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Introduction

Radiotherapy is still widely and routinely used in the treatment of breast cancer, mainly as adjuvant treatment post lumpectomy or post mastectomy. Bourgier et al. [1] reviewing 75 articles found that 50 Gy in two Gy fractions plus a “booster dose” of 16 Gy in two Gy fractions “is still the standard of care”. For patients with special risk factors, they conceded that accelerated fractionation (AF) or accelerated partial breast irradiation (APBI) may be considered. To ensure optimal planning for whole breast radiotherapy of any kind (WBRT), the wound margins should be marked with clips by the surgeon. Kirby et al. [2] showed that five markers may be optimal: one deep, and four radial; computed tomography (CT) imaged, clip based delineation of the tumor bed was found adequate. A useful free downloadable program is called Adjuvant! Online that gives the oncologist and the patient information on what the likely benefits of adjuvant radio-chemotherapy may be for a given patient. For a busy oncologist and an inquisitive patient, this is a convenient tool.

Accelerated partial breast irradiation (APBI): the new gold standard for early breast cancer?

Azria and Bourgier [3] suggest that partial breast irradiation may be the new standard for selected patients conforming to the ASTRO and GEC-ESTRO guidelines; the guidelines are readily available on the Internet. Since APBI is of fairly recent use, data younger than five years were preferred.

Brief historical perspective: radiotherapy is an integral part in the evolution of breast conservation

The trend towards greater conservatism of breast cancer treatment using radiotherapy as adjunct to surgery began with Prof. Robert McWhirter [4], a Scottish and Forbes professor of Medical Radiology (Edinburgh University). He obtained diplomas in both surgery and radiology (under the famed radiotherapist Ralston Paterson) and pioneered the establishment of good radiology and radiotherapy departments. He proved in the 1950’s to the skeptically aggressive surgeons at the time, that simple mastectomy followed by well applied radiotherapy gave results equal to that of radical mastectomy.

Umberto Veronesi et.al. [5] pioneered breast conservation therapy, eliminating the need for mastectomy in many women with early stage breast cancer. They showed that quadrantectomy plus axillary dissection was equivalent to mastectomy plus axillary dissection, provided that quadrantectomy was supplemented by a full course of radiotherapy.

Bernard Fisher (USA) [6] pioneered the more conservative “lumpectomy” for noninvasive and invasive early stage breast cancers. Project 13-06 of the NSABP compared modified radical mastectomy vs lumpectomy alone to lumpectomy plus radiotherapy for Stage I-II breast cancers. They concluded in the 1990’s that “all the evidence continues to justify the use of lumpectomy plus radiotherapy for the treatment of invasive breast cancer”.

In 1998 {study B-17} Fisher et al. [7] showed that the above held true for intra-ductal carcinoma. Lumpectomy plus radiotherapy reduced the incidence of ‘in breast tumor recurrence’ (IBT) from 13.4% to 8.15% for non-invasive cancers \(p = 0.007\) and from 13.4% to 3.9% for invasive cancers \(p < 0.0001\) after eight years of follow-up.

Is a six week course of radiotherapy always necessary? Veronesi et al. [8] introduced Intra-operative radiotherapy (IORT) for breast cancer with an electron beam using a newly developed mobile linear accelerator that could be pushed into the theater. They used a single dose of 21 Gy to the tumor bed instead of using a five-week course of radiotherapy. The principle of single dose APBI was born and radiobiologically makes sense only for microscopic residual disease.

Historically then, radiotherapy has been the central player in the quest for ever greater breast conservative therapy and for palliation of brain and bone metastases.
Is radiotherapy really effective?

Standard tangential fields: specific studies by Fletcher [9,10] determined that the radiation dose needed to eradicate subclinical deposits of cancer cells 90%-95% of the time is about 45 Gy to 50 Gy; larger doses are needed for clinically obvious tumors. This is so for standard tele-therapy with Cobalt units or linear accelerators. For the new trend to administer intra-operative adjuvant radiotherapy to eradicate microscopic residual disease for early cancers, electrons (from linear accelerators), brachy-therapy units, either radioactive isotopes or 50 kV X-ray units, were developed.

Dose distribution issues with the new spherical applicators.

Table 1 shows the non-homogeneous dose distribution around spherical applicators with a central point source—the dose ranges from “overkill” at the surface of the applicators to “barely sufficient” at 20 mm away (Table 1). This is a matter of concern for radiation oncologists. However, new studies show that the radiation dose is probably adequate up to about 23 mm from the applicator surface for balloon type applicators with an Ir\(^{192}\) point source. Herskind et al. [11] proposes the concept of a “sphere of equivalence” around spherical applicators; i.e. a dose equivalent in to that of a dose of 60 Gy in two Gy fractions up to about 25 mm from the applicator surface. They argue inter alia that there is evidence that immediate post-op irradiation is more effective because it arrests cell growth that would otherwise result from the growth-stimulating ‘soup’ of factors released by the surgery. Delayed radiotherapy loses this advantage.

For a 40 mm diameter ball type applicator, the effective dose 20 mm from the surface is 30.0 Gy (EQ2) which is still enough to reduce the number of viable cells to about one surviving mammary cell out of 1,000 [12]. For micro-tumors of four\(^3\) mm, the dose required to kill 50% of the tumors is 45.75 Gy [13].

It is interesting to note that for a 40-mm diameter sphere and the dose prescribed at ten mm the volume effectively irradiated is about 42 cm\(^3\) or ten times the volume of a two-cm diameter tumor which is three time more than tissue (12 cm\(^3\)) removed by the surgeon for a two-cm diameter tumor (volume about four cm\(^3\)). More information about volumes and dose distributions with respect to a major clinical trial is discussed by Smit [14].

