus together with the hypogastric nerves. Both of them constitute the inferior hypogastric plexus. Actually, the inferior hypogastric plexus is an anastomotic autonomic pelvic plexus which is composed from sympathetic (hypogastric) and parasympathetic (splanchnic) nerves in the caudal-lateral part of cardinal ligament. From the inferior hypogastric plexus originates fibres directed to the rectum, uterus, and bladder reliable for the ano-rectal, sexual, and bladder function [11, 12].

**Identification of autonomic pelvic plexus and introduction of NSRH**

The surgical concept of the identification and preservation of the pelvic autonomic nerves was first introduced by Japanese gynaecologists. In the 1960s, Kobayashi working at the University of Tokyo published an extensive description of a modified Okabayashi operation, in which the autonomic nerves were identified and pushed aside before dissection of the cardinal ligament. This publication was in Japanese and the technique spread only throughout Japan [13-15]. Sakamoto, who had been an apprentice of Kobayashi, published the first paper in English in the 1980s. He meticulously described a nerve sparing surgical radical hysterectomy, which he named the “Tokyo method” [16]. He stressed the significance of preservation of the autonomic nerves of the pelvis during radical pelvic intervention to avoid postoperative sexual, bladder, and rectal dysfunction. The objective was to preserve the inferior hypogastric plexus without compromising the radicality of hysterectomy as it was introduced from Wertheim [17], the pioneer of the radical hysterectomy, and was later modified by Meigs [8]. Thereafter, modifications of the technique were made by other gynaecologists in Japan, emphasizing the preservation of the distal part of the inferior hypogastric plexus (bladder branch), to avoid urinary dysfunctions [16, 18-20]. In addition, thorough exploration of the anatomy of the sympathetic and parasympathetic nerves of the pelvis was performed in the same period, in an effort to molleriate the anatomic recognition of the autonomic pelvic plexus, improving thereby the outcomes related with pelvic organs dysfunction [12, 21-23].

The last two decades, European oncologic centers gave their descriptions of the nerve-sparing technique of radical hysterectomy. Höckel et al. in 1998 was the first who described the nerve-sparing technique with liposuction of cardinal ligament, in order to present a clear identification of the inferior hypogastric plexus. High-resolution magnetic resonance imaging has also been used preoperatively, for the better investigation of the pelvic anatomy. Total mesometrial resection (TMMR) was the base of the nerve-sparing technique, according to Höckel’s report [24]. The technique however, has been revised by the same author later in an effort to optimize the mesometrial dissection,
based on embryological topographic anatomy, in order to omit the adjuvant treatment of patients with cervical cancer with free surgical margins, even if with high-risk prognostic factors for recurrence disease [25].

Trimbos et al. gave almost a similar description of NSRH with that has been reported by Japanese’s school, emphasizing the feasibility of the nerve-sparing technique in European female population [26]. According to Trimbos’s technique, surgical points of unambiguous interest, performing NSRH, were the early identification of hypogastric nerve at the level of uterosacral ligaments, as well as the preservation of the proximal and distal part of inferior hypogastric plexus in the cardinal ligaments.

Recently, Raspagliesi et al. reported a study of 23 patients with cervical cancer who were treated with the nerve-sparing technique Piver III radical hysterectomy. In this study, an anatomical description of the autonomic pelvic plexus was given and the introduction of NSRH technique, using a cavitron ultrasonic surgical aspirator (CUSA), for the removal of the parametrial tissue, was presented [11].

According to the former descriptions from Japanese and European centres, critical points performing NSRH, are the protection of the origin of the hypogastric nerves in the lateral parts of uterosacral ligaments and the caudal-lateral part of paracervix (parametrium), which encloses the inferior hypogastric plexus. Moreover, the protection of the distal part of inferior hypogastric plexus is also of principal significance, since it encloses the bladder branch (motoric innervation of the bladder).

A brief description of NSRH is as following: Utero-sacral ligament is constituted by two layers: the medial and lateral. The lateral part contains the hypogastric nerve. The two layers of the uterosacral ligament are separated carefully by blunt dissection. By this means, the medial layer can be dissected during NSRH, leaving the lateral part intact without scarification of the enclosed hypogastric nerve. Caudal-lateral part of paracervix (parametrium) includes the main part of the inferior hypogastric plexus. Because of this, the cranio-medial part of paracervix can be dissected although some of the fibres will be scarified. The distal part of the inferior hypogastric plexus lies deeper in the lateral wall of the vagina and in the caudal-dorsal part of the vesico-uterine ligament. Identification of the ureter and of the inferior hypogastric plexus contributes to preserve as much as possible from the plexus. Restricting the colpectomy in the upper part of the vagina (no more than two cm), the majority of the fibres from the inferior hypogastric plexus which run along to the lateral wall of the vagina and of the bladder remain un-cut, preserving the innervation of the urinary bladder.

NSRH via laparoscopy

Laproscopic exposition of the inferior hypogastric plexus and NSRH were also performed successfully in European centers the last decade. The advantage of the magnification of the laparoscope permits the clear identification and the protection of the neural part of cardinal ligament, which includes the inferior hypogastric plexus. Possover et al. reported for the first time in Germany a description of nerve-sparing procedure during laparoscopic-assisted radical vaginal hysterectomy (LARVH) type III [27]. According to this description, the middle rectal artery was used as a landmark to separate the neural from the vascular part of the cardinal ligaments. With this landmark, after the clear exposition of cardinal ligament performing pelvic and paracervix lymph-node dissection, and with the merit of laparoscopic magnification, the medial part of the cardinal ligament can be safely dissected including only the vascular part of the paracervix. Vaginal-assisted laparoscopic nerve-sparing radical hysterectomy (LNSRH) has been also reported by other authors [28]. Recently, a description of the total LNSRH was presented [29, 30]. Thirty-two patients with cervical cancer underwent LNSRH with pelvic lymphadenectomy [30]. According to the technique, the superior hypogastric plexus was identified in pre-sacral space at the level of promontory. Hypogastric nerve was identified bilaterally along to the lateral sides of uterosacral ligament. The former procedure has been performed after pelvic lymphadenectomy and the identification of the ureter at the level of common iliac artery. Thereafter, the inferior hypogastric nerve was prepared towards the uterine artery (cardinal ligament) by blunt removal of the hypogastric nerve from the lateral sheet of the uterosacral ligament, as well as from the caudal-lateral part of the cardinal ligament. After the identification of inferior hypogastric plexus, radical resection of the cardinal ligament and uterosacral ligament was performed, without scarification of the inferior hypogastric plexus.

Bladder dysfunction after NSRH

Dysfunction of the pelvic organs is very common after radical hysterectomy [31, 32]. Postoperative bladder dysfunction has an incidence as high as 20% after radical hysterectomy [5, 33, 34], with further aggravation in the patients’ QoL [35, 36]. Nowadays, there is a large effort to reduce the postoperative morbidity (ano-rectal, sexual, and bladder dysfunction) after radical hysterectomy without compromising the oncologic standard. NSRH has been widely introduced in the last two decades, since it comprises the ability to satisfy the previous criteria.

In a retrospective analysis by Raspagliesi et al., 110 patients with cervical cancer (FIGO Stage I(A2-III)) were managed with type II, type III and nerve-sparing type III radical hysterectomy [37]. The objective was to evaluate the early bladder dysfunction (within three months after the operation) and the perioperative outcomes between the three groups of the study. The authors demonstrated that type II and NSRH are comparable, concerning the bladder dysfunction, and the perioperative complications, compared to type III radical hysterectomy. None of the patients treated with type II radical hysterectomy were discharged with self-catheterism, 7% after NSRH, and 55% after radical type III hysterectomy [37].
Kato et al., in a series of 32 patients with FIGO Stage IB-IIIB, of locally advanced cervical cancer (tumor more than 20 mm), performed radical hysterectomy and pelvic lymphadenectomy [23]. In case of unilateral spread of the tumor beyond the cervix, unilateral preservation of the autonomic pelvic plexus of the uninvolved parametrial side was performed [unilateral nerve-sparing (UNS) radical hysterectomy]. On the other hand, for the patients with medial position of cervical tumor (confined only to the cervix), bilateral nerve-sparing (BNS) radical hysterectomy was performed. Although the authors reported that all patients voided spontaneously without the need of postoperative self-catheterization, the mean duration of postvoid residual volume of less than 50 ml was significantly longer in the UNS (11.5 days), compared to BNS group of patients (5.3 days). The authors concluded that postoperative bladder dysfunction is more common after UNS radical hysterectomy [23]. Recently, Skret-Magierlo et al. confirmed the previous results [38]. Fujii et al., in a similar analysis of postoperative duration of bladder recovery after NSRH, showed an average of 14 days for postvoid residual urine volume (<50 ml), 11 days to obtain a sensation of bladder fullness, and 12 days to obtain satisfaction of micturition [12]. Moreover, the same authors demonstrated that all the patients had full bladder recovery 21 days after the operation [12].

Reports related to the “late” postoperative outcomes, such as bladder dysfunction (more than six months of follow-up period) are not so common in the bibliography. In one of them, 22 patients with cervical cancer were treated with systematic NSRH [22]. After one year follow-up period, none of them demonstrated urinary incontinence, while only two complained for diminished bladder sensation. These findings were significantly lower compared to that of the group of patients who underwent conventional radical hysterectomy (Sakuragi et al., 2005). Similar results concerning the late morbidity following NSRH was shown by Cibula et al. [39].

Studies designed to investigate postoperative bladder dysfunction of patients managed laparoscopically, have shown comparable results with those published by laparotomic trials. Performing LAVRH type III with additional preservation of autonomic pelvic plexus, Possover et al. showed that the suprapubic drainage was removed significantly earlier (11 days) in case of preservation of autonomic pelvic plexus compared to the group of patients without nerve-sparing approach (21 days) [27]. Kavallaris et al., performing total laparoscopic nerve-sparing radical hysterectomy, showed that all patients (n = 32) spontaneously voided in the third postoperative day. In the same day, ultrasound evaluation (two different measurements) of all patients showed that none of them had a postvoid residual urine volume >50 ml [30].

Sakuragi et al. evaluated the disease-free survival in 27 patients with cervical cancer (FIGO Stage IB-IIB). In this study, 22 patients were treated with systematic NSRH and compared with five patients, who underwent conventional radical hysterectomy [22]. The results showed a cumulative disease-free survival rate in 24 months 95.5% for the nerve-sparing group and 100% for the conventional group of treatment. Only one patient (IIB) of NSRH group presented with recurrence disease (pelvis), 13 months postoperatively and was successfully treated with radiotherapy. The non-randomized nature of the study, the short follow-up period, and the small sample size of the control group (n = 5), has limited the results of this series [22].

Querleu et al., managed 95 patients with modified radical hysterectomy using combined laparoscopic and vaginal approach. Forty-seven patients were managed with laparoscopic nerve-sparing procedure (average follow-up 26 months), while 48 patients were not (average follow-up 41 months) [28]. The authors concluded that because of high recurrence rate of patients with FIGO Stage IB1, with tumor diameter > two cm, nerve sparing technique is not acceptable for this group of patients. However, for patients with IA2-IB1 cervical cancer (tumor diameter < two cm), the outcomes related to the recurrence disease was excellent with or without nerve-sparing technique [28].

Recently, Van den Tillaart et al. reported the safety of NSRH in cervical cancer with Stage IA-IIA [40]. In this cohort, 122 patients underwent NSRH and were compared with 124 patients treated conventionally. Both groups were well-balanced concerning FIGO staging. Local recurrence, within 24 months after the operation, was more common in NSRH group (8.3%) compared to the conventional group (4.9%), however did not reach significant difference [OR = 1.7 (95% CI; 0.6-4.9), \( p = 0.27 \)]. Moreover, the estimated five-year overall survival did not significantly differ between the two groups of the study (\( p = 0.4 \)) [40].

In conclusion, NSRH is a feasible technique in patients with cervical cancer. It can be preformed via laparotomy or laparoscopy by an expert surgeon specialized in gynecological oncology and comprises an effective method concerning the postoperative bladder recovery and the patients’ wellness. Concerning the survival outcomes, nerve-sparing technique appears to be a suitable technique for the early-stage cervical cancer especially IA2-IB1 with tumor less than two cm. Further randomized trials are required to confirm the former results.

References

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Address reprint requests to:
D. ZYGOURIS, M.D.
Dimitsanas Street 40
11522 Athens (Greece)
e-mail: zyg14@hotmail.com
Sentinel node mapping with radiotracer alone in vulvar cancer: a five year single-centre experience and literature review

S. Bogliolo1,4, P. Marchiole1,5, P. Sala1, E. Giardina1, G. Villa2, E. Fulcheri3, M. Valenzano Menada1

1 Department of Obstetrics and Gynecology, University of Genoa, Genoa
2 Service of Nuclear Medicine, University of Genoa, Genoa
3 Department of Histology and Pathology, University of Genoa, Genoa
4 Department of Obstetrics and Gynecology, Foundation I.R.C.C.S. Policlinico San Matteo, Pavia
5 Department of Obstetrics and Gynecology, Villa Scassi Hospital-ASL 3, Genoa (Italy)

Summary

Purpose of investigation: The pathologic status of lymph node represents the most important prognostic factor in vulvar cancer patients, but a complete groin dissection is associated with high post-operative morbidity. Sentinel lymph node (SLN) could be representative of the totality of regional lymph nodes and consequently its biopsy might have a significant impact on clinical management in vulvar cancer patients. Materials and Methods: From January 2006 to December 2010 45 patients with vulvar carcinoma are evaluated. Preoperative lymphatic mapping with technetium–99m–labeled nanocolloid was performed in all patients, followed by radioguided intraoperative detection. The detection rate is 100% of patients. All the SLNs were dissected separately for histopathological evaluation and a routine inguinofemoral lymphadenectomy was performed. Results: Nine patients had positive SLNs. In the remaining 36 patients with negative SLNs, one of them showed positive non-SLNs at histological examination. It was the only false negative case in the present series. Conclusions: Based on literature review, lymphoscintigraphy and sentinel node biopsy under gamma-detecting probe guidance offer a reliable and careful method to identify sentinel node in early vulvar cancer. Taking certain guidelines, SLN biopsy seems to be a safe alternative to inguinofemoral node dissection in order to reduce morbidity of surgical treatment.

Key words: Vulvar cancer; Sentinel-node biopsy; Squamous cells carcinoma; Radiotracer; Groin dissection.

Introduction

Vulvar cancer is a rare event in woman. It is responsible for three to four percent of all female neoplasia and for 0.3% of all female cancer deaths [1]. However in the United States 3,900 new cases and 920 deaths annually are reported [1, 2]. The mean age of presentation is about 70 years, affecting elderly women overall. The percentage of young patients is little in number, but has increased fourfold, caused by its association with human papillomavirus (HPV) infections [3]. An HPV association is present in about 40-60% of cases of vulvar cancer, and HPV 16 is the most frequent type of virus related to the tumoral lesions [4, 5]. Frequently the older patients have no evidence of pre-cancerous lesion before diagnosis and they already have positive lymph node.

The majority of vulvar carcinomas are squamous cell carcinomas (SCC), which account for 90% of all vulvar carcinomas [6]. The pattern of spreading is principally via lymphatic channels to the inguinofemoral lymph nodes stations, especially when the depth of invasion exceeds one mm in the primary tumour [7]. The surgical approach with radical vulvectomy and bilateral inguinofemoral lymphadenectomy represent the standard treatment for patients affected by vulvar squamous cell carcinoma. While these techniques were performed, a long-term survival of approximately 70% resulted, with over 90% of Stage I patients alive in five years [8]. During lymphadenectomy, the Gynecologic Oncology Group (GOG) recommended a complete asportation of superficial and deep inguinal nodes, indeed the superficial inguinal node dissection alone is associated with a higher incidence of groin recurrence [9]. In effect, the rate of lymph node metastases in early-stage disease ranges from 25% to 35% of cases [10]. However, a large percentage of patients (ranging from 65% to 75%) will be at risk for significant morbidity when in absence of node metastasis. At short-term after vulvar radical surgery, the most important sequels remain infection and wound breakdown in 20% to 40% of patients, but at long-term the most significant consequence is lymphedema of the legs, present.
in 30% to 70% of patients [11]. Sentinel lymph node (SLN) biopsy seems to reduce incidence of long-time complication. In 1994 Levenback et al. were the first who applied SLN in squamous cell vulvar cancer [12-14].

The present study aims to assess the feasibility, efficacy, and accuracy of sentinel node detection with radiotracer alone, comparing it to the results reported in current literature.

Materials and Methods

From January 2006 to December 2010 a total of 48 women affected by tumour of the vulva were observed at the Department of Gynaecology of University of Genoa, Italy. Before initiation of the study, local Institutional Review Boards had approved the protocol. In 45 (94%) of these patients, the SLN technique was performed. Preoperatively, a diagnostic workup with complete anamnesis, physical and gynaecological examination, complete blood count and metabolic profile, electrocardiogram, thoracic x-ray, and abdomino-pelvic computer tomography were performed for all patients. The inclusion criteria were as follows: histologically confirmed diagnosis of invasive squamous vulvar cancer, no prior chemotherapy or radiotherapy, tumour lesions T1–T2, diameter of lesion ≤ four cm, clinically negative groins, absence of distant metastases, performance status < two according to the World Health Organization (WHO).

Exclusion criteria included: tumour lesions T3–T4,diameter of lesion > four cm and clinically positive groin nodes or distant metastasis.

The median age was 75.5 (range 53-86 years). At the presentation the symptoms were: itch in vulvar region (39% of cases), bleeding (30% of cases), vulvar pain (27% of cases), and only two patients presented only a small mass in genital area without other symptoms. All patients were informed of sentinel node procedure and explained the procedure in detail before the acceptation. At the time of surgery, the SLN was detected before groin dissection, using a gamma probe. In every patient all the non-sentinel inguinofemoral nodes (NSLN) were removed, independently from the status of the sentinel node.

At the clinical FIGO staging, 14 patients were classified as T1 Stage and 31 as T2 Stage.

The lesion was located medially in 32 cases and laterally in 13 cases. For midline lesion the authors imply a tumour whose median margin was within one cm from the midline. Table 1 presents the patients’ characteristics.

In all cases a lymphoscintigraphy was performed 24 hour before surgery: colloid particles of human albumin were labelled with Tc99 and injected near the tumour site. For every patient 40 MBq in a volume of 10 ml were injected subcutaneously by four perilesional injections, according to biopsy results. After 30-60 minutes, planar scans of the vulvar and inguinal areas in anterior and lateral projections were obtained. At the end of the last scan, a cutaneous marker with a suitable pen was signed in correspondence to the first lymph node chronologically revealed by the gamma-camera.

At the time of surgery, the SLN was detected before groin dissection, using a gamma probe, inserted in a sterile glove. The sentinel node was detected at the probe projection, then an inguinal incision was made to excise the lymph node labelled as SLN. The sentinel node was evaluated outside the lesion to confirm the radioactivity level. After sentinel node biopsy, the area was checked again with the gamma probe for residual radioactivity. The nodes radioactivity level was evaluated, when more radioactive nodes were detected in the same area.

The highest tracer uptake node was named as first sentinel node, all the others were considered second nodes. The first sentinel node and the second nodes were sent separately at histological examination. When no residual radioactivity was detected, the superficial and deep inguinal lymphadenectomy was performed as usual.

Surgical vulvar treatment consisted of a standard radical vulvectomy for 32 patients or hemivulvar excision in the remaining 13 patients.

Histological evaluation

The sentinel node and the other removal node were histologically examined. Every node was cut to obtain three- to four-mm thick sections and then fixed with formalin. Every sections was coloured with haematoxylin and eosin and, if there was a doubt, they were coloured again with cytokeratin 1% AE1:AE3 antikeratin solution for immunohistochemical reaction.

Results

In all 45 patients, lymphoscintigraphy detected at least one sentinel node, identified as “hot” lesion by the gamma probe. A total number of 77 groin were investigated. The final number was 100 sentinel nodes among 669 nodes removed. The mean number of node per groin was 8.7, while the mean number of sentinel node was 1.3 per groin. The detection rate respectively “per” patient and “per” groin was 100% and 98.5%, respectively.

In every patient all the non-sentinel inguinofermal nodes (NSNLN) were removed, independently from the status of the sentinel node.

In all 13 patients with unilateral lesion, the sentinel node was always found ipsilateral to the primary lesion: only an ipsilateral lymphadenectomy was performed for these patients, because no contralateral lymphatic drainage was demonstrated.

For 32 patients with midline lesion, a “hot” sentinel node was identified in both groins only five times: in the first
one both the sentinel nodes were metastatic but not the other inguinal nodes; in the other four case the two sentinel nodes were negative as the remaining nodes. In the other 27 patients with midline lesion, the sentinel node was found for 16 times on the right side and for 11 times on the left (Table 2). In all cases of midline lesion the bilateral groins, lymphadenectomy was performed.

At pathologic examination, 17 out of 100 sentinel nodes were positive for metastases. These 17 nodes belonged to nine different patients (Table 3).

In patient n.1 of Table 3, the right sentinel node was the only positive one and it was affected by micro-metastases. In this patient, the inguinal femoral nodes of the right side were removed, according to intraoperative radioactivity detection. Right NSLN were all negative for metastases. In spite of this fact, the patient had a contralateral recurrence two years later, with left inguinal nodes positive for metastases. It is possible that left inguinal nodes were positive at first time of surgery, but the authors did not find signal of any left sentinel node during surgery.

In patient n.5 of Table 3, the authors found a group of nodes identified as SLN at time of surgery. During histologic evaluation, this group was composed by six different nodes and four of these were involved with metastatic spread. Also ipsilateral NSLN were interested by wide metastases.

In a single case out of 45, there was a false negative. This patient was treated with hemivulvectomy and bilateral inguinofemoral lymphadenectomy in January 2006: at gamma probe, a right SLN was found intraoperatively. At histological evaluation, this sentinel node was negative, but another superficial right NSLN was positive.

On the left side no SLN was detected during surgery; a complete groin dissection was performed and final histology found one involved node. After radiotherapy, a vulvar recurrence appeared six months later and further surgery was done with radical wide excision and pelvic lymphadenectomy. Because of distant metastases and neoplastic spread, this patient died six months later.

No difference in sentinel node detection was revealed between patients who have previous vulvar surgery and those with an intact lesion.

The sentinel node detection rate in all patients was 100%, with sensitivity of 90%, and negative predictive value of 97% (Table 4).

### Discussion

SLN mapping offers a promising minimally invasive alternative for surgical staging of vulvar cancer. Inguinal node status is considered the most important prognostic factor: groin recurrences are fatal in the most part of women. Actually, radical inguino-femoral lymph node dissection may be accompanied by an impressive morbidity; early postoperative complication as wound infection and wound breakdown or long-term morbidity, as lymph cysts, groin swelling, edema of the mons pubis, and the lower limb are reported in up to 85% of patients [15]. Chronic lymphedema is reported to occur in up to 30% of the patients [16]. Preoperative non-invasive or minimal invasive methods such as palpation, ultrasound, and ultrasound-guided fine-needle aspiration, as well as magnetic resonance imaging (MRI) and computed tomography (CT) or positron emission tomography (PET) -CT are not sensitive enough to exclude micrometastases in lymph nodes [17].
The SLN concept, validated in melanoma and breast cancer, affords the possibility of avoiding unnecessary lymphadenectomies. SLN technique could provide an opportunity to clearly identify the nodes most likely to harbour metastases. The best method to identify sentinel nodes seems the use of the hand-held gamma probe with preoperative lymphoscintigraphy, according to de Hullu et al. and De Cesare [18, 19]. Using preoperative lymphoscintigraphy and intraoperative gamma-probe, the present authors reached a 100% identification rate in this series (Table 5). It seems clear that lymphoscintigraphy ahead of surgery aids in the process of node localization and significantly lowers the learning curve against dye alone technique [20]. Especially in the case of medially situated tumors, where the lymphatic drainage could be unilateral or bilateral, lymphoscintigraphy offers a reliable preoperative estimate of the location and number of the SLNs [18, 19, 21-29]. As seen in the present study, the primary site of the tumor affects the SLN identification rate: the only true false negative case was in a patient with a medial tumor. Levenback et al. demonstrated that the identification rate is higher for unilateral than for midline tumors (90% vs 69%, respectively) [14]. Hampl et al. reported three cases with false negative SLNs in tumors located in the midline and a single SLN detected, one on each side [30]. Nonetheless, the incidence of midline lesion in the present series points indicates the problem to treat this kind of lesion. Theoretically, surgeons have to investigate both groins, also when bilateral drainage is not demonstrated. Louise-Sylvestre et al. reported three of 13 patients with midline lesions and unilateral drainage; in fact with disease in the contralateral nodal basin [38]. Comparing to other studies, it appears that the further the lesion is localized from the midline, the less likely an SLN will be identified in the contralateral groin [39].

The same problem is faced with multifocal lesions: they must be considered as midline lesions and sentinel node identification must be performed in both groins, to avoid false negative of SLN biopsy [40].

According with Ennik et al., as in the present series, there was no evident difference in sentinel node detection among patients who have previously vulvar surgery and patients with intact lesion [35].

A false negative case is present in this series: Raspagliesi et al. were the first reporting a false negative case using lymphoscintigraphy [41]. The present authors' failure could be given to different reasons:

- at histological evaluation metastases have been found in bilateral inguinofemoral nodes. It indicates that metastatic spread could be change the anatomic conformation of lymphatic channels; superficial nodes could be totally replaced by metastases and a lymphatic stasis - that did not allow radiotracer spread- could be created;
- histological evaluation demonstrated a premature lymphovascular spreading;
- tumour characteristics, as midline position, large dimension (three cm of highest diameter) associated to high body mass index (BMI = 42,2) of the patient may have affected SLN detection.

As reported by other authors in breast cancer [42] or in vulvar cancer patients [28, 38], these features may render more difficult the detection of SLN.

Table 5. — Literature review.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>N° of patients</th>
<th>Detection techniques</th>
<th>Detection rate (%)</th>
<th>N° of false negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levenback et al.</td>
<td>1994-01</td>
<td>52</td>
<td>Blue dye</td>
<td>75</td>
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<td>10</td>
<td>Radiosotope</td>
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<td>0</td>
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<td>De Hullu et al.</td>
<td>1998-04</td>
<td>59</td>
<td>Combined</td>
<td>100</td>
<td>0</td>
</tr>
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<td>1999</td>
<td>51</td>
<td>Blue dye</td>
<td>82</td>
<td>2</td>
</tr>
<tr>
<td>De Cicco et al. [23]</td>
<td>2000</td>
<td>37</td>
<td>Radiosotope</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Sideri et al. [24]</td>
<td>2000</td>
<td>44</td>
<td>Radiosotope</td>
<td>100</td>
<td>0</td>
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<tr>
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<td>2002</td>
<td>26</td>
<td>Combined</td>
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<tr>
<td>Puig-Tintore et al. [26]</td>
<td>2003</td>
<td>26</td>
<td>Combined</td>
<td>96</td>
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<td>Moore et al. [27]</td>
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<td>21</td>
<td>Combined</td>
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<td>Merisio et al. [28]</td>
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<td>20</td>
<td>Radiosotope</td>
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<td>Nyberg et al. [29]</td>
<td>2007</td>
<td>47</td>
<td>Combined</td>
<td>98</td>
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<td>Hampt et al. [30]</td>
<td>2008</td>
<td>127</td>
<td>Combined</td>
<td>98</td>
<td>3</td>
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<tr>
<td>Achimas-Cadariu et al.</td>
<td>2009</td>
<td>59</td>
<td>Radiosotope</td>
<td>94</td>
<td>0</td>
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<tr>
<td>Levenback et al. [32]</td>
<td>2009</td>
<td>515</td>
<td>Blue dye vs Radiois</td>
<td>79 vs 96</td>
<td>-</td>
</tr>
<tr>
<td>Lindell et al. [33]</td>
<td>2010</td>
<td>77</td>
<td>Blue dye vs Radiois</td>
<td>94 vs 98</td>
<td>2</td>
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<td>Radziszewski et al. [34]</td>
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<td>62</td>
<td>Blue dye vs Radiois.</td>
<td>76 vs 99</td>
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<td>2011</td>
<td>65</td>
<td>Combined</td>
<td>94</td>
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<td>Levenback et al. [36]</td>
<td>2012</td>
<td>452</td>
<td>Combined</td>
<td>92.5</td>
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<tr>
<td>Zekan et al. [37]</td>
<td>2012</td>
<td>25</td>
<td>Radiosotope</td>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>

* Combination of three reports [12, 13, 14]; †Combination of two reports [18, 19].
In this time the present authors are in accordance to the recently published statement of the expert-panel which has been formed during the 6th Biennial International Sentinel Node Society Meeting in February 2008. SLN biopsy could be performed only in patients with tumours smaller than four cm and with no clinical suspicious nodes. In case of a midline tumor, a SLN should be identified in both groins. If the SLN is not identified or any doubt exists regarding the correlation between a preoperative lymphoscintigram and the operative findings, then the SLN biopsy procedure should be abandoned and lymphadenectomy performed.

Gynecologic oncologists should perform a certain learning curve (at least ten groin dissections), in a contest of multidisciplinary group, with successful identification of the SLN and no false negative, before recommending SLN biopsy alone [34,40].

In the end, ultrastaging may be recommended for all removed SLNs, especially to identify micrometastases; it has been shown that when SLN harbor only micrometastases (< two mm focus of metastatic cells), non-sentinel nodes from the same inguinal basin will be negative for metastatic disease [43]. When a micrometastases is detected in a SLN, a complementary groin dissection or postoperative inguinal radiotherapy must be performed.

The recent creation of multicentric trials gave us more reliable results. In this regard, a very important European study was conducted in different centres of Netherlands, Belgium, Italy, and Germany: it suggested that sentinel node dissection, performed by a quality-controlled multidisciplinary team, should be part of the standard treatment in selected patients with early-stage vulvar cancer [40]. Indeed this observational study (GROINSS-V) prove that in a manner of 403 patients with FIGO I and II Stages with squamous cell carcinomas of the vulva ≤ four cm in size, in case of a negative sentinel node, a complete inguino-femoral lymphadenectomy can be omitted. The data analysis showed that, in a follow-up after two years, in the presence of unifocal tumor, the percentage of inguinal relapse was 2.3% (six groin recurrences in 259 patients) and a three-year survival rate of 97% [40]. However, Oonk et al. showed that the prognosis for patients with sentinel node metastasis larger than two mm is poor and they suggested new treatment regimens for these patients [44].

The result of GOG protocol 173, begun in 2000, were presented by Leenback et al. at the annual meeting of the American Society of Clinical Oncology (ASCO) in 2009 [32]. In 411 patients with vulvar cancer and clinically negative groin status, 282 patients had negative nodes and 129 patients had positive nodes. The study showed a sensitivity of 89.9%, a negative predictive value of 95.6% and a false-negative predictive value of 4.4%.

In terms of morbidity related to surgical procedure, two studies showed that the SLN biopsy was associated with significantly less short-term and long-term morbidity, in comparison to complete lymph node dissection.

At the analysis of short-term morbidity, Van der Zee et al. reported that wound breakdown occurred in 11.7% and cellulitis in 4.5% in SLN patients vs respectively, 34% and 21.3% in inguinal lymphadenectomy patients ($p < 0.001$) [40]. Also, the time of hospitalisation is significantly shorter in event of SLN biopsy when compared to inguinal lymphadenectomy (8.4 days vs 13.7 days ($p < 0.001$) [40]. At the analysis of long-term morbidity, Van der Zee et al. showed that the incidence of lymphedema and recurrent erysipelas decreased significantly from 25.2% to 1.9% and from 16.2% to 0.4%, respectively, in SLN biopsy patients vs inguinal lymphadenectomy ($p < 0.001$) [40]. Relative to rate of lymphedema, Johann et al. also reported that this rate decreased from 39% to 13% in SLN biopsy patients [45].

Conclusion

The authors believe that sentinel node procedure in the management of early-stage vulvar cancer could decrease morbidity without compromising groin recurrence or survival rates, while taking certain guidelines into account.

References


Address reprint requests to:
S. BOGLIOLO, M.D.
Department of Obstetrics and Gynecology,
Foundation I.R.C.C.S. Policlinico San Matteo,
19, p.le Golgi, 27100, Pavia (Italy)
e-mail: s.bogliolo@smatteo.pv.it
Introduction

Although great advance has been made in the understanding and therapy of ovarian cancer, it still remains the sixth most common leading-death cancer in women in the world [1]. Ovarian carcinoma can be classified into three broad subgroups such as epithelial, stromal, and germ cell tumors, each of them with different etiologies and clinical behavior. Epithelial ovarian cancer is the most common, accounting for more than 85% of all cases of ovarian cancer. Despite progress in platinum-based chemotherapy have resulted in improved survival, patients typically experience disease relapse within two years of initial treatment and develop platinum resistance [2]. Greenlee et al. have stated that the diminishing response rate for platinum-based drugs is due to the development of drug resistance, resulting in only 30% of five-year survival rate among patients with advanced ovarian cancer [3].

Currently, extensive studies have illustrated the existence and importance of mechanism of chemotherapeutic resistance mediated by means of short noncoding RNA [4-6]. Miller et al. has identified that miR-221/222 expression and HER2/neu overexpression in primary breast tumors that are generally resistant to tamoxifen therapy [7]. Moreover, it has been verified that miR-214 [8] and has-mir-27a [9] can induce platinum-based drug resistance in human ovarian cancer.

Here, the present authors used a cell culture model to determine the miRNA expression profile of a platinum-resistant cell line that was subsequently validated in primary human ovarian cancers.

Materials and Methods

Patients and specimens

Ovarian cancer COC1 and COC1/DDP cell-line were collected from Chinese center for typical culture collection. Specimens for real-time quantitative polymerase chain reaction (qRT-PCR) were collected from 86 patients with ovarian serous carcinoma. All patients treated with paclitaxel and carboplatin were followed up for six months. According to the National Comprehensive Cancer Network (NCCN) ovarian cancer practice guideline [10], chemotherapy resistance was defined if patients were observed tumor recurrence within six months after chemotherapy, whereas sensitive chemotherapy was considered if tumor recurrence was not reported within or more than six months after chemotherapy. In this study, 20 cases were in chemotherapy resistant group and 20 cases were in chemotherapy sensitive group. Characteristics of specimens derived from patients with ovarian cancer are illustrated in Table1.