Radiation delivery systems used and the associated clinical results.

A. Conventional linear accelerators.

These have developed enormously over the last decade as will become clear. They offer a choice of photon energies (usually two) and several electron energies. Electrons have the very useful quality that the depth of penetration of the radiation energy is dependent on the energy. For instance a six MeV beam will penetrate approximately 20 mm (about 1/3 of the nominal energy in centimeters) while the tissues underneath are spared; photons are far more penetrating. The new accelerators have built in position checking ability, multi-leaf collimators etc.

Linear accelerator based radiotherapy is used in several forms: whole breast radiotherapy (WBRT) which can be conventionally fractionated over 33 days or hypo-fractionated over 16 days. Obviously this is also accelerated fractionation (AF).

Results of WBRT: 50 Gy in 25 fractions +/- a booster dose of ten to 16 Gy in two Gy fractions Is the universally accepted standard therapy as stated above. This approach gives good results with the local recurrence rate at ten years about six percent, Bartelink et al. [15]. For early breast cancers, this figure may be as low as 2.5%.

Hypo-fractionated WBRT: a large study of this abbreviated type of conventional radiotherapy comes from Ontario, Canada - (The Ontario Clinical Oncology Trial), Ashworth et al. [16], who retrospectively analyzed data for > 41,000 post-lumpectomy patients who received either 16 or 25 fractions; the results were equivalent and resulted in about 70% of the patients surveyed being so treated. In an excellent review, Yarnold et al. [17] concluded that the results of recent randomized trials justify the routine use of “modest hypo fractionation” for adjuvant whole breast irradiation. Regimens used include the United Kingdom schedule of 40 Gy in 15 fractions (EQ2 dose,\(\alpha/\beta\) ratio of 4.0) is 44.4 Gy. The authors prefer 42.5 Gy in 16
fractions (EQ2 = 47.14 Gy). Whelan et al. [18] compared 13-16 fractions (3.2 Gy or 2.65 Gy) given to 622 patients treated by hypo-fractionation to 612 patients treated by the standard EBRT regimen, the results were the ‘the same’.

From the current information, one could conclude that a hypo-fractionated regimen is acceptable for the routine treatment of patients that would normally receive 50 Gy in two Gy fractions. Most centers use a central “boost of ten to 16 Gy in two Gy fractions; Hypo-fractionated boosts would add another three days to the regimen if used.

**Accelerated partial breast irradiation (APBI) by means of a linear accelerator:**

Multileaf collimators and intensity modulation of photons made conformal therapy of post-lumpectomy sites- not cavities- practical. Conformal therapy has the advantage that the wound can be closed and no further interventions/operations are needed.

Bondiau et al. [19] showed during a dose escalation study (highest 25 Gy in three fractions; EQ2 = 51.3 Gy) that for patients who do not qualify for breast conserving therapy, neo-adjuvant chemotherapy followed by highly conformal stereotactic radiotherapy followed by surgery could yield pathology based complete responses (pCR) of 36% with the ability to achieve a 92% breast conservation rate in this cohort. This did not result in any complications relating to the surgery (lumpectomy eight weeks after the last chemotherapy dose). One case of serious skin toxicity occurred due to the radiotherapy. The authors find that a phase II trial will be justified. This is an interesting approach and could in principle extend the number of patients that could avoid mastectomy. This approach should preferably be within the context of a clinical trial.

**Special, dedicated mobile electron accelerators were developed:**

Examples are the “Mobetron” and the Novac -7. These systems are expensive. Veronesi et al. [8] first used intra-operative electron therapy (IORT), where the electron applicator is placed in virtual contact with the flat, opened up, wound surface. The dose of 21 Gy is given in one single session (EQ2 = 80 Gy; α/β=4) A single dose of 18 Gy would be equivalent to 66 Gy. Results after quadrantectomy were reported by Veronesi et al. [20] on 1,822 patients so treated (January 2000 to December 2008). The tumors were invasive carcinomas < 2.5 cm in diameter treated by quadrantectomy followed by intra-operative electron therapy (ELIOT). The results were as follows: the mean follow-up period was 36.1 months, 42 women (2.3%) developed local recurrence, 24 (1.3%) developed a new primary in the ipsilateral breast, 26 (1.4%) developed distant metastases; 46 (2.5%) died of carcinoma, the other of other causes. The five-year survival rate was 97.4% and the ten- year survival rate was 89.7%. Complications: fat necrosis, was observed in 4.2% of the patients but usually caused no problems, and 1.8% had significant fibrosis. They concluded that the results appeared promising.

Leonardi et al. [21] reported the results of an electron (ELIOT) APBI trial in 2013. The guidelines of the European Society for Therapeutic Radiology and Oncology (“GEC-ESTRO”) were used to stratify the patients with early breast cancer. The same 1,822 patients were analyzed, and of these, 573 candidates fell into the “good candidates group. The five-year in breast recurrences for this group was 1.9% and in the two less favorable groups, 7.4% and 7.7%, respectively. The GEC ASTRO guidelines separated the low risk group from the two higher risk groups, but not the latter from each other. (Compare the above results to the in- breast recurrence rate for conventional whole breast irradiation of about six percent for early stage tumors).

Leonardi et al. [22], using the same 1,822 patients following the American Society for Therapeutic Radiation Oncology (ASTRO) guidelines which identifies three risk groups, although slightly different to the European guidelines. They concluded that the ASTRO guidelines differentiated better than the GEC ESTRO guidelines between the risk groups.

Fibrosis: Rampinelli et al. [23] compared APBI (ELIOT) and whole external breast radiotherapy (EBRT) with reference to the incidence and severity of fibrotic changes induced in the lungs; 178 patients were, prospectively studied. The dose used was 21 Gy as a single dose prescribed at the 90% isodose vs a 50 Gy dose EBRT plus a ten Gy boost in two Gy fractions. Pulmonary fibrosis was assessed in 83 in the EBRT arm and 96 in the ELIOT arm. All patients had infiltrating carcinoma with lesions < 2.5 cm in diameter. Of 42 patients who had developed pulmonary fibrosis, 38 or 90% were in the ELIOT arm and four (about 10%) in the ELIOT arm (p < 0.0001). Of these, 26 were grade 1 (one in ELIOT), 15 were grade 2 (three in ELIOT), and only one was grade 3.

**Safety of the mobile linear accelerators:** Giocca et al. [24] found that the mobile linear accelerators were safe for use in the operating room, i.e. did not need dedicated shielded rooms- a major advantage (also shared by the 50kV X ray units- see later). The safety level depended to some extent on patient load. No extra shielding was required.

**Robotic linear accelerators like the “cyberknife” for body stereotactic radiotherapy** (Body SRT): a very high dose of radiation can be delivered to a very small volume of tissue for example lesions in the brain lung or liver. Very high doses confined to very small volumes of tissue (< ten cm³) causes relatively little damage to surrounding structures. There are not many of these in use; modern linear accelerators can do much the same.

**Proton therapy:** These units are physically huge very costly and cumbersome; apart from that they can do what modern linear accelerators can do, in some cases with more sparing of normal tissues.
B. Lumpectomy cavity irradiators:
An excellent review of the available APBI techniques is offered by Njeh et al. [25]. It gives excellent pictorial details of the hardware available and also the clinical results up to 2010. A book on the subject of APBI was recently published [26].

C. Spherical applicators with radionuclide point sources.
Inflatable balloon systems using a central catheter with a central Ir $^{192}$ point source include the Mammosite balloon which is inserted into the lumpectomy cavity, the wound is closed around the catheter and the total dose is delivered in ten fractions over five days. Thereafter the balloon is deflated and removed with relatively little discomfort.

Clinical results: This scheme demands that at least six hours must elapse to allow repair of sub-lethal radiation damage. This is a nuisance for department and patient alike.

Some institutions compared three different methods, like the William Beaumont Hospital group. A recent report on 2,127 patients so treated used three types of APBI: interstitial, balloon type and 3D conformal radiotherapy. The median age of the patients was 65 (32-94) years, median tumor size ten mm (0-45mm) and median follow-up 60.6 months. Intra-breast tumor recurrence (IBTR) was observed in 2.8%, regional node failure in 0.6% and metastatic disease in 1.6% of the entire cohort. The IBTR was not significantly different between the ASTRO and CS ESTRO guideline groups (suitable’ ‘cautionary’ or ‘unsuitable’) These results are impressive even if the guidelines failed to separate the population into categories [27]. A similar trial is ongoing- The NSABP 39/RTOG 0413 that will compare ‘standard radiotherapy” 60 Gy in 30 fractions, Iridium type balloon MammoSite” and 3-D conformal radiotherapy. The trial aims to include 4,700 patients.

Inflatable balloon devices using multiple catheter arrangements for improved dosimetry.
Examples are the Strut Adjusted Volume Implant “SAVI” and ClearPath and the Mammosite Multi. Some data are available in this respect. A study by Sato et al. [28] reported on 184 patients so treated in Japan. A total of 120 patients with pN0 tumors and mean age 55 years who had at least one year’s follow-up were reported on. APBI was initiated on the same day as the surgery, and eight fractions of four Gy each were given over five to six days with a 2- mm margin coverage. The ten-year risk of breast tumor recurrence (IBTR) was calculated using a web-based tool IBTR! The median follow-up period was 3.1 years (1.1 -4.4 years range); 96% of tumors were less than two cm in diameter and 89.4% were ER+.

Hormone treatment was used in 86% of the patients and adjuvant chemotherapy in 20% of the patients. They estimated the ten-year risk of breast tumor recurrence (IBTR) was calculated using a web-based tool IBTR! The median follow-up period was 3.1 years (1.1 -4.4 years range); 96% of tumors were less than two cm in diameter and 89.4% were ER+. Hormone treatment was used in 86% of the patients and adjuvant chemotherapy in 20% of the patients. They estimated that if all the patients in this group would have received WBRT, the IBTR would be 1.1 to 2.8; for the study patients only one IBTR was observed (< 1%) and none occurred in the tumor bed. The authors concluded that multi-catheter APBI would give results equal to WBRT.

Spherical applicators with miniature X ray with 50 kV “point” sources of radiation.
The radiation source for these devices is a miniature X ray machine (easily shielded) and not a radionuclide like Ir192. The 50 kV sources are mobile and easily shielded, and can be pushed into the theater; they should be considerably cheaper than mobile linear accelerators.

Multicatheter types: An example is Axxent. The dose can be fractionated over days via multiple catheters that enables more satisfactory dose distributions yet retaining the benefit of the balloon type of device.

Rigid hollow ball types like “Intrabeam” uses hollow ball type applicators. The total dose must be delivered in a single session. Grobmyeyer et al. [29] reported their results in 78 patients so treated. The relative biological effectiveness (RBE) of 50 kV X rays is = one at the surface and two at 20 mm so that the nominal dose must be multiplied by this factor. An applicator of suitable size is inserted into the lumpectomy cavity and the tumor bed is then irradiated. The total operative time including the lumpectomy, sentinel lymph node dissection, and the Intrabeam (IB) treatment ranged from 79 minutes to 232 minutes with a mean time of 132 minutes. At 12 months follow-up, the cosmetic results were reported to be good to excellent in 92% of the patients and no local recurrences were seen in the follow up period (November 2010 to October 2012). The costs calculated for the Intrabeam device came to USD 1,857.00, which is far less than a course of conventional tele-therapy at USD 9,658.00. They concluded that the safety, ease of use, and reduced costs argues for more widespread use of the method. Experience is limited at this stage; more data must be obtained.