Cell lines and cell culture

The human ovarian cancer cell line of COC1 and COC1/DDP were cultured in medium RPMI 1640 containing 10% newborn calf serum and 40 μg/ml gentamicin at 37°C in a 5% CO2 atmosphere. COC1 and COC1/DDP cells at logarithmic growth phase (80%-90%) were harvested and seeded into 250 ml flask at the density of 5 x 10⁶/ml for 24 hours.

RNA extraction

The small and total RNA fractions were isolated using the miRNeasy mini kit and TRI reagent, respectively. Both proce-
dures were followed according to the manufacturer’s recommendations. Quantity and purity of the RNA were tested using a nanodrop spectrophotometer. RNA integrity was determined by running agarose gel electrophoresis.

**miRNA microarray hybridization**

One μg total RNA from each sample were labeled with Hy3 and Hy5 fluorescent label, respectively, with the help of the miRCURY array power labeling kit following the instructions. The Hy3 and Hy5 labeled reference RNA sample were mixed pairwise and hybridized to the miRCURY LAN array, which contained 1,891 capture probes targeting all of the miRNAs for all the species registered in the miRBase (Version 16.0) at the Sanger Institute. Hybridization signals were detected by biotin binding of a streptavidin alexa 647 conjugate using a scanner 4000B. The images were quantified by GenePix 6.0 software.

**miRNA microarray data analysis**

Microarray data were analyzed in R using the linear models for microarray data package. Poor quality (flagged) spots were excluded from the analysis. The array results were background-corrected using the Normexp method [11]. The intensities were then log2-transformed and normalized, using the LIMMA implementation in quantile normalization. Subsequently, the normalized microarray data were managed and analyzed by scatter plot, volcano plot, and MEV software (version 4.6).

**qRT-PCR**

The expression level of miR-141-3p in COC1 vs. COC1/DDP group as well as chemotherapy resistant group (n=20) vs. chemotherapy sensitive group (n=20) were detected by qRT-PCR. Here, the authors only validated the most significant miRNA namely miR-141-3p. To quantify miRNA, the expression of the miR-141-3p included in the TaqMan MicroRNA assays human panel kit was examined according to the manufacturer’s protocol. To validate the results, the TaqMan kit specified that the quantification of miR-141-3p be used, with normalization to the U6 small nuclear RNA (U6 snRNA). RNA (20 ng) from each sample was reverse transcribed using a TaqMan human microRNA assays kit. To quantify the miRNA, 20 μl of RNA from each sample was reverse transcribed into complementary DNA (cDNA) using a PrimeScript RT reagent kit. The resulting cDNA was amplified by PCR using TaqMan microRNA assay primers with the TaqMan universal PCR master mix and analyzed with a 7500 ABI sequence detector system according to the manufacturer’s instructions. The non-coding RNU6B (U6 control) was used as housekeeping control: F: 5’GCTTCGGCAGCACATATAC-TAAAAT3’; R: 5’CGCTTCACGAATTGGCTGTCAT3’. In addition, gene-specific primer (GSP) of hsa-miR-141-3p was F: 5’GGGTAAACACTGTCTGGTAA‘; R: ‘TGCGTG TCGTG-GAGTC3’. Reaction substrate was dissolved in diethyl-pyrocarbon-(DEPC)-treated water in the ratio of 1:10, 1:100, 1:1000 and 1:10000.

**Statistical analysis**

The relative gene copy number was estimated by real time PCR using the ΔΔCt method [12]. Statistical analysis was performed by using the SPSS statistics software package (SPSS). All results were expressed as mean ± SD were evaluated for statistical significance using Student’s t test. A p value < 0.05 was used for significance.

**Results**

**Distinct miRNA signatures in ovarian cancer COC1/DDP, compared with COC1**

To identify miRNAs differentially expressed in COC1/DDP compared with the COC1, the authors used a customized miRNA microarray that contained more than 1800 miRNAs (Figure 1). Seventeen miRNAs were differentially expressed between COC1 and COC1/DDP. The up-regulated miRNA (n=13) were hsa-miR-1290, hsa-miR-636, hsa-miR-1973, hsa-miR-3687, hsa-miR-3621, hsa-miR-200c-3p, hsa-miR-195-5p, hsa-miR-96-5p, hsa-miR-141-3p, hsa-miR-491-3p, hsa-miR-3135a, hsa-miR-149-3p, and hsa-miR-3149 (Table 2). The downregulated miRNAs (n = 4) were hsa-miR-933, hsa-let-7g-5p, hsa-let-7i-5p, hsa-miRPlus-G1246-3p (Table 3). Of these miRNAs, hsa-miR-141-3p was the most significantly different ex-
pressed miRNA between COC1/DDP and COC1 group, and may have a potential role in the mechanism of platinum-resistance in ovarian cancer.

Validation of the microarray results

The method of qRT-PCR was used to validate the microarray results in COC1/DDP and COC1 cell-lines. The authors selected the most frequently hsa-miR-141-3p for qRT-PCR test. Of note, the expression level of hsa-miR-141-3p was significantly higher in COC1/DDP rather than in COC1 cell-lines (14.0433 ± 4.4895 vs. 1.7833 ± 0.7213, p < 0.05, Figure 2). U6 amplification plot and hsa-miR-141-3p amplification plot are clearly illustrated in Figure 3.

The expression level of hsa-miR-141-3p between chemotherapy resistant and chemotherapy sensitive group

In order to detect the different expression level of hsa-miR-141-3p between chemotherapy resistant (n=20) and chemotherapy sensitive group (n=20), the authors also used qRT-PCR. Similar to the result of comparing COC1/DDP and COC1 cell-lines, hsa-miR-141-3p was more frequently upregulated in chemotherapy resistant group rather than in chemotherapy sensitive group (9.56 ± 1.04 vs. 1.59 ± 0.91, p < 0.05)

Discussion

To date, numerous findings have confirmed a critical role of miRNA as powerful diagnostic and prognostic indicators of human ovarian cancer [10, 13-15], resulting in the development of novel approaches to ovarian cancer management [8]. Despite the well-established role of miRNA in cancer [16, 17] and the understanding of the molecular mechanisms involved in the development of chemotherapy-resistant cancer cells, the role of miRNA in cancer drug resistance is still largely unexplored.

In this present study, the authors provided data indicating the importance of altered expression of miRNA in the acquisition of ovarian cancer cells resistance to platinum-based
drugs. Their findings showed that upregulation of has-miR-1290, has-miR-636, has-miR-1973, has-miR-3687, has-miR-3621, has-miR-200c-3p, has-miR-195-5p, has-miR-96-5p, has-miR-141-3p, has-miR-491-3p, has-miR-3153a, has-miR-149-3p, has-miR-3149, and downregulation of has-miR-933, has-let-7g-5p, has-let-7i-5p, has-miRPlus-G1246-3p were in COC1/DDP compared to COC1 cell lines. Of which, let-7 family [18] and miR-200c family [19] and miR-141 [20] have been widely reported in the development of tumorigenesis.

Ectopic expression of miR-200 family (miR-200a, miR-141, miR-200b, miR-200c, miR-429) expression has been detected in human ovarian cancer [21-23]. Of which, miR-200c was found directly target the mRNA of the E-cadherin transcriptional repressors ZEB1 (TCF8/δEF1) and ZEB2 (SMAD-interacting protein 1 [SIP1]/ZFXH1B). Altered expression of miR-200 resulted in up-regulation of E-cadherin in cancer cell lines [24]. Li et al. have demonstrated that the gradually increment of upregulated miR-141 and miR-200c expression was detected in normal ovarian tissue, borderline ovarian tumors, and ovarian cancer, respectively. However, expression of miR-200c was downregulated in ovarian metastatic carcinoma, poorly differentiated ovarian carcinoma and ovarian clear cell adenocarcinoma. In addition, poor prognosis of ovarian cancer was found in miR-200c downregulated group compared to upregulated counterpart, suggesting that miR-200c can be severed as a potential prognostic indicator in the clinical practices [25]. In this study, miR-200c-3p, one of miR-200 membership, was detected to be upregulated in COC1/DDP group, which indicates that miR-200c-3p may be associated with the development of platinum-resistance in ovarian tumors.

Furthermore, PTEN (antioncogene) as one of the target genes of miR-141 was found to inhibit tumor cell growth, migration and invasion through regulating the P13K/PKB signaling pathway. Indeed, PTEN can induce the cell cycle arrest at G1 stage and initiate apoptosis. Lost function of PTEN can result in cell over-proliferation and elicit tumorigenesis [26]. Importantly, in this present study, the authors identified that miR-141 was upregulated in COC1/DDP compared to that in COC1 group, indicating that miR-141 may have a potential role in the mechanism of platinum-resistance in ovarian cancer cell line.

Takamizawa et al. have reported that Let-7 can inhibit the growth of lung carcinoma A549 cell in vitro. Hence the Let-7 expression level is considered as an independent prognostic factor in lung cancer [27]. The present findings further demonstrate that has-let-7g-5p and has-let-7i-5p (Let-7 family) were downregulated in ovarian cancer COC1/DDP compared to COC1. Conversely, Van et al. have stated that let-7g was upregulated in platinum-resistant ovarian cancer cell line [28]. Looking at this discrepancy of the results, the present authors elucidate that the expression of miRNAs may have a highly association with some factors such as different tumor stage, primary or recurrent cancer and various platinum-based drugs.

The present authors will concentrate on studying the role of miR-141-3p and its target genes in platinum-resistant ovarian cancer because of several reasons: 1) miR-141-3p was found to be most frequently up-regulated in COC1/DDP compared to COC1 (a fold-change of 31.01228); 2) most studies have reported the mechanism of miR-141 in regulating tumor apoptosis. Yet, less evidence has shown its role in platinum-resistance; 3) the molecular function of other two miR-141-3p target genes, BRD3 and UBAP1, still remains unclear.

Conclusions

Altogether, the present authors have identified other miRNAs that are differentially expressed in platinum-resistant cell lines and chemotherapy resistant group. Some of these miRNAs could emerge as potential biomarkers of platinum-resistant tumors. Identification of target genes of these miRNAs will further enhance our knowledge of platinum resistance and facilitate design of new therapeutic agents targeting these proteins.

Acknowledgements

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References


Address reprint requests to:

H. YING, M.D
Department of Gynecology and Obstetrics,
Shengjing Hospital of China Medical University,
No.36, Sanhao Street, Heping District,
Shenyang, Liaoning Province, 110004 (China)

e-mail: huanchunying@hotmail.com
Prevalence of endometriosis in epithelial ovarian cancer. Analysis of the associated clinical features and study on molecular mechanisms involved in the possible causality

F. Machado-Linde1, M.L. Sánchez-Ferrer1, P. Cascales2, A. Torroba3, R. Orozco1, Y. Silva Sánchez1, A. Nieto1, G. Fiol1

1Department of Gynecology and Obstetrics, University Hospital Virgin Arrixaca, Murcia
2Department of General Surgery, University Hospital Virgin Arrixaca, Murcia
3Department of Pathology, University Hospital Virgin Arrixaca, Murcia
4Department of Gynecology and Obstetrics, Hospital Torrecardenas, Almeria (Spain)

Introduction

Endometriosis is an estrogen-dependent inflammatory disease defined by the presence and growth of foci in endometrial tissue (stroma and glands) outside the uterine cavity [1]. The most common anatomic site of endometriosis is the pelvic peritoneum and the ovarian surface, which leads to adhesions, chronic pelvic pain, and infertility [2]. The prevalence of endometriosis is 7% to 15% among women of childbearing potential, but as high as 25% to 30% in infertile women and up to 40% to 70% in women with chronic pelvic pain [3]. The exact etiopathology is unknown. The theory most widely accepted is Sampson’s classic theory on menstrual reflux through the fallopian tubes [4]. Although considered a benign disease, endometriosis presents some characteristics that resemble malignant neoplasms, such as the development of local and distant sites and the invasion of other target organs [5]. Additionally, several studies have consistently shown that endometriosis is associated with a higher risk of epithelial ovarian cancer [6-12]. This association is predominantly related to the clear-cell and endometrioid histologic subtypes of ovarian cancer [13]. However, not all authors confirm the association of endometriosis with ovarian cancer [14]. This study focused on the prevalence of endometriosis in patients who underwent surgery for ovarian cancer at the present hospital between 1971 and 2010.

Material and Methods

The study reviewed the anatomic pathology (AP) reports and medical histories of all patients operated for ovarian cancer (n = 496) from 1971 to 2010 in the Obstetrics and Gynecology Department of the Hospital Clínico Universitario Virgin de la Arrixaca (Murcia, Spain). Patients with ovarian tumor of low malignant potential were excluded from this study. The ovarian cancers were histologically classified according to the World Health Organization classification of ovarian tumors [15]. Concomitant endometriosis was determined by a review of the AP reports and the presence of glandular epithelium accompanied by endometrial stroma as described in the reports. The association of ovarian cancer and endometriosis was defined according category C by Van Gorp et al. [16]: C) ovarian cancer with concomitant endometriosis at any site in the pelvis: endometriosis in both ovaries, in the contralateral ovary, extragonadal, or unspecified lateralization or lesion site. The following variables were also collected from the patients’ medical history: age, parity, menopausal status at the time of surgery, and disease stage. Patients were staged according to the International Federation of Gynecology and Obstetric (FIGO) criteria [17]. Patients were grouped into two categories according to surgical staging: early stage (FIGO Stages I

Summary

Purpose of investigation: To determine the prevalence of endometriosis in patients with epithelial ovarian cancer and explore the differences between women with endometrioid and clear-cell histologic subtypes with and without associated endometriosis. Materials and Methods: The medical charts of 496 patients with epithelial ovarian cancer at the Hospital Virgin de la Arrixaca (Murcia, Spain) between 1971 and 2010 were reviewed. Results: Endometriosis was present in 27 (5.4%) of the 496 cases (p < 0.001), and was associated with the endometrioid histotype in 13/45 cases (29%) and with the clear cell histotype in 7/22 (32%). The prevalence of an association with endometriosis according to histologic type was 28.8% (13/45) for endometrioid carcinoma and 31.8% (7/22) for clear-cell carcinoma. Conclusion: Both endometrioid and clear-cell ovarians tumours are associated with pelvic endometriosis. Patients with endometriosis associated ovarian cancer differ from non-endometriosis associated ovarian cancer in their clinical characteristics.

Key words: Endometriosis; Ovarian cancer.
and II) or advanced stage (Stages III and IV). The authors performed a descriptive study, in which numeric variables were described as mean ± SD. The qualitative variables are summarized as frequencies and percentages. For the comparative studies, the authors used the Pearson chi-squared test to analyze associations between qualitative variables and the Student t test for comparisons of means. Statistical significance was set at $p < 0.05$. SPSS 18.0 was used for the statistical analysis.

**Results**

A total of 496 cases of ovarian cancer were treated at the present hospital over 39 years; endometrioid and clear-cell histologic subtypes were identified in 45 (9.2%) and 22 patients (4.4%), respectively. A significant association between ovarian cancer and endometriosis was found in 27 cases (5.4%) ($p < 0.001$). Among the 45 patients with endometrioid carcinoma, association with endometriosis was found in 13 (28.8%), which accounted for 26.6% of all ovarian cancers in the series and 48.1% ($p < 0.079$) of all ovarian cancers associated with endometriosis. Regarding clear-cell carcinoma, the presence of endometriotic tissue was observed in 7/22 cases (31.8%), specifically in 1.4% of all cancers presenting with endometriosis. In clear-cell carcinoma patients, 85.7% of patients with endometriosis and 78.6% of those without endometriosis were menopausal. Nulliparous patients accounted for 24.2% of patients with endometrioid carcinoma but no endometriosis and 30.8% of patients with endometrioid carcinoma and endometriosis. In clear-cell carcinoma patients, 26.6% of those without endometriosis had no offspring, compared to 42.8% in those with endometriosis. Lastly, 42.3% (11/26, unknown stage in six cases) of patients with endometrioid carcinoma not associated with endometriosis presented an early stage of the disease (FIGO Stage I/II) versus 75% (9/12, unknown stage in one case) of cases associated with endometriosis. In patients with clear-cell carcinoma, 53% (7/13; unknown stage in two cases) presented early stage when the cancer was not associated with endometriosis compared to 83% (5/6; unknown stage in one case) when associated with endometriosis. A total of 2/7 (28%) patients diagnosed with other tumors associated with endometriosis presented early stage (Table 1 summarizes the results).

**Discussion**

The incidence of endometriosis in ovarian cancer in the present series was 5.4% ($p < 0.0001$), a figure within the range reported by other authors (4%-29%) [18]. These findings are difficult to interpret because the actual incidence of endometriosis is unknown. The true incidence is generally agreed to be around 10% of women of childbearing potential [3] and, therefore, the association of endometriosis with ovarian cancer would be in a similar range to that of the general population. In the present series, as in most published series, the histologic types of epithelial ovarian cancer most commonly associated with endometriosis were endometrioid carcinoma (48%) and clear-cell carcinoma (25.9%) [19, 20]. The association with certain histologic types is the most relevant evidence in establishing a causality relationship between ovarian cancer and endometriosis. Although this may seem to be proven, there are two important aspects to consider before this evidence is applied to daily clinical practice in patients with endometriosis. Firstly, the endometrioid and clear-cell histologic types of ovarian cancer are rare, in the

<table>
<thead>
<tr>
<th>Factors</th>
<th>OCE</th>
<th>CC6CAE</th>
<th>CCNAE</th>
<th>EAE</th>
<th>ENAE</th>
<th>OAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± standard deviation)</td>
<td>51 (±13)</td>
<td>51 (±13.9)</td>
<td>55.1 (±11)</td>
<td>55 (±12.9)</td>
<td>53.8 (±15.9)</td>
<td>50.9 (±11.5)</td>
</tr>
<tr>
<td>FIGO, Stage I/II</td>
<td>83%</td>
<td>53%</td>
<td>75%</td>
<td>42.3%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>55.5%</td>
<td>85.7%</td>
<td>78.6%</td>
<td>53.8%</td>
<td>37.5%</td>
<td>28.6%</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>42.8%</td>
<td>26.6%</td>
<td>30.8%</td>
<td>24.2%</td>
<td>42.9%</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** OCE: ovarian cancer associated to endometriosis; C6CAE: clear cell associated to endometriosis; CCNAE: clear cell not associated to endometriosis; EAE: Endometrioid associated to endometriosis; ENAE: Endometrioid not associated to endometriosis; OAE: another (histologic subtype) associated to endometriosis.
present series accounting for 4.4% and 9% of total cases. When related to the total sample, association with endometriosis was even more rare: 2.6% of endometrioid type and 1.4% of clear-cell type of ovarian cancers treated in this series. Secondly, although some evidence already indicates that atypical endometriosis may be the precursor of these two histologic types of ovarian cancer, it is still not conclusive [21]. Some authors have identified a mutation of the ARID1A tumor-suppressor gene in endometrioid and clear-cell carcinoma samples and in adjacent endometrial tissues [22, 23]. These genetic alterations have also been observed in endometrioid tissues not associated with ovarian cancer, particularly in endometriosis [24]. However, confirmation that the endometrial tissue in which these molecular abnormalities develop is a premalignant lesion would require comparing them to samples from the same patients who develop ovarian cancer years later. Wiegand et al. [25] reported the case of a patient diagnosed with atypical endometriosis who showed reduced BAF250 expression and presented endometrioid carcinoma at the same site two years later, although the lesion was possibly already present and simply unnoticed. Ayhan et al. [26] recently reported that in 31 of 47 cases of endometrioid and clear-cell carcinoma associated with endometriosis, they found a decrease in ARID1A immunoreactivity in the carcinoma and epithelium of the endometriotic cyst adjacent to the carcinoma, but not in the epithelium of the cyst not adjacent to the tumor.

In the present series, the authors found no statistical differences between the endometrioid and clear-cell histologic types in the clinical parameters analyzed: age, menopausal status, and parity. However, they observed that the percentage of nulliparous women was higher among patients with ovarian cancer associated with endometriosis (for both clear-cell and endometrioid subhistologic types) than among those with no associated endometriosis. These data are consistent with those published by other authors. Nevertheless, it is not clear if sterility or endometriosis is the true risk factor, because endometriosis is itself a cause of sterility [27, 28]. A case-control study by Nagle et al. [29] compared endometrioid and clear-cell carcinoma, but not in relation to endometriosis association, and found a higher risk for both histologic types in patients with endometriosis and a lower risk in patients with at least one term pregnancy. Therefore, it is unclear if this lower parity-related risk can be attributed to the protective effect of pregnancy on ovarian cancer or if the presence of endometriosis, known to cause sterility, raises the risk [30, 31]. Similar to reports published by other authors [27, 32], patients with endometrioid carcinoma were somewhat younger than those who presented clear-cell carcinoma, a difference that was more pronounced in endometrioid carcinoma associated with endometriosis in the present study. In terms of menopausal status, both Nagle et al. [29] and the present studies found a higher number of menopausal patients, but the authors observed that the percentage was higher when both histologic types were associated with endometriosis (53% vs. 37.5% in endometrioid and 85% vs. 78% in clear-cell). Orezzoli et al. [33] studied clear-cell carcinoma based on an association with endometriosis and observed a higher percentage of menopausal and nulliparous patients among patients with clear-cell carcinoma associated with endometriosis, compared to those not associated with endometriosis.

In the present series, patients with endometrioid carcinoma presented less advanced (Stages I/II) disease compared to patients diagnosed with clear-cell carcinoma. These findings are similar to other publications that report a more somber prognosis for clear-cell carcinoma [34]. Thus, the present authors observed that the percentage of patients in early stage was higher in both histologic types when concomitant with endometriosis. Other authors also reported a better prognosis for cancer associated with endometriosis [29, 35].

In a Swedish cohort study, Melin et al. [36] studied the repercussions of endometriosis on the prognosis of various malignant diseases. They reported greater survival among patients with endometriosis, particularly in patients with breast or ovarian cancer; however, the presence of endometriosis casts a shadow over the prognosis of patients with melanoma. Nonetheless, the repercussions of endometriosis coexistence with ovarian cancer on prognosis are still debated. Cuff et al. [28] studied clear-cell and endometrioid carcinomas, but found no significant relationship between disease-free survival and the presence of endometriosis. In this regard, Katagiri et al. [37] considered the finding of the ARID1A gene mutation in clear-cell carcinoma to be a negative prognostic factor, as its presence determines a higher rate of resistance to platinum-based chemotherapy and a shorter disease-free period. Furthermore, as the present authors have stated, some findings [22, 24] reveal an important correlation between the ARID1A mutation and endometrioid and clear-cell ovarian carcinomas and between these two types of carcinomas and atypical endometriosis. Consequently, the current findings are controversial. The presence of the ARID1A gene mutation may be related to poorer response to treatment in clear-cell carcinoma [35] or to a more promising prognosis in clear-cell and endometrioid carcinomas associated with endometriosis [33]. Other authors have found no endometriosis-related differences in the prognosis of these ovarian cancer histiotypes [32].

Conclusion

In conclusion, although the present study was descriptive and retrospective and, therefore, had all the limitations common to these types of studies, the series confirms a significant association between endometrioid or clear-cell ovarian cancer and endometriosis. Despite this, there is currently no evidence to suggest a change in the treatment of endometriosis in clinic practice.
References


Address reprint requests to:
M.L. SÁNCHEZ FERRER, M.D.
Department of Gynecology and Obstetrics,
University Hospital Virgen Arrixaca
30120 Murcia (Spain)

E-mail: marisasanchezferrer1@gmail.com
Laparoscopic ovarian transposition in young women with cervical squamous cell carcinoma treated by primary pelvic irradiation

H. Shou, Y. Chen, Z. Chen, T. Zhu, J. Ni

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

Summary

Objective: To report the authors’ experience with laparoscopic ovarian transposition and ovarian function preservation in young women with cervical squamous cell carcinoma treated by primary pelvic irradiation. Materials and Methods: Twenty-seven premenopausal patients were treated with radiotherapy for a cervical squamous cell carcinoma. Laparoscopic ovarian transposition to paracolic gutters with uterine conservation with pelvic common iliac lymph node and para-aortic lymph node sampling were performed in ten patients at the same time of laparoscopic ovarian transposition. Preservation of ovarian function was assessed by patients’ symptoms and serum follicle-stimulating hormone level. Results: Bilateral or unilateral laparoscopic ovarian transposition was performed in 27 patients: 22 cases Stage IIB, one case Stage IIIA, and four cases Stage IIIB. No immediate intraoperative or postoperative complications were observed. Two of the ten patients were confirmed by lymph node metastases. One patient was lost to follow-up. Ovarian preservation was achieved in 18 (69.2%) of 26 patients. No patient was detected with ovarian metastasis at follow-up. Conclusions: Laparoscopic ovarian transposition is a safe and effective procedure for preserving ovarian function. This procedure may be considered in premenopausal women who need to undergo pelvic irradiation for cervical squamous cell carcinoma, especially for those less than 40 years of age. Otherwise, para-aortic lymph node or common iliac lymph nodes sampling at the same time of laparoscopic ovarian transposition may preferably guide radiation therapy.

Key words: Ovarian transposition; Squamous cell carcinoma; Pelvic irradiation; Laparoscopy.

Introduction

Recent studies on the treatment of cancer have focused on the quality of life (QOL) of patients. The total number of patients with cervical carcinoma includes an increasingly large percentage of young women [1], and preservation of ovarian function is thought to be particularly important to the physiologic and psychosexual well-being of these patients. The loss of ovarian function in such young women is one of the usual consequences of chemotherapy and radiotherapy, causing climacteric symptoms, which often seriously impair their QOL [2]. The conventional method of treatment has been radiological for middle and advanced stage cervical cancer. When the ovaries are preserved in a normal position, ovarian function can easily be lost due to radiation. A review of published studies indicated that the ovarian metastasis rate from uterine cervical cancer is very low, especially in cervical squamous cell carcinoma. Therefore the authors performed a transposition of the ovaries through laparoscopy in patients with cervical squamous cell carcinoma, so that they would be outside of the field of radiation during postoperative radiotherapy.

Materials and Methods

Using a retrospective review of the medical records, the authors identified all women who underwent laparoscopic ovarian transposition at Zhejiang Cancer Hospital between August 2008 and August 2011. Patients and tumor characteristics as well as treatment plan and follow-up data were collected (Tables 1 and 2). Thirteen women younger than 45 years were included. The mean age of the patient population was 34.6 years (range, 25-44); 22 patients were younger than 40 years. Twenty-two cervical cancers were Stage IIB, One was Stage IIIA and another four were Stage IIIB (FIGO staging system). All the histological types are squamous cell carcinoma. All had received pelvic radiotherapy and concurrent cisplatin-containing chemotherapy and brachytherapy. Laparoscopic ovarian transposition was completed before pelvic irradiation. All had a history of regular menstrual cycle and no vasomotor symptoms before the ovarian transposition. Ten patients, who had probable positive lymph node with preoperative radiologic imaging and in which lymph node puncture biopsy was difficult to perform, underwent pelvic common iliac lymph node and para-aortic lymph node sampling at the same time of laparoscopic ovarian transposition. A random follicle-stimulating hormone (FSH) level was obtained before the procedure. The surgery was not performed if the patient had (1) evidence of carcinomatosis, ovarian, or tubal metastasis on imaging study and (2) amenorrhea and/or vasomotor symptoms and serum FSH level greater than 40 IU/L. The patients were informed of the risks and benefits, with a description of the procedure, and informed consen-
sents were obtained. After therapy completion, all patients were evaluated by a radiation oncologist and gynecologic oncologist after one month, followed by evaluations at three-month intervals for two years and then every six months thereafter. On follow-up, ovarian function was evaluated by the presence or absence of postmenopausal symptoms and by the measurement of FSH. Transient ovarian failure may last for a long time, and the authors defined ovarian failure as FSH levels elevated (> 40 U/L) twice apart (in three- to six-month interval) two years after complete treatment of cancer. The local ethics committee at Zhejiang cancer Hospital approved the study.

### Surgical procedure

The operation was performed under general anesthesia. A total of three trocars were used. First, a ten-mm trocar was inserted through an umbilical incision, and the endoscopic camera was then introduced. Two extra five-mm trocars were placed in each lumbar quadrant. All pelvic and abdominal structures were inspected in a clockwise fashion before proceeding with the procedure. If adhesions were noted around the uterus, ovaries, pelvic cavity, and thought to interfere with the procedure, then adhesiolysis was performed. The utero-ovarian ligaments were divided. The ovaries were then mobilized on the infundibulopelvic ligaments. The peritoneum under and lateral to the infundibulopelvic ligaments were incised enough to reach the level of iliac crest, under direct vision of the ureter. The ovaries were fixed in the paracolic gutters at the level of pelvic brim with a sufficient angle to maintain good blood supply. The upper and lower poles of the ovaries were marked with metal clips. Pelvic common iliac lymph node and para-aortic lymph node sampling was performed in patients with probable positive lymph node at the same time of laparoscopic ovarian transposition.

### Results

The mean operative time was 125 minutes (range, 70-170) and average blood loss was 59 ml (range, 10–100) (Table 3). Twenty-one patients were thought to be appropriate for bilateral ovarian transposition; in the re-

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**Table 1. — Patient characteristics.**

<table>
<thead>
<tr>
<th>Variable (n. = 27)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at operation (range)</td>
<td>34.6 years (25–44)</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>5</td>
</tr>
<tr>
<td>&lt; 40 years</td>
<td>22</td>
</tr>
<tr>
<td>Cancer Stage^a (n. of cases)</td>
<td>27</td>
</tr>
<tr>
<td>IIB</td>
<td>22</td>
</tr>
<tr>
<td>IIIA</td>
<td>1</td>
</tr>
<tr>
<td>IIIB</td>
<td>4</td>
</tr>
</tbody>
</table>

^a All the histological types were squamous cell carcinoma and Stage for cervical cancer according to International Federation of Gynecology and Obstetrics (FIGO) classification.

**Table 2. — Summary of patients characteristics, treatment, and outcome.**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age, yrs</th>
<th>Diagnosis</th>
<th>Surgical procedure</th>
<th>Lymph node metastasis</th>
<th>Elevated FSH</th>
<th>Ovarian benign lesion</th>
<th>Ovarian metastasis</th>
<th>Survival</th>
<th>F/U</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>IIB</td>
<td>BOT+PLNS+PALNS</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
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<td>BOT</td>
<td>-</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
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<td>BOT</td>
<td>-</td>
<td>NO</td>
<td>Ovarian cyst</td>
<td>NO</td>
<td>YES</td>
<td>31</td>
</tr>
<tr>
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<td>BOT</td>
<td>-</td>
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<td>NO</td>
<td>NO</td>
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<td>26</td>
</tr>
<tr>
<td>5</td>
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<td>IIB</td>
<td>BOT</td>
<td>-</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
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<td>UOT</td>
<td>-</td>
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</tr>
<tr>
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<td>UOT</td>
<td>-</td>
<td>NO</td>
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<td>NO</td>
<td>YES</td>
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<tr>
<td>8</td>
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<td>YES</td>
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<td>NO</td>
<td>NO</td>
<td>YES</td>
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<td>9</td>
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<td>BOT</td>
<td>NA</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>25</td>
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<tr>
<td>10</td>
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<td>IIIA</td>
<td>BOT+PLNS+PALNS</td>
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<td>YES</td>
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<td>NO</td>
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<tr>
<td>11</td>
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<td>IIB</td>
<td>BOT</td>
<td>-</td>
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<td>NO</td>
<td>NO</td>
<td>YES</td>
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<td>12</td>
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<td>BOT+PLNS+PALNS</td>
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<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>38</td>
<td>IIB</td>
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<td>NO</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
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<td>IIB</td>
<td>UOT+PLNS+PALNS</td>
<td>NO</td>
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<td>26</td>
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</table>

BOT: bilateral ovarian transposition; UOT: unilateral ovarian transposition; PLNS: pelvic common iliac lymph node sampling; PALNS: para-aortic lymph node sampling; NA: not available; F/U: follow-up in months.
Laparoscopic ovarian transposition in young women with cervical squamous cell carcinoma treated by primary pelvic irradiation

Table 3. — Results.

<table>
<thead>
<tr>
<th>Operation time, mean (range)</th>
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<tr>
<td>Estimated blood loss, mean (range)</td>
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<tr>
<td>Hospital stay, mean (range)</td>
<td>4.9 days (3–8)</td>
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<td>Complications</td>
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<td>Lymph node Metastasis, n. of cases (%)</td>
<td>2 (20%)</td>
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<tr>
<td>Ovarian function after treatment</td>
<td></td>
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<td>Menopausal, n. of cases (%)</td>
<td>8 (30.8%)</td>
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<td>Bilateral ovarian transposition</td>
<td>6</td>
</tr>
<tr>
<td>Unilateral ovarian transposition</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 40 years</td>
<td>4</td>
</tr>
<tr>
<td>&lt; 40 years</td>
<td>4</td>
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<tr>
<td>Functional, n. of cases (%)</td>
<td>18 (69.2%)</td>
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<td>Bilateral ovarian transposition</td>
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<td>17</td>
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</table>

main six patients unilateral ovarian transposition was performed. Ten patients had undergone pelvic common iliac lymph node and para-aortic lymph node sampling at the same time. Two patients were finally proved to have common iliac lymph node metastasis. The procedure was completed by laparoscopic approach in all 27 women as planned without conversion to laparotomy. There were no immediate intraoperative or postoperative complications reported in the first 30 days after surgery. The mean duration of hospitalization was 4.9 days (range three to eight). Pelvic radiotherapy were planned to be carried out immediately in all patients after surgery. Conventional whole pelvic radiation therapy was performed in all cases. Twenty-six cases had undergone pelvic radiotherapy, concurrent cisplatin-containing chemotherapy, and brachytherapy. Extended-field irradiation of para-aortic lymph nodes was carried out in the two patients with lymph node metastasis. One case (patient 9) discontinued pelvic radiotherapy halfway because of intestinal fistula due to tumor necrosis. The patient did not adhere to the study protocol, and no timed postoperative FSH level was assessed in this patient.

In addition to the evaluation and surveillance for their cancer, women were asked about their menopausal symptoms. Serum FSH levels were checked in the three- and six-month intervals after completion of planned treatment. The mean follow-up was 34.4 months ranging from 25 to 59 months. Eighteen patients (69.2%) had FSH levels less than 40 IU/L, four of these patients underwent unilateral ovarian transposition and 17 of them were less than 39 years of age. None of the patients described reported menopausal symptoms. Of the eight patients who had elevated FSH level after completion of their cancer treatments, four patients were over 40 years of age. None of the patients described reported menopausal symptoms. Serum FSH levels were checked in the three- and six-month intervals after completion of planned treatment. The mean follow-up was 34.4 months ranging from 25 to 59 months. Eighteen patients (69.2%) had FSH levels less than 40 IU/L, four of these patients underwent unilateral ovarian transposition and 17 of them were less than 39 years of age. None of the patients described reported menopausal symptoms. Of the eight patients who had elevated FSH level after completion of their cancer treatments, four patients were over 40 years of age. Nevertheless, there were two patients (patient 3 and 14) who had an ovarian cyst. Therapy was not given because these patients were asymptomatic. All the patients survived at follow-up and recurrence did not occur, except for patient 9. This patient who did not complete radiotherapy and had undergone chemotherapy and brachytherapy, eventually went into renal failure.

Discussion

Preservation of ovarian hormonal function in women undergoing treatment for cancer is receiving increasing attention. Improved long-term survival will mandate that strategies be developed to minimize or avoid this important morbidity.

Although concurrent chemoradiotherapy results in a good outcome for patients with middle and advanced stage cervical cancer [3], exposure to radiation can lead to early ovarian failure. In the literature, radiation doses of less than three Gy to the ovary led to ovarian failure in 11% of women, more than three Gy in 60% of women, and over five Gy were sufficient to sterilize the ovary [4]. Typically, within four to six weeks after radiation, serum E2 levels decline, whereas FSH levels progressively increase and menopausal symptoms become evident. Women with ovarian failure experience vasomotor hot flushes, urogenital dysfunction, and emotional disturbances, and risk of osteoporosis at long term. Since the publication of the Women Health Initiative (WHI) study [5], there has been a major shift to minimize the use of hormone replacement therapy (HRT) because of the associated increased risk of thromboembolic phenomena, cerebrovascular accidents, and breast cancer. Ovarian transposition outside the pelvic irradiation field has been shown to reduce the ovarian irradiation dose by 90% to 95% compared with the untransposed ovary [6].

Laparoscopic surgery has been one of the important innovations in modern-day surgery, allowing the patient to have a rapid recovery. The published laparoscopic experience with ovarian transposition has been limited to few small series [7-9]. The transposed ovary can induce complications such as torsion of ovarian pedicle, bleeding, and symptomatic ovarian cyst [10]. Although the risk of ovarian metastasis has been thought to be rare and negligible, ovarian metastasis on transposed ovary in patients treated for the cervix carcinoma has been reported [11-13]. A review of published studies indicated that the incidence of ovarian metastasis from uterine cervical cancer is less than 0.5% of squamous cell carcinoma and 1.4% of adenocarcinoma [13]. Shimada et al. [11] demonstrated that ovarian metastasis occurred more frequently among patients with adenocarcinoma than among those with squamous cell carcinoma (5.31% vs. 0.79%). Outcome for patients with ovarian metastasis was very poor and not related to FIGO Stage and histological type. Yamamoto et al. [12] analyzed the risk
factors of ovarian metastases in Stage IB–IIIB cervical carcinoma. Ovarian metastasis was identified in two of 485 (0.4%) patients with squamous cell carcinoma and in 12 of 146 (8.2%) patients with non-squamous tumors of the cervix; however histologic type and blood vessel invasion were significant independent risk factors for ovarian metastases, as revealed by multivariate logistic regression analysis.

The present authors aimed to evaluate the feasibility, the complication rates, and the functional outcome after laparoscopic ovarian transposition before pelvic irradiation in their patients younger than 45 years of age. Because ovarian metastasis occurred more frequently among patients with adenocarcinoma than among those with squamous cell carcinoma, the authors collected 27 women younger than 45 years with squamous cell carcinoma. They have found laparoscopic ovarian transposition to be a feasible procedure in their patient population with a short operative time and with minimal complications. Metal clips are easily identified to locate the ovaries. In this study, patient 3 and 14 developed an ovarian cyst. Therapy was not made in one of these patients that was asymptomatic. There was also no case of ovarian metastasis or recurrence in 26 patients who underwent ovarian transposition during follow-up.

The success of ovarian function preservation after transposition is variable in the literature. Successful preservation of ovarian function after ovarian transposition is dependent on two factors: the dose of radiation received by the ovary and the age of the patient [9, 14, 15]. Pahisa et al. [16] reported a 72.7% success rate in 11 patients after a mean follow-up of 44 months. Al-Badawi et al. [7] reported an ovarian preservation rate of 65% at a mean follow-up of 33 months. The reported age of ovarian transposition was usually less than 45 years, Han et al. [17] suggested that the rate of ovarian preservation is low after 40 years, Ovarian transposition should be performed only in patients less than 40 years of age. Hwang et al. [18] reported that the rate of normal ovarian function after lateral ovarian transposition after adjuvant radiation was 65.5% in < 40 years of age, and 35.5% in > 40 years of age.

In the present series, 69.2% of the patients maintained normal FSH levels at a mean follow-up period of 34.4 months; 80% of the five patients over 40 years of age experienced ovarian failure. Otherwise, the authors noted that four patients had normal ovarian function after irradiation in six unilateral ovarian transposition’s patients. Therefore, unilateral ovarian transposition perhaps is an effective procedure and can cut down the risk of ovarian transposition. This finding was nearly identical by Clough et al. [14].

For patients with known metastases to the para-aortic lymph nodes or common iliac lymph nodes, para-aortic radiation is probably necessary [19]. In this study, the authors performed pelvic lymph node or para-aortic lymph node sampling in ten patients at the same time of laparoscopic ovarian transposition. Two of the patients had confirmed metastases, therefore extended-field irradiation of para-aortic lymph nodes was necessary.

In conclusion, laparoscopic transposition of ovaries outside the irradiation field is a safe and effective procedure for the preservation of ovarian function. The result of the present study shows that ovarian metastasis in patients with cervical and vaginal squamous cell carcinoma is extremely rare. Therefore, radiotherapy without transposition of the ovaries for cervical squamous cell carcinoma is not recommended as a treatment for young patients, especially for those less than 40 years of age. Unilateral ovarian transposition perhaps is an alternative method. Para-aortic lymph node or common iliac lymph node sampling at the same time of laparoscopic ovarian transposition may preferably guide radiation therapy. More studies are needed to determine the long-term health and QOL benefits of ovarian preservation in young cancer patients.

Acknowledgments

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References

Laparoscopic ovarian transposition in young women with cervical squamous cell carcinoma treated by primary pelvic irradiation


Address reprint requests to:
Y. CHEN, M.D.
Department of Gynecology Oncology,
Zhejiang Provincial Cancer Hospital,
38 Guangji Road, Hangzhou,
310022 (China)
e-mail: hfshou@126.com
The prognostic significance of pretreatment [18F]FDG-PET/CT imaging in patients with uterine cervical cancer: preliminary results

S.H. Cho¹, J.Y. Lim¹, S.N. Kim¹, S. Hong³, H.W. Chung¹, Y. So³, W.Y. Kim⁴, S.J. Lee¹

¹Department of Obstetrics and Gynecology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul
²Department of Radiation Oncology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul
³Department of Nuclear Medicine, Konkuk University Hospital, Konkuk University School of Medicine, Seoul
⁴Department of Pathology, Konkuk University Hospital, Konkuk University School of Medicine, Seoul (Korea)

Summary
Purpose of Investigation: To evaluate the prognostic significance of positron emission tomography/computed tomography (PET/CT) in patients diagnosed with cervical cancer. Materials and Methods: Patients with cervical cancer in FIGO Stages IB1 to IVB were imaged with PET/CT prior to treatment during one of the staging work-ups. The patients were observed for a median of 31.4 months (range, six to 89 months) after the initial treatment. The standardized uptake value (SUV) max of the primary cervical tumor mass was compared with the prognostic factors. Results: A total of 81 patients who were primarily treated with radical hysterectomy (RH, n=45) or concurrent chemoradiation (CCRT, n=36) were analyzed. Multivariate analysis indicated that larger tumor size (> 4 cm, OR 8.694, 95% CI, 1.638-46.146), deep stromal invasion (≥ 1 cm, OR 7.249, 95% CI, 1.141-46.039) by the primary tumor, and pathologically confirmed pelvic lymph node involvement (positive, OR 14.586, 95% CI, 2.072-102.674) were significantly associated with recurrence after treatment. However, pretreatment SUVmax was not a significant independent predictor of disease recurrence (OR 1.058, 95% CI, 0.255-4.398). Conclusion: [18F]Fluorodeoxyglucose (FDG) uptake by the primary tumor showed a significant association with several risk factors that have been identified as treatment predictors. However, a high pretreatment SUVmax was not predictive of recurrence in uterine cervical cancer patients.

Key words: FDG-PET/CT; SUVmax; Uterine cervical cancer; Recurrence; Prognostic factor.

Introduction
Cervical cancer is the most frequently diagnosed gynecological cancer in Korea [1-2] and the second most common malignancy among women worldwide [3]. The International Federation of Gynecology and Obstetrics (FIGO) reported five-year recurrence and five-year overall mortality rates for cervical cancer of 28% and 27.8%, respectively [2, 4]. The pretreatment evaluation of patients with cervical cancer is important for determining optimal treatment and predicting the patient’s prognosis. Although accurate clinical staging is the most predictive factor of treatment outcome, several clinical and pathological risk factors have been identified to support this prediction. Risk factors include age, histological tumor type, pelvic lymph node metastasis, involvement of the resection margin and parametrium, deep cervical stromal invasion, bulky tumor size, and lymph-vascular space invasion (LVSI) [3, 5-12]. Several studies have attempted to accurately predict the outcome of cervical cancer patients.

[18F]Fluorodeoxyglucose positron emission tomography (FDG-PET) is an imaging technique based on the increased uptake of glucose that is characteristically observed in malignant lesions. Previous studies have demonstrated the efficacy of FDG-PET and PET/CT in the pretreatment and post-treatment evaluation of cervical cancer patients [13-15]. Several studies have shown that FDG uptake in primary cervical cancer is predictive of survival in patients undergoing radiotherapy [16-17]. Recent clinical studies in patients with cervical cancer have demonstrated that high pretreatment FDG uptake measured as a standardized uptake value (SUV) in the primary tumor should be considered a risk factor for disease recurrence after treatment [2, 12, 16, 18].

In this study, the authors evaluated FDG uptake, which was measured as the SUVmax, in primary cervical cancer to determine its association with the prognoses of cervical cancer patients in FIGO Stages IB1 to IVB. It is unknown whether routine performance of FDG-PET/CT before treatment will have prognostic effects independent of treatment modalities in patients with various stages of cervical cancer.
Materials and Methods

Patients

The present hospital has been a referral center for patients with cancer since August 2005. All patients who are diagnosed with invasive cervical cancer routinely undergo FDG-PET/CT imaging before treatment at this institute. The authors analyzed all clinical, histological, and imaging data from the patients who were treated for invasive cervical cancer between August 2005 and December 2012. They restaged 12 patients in FIGO Stages IIA to IIA1 (four patients) and IIA2 (eight patients) according to the revised FIGO staging system [19] after reviewing their records. Patients were excluded from the analysis if any of the following criteria were present: (1) PET/CT performed at other hospitals, (2) follow-up period of < three months, (3) refusal of PET/CT imaging, (4) no chemotherapy during radiotherapy because of advanced age and/or medical problems, (5) no standard intracavitary irradiation after external irradiation because of non-response, and (6) referral requests to other treatment facilities. Stages IA1 and IA2 disease. This study was approved by the institutional review board at Konkuk University Hospital.

PET/CT imaging and analysis

The patients fasted for at least six hours, and their blood glucose concentrations were evaluated before the PET studies (< 120 mg/dl for non-diabetic patients and < 200 mg/dl for diabetic patients). In the resting state, the patients were intravenously injected with FDG (4.8 MBq/kg body weight). PET/CT images were acquired 60 minutes later using a scanner. The axes from the PET and CT systems were mechanically aligned. ACT from the skull base to the mid-thigh (without i.v. contrast) was performed for attenuation correction and anatomic localisation using a standardised protocol of 120 kV, 50 mA, a 0.75-sec tube-rotation time per rotation, a 1.5-pitch, and a five-mm section thickness. Immediately after the CT, PET images were acquired for 2.5 minutes per frame using a conventional three-dimensional protocol.

The FDG-PET/CT images were assessed by two experienced nuclear medicine physicians. The SUVmax was used quantitatively to determine the FDG-PET/CT activity. SUVmax was defined as the maximum tumour concentration of FDG divided by the injected dose corrected for the patient’s body weight. To obtain the SUVmax, a transaxial image representing the highest tumour uptake was carefully selected, and a circular region of interest was placed in the FDG accumulating area.

Treatment

1) Radical hysterectomy (RH) with pelvic lymph node dissection (PLND): After pathological identification, the patients underwent physical examinations, intravenous pyelography (IVP), cystoscopy, rectosigmoidoscopy, chest X-ray, MRI or CT of the pelvis, PET/CT, and other routine laboratory tests prior to surgery. Each patient underwent a class III RH with PLND. All of the specimens were assessed by two pathologists who specialize in gynecological oncology. The authors categorized the patients by pathological risk factors into a high-risk group and an intermediate-risk group. The high-risk group included the patients who had positive pelvic lymph nodes, microscopic parametrial extension, or positive vaginal resection margins [20-21]. The intermediate group included the patients with stromal invasion of ≥ 1 cm, LVSI, or a large tumor (the largest diameter was ≥ 4 cm) [8, 22]. The patients with one or more of the high-risk factors received adjuvant pelvic radiotherapy (RT) and concurrent chemotherapy (weekly cisplatin, 40 mg/m²/week). The patients who had two or more intermediate risk factors received adjuvant RT alone [18]. Radiotherapy was delivered at 1.8 Gy per fraction using a 15 MV photon from a linear accelerator once a day for five days a week. A four-field box technique was used. The median dose to the entire pelvis was 50.4 Gy, and the median treatment time was six weeks.

2) Concurrent chemoradiation (CCRT): Radiation was delivered as a combination of external beam radiotherapy and high-dose-rate (HDR) intracavitary brachytherapy. The radiation field encompassed a volume that included the whole uterus, primary mass, paracervical, parametrial, and uterosacral regions, and the external iliac, hypogastric, and obturator nodes. Radiotherapy was delivered at 1.8 Gy per fraction using a 15MV photon from a linear accelerator once a day, five days a week. A four-field box technique was used. The median dose to the entire pelvis was 50.4 Gy. A midline block was used after 41.4 Gy. A parametrial boost of 60 Gy was delivered to the thickened parametrium. The metabolic treatment time was seven weeks. After receiving external beam radiotherapy to the pelvis, the patients received intracavitary brachytherapy two times per week using an 192Ir high dose brachytherapy unit. The patients with Stage IIB cervical cancer received 35 Gy in seven fractions to point A, and the patients with Stage IIA or greater received 30 Gy in six fractions. The patients were concurrently given chemotherapy (cisplatin-based chemotherapy, with weekly cisplatin or 5-FU + cisplatin).

The patients had follow-up examinations approximately every three months for the first two years, every four months for the next three years, and every year thereafter. The authors defined the disease-free interval as the time from the initial treatment to relapse as noted on images or the last follow-up visit.

Statistics

The clinical and pathological factors and outcome data were analyzed for correlation with the SUVmax. The data were analyzed using Student’s t-test or the Wilcoxon rank-sum test to compare the SUV values in the different subgroups. Disease-free survival was calculated using the Kaplan–Meier method. A logistic regression model was used for the multivariate analyses. Variables that were shown to be significant in the univariate analysis were selected for the logistic regression model. A p-value of < 0.05 was considered significant. The dBSTAT 5.0 software was used for the statistical analysis.

Results

Patient characteristics

During the study period, 264 cervical cancer patients were diagnosed in the present hospital or transferred to this institute from other hospitals. Of these, 42 patients were transferred after PET imaging was performed at other institutes. Among the 222 patients recommended for PET/CT, 60 patients (mostly Stage IA1 or IA2 disease) refused the PET/CT imaging; 138 patients requested a transfer to other institutes for treatment or did not have CCRT during RT because of advanced age and/or medical problems. Six patients were excluded because of a short follow-up period. A total of 81 patients met the eligibility criteria for this study and were included in the analysis. The patient characteristics are summarized in Table 1. The mean age of the patients was 53.1 ± 14.4 years. The median follow-up was 31.4 months (range, 6-89 months). The median SUVmax was 9.7 (range, 3.0-20.6).

1) Patients primarily treated with radical hysterectomy and pelvic lymph node dissection (n=45). Table 2 shows the characteristics and clinical and pathological findings of the
The prognostic significance of pretreatment [18F]FDG-PET/CT imaging in patients with uterine cervical cancer: preliminary results

32 patients who were primarily treated with RH and PLND. The mean age of the patients in this group was 50.1 ± 12.6 years. The median SUVmax was 8.9 (range, 3.0 - 19.9).

After primary treatment, adjuvant CCRT was performed in 15 of the patients (33.3%) with one or more of the high-risk factors according to pathologic reports.

2) Patients primarily treated with concurrent chemoradiation (n=36). Table 3 summarizes the characteristics of the patients who were primarily treated with CCRT. The mean age and median SUVmax of the patients with cervical cancer treated with CCRT were higher compared with the patients treated with radical surgery.

Correlation between SUVmax and the clinical and pathological parameters Table 4 shows the SUVmax relative to the pathological parameters. The median SUVmax for all of the patients was 8.9 (range, 3.0 - 19.9). The mean preoperative SUVmax was significantly higher in the patients with pathologically confirmed pelvic lymph node involvement (p = 0.030), deep cervical stromal invasion (≥ 1 cm, p = 0.004), clinically estimated large tumors (> 4 cm, p = 0.019), and treatment with primary CCRT (p = 0.049). However, no difference was observed in the SUVmax values of the patients with pelvic lymph node involvement detected by patients who were primarily treated with RH and PLND.

The mean age of the patients in this group was 50.1 ± 12.6 years. The median SUVmax was 8.9 (range, 3.0 - 19.9). After primary treatment, adjuvant CCRT was performed in 15 of the patients (33.3%) with one or more of the high-risk factors according to pathologic reports.

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### Table 1. — Patient characteristics.

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<th>Characteristics</th>
<th>No. of patients</th>
<th>%</th>
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</table>

### Table 2. — Patients primarily treated with radical hysterectomy and pelvic lymph node dissection (n=45).

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<th>Characteristics</th>
<th>No. of patients</th>
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<tr>
<td>Age at diagnosis (years)</td>
<td>50.1 ± 12.6</td>
<td>8.9 (3.0-19.9)</td>
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<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB1</td>
<td>29 (64.4)</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>IB2</td>
<td>7 (15.6)</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>IIA1</td>
<td>4 (8.9)</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>5 (11.1)</td>
<td>15.2</td>
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<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>29 (64.4)</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>14 (31.1)</td>
<td>9.3</td>
<td></td>
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<tr>
<td>Adenosquamous carcinoma</td>
<td>1 (2.2)</td>
<td>10.1</td>
<td></td>
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<tr>
<td>Carcinoid</td>
<td>1 (2.2)</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Mean largest tumor diameter (PET/CT, MRI, CT), cm</td>
<td>3.4 ±1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean largest tumor diameter (pathology), cm</td>
<td>4.4 ± 2.3</td>
<td></td>
<td></td>
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<tr>
<td>Pelvic lymph node involvement</td>
<td>15 (33.3)</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>Microscopic parametral invasion</td>
<td>9 (20.0)</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion (≥1cm)</td>
<td>26 (57.8)</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>LVSI</td>
<td>18 (40.0)</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Vaginal resection margin</td>
<td>11 (24.4)</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Adjuvant treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>20 (44.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>10 (22.2)</td>
<td></td>
<td></td>
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<tr>
<td>Concurrent chemoradiation</td>
<td>15 (33.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>2 (5.0)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Death related to cervical cancer</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LVSI = lymph-vascular space invasion

### Table 3. — Patients primarily treated with concurrent chemoradiation (n=36).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients</th>
<th>SUVmax (%)</th>
<th>(mean)</th>
</tr>
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<tbody>
<tr>
<td>Age at diagnosis (years)</td>
<td>58.9 ± 14.9</td>
<td>10.8</td>
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<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB1</td>
<td>8 (22.2)</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>IB2</td>
<td>2 (5.6)</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>IIA1/IIA2</td>
<td>0/3 (8.3)</td>
<td>-/10.3</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>16 (44.4)</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>IIIA/IIIB</td>
<td>0/4 (11.1)</td>
<td>-/12.1</td>
<td></td>
</tr>
<tr>
<td>IVA/IVB</td>
<td>0/3 (8.3)</td>
<td>-/9.4</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>28 (77.8)</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>7 (19.4)</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>1 (2.8)</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td>Mean largest tumor diameter (PET/CT, MRI, CT), cm</td>
<td>4.1±1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node involvement (PET/CT)</td>
<td>8 (22.2)</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>Parametrial invasion (PET/CT, MRI, CT)</td>
<td>15 (41.7)</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td>1 (2.8)</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Persistence</td>
<td>4 (11.1)</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>Death related to cervical cancer</td>
<td>3 (8.3)</td>
<td>9.1</td>
<td></td>
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</tbody>
</table>
PET/CT ($p = 0.874$). The parameters of pathologically confirmed parametral invasion, LVSI, and a positive vaginal resection margin were not associated with differences in the SUVmax ($p = 0.362$, $p = 0.355$, and $p = 0.256$, respectively). Age, FIGO Stage, and cell type were also not associated with pretreatment SUVmax in this study.

**FDG- PET/CT and prediction of recurrence**

Three cases of recurrent disease and four cases of persistent disease were recorded over the study period (Table 1). A receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off values of SUVmax for predicting recurrence. Based on the ROC curve analysis, the patients were divided into two groups according to the SUVmax (< 7.0 and ≥ 7.0). This study demonstrated that recurrent or persistent disease did not occur in the patients with SUVmax values of < 7.0. The log-rank test showed that the patients with high SUVmax values had significantly lower disease-free survival rates compared with patients with low SUVmax values ($p = 0.038$, Figure 1).

The authors analyzed the SUVmax and clinical and pathological parameters using the logistic regression test model and found that large tumor size (> 4 cm, OR 8.694, 95% CI 1.638-46.146, $p = 0.011$), deep cervical stromal invasion (≥ 1 cm, OR 7.249, 1.141-46.039, $p = 0.036$), and pathologically confirmed pelvic lymph node involvement (OR 14.586, 95% CI 2.072-102.674, $p = 0.007$) were significantly associated with recurrence (Table 5). However, the pretreatment SUVmax was not a significant independent predictor of disease recurrence (OR 1.058, 95% CI 0.255-4.398, $p = 0.938$).

**Discussion**

The purpose of this study was to determine whether metabolic activity in the primary tumor, measured as the FDG SUVmax, has prognostic significance in patients with uterine cervical cancer. This study attempted to include all of...
the cervical cancer patients without selection based on routine diagnostic work-up and treatment at a single institution. Several previous studies have shown that FDG uptake by the primary tumor has a significant association with recurrence in patients with uterine cervical cancer. Most of these studies included cervical cancer patients who underwent RT [16, 23] or RH treatments [2, 12, 18, 24]. The present study reports the value of pretreatment FDG-PET/CT independent of treatment modality in predicting the risk of recurrence in patients with Stage IB1 to IVB cervical cancer.

The present authors defined the cut-off values for SUVmax as < 7.0 and ≥ 7.0. A statistically significant difference in disease-free survival was observed between the patients with SUVmax values of < 7.0 and with SUVmax values of ≥ 7.0 (p = 0.0381). While the patients with SUVmax values of < 7.0 did not experience recurrent or persistent disease, the patients with SUVmax values of ≥ 7.0 presented with three instances of recurrent disease and four instances of persistent disease (RH, n=2, and CCRT, n=5).

Definitive cut-off values for the SUV could indicate poor prognosis or a higher probability of recurrence have not been established. Kidd et al. [25] demonstrated that a SUVmax of < 5.2 is associated with an excellent five-year overall survival rate. A cut-off value of 13.4 [18] has been reported in another study. Although the SUVmax may be a risk factor that indicates poor prognosis, the cut-off values might not be applicable at different institutions and should be established at a single institution according to specified criteria. The present authors expected the pretreatment SUVmax to be an independent risk factor of recurrence. However, logistic regression test models indicated that SUVmax was not significant as a predictor of recurrence (p = 0.938, Table 5). Although several studies have suggested that pretreatment FDG uptake in primary cervical cancer is a prognostic factor that is related to recurrence, Crivellaro et al. [26] have demonstrated that SUVmax, SUVmean, and metabolic tumor volume of the cervical uptake are not predictors of recurrence. The results of the present study were consistent with those of Crivellaro’s.

The principal finding of this study is that the clinically estimated tumor size (by PET/CT, MRI, and CT) is significantly associated with primary tumor FDG uptake and recurrence in patients with various stages of cervical cancer (Table 5). Deep cervical stromal invasion (≥ 1 cm) by the primary cervical tumor and pathologically confirmed pelvic lymph node involvement after RH were also predictors of recurrences with high primary tumor FDG uptake. Although a previous study demonstrated that FDG-PET detects abnormal lymph node regions more frequently than CT and that the results obtained from PET can better predict survival than CT in cervical cancer patients [27], the present study showed no differences in FDG uptake (Table 4). Signorelli et al. [28] demonstrated that PET/CT had a low sensitivity in depicting nodal metastases and had a minimal clinical impact in the pretreatment planning of Stage Ib1-IIa < four cm cervical cancer. Age, FIGO Stages, microscopic parametrial invasion, LVSI, and vaginal resection margin involvement were not associated with significant differences in FDG uptake in the univariate analysis, despite the fact that these factors are well-known to be predictive of recurrence. In the present study, patients treated with CCRT had greater FDG uptake in the primary tumor compared with the patients treated with RH (p = 0.049, Table 4). However, treatment type was not a risk factor for recurrence based on the multivariate analysis (p = 0.195, Table 5). Although the lack of associations may be attributed to the low number of patients analyzed and study design, the present authors cannot exclude the possible limitations of detecting the risk factors with PET/CT. Therefore, their results suggest that a low SUVmax should not necessarily be considered a good prognostic factor and low SUVmax values should be correlated with other risk factors to determine prognosis.

This study contained several limitations. First, the study was retrospectively performed with a relatively small number of patients (n=81) at a single institution. Second, these findings may represent only patients from the present institution and may not be applicable to other institutions. Third, although the authors attempted to include all of the patients during the study period for complete enumeration analysis, many patients were excluded based on the exclusion criteria; this exclusion may have created biases.

Conclusion

Pretreatment tumor FDG uptake was significantly associated with several clinical and pathological prognostic factors for recurrence in patients with Stage IB1 to IVB cervical cancer who were treated with RH or CCRT. Although the multivariate analysis indicated that a high pretreatment SUVmax (≥ 7.0) was not predictive of recurrence, this value could be an important factor to consider in the follow-up of cervical cancer patients because of the association with prognostic factors for recurrence. Moreover, a lower pretreatment SUVmax should not be considered predictive of a good prognosis due to the possible limitations of FDG-PET/CT in detecting risk factors.

Acknowledgements

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References


Address reprint requests to:
S.J. LEE, M.D., Ph.D
Department of Obstetrics and Gynecology,
Konkuk University Hospital
Konkuk University School of Medicine
4-12, Hwayang-dong,
Kwangjin-gu, Seoul (Korea)
e-mail: lsj671121@gmail.com
The role of mTOR signaling pathway in premalignant and malignant cervical lesions

M. Andrikopoulou¹, N. Salakos¹, E. Deligeoroglou¹, A. Pafiti², I. Boutas¹, N. Nikitakis³, A. Sklavounou³, G. Creatsas¹

¹ 2nd Department of Obstetrics and Gynecology, University of Athens, Medical School, Aretaieion Hospital, Athens
² Department of Pathology, University of Athens, Medical School, Aretaieion Hospital, Athens
³ Department of Oral Medicine and Pathology, University of Athens, Dental School, Athens (Greece)

Summary
Purpose of the study: Aberrant activation of the Akt/mTOR/pS6 signaling pathway has been identified in various types of cancer and is under investigation in cervical cancer. The purpose of this study was to assess the expression of the phosphorylated/activated forms of Akt (upstream molecule), 4E-BP1 and pS6 (downstream molecules) in biopsy samples of cervical low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), and squamous cell carcinoma (Ca) compared to normal cervical epithelium. Material and Methods: The study included 38 cases diagnosed as LSIL, 31 cases as HSIL, 29 cases as Ca, and eight control cases from normal cervix. Immunohistochemistry was used to assess the expression of pAkt, p4E-BP1 and pS6. Results: Statistical analysis revealed significant differences between HSIL and Ca groups compared to controls regarding intensity, positivity, and total scores for all three molecules (p < 0.001). A trend for higher expression with increasing grade of dysplasia was demonstrated. Conclusion: These results strongly support the view that the mTOR signaling pathway is involved in cervical carcinogenesis.

Key words: Cervical cancer; mTOR signaling pathway; LSIL; HSIL.

Introduction
Squamous cell carcinoma (Ca) of the uterine cervix is the most common malignant tumor of the genital tract worldwide [1]. In recent years, advances in molecular biology have led to the identification of several carcinogenesis-related signaling pathways. A more profound understanding of underlying molecular mechanisms involved in the initiation and progression of Ca of the cervix will offer insight into diagnosis and treatment.

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase of the phosphatidylinositol-3-kinase (PI3K)/AKT signaling pathway which regulates cell growth, proliferation, and protein synthesis. Aberrant activation of upstream and downstream molecules of the Akt/mTOR/pS6 signaling pathway has been identified in various types of human cancer, including head and neck squamous cell carcinoma, prostate cancer, ovarian and gastrointestinal cancer [2-4]. The serine/threonine kinase mTOR pathway is an appealing therapeutic target because of the availability of potent inhibitors such as rapamycin and its analogues which have been utilized in clinical trials for malignancies [5, 6].

Akt, a serine-threonine kinase is considered a key molecule that functions as a downstream target and effector of phosphatidylinositol 3-kinase (PI3K). It regulates normal and cancer cell growth and fate decisions [7]. Akt phosphorylates and inhibits the tuberous sclerosis complex (TSC), thus acting as a positive upstream regulator of mTOR, which in turn, regulates a number of downstream molecules, such as ribosomal protein S6 kinase 1 (pS6K) and eukaryotic translation initiation factor 4E binding protein (4E-BP1). The activation of these downstream targets plays a critical role in controlling fundamental cell processes such as cell survival, cell cycle regulation, proliferation, protein synthesis, and angiogenesis [8].

The exact role of mTOR signaling pathway in cervical cancer is not fully understood and is currently under investigation. Overexpression of the mTOR molecule, as well as Akt and pS6, major upstream and downstream factors of the pathway, has been shown in cervical cancer and has been correlated with poor prognosis and resistance to chemotherapy and radiotherapy [9, 10]. However their role in low grade squamous intraepithelial lesions (LSIL) and high grade squamous intraepithelial lesions (HSIL) has not been evaluated.

The purpose of this study was to assess the expression of the phosphorylated (activated) forms of Akt, 4E-BP1, and pS6 in biopsy samples of cervical LSIL, HSIL, and Ca compared to normal squamous epithelium of the cervix.

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Materials and Methods

Patients and tumor samples

Ninety-eight formalin fixed, paraffin-embedded tissue samples were retrieved from the archives of the Pathology Department of the Aretaieion University Hospital Athens Medical School. Only specimens with sufficient tissue were selected. Of 98 patients with premalignant and malignant cervical lesions, 38 cases were diagnosed as LSIL, 31 cases as HSIL, and 29 cases as Ca of the cervix. Ten cases of the latter group were retrieved from the files of the Pathology Department of Agios Savvas Cancer Hospital, Athens. Eight control cases of normal cervical epithelium were also included in the study. Representative hematoxylin and eosin sections of each lesion were reviewed and diagnosis was confirmed in all cases.

Immunohistochemistry

Five micron-thick serial sections of formalin-fixed and paraffin-embedded tissues were immunostained using a fully automated immunohistochemistry system, by applying a polymer detection system. For epitope retrieval a high temperature technique with citrate buffer was utilized. The sections were incubated in 3% hydrogen peroxide to neutralize endogenous peroxidase activity; treated with a protein block to reduce non-specific binding of primary and polymer; incubated with primary antibodies; and treated with a post primary block, containing 10% (v/v) animal serum in tris-buffered saline, to enhance penetration of the subsequent polymer reagent. Consequently, poly-HRP anti-mouse/rabbit IgG reagent containing 10% (v/v) animal serum in tris-buffered saline was applied to localize the primary antibody and the reaction product was visualized by incubation with the substrate/chromogen, 3,3′-diaminobenzidine (DAB) and substrate buffer (polymer), as a brown precipitate. Finally, the sections were counterstained with hematoxylin (0.02%).

The following primary antibodies were used: rabbit monoclonal antibody against phosphorylated Akt (1:50, p-Akt, phosphorylated at serine 473, #4060), rabbit monoclonal antibody against phosphorylated 4E-BP1 (1:100, 4E-BP1, phosphorylated at threonin 37/46, #2855), and rabbit polyclonal antibody against phosphorylated pS6 protein (1:400, phospho-pS6, phosphorylated at serine 235/236, #2211). Appropriate positive control cases were used for all antibodies. As negative control, sections were treated with phosphorylated buffered saline (PBS) with omission of the primary antibody.

The immunostains were reviewed by two independent evaluators. The interobserver variability was very low (< 5% of cases). In cases in which there was initial disagreement, stains were re-evaluated by the aforementioned investigators using a multiobserver microscope and discussed until consensus was reached. Both cytoplasmic and nuclear immunostaining were evaluated for all three molecules.

Immunohistochemical reactivity for pAkt, 4E-BP1 and phospho-pS6 stains was graded in a semi-quantitative manner according to the percentage of positive epithelial cells: (0) 0%, (1) <20%, (2) 20-50% and (3) >50%, and the intensity of staining: (0) negative, (1) weak, (2) moderate, or (3) strong, as compared to the negative control tissues. Moreover, a combined score of immunohistochemical positivity (0, 2-6) was calculated for each case by adding the individual scores for percentage of cells (0-3) and intensity of staining (0-3).