There was lively correspondence after the publication by Vaidya et al. [30] of their results of the “‘TARGIT” (Intrabeam) trial. Reitsamer et al. [31] at the time felt that the follow-up is much too short to be overconfident. Smith et al. [32] felt that the doses were inadequate and just delayed recurrences. Haviland et al. [33] warn that recurrence may occur many years later and that conclusions are immature. Cameron et al. [34] disagrees that the TARGIT trial data give “robust and mature” evidence. They concur with the ASTRO consensus statement that on PBI “That women should be informed about the much longer track record of safety and efficacy of post-operative whole breast irradiation”. They like wise would like
to see mature outcome data from TARGIT A before it can be regarded as safe. The rebuttal by Vaydia et al. [30] however, has good arguments to support their conclusions, especially that older women 60 years and older will be spared the rigors of a protracted course of radiotherapy, yet will probably enjoy the full benefit of WBRT by availing themselves of the intra-operative APBI technique.

**Interstitial volume implants.** These are still being done, but it implies a second surgical procedure after a lumpectomy or a quadrantectomy. Guide tubes are placed and later filled with Ir192 wires, or by LDR (low dose rate) or HDR after-loading systems. The patients need to be isolated for either the manual or LDR afterloading.

### Complications

Many articles addressed the problem of cardio-toxicity due to radiotherapy. Nowadays the anterior descending coronary artery (ADCA) should routinely be identified and specifically excluded from radiation. Mediastinal irradiation may induce cardiomyopathy, damage to valves, pericarditis, etc. Multidisciplinary teams are advised when the mediastinum needs to be irradiated.

### Developments that may impact on the future use of radiotherapy

ER, PR and Her 2 receptors are integrated with tumor grade to define new classes of breast cancer with different prognoses. These groups or classes may require different therapeutic approaches. The **luminal** cells are the cells lining the breast ducts. Examples are:

- **Luminal A**, (ER+ and low grade, 36% of tumors - for hormonal Rx alone?);
- **Luminal B**, (ER+ and high grade, Subtype 1: HER2 - 26% of tumors), Subtype 2: ER+ and high grade, HER 2 +; 19% of tumors);
- **ERBB2/HER2+** (non-luminal) with amplified HER2/neu (19% of tumors);
- Normal breast-like tumors;
- **Basal like ER-, PR-**, HER2- or triple negative breast tumors (TNBT). **most BCRI tumors are triple negative** (12.9% of tumors);
- **Luminal ER-/AR+; androgen responsive subtype** that may respond to the anti-androgen bicalutamide (androgen receptor: AR) after failure to respond to tamoxifen/aromatase inhibitors and
- **Claudin – low**, frequently triple negative, lacking E-cadherin expression, often with lymphocyte infiltrate.

Yanagawa et al. [35] evaluated 363 tumors and found the percentage distribution as given; above and it seems clear that many more factors may have to be taken into account when selecting patients for treatment; some may not need radiotherapy, others may well need a redefined hormonal treatment or a combination of hormonal (including androgens), chemotherapy, and radio-therapeutic approaches. These findings may signify the need for a whole host of new clinical trials.

**Proteomics:** Somiari et al. [36] pointed out that proteomics may offer bedside diagnostic tests to determine the presence of malignant breast tissue- a possible paradigm shift in early diagnoses and it obviously may have a major impact on diagnosing recurrent tumor. All these developments will eventually impact further how radiotherapy is going to be used in the future; for the moment it remains a powerful therapeutic modality for breast cancer. Imaging techniques to detect viable tumor cells may come in very handy, like positron emission tomography/CT scans and functional magnetic resonance imaging.

### References

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Introduction

Uterine sarcomas are a rare gynecologic malignancy. Recently, some authors have reported some increase in their incidence [1]. FIGO has also published a revised staging in 2009. Some authors reported a good prognosis for some tumors, even when a fertility-sparing approach was used for young women in some cases. The authors analyzed all cases that occurred in the present centre and reviewed the literature available to date. The challenging management of mesenchymal cancer of corpus uteri (CU) requires experienced equipes and multidisciplinary approach. A pivotal role is played by the pathologist, which should have a specific experience for mesenchymal diseases to make a correct diagnosis.

Epidemiology and risk factors

The incidence per year in 1995 among American women was 17 per million, accounting for two to five percent among all uterine malignancies [2].

Investigating the Surveillance, Epidemiology, and End Results (SEER) database for women with invasive uterine neoplasm diagnosed in the period 1988-2004, 76,953 women were stratified by histology into endometrioid, sarcoma and clear cell type. Sarcomas rates were nine percent in whites and 26% in blacks [3].

In 1989-1999, 2,677 women underwent a uterine sarcoma diagnosis. White women were significantly older at the time of diagnosis compared to blacks (64.2 vs 62.7, \( p < 0.05 \)). The age-adjusted incidence rate of uterine sarcoma in black women was nearly twofold greater than that of white women (7.0/105 vs 3.6/105, \( p < 0.05 \)) [4].

Tamoxifen adjuvant therapy has been associated to an increased risk of sarcomas. Role of tamoxifen as an agent promoting the development of endometrial carcinomas is well-established, based on evidences from different studies. According to literature, women with long-term use of tamoxifen are more likely than non-users to develop a uterine sarcoma, with sarcomas making up more than ten percent of all uterine malignancies in this group of patients [5].