Statistical analysis

Differences in immunohistochemical scores (intensity and positivity) for pAkt, 4E-BP1 and phospho-pS6 between all four groups (LSIL, HSIL, Ca, control) were assessed using Fisher’s Exact test. Total scores between groups for all three antibodies were evaluated using Kruskal Wallis test (following Bonferroni correction). Correlation between the molecules was investigated using Kendall’s tau correlation coefficient. Non-parametric test for trend was also used to identify possible trend between grade of dysplasia and expression of the molecules. A level of $p \leq 0.05$ was considered to be statistically significant.

Results

All three molecules expressed both cytoplasmic and nuclear immunostaining.

p-Akt

All 38 LSIL cases studied (100%) were positive for p-Akt expression. Seventeen cases (44.7%) showed p-Akt immunopositivity in <20% of epithelial cells, while 14 (36.8%), and seven (18.4%) cases exhibited staining in 20-50% and >50% of epithelial cells, respectively; the average score for the percentage of positive epithelial cells for p-Akt was 1.73. On the other hand, the average score for staining intensity was 1.60, corresponding to 16 (42.1%) cases that stained weakly, 19 (50%) moderately, and three (7.9%) strongly. The average combined score for p-Akt immunohistochemical positivity in the LSIL group was 3.00.

With respect to p-Akt staining, all 31 HSIL cases were positive. Regarding the percentage of positive cells, 13 cases (41.9%) received a score of 3 and 14 cases (45.2%) a score of 2 for positivity (average score: 2.29). The average intensity score was 2.06; six cases (19.4%) received a score of 1, 14 cases (45.2%) a score of 2, and 11 cases (35.5%) a score of 3. The average combined score for p-Akt immunohistochemical positivity in HSIL was 5.00.

In addition, all 29 cervical Ca cases (100%) were positive for pAkt expression. Seventeen cases (58.6%) showed immunoreactivity for >50% of cancerous cells whereas ten cases (34.5%) for 20-50% of cells. In addition, 16 cases (55.2%) received a score of 2 for intensity and six cases (20.7%) a score of 3. The average positivity, intensity, and total scores were 1.96, 2.51 and 5.00, respectively.

On the other hand, regarding the control group, three cases (37.5%) were negative for pAkt expression, four cases (50%) received a score of 1 and one case (12.5%) a score of 2 for both positivity and intensity. The average combined score for p-Akt immunostaining in the control group was 2.00.

Regarding intensity and positivity significant differences between HSIL and Ca groups compared to controls were detected ($p < 0.001$). In terms of total scores, the group of HSIL and Ca received significantly higher scores for p-Akt expression compared to the control group ($p < 0.001$). Interestingly, the total score for p-Akt expression was significantly higher in the group of Ca compared to the LSIL group and in the HSIL group compared to the LSIL group ($p < 0.0001$).

The results for p-Akt were summarized in Table 1, Figures 1 and 4.
The role of mTOR signaling pathway in premalignant and malignant cervical lesions

Figure 1. — pAkt staining percentage and intensity in the control, LSIL, HSIL, and Ca groups.

Figure 2. — p4E-BP1 staining percentage and intensity in the control, LSIL, HSIL, and Ca groups.

Figure 3. — pS6 staining percentage and intensity in the control, LSIL, HSIL, and Ca groups.
Figure 4. — Immunohistochemical expression of phosphorylated Akt (pAkt) in selected cases of A) normal mucosa, B) LSIL, C) HSIL, and D) Ca of the uterine cervix. Diffuse and strong staining is observed in B, C, and D, while A is negative (A and C: x400 magnification, B and D: x200 magnification).

Figure 5. — Immunohistochemical expression of phosphorylated 4E-BP1 (p4E-BP1) in selected cases of A) normal mucosa, B) LSIL, C) HSIL, and D) Ca of the uterine cervix. Diffuse and strong staining is observed in B and C and D, while A is negative (A and B: x100 magnification, C and D: x400 magnification).

Figure 6. — Immunohistochemical expression of phosphorylated pS6 (phospho-pS6) in selected cases of A) normal mucosa, B) LSIL, C) HSIL, and D) Ca of the uterine cervix. Focal and strong staining is observed in B and C, diffuse and moderate staining is seen in D, while A is negative. (A and D: x400 magnification, B and C: x200 magnification).
The role of mTOR signaling pathway in premalignant and malignant cervical lesions

P 4E-BP1

All 98 premalignant and malignant lesions (100%) were positive for 4E-BP1 expression. With regards to the LSIL group, 15 cases (39.5%) showed immunoreactivity in <20% of epithelial cells, another 15 cases (39.5%) in 20% to 50% of epithelial cells and eight cases (21.1%) showed positive staining in >50% of epithelial cells. The average positivity, intensity, and total scores for p4E-BP1 in the LSIL group were 1.18, 1.78, and 4.00, respectively.

In the HSIL group, 15 cases (48.4%) received a score of 2 and 13 cases (41.9%) a score of 3 for positivity (average score: 2.32). The average intensity score was 2.13, with six cases (19.4%) receiving a score of 1, 12 cases (38.7%) a score of 2, and 13 cases (41.9%) a score of 3. The average total score for p 4E-BP1 immunohistochemical positivity in HSIL was 5.00.

In the cervical Ca group, all 29 cases were positive. In terms of positivity, 13 cases (44.8%) received a score of 3 and another 13 cases (44.8%) a score of 2. With regards to intensity, 11 cases (37.9%) received a score of 3 and 13 cases (44.8%) a score of 2. The average positivity, intensity, and total score for p 4E-BP1 were 2.34, 2.20 and 5.00, respectively.

In contrast, in the control group, two cases (25.0%) were negative, five cases received a score of 1 and one case (12.5%) a score of 2 for both positivity and intensity. The average total score for p 4E-BP1 immunostaining in the control group was 2.00.

Statistical analysis revealed significant differences between HSIL and Ca study groups compared to controls regarding intensity (p < 0.001), and positivity (p < 0.001). Differences in total scores between HSIL and controls as well as Ca group and controls were significant (p < 0.001 respectively). In contrast, no significant differences in total scores between LSIL and HSIL and between LSIL and Ca group were noted. The results for p 4E-BP1 are summarized in Table 2, Figures 2 and 5.

Phospho-pS6 (pS6)

Ninety-six of the 98 cervical premalignant and malignant cases (98%) were positive for pS6 expression. With regards to the LSIL group, 14 cases (36.8%) showed immunoreactivity in <20% of epithelial cells, while 19 (50%) and three (7.9%) showed immunostaining in 20-50% and >50% of epithelial cells, respectively; only two LSIL cases (2%) were negative. The average score for the percentage of positive epithelial cells for phospho-pS6 in the LSIL group was 1.60.

Regarding phospho-pS6 staining intensity in 13 (34.2%) cases was weak, in 14 (36.8%) was moderate, and in the remaining nine (23.7%) it was high; the average score for staining intensity was 1.78. Finally, the average total score for phospho-pS6 immunostaining in LSIL was 3.50.

All HSIL cases were positive for phospho-pS6 staining (100%). Sixteen cases (51.6%) demonstrated immunoreactivity in 20-50% of epithelial cells, eight cases (25.8%) were positive in <20% of cells, while seven cases (22.5%) showed
positive in >50% of cells; the average positivity score was 2.03. On the other hand, the average intensity score was 2.22 corresponding to seven cases (22.5%) which received a score of 1, 13 cases (41.9%) which received a score of 2, and 11 cases (35.4) a score of 3. The average total immunohistochemical score for phospho-pS6 in HSIL was 4.00.

All cases of cervical Ca (100%) were positive for immunoreactivity, most of them (41.4%), receiving a score of 2 in >50% of tumor cells and six cases (20.7%) a score of 3. In terms of stain intensity, 11 cases showed moderate immunoreactivity, four cases (13.8%) high, whereas 14 cases low staining (48.3%). The average positivity, intensity, and total scores for phospho-pS6 in cancer cases were 1.82, 1.65, and 3.00, respectively. Finally, all control cases were positive and the positivity, intensity, and total scores were 1.78, 1.78, and 3.56, respectively.

Statistical analysis revealed significantly higher levels for phospho-pS6 positivity and intensity in LSIL, HSIL, and Ca study groups compared to controls. Similarly, the total scores for phospho-pS6 immunoexpression were significantly lower in the control group compared to the LSIL, HSIL and Ca groups (p < 0.001). However, there was no statistically significant difference in phosphor-pS6 expression between LSIL and HSIL or HSIL and Ca group. The results for phospho-pS6 immunostaining are summarized in Table 3, Figures 3 and 6.

In addition possible correlation between the three molecules in all cervical lesions was assessed in terms of percentage of positive cells, intensity of immunoreactivity and total scores using Kendall’s tau test. All molecules were positively correlated to each other, as expected since they all serve as upstream and downstream effectors of the same signaling pathway (Table 4). Furthermore an increasing trend in intensity, percentage of positive cells as well as total score with increasing grade of dysplasia was observed in all cases at a statistically significant level (Table 5).

### Discussion

The mTOR pathway is well known to be a critical regulator of cell proliferation, growth, and translation. The deregulation of this signaling pathway has been involved in many types of cancer [2, 11] but the exact role in cervical cancer has not yet been identified and is an area of ongoing research [8, 12, 13].

In the present study, the expression of pS6 and 4E-BP1 which are downstream molecules activated by mTOR molecule was evaluated in an attempt to affirm the state of constitutive activation of the pathway.

Furthermore, the expression of Akt which serves as a positive upstream regulator of mTOR and a key player of tumor cell survival was also assessed [7]. Since phosphorylation of Akt, 4E-BP1 and pS6 is necessary for their activation, the phosphorylated levels of these molecules were examined.

To evaluate Akt activation status, an antibody recognizing Akt phosphorylated at serine 473 was used. Since Akt phosphorylation at Ser-473 involves an mTOR-containing protein complex (mTORC2), it is also a marker of mTOR activity [14, 15]. Activated Akt dissociates from the plasma membrane and exerts its activity by phosphorylating both cytoplasmic and nuclear downstream effectors, including mTOR [16]. By doing so, Akt regulates a number of critical biological functions, such as cell growth and survival, apoptosis and metabolism.

Regarding pAkt expression, it was interesting to note that premalignant and malignant cervical lesions showed pAkt positivity at a statistically significant level compared to normal cervical biopsies. Specifically, the pAkt expression gradually increased with increasing degree of malignancy (p < 0.001). These results, taken together, suggest that pAkt may be an important indicator of the biological activity of the molecule in the context of cervical carcinogenesis and could be considered as a predictive marker of progression of a low grade cervical lesion to high grade dysplasia and cancer.

Even though the expression of pAkt is frequently observed in various human cancers, only a few studies on its role in cervical cancer and premalignant lesions have appeared in the literature. A positive correlation between pAkt activation and high resistance to radiation and chemotherapy of cervical cancer cells has been reported. [9, 10, 17, 18].
and that 4E-BP1 plays a significant role in maintaining the growth and survival of HPV associated cancer cells, been shown that E7 viral oncoprotein is directly linked in the pathogenesis of cervical carcinoma. Since it has HPV variants is the single most important etiologic factor in the mTOR signaling pathway in cervical cancer pathogenesis.

4E-BP1 is another major downstream target and effector of the mTOR pathway. Activation of mTOR leads to phosphorylation of 4E-BP1 which in turn disrupts its interaction with eukaryotic initiation factor 4E (eIF4E); thus, leading to up regulation of translation in cancer cells [21, 22].

The present study showed significantly higher expression of pS6 and 4E-BP1 in premalignant and malignant cervical lesions in comparison to normal cervical biopsies. Be navente et al. [21] investigated the prognostic significance of 4E-BP1 in the clinical outcome of patients with cervical carcinoma and they demonstrated that 4E-BP1 activation is associated with a poor prognosis in cervical carcinoma treated with postoperative radiotherapy. With regards to pS6k and mTOR expression in cervical cancer lesions, the morphoproteomic analysis of Feng et al. [13] strongly suggested a constitutively activated and overexpressed mTOR pathway, which is in agreement with the present findings. Similarly, Jing et al. [12] using cervical cancer cell lines, concluded that mTOR and phosphor-pS6k are increased in cancer cells compared to normal cervical cells. Furthermore, it has been suggested that mTOR activation may be a poor prognostic marker for response to radiotherapy in patients with cervical cancer [23].

It is easily understood, as expected, that pS6k activity evaluated in the aforementioned studies, as well as the expression of its substrate, pS6, assessed in the present study, were found both increased in premalignant and malignant cervical lesions.

The present study demonstrated upregulation of the three molecules in cervical cancer, LSIL, and HSIL, thus implying that they may be actively involved in the early stages of cervical carcinogenesis. It was demonstrated that pAkt, pS6, and p4E-BP1 were highly positively correlated and that there was a trend for higher expression of all three molecules with increasing grade of dysplasia. These results, taken together, enhance the view of the potential role of the mTOR signaling pathway in cervical cancer pathogenesis. On the other hand, it is well established that high risk HPV variants is the single most important etiologic factor in the pathogenesis of cervical carcinoma. Since it has been shown that E7 viral oncoprotein is directly linked to the growth and survival of HPV associated cancer cells, and that 4E-BP1 plays a significant role in maintaining a high level of E7 protein expression [24], it would be of interest to further investigate a correlation between HPV positive LSIL, HSIL, and Ca cervical lesions with the mTOR signaling pathway. This is a project of ongoing research in the present laboratory and the results will be published shortly.

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Address reprint requests to:
M. ANDRIKOPOULOU, M.D.
3501 St. Paul Street, apt 413
Baltimore, MD, 21218 (USA)
e-mail: maria_andrik@hotmail.com
Effect of lentivirus mediated cyclooxygenase-2 gene shorthairpinRNA on invasiveness of endometrial carcinoma

Y.T. Xiao, L.M. Luo, R. Zhang
Obstetrics and Gynecology reported Hospital of Fudan University, Shanghai (P.R. China)

Summary
Objective: This study aims to explore the effects of lentivirus mediated cyclooxygenase-2 gene shorthairpinRNA (COX-2-shRNA) on invasiveness of endometrial carcinoma HEC-1B cells. Materials and Methods: Double-stranded DNA oligonucleotide of COX-2-shRNA was designed and synthesized, and the recombinant lentiviral vector COX-2-shRNA (LV-COX-2-ShRNA) was constructed. LV-COX-2-ShRNA, pHelper 1, and pHelper 2 were transferred into 293T cells, followed by lentiviral packaging. The virus titer was tested according to expression level of GFP in 293T cells. HEC-1B cells were infected with recombinant lentivirus. The silencing of COX-2 gene was assessed by real-time PCR and western-blot, and the in vivo invasiveness of HEC-1B cells was analyzed by transwell invasion assay. Results: Recombinant lentiviral vector expressing siRNA targeting COX-2 gene was successfully constructed to harvest the recombinant lentivirus with the concentrated virus suspension titer of 5×10^7 TCID/ml. Compared with control group, the inhibitory rate of COX-2 expression in HEC-1B cells in siRNA group were 61.87% and 67.48% at mRNA and protein level, respectively. The mean number of cells penetrating matrigel was 16.6, which was significantly less than the control group 50.2 and non-specific siRNA infection group 47.2, the invasion inhibition rate being 64.8% (p < 0.01). Conclusion: RNA interference can inhibit the invasiveness of HEC-1B cells.

Key words: RNA interference; Cyclooxygenase-2; Endometrial carcinoma.

Introduction
Cyclooxygenase-2 (COX-2) is an important rate-limiting enzyme transformation of arachidonic acid to prostaglandin, with abnormally increases expression in a variety of epithelial tumors such as colorectal cancer, esophageal cancer, lung cancer, gastric cancer, bladder cancer, cervical cancer, etc. [1]. Endometrial carcinoma is the second malignant tumor in gynaecology, which seriously threatens the life and health of elderly women. At present, the prognosis of advanced endometrial carcinoma is not ideal, and how to improve the survival rate still needs further research.

In the present authors’ previous studies, it was confirmed by in vitro experiments that, COX-2 selective inhibitor celecoxib can inhibit the proliferation of HEC-1B cells, induce the apoptosis, and reduce the cell invasiveness. In addition, the animal model of human endometrial carcinoma has been established successfully. It is further confirmed by in vivo experiments that, celecoxib can inhibit the growth of endometrial carcinoma in nude mice, and the mechanism may be related to the down-regulation of COX-2 mRNA expression in cells [2]. RNA interference technique is research means to specifically block the expression of some genes, with wide application in treatment of tumor, virus infection, genetic diseases, and other diseases [3-7]. Invasion and metastasis are important biological characteristics of malignant tumor, which are also the main reasons of death in patients with endometrial cancer. How to inhibit the metastasis and how to treat patients with advanced metastasis endometrial cancer are difficult clinical problems. This study aims to inhibit the expression of COX-2 in endometrial carcinoma cells by lentivirus mediated RNA interference technique, as to observe the variation of endometrial carcinoma invasiveness and explore the relationship between the COX-2 and the invasion of endometrial carcinoma.

Materials and Methods
Cell culture
Human endometrial adenocarcinoma HEC-1B cells were adhering grown and routinely cultured in RPMI-1640 medium containing 10% inactivated fetal bovine serum, at 37°C, 5% CO2 culture box, and the cultured medium was changed every two to three days, which were digested and passaged with 0.25% trypsin/EDTA, when the cells reached 80%

Preparation of lentiviral vector [8]
In the GeneBank, human COX-2 gene coding sequence of NM-000963 as the analysis sequence, four pairs oligonucleotide sequences of shRNA targeting COX-2 gene were designed and synthesized, and Basic Local Alignment Search Tool (BLAST) was used to perform sequence alignment of the selected interference sequence and the corresponding genomic data (human,
mouse, rat), which was certain that the selected gene sequence had no homology with other genes. Expression plasmids were constructed for each siRNA, respectively, which were transfected into 293T cells, and according to the inhibition rate of COX-2 gene, it was determined that the most effective target sequence was 5'- CGTTGCACTGAACTGTAGA-3', then according to which the DNA oligonucleotide of its shRNA was designed and synthesized as 5'-TaagTTGCACTGAACTGTAGAATTCAAAGATCT-TACAGTTCAGTGACGGTATC-3'. Core sequence of negative control was 5'-TTTCTCCGAACGTGTCACGTTTCAAGA-3', and downstream primer 5'-TTATTCCTCATGCCACGTGATTC-3'. Oligonucleotides were annealed to form double-stranded DNAs, which were connected with a PGCL-GF linear vector through T4 ligase and enzyme digestion by Age I and EcoR I to form the recombinant lentivector expressing shRNA, and which were verified by enzyme digestion. Fresh Competent Escherichia coli cells were prepared by calcium chloride and transformed into E.coli DH5α. The recombinant positive clones were screened from PCR and sequencing analysis. Positive clones primers were identified by PCR, with the upstream primer 5'-GTGTCACTAGGGGAAACAC-3', and downstream primer 5'-TTATTCCTCATGCCACGTGATTC-3'.

**Lentiviral packaging and titer determination [9]**

The recombinant lentivector plasmid of COX-2 shRNA and two auxiliary packaging plasmid pHelper 1 and pHelper 2 were transfected into 293T cells, eight hours after transfection, the medium being changed to the complete medium, then after 48 hours incubation, supernatant with slow virus particles was collected, the high titer lentivirus concentrate was detected in 293T cells and virus titer was calibrated. The virus concentrate was collected in sample cup to transfect the 293T cells after diluted in ten times series hole. The number of fluorescent cells with a proportion of about 10% in holes was calculated, which value was multiplied by the titer value of the virus stock obtained from the corresponding dilution.

**Grouping and infection of cells**

Cells were divided into three groups: experimental group shRNA-COX-2, negative control group COX-2-NC, and blank control group COX-2-CON. The target cells in logarithmic growth phase were digested by trypsin to prepare cell suspension, and the suspension with about 1.2 x 10⁷ cells was inoculated in 12-well plates and cultured at 37°C, 5% CO₂ incubator. When the cells were grown to reach 70% confluence, according to the Multiplicity of infection (MOI) value and experimental design group, RNAi lentivirus particles were added to target cell to carry out infectious experiment, and after 48 hours, RNA and protein were extracted from the cells with the success rate of 80% or more to carry out RT-PCR and Western blot detection experiment, so as to determine the interference effect of the target.

**Detection of COX-2 mRNA by RT-PCR**

HEC-1B cells of above three groups was collected and washed in PBS for three times, and total RNA was extracted according to kit operation manual, which was quantitative determined by UV spectrophotometry. PCR reaction conditions were denaturation for three minutes at 94°C, 94°C 30 seconds, 55°C 30 seconds, 72°C 45 seconds, and 72°C five minutes.

Products amplified by PCR were confirmed by 1.5% agarose gel electrophoresis, ethidium bromide (EB) staining, observation and analysis of ultraviolet projection instrument. All experiments were repeated three times.

**Determination of invasion assay by transwell chamber method**

Transwell chamber was coated by 50 mg/l Matrigel 1:8 dilution liquid at the bottom, air-dried at 4°C, sterilized under ultraviolet lamp for two hours, suctioning residual liquid, 50 ul serum-free medium containing 10 g/l BSA being added to each well, which were placed into 37°C incubator for 30 minutes, then the Transwell chamber were put into the 24-hole culture plate, adding 400 ul 1:1 mixed conditioned medium (HEC-1B cell supernatant) and complete medium at outside of the chamber, and adding in the chamber 200 ul tumor cell suspension with the cell number of 2 x 10⁵/ml and culture media containing 10 g/l BSA and 1% volume fraction of fetal bovine serum RPMI 1,640, after 24 hours, the Transwell chamber was removed and cleaned by PBS, the upper cell of the micro-porous membrane was wiped with a cotton swab, followed by fixing in 80% ethanol and stained by Giemsa. Under a microscope, the cells invaded to below the membrane were counted, and under a 100 times microscope, the number of passed membrane cells in five views was counted, from which the mean was derived.
Effect of lentivirus mediated cyclooxygenase-2 gene shorthairpinRNA on invasiveness of endometrial carcinoma

Statistical analysis
The results were analyzed with SPSS11.5 software, all data are used x ± s, two samples were compared using t test, the comparison of multiple mean using analysis of variance, and the difference has statistics significance as \( p < 0.05 \).

Results
Preparation and identification of lentiviral vector
COX-2 shRNA recombinant lentiviral vector plasmid was transformed into Escherichia coli DH5a, recombinant positive clones were identified by PCR, PCR positive products were cloned recombinant bacteria of 343 BP, and after double enzyme digestion, the pGC-L empty vector PCR fragment of 306 BP without any insertion was taken as control, which showed that the identification result corresponded with expectation (Figure 1). The sequencing result showed that the insertion of fragment was proved to be the same as designed COX-2 shRNA nucleotide sequence, and there was no mutation, deletion, and anomaly insertion, which indicated that recombinant lentiviral vector of COX-2 gene shRNA had been successfully constructed.

Recombinant lentivirus titer determination and infection
According to the expression level of GFP in 293T cells, using hole-by-hole dilution method, it was detected that the lentivirus concentration titer was \( 5 \times 10^7 \) Tu / ml, which indicated that a large number of plasmid had been transfected into 293T cell, and the virus had been successful packaged. As the MOI was 10, according to the percentage of cells under the fluorescence green microscope, it was determined that the infection rate of the recombinant lentiviral gene knockdown group cells can emit green fluorescence (RNAi) and negative control group cells (NC) infection rate was above 80%, whereas the blank control group (PC) was not observed with green fluorescence protein expression in cells (Figure 2).

Expression of COX-2 mRNA in HEC-1B cells infected by lentivirus
RT-PCR results showed that expression of COX-2 mRNA in RNAi group only was 38.13% of negative control group (NC), that was the inhibition rate being 61.87%, while no significant difference was COX-2 mRNA expres-
sion between the negative control group and blank control group \((p > 0.05)\), which compared with the negative control group, signified that the experimental group had an obvious inhibiting effect \((p < 0.05)\) on the mRNA gene expression of COX-2 in HEC-1B cells (Figure 3).

**Protein expression of COX-2 in HEC-1B cells after infection of lentivirus**

Western blot results showed that expression of COX-2 mRNA in RNAi group only was 32.5% of negative control group (NC), with the inhibition rate being 67.48%, while no significant difference in COX-2 mRNA expression between the negative control group and blank control group \((p > 0.05)\) was observed, which signified that compared with the negative control group, the experimental group had an obvious inhibiting effect \((p < 0.05)\) on the mRNA gene protein expression of COX-2 in HEC-1B cells (Figure 4).

**Effect of RNA interference on invasiveness HEC-1B cell**

Experimental results of invasion assay showed that the average number of HEC-1B cells in RNAi group penetrating through the artificial basement membrane was 16.6, \(s = 0.7\), which was significantly less than the negative control group \((47.2, s = 0.6)\) and control group \((50.2, s = 0.7)\), invasion inhibition rate being 64.8%, and the difference was statistically significant \((p < 0.01, \text{Figure 5})\).

**Discussion**

Recombinant lentivirus as transgenic vector, compared with the former chemical and physical method, adenovirus vector, and retroviral vector method, could not only ensure the safety, but also have high transduction efficiency, being capable of transducing mitotic cells and non-dividing cells, achieving a lasting expression stability, simultaneously having minimal immunogenicity, which does not easily produce neutralizing antibodies and other advantages, therefore the recombinant lentivirus was the current most appropriate in vivo transgenic vector even for gene therapy \([10]\).

Singh et al. \([11]\) had subcutaneously inoculated the human breast cancer cell line MCF-10A transfecting with COX-2 into nude mice to establish breast cancer model, which results showed that increased expression of COX-2 in tumor tissue of nude mice could enable the bone metastasis rate increasing significantly, indicating that COX-2 can promote the invasion and metastasis of breast cancer. Ogino et al. had detected in 662 cases of patients with colon cancer (Stage I-IV) by immunohistochemical method, and the results showed that cyclooxygenase-2 (COX-2) was overexpressing in 83% (548 cases) patients, which indicating that high expression of COX-2 is an important risk factor for colon cancer, and further study on the survival rate found that the higher the COX-2 expression in patients, the lower the survival rate \([12, 13]\). Many clinical studies have demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) can reduce incidence of colorectal cancer and the number and size of familial adenomatous polyp patients, which main mechanism realized through inhibition of COX, especially the inhibition of COX-2. Strillacci et al. \([15]\) reported that RNA interference could successfully inhibited the proliferation and invasion of colon cancer cells. Recent studies have found that RNA interference could inhibit the growth and metastasis of a variety of other malignant tumor \([16-21]\).

The present study applied RNA interference to inhibit COX-2 expression, which on the one hand, further clarified the roles of COX-2 in tumorigenesis and tumor progression, on the other hand, investigated the use of RNA interference technology as the strategy and method for gene therapy of tumor. Inhibition of RNA interference on COX-2 gene expression, also affected some biological characteristics of endometrial cancer cells. Tumor cells must destroy the extracellular matrix composed of extracellular matrix and basement membrane in the invasion process,
Matrigel gum in the experiments is very similar to extra-cellular matrix components, which can effectively mediate the tumor cells in vitro invasion process. Cell invasion assay results showed that mean cell number of siRNA group HEC-1-B cells penetrating through the artificial basement membrane of Transwell chamber was 16.3, which was significantly less than the negative control group (48.5), and control group (50.3); the invasion inhibition rate was 66.4% and the difference had statistical significance. Results of the present study demonstrated that, RNA interference could reduce the invasion of HEC-1-B cells in endometrial adenocarcinoma. Compared with control group, the expressions of COX-2 mRNA and protein in siRNA group were significantly inhibited. This further suggested that, RNA interference might down-regulate COX-2 expression in HEC-1-B cells, thus reduce the invasion of tumor cells, which had provided experimental basis for biological treatment of endometrial carcinoma.

To sum up the aforementioned, through transferring the constructing shRNA eukaryotic expression vector targeting COX-2 into cells can highly and specifically inhibit COX-2 expression in HEC-1B cells, thus reduce the invasion of tumor cell invasion. It is feasible and effective for prevention and treatment of tumors with high COX-2 expression through application of RNA interference. However, these results are confirmed only at the cellular level, and there is no in vivo research report, hence further study is still required.

References

Primary peritoneal cancer: study of 14 cases and comparison with epithelial ovarian cancer

Department of Obstetrics and Gynecology, Osaka City University Graduate School of Medicine, Abeno-ku, Osaka (Japan)

Summary
Purpose of investigation: Primary peritoneal carcinoma (PPC) is histologically similar to ovarian serous carcinoma, but its biochemical features remain obscure. The authors investigated and compared clinical findings, treatments, and outcomes of patients with PPC and those with epithelial ovarian cancer (EOC). Materials and Methods: The authors retrospectively reviewed data from 14 patients with PPC and 219 patients with EOC treated at the present hospital from January 2005 to December 2012, including demographic data, pathologic findings, treatments, and outcomes. Results: Patients with PPC were significantly older (62.6 ± 8.4 years) than those with EOC (56.3 ± 11.3 years) (p = 0.045). There was no significant difference in serum CA-125 levels. The five-year survival rates did not differ significantly between patients with PPC (61.1%) and those with EOC (60.3%; p = 0.78); nor between patients with PPC and those with Stage III serous EOC (43.8%; p = 0.40). Conclusions: Treatment strategies for EOC applied to PPC apparently led to similar survival patterns among the two patient groups. Cytoreductive surgery combined with pre/postoperative platinum-containing chemotherapy may be effective for PPC patients.

Key words: Primary peritoneal cancer; Epithelial ovarian cancer; Cytoreductive surgery; Platinum-containing chemotherapy.

Introduction
Primary peritoneal carcinoma (PPC) was first described by Swerdlow in 1959 [1]. PPC is a malignancy with diffuse involvement of the peritoneal surfaces, involving mostly the omentum with minimal or no ovarian involvement [1, 2]. Histopathologic and clinical similarities have been reported between PPC and ovarian serous carcinoma [3–6]. PPC has been reported in women who underwent oophorectomy for benign disease or prophylaxis [7]. Both differences [3, 8, 9] and molecular similarities [10] have been reported between PPC and ovarian serous carcinoma, although the clinicopathologic features and etiology of PPC are somewhat obscure. As PPC begins from intra-abdominal disease, it is at Stage ≥ 3 at presentation, which is the main reason for its poor five-year survival rate. Management of PPC shadows that of epithelial ovarian carcinoma (EOC), with initial debulking surgery followed by adjuvant platinum-containing chemotherapy. However, optimal cytoreduction is more difficult in PPC than in EOC, as PPC tends to have widespread peritoneal disease.

This study investigated clinical findings, treatment, and prognosis of patients with PPC compared with those with EOC, to improve recognition of this disease.

Materials and Methods
Patients
This retrospective study included 14 patients with PPC and 219 patients with EOC, who were treated at the present hospital between January 2005 and December 2012. Each patient provided written informed consent. Both sets of patients were treated with debulking surgery combined with adjuvant/neoadjuvant chemotherapy.

Definitions
PPC was defined by the following Gynecologic Oncology Group’s (GOG’S) inclusionary criteria for PPC [11]: (1) both ovaries must be either physiologically normal in size or enlarged by a benign process; (2) involvement in extraovarian sites must be greater than involvement on the surface of either ovary; (3) microscopically, the ovarian component must be one of the following: (a) non-existent, (b) confined to ovarian surface epithelium with no evidence of cortical invasion, (c) involve ovarian surface epithelium and underlying cortical stroma but with no given tumor size ≥ 5 × 5 mm, or (d) any tumor < 5 × 5 mm within ovarian substance associated with or without surface disease; (4) histological and cytological tumor characteristics must be predominantly of the serous type, i.e., similar or identical to that seen in ovarian serous papillary adenocarcinoma. Diagnoses were based on original pathologic reports, reviewed by two certified pathologists.

Data and treatment
Clinical data were obtained from medical records of each patient including age at diagnosis, presenting symptoms, operative findings, whether primary cytoreductive surgery was optimal or suboptimal, preoperative CA125 levels, International Federation of Gynecology and Obstetrics (FIGO) stage based on clinical/surgical examinations and cytology results, type of chemotherapy, number of treatments, follow-up, and survival.
courses, date of tumor recurrence or progression, date of last follow-up, and date of death. Optimal surgery was defined as presence of residual tumor < 1.0 cm in size after surgery. Suboptimal surgery was defined as presence of residual tumor ≥ 1.0 cm after surgery. Tumors were staged according to FIGO criteria for ovarian cancer. Treatment for both PPC and EOC usually consisted of initial debulking surgery followed by adjuvant chemotherapy; patients who seemed unsuitable for initial surgery received a few cycles of neoadjuvant chemotherapy. Standard surgery included total hysterectomy with bilateral salpingo-oophorectomy, partial omentectomy, and lymphadenectomy. For those with large residual disease and/or poor medical condition at surgery, lymphadenectomies were omitted. The chemotherapy regimen consisted of paclitaxel + carboplatin or docetaxel + carboplatin. The authors compared characteristics and prognosis of 14 patients with PPC to those of 219 patients with EOC; the 14 patients with PPC were also compared with 41 patients with Stage III EOC, which histologically shows as serous adenocarcinoma or poorly differentiated adenocarcinoma (i.e., the histology type most similar to PPC).

Statistics

Clinicopathological data were analyzed by Fisher’s exact test and the χ² test. Survival and disease-free survival probabilities were analyzed using the Kaplan–Meier method. SPSS 21.0 was used for data analyses. A \( p < 0.05 \) was considered to be significant.

Results

Clinical characteristics of all 14 patients with PPC are detailed in Table 1. Among presenting complaints, ten patients (71.4%) had abdominal distention, and one each (7.1%) had abnormal genital bleeding, appetite loss, or deterioration of dermatomyositis; one (7.1%) had no symptoms but was singled out in a screening. Four of the 14 (28.6%) could undergo optimal surgery; two (14.3%) had a few cycles of neoadjuvant chemotherapy consisting of paclitaxel + carboplatin before undergoing optimal surgery. Follow-up range for PPC patients was 11–96 months (median: 55 months), with 9/14 (64.3%) patients followed for at least five years or until death. By August 31st, 2013, 5/14 (35.7%) were alive free of disease, 5/14 (35.7%) were alive with disease, and 4/14 (28.6%) had died of disease.
The mean age of patients with PPC (62.6 ± 8.4 years) was significantly older than for patients with EOC (56.3 ± 11.3 years, \( p = 0.045 \); Table 2). All PPC patients had Stage III disease; whereas among the EOC patients, 82 had Stage I, 20 had Stage II, 94 had Stage III, and 23 had Stage IV (\( p = 0.001 \)). Among histologic types, patients with PPC had 12 serous adenocarcinomas and two poorly differentiated adenocarcinoma; those with EOC had 60 serous adenocarcinomas, 37 mucinous adenocarcinomas, 46 endometrioid adenocarcinomas, 65 clear-cell adenocarcinomas and 11 poorly differentiated adenocarcinomas (\( P<0.001 \)). Initial CA125 levels did not differ significantly.
Between PPC patients and those stage III EOC patients with serous adenocarcinoma or poorly differentiated adenocarcinoma as histologic type, mean age, clinical stage, histologic type and initial CA125 level were not significantly different (Table 3).

The two groups did not significantly differ in overall survival ($P = 0.78$; Figure 1) or disease-free survival ($P = 0.73$; Figure 2). Five-year survival was 61.1% for PPC and 60.3% for EOC. Median time to recurrence was 670 days for PPS and 811 days for EOC.

Overall survival of patients with PPC and those with Stage III EOC and serous adenocarcinoma or poorly differentiated adenocarcinoma histology did not significantly differ ($P = 0.40$; Figure 3); five-year survival was 61.1% for PPC and 43.8% for Stage III EOC. Disease-free survival did not significantly differ between PPC patients and Stage III EOC patients ($P = 0.55$; Figure 4). Median time to recurrence was 670 days for PPS and 532 days for Stage III EOC.

Discussion

The clinical and histologic features of PPC are similar to those of EOC, and patterns of spreading, response to chemotherapy, and prognoses are almost the same as EOC [11, 12]. However, PPC can occur after prophylactic oophorectomy, and has been encountered even in a male patient [13]. Histologically, most PPS has serous adenocarcinoma, but other types have also been reported [14, 15]. The etiology, pathogenesis, cell of origin, and clinicopathologic features of PPC remain obscure.

In this study, compared with all EOC patients, PPC patients were older at diagnosis and all had advanced disease (Stage III), which concords with previous studies in terms of age [16, 17] and general disease stage [15]. As PPC begins from intra-abdominal disease, presenting stage must be at least III. Serum CA125 level was elevated in all PPC patients, but did not significantly differ from EOC patients. Most PPC patients had serous adenocarcinoma histology; whereas EOC specimens varied (clear-cell carcinoma; 29.7%, serous adenocarcinoma; 27.4%, endometrioid adenocarcinoma; 21.0%, mucinous adenocarcinoma; 16.9%, poorly differentiated adenocarcinoma; 5.0%). Overall survival and progression-free survival did not significantly differ between the two patient groups. About 30% of EOC patients had clear-cell carcinoma, which has a poor prognosis, and about 60% of EOC patients with other than clear-cell carcinoma had Stage ≥3, which explains why the two types had similar prognoses despite all PPS patients having advanced disease.

When PPC patients and patients with Stage III EOC with serous- or poorly-differentiated adenocarcinoma (i.e., the EOC histology most similar to that of PPC) were compared, mean age, clinical stage, histologic type, initial CA125 level, and prognosis did not significantly differ.

Comparative prognoses of PPC and EOC are controversial. Some studies report PPC patients to have significantly worse survival rates than those with EOC [18, 19]; others report that their survival rates are not significantly different [14, 15, 18, 20].

Management of PPC has shadowed that of EOC, with initial debulking surgery followed by adjuvant platinum-containing chemotherapy. Although degree of cytoreductive surgery is reportedly a significant prognostic factor in PPC [21], optimal cytoreduction is more difficult in PPC than in EOC, as PPC tends to develop widespread peritoneal disease. In this study, the optimal cytoreductive rate was only 28.6%, but has been reported as 33–70% [8, 12, 14, 15, 20]. Only 2/14 of the present patients received neoadjuvant chemotherapy, both of whom then underwent optimal cytoreductive surgery. Neoadjuvant chemotherapy might help achieve higher rates of successful cytoreductive surgery.

A standard treatment for PPC has not been established, although such treatment would appear to be similar to that for EOC, as is its prognosis.

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Address reprint requests to:
T. FUKUDA, M.D.
Department of Obstetrics and Gynecology,
Osaka City University Graduate School of Medicine,
1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585 (Japan)
e-mail: takeshif@med.osaka-cu.ac.jp
Combination therapy of liposomal paclitaxel and cisplatin as neoadjuvant chemotherapy in locally advanced cervical cancer

Y. Li, X. Wang, J. Li, W. Ding

Department of Gynecologic Oncology, Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center of Cancer Medicine, Guangzhou (People’s Republic of China)

Summary

Objectives: To investigate the efficacy and toxicities of combination therapy of liposomal paclitaxel and cisplatin as neoadjuvant chemotherapy (NACT) in locally advanced cervical cancer. Materials and Methods: The authors retrospectively reviewed the clinical records of patients with cervical cancer who received NACT with liposomal paclitaxel and cisplatin at Sun Yat-sen University Cancer Center from April 1, 2008 to December 31, 2012. Liposomal paclitaxel and cisplatin was administrated intravenously at a dose of 175 mg/m² and 75 mg/m², respectively. Results: The total response rate was 86.1% (62/72) including a complete response and partial response rate of 27.8 % (20/72) and 58.3% (42/72), respectively. Stable disease was observed in 12.5 % (9/72) of patients and progressive disease in 1.4 % (1/72). Hematological toxicities were the major dose-limiting toxicities. Grade 3/4 neutropenia and anemia developed in 18.1% (13/72) and 6.9% (5/72) of patients, respectively. Peripheral neuropathy occurred in 6.9% (5/72) of patients (all grade 1). Conclusion: the study findings support further evaluation of liposomal paclitaxel with cisplatin as an additional chemotherapy regimen which may be efficacious and tolerable in the NACT of cervical cancer.

Key words: Cervical cancer; Neoadjuvant chemotherapy; Liposomal paclitaxel; Locally advanced.

Introduction

Cervical cancer is still the second most common malignancy in women in most low- and middle-income countries [1]. Despite the application of screening method for early disease, numerous patients present with locally advanced diseases (Stages Ib2-Ia) [2] especially in developing countries. Prognosis of this group still needs to be improved. Treatment modality for locally advanced cervical cancer remains controversial. Concurrent radiation therapy and chemotherapy is commonly recommended [3]. However, some patients could still recur after the treatment. Furthermore, in developing areas, radiation facility may not be available. Another treatment choice is preoperative neoadjuvant chemotherapy (NACT) for two to three courses followed by radical hysterectomy [4]. Several cisplatin-based chemotherapy regimens, including combination of paclitaxel and cisplatin, have been shown to be effective against cervical cancer as NACT [4, 5].

Paclitaxel is hydrophobic and possesses a very low solubility in conventional aqueous vehicles. The preparation approved for clinical use solubilizes paclitaxel in mixture of polyethoxylated castor oil and ethanol. This vehicle may cause severe hypersensitivity reactions in humans and peripheral neuropathies [6-8].

Liposomes are a drug delivery system with diameter from 250Å to more than 20 µm and offer a flexible platform to encapsulate both lipophilic and hydrophilic drugs. It has been used to enhance the therapeutic efficacy and reduce the toxicity of several anticancer agents, including doxorubicin. Liposomal paclitaxel is a liposome-encapsulated formulation of paclitaxel. Paclitaxel liposome was found to be a viable alternative of paclitaxel because of its improved toxicological and pharmacological characteristics [9,10]. Pharmacokinetic studies in animals [10-12] have shown that liposomal paclitaxel has a longer elimination half-life and a larger volume of distribution, as compared with paclitaxel [10-12]. The concentration of liposomal paclitaxel in tissues is dramatically higher than that of paclitaxel, especially in the reticuloendothelial system, such as lymph nodes, liver, and spleen. Recent clinical trials have shown that liposomal paclitaxel and paclitaxel has similar efficacy in breast, gastric, and non-small cell lung cancer and liposomal paclitaxel has less adverse effects than paclitaxel [13-15]. In this study, the authors aim to investigate the efficacy and toxicities of combination therapy of liposomal paclitaxel and cisplatin as NACT before surgery in patients with locally advanced cervical cancer by retrospectively reviewing their data.
2012. The patient list was obtained from the database of Sun Yatsen University Cancer Center. Patient hospital records were reviewed to obtain demographic data including age, diagnosis, weight, height, tumor size, stage, histology, abdominal and pelvic computed tomography (CT) or magnetic resonance imaging (MRI), results of routine blood test, and treatment data including total cycles, doses of liposomal paclitaxel and cisplatin, date of chemotherapy delivery, and toxicities of chemotherapy. Approval was granted from the institutional review board prior to the review of all the clinical records.

**Inclusion Criteria**
Patients who met the following criteria were included in the study: (1) a pathologically confirmed diagnosis of cervical cancer; (2) Stage IB2 - II B disease except for Stage IA1 tumor; (3) two or three courses of combined therapy with liposomal paclitaxel and cisplatin; (4) absence of prior anti-cancer therapy.

**Treatment**
Liposomal paclitaxel was administrated by intravenous infusion over three hours at a dose of 175 mg/m² on day 1. Cisplatin was administrated intravenously at a total dose of 75 mg/m², which was infused over six hours on day 1 or was divided into two or three doses and given over three hours on day 1 to 2 or 3. The treatment was repeated every three weeks. Usually after each cycle, blood count was performed twice weekly. Granulocyte colony-stimulating factor (G-CSF) was administrated if grade 3 or 4 neutropenia developed. All patients received radical hysterectomy three to four weeks after the last course of NACT.

**Evaluation of Treatment Response and Adverse Effects**
Treatment evaluation was made based on a modification of the Response Evaluation Criteria in Solid Tumors (RECIST) [16]. Complete response (CR) was defined as the disappearance of measurable disease. Partial response (PR) was defined as a reduction of ≥50% in the sum of the products of the maximum and perpendicular diameters of measurable lesion. Progressive disease (PD) was defined as ≥25% increase in the sum of the products of maximum and perpendicular diameters of measurable lesion, or the appearance of new lesions. Stable disease (SD) was a steady state of response less than a PR or progression. Adverse effects were graded according to the National Cancer Institute (NCI) toxicity criteria [17].

To investigate the impact of different cisplatin administration method on renal function, creatinine clearance (CrCl) before chemotherapy and after two cycle of chemotherapy was calculated by the Cockcroft-Gault equation [18,19], which is as follows: 

\[
\text{CrCl (ml/min)} = \frac{(140 - \text{age (years)}) \times \text{weight (kg)} \times 0.85}{\text{serum Cr (mg/dl) \times 72}}
\]

Statistical analysis was performed using the SPSS 16.0. Chi-square test was used to compare the difference of CrCl decrease. A p value of < 0.05 was considered statistically significant.

**Results**

**Patient characteristics**
A total of 72 consecutive patients were identified. The median age was 48 years (range 29-64). The number of patients with Stage Ib2, Iia2, and Iib disease was 33, 21, and 18, respectively. Sixty-three patients had squamous cell carcinoma, eight adenocarcinoma, and one adenosquamous cell carcinoma. The median tumor size at diagnosis was five cm (range 3-7) and it was greater than six cm in 12 patients. Fifty-six patients received two cycles and 16 patients three cycles of liposomal paclitaxel and cisplatin combination therapy as NACT. The total dose of cisplatin was administrated on day 1, or given dividedly on day 1 - 2, or day 1 - 3 in 13, 34, and 25 patients, respectively (Table 1).

**Table 1. — Patient characteristics (total n = 72).**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Years/No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48</td>
<td>29–64</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ib2</td>
<td>33</td>
<td>45.8</td>
</tr>
<tr>
<td>Iia2</td>
<td>21</td>
<td>29.2</td>
</tr>
<tr>
<td>Iib</td>
<td>18</td>
<td>25.0</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>63</td>
<td>87.5</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>8</td>
<td>11.1</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Number of cycles</td>
<td>2</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>PDD administration schedule</td>
<td>Total dose on day 1</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Total dose dividedly on day 1 - 2</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Total dose dividedly on day 1 - 3</td>
<td>25</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>Median</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>3-7</td>
</tr>
<tr>
<td></td>
<td>&lt; 6</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>≥ 6</td>
<td>12</td>
</tr>
</tbody>
</table>

**Treatment response**
All patients were assesseable for treatment response. The total response rate was 86.1% (62/72) including a CR and PR rate of 27.8% (20/72) and 58.3% (42/72), respectively. Pathologically confirmed CR was noted in 9.7% (7/72) of patients, and pathologically near complete remission (a few tumor cells were still seen after NACT), stable disease, and progressive disease in 13.9% (10/72), 12.5% (9/72), and 1.4% (1/72) of patients, respectively. The response rate was 84.8%, 85.7%, and 88.9% in patients with Stage Ib2, Iia2, and Iib disease, respectively. In 63 patients with squamous cell type, eight patients with adenocarcinoma, and one patient with adenosquamous cell type, the response rate was 85.7% and 87.5%, 100%, respectively. In 12 patients with tumor size equal to or greater than six cm and 60 patients with tumor size less than six cm, the response rate was 91.7% and 85.0%, respectively. The response rate was 84.6% (11/13), 88.2% (30/34), and 84.0% (21/25), in patients receiving total dose of cisplatin on day 1, or divided doses on day 1 – 2, or divided doses on day 1 – 3, respectively (Table 2).
Combination therapy of liposomal paclitaxel and cisplatin as neoadjuvant chemotherapy in locally advanced cervical cancer

Adverse effects

All patients were assessable for adverse effects. Hematological toxicities were the major dose-limiting toxicities. Grade 3/4 neutropenia developed in 18.1% (13/72) patients. Grade 3/4 anemia occurred in 6.9% (5/72) patients. Packed red blood cells were transfused in one patient. Grade 1/2 thrombocytopenia was observed in 8.3% (6/72) patients. No grade 3/4 thrombocytopenia occurred. Nausea/vomiting was frequent with a total incidence of 58.3% (42/72), but was not dose-limiting. Peripheral neuropathy developed in 6.9% (5/72) patients (all grade 1). Other toxicities included alopecia, liver and renal impairment. Alopecia happened in 65.3% (47/72) patients, with grade 1 and 2 in 41.7% (30/72) and 23.6% (17/72) patients, respectively. Grade 3 liver impairment occurred in 4.1% (3/72). No hypersensitivity reaction was noted (Table 3). There was no treatment related death.

**Table 2. — Treatment response (total n = 72).**

<table>
<thead>
<tr>
<th>Factors</th>
<th>CR+ PR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>PD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62 (86.1)</td>
<td>20 (27.8)</td>
<td>42 (58.3)</td>
<td>9 (12.5)</td>
<td>1 (1.4)</td>
</tr>
</tbody>
</table>

**Stage**

- Ib2 (n = 33)  
  - CR+ PR: 28 (84.8%)  
  - CR: 12 (36.4%)  
  - PR: 16 (48.5%)  
  - SD: 4 (12.1%)  
  - PD: 1 (3.0%)  

- Ila2 (n = 21)  
  - CR+ PR: 18 (85.7%)  
  - CR: 4 (19.0%)  
  - PR: 14 (66.7%)  
  - SD: 3 (14.3%)  
  - PD: 0  

- Iib (n = 18)  
  - CR+ PR: 16 (88.9%)  
  - CR: 4 (22.2%)  
  - PR: 12 (66.7%)  
  - SD: 2 (11.1%)  
  - PD: 0  

**Histology**

- Squamous (n = 63)  
  - CR+ PR: 54 (85.7%)  
  - CR: 18 (28.6%)  
  - PR: 36 (57.1%)  
  - SD: 8 (12.7%)  
  - PD: 1 (1.6%)  

- Adenocarcinoma (n = 8)  
  - CR+ PR: 7 (87.5%)  
  - CR: 2 (25%)  
  - PR: 5 (62.5%)  
  - SD: 1 (12.5%)  
  - PD: 0  

- Adenosquamous (n = 1)  
  - CR+ PR: 1 (100%)  
  - CR: 0  
  - PR: 1 (100%)  
  - SD: 0  
  - PD: 0  

**Tumor size (cm)**

- ≥6 (n = 12)  
  - CR+ PR: 11 (91.7%)  
  - CR: 2 (16.7%)  
  - PR: 9 (75%)  
  - SD: 1 (8.3%)  
  - PD: 0  

- < 6 (n = 60)  
  - CR+ PR: 51 (85.0%)  
  - CR: 16 (26.7%)  
  - PR: 35 (58.3%)  
  - SD: 8 (13.3%)  
  - PD: 1 (1.7%)  

**PDD administration schedule**

- Total dose on d 1 (n = 13)  
  - CR+ PR: 11 (84.6%)  
  - CR: 5 (38.5%)  
  - PR: 6 (46.2%)  
  - SD: 2 (15.4%)  
  - PD: 0  

- Total dose dividedly on d 1-2 (n = 34)  
  - CR+ PR: 30 (88.2%)  
  - CR: 6 (17.6%)  
  - PR: 24 (70.6%)  
  - SD: 4 (11.8%)  
  - PD: 0  

- Total dose dividedly on d 1-3 (n = 25)  
  - CR+ PR: 21 (84.0%)  
  - CR: 9 (36.0%)  
  - PR: 12 (48.0%)  
  - SD: 3 (12.0%)  
  - PD: 1 (4.0%)  

**Table 3. — Adverse effects (total n = 72).**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Total (%)</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>58.3</td>
<td>11</td>
<td>15.3</td>
<td>18</td>
<td>25.0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4.2</td>
<td>3</td>
<td>4.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Hematological             |           |      |      |      |      |
| Anemia                    | 59.7      | 21   | 29.1 | 17   | 23.6 |
| Neutropenia               | 56.9      | 8    | 11.1 | 20   | 27.7 |
| Thrombocytopenia          | 8.3       | 4    | 5.5  | 2    | 2.8  |

| Others                    |           |      |      |      |      |
| Alopecia                  | 65.3      | 30   | 41.7 | 17   | 23.6 |
| Peripheral neuropathy     | 6.9       | 5    | 6.9  | 0    | 0    |
| Allergy reaction          | 0         | 0    | 0    | 0    | 0    |
| Liver impairment          | 4.2       | 3    | 4.2  | 0    | 0    |

G: grade.

Adverse effects

All patients were assessable for adverse effects. Hematological toxicities were the major dose-limiting toxicities. Grade 3/4 neutropenia developed in 18.1% (13/72) patients. Grade 3/4 anemia occurred in 6.9% (5/72) patients. Packed red blood cells were transfused in one patient. Grade 1/2 thrombocytopenia was observed in 8.3% (6/72) patients. No grade 3/4 thrombocytopenia occurred. Nausea/vomiting was frequent with a total incidence of 58.3% (42/72), but was not dose-limiting. Peripheral neuropathy developed in 6.9% (5/72) patients (all grade 1). Other toxicities included alopecia, liver and renal impairment. Alopecia happened in 65.3% (47/72) patients, with grade 1 and 2 in 41.7% (30/72) and 23.6% (17/72) patients, respectively. Grade 3 liver impairment occurred in 4.1% (3/72). No hypersensitivity reaction was noted (Table 3). There was no treatment related death.

**Table 4. — The impact of different methods of cisplatin administration on renal function.**

<table>
<thead>
<tr>
<th>PDD administration schedule</th>
<th>N</th>
<th>CrCl decrease ≥ 10 ml/min</th>
<th>CrCl decrease ≥ 20 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>CrCl decrease</td>
<td>N</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>N</td>
<td>%</td>
<td>After two cycles</td>
<td>After two cycles</td>
</tr>
</tbody>
</table>

| PDD on day 1                | 13    | 7                         | 53.8                      | 4 | 30.7 |
| PDD on day 1-2 or 1-3       | 59    | 22                        | 37.3                      | 11 | 18.6 |

PDD: cisplatin; CrCl: creatinine clearance.

CrCl decrease of greater than or equal to ten ml/min after two cycles of chemotherapy occurred in 53.8% (7/13) and 37.3% (22/59) patients, respectively, in group receiving cisplatin on day 1 and in group receiving cisplatin on day 1-2 or 1-3. The difference was not significant (p > 0.05).
CrCl decrease of greater than or equal to 20 ml/min after two cycles of chemotherapy occurred in 30.7% (4/13) and 18.6% (11/59) patients, respectively, in group receiving cisplatin on day 1 and in group receiving cisplatin on day 1-2 or 1-3. The difference was also not significant (p > 0.05) (Table 4).

Discussion

The present study suggested that combination therapy of liposomal paclitaxel and cisplatin is effective as NACT before surgery in patients with locally advanced cervical cancer. The toxicities are controllable.

In the literature, several regimens have been reported to be effective against locally advanced cervical cancer in the NACT setting with a response rate of 78% - 95% [5]. Combination therapy of paclitaxel and cisplatin is one of the effective regimens reported. The response rate is usually 72% - 90% [20-22]. In one study, paclitaxel and cisplatin regimen was given to 43 patients with Stage IB2 to IIB cervical cancer before surgery. Paclitaxel 60 mg/m² was administered intravenously over three hours, followed by cisplatin 60 mg/m², also administered intravenously. The chemotherapy was administered every ten days and for three courses. The total response rate was 90.7% (39/43), including a complete response rate of 39.5% (17/43) and a partial response of 51.2% (22/43). Stable disease occurred in 9.3% (4/43) of patients [22].

There is only limited report on the use of liposomal paclitaxel in cervical cancer. One study compared the efficacy and toxicities of liposomal paclitaxel or paclitaxel combined with cisplatin or carboplatin in concurrent chemoradiotherapy. Both paclitaxel and liposomal paclitaxel was administrated at a dose of 135 mg/m², cisplatin 80 mg/m², and carboplatin AUC (area under curve) 4 – 6. Treatment repeated every 21 days for two to three cycles. Radical radiotherapy was given to both groups at the same time. Seventy-one cases were included in paclitaxel group and 91 in paclitaxel liposome group. The overall response rate was 90.1% and 89.0%, in paclitaxel and paclitaxel liposome group, respectively. The one-year cumulative survival rate was 91.4% for paclitaxel group and 89.2% for paclitaxel liposome group. Both overall response rate and one-year cumulative survival rate is not significantly different between two groups [23]. However, in that study, treatment efficacy is the combined result of chemotherapy and radiotherapy. One cannot see the exact effect of chemotherapy.

In the current study, liposomal paclitaxel combined with cisplatin was given to patients with locally advanced cervical cancer as NACT. The overall response rate was 86.1% (62/72), including a CR rate of 27.8% (20/72), and a PR rate of 58.3% (42/72). The response rate in the present study was similar to that (72% - 90%) of paclitaxel combined with cisplatin reported in the literature [20-22]. The pathological CR rate was 13.9% (10/72) in the current study, which was close to that (11% -20%) reported in the NACT of locally advanced cervical cancer by cisplatin or carboplatin based regimens in the literature [5]. Pathological CR rate after NACT or chemoradiotherapy was shown to be associated with favorable prognosis [24, 25].

The toxicities of conventional paclitaxel were mainly hematological toxicities, neuropathy, and hypersensitivity reactions. The latter two adverse effects were caused by the dehydrated ethanol and polyethylene glycol [Cre-mophor EL] used to dissolve paclitaxel [6]. Liposomal paclitaxel is designed to not only obviate the hypersensitivity reactions associated with the Cre-mophor EL vehicle, but also decrease the toxicities that arise from the drug’s pharmacological action [9, 26]. A reduction in toxicities leads to a substantial elevation of the maximum tolerated dose [26]. Several clinical trials showed that liposomal paclitaxel has less hypersensitivity reactions and neuropathy, and similar other toxicities, such as hematological toxicities, when compared to paclitaxel [13-15,23]. In one prospective study, liposomal paclitaxel or paclitaxel plus cisplatin regimen was given to a total of 100 patients with non-small cell lung cancer. Liposomal paclitaxel or paclitaxel was administrated at a dose of 150 mg/m², and cisplatin 75 mg/m² every 21 days. There was no significant difference as of grades 3 and 4 toxicity including hematological toxicities and nausea/vomiting between the two arms. Peripheral neuropathy occurred less frequently in the liposomal paclitaxel plus cisplatin group than in the paclitaxel plus cisplatin group (8% vs. 28%) [15].

In the current study, liposomal paclitaxel was administrated at a dose of 175 mg/m², and cisplatin 75 mg/m². The toxicities were generally controllable. Hematological toxicities were the major dose-limiting toxicities. Grade 3/4 anemia and neutropenia was observed in 6.9% and 18.1% patients, respectively. No grade 3/4 thrombocytopenia happened. Other frequent adverse effects were vomiting and alopecia, which was 58.3% and 65.3%, respectively. Peripheral neuropathy occurred in 6.9% patients, all were grade 1. No allergy reaction was noted.

One of the major adverse effects of cisplatin is renal impairment. Administering the total dose of cisplatin per cycle dividedly may decrease the impact of cisplatin on renal function. In this study, the impact of different method of cisplatin infusion on CrCl was evaluated by comparing the CrCl decrease after two cycles of chemotherapy. CrCl decrease of ≥ 10 ml/min occurred in 53.8% (7/13) and 37.3% (22/59) patients, and CrCl decrease of ≥ 20 ml/min in 30.7% (4/13) and 18.6% (11/59) patients, respectively, in group receiving total dose of cisplatin on day 1 and in group receiving divided dose of cisplatin on days 1–2 or 1–3. Although the difference was not significant, the tendency indicated that the group receiving total dose of cisplatin on day 1 may suffer more renal impairment than the group receiving cisplatin dividedly on days 1-2 or 1-3.
Dividing the total dose of cisplatin per cycle to two or three doses and administering on days 1-2 or 3 may decrease renal toxicities, but may also compromise the antitumor effects. In this study, the response rate was 84.6%, 88.2%, and 84.0% in patients with total dose of cisplatin per cycle administered on day 1, or dividedly given on days 1–2, or on days 1–3, respectively. It seems that the response rate in different group was close.

One should be aware of the limitations of this study. This is a retrospective study and is subject to the limitations of this type of study. The number of cases is small. The results were from one center. Thus, the interpretation of the results should be made with caution.

In conclusion, the study findings support further evaluation of liposomal paclitaxel with cisplatin as an additional chemotherapy regimen which may be efficacious and tolerable in the NACT of cervical cancer.

References


Address reprint requests to: YANFANG LI, M.D., Department of Gynecologic Oncology, Sun Yat-sen University Cancer Center, 651 Dongfeng Road East, Guangzhou, 510060 (People’s Republic of China) e-mail: liyf@syyucc.org.cn
Introduction

Pyometra is the accumulation of pus in the uterine cavity. It occurs in the absence of inherent drainage of the uterus as a result of a closed uterine cervix, or, as is the case with cancers or the presence of foreign bodies, in the presence of a causal factor that leads to inflammation. It is generally seen in post-menopausal women, with an incidence of 0.2% [1]. Its incidence is 1.5% - 4% in patients with malignant lesions [2,3]. Pyometra should always be considered in post-menopausal patients who have nonspecific symptoms, such as discharge, bleeding, fever, enlarged uterus, and lower abdominal pain, because it may accompany malignancy and, when the diagnosis is delayed, morbidity and mortality increase [4].

The purpose of this study was to describe the clinical and histopathological characteristics of a cohort of 12 patients with pyometra and to highlight the increased incidence of gynecological malignancy in patients with pyometra.

Materials and Methods

Between 2009 and 2013, 12 patients diagnosed with pyometra were treated in the gynecology and gynecologic oncology clinics of the present hospital, which is a tertiary treatment center. The diagnosis of pyometra was confirmed by the presence of accumulated pus from the cervix. Accompanying lesions were investigated using ultrasound (US) and, when indicated, magnetic resonance imaging (MRI).

All patients underwent cervical dilation, endometrial curettage, and, when indicated, cervical biopsy. Medical records were reviewed, and specific data were collected, including patient age, symptoms, systemic diseases, radiological investigations (US, MRI), microbiological results, treatment modality, histopathological results, and blood work, including hemoglobin, white blood cell count, erythrocyte sedimentation rate, C-reactive protein, procalcitonin, and Ca 125.

Data were shown as mean ± SD for metric discrete variables, and the number and percentage of cases were used as nominal variables.

Results

Twelve patients were enrolled in this study (Table 1). All patients were post-menopausal and multiparous. The patients’ mean age was 70.83 ± 6.978 years (min=61, max=82). Five patients (41.6%) came to the emergency department, and four patients (58.4%) came to the polyclinic of gynecology. The most common presenting symptom was vaginal discharge (n=8, 66.6%), followed by fever (n=6, 50%) and bleeding (n=4, 33.3%).

Blood work included the following: mean hemoglobin, 11.47 ± 1.56 g/dl; mean white blood cell count, 13.06 ± 5.2 µL, mean erythrocyte sedimentation rate, 49.8 ±0.96 mm/h, C-reactive protein, 68.27 ± 59.07 mg/dl, and Ca 125, 56,57 ± 34.86 U/ml. Procalcitonin levels were less than 0.5 ng/ml in 11 patients (91.6%), and only one patient had a procalcitonin level of 83 ng/ml.

The most commonly seen accompanying systemic disease was hypertension (n=9, 75%), followed by diabetes mellitus (n=5, 41.6%). Among the purulent fluid specimen cultures, eight (66.6%) showed growth, whereas four (33.3%) did not show any growth. Among the eight patients, there were mixed infections. Results of cultures showed growth of Escherichia coli (six patients), Bacteri-

odes fragilis (four patients), and Enterococcus faecalis (four patients).

All patients underwent US, which most commonly showed moderate distention of the uterus with anechoic fluid collection in the uterine cavity (n=6, 50%), followed by echogenic fluid collection in the uterine cavity (n=3, 25%). The majority of the patients also underwent pelvic MRI (n=10, 83.3%). Based on pelvic MRI, all patients were reported to have varying amounts of fluid collection in the uterine cavity, and eight of nine cancer patients (88.8%) were presumed to have malignancy (Figure 1).

Only one patient did not have a biopsy result or radiological finding that would indicate uterine or cervical malignancy except the development of pyometra and leiomyoma; this patient, who was surgically treated for leiomyoma, was diagnosed with uterine leiomyosarcoma based on a frozen section procedure.

For the removal of purulent fluid with dilation and the probability of malignancy, three patients (25%) underwent cervical biopsy and endometrial dilatation and curettage, and the other nine patients (75%) underwent dilatation and curettage alone, along with suitable antibiotic therapy.
Ten of 12 patients treated with antibiotic therapy underwent hysterectomy and bilateral salpingo-oophorectomy. Of the patients who underwent surgery, eight (80%) had malignancy and two (20%) had leiomyoma as indicated. Suspected tumoral sections of the operated patients were examined using frozen section during the operation and, as a result, all patients who were found to have a malignancy and one patient who had no finding that would indicate malignancy preoperatively showed malignancy in their frozen section examination. Of the patients, nine (75%) were found to have gynecologic malignancy [(endometrial cancer, n=5, 41.6%), (cervical cancer, n=3, 25%), (uterine leiomyosarcoma, n=1, 8.3%) and, in three (25%) subjects, the cause of pyometra was reported to be benign pathologies, among which the most common were leiomyomas (n=2, 66.6%). One patient could not be operated because of impaired general status despite the lack of a diagnosis of endometrial cancer. Therefore, she received radiotherapy after that infection was controlled with drainage and antibiotic therapy. All patients were alive at the one-year follow-up visit except for one patient, who died six months after the diagnosis because of systemic problems.

Discussion

Pyometra is a rare condition often seen during the postmenopausal period. The most common causes are traumatic damage of the cervix, congenital anomalies of the genital tract during the pre-menopausal period, and malignancy, radiation, and atrophic cervicitis during the post-menopausal period [1-5].

Pyometra should be treated with caution in elderly patients because it may indicate a malignancy during the post-menopausal period, and it may cause life-threatening complications, such as spontaneous rupture and septicaemia [4].

In this study, among 12 patients, five were diagnosed with endometrial cancer (41.6%), three with cervical cancer (25%), and one with uterine leiomyosarcoma (8.3%). Thus, 75% of the patients had a malignant lesion of the uterus or the cervix. In the literature, the majority of the case series were reported to have a malignant uterine lesion as the cause of pyometra [1-3]. Muram et al. reported a malignancy rate of 72%, similar to the present results. However, in that study, 38.4% of the patients in whom pyometra was shown to be related to malignancy had been previously diagnosed with cervical cancer and had received radiotherapy [1]. In the study by Chan et al., 23% of patients with post-menopausal pyometra had gynecologic cancer, and 50% of patients with malignancy were those who had been previously diagnosed, those who had developed cervical stenosis following radiotherapy, or those with cervical cancer with tumor recurrence [5]. In the present series, none of the patients examined for pyometra had been previously diagnosed with gynecologic malignancy, which probably resulted from the decreased incidence of complications related to radiotherapy because of recent advances in radiotherapy techniques. Although in the Chan et al. study, the most commonly detected malignancy was cervical cancer; in the present study, the corresponding malignancy was endometrial cancer.

Examination of previous studies showed that the condition that caused pyometra could be diagnosed in 11% to 45% of the cases [1-3]. US is a useful method for investigating pyometra. Hypoechoic fluid collection, which was described as an US finding in pyometra, was present in most of the present subjects [4]. In this series, 11 of 12 patients (91.6%) were preoperatively diagnosed. In one patient, the results from examination, biopsy, US, and MRI did not indicate a factor that could lead to pyometra, and endometrial cancer was diagnosed based on frozen section procedure. Currently, advancements in radiological techniques and increased experience in this field have inevitably increased the rate of accurate preoperative diagnosis.

In conclusion, pyometra detected during the postmenopausal period is considered a complication caused by gynecological malignancy, until proven otherwise. In the present authors’ opinion, although the malignancy may not be detected using biopsy or imaging methods, follow-up for malignancy screening should be continued after treatment of the infection. Or, if a patient diagnosed with pyometra will undergo surgery for any gynecologic reason, it should be performed at a facility where gynecologic oncologic surgery can be performed and the endometrium can be evaluated with frozen section procedure.