One study evaluated the association between tamoxifen use and the risk of developing uterine sarcomas and endometrial carcinomas in a cohort of Israeli women diagnosed with breast cancer from 1987-1988. There were four uterine sarcomas among the tamoxifen users but none among non-users [6]. Risk of corpus uteri (CU) cancer after breast cancer was 2.5 (CI: 1.1-4.7) for adenocarcinomas and 29 (CI: 3.5-104.9) for malignant mixed Mullerian tumor (MMMT) in women treated with tamoxifen. The strongly increased risk for developing MMMTs among these women does make close surveillance mandatory [7].

In conclusion, from a population-based evidence, use of tamoxifen appears to be associated with an overall fourfold relative risk for MMMTs, which increased to eightfold among long-term breast cancer survivors, compared with the twofold risk for endometrial adenocarcinomas [8].

A history of breast cancer can be found in many patients with sarcomas. Among 52,109 women diagnosed with CU cancer, 1922 had a history of breast cancer in a large, multicenter study [9].

The proportional incidence of uterine papillary serous cancer (UPSC) and sarcoma was significantly higher in women with a breast cancer history. These findings highlight the association of breast cancer with high-risk corpus cancer subtypes.

Pelvic radiation is found in the natural history of 10%-25% of women with a sarcoma of the uterus [10]. Relative risk after radiation is estimated to be 5.38 with an interval of usually 10 to 20 years [11].
Classification

According to World Health Organization (WHO) classification, uterine sarcomas are divided into non-epithelial and epithelial non-epithelial tumors. Non-epithelial tumors could be homologous or heterologous whether they express tissues that are native to the uterus or not. Homologous tumors are endometrial stromal sarcoma (ESS) (low-grade-LG and high-grade-HG) and such smooth muscle tumors as leiomyosarcoma (LMS) and leiomyoma (LM) variants and benign metastasizing smooth muscle cell (SMC) tumors. Heterologous tumors show extra-uterine tissues in their context, which could be bone, cartilage or striated muscle. Epithelial/non-epithelial tumors include mixed Mullerian tumors (MMT) (homologous also known as carcinosarcomas (CS) and heterologous) and adenosarcoma.

Current opinion is that tumors with epithelial composition-like carcinosarcomas should not be included among sarcomas but should rather be considered as carcinomas [12]. CS is better thought as an aggressive metaplastic adenocarcinoma of the endometrium. Evidences supporting this view refer to their behavior resembling a carcinoma rather than a sarcoma. They show a lymphatic spread rather than diffusion via blood stream (typical of sarcomas). Furthermore they respond to chemotherapy agents that are effective in treating adenocarcinomas (paclitaxel, cisplatin, carboplatin) but are not active on sarcomas. The sarcomatous component is thought to be secondary to a dedifferentiation suggesting a common clonal endometrial origin of both carcinomatous and sarcomatous elements. Although CS cases were included in this study, WHO still classifies them as sarcomas.

Staging

FIGO 2009 revised staging for uterine cancer. CU cancer includes MMT. Uterine sarcomas have two different sub-stagings for LMS/ESS and adenosarcomas (Figure 1).

LMS

LMS shows cells with a smooth muscle differentiation. It accounts for the 25%-36% of uterine sarcomas and one percent of all uterine malignancies [13]. Smooth muscle tumors of uncertain malignant potential (STUMP) are considered intermediate between benign leiomyomata and LMS. They show an unpredictable behavior, from highly lethal to relapse at 25 years.

Pathologic and biologic features

To define a LMS on its morphological ground, criteria adopted should include the number of mitosis per field (that was the main previous criteria), but even coagulative tumor cells necrosis and cytological atypia need to be included. The previous criteria were raising confusion regarding the histology of this tumor, since the peculiarity of the disease is a varying clinical presentation. It could show a very aggressive outcome in terms of spread and aspect, whether it underlies a benign pathology and falsely subtlety in malignancies.

Necrosis, atypia, and mitotic index define the Broders four-level system, which is a prognostic model for LMS. Level 1 is considered LG; 2, 3, and 4 as HG [14]. Grade 1 tumors show diffuse, mild cytological atypia, abundant eosinophilic cytoplasm, and a fascicular growth pattern. Grade 2 tumors possess more nuclear irregularity with a greater degree of nuclear variation in size and shape. Grade 3 and 4 tumors demonstrate moderate to marked nuclear irregularity.

Typical LMS is positive for actin, desmin, and caldesmon but even for such epithelial markers as CAM 5.2 and AE1/AE2. c-Kit proto-oncogene expression was found in all histological types of uterine sarcomas in one series published in 2004 and it was suggested that further investigation on c-kit tyrosine kinase inhibitor as imatinib mesylate role in these mesenchymal tumors should be done [15]. c-Kit is also mandatory to differentiate LMS from gastrointestinal stromal tumors (GIST). Estrogen receptor (ER) and progesterone receptor (PR) expression were reported in 57% of LMS [16] and in 57% [17] and 43% of cases [18]; microarray tissue analysis, showed immunoreexpression of ER and PR in 40% and 48% of LMS and in 78% and 88% of leiomyomata [19, 20]. Many studies agree in reporting an increased expression of MIB-1 (Ki-67) [21, 22], overexpression of p53 (16; 23; 24) and loss of PR expression [16]. MIB-1, p53 and steroid receptors can be useful in differentiate LMS from cellular leiomyomas and STUMP [25].

LMS typically have complex cytogenetic abnormalities. Karyotypes show both numerical and structural aberrations. These aberrations are often unstable, resulting in significant variation from metaphase to metaphase. Quade et al. examined archival materials from 16 LMS and 13 benign LMS by polymerase chain reaction (PCR) for 26 microsatellite polymorphisms. Interestingly, eight of 14 (57.2%) informative LMS had loss of heterozygosity (LOH) for at least one marker on chromosome 10 and involved both chromosomal arms in 45.5% (5 of 11). In contrast to LMSs, LOH for chromosome 10 was not found in 13 benign LMSs. Missatellite instability was found infrequently in LMSs and not detected in LM. Clinico-pathological features (e.g.: atypia, necrosis and clinical outcome) did not appear to correlate with LOH for chromosome 10. In contrast to other chromosomes studied, LOH on chromosome 10 was frequent in LMS and absent in benign LM [26].

LMS arises de novo. Many studies confirmed that LM would not turn malignant. Despite this support, rapidly growing myomas are quite common indications for such surgeries as myomectomy or hysterectomy. Still, the incidence of sarcomas in a series of hysterectomy performed for LM was very low (0.5%) [27].
In contrast, a useful tool, as hierarchical cluster analysis, applied in the study by Hodge and Morton [28], raised the fascinating idea that LMS do indeed derive from LM, and that the discrepancy in their frequency lies in the fact that only rare histologic and karyotypic variants of LM are amenable of malignant progression. Mittal and Joutovsky (GO 2007) [29] investigated LM subsets, suggesting that the ability to progress into LMS could be more likely with cellular and symplastic LM.

Macrosopically, a LMS shows as a single mass, soft, fleshy, yellow-brownish in the context of a uterus that could often present as fibromatous (Viereck et al. 2002) [30]. Usually, the malignant mass is rather a single lesion than multiple, other lesions being benign fibroids. Some studies have focused on the diverse histological patterns that these tumors could display. They could show, in fact, either as such a “classic” form [31, 32] or LMS variants. Variants include epithelioid, differentiated, myxoid, intravenous, osteoclast-like giant cells in SMT, LMS with a clear cell component, and LMS with liposarcomatous differentiation [33].

Clinical presentation

The clinical presentation of LMS resembles that of fibroids. In one series, the most common presentation symptom was abnormal uterine bleeding (AUB) in the 45%-86% of cases [34]. Pelvic pain was found to be relevant in 20% to 50% of cases in another series [35]. Some authors recommend regularly checking the largest myoma in a polimyomatous uterus [36]. In 95% of cases LMS appeared, in fact, to be the largest or the unique mass of the uterus. Imaging techniques for LMS seem not to discriminate from benign to malignant lesions at the state of the art. A prospective study among 298 women, who had a uterine smooth muscle tumor (SMT) diagnosis during the decade 1990-2000, evaluated the role of combining dynamic magnetic resonance imaging (MRI) with serum markers, as lactate dehydrogenase (LDH) isoenzymes. The authors reported 100% specificity, 100% positive predictive value, 100% negative predictive value, and 100% diagnostic accuracy for LMS. This study shows the feasibility of a preoperative diagnosis for LMS. One of the latest techniques for fibroids care is MRI-guided ultrasound (US), which does not allow obtaining samples of the tissue lesion after treatment. That is one additional reason to promote the search for a preoperative diagnosis of the lesion.

Treatment and prognostic features

LMS are confined to the uterus in the majority of cases. Ovarian and lymphatic spread is uncommon in patients without extra-uterine disease [14]. Thus, oophorectomy and lymph node resection in patients with disease limited to the uterus should not be a standard procedure. In one series only five out of 101 women with uterine LMS had lymph node involvement and ovarian metastases were present in four out of 108 [37]. In pre-menopausal patients with LG of LMS the ovaries can be preserved [38].

Another series of 208 women showed lymph node metastasis in four out of 36 patients who had a lymph node biopsy [39]. When LMS is localized out of the uterus, it usually extends to the pelvic cavity.

Lymph node invasion does not predict whether or not there are or there will be distant metastases. The tumor may spread via blood stream and show a negative lymph node sampling.

Most common localization for metastases is lung, followed by liver, kidney, brain, and bone [40].

Thyroid is considered an uncommon site of metastasis; the most recent report to date cites only three previous cases [41].

The reported five-year survival rates range from 4% to 74% [42, 43] for all Stages together and as high as 81% for Stage I disease. This wide variation relates to the use of small samples, failure to use standard pathologic criteria, lack of a standardized staging, various proportion of low- and high-stage patients in different series, and long periods accumulating patients with different treatment approaches during these intervals. A retrospective study of 1,396 patients (1988-2003) found a five-year survival rate of 65.7%, similar to the rates found in the most recent series published [44]. Disease stage is a strong prognostic factor in nearly all multivariate analyses, with better survival rates for Stages I and II [39, 43, 45-47]. Patient age has been identified as a strong, independent prognostic factor in favor of younger patients attaining a better prognosis. Earlier studies indicated that premenopausal patients had better outcomes than postmenopausal patients. However, more recent analyses have not identified an independent prognostic benefit associated with menopausal status when patient age is taken into account. Tumor grade is considered as a prognostic factor in several studies, but other series do not consider it as influent on the outcome. Probably, it reflects the lack of using unique grading criteria in earlier studies. Race is also an independent prognostic factor. Various series showed a higher incidence in Afro-American women [48, 49]. Brooks et al. in 2004 [4] reported a threefold higher risk among black women. Silverberg et al. (1971) [50] in a series of 34 patients reported that 11 out of 21 Afro-American patients died from the disease while only one out of nine white women deceased. The importance of primary surgical management of LMS were confirmed in multivariate analyses [44] Sagae et al. also reported that the presence of known residual disease after initial surgery was associated significantly with the risk of recurrence or death [45]. In a series of 46 patients with uterine sarcomas that included 14 patients with LMS, Marchese et al. noted that complete surgical resection was essential for long-term survival surgery for LMS [51].