References


Address reprint requests to:
O.S. KERIMOGLU, M.D.
Selçuk University Selçuklu Medicine Faculty, Obstetrics and Gynecology Department
Selçuk Üniversitesi Selçuklu Tip Fakültesi
Kadim Hastahıkları ve Doğum Anabilim Dalı.
Selçuklu, Konya (Turkey)
e-mail: ozlemsecilmis@hotmail.com
Expressions of survivin, P16$^{\text{INK4a}}$, COX-2, and Ki-67 in cervical cancer progression reveal the potential clinical application

W.Q. Zhou¹, Q.Y. Sheng², Y.H. Sheng³, W.J. Hou², G.X. Xu², Y.M. Wu³, H. Lu²

¹Department of Gynecology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai
²Department of Obstetrics and Gynecology, Central Hospital of Fengxian District, Shanghai
³Department of Pathology, Central Hospital of Fengxian District, Shanghai (China)

Summary

Purpose of investigation: To explore the significance of survivin, P16$^{\text{INK4a}}$, COX-2, and Ki-67 expressions for prediction of cervical cancer progression. Materials and Methods: A retrospective study was performed in 129 cases including 24 squamous carcinoma of the cervix (SCC), 70 cervical intraepithelial neoplasias (CIN), 15 cervical condyloma acuminatum (CCA), ten chronic cervicitis (CC), and ten normal cervix (NC). Protein expressions were evaluated using immunohistochemistry. Results: Survivin, P16$^{\text{INK4a}}$, COX-2, and Ki-67 were highly expressed in SCC and CIN compared with others. Their expression rates were gradually increased in CIN I, CIN II, CIN III, and SCC groups, showing 72.00%, 88.00%, 90.00%, and 95.83% for P16$^{\text{INK4a}}$, 68.00%, 84.00%, 95.00% and 100.00% for COX-2, 76.00%, 96.00%, 100.00%, and 100.00 for Ki-67, respectively. There were significant correlations between survivin and P16$^{\text{INK4a}}$, COX-2, Ki-67, as well as P16$^{\text{INK4a}}$ and Ki-67. Conclusion: Survivin, P16$^{\text{INK4a}}$, COX-2 and Ki-67 play critical roles for development and progression of cervical cancer.

Key words: Cervical cancer; Survivin; P16$^{\text{INK4a}}$; Cyclooxygenase-2; Ki-67; Immunohistochemistry.

Introduction

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. It is prevalent among women worldwide and performs a high mortality for a long time [1]. It is reported that there are 529,800 new cases all over the world, of which around 80% are diagnosed in developing countries [2, 3]. Cervical cancer has been reported to be largely associated with human papillomavirus (HPV). As reported, at least 93% of invasive cervical cancers were infected by HPV [4]. However, the exact mechanism is still controversial.

As is known, the progression of cervical cancer involves many oncogenes and cancer suppressor genes, which finally lead to abnormal tumor proliferation in cervix uteri [5, 6]. Of them, survivin as one of novel inhibitors of apoptosis proteins playing a key role in cervical intraepithelial neoplasia (CIN) and squamous cervical carcinoma (SCC) [7]. P16$^{\text{INK4a}}$ and Ki-67 were indicated to be useful biomarkers of cervical neoplasia [8]. Additionally, as outlined in a previous study, cyclooxygenase-2 (COX-2) was reported to contribute to preventing epithelial malignancies [9].

Nowadays, the literatures clarifying the correlation of survivin, P16$^{\text{INK4a}}$, COX-2, and Ki-67 are relatively rare. Thus, survivin, P16$^{\text{INK4a}}$, COX-2, and Ki-67 were selected to assess their correlation in cervical cancer onset and progression in the present study. The authors aimed to explore the potentially predictive values of survivin, P16$^{\text{INK4a}}$, COX-2, and Ki-67 in the progression and development of cervical cancer.

Materials and Methods

Objects

This study was approved by the Ethics and Clinical Research Committee of the Faculty of Medicine of the present University. Patients with cervical cancer were from Shanghai Fengxian District Central Hospital in China, ranging from 2005 to 2010. The total 129 patients contained 70 cases of cervical intraepithelial neoplasm (CIN) (CIN I: 25, CIN II: 25, CIN III: 20), 24 cases of squamous cell carcinoma (SCC), 15 cases of cervical condyloma acuminatum (CCA), and ten cases of chronic cervicitis (CC). Normal cervical (NC) tissues were separated from ten healthy individuals as control. The mean age of the total patients was 39.13 ± 7.07 years and the mean age of group CIN I, CIN II, CIN III, SCC, CCA, CC, and NC was 37.28 ± 5.64, 38.64 ± 6.15, 37.52 ± 6.41, 45.83 ± 7.19, 37.60 ± 3.33, 37.90 ± 5.80, and 36.60 ± 7.96 years, respectively. All cases did not undergo radiotherapy or chemotherapy before diagnosis.

Electronic colposcopy

The electronic colposcope test was performed by full-time physicians. Before test, the patients with vaginitis were excluded. In the preliminary observation, the cervix secretion of every individual was wiped off with normal saline cotton ball to observe the cervical characters including morphology, size, pathological feature, color, and luster. Then the patients were treated with 3% acetic acid to inspect the vessels by a green filter. After that, patients were conducted to receive the iodine test, followed by being...
graded using Reid colposcopic scores. For the patients with abnormal or suspicious image areas, about four to five biopsy samples were obtained, fixed in 10% neutral formalin solution, and then sent to pathology department for examination. The endocervical curettage was carried out if necessary and the image of abnormal areas were collected for further investigation.

**Hematoxylin/eosin (HE) staining**

The tissue specimens from normal and disease cervix uteri were embedded in paraffin. Four-μm serial sections were cut and dried at 60°C overnight. After dewaxing by dimethylbenzene, sections were stained with HE.

**Immunohistochemistry**

The immunohistochemical procedure was performed according to the method previously reported [10]. The primary antibodies used in this assay as followed: mouse monoclonal antibody p16INK4A (1:100 dilution), mouse monoclonal antibody Survivin (M-068) (1:100 dilution), mouse monoclonal antibody COX-2 (M-0715) (1:100 dilution), and mouse monoclonal antibody Ki-67 (M-0693) (1:100 dilution). The sections from the same sample were divided into two groups. The negative control group had phosphate buffered saline (PBS) added instead of primary antibodies. Then the sections were stained with HE for one minute and observed by microscope.

**Positive results analysis**

The positive cell gathered region of each sample was analyzed randomly by light microscope under high power fields. The total of 1,000 cells were observed. The percentage of positive cells was calculated as following:

Positive expression rate (%) = the score of immunoreactivity was in the light of two criterions. One criterion was in accordance with the degree of positive cells coloring and the immunoreactivity was divided into four grades: 0 (negative- no stained cells), 1 (weak positive- pale-yellow cells), 2 (moderate positive –Brown cells), and 3 (strong positive- Sepia cells). Another criterion was based on the proportion of positive cells and scored as follows: 0 (< 5%), 1 (5% ~ 25%), 2 (26% ~ 50%), 3 (51% ~ 75%), and 4 (> 75%). The final results were cumulative by the two criterion scores and defined as 0: negative (-), 1–4: weak positive (+), 5–8: moderate positive (++), 9–12: intense positive (+++). P16INK4a and survivin positively expressed cells were defined as the cells with brown particles in nucleus or cytoplasm, while COX-2 and Ki-67 positive cells were defined as the cells with brown particles only in cytoplasm and nucleus, respectively.

**Statistical analysis**

All the data were analyzed by SPSS 18.0 software. Count data were analyzed with rates or proportions. Significant differences between different groups were evaluated using chi-square. The difference and correlation of graded scores were analyzed by non-parametric Wilcoxon test and Spearman rank correlation analysis, respectively. The statistically significant difference was defined as \( p < 0.05 \).

**Results**

**Survivin expression in cervical tissue**

All normal cervical tissues showed negative expression for survivin. Most of the koilocytes in CCA group showed survivin positive expression. Figure 1 shows cell nuclear staining and cytoplasm staining for survivin, especially in nuclear staining of the koilocytes in groups of CIN II, CIN III, and SCC group.

As shown in Table 1, there was statistically significance of survivin expression rate in NC, CC, CCA, CIN, and SCC group \( (p < 0.05) \). The positive staining for survivin among CIN I, CIN II, CIN III, and SCC groups were not statisti-
Expressions of survivin, P16\(^{INK4a}\), COX-2, and Ki-67 in cervical cancer progression reveal the potential clinical application

Significantly (\(p > 0.05\)). The Spearman rank correlation coefficient (\(r = 0.486, p = 0.000\)) showed that graded expression of survivin in each group were positively correlated. The degree difference of survivin expression between CIN I and CIN II, CIN III, and SCC groups were statistically significant (\(z = -3.323, p = 0.001; z = -2.265, p = 0.023; z = -2.009, p = 0.045\)).

P16\(^{INK4a}\) expression in cervical tissue

P16\(^{INK4a}\) was not stained in the cervical tissues. P16\(^{INK4a}\) was expressed in both cytoplasm and nuclear. P16\(^{INK4a}\) was stained in the lower 2/3 of epithelium cells in CIN II and CIN III group, which correspond with the histopathology results (Figure 2). The staining trend of P16\(^{INK4a}\) was similar with survivin from CIN to SCC (Figure 1). CIN and SCC group showed strong P16\(^{INK4a}\) staining in squamous koilocytes almost accompanied with HPV infection.

The differences of P16\(^{INK4a}\) expression rates in NC, CC, CCA, CIN, and SCC group were statistically significant (\(p < 0.05\)). There were no statistically significance of the positive expression rates among CIN I, CIN II, CIN III, and SCC groups (\(p > 0.05\)). The correlation analysis of group levels expression, Spearman rank correlation coefficient \(r = 0.706, p = 0.001\), were positively correlated. The comparisons of survivin expression levels among CIN I, CIN II, CIN III, and SCC were statistically significant (\(z = -2.015, p = 0.0044; z = -2.919, p = 0.004; z = -4.241, \) and \(p = 0.001\)). There was a significant difference between CIN II group and SCC group (\(z = -3.004, p = 0.003\), Table 2).

COX-2 expression in cervical tissue

COX-2 was localized in cytoplasm and mainly expressed in cancer and atypical hyperplasia cells. Results showed that it could be observed in regenerately vascular endothelial cells of cervical tissue (Figure 3).

The differences of COX-2 expression rates among NC, CC, CCA, CIN, and SCC group were statistically significant (\(p < 0.05\)). Compared analysis of COX-2 expression rate in CIN I, CIN II, CIN III, and SCC group, only CIN I with CIN III group (\(p = 0.030, p = 0.004\)), and CIN I with SCC group were significantly different, while the other groups had no statistically differences (\(p > 0.05\)). The correlation analysis of group levels expression, in which the Spearman rank correlation coefficient (\(r = 0.728 \) and \(p = 0.001\)) were positively correlated (Table 3). Compared with CIN (CIN I, CIN II, CIN III) group with the SCC group, the difference was significant (\(z = -2.373, p = 0.018\), Table 3).

Ki-67 expression in cervical tissue

The normal cervical epithelium, cervical glandular epithelium and metaplasia showed scattered staining particle of Ki-67 around cell nucleus. Ki-67 was positively expressed in nucleus of the cervical warts koilocytes. In cervical intraepithelial lesions and SCC tissues, Ki-67 staining

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Positive Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>CC</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td>CCA</td>
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<td>9</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>40.00</td>
</tr>
<tr>
<td>CIN I</td>
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<td>6</td>
<td>0</td>
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</tr>
<tr>
<td>CIN II</td>
<td>25</td>
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<td>5</td>
<td>14</td>
<td>4</td>
<td>92.00</td>
</tr>
<tr>
<td>CIN III</td>
<td>20</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>85.00</td>
</tr>
<tr>
<td>SCC</td>
<td>24</td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>5</td>
<td>79.17</td>
</tr>
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</table>

Table 1. — Survivin expression in cervical carcinoma of each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Positive Rate (%)</th>
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<tbody>
<tr>
<td>NC</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>CC</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10.00</td>
</tr>
<tr>
<td>CCA</td>
<td>15</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>33.33</td>
</tr>
<tr>
<td>CIN I</td>
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<td>7</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>72.00</td>
</tr>
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<td>CIN II</td>
<td>25</td>
<td>3</td>
<td>8</td>
<td>10</td>
<td>4</td>
<td>88.00</td>
</tr>
<tr>
<td>CIN III</td>
<td>20</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>90.00</td>
</tr>
<tr>
<td>SCC</td>
<td>24</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>13</td>
<td>95.83</td>
</tr>
</tbody>
</table>

Table 2. — P16\(^{INK4a}\) expression in cervical carcinoma of each group.
degree enhanced and the distribution was extended ranging from the upper 2/3 of cervical epithelium to the full-thickness (Figure 4).

There were statistically significant differences of Ki-67 positive expression rates between NC and CCA group ($p < 0.05$), while compared with CCA and CC group; the difference was not significant ($p > 0.05$). The statistically significant difference of the positive expression rates were shown between CIN I, CIN II, CIN III, SCC and NC, CC, CCA groups ($p < 0.05$). Through correlation analysis of graded Ki-67 expression, the correlation coefficient was calculated as $r = 0.817, p = 0.001$, which showed the data were positively correlated. The comparisons of Ki-67 graded expression among CIN I, CIN II, CIN III, and SCC group were statistically significant ($z = -2.998, p = 0.003; z = -3.322, p = 0.001; z = -5.595, p = 0.001; z = -3.322, p = 0.001; z = -4.364, p = 0.001$) except the comparison between CIN III and SCC group ($z = -10378, p = 0.168$) and there was a significant difference between CIN group and SCC group ($z = -20373, p = 0.018$, Table 4).

The correlation analysis of survivin, $P16^{INK4a}$, COX-2 and Ki-67 expression in CIN and SCC group

As shown in Table 5, the correlations were significant between survivin and $P16^{INK4a}$, COX-2, Ki-67 expression ($p < 0.05$). There was statistical significant between $P16^{INK4a}$ and Ki-67 expression ($p < 0.001$). The expression of COX-2 was relevant with $P16^{INK4a}$ and Ki-67 expression, but the expression differences were not significant statistically ($p > 0.05$).

Discussion

The development of cervical cancer is a long-term process and the pathogeny seems to be diversified. Of this, the imbalance of the cell proliferation and apoptosis is one of the
Expressions of survivin, P16INK4a, COX-2, and Ki-67 in cervical cancer progression reveal the potential clinical application

Main factors leading to cervical cancer. The cell factors, affecting cellular proliferation and apoptosis, were considered to be functional in the formation and development of tumor, such as survivin, P16INK4a, COX-2, and Ki-67. In this paper, the authors applied HE and immunohistochemical staining to explore the relationship of these factors with cervical cancer.

Survivin, a member of apoptosis inhibitor family, is recently known to be relevant to cancer onset and progression [12]. Survivin inhibits apoptosis and ensuring normal cell division by regulating G2/M phase of cell-cycle. Over-expressed survivin sequesters physiologically relevant caspase on microtubules to default apoptosis [13]. There were evidences that the expression of survivin was elevated in CIN and SCC tissues along with tumor progression [14, 15]. Survivin was considered to be an independent prognostic predictor for the development and prognosis of cervical cancer [16]. As shown in the present result, survivin was not expressed in NC group, but there was an increasing expression in cells from CC, CCA to CIN and SCC. Survivin expression in CIN II, CIN III, and SCC was significantly higher than that in normal tissues. In addition, the survivin staining was observed in cytoplasm of all groups except for NC. CIN II, CIN III and SCC group showed positive staining in both cell nucleus and cytoplasm. It indicates that in the early-stage cervical cancer, survivin mainly expressed in cytoplasm of immature squamous cells. As the cervical cancer developed, survivin was gradually expressed in cell nucleus. The nucleus expression of survivin might be the biomarker for the prediction of cervical cancer.

P16INK4a, a tumor suppressor protein, plays a key role in preventing cell proliferation by specifically inhibiting cyclin D-dependent kinases [17]. Previous studies reported that P16INK4a was increasingly expressed in HPV infected cervical cells to regulate the increasing expression of viral oncopgenes [18]. A strong expression of P16INK4a was detected in all the cervical cancer cases with a high-risk HPV positive typing [19]. The degree of P16INK4a expression in cervical tissue had a indicative effect on the severity of cervical neoplasia ($p < 0.001$) [8]. These conclusions are consistent with the present results. Therefore, P16INK4a can be regarded as a specific marker in HPV-induced cervical neoplasia, which can help to identify the severity of diseases [20]. COX-2 which is responsible for prostaglandins synthesis, is prevalent in normal cells but COX-2 expression can be upregulated in tumor formation. The elevated expression of COX-2 directly leads to an increasing level of prostaglandins. It is reported that prostaglandins may possess multiple function in cancer development [21, 22], for instance prostaglandin E2 up-regulate apoptosis protein Bcl-2 to promote tumor

Table 5. — Correlation analysis of Survivin, COX-2, Ki-67 and P16INK4a.

<table>
<thead>
<tr>
<th></th>
<th>Survivin</th>
<th>COX-2</th>
<th>Ki-67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivin</td>
<td>-</td>
<td>0.330</td>
<td>0.319</td>
</tr>
<tr>
<td>P16INK4a</td>
<td>0.246</td>
<td>0.197</td>
<td>0.356</td>
</tr>
<tr>
<td>Ki-67</td>
<td>-</td>
<td>0.212</td>
<td>-</td>
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</table>

Figure 4. — Positive staining for Ki-67 in CIN and SCC. a: CIN I (I H C×200), b: CIN II (I H C×200), c: CIN III (I H C×200), d: SCC (I H C×100).
growth [23]. COX-2 may enhance tumor invasion and metastasis by suppressing cell apoptosis [24]. As outlined in previous studies, COX-2 expressed in cervical cancer cells but it was undetectable in normal cervical tissues [9]. The similar result was found in the present work that there was no COX-2 expression in NC. The degree of COX-2 staining had positive correlation with degree of cervical neoplasia from CIN to SCC (r = 0.460). In the progression of cervical cancer, COX-2 expression was a common molecule event.

Like survivin, Ki-67 is another cell-cycle regulatory protein which has been found to be involved in cervical carcinoma [25]. Ki-67 expressed in all active phases of normal cell cycle except for G0 [26]. The Ki-67 expression is defined as a proliferation marker for assessment of cervical cancer [27-29]. It was reported that the detection of Ki-67 protein showed highest accuracy in recognizing cervical cancer [30] and progression prediction in CIN [31]. Moreover, another research showed that the expression of Ki-67 detected in low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), and SCC cases were 25%, 68%, and 65.5%, respectively [32]. The change of Ki-67 expression correlates with the severity of the lesion, which is helpful for diagnosis in cervical cancer development [32]. In the present study, Ki-67 staining in CIN I, CIN II, CIN III, and SCC was 76%, 96%, 100%, and 100%, respectively. CIN and SCC showed stronger staining than NC, CC, and CCA. CIN I to III had an increasing trend in Ki-67 expression but there was no statistical significance between CIN III and SCC (p > 0.05). Therefore the present authors speculated that with the increase of level of CIN, cell proliferation activity gradually enhanced and Ki-67 index was the crucial value in cervical cancer progression.

The development and progression of cervical cancer is characterized for deregulated cell cycle and abnormal cell proliferation [33, 34]. Potential biomarkers, P16\(^{\text{INK4a}}\), Ki-67, and survivin and COX-2 were all associated with cervical lesions in different degrees and could predict the progression of cervical cancer [35]. The dysregulation of survivin, P16\(^{\text{INK4a}}\), COX-2, and Ki-67 expression increased the risk of cervical cancer. In the present study, the correlation of survivin expression with P16\(^{\text{INK4a}}\), Ki-67, and COX-2 were statistically significant (p < 0.05). Survivin has multiple bio-functions in cell cycle, proliferation and apoptosis [36-38]. P16\(^{\text{INK4a}}\) and COX-2 were considered to induce cell cycle arrest leading to apoptosis in cancer [39, 40]. Ki-67 was detected to be only in proliferating cells and strictly associated with cell cycle [41]. Survivin might control apoptosis with interaction with P16\(^{\text{INK4a}}\), COX-2, and Ki-67. Survivin competitively interacted with the Cdk4/P16\(^{\text{INK4a}}\) for initiating cell cycle entry [37]. Survivin had positive correlation with COX-2 in endometrial carcinoma. It was speculated that survivin and COX-2 enhanced each other’s function in the same molecule pathway [42]. Survivin and Ki-67 simultaneously present in melanomas [43] suggested that survivin might have positive interaction with Ki-67.

The correlation of P16\(^{\text{INK4a}}\) and Ki-67 was significant (r = 0.356, p < 0.001). As a previous study reported, the degree of P16\(^{\text{INK4a}}\) and Ki-67 expression showed close association with severity of cervical neoplasia (p < 0.001) [8]. Detection of both P16\(^{\text{INK4a}}\) and Ki-67 can help to improve the diagnostic accuracy of HSIL and SCC [44]. A large amount of P16\(^{\text{INK4a}}\) positive cells in CIN I showed Ki-67 expression and CIN II, CIN III and carcinoma cases also showed co-expression of Ki-67 and P16\(^{\text{INK4a}}\) in different degrees [45]. All these implied that the Ki-67 and P16\(^{\text{INK4a}}\) might have synergistic effect on cell cycle and proliferation in cervical cancer progression.

Overall, the progression and development of cervical cancer involved multi-factors and pathways. Potent evidence indicated that survivin, P16\(^{\text{INK4a}}\), Ki-67, and COX-2 played essential roles in cervical cancer progression. Although the mechanism of the interaction for survivin, P16\(^{\text{INK4a}}\), Ki-67, and COX-2 was still unclear, these factors showed strong correlation with the lesion progression. So the present authors speculated that the comprehensive assessment of survivin, P16\(^{\text{INK4a}}\), Ki-67, and COX-2 might have potential predictive value in lesion progression of cervical cancer.

References

Expressions of survivin, p16\(^{INK4a}\), COX-2, and Ki-67 in cervical cancer progression reveal the potential clinical application


Address reprint requests to: W.Q. ZHOU, M.D.
Department of Gynecology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, No.536 Changle Rd., Shanghai, 200040 (China)
e-mail: zhouweiqiangzwq@hotmail.com
Epidemiology of ovarian cancer in North Sardinia, Italy, during the period 1992-2010

E.T. Tanda¹, M. Budroni², R. Cesaraccio², G. Palmieri³, G. Palomba³, G. Capobianco⁴, M. Dessole⁴, S. Dessole⁴, A. Cossu¹

¹ University of Sassari, Medicine and Surgery, Sassari
² Service of Epidemiology, A.S.L. 1, Sassari
³ Institute of Biomolecular Chemistry, Cancer Genetics Unit, C.N.R., Sassari
⁴ Gynecologic and Obstetric Clinic, Department of Surgical, Microsurgical and Medical Sciences, University of Sassari, Sassari (Italy)

Summary

Introduction: The aim of this study was to analyze and describe the incidence and mortality trends of ovarian cancer in North Sardinia, Italy, in the period 1992–2010. Materials and Methods: Data were obtained from the tumor registry of Sassari province which makes part of a wider registry web, coordinated today by the Italian Association for Tumor Registries. Results: The overall number of ovarian cancer cases registered in the period under investigation was 600. The mean age of the patients was 62 years. The standardized incidence and mortality rates were 11.2/100,000 and 5.1/100,000 respectively. A substantially stable trend in incidence and mortality of ovarian cancer was evidenced. Relative survival at five years from diagnosis was 44.2%. Conclusions: The incidence and mortality trends of ovarian cancer in North Sardinia remained relatively stable in the last decades, while prognosis remains relatively poor.

Key words: Ovarian cancer; Incidence; Mortality; Sardinia, Italy.

Introduction

Ovarian cancer is one of the most common malignant neoplasias in women worldwide, with more than 238,000 new cases estimated in 2012; furthermore, more than 151,000 deaths estimated make ovarian cancer one of the most lethal gynecologic malignancies [1]. Large part of cases are registered in developed countries, except for Japan, with higher incidence rates in Northern Europe and United States, intermediate rates in western Europe, and lower rates in developing countries. In high incidence areas, the lifetime risk of ovarian cancer is 1-2% [2].

In spite of many therapeutic progresses obtained combining aggressive surgery and chemotherapy [3], the prognosis of ovarian cancer remains poor, with a mean five-year survival rate of about 40% [2]. Unfortunately, the majority of cases are diagnosed at an advanced stage, and the five-year survival in Stages III and IV drops to 30% and 20%, respectively [4]. The lack of a precise clinical manifestation, along with the absence of effective screening programs may explain the low number of cases diagnosed at an early stage.

Previous reports investigated the epidemiological characteristics of ovarian cancer in North Sardinia, Italy in the past [5-6]. The aim of this population-based study was to analyze and describe the incidence and mortality trends of ovarian cancer in the period 1992–2010, and to compare them with those of previous reports, in order to investigate the epidemiological evolution of the disease in the area.

Materials and Methods

The epidemiological data presented in this article were obtained from the “Cancer Registry of the Province of Sassari”. This registry was created in 1992 by the local health agency for the epidemiological surveillance of tumors in the province. In 1999, it became part of a wider web of tumor registries, coordinated by the Italian Association for Tumor Registries (Associazione Italiana Registri Tumori, AIRTUM). The association coordinates 34 registries in the country, collects and publishes data, and collaborates with international organizations in the field.

Every registry collects data on tumoral diseases affecting inhabitants in the territory of jurisdiction through the local hospitals and healthcare services, as with other registries (e.g., death registries). Demographic, clinical, pathological, and prognostic data are collected for each case of cancer and are registered in a digital database. This database was the data source for the present population-based report and for other reports published in the past, depicting the burden of the principal malignant tumors in the area [7-11].

The demographic characteristics of the patients affected by ovarian cancer were collected. Crude incidence and mortality rates per 100,000 inhabitants per year were calculated, as were standardized rates adjusted for European age-population standards. A comparison between incidence and mortality in the province of Sassari and those in other Italian provinces was performed. Additionally, the cumulative risk of developing the disease and of dying between zero and 74 years of age was estimated. The age-class distribution and time-trends of incidence, mortality and histology were evaluated. Finally, relative five-year survival was calculated with the Ederer method.
Results

The overall number of cases of ovarian cancer registered in the period under investigation was 600. Diagnosis was obtained by histological or cytological reports in 501 cases (83.5%) and using other information sources (clinical reports, radiological referrals, death certifications, etc) in 93 cases (15.5%); in six cases (1%) the modality of diagnosis was not known. The mean age of the sufferers was 62 years.

The cumulative risk of developing the disease between zero and 74 years of age was 0.91%.

Among the 501 tumors that had a histological or cytological diagnosis, 130 (25.9%) were serous carcinomas, 92 (18.4%) were mucinous carcinomas, 45 (18.4%) were endometrioid carcinomas, and 144 (28.8%) were other histotypes, while in the remaining 90 (18%) cases the exact histologic subtype was not specified.

The crude and standardized incidence rates of ovarian cancer in the period under investigation were 14.1/100,000 and 11.2/100,000, respectively. Table 1 shows the distribution of cases in percentages in relation to age, while Table 2 shows the distribution of incidence rates per age-class. Peak incidence occurred at 60-74 years. Figure 1 depicts the trend of incidence rates in the period 1992–2010; there were no substantial modification registered, with incidence rates oscillating between 9.01/100,000 and 14.17/100,000. Analysis of the trend of mean age at disease onset for the same period of time did not reveal any relevant changes. Furthermore, no substantial modifications of the proportions of the histological types mentioned before were found. Table 3 shows the comparison of the incidence and mortality in the province of Sassari with those in other Italian provinces.

There were 307 deaths registered in the period under investigation. Crude overall mortality rate was 7.2/100,000, while standardized mortality rate was 5.1/100,000. Mean age at death was 69.9 years. The cumulative risk of death between zero and 74 years of age was 0.4%.

### Table 1. — Age-class incidence distribution of ovarian cancer in North Sardinia, 1992-2010.

<table>
<thead>
<tr>
<th>Age class</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>0</td>
</tr>
<tr>
<td>15-29</td>
<td>4</td>
</tr>
<tr>
<td>30-44</td>
<td>11.9</td>
</tr>
<tr>
<td>45-59</td>
<td>25</td>
</tr>
<tr>
<td>60-74</td>
<td>34.3</td>
</tr>
<tr>
<td>75+</td>
<td>24.8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Incidence / 100,000</th>
<th>Mortality / 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>5-9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-19</td>
<td>2.8</td>
<td>0</td>
</tr>
<tr>
<td>20-24</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td>25-29</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>30-34</td>
<td>5.3</td>
<td>0.9</td>
</tr>
<tr>
<td>35-39</td>
<td>4.2</td>
<td>0.9</td>
</tr>
<tr>
<td>40-44</td>
<td>12.3</td>
<td>1.3</td>
</tr>
<tr>
<td>45-49</td>
<td>13.6</td>
<td>3.8</td>
</tr>
<tr>
<td>50-54</td>
<td>19.8</td>
<td>5.1</td>
</tr>
<tr>
<td>55-59</td>
<td>21.8</td>
<td>10.9</td>
</tr>
<tr>
<td>60-64</td>
<td>27.1</td>
<td>13.4</td>
</tr>
<tr>
<td>65-69</td>
<td>28.2</td>
<td>15.5</td>
</tr>
<tr>
<td>70-74</td>
<td>41.5</td>
<td>27.3</td>
</tr>
<tr>
<td>75-79</td>
<td>43.6</td>
<td>30.8</td>
</tr>
<tr>
<td>80-84</td>
<td>39.3</td>
<td>32.8</td>
</tr>
<tr>
<td>85+</td>
<td>45.5</td>
<td>46.5</td>
</tr>
</tbody>
</table>

### Table 3. — Comparison of incidence and mortality rates of ovarian cancer in North Sardinia with those of other Italian provinces.

<table>
<thead>
<tr>
<th>Province</th>
<th>Incidence / 100,000</th>
<th>Mortality / 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alto Adige</td>
<td>14.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Biella</td>
<td>12.8</td>
<td>7.2</td>
</tr>
<tr>
<td>Ferrara</td>
<td>10.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Firenze</td>
<td>13.4</td>
<td>6.7</td>
</tr>
<tr>
<td>Friuli V.G.</td>
<td>11.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Genova</td>
<td>15</td>
<td>6.7</td>
</tr>
<tr>
<td>Macerata</td>
<td>12</td>
<td>7.3</td>
</tr>
<tr>
<td>Modena</td>
<td>12.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Napoli</td>
<td>9.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Parma</td>
<td>16.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Ragusa</td>
<td>11.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Reggio Emilia</td>
<td>12.4</td>
<td>6</td>
</tr>
<tr>
<td>Romagna</td>
<td>12.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Salerno</td>
<td>11.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Sassari</td>
<td>11.2</td>
<td>5.1</td>
</tr>
<tr>
<td>Torino</td>
<td>13.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Trento</td>
<td>11.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Umbria</td>
<td>13.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Varese</td>
<td>17.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Veneto</td>
<td>10.8</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Figure 1. — Incidence and mortality rates trends of ovarian cancer in North Sardinia, 1992-2010.
the age-class distribution of mortality rates. There was a slight increase in mortality after the sixth decade of life. As shown in Figure 1, a substantially stable trend of mortality rates between 1992 and 2010 was reported, oscillating between 4.85/100,000 and 7.59/100,000, with a slight increment between 2008 and 2010. Finally, relative survival at five years from diagnosis was 44.21%.

Discussion

Ovarian cancer is one of the most incident cancer of the female reproductive tract. More than 238,000 new cases and more than 151,000 deaths were estimated in the world in 2012 [1]. Most of ovarian cancer cases are sporadic, and only 5% to 10% of ovarian cancers are familiar; mutations in the BRCA1 and BRCA2 genes are responsible for the majority of hereditary ovarian cancers. Epidemiologic and molecular-genetic studies have identified both risk and protective factors for the disease [12-15]. Increasing age, family history of breast or ovarian cancer, nulliparity, early menarche, and late menopause are known risk factors [16-18] for ovarian cancer, while the impact of tobacco and alcohol consumption are still under investigation [19-20]. On the other hand, oral contraceptives, pregnancy, multiparity, breastfeeding, hysterectomy, and tubal ligation appear to decrease ovarian cancer risk [16-18].

Ovarian cancer is considerably more common in developed areas of the globe; about 91% of the new cases and 89% of the deaths estimated in 2012 worldwide occurred in those areas. Most of the cases of ovarian cancer are diagnosed at a late stage, and this causes a higher mortality. However, when ovarian cancer is detected in an early stage, the survival at five years is quite good.

In Europe in 2012 there were estimated more than 44,577 new ovarian cases and the standardized incidence rate of the disease was 9.4/100,000 [1]. High risk European regions include Northern countries (Scandinavia) and Eastern countries (e.g. Poland, Bulgaria) [1]. More than 30,079 deaths were estimated in 2012 in Europe (standardized mortality rate: 5.1/100,000) [1].

In Italy it has been estimated that approximately 5,911 new cases of ovarian cancer occurred in 2012 [1]. These figures make ovarian cancer the sixth most frequent malignancy in women and the second most frequent gynecological malignancy. Incidence rates showed a moderate reduction during the two last decades (-2.2% per year) in the country [21]. Furthermore, a decreasing trend in incidence rates is observed, moving from northern to southern Italian regions. The global mortality rate in Italy is 6.4%, and it shows a moderate reduction in recent years; the reduction of mortality rates is smaller than the one seen for the incidence rates (-1.3% per year) and a decreasing trend is observed from North to South. The five-year survival of patients with ovarian cancer has improved in Italy in the last decades, and it is currently estimated at approximately 38% [21]. Concerning Sardinia, estimated incidence rates were 4.27/100,000, 11.99/100,000, and 9.2/100,000 in the periods 1974-1985, 1992-2001, and 2005-2009, respectively [6, 21]. The standardized incidence rate the present authors calculated for the period 1992-2010 was 11.2/100,000, confirming the steady trend of incidence previously reported. Furthermore, this rate was inferior to those of several other Italian regions, especially the northern ones (Table 3).

Concerning mortality, standardized mortality rate in North Sardinia was 5.1/100,000, being one of the lowest in Italy; also the cumulative risk of death from the disease was extremely low (0.4%). With regards to survival, the relative five-year rate estimated in the present region was 44.21%. This figure was slightly superior to that estimated for the entire country.