The role of adjuvant pelvic RT has become intriguing. In a case-control study of 31 cases and 31 controls performed
at Mayo Clinic, there was no statistically significant improved survival between cases and controls, but it significantly reduced the rate of pelvic recurrence [39].

Yoney et al. (2008) [52] in a retrospective analysis of 105 patients favor a treatment that includes radical surgery and adjuvant RT alone at 54 Gray or with chemotherapy. Adjuvant CTX would be mandatory if we consider that LMS have a very high-rate of early metastases, still there are no proven benefits in literature.

Endometrial stromal sarcoma (ESS)

ESSs are most commonly seen in pre-menopausal women, but age at presentation may range from 20 to 80 year. It accounts for 0.2% of all uterine malignancies, 15% of uterine sarcomas, with a prevalence of 0.19/100000 women (> 20 years) [3, 4]. Median age at diagnosis is 47 years. In the series by Brooks, race disparity was not really significant as in LMS (a incidence rate of 0.623 in whites and 0.583 in blacks) [4].

Pathologic and biologic features

ESS may possibly arise from uterine stroma, adenomyosis or endometriosis. It resembles cells from the endometrial stroma during the proliferative phase of the menstrual cycle, showing small round or elongated, often hyperchromatic cells exhibiting varying degrees of atypia. Immunohistochemistry shows reactions for vimentin, inhibin, CD99 (MIC2) [53], and keratins [54]. The most reliable tool to distinguish ESS from SMT is CD10 [55] in contrast to the h-caldesmon, CD44 positivity, and widespread actin positivity in smooth muscle lesions. ER and PR positivity reflects the response to progestagens typical of normal stromal cells, identifying LG lesions [56-60].

ESSs are divided into three entities: stromal nodule, LGESS and the undifferentiated ESS, formerly known as HGEES (WHO). An infiltrating margin and vascular space invasion separates ESS from benign stromal nodule [61]. The mitotic rate alone cannot indicate whether a ESS is HG or LG and therefore has no chance to suggest a poor outcome or a more aggressive behavior [62-64]. Some years ago ESSs included diverse entities, LG, HG, stromatosis, and endolymphatic stromal myosis (SESM). The LGESS includes nowadays many tumors that would have been considered once as HG. LG is, nowadays, a tumor that shows morphologic aspects of endometrial stroma, while the HG or undifferentiated are anaplastic tumors without endometrial differentiation.

Grossly, ESS resembles pale yellow rubbery growths extending through the myometrium into lymphatic and venous channels. Therefore, evaluating an hysterectomy specimen, close attention should be given to vessels to the broad ligaments and adnexa [65].

Treatment and prognostic features.

The surgical approach for ESS consists of primary surgery and surgery for restaging or recurrences. Another option is fertility-sparing surgery in younger women that expressed the wish for an offspring. Primary surgery consists of a total hysterectomy, bilateral salpingo-oophorectomy (BSO), and lymphadenectomy (LND). Surgical options may be different, considering the stage and the grade. The surgical plans for LGESS confined to the uterus, Stages I-II, should include BSO, but literature shows different results in affecting survival. Li et al. [66] argue that progestins have no defined value in adjuvant settings and no in vitro studies confirmed the hormonal induced proliferation in LGESS. Furthermore, there is a wide variation in recurrences if the ovaries are retained (0%-100%), and these series include all stages, all ages, and HG [67-70]. If we consider the data extrapolated from other hormonal responsive gynecologic cancers, BSO is very unlikely to affect survival [71]. Nevertheless there is some authors [70, 72] that still recommend performing BSO. Progestins (GnRH analogs, aromatase inhibitors) cause regression/stabilization of recurrent LGESS [73] and the expression of ER/PR in LGESS suggests hormonal responsiveness [74]. In the multicenter case-control study 1976-2002 by Li et al. [66] there were no differences in the pattern of recurrence among patients where BSO was not performed, and eventually no disease recurred in the ovaries. The only independent risk factor was an older age at diagnosis. Immunohistochemistry was positive for ER and PR in all cases. All recurrences were ER and PR positive. Gadducci et al. [75] considered 12 patients younger than 50 years with LGESS Stage I who underwent total abdominal hysterectomy (TAH). The rates of recurrent disease were 33.3% with BSO and 16.7% without BSO. Amant et al. [76] included 18 premenopausal patients Stage I-II LGESS with TAH in their study. Their rates of recurrence were 25% with BSO and 17% without BSO; these results overlap. While BSO should always be performed in primary surgery for HGESS, it could be discussed for LG. Young women could retain their ovaries when a diagnosis of LGESS is made as an incidental finding on a hysterectomy for a benign indication. The decision to perform a BSO should then be taken on an individual basis and discussed with the patient.