From an etiological point of view, a fraction of ovarian cancer cases recognizes genetic factors in their pathogenesis [22]. Germline mutations in either BRCA1 or BRCA2 genes occur in approximately 10% of unselected women with ovarian cancer and, on the other side of the coin, women with inherited BRCA1/2 mutations are at significant risk of developing ovarian cancer [23]. The lifetime risk of developing ovarian cancer in women who carry a germline BRCA mutation has been estimated to be of 40-60% for BRCA1 and 11-27% for BRCA2 [24]. A meta-analysis of 22 studies with over 8,000 disease probands has defined the incidence for ovarian cancer to be approximately 39% for BRCA1 and 11% for BRCA2 [25]. In North Sardinia, less than 10% of breast cancer families presented an association with ovarian cancer (at least one affected family member) [26]. Nevertheless, presence of ovarian cancer was demonstrated to significantly increase the occurrence of BRCA1/2 germline mutations in Sardinian breast cancer families [26-27]. Finally, BRCA2 mutations were notably more recurrent than BRCA1 mutations in breast cancer families from North Sardinia [26-27]. Gathering all these findings, one could speculate that: a) the poor association with ovarian cancer may explain the relatively low prevalence of BRCA1/2 germline mutations in Sardinian population; and b) the very low proportion of BRCA1 mutations may account for the very limited number of breast cancer families with association to ovarian cancer in such a population. As a confirmation of this, breast cancer families originating from South Sardinia, where BRCA1 mutations were instead demonstrated to be much more prevalent, present markedly higher rates of association with ovarian cancer [28].

Conclusions

The incidence and mortality trends of ovarian cancer in North Sardinia remained relatively stable in the last decade. Mortality and cumulative risk of death from the disease were low. Furthermore, survival of patients with ovarian cancer was relatively good in the area, sanctioning the adequacy of the preventive and clinical measures employed in.
the management of the disease. As for other malignancies, concurrence of different environmental factors and genetic backgrounds may determine the incidence of ovarian cancer. This is particularly true in Sardinia, whose population shows genetic peculiarity due to geographical isolation and strong genetic drift.

Acknowledgements

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References

Prevalence of human papillomavirus and the correlation of HPV infection with cervical disease in Weihai, China

L. Yang, S.Z. He, X.Y. Huang, H.N. Liu, J.Y. Tao

Department of Obstetrics and Gynecology, Weihai Municipal Hospital, Dalian Medical University, Weihai (China)

Summary

Objective: This study investigates the human papillomavirus (HPV) infection rate in female genital tracts, as well as the HPV genotype distribution and HPV correlation with cervical disease in Weihai, Shandong Province, China. Materials and Methods: A random sample of 9,460 volunteers was simultaneously screened using gene chips and examined by ThinPrep liquid-based cytology test (TCT). Cervical biopsy samples were collected from women with positive HPV-DNA and abnormal TCT for pathological diagnosis. Results: The overall HPV prevalence was 6.93% (656 of 9,460). A total of 753 subjects were infected with HPV subtypes (including multiple HPV infections). Of those with infections, 688 were infected with high-risk (HR) types (91.37%), and 65 were infected with low-risk subtypes (8.63%). The single-infection rate was 63.1%. The prevalence rates of HPV in women aged 20 to 39 years and 40 to 59 years were 7.29% and 6.71%, respectively. The most common genotype was HPV16. The HR genotypes were associated with cervical diseases such as atypical squamous cells of undetermined significance (ASCUS) (37.9%), atypical squamous cells high grade (ASC-H) (42.5%), low grade squamous intraepithelial lesion (LSIL) (50%), and high grade squamous intraepithelial lesion HSIL (66.7%). Cervical biopsy results show that the HPV detection rate increased in the following biopsy samples: cervical intraepithelial neoplasia (CIN) I (74.11%), CIN II (84.31%), CIN III (90.32%), and squamous-cell carcinoma (SCC) (100%). Conclusions: The HPV infection rate with associated cervical disease in Weihai is equal to those in foreign countries but is lower than the average rate in China. The prevalence of HPV was higher in young people. The most common HPV genotype was 16, followed by 52 and 58. HR HPV is the most probable infection factor for cervical diseases.

Key words: Human papillomavirus (HPV) Genotype; Cervical diseases; Liquid-based cytology.

Introduction

Human papillomavirus (HPV) particles are double-stranded DNA viruses that are widely distributed in humans and animals. The virus does not cause cross-infection between species, and the infection is limited and non-systemic. Through epithelial tropism, HPV infects a person’s skin or mucosal epithelial cells and causes the infected site to exhibit benign and malignant lesions. Cervical cancer (CC) is mostly linked to HPV infection [1, 2]. In particular, high-risk (HR) HPV, which has 13 types, has been a major causative factor in the development of cervical intraepithelial neoplasia (CIN) and CC [3, 4]. An important requirement for a diagnostic and therapeutic approach is that the testing and identification of HR HPV types should be highly sensitive and specific. Vaccines are vital to the prevention and treatment of CC [5]. The HPV vaccine is highly specific. However, the epidemiological distribution of HPV genotypes varies in different countries and regions. Thus, the authors investigated HPV infection in women of childbearing age in Weihai, Shandong Province, China and determined the relationship of the infection with cervical lesions.

Materials and Methods

Subjects

Between October 2008 and April 2009, 9,460 volunteers were randomly selected from the urban city of Weihai and the counties of Wendeng, Rushan, and Rongcheng in Shandong Province, China. In each county, women from towns or villages were randomly selected. An HPV virus check was conducted in married women, particularly in terms of their sexual life (except for hysterectomy, cervical surgery, and menstrual period). All women, who were 20 to 60 years of age, agreed to participate in the study. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Weihai Municipal Hospital. Written informed consent was obtained from all participants.

Questionnaire interview

Questionnaire survey: an epidemiological survey that included demographic and behavioral indicators was conducted. The demographic indicators included age, address, economic income, educational level, menstrual marital status, occupation, and spouse profession. The behavioral informatics indices included age at first sexual experience, number of sexual partners, contraceptive methods, number of pregnancies, mode of delivery, menopause, and smoking. The questionnaire survey included a self-administered questionnaire and an interview.

Specimen collection and gynecological examination

Gynecologists who passed the unified training performed the routine gynecological examination and sample collection and obtained
the detailed records of the conditions of the vulva, vagina, cervix, uterus, accessories, and vaginal discharge. At the same time, samples of exfoliated cervical cells were collected using a cervical brush. From the location of the cervical squamous columnar junction, the sampler was rotated for three to five full circles in a clockwise direction, brushed into a three-ml cervical cell preservation solution, and stored at 4°C until use.

**HPV determination**

The cells were centrifuged for five minutes at a relative centrifugal force of 9,660×g. Afterward, the brush and supernate were removed, and the sediments obtained were extracted via alkali lysis using DNA extraction kits.

**HPV genotyping via the Hybrio HPV GenoArray test**

HPV genotype detection was conducted using an HPV GenoArray test kit. The kit used both DNA PCR amplification and Hybrio’s proprietary flow-through hybridization technique to simultaneously identify 21 HPV genotypes, including 13 HR types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), two probable HR (PHR) types (53, 66), and five low-risk (LR) types (6, 11, 42, 43, 44, and cp8304(81)). The assay was performed according to the manufacturer protocol. In brief, PCR was performed on a PCR system apparatus [6] using a reaction volume of 25 μl, which contained one μl of the DNA template, 23.25 μl of a PCR mix, and 0.75 μl of DNA Taq polymerase.

**TCT**

The liquid-based thin-layer cytology test (TCT) adopted the Bethesda classification system as the cytologic diagnostic criteria, as follows: negative, atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells high grade (ASC-H), atypical glandular cells (AGS), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), and squamous-cell carcinoma (SCC).

**Colposcopic biopsy and histopathology**

Women with abnormal TCT results (≥ ASCUS) underwent colposcopic examination. The columnar junction and transformation zone were observed to assess the lesion, and a punch biopsy was performed. Pathological examination was divided into inflammation, CIN I, CIN II, CIN III, and invasive cervical carcinoma.

**Statistical analysis**

After reviewing the questionnaire, data were key-entered twice and analyzed using the SPSS 15.0 software. Statistical analysis consisted of (1) the frequency table, which describes the demographic characteristics and other related indicators based on the mean; and (2) the description of the overall infection rate and different characteristics, as well as different types of HR HPV infection.

**Results**

**Overall HPV infection**

A total of 9,460 individuals were included in this study. The number of people from rural areas was 4,130, whereas those from the urban areas was 5,330. Of the total, the number of HPV-positive subjects were 656 and the positive rate was 6.93%. Twenty-one subtypes were detected in 753 cases. HR HPV was detected in 688 subjects (91.37%), whereas the low-risk (LR) type was found in 65 cases (8.63%). The number of HPV-positive cases in the rural population was 289 (infection rate, 7.0%), and that in the urban population was 367 (infection rate, 6.89%). No significant difference was found in the infection rates ($p = 0.86$).

**Distribution of different HPV genotypes**

HPV16 was the most prevalent type (168 cases, 22.31%), followed by HPV58 (95 cases, 12.61%) and HPV52 (95 cases, 12.61%), which were HR HPVs. Meanwhile, the prevalence rate of LR HPVs did not exceed 2% (Table 1).

**Multiple HPV infection rate**

Table 2 shows the proportions of HPV single infection and multiple infections. From a total of 656 positive cases, 414 (63.1%) involved single infections, whereas 242
Prevalence of human papillomavirus and the correlation of HPV infection with cervical disease in Weihai, China

(36.9%) had multiple infections. Double infection was most common type (23.9%) of multiple infections.

HPV infection in different age ranges
The prevalence rates of HPV among women aged 20 to 29 years and 29 to 39 years were 7.29% and 7.28%, respectively. These values were slightly higher than those among the 40 to 49 years and 50 to 59 years age groups (6.98% and 6.43%, respectively) (Figure 1).

HPV infection and liquid-based cytology relationship
The liquid-based cytology results for 563 cases were higher than those of ASCUS. A total of 218 HPV-positive patients were identified (positive rate, 38.7%), whereas 345 patients were HPV-negative (negative rate, 61.3%). The rate of HPV infection increased with the severity of the lesions (Table 3).

TCT ≥ ASCUS, HPV subtype distribution
In TCT ≥ ASCUS, the total number of HPV-positive detection was 299. Of these, type 16 was the most frequently detected (83 times) and accounted for 27.76%, followed by types 52 (13.71%), 33 (11.04%), and 18 (30 cases, 10.03%) (Table 4).

Discussion
This study is a large-scale evaluation of the reproductive-tract HPV infection rate and distribution characteristics based on the investigation of married women in Weihai, which is located on the eastern part of Shandong Province in China. HPV is a common sexually transmitted disease pathogen and has an important function in the pathophysiology of CC and cervical diseases. Numerous studies have been conducted on this topic. However, the reported HPV infection rates significantly varied. The present investigation showed that the HPV infection rate among married women in Weihai was 6.93%. This value
was slightly lower than those in Shanxi (14.8%) [7], Shenzhen (11.3%) [8], Shenyang (17%) [9], and Zhejiang (13.3%) [10], but is within the global level (5.0% to 10.0%) [11]. Single infection accounted for 63.1% of all cases and is the most common condition. Meanwhile, the correlation of multiple infections is under investigation. Lee et al. [12] concluded that single HPV infections increase the risk of CC by 19.9 times, whereas multiple HPV infections increase the risk by 31.8 times.

Most population screening studies showed a declining prevalence of HPV infection with increasing age [13, 14]. However, the report for Guangdong Province, China is different [12]. The present data showed that the incidence of HPV infection was slightly higher in younger women. In the present investigation, the most common genotype was HPV-16, followed by HPV-52, HPV-58, HPV-68, HPV-33, HPV-56, HPV-51, HPV-31, HPV-18, and HPV-59. The first five genotypes accounted for 55.67% of the total infections, and all were HR types. This condition was similar to that found in East Guangdong [15, 16]. Based on total infections, and all were HR types. This condition was 59. The first five genotypes accounted for 55.67% of the HPV-33, HPV-56, HPV-51, HPV-31, HPV-18, and HPV-59. The first five genotypes accounted for 55.67% of the total infections, and all were HR types. This condition was similar to that found in East Guangdong [15, 16]. Based on the analysis of De Sanjosé et al. [11], HPV-16 and HPV-18 are prevalent worldwide and account for 32% of all CC causal factors, 87% of cervical diseases, and 75% of invasive CC. HPV-45 and HPV-58 are also related to CC. The investigated sequence in Weihai in terms of decreasing prevalence is HPV-16, HPV-52, and HPV-58, with HPV-18 is the ninth most prevalent subtype. Most statistical evidence in Asia shows that although HPV-16 is the most common genotype, HPV-52 and HPV-58 are also highly prevalent. In Japan, the HPV-52 genotype is the second most prevalent type. In South Korea, HPV-33 and HPV-58 rank second and third in prevalence, respectively [17, 18]. The situation in Weihai is similar to those in Japan and South Korea, mainly because of the close contact between the population of Weihai and those of these two countries.

HR HPV mainly results in squamous intraepithelial lesions and CC. This study demonstrated that the HPV infection rate increased with worsening patient condition (ASCUS 37.9%, ASC-H 42.5%, LSIL 50%, and HSIL 66.7%). HR HPV-16 constituted the largest proportion (27.76%), followed by HPV-52 and HPV-18. These results indicate that the HPV-52 and HPV-18 may also lead to CC or diseases. The cervical biopsy results show that the detection rate of HPV increased in the severe lesions (CIN I, 74.11%; CIN II, 84.00%; CIN III, 90.00%, SCC 100%). Thus, the HR HPV infection rate was positively related to cervical disease deterioration, particularly in high CIN and CC. A number of researchers have shown that CIN I, CIN II, and CIN III exhibit different disease deterioration levels that are associated with the presence of HPV DNA. CIN I is in the free-mode form in benign lesions. The DNA virus does not bind to the host chromosome but simply invades the bottom of squamous cells with low reproduction rate. Meanwhile, CIN III is in the integrated form. In this disease, the DNA virus integrates and binds with the host chromosome and results in cancer formation and invasion of the bottom of squamous cells. Persistent HR HPV infections during CIN I worsen the condition and can lead to CC. The HPV test is clearly useful in the early diagnosis and early treatment of CC as well as in the evaluation of therapeutic effects. This investigation is the first to provide data on HPV infection and HPV genotype distribution in Shandong Province, China. Scientific HPV investigation, HR HPV detection, and research on HPV genotype distribution provide vital data for HPV vaccine research in the present country [19]. This study may contribute to research on CC prevention and can be used in designing effective clinical treatments.

References

Prevalence of human papillomavirus and the correlation of HPV infection with cervical disease in Weihai, China


Address reprint requests to:

S. HE, M.D.
Department of Obstetrics and Gynecology,
Weihai Municipal Hospital,
Dalian Medical University,
No. 70 Heping Road Huancui District,
Weihai 264200 (China)
e-mail: ylszcn@163.com
Correlation analysis of hormone receptors and the expressions of HER-2 and Ki-67 in breast cancer

Q. Liu1,2, X.Z. Wang1,2, D.B. Mu1, T.Y. Li1, Y.S. Liu1, Z.Y. Yu1

1 Department of Surgery II, Shandong Cancer Hospital, Shandong Breast Center of Prevention and Treatment, Ji’nan
2 Shandong Academy of Medical Sciences, Jinan University, Ji’nan (China)

Summary

Objective: This study aims to investigate the correlation and clinical significance of hormone receptors and the expressions of HER-2 and Ki-67 in breast cancer primary lesions and lymph node metastatic tissues. Methods: 83 cases were studied, who were performed breast cancer surgeries and confirmed the ipsilateral axillary lymph node metastasis by the postoperative pathological diagnosis. Immunohistochemical method was used to simultaneously detect the expressions of ER, PR, HER-2 and Ki-67 in the primary lesions and lymph node metastases. Results: ER exhibited the expression concordance rate as 85.5% in primary lesions and metastases, with significant difference (P = 0.039); the expression concordance rates of PR and HER-2 in primary lesions and metastases were 90.4% and 89.2%, respectively, without significant difference (P = 0.289, 0.180); between the Ki-67-highly-expressed primary lesions and Ki-67-lowly-expressed metastases, the expressions of ER in primary lesions and metastases exhibited statistical significance, with P as 0.031. Conclusions: The primary lesions and lymph node metastases had higher consistency, while there was still about 10% patients showed differentiated expression. The simultaneous detection of breast cancer primary lesions and lymph node metastases was still very necessary.

Key words: breast cancer; ER; PR; HER-2; Ki-67; immunohistochemistry; correlation analysis.

Introduction

Breast cancer is the most common malignancy in female, and the molecular-level understanding of the biological behavior of breast cancer would contribute to the clinical diagnosis and treatment. The occurrence and development of tumor is a complex, multifactorial and multi-step process, with tumor heterogeneity as one of the most important factors that affect cancer treatment and prognosis. The expressions of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (HER-2) in breast cancer have important guiding significance towards the molecular typing of breast tumors, the prognosis judgment and treatment options. The high expression of ER and PR in breast cancer indicate a higher degree of histological differentiation, and the endocrine therapy would have good results; HER-2 plays an essential part in regulating cell proliferation, adhesion, motility, and survival [1, 2]. Trastuzumab, an anti-HER-2 monoclonal antibody, is one such therapy that has had a dramatic impact on the treatment of HER-2-positive breast cancer [3, 4]. The previous adjuvant therapy of clinical treatment of breast cancer were mainly judged on the basis of disease stage, expression status of primary lesions’ hormone receptors and HER-2, while normally ignored the expressions of the above 3 factors in the metastases, and whether the changes would affect the patient’s treatment and prognosis was also ignored. Many studies about the differentiated expressions of ER, PR and HER-2 in breast cancer primary lesions and metastases were not the same. Some scholars believed that the ER [4-8] and PR [6-9] of the primary lesion and metastases in the same breast cancer patient were inconsistent, while some other literature reported the inconsistency of HER-2 in primary lesion and metastases, and this result was not uniform [10-13, 14], proposing the necessity of biological characteristics re-evaluation towards the recurrent lesion through further biopsy of the breast cancer metastases. In recent years, some scholars also advocated that ER, PR and HER-2 immunohistochemistry should be performed not only to all breast cancer primary lesions, but also to the metastases, after that the treatment programs could be selected which would take both the original lesions and metastases into account [15].

Ki-67 (Nuclear-associated antigen) is the nuclear antigen associated with cell proliferation, playing an important role in tumor progression, and its expression changes with the changes of cell cycle. Many studies have shown that the development, metastasis and prognosis of tumor are related to the expression levels of Ki-67, and Ki-67 has been noted as a very important indicator towards the molecular subtypes, prognosis and treatment prediction of breast cancer clinically [16, 17]. How is the expression of Ki-67 in breast cancer metastases and its relationship with the expressions of ER and PR still need further study.
In this study, we detected the expressions of ER, PR, HER-2 and Ki-67 in 83 cases of primary lesions and metastatic tissues of breast cancer patients, to investigate their clinical significance in the treatment and prognosis of breast cancer.

Materials and Methods

Clinical data

236 cases of breast cancer were performed surgical treatment in the 2nd Clinical Section, Shandong Tumor Hospital, from Jan. 2012 to Sep. 2012, among who there were 83 cases were pathologically diagnosed as ipsilateral axillary lymph node metastasis, aging from 24 to 73 years, with a median age of 47 years old. According to the 2003 edition of TNM staging, 48 patients were in clinical stage II, 27 patients were in stage III and 8 patients were in stage IV. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Shandong Cancer Hospital. Written informed consent was obtained from all participants.

Experimental methods

The primary lesions of breast cancer and axillary lymph node specimens were cut postoperatively, fixed in 10% formalin, at the same time, the primary lesions and pathologically-confirmed axillary lymph node metastases were cut and performed 4μm serial sections. The above sections were dewaxed with xylene and dehydrated with graded ethanol on the same slide and under the same conditions and time. Then used 3% H2O2 to treat the sections and washed with PBS thoroughly, then performed the antigen hotfix with microwave oven, ER, PR and HER-2 were repaired with citrate buffer, and Ki-67 was repaired with EDTA buffer. After the hotfix, cooled the slides to the room temperature, washed the slides with PBS and added biotinylated secondary antibody (Fujian Maixin Biotechnology Development Co., Ltd., Fuzhou, China), incubated at the room temperature and then rinsed with PBS thoroughly. The horseradish peroxidase-labeled streptavidin was then added for the incubation at the room temperature, then rinsed with PBS thoroughly, DAB staining (Fujian Maixin Biotechnology Development Co., Ltd., Fuzhou, China), hematoxylin restaining and neutral gum mounting.

The known positive section was set as the positive control, the primary antibody was replaced by PBS for the negative control, then observed and photographed with a microscope.

Results judgment

5 high power fields were randomly selected in both primary lesions and metastases, counting more than 500 cells. The nuclear staining was judged as positive [18], and scored according to the number of positive cells: non-positive cell, 0 point; 1% ~ 30% positive cells: 1 point; 31% ~ 70% positive cells: 2 points; 71% ~ 100% positive cells: 3 points; then according to positive staining intensity, light brown was recorded as 1 point, brown as 2 points, dark brown as 3 points. The total points of each slice was the accumulation of the above 2 scores, 0 point meant negative, 1 ~ 2 points was labeled as (+), 3 ~ 4 as (2+), 5 ~ 6 as (3+), among which the above “(+)” results of ER, PR and Ki-67 were recorded as positive, while the “(3+)” results of HER-2 were recorded as positive, and the suspicious HER-2-positive (2+) patients should be performed FISH analysis, and the positive FISH results would result in HER-2 positive, the negative FISH result would result in HER-2 as negative (Figure 1).

The expression of Ki-67 was based on the identified criteria in 2011 St.Gallen Breast International conference [19], Ki-67 <14% referred to the low expression, ≥ 14% referred to the high expression.

Statistical analysis

The experimental data were analyzed by SPSS17.0 statistical software, the counting data were expressed as the percentage, intergroup data were compared with X2 test, and the test level was set as α = 0.05.
Results

Expressions of ER, PR and HER-2

The positive and negative expression of ER in primary lesions were 55 cases and 28 cases, respectively, while 47 positive cases and 36 negative cases in metastases, the expression of ER was consistent in primary lesions and metastases, namely negative/negative or positive/positive were 26 cases and 45 cases, with a total of 71 cases, the concordance rate was 85.5%; the positive and negative expression of PR in primary lesions were 55 cases and 28 cases, respectively, while 49 positive cases and 26 negative cases in metastases, the expression of PR was consistent in primary lesions and metastases, namely negative/negative or positive/positive were 49 cases and 26 cases, with a total of 75 cases, the concordance rate was 90.4%; the positive and negative expression of HER-2 in primary lesions were 16 cases and 67 cases, respectively, while 11 positive cases and 72 negative cases in metastases, the expression of HER-2 was consistent in primary lesions and metastases, namely negative/negative or positive/positive were 65 cases and 9 cases, with a total of 74 cases, the concordance rate was 89.2%. Statistical analysis revealed that there was statistically significant difference in the ER expression between primary lesions and metastases, \( P = 0.039 \), while no significant differences in the PR and HER-2 expression between primary lesions and metastases, \( P \) values were 0.289 and 0.180, respectively (Table 1, 2).

Correlation of Ki-67 expression and ER, PR and HER-2

Among the primary lesions, 40 cases showed low expression of Ki-67, while 43 cases showed high expression. In the low-expression cases, 2 cases were negative/positive ER expression, while the expressions of PR and HER-2 were exactly the same in primary lesions and metastases, namely all were negative/negative or positive/positive; 4 cases were negative/positive ER expression in primary lesions and metastases, 2 cases for PR and 4 cases for HER-2. In the primary-lesion-highly-expressed Ki-67 patients, the negative/positive PR and HER-2 expression patients were all 2 cases, and ER expression was exactly the same in primary lesions and metastases, all were negative/nega-

Table 1. — Expression status of ER, PR and HER-2 in breast primary lesions and lymph node metastatic tissues of the 83 cases

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Primary lesion</th>
<th>Metastatic tissues</th>
<th>Concordance numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Positive</td>
<td>55</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>28</td>
<td>36</td>
</tr>
<tr>
<td>PR</td>
<td>Positive</td>
<td>55</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>HER-2</td>
<td>Positive</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>67</td>
<td>72</td>
</tr>
</tbody>
</table>

Table 2. — Correlation analysis of ER, PR and HER-2 expression in primary lesions and lymph node metastatic tissues of the 83 breast cancer cases

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Primary lesions</th>
<th>Metastatic tissues</th>
<th>Summary</th>
<th>( P )</th>
<th>( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Positive</td>
<td>45</td>
<td>10</td>
<td>55</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>26</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>Positive</td>
<td>49</td>
<td>6</td>
<td>55</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>26</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>HER-2</td>
<td>Positive</td>
<td>9</td>
<td>7</td>
<td>16</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
<td>65</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — Correlation analysis of Ki-67 expression in primary lesions and the changes of ER, PR and HER-2 between primary lesions and metastases

<table>
<thead>
<tr>
<th>Ki-67</th>
<th>Primary lesions</th>
<th>Metastases</th>
<th>Summary</th>
<th>( P )</th>
<th>( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Low expression</td>
<td>Positive</td>
<td>28</td>
<td>4</td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
<td>6</td>
<td>0.031</td>
</tr>
<tr>
<td>PR</td>
<td>Low expression</td>
<td>Positive</td>
<td>17</td>
<td>6</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>0</td>
<td>20</td>
<td>0.500</td>
</tr>
<tr>
<td>HER-2</td>
<td>Low expression</td>
<td>Positive</td>
<td>9</td>
<td>4</td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
<td>18</td>
<td>0.500</td>
</tr>
<tr>
<td></td>
<td>High expression</td>
<td>Positive</td>
<td>7</td>
<td>5</td>
<td>0.453</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>
tive or positive/positive; 6 cases expressed ER as negative/positive or positive/negative in primary lesions and metastases, 6 cases showed PR, and 7 cases showed HER-2. The metastatic low expression of Ki-67 had 43 cases, while 40 cases with high Ki-67 expression. Among the low expression cases, 2 cases exhibited HER-2 expression as negative/positive, respectively, while ER and PR expression did not change. The positive/negative expression of ER, PR and HER-2 were 6, 2 and 1 case, respectively. In the metastatic high Ki-67 expression cases, negative/positive expression of ER and PR were 2 and 3 cases, respectively, while HER-2 expression did not change. The positive/negative expression of ER, PR and HER-2 were 4, 4 and 5 cases, respectively. Statistical analysis revealed that there was statistically significant difference in the ER expression in primary lesions and metastases only in the high Ki-67 expression primary lesion cases and low Ki-67 expression metastases cases, $P = 0.031$ (Table 3, 4).

### Discussion

Breast cancer has the characteristic of heterogeneity, because of the abnormal expression of a series of breast cancer-related genes, the different combinations of gene expression could prompt the prognosis and predict therapeutic effects. The usage of immunohistochemical detection of ER, PR, HER-2 and Ki-67 could perform the molecular typing of breast cancer, which would facilitate the development of treatment strategies [20]. Endocrine therapy and targeted therapy play an important role in the combined treatment towards breast cancer. In normal circumstances, endocrine treatment would be effective towards the hormone receptor-positive patients, with low probability of recurrence and metastasis [21]. HER-2-positive tumors have highly malignant degree, and the disease normally would progress quickly. Trastuzumab-targeted therapy is considered as the standard therapy in HER-2 positive cases [22], patients who were performed the targeted therapy exhibited the significantly improved prognosis. Currently, lymph node metastasis is still an important indicator towards the prognosis prediction of breast cancer. The researches about the clinical significance of relevant immunohistochemical indicators in metastases, whether the changed results could change our conventional treatment plans and optimize treatment strategies, so that patients could get the best treatment, are the issues worthy of exploration in current breast cancer treatment.

Recent studies have found that the expressions of ER, PR and HER-2 had differences between breast primary lesions and lymph node metastases. In 2011, Deng [23] found that there was statistically significant difference in ER expression between primary lesions and recurrent metastases, the change rate was 66.67%; while there were no significant differences in PR and HER-2 expression between primary lesions and recurrent metastases, the change rate were 17.78% and 13.33%, respectively. Zhao [24] reported that there were no significant difference in ER, PR and HER-2 expression between primary lesions and lymph node metastases. This study found that there was significant difference in ER between primary lesions and metastases of breast cancer, while no significant difference in PR and HER-2. Monaco [25] found that the concordance rates of ER and PR expression in primary lesions and metastases were 81% and 65%, and in this study, the concordance rate were 85.5% for ER and 90.4% for PR, slightly higher than the above study. The differences in the results might involve many subjective and objective factors, such as patient selection, sample collection time, sampling sites and methods, laboratory reagents, methods and evaluation standards, etc. The specimens in this study were obtained from surgical resection, the immunohistochemistry of primary lesions and metastases were at the same time, on the same slide, under the same experimental conditions and judged by the same chief physician. Towards the patients with inconsistent expression between primary lesions and metastases, if the primary lesions exhibited negative ER and PR while positive in metastases, the endocrine therapy would be ad-

### Table 4. Correlation analysis of Ki-67 expression in metastases and the changes of ER, PR and HER-2 between primary lesions and metastases

<table>
<thead>
<tr>
<th>Ki-67</th>
<th>Primary lesions</th>
<th>Metastases</th>
<th>Summary</th>
<th>$P$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Low expression</td>
<td>Positive</td>
<td>27</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>0</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>High expression</td>
<td>Positive</td>
<td>18</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>PR</td>
<td>Low expression</td>
<td>Positive</td>
<td>33</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>0</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>High expression</td>
<td>Positive</td>
<td>15</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>3</td>
<td>18</td>
<td>40</td>
</tr>
<tr>
<td>HER-2</td>
<td>Low expression</td>
<td>Positive</td>
<td>5</td>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>2</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>High expression</td>
<td>Positive</td>
<td>4</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>0</td>
<td>31</td>
<td>40</td>
</tr>
</tbody>
</table>
ministrated on the basis of conventional chemotherapy and radiotherapy, whether it could improve the efficacy of clinical practice still needed further verification.

The detection of HER-2 includes protein-level immuno-histochemistry and gene-level FISH test, ASCO / CAP suggested that if the immunohistochemical results of HER-2 was (2 +), the FISH should be performed [26]. Lower [14] carried out the research on 382 recurrent patients of breast cancer, finding that 254 cases (66%) exhibited the concordance of HER-2 in primary lesions and metastatic sites, 90 cases had positive results in primary lesions and negative in metastases, while 37 cases were just the opposite. In 2011, Monaco [25] reported that the concordance rate of HER-2 in breast cancer primary lesions and metastases was 71%. In this study, it was found that the expression concordance rate of HER-2 in primary lesions and metastases was 89.2%, which was a high concordance rate, and the inconsistent cases were mostly positive in primary lesions and negative in metastases, which might indicate that some mechanism might inhibit the expression of HER-2 during the cancer in vivo metastasis to the lymph nodes. Thus, towards the patients with HER-2 expression as positive/negative, if they were administrated the targeted therapy based on the HER-2 expression in primary lesions, whether the clinical efficacy would be the same as positive/negative; while as for the patients with HER-2 expression as negative/positive, whether the clinical treatment was deficiencies, and whether the patients had missed opportunities of targeted therapy, the above still need further study.

Ki-67 antigen is a nuclear antigen present in proliferating cell nucleus, with short half-life, it begins the expression in G1 phase of the cell cycle, and reaches the highest peak in M phase, and would rapidly degrade after the completion of the cell cycle. As a cell proliferation marker, it’s the reliable indicator of reflecting cell proliferation [27]. In the early stage of breast cancer, Ki-67 expression occurs a high proportion in lymph node metastasis, with poor prognosis. Tawfiq [28] found that detection of Ki-67 expression status in lymph nodes was superior to evaluate the expression of Ki-67 in primary lesions, and would help to select the treatment options. In 2011, St.Gallen Breast International Conference formally proposed Ki-67 as an important reference index of molecular classification of breast cancer. In this study, the correlation of different expression levels of Ki-67 and ER, PR and HER-2 expression in primary lesions and metastases of breast cancer patients were explored, finding that in primary lesions with high Ki-67 expression and metastasis with low Ki-67 expression, the ER expression exhibited statistical significance between primary lesions and metastases, therefore, when use immunohistochemistry to detect the primary lesions of breast cancer patients, it should not neglect the Ki-67 expression status in lymph node metastases. In differences Ki-67 expression levels, PR and HER-2 expression also showed differences between primary lesions and metastases, although no statistically significant was found yet, the tumor with high Ki-67 expression had biological behavior of high proliferation, during the administration of hormone therapy or targeted therapy, the chemotherapy was also the measures which should not be ignored.

Conclusions

Hormone receptors and the expressions of HER-2 and Ki-67 of primary lesions and lymph node metastases in breast cancer had higher consistency, while there were still about 10% patients showed differentiated expression. The simultaneous detection of breast cancer primary lesions and lymph node metastases was still very necessary. Management of breast cancer still requires particular attention to metastatic site, long term follow-up after breast cancer therapy, more aggressive adjuvant therapy may be useful in different hormone receptors and the expressions of HER-2 and Ki-67 of metastatic lesions, and to access to the best effect.

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Address reprint requests to:
Z.Y. YU,
Department of Surgery II,
Shandong Cancer Hospital,
Shandong Breast Center of Prevention and Treatment
Ji’nan 250117 (China)
e-mail: zhionguyen@163.com
Case Reports

Leiomyosarcoma: a rare malignant transformation of a uterine leiomyoma

G. Di Luigi, A. D’Alfonso, F. Patacchiola, L. Di Stefano, P. Palermo, G. Carta
Department of Obstetrics and Gynecology, University of L’Aquila, L’Aquila (Italy)

Summary
The malignant transformation of a uterine leiomyoma is still debated and, if it occurs, it is very rare. The case of a patient affected by one small leiomyoma is described. Diagnosis was made postoperatively on histopathological examination. The case reported here is meant to underline the need to keep all uterine myomas in check since the transition into leiomyosarcomas (LMSs) may occur with an evolution over a time period which has not been established so far. Specific receptors for luteinizing hormone / human chorionic gonadotropin (LH/hCG) have also been identified in the myometrium of several animal species, including humans. Conventional LMSs express estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR) in 30-40% of cases. In comparison with other more common uterine malignancies, uterine LMSs bear some resemblance to type 2 endometrial carcinomas and high-grade serous carcinomas of ovary/fallopian tube origin, based on their genetic instability, frequent p53 abnormalities, aggressive behavior, and resistance to chemotherapy. It could be useful to understand with further researches if hormonal stimulation could be a contributing factor of uterine leiomyoma transformation into LMS. Until today the oncogenic mechanisms underlying the development of uterine LMSs remain elusive.

Key words: Leiomyosarcoma; Uterus; Leiomyoma; Ovarian stimulation.

Introduction
Uterine sarcomas are rare tumors that account for approximately 1% of female genital tract malignancies and 3% to 7% of uterine cancers [1]. Although the aggressive behavior of most cases is well recognized, their rarity and histopathological diversity has contributed to the lack of consensus on risk factors for poor outcome and optimal treatment [2]. Histologically, uterine sarcomas were first classified into carcinosarcomas, accounting for 40% of cases, leiomyosarcomas (LMSs) (40%), endometrial stromal sarcomas (10% to 15%), and undifferentiated sarcomas (5% to 10%).

In most instances, uterine smooth muscle tumors (USMTs) are readily diagnosed as either benign or malignant. Rare patients whose smooth muscle tumors fail to meet LMS diagnostic criteria will experience recurrence, and occasional cases of LMS patients experience a protracted clinical disease course [3]. For these reasons a new classification is catching on: smooth muscle tumors of uncertain malignant potential (STUMP) are a heterogeneous group of neoplasms, from both the histological and clinical point of view. Due to the rarity of these tumors, the literature on the topic is limited; a consensus on their diagnosis, malignant potential, monitoring, and treatment has still not been reached [4-8]. The clinical behaviour of these neoplasms is also poorly understood. The majority of cases follow a benign clinical course, however a few can metastasize as either tumor of low malignant potential or LMSs [9]. Until today leiomyomas and LMSs are believed to develop independently and are not progressive. This belief is based on population studies, as well as genetic and cytogenetic profiles of these tumors and the fact that they lack shared mutations and transformations. LMSs are usually asymptomatic until they reach a size large enough to cause pain or bleeding. In cases in which the initial radiographic impression is not worrisome, rapid growth during the interval between imagings is an indication for resection. Most LMSs occur in perimenopausal and postmenopausal women; the average age of a woman with LMS is 50 years. However, premenopausal women may develop them. Most LMSs metastasize within two years of diagnosis. In this case report the authors would like to highlight that malignant transformation of uterine leiomyoma can occur over the years.

Case Report
A 43-year-old female patient presented at the gynecology clinic complaining of a left anechoic adnexal cyst. She was a non-smoker and had a body mass index within the normal range. The patient attained menarche at the age of 14 years and had a regular cycle with four-day flow every 28 days. In the year 2000 a
transvaginal pelvic ultrasound revealed a small (1.0 × 0.7 cm) solitary intramural leiomyoma of the posterior wall of the uterine body. In the year 2002 she had undergone one successful IVF cycle (a long stimulation protocol with Decapeptyl 3.75 mg + Menogon 75 UI + Profasi HP 5,000 UI) for primary infertility of male origin. She responded well to stimulation and produced eight eggs. Three embryos were transferred. This treatment was successful and a twin gestation was achieved (one boy and one girl). In the year 2006 she conceived spontaneously and she delivered a healthy girl at term. The patient was asymptomatic and physical examination was normal. She had no prior history of vaginal bleeding or abdominal pain. Preoperative assessment tests were all normal, including a full blood count, urea, glucose, clotting screen, hepatitis B-C status, and tumor markers. Transvaginal ultrasound examination confirmed a solitary left anechoic adnexal cyst (25 x 15 mm), an intramural leiomyoma (two cm in diameter, slightly larger than the previous ultrasound performed in the year 2000) of the posterior wall of the uterine body and no evidence of intra-abdominal fluid collection was detected. The magnetic resonance imaging confirmed these data. The patient underwent a left monolateral laparotomic salpingectomy without complications. Intraoperative findings showed a normal upper abdomen and no palpable lymph nodes. An extemporaneous histologic examination of the cyst was performed. While waiting for the outcome of histology, the surgical team decided to remove the uterine myoma. The pathological examination on frozen section revealed an hydrosalpinx with tubal endometriosis, but the final histopathological results of the uterine specimen showed an unexpected “cellular leiomyoma with a central area of malignant transformation into LMS (more than ten mitoses per ten high power fields) desmin +, actin +, p16 +, Ki67 +, in 1% of the neoplastic cells”. Two months later the patient underwent total abdominal hysterectomy; the histologic examination showed no presence of leiomyosarcomatous tissue. No further treatments were performed and until today the patient is asymptomatic and physical examination is normal.

Discussion

Analyzing the evolution of the case under examination, and in light of the several US scanning checks carried out over the years on the patient, it is likely that the very small-sized intramural myoma may have undergone malignant transformation. After excluding carcinosarcoma, LMS has become the most common subtype of uterine sarcoma. However, it accounts for only 1–2% of uterine malignancies. Most occur in women over 40 years of age who usually present with abnormal vaginal bleeding (56%), palpable pelvic mass (54%), and pelvic pain (22%). Signs and symptoms resemble those of the far more common leiomyoma and preoperative distinction between the two tumors may be difficult. Only very rarely does a LMS originate from a leiomyoma. The minimal pathological criteria for the diagnosis of LMS are more problematic and, in such cases, the differential diagnosis has to be made, not only with a variety of benign smooth muscle tumors that exhibit atypical histologic features and unusual growth patterns but also with STUMP. The diagnosis of these tumors is often challenging, as interpretative difficulties and subjectivity can be encountered when analysing any of the three histological features: cytologic atypia, mitotic index, and coagulative tumor cell necrosis. These three features are called the Stanford criteria and were developed by Bell et al. [5].

Cytologic atypia

The adjacent nonneoplastic myometrium may be used as an internal control for the patient’s baseline smooth muscle histology. Cytologic atypia is assessed by determination of nuclear size, examination of membrane contours, and evaluation the prominence and number of nucleoli. The mitotic index aids in classifying the tumor.

Mitotic index

By itself, the mitotic index is not an independent predictor of malignancy. Mitotically active leiomyomas are well studied and reported. These are defined as smooth muscle tumors with up to 20 mitoses/ten high power fields (HPF), but they are devoid of atypia and tumor cell necrosis. Although this feature alone does not denote malignancy, when other worrisome features are present, mitotic activity becomes extremely important in assessing malignant potential. To measure the mitotic index, find the most mitotically active area of the tumor (but avoid areas adjacent to hyalinized necrosis) and count ycn HPF (×40).

Coagulative tumor cell necrosis

Of the three features discussed, coagulative tumor cell necrosis seems to be the most predictive histologic feature of malignancy. Coagulative tumor cell necrosis is characterized by an abrupt change of viable myocytes adjacent to necrotic myocytes without an intervening sclerotic edge. Features predictive of malignancy include tumor cell necrosis (regardless of other features), infiltrative borders (recognized by the adjacent normal myometrium splayed and separated by the myxoid stroma), and mitoses greater than two per ten HPF. Some early reports of myxoid smooth muscle tumors found that tumors characterized by a mitotic index of zero per ten HPF, bland cytology, and infiltrative borders were associated with poor outcomes [10]. Although these findings have not been reproduced to date, they should probably be diagnosed with care; a STUMP category may be most appropriate. The term STUMP is sometimes applied to cases in which there are indeterminate features of malignancy or a combination of features that are unusual and therefore are not reported extensively in the literature. This term should be reserved for cases in which the malignant potential really is unknown, and it should be used sparingly. STUMP is essentially a non-diagnosis, and it is fraught with frustration for clinicians and patients. Most studies of STUMP report benign outcomes, which probably reflect the fact that the term is overutilized. If the tumor has features that are usually benign but if rare cases of recurrence are known, the term “low recurring potential” may be preferable to STUMP, because that term conveys more information about the predicted and known malignant potential [5, 11].

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Expression and role of the luteinizing hormone/human chorionic gonadotropin (LH/hCG) axis in extragonadal tissues

It is not yet clear the role of ovarian stimulation in the development of uterine sarcomas. The presence of LH/hCG-Rs was shown for the first time by Reshef et al. [20] in the uterus of nonpregnant women by immunohistochemistry, an observation subsequently confirmed using different techniques [21, 22]. By acting through the transduction mechanisms described above, LH and hCG regulate ovarian steroidogenesis, but have also been shown to exert various effects on nongonadal tissues, such as endometrium, myometrium, and fallopian tubes. LH/hCG-Rs have been identified in epithelial and stromal cells of the endometrium, as well in smooth muscle cells of myometrium and uterine vessel. The expression of LH/hCG-R varied during the women’s cycle phase, with the maximal expression occurring during the luteal phase [23]. Specific receptors for LH/hCG have also been identified in the myometrium of several animal species, including humans [24]. In this tissue, LH/hCG apparently acts through the LH/hCG-R-dependent activation of both the c-AMP and phospholipase C transduction pathways [24, 25]. It was proposed that the triggering of adenyl cyclase could determine an activation of COX-2, which in turn should induce an increase of the synthesis of either prostaglandin (PG)E2, with an ensuing muscle relaxation, or PGF, which determines the contraction of the uterine musculature [26].

Immunohistochemistry and molecular biology

Several immunohistochemical and molecular genetic studies on uterine LMSs have been reported [4, 12–18]. LMSs usually express smooth muscle markers such as desmin, h-caldesmon, smooth muscle actin, and histone deacetylase 8 (HDAC8). Conventional LMSs express estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR) in 30–40% of cases. Whereas a variable proportion of uterine LMSs has been reported as being immunoreactive for c-KIT, no c-KIT mutations have been identified [19]. Recent studies have shown statistically significant higher levels of Ki67 in uterine LMSs compared with benign smooth muscle tumors [14–18]. Mutation and overexpression of p53 have been described in a significant minority of uterine LMSs (25–47%) but not in leiomyomas [14, 17, 18]. Overexpression of p16 has been described in uterine LMSs and may prove to be a useful adjunct immunomarker for distinguishing between benign and malignant uterine smooth muscle tumors [12–14]. Overall, uterine LMS is a genetically unstable tumor that demonstrates complex structural chromosomal abnormalities and highly disturbed gene regulation which likely reflects the end-stage of accumulation of multiple genetic defects. In comparison with other more common uterine malignancies, uterine LMSs bear some resemblance to type 2 endometrial carcinomas and high-grade serous carcinomas of ovary/fallopian tube origin, based on their genetic instability, frequent p53 abnormalities, aggressive behavior, and resistance to chemotherapy.

References


Address reprint requests to:
G. DI LUIGI, M.D.
Department of Obstetrics and Gynecology
University of L’Aquila
U.O. Ginecologia ed Ostetricia DU
Ospedale Civile “San Salvatore”
67100 Coppito (Italy)
e-mail: giandiluigi@hotmail.com
Small cell carcinoma of the ovary of the hypercalcemic type (SCCOHT) – case report

J. Lubin¹, M. Pawałowska¹, A. Markowska², A. Bielas

¹ Gynecology Department, Poznań University of Medical Sciences, Poznań
² Perinatology and Gynecology Department, Poznań University of Medical Sciences, Poznań (Poland)

Summary
Small cell carcinoma of the ovary of the hypercalcemic type (SCCOHT) is a very rare malignant disease, seen mostly in young women, with a very poor prognosis. There is no standard treatment for patients with this disease and most literature is limited to short series or case reports. This report describes the case of a 34-year-old woman with aggressive course of SCCOHT and poor outcome. What proved difficult was the process of establishing the diagnosis due to non-specific first symptoms of disease and consequently the combined treatment of surgery and chemotherapy with concurrent side effects.

Key words: Ovarian cancer; Small cell carcinoma of hypercalcemic type.

Introduction

Primary small cell carcinoma of the ovary of the hypercalcemic type (SCOC – small cell ovarian cancer), is a rare form of cancer derived from this organ (about 1% of ovarian cancers). It belongs to the primary neuroendocrine tumors, and its presence is usually sporadic, with no familial circumstances. The tumor is usually diagnosed in adolescence and young adulthood, with the mean age of the patient being 24.3 to 34.5 years [1, 2], although incidence has been reported in both the first and the eighth decade of life [2, 3]. Tumors are usually solid and located almost exclusively unilaterally [4]. Cystic components of the tumor are observed as well as extensive areas of necrosis [5]. The initial symptoms are non-specific, consequently more than half of the cases are diagnosed in advanced stages of the disease [4]. Patients usually present with abdominal pain, a palpable mass/tumor in the abdomen, ascites, nausea or vomiting, and difficulty passing stool. Clinical symptoms of the cancer are non-specific beyond those symptoms associated with paraendocrine hypercalcemia. Extraovarian spread during surgery is observed in about half of the cases [5].

Case Report

A 34-year-old woman presented to the physician in December 2010 due to edema of the left groin and the left lower limb, as well as frequent urination. In the performed computer tomography scan of the abdomen and pelvis (December 12, 2010), enlarged left paraaortic lymph nodes up to 35mm in size and enlarged lymph nodes of the left common iliac artery measuring 20 x 10 x 10 mm were found. In January 2011, a fine needle biopsy of the left inguinal lymph node was performed. In the biopsied tissue sample (measuring 20 x 10 x 10 mm), neoplastic cells were not found, instead a fragment of adipose and fibrous tissue with a small focal area of bloody hemorrhage was observed. Additional diagnostic studies of the colon were performed – rectoscopy and colonoscopy of which neither displayed any pathology. March 2011, an abdominal ultrasonography (USG) was performed three times, which demonstrated enlarged retroperitoneal lymph nodes along the abdominal aorta, the left renal artery, bilateral common iliac arteries, the left external iliac artery, and the lymph nodes of the left groin (changes measuring up to 51 mm). The radiologist suggested, based on the topographic changes, non-Hodgkin’s lymphoma as the initial diagnosis. In April 2011, a gynecologic examination was performed in which a tumor of the left ovary measuring about 50 mm was found. Simultaneously, laboratory results had shown elevated levels of LDH (1,794 U/L; normal range below 480 U/L), CEA (6.9 ng/ml; normal range 0.0 - 3.0 ng/ml), ESR (32 mm/h; normal range 3.0 - 15 mm/h). It is worth mentioning that the values of the tumor markers CA 125, CA 19.9, AFP, and beta-HCG were within normal limits. In April, the excision of the left-sided uterine adnexa was performed and a fragment of a lymph node was taken for testing. The enlarged lymph nodes along the major vessels described in the abdominal USG examination were not removed. The resected tumor had a diameter of about 80 mm and the cross-section was of a solid character. The intraoperative histopathologic examination revealed the presence of high-grade malignant cells that may correspond to lymphoid cells or a poorly differentiated tumor. The histological picture, on the basis of the immunohistochemical results, corresponded to, small cell carcinoma of the ovary of the hypercalcemic type G3, pT2 FIGO, pN0 [tumor composed of poorly differentiated cells with a very high proliferative index (Ki67 over 95%), immunophenotype: CKAE1/AE3 +, EMA +, CK7 +, CD3 -, CD20-, WT1-, LCA-, CA125-, CHR + SYN + (single cells), and CD30-. In the final conclusion, the pathologist stated that the small cell carcinoma of the ovary of the hypercalcemic type, is the primary tumor of the ovary. The neoplasm was diagnosed as Stage IIIA according to FIGO classification. Shortly after the procedure the patient received chemotherapy, paclitaxel (175mg/m2) and car-

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boplatin (AUC 6). During ultrasound monitoring with a vaginal probe, a cluster of enlarged lymph nodes was detected in the left lower pelvis. During physical examination, soft tissue swelling in the left groin and the left thigh were noted. Additionally, there was a decrease in the size of the affected lymph nodes from 65.4 mm to 41 mm, and a reduction in the soft tissue swelling of the described area was noted after two courses of treatment with taxanes and platinum derivatives. A high toxicity reaction was noted in the patient due to the regimen of chemotherapeutics (neutropenia and thrombocytopenia were observed), requiring pegfilgrastim and steroid therapy. In August 2011, after the third course of treatment, in the face of persistent hematologic toxicity (thrombocytopenia, anemia, neutropenia), a trephine biopsy was performed to evaluate the bone marrow. The material obtained from the trephine biopsy was found to contain no metastatic cells. The immunohistochemical study demonstrated: MPO + about 60 - 70%, cytokeratin AE1/AE3, and CK7 negative. It was decided to use taxane, at a dose of 135 mg/m2 with cisplatin, at a dose of 75mg/m2 (75% of the recommended dose). In August 2011, in USG examination of the lower pelvis, an increase in the size of the monitored lymph node mass was observed. September 2011, the patient received the second line of treatment, and was treated with two courses of cytostatics - etoposide 200 mg/m2 and cisplatin 75mg/m2 (75% of the recommended dose). After the administration of the above treatment, again a progression was observed in the lesser pelvic lymph nodes, as well as an increase in the swelling of the left groin and left thigh. Nonetheless, a regression was observed in the lymph nodes of the abdominal cavity, in comparison to studies performed previous to the application of the second line of treatment with cytostatics, therefore it was decided to implement the next course of treatment according to the existing scheme. In early November 2011, the patient was hospitalized due to a significant degree of weakness, severe pain in the left lumbar region of the spine, and severe swelling of the left lower limb. In laboratory studies, neutropenia, hypomagnesaemia, and hyponatremia were found. In imaging studies - USG examination of the abdomen, a swelling of the tissues of the abdominal wall with the diameter of 25 mm was revealed. The paraaortal lymph nodes had increased in diameter to 47 mm and fluid was detected in the abdominal cavity. In the chest radiograph, fluid was found in both pleural cavities. In view of the ineffectiveness of the current treatment with cytostatics and the poor general condition of the patient, it was decided to discontinue chemotherapy. The patient died on November 21, 2011.

Discussion

Primary small cell carcinoma of the ovary is uncommon neoplasm. The present case proved that the diagnosis and treatment is really difficult and the outcome is poor.

There is no consistent information regarding the effectiveness of determining CA 125 in the diagnosis and monitoring of this form of ovarian cancer. Elevated levels of CA 125 in serum is observed in approximately 76.9% of cases, however, compared with serous carcinomas, these values are relatively low. The average concentration of this marker prior to treatment is estimated to be about 176 U/ml. It seems that there is no correlation between the level of the CA 125 marker and the size of the tumor or the concentration of calcium ions in the blood [1].

In a study of 150 cases of SCOC [4] it was found that 63% of patients with hypercalcemia in the serum were largely asymptomatic. The typical symptoms of hypercalcemia include: renal manifestations (polyuria, nephrolithiasis), lack of appetite, constipation, vomiting, abdominal pain, pancreatitis, hypertension, and symptoms of the central nervous system such as headache and disorientation. Some researchers believe that in women with high levels of calcium in the blood, determination of Ca 2+ in the course of treatment may be a useful tool to evaluate the effectiveness of therapy [6, 7].

The first description of ovarian small cell cancer dates back to the beginning of the 1980’s, [8] and despite the progression of time, the prognosis of these patients is still very poor. The majority of patients, despite intense cancer treatment, die within two years of diagnosis (for Stage III disease according to FIGO, average life expectancy of patients is approximately six months [2]). Young et al. [4] presented data indicating the possibility of long-term survival among patients whose tumor, at the time of diagnosis, was limited to only one ovary (FIGO IA). Favorable prognostic factors include, patients over the age of 30, normal preoperative serum calcium levels, tumor size less than ten cm and the absence of large cells in the histological picture. From the series Harrison et al. [2], of 17 patients described, ten were diagnosed with Stage I of the disease; seven of them had a disease free period estimated to average 40 months after completion of therapy.

Due to the relatively low incidence of this cancer, and the variety of therapeutic regimens used in oncologic centers, a generally accepted standard of management has not yet been developed. Treatment is based on the execution of primary surgery, chemotherapy, secondary cytoreduction, and radiotherapy.

Surgery to remove the affected adnexa is necessary for the diagnosis and the assessment of the severity of the disease, but there is no unified position on the extent of the surgery in this group of patients. Unilateral removal of the adnexa or exclusively of the ovary is carried out in about 70% of cases [1]. Despite the generally very young age of patients, and the desire to maintain their fertility, because of the very dynamic course of the disease, with a short life expectancy, many surgeons do not implement conserving surgery but instead remove the uterus, the second adnexa, and the greater omentum [9].

In some women, the diversity of chemotherapy regimens used, the varying number of cycles of chemotherapy administered, and the addition of radiotherapy applied to the abdomen and pelvis, account for the considerable difficulty in evaluating the effectiveness of adjuvant therapy.

The analysis of literature covering a total of 136 cases of SCOC [1] proved that the greatest efficacy of chemotheraphy regimens were those containing cisplatin or carboplatin, etoposide, and vinca alkaloids. In this study, in about 65% of the women who originally obtained complete remission, recurrence was found at approximately 11.5 months after the culmination of the first line of treatment.

Senekjian et al. [10] reported the results of treatment of five patients with SCOC receiving chemotherapy with VPCBAE
- vinblastine, cisplatin, cyclophosphamide, bleomycin, adriamycin (doxorubicin), etoposide. Four of the five women died within 11-18 months of diagnosis of the disease, whereas in one patient (FIGO IA), a 29-month disease-free period was achieved. During the course of the treatment, all the patients reported a high degree of hematologic complications, requiring an adjustment of dose in three of the cases. Additionally, severe neuropathy was present in one of the patients.

In a series of 17 patients described by Harrison et al. [2], all of the patients, after the initial surgery, received adjuvant chemotherapy, seven had additionally undergone radiotherapy. Eight women (five FIGO I, three FIGO III) received cisplatin with etoposide with or without bleomycin, and five of them (four FIGO I, one FIGO III) received radiotherapy at a later phase of the treatment. In women with Stage I disease, the median time observed without recurrence was 51 months. Two patients with FIGO Stage III died at six and seven months after the diagnosis of the disease. One of the patients that had been treated with additional radiotherapy, had achieved a disease-free period of over six months. Another four patients (three FIGO III, one FIGO IC) were treated with paclitaxel and carboplatin – in two of them, tumor recurrence was found after nine and seven months; in the remaining two, disease progression was already observed during the course of the treatment. The next three patients who were diagnosed with Stage IC disease received cisplatin, etoposide, paclitaxel, and carboplatin, two of which later receive additional radiotherapy treatment. In this subgroup, two women had attained a disease-free period of at least 16 and 54 months, only one achieved a partial response to the chemotherapy applied.

The study of Pautier et al. [9] included 27 patients who underwent surgical cytoreduction (with the excision of both adnexa, the uterus, the greater omentum, and the pelvic and para-aortic lymph nodes) before/during/and after the completion of chemotherapy and receiving four to six cycles of chemotherapy according to the PAVEP regimen (cisplatin, adriamycin, etoposide, cyclophosphamide). Of the 18 women who achieved complete clinical remission, ten received one course of high-dose chemotherapy of consolidating CARBOPEC (carboplatin, etoposide, cyclophosphamide) and subsequently received autologous hematopoietic stem cell transplantation (AH SCT). The results of such intensive treatment are encouraging - among the ten patients treated with high-dose chemotherapy (HDCT), at a mean follow-up of 37 months, seven patients still remained in complete remission, as opposed to five of the eight not subjected to HDCT, which were diagnosed with recurrent disease. One- and three-year survival for all patients included in the study were 58% and 49% respectively. It is worth noting that in the evaluated group of 17 patients, only five had Stage I disease according to FIGO.

Some researchers emphasize the important role of radiotherapy in the treatment of SCOC. Both Harrison et al. [2] and Young et al. [4] showed that the majority of women with long-term disease-free periods, in addition to chemotherapy, received radiation therapy within the pelvic and abdominal cavities. Favorable results of irradiation are explained by the fact that most frequently recurrence of disease occurs in the pelvis and the peritoneum.

Baeyens et al. [7] described a 19-year old female, kidney transplant patient, in which during the initiation of the transplantation, after exploration of the abdominal cavity, nodular changes were found in the right ovary, which proved to be SCCOHT. During the procedure, the right adnexa was excised, and all macroscopic residual tumors were removed from the abdomen (FIGO IA). The transplantation of the kidney was abandoned. Due to kidney failure, it was decided to suspend chemotherapy treatment, and the abdominal cavity was irradiated with a dose of 30 Gy and the pelvis with a dose of 44.8 Gy, yielding a greater than ten-year disease-free period.

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Address reprint requests to:
J. LUBIN, M.D., PhD
Department of Gynaecology Poznan
University of Medical Sciences
ul. Szmarzewskiego 82/84
60-569 Poznań (Poland)
e-mail: jola.lubin@gmail.com
Endometrial cancer in a patient with rheumatoid arthritis

G. Androutsopoulos¹, G. Adonakis¹, E. Terzakis¹, E. Geropoulou², G. Decavalas¹

¹Department of Obstetrics and Gynecology, University of Patras, Medical School, Rion
²Department of Pathology, University of Patras, Medical School, Rion (Greece)

Summary

Background: Rheumatoid arthritis is a chronic, systemic, and autoimmune disease. In patients with rheumatoid arthritis, there is increased risk for site-specific malignancies. The authors present a case of endometrial cancer in a patient with rheumatoid arthritis and a review of the current literature. Case: The patient, a 60-year-old, postmenopausal Greek woman suffering from rheumatoid arthritis, presented with a complaint of abnormal uterine bleeding. She underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphadenectomy. Histopathology revealed endometrial cancer. The final diagnosis was Stage Ib endometrial cancer endometrioid type. She underwent postoperative adjuvant radiotherapy. She remains without evidence of disease, 16 months after initial surgery. Conclusion: Although the present patient was diagnosed at early-stage disease and remains well 16 months after initial surgery, she needs a multidisciplinary treatment approach in order to achieve prolonged survival.

Key words: Rheumatoid arthritis; Endometrial cancer; Surgery; Radiotherapy.

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, and autoimmune disease [1]. It is characterized by symmetrical joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality [1-3].

It affects approximately 0.5-1% of the population worldwide, including all ethnic groups [2]. However, it is more common in women (female/male ratio is 2.5:1) [2].

The etiology of RA is thought to be a complex with both genetic and non-genetic (hormonal, immunologic, and environmental) factors influencing susceptibility, severity, and response to therapies [2,4].

In patients with RA, there is increased risk for site-specific malignancies [5]. Also there is a concern whether the inflammatory disease or its treatment might increase the risk of cancer [5,6].

The authors’ aim was to present a case of endometrial cancer (EC) in a patient with RA and a review of the current literature.

Case Report

The patient, a 60-year-old, gravida 5, para 2 postmenopausal Greek woman presented to the Department of Obstetrics and Gynecology of the University of Patras Medical School, with a complaint of abnormal uterine bleeding. She was suffering from RA for the last three years and received leflunomide, corticosteroids, and methotrexate. Her surgical history was unremarkable. She had no history of hormone replacement therapy and her family history revealed no evidence of cancer among the first-degree relatives.

On gynecologic examination there were no findings. Preoperative computer tomography (CT) of the abdomen and pelvis, and abdominal ultrasound (U/S) revealed irregular endometrial thickening. Chest X-ray, intravenous pyelography (IVP), colonoscopy, and urethrocytoscopies were normal. Dilation and curettage revealed EC. Preoperative CA-125 was normal.

She underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphadenectomy. The histopathology revealed EC endometrioid type, grade 2 (Figure 1). The endometrial tumor invaded more than half of myometrium. The ovaries, pelvic and para-aortic lymph nodes were normal. The peritoneal washing smear was negative for malignant cells. Immunostaining revealed that tumor cells were positive for ErbB-2 receptors (Figure 2). The final diagnosis was Stage Ib EC endometrioid type according to the FIGO staging system 2009 [7].

The patient underwent postoperative adjuvant radiotherapy. She received 5,000 cGy of external pelvic radiotherapy. Moreover, she continues medication with hydroxychloroquine and corticosteroids in order to achieve control of RA.

Follow up 16 months after initial surgery, with CT of the chest, abdomen and pelvis, abdominal U/S, chest X-ray, IVP, colonoscopy, and urethrocytoscopies, revealed no evidence of recurrence.

Discussion

RA is a common autoimmune disease with dysregulated lymphocytes reacting against self-antigens by producing autoantibodies and the normal immune function is suppressed [8]. Dysregulation of the host’s immune surveillance is a recognized cause of human cancer [8,9]. The subsequent risk of cancer after diagnosis of RA has been studied extensively.

In patients with RA, there is increased risk for site-specific malignancies [5, 8, 10, 11]. The most common malignancies are: Hodgkin lymphoma (SIR 4.06), non-Hodgkin lym-
phoma (SIR 2.34), squamous cell skin cancer (SIR 1.89), lung cancer (SIR 1.73), non-thyroid endocrine glands cancer (SIR 1.62), kidney cancer (SIR 1.53), leukaemia (SIR 1.44), and prostate cancer (SIR 1.44) [5, 8, 10, 11].

The increased risks for lymphomas and squamous cell skin cancer are in accordance with the spectrum of cancers observed after immunosuppression [8, 10]. Moreover, EC is relative uncommon in patients with RA [5,8,11].

There is a concern whether RA or its treatment might increase the risk of cancer [5,6]. There are known associations between autoimmune diseases, chronic inflammatory diseases, and cancer [9, 12, 13]. Immunosuppression is a known risk factor for many cancers, as noted after organ transplantation and infection by immunodeficiency viruses [8]. Moreover, patients with RA are often subject to prolonged treatment with disease-modifying antirheumatic drugs (DMARDs) [5]. These drugs act by directly modifying the immunologic pathways involved in the pathogenesis of RA [5]. Perhaps, the immunosuppressive effect of DMARDs in patients with RA may predispose to the development of malignancies [5, 10].

Although EC is relative rare in patients with RA, the present patient developed EC three years after initial diagnosis of RA. During that period she received leflunomide, corticosteroids, and methotrexate in order to achieve control of RA. Perhaps immunosuppressive treatment for RA predisposed her to develop EC.

Surgery is the primary treatment for patients with EC [14]. For most of them, systematic surgical staging is the baseline therapy and allows clear decision for stage related postoperative adjuvant therapy [16]. Systematic surgical staging includes: total hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy, and complete resection of all disease [16, 17].

Appropriate surgical staging provides prognostic and therapeutic benefits for women with EC [14, 16, 17]. It facilitates targeted therapy to maximize survival and to minimize the effects of undertreatment (recurrent disease, increased mortality) and potential morbidity associated with overtreatment (radiation injury) [16, 17]. The present patient underwent systematic surgical staging according to current treatment protocols for EC [14-20].

In EC patients at increased risk for recurrence or with advanced stage disease, required more aggressive management with postoperative adjuvant radiotherapy and/or chemotherapy [14, 15, 17, 19]. Postoperative adjuvant radiotherapy includes external pelvic radiotherapy and/or brachytherapy.

External pelvic radiotherapy in EC patients with early stage disease, reduces the risk of local recurrences but has no impact on overall survival [16, 17, 21-23]. However, it is associated with significant morbidity and a reduction in quality of life [17, 21, 23]. It is used only in high-risk EC patients or at advanced stage disease [17, 24, 25]. The present patient underwent postoperative external pelvic radiotherapy [15, 18-20].

Vaginal brachytherapy in EC patients with early stage disease, also reduces the risk of local recurrences but has no impact on overall survival [17, 23]. Moreover, it is well tolerated and associated with less side effects than external pelvic radiotherapy [17, 23]. It is the adjuvant treatment of choice for high-intermediate risk EC patients [17,23,24].

Adjuvant chemotherapy is the mainstay of treatment for EC patients with locally advanced or metastatic disease [14, 15, 17, 26]. The most active chemotherapeutic agents are: taxanes, anthracyclines, and platinum compounds [26, 27]. Although they achieve high response rates, they have only
modest effect in progression-free survival and overall survival [17,26].

Molecular targeted therapies have still shown modest effect in unselected EC patients [26]. They usually target the inhibition of EGFR, VEGFR, and PI3K/PTEN/AKT/mTOR signal pathways [28]. Perhaps they may be clinically active as adjuvant therapy in well-defined subgroups of type II EC patients with EGFR and ErbB-2 overexpression [17, 29-32].

Prognostic factors for EC are: age at diagnosis, stage, grade, histologic type, ploidy, and receptor status [20, 33]. However, cancer patients with RA have dismal prognosis compared with patients without RA [34]. The high mortality rates are independent of age at diagnosis and stage of the disease [34]. It is obvious that those patients need a multidisciplinary treatment approach in order to achieve prolonged survival.

References


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The challenge in the fight against tumors began in ancient times and still continues today. As referred by the Author of this Book, history is generally written by the winner, however the counterpart, the tumor, unfortunately has not yet been conquered, even if numerous battles have been won while offering surprising results.

Since its debut medical treatment of tumors was utilized as adjuvant therapy for both surgical and radiotherapy and from several decades has become the primary treatment for many of them.

The history of medical treatment of tumors presented in this Book of elevated scientific value enables us to traverse all the phases of its progress. Each chapter discusses different aspects of chemotherapy, the successes obtained, and also the frustrations of the researchers when their expectations were disappointed.

The initial tumor observations, the dawn of new medical drugs, the milestones obtained, the formation of dedicated study groups with the aim to deepen the knowledge of each, to the application of mathematical models for the study of variants that lead to drug resistance, are clearly and carefully described. All of these aspects render this Book useful, not only to the experts, but also to those about to embark in oncological studies.

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Study and research are frequently characterized by an overwhelming anxiety to obtain results and there is no time dedicated to reflection. This textbook, however, invites us to stop and reflect on the state of the art of medical oncological therapy and subsequently to resume the challenge right from the very point where previous researchers have led us to, in order to continue with the battle against cancer, which still cannot be considered won.

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Foreword

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

This book, written by 32 international acknowledged experts, with rich and clear illustrations, offers an expert guide to all aspects of this neoplasia.

From the epidemiology, through risk, management in early and advanced stages, pediatric neoplasia, to the quality of life, the author explores all the possible aspects of this disease and all the implications that affect the outcome.

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

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

M. MARCHETTI

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

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

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

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

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

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

Contents


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

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


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

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

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


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


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

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

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

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