ESS was first designed as endolymphatic stromal myosis (ESM), underlining its strong tendency to invade lymphatic tissue. The benefit for LND is first in a more accurate staging; Reich et al. [77] recommend to perform it while Ripsel et al. [78] found 33% (5/15) of nodal metastases at some point in ESS evolution. On the other hand, Chang et al. [68], Gadducci et al. [75], Amant et al. [76] argue as ESS has tendency to recur at different sites. Amant et al. [76] found only three percent (1/31) of retroperitoneal recurrences. In the series by Li et al. [79] 2/3 ESS with node negative after radical hysterectomy had distant metastases at 12/39 months.
Considering the multi-institutional review 1972-2004 by Leath III et al. [80], 72 patients with LG and 31 with HG, LND was performed in 16 patients at all stages. The rates of positive pelvic nodes were nine percent in LG, and 18% in HG (p = 0.44). Aortic nodes were involved in 0% of cases for LG and 15% for HG (p = 0.12). The series 1972-2003 by Geller et al. [81] presented 19 LG and nine HG, of which underwent complete LND. Survival rates favored the LND, but as a retrospective study, cases with extraterine disease had no LND. As with BSO, LND in early stages should then be taken on an individual basis and discussed with the patient.

Surgery for restaging means performing BSO and/or LND in patients who had a previous hysterectomy. BSO for LGESS in patients approaching menopause is of less concern, if it was not performed, primarily they may not need it. Lymph node sampling is rarely performed, since ESS is often diagnosed after surgery for benign condition. Prognostic significance of nodal metastases in LGESS is still unknown [82]. In advanced or recurrent ESS, the most common option for salvage therapy is surgery [79]. A secondary or tertiary debulking surgery is often required [76]. If primary surgery was suboptimal, a secondary debulking surgery is mandatory [52]. The strongest independent prognostic factor in the series by Nordal et al. [48] pts 1976-1985 71% Stage I; 46 pts TAH and BSO [83] was positive resection margins. Evidences in literature about fertility-sparing surgery are few. Lissoni et al. [84] present six nulliparous women, median age 27 years, median follow-up 51 months in the period 1982-1996 who underwent laparotomic myomectomy for ESS. There were three pregnancies (37%) with two spontaneous deliveries. Two patients underwent a second surgical procedure: a resection of a pedunculated lesion seen during first surgery and a myomectomy 31 months after first procedure. They reported that all patients were alive and well and had no recurrences.

For HG it should be considered that it could be strongly residual disease after removal of the mass. A possible conservative strategy for ESS surgery should be guided by some criteria, as: a tumor completely resected (free margins > two mm), a woman who strongly desires fertility, some criteria, as: a tumor completely resected (free margins > two mm), a woman who strongly desires fertility, a myomectomy 29 months after first procedure. They reported that all patients were alive and well and had no recurrences.

In the classic series by Salazar et al., 1980, the five-year survival for ESS was 55% among Stage I, 12% for Stages II-IV [85].

**MMT (CS and AS)**

CS account for 1.5% of all gynecologic malignancies. CS shows a prevalence of 0.82/100000 women (> 20 years). Mean age at diagnosis is 65 years. Race seems to play a major role in CS etiology, with a fourfold higher incidence among Afro-American women when compared to Caucasians [3, 4].

**Biology and pathology**

CS is a biphasic mixture of malignant epithelial, usually endometrioid adenocarcinoma, and malignant stromal component [86, 87]. The latter can be undifferentiated or resemble a differentiated stromal sarcoma with a heterologous component, with, either benign or malignant, rhabdoid, cartilage, bone or adipose elements [88].

Epithelial and stromal elements can merge but in most cases they are separated. Many present with a HG stroma but a LG stromal component can be seen in the 16% of cases [86, 88, 89]. The apparent fusion of epithelial and stromal components brought to studies that showed how stromas had an epithelial immunohistochemical profile and a similar reactivity to p53 [90]. It is widely accepted that the tumor represents a metaplastic change of a carcinoma in a sarcomatous malignant component in 85%-95% of cases [86, 89, 91, 92]. The epithelial Müllerian component plays a major role in survival. Recurrences are more often carcinomatous, endometrioid or serous papillary subtype; nevertheless, they could show a sarcomatous or mixed histology.

CA125 is a marker that results preoperatively elevated and seems to have a prognostic value during follow-up [93].

**Therapy**

Recurrence rate for Stages I and II is 50%. Distant metastases constitute the 50%-80% of all. The most common sites of metastases are the omentum and the lungs. Risk factors associated to a worst prognosis are adnexal involvement, lymph node metastases, and HG tumor. Five-year survival rate is lower than 20%

Primary surgery in carcinosarcomas should include an exploratory laparotomy, pelvic washing, whole peritoneal cavity surgical staging, omentectomy, multiple lesion biopsies, mass debulking, para-aortic lymphadenectomy (PALA), and pelvic lymphadenectomy (PLND).

Sixty-two patients, consecutively treated in 1974-1995 with Stages I-II showed extra-uterine spread in 61% of cases. Among them, 81% underwent a gynecologic oncology referral; PLND and PALA were performed in 89% and 42% of cases, respectively [94].

LND, even in early Stages (I-II), is currently recommended. Fronting a higher morbidity (age-related, obesity, and hypertension) [95, 96], there are still benefits derived from LND. Undoubtedly, 15%-20% of patients show node metastases at the time of diagnosis [97], six percent will have the first recurrence at the para-aortic lymph nodes [98] and it showed to be a prognostic factor on multivariate analysis [99].
One hundred thirty-three out of 206 consecutively treated patients (1991-2000) underwent LND: an average of 19 nodes were dissected [9-74]. Higher rates of complications occurred when more than 14 nodes were removed [95].

Conclusion

The management of uterine sarcomas requires a multidisciplinary approach or tumor board before commencing the treatment. Until last decade, these tumors had been grouped together with other tumors generally described under the name of uterine sarcomas. This has been limiting current knowledge considering that the latter are different in terms of etio-pathology, genetics, behavior, and treatment. Old studies are therefore of limited use for meta-analysis. Gynecologists instead of a soft-tissue sarcomas expert have classically treated these tumors. Current views suggest to consider LMS as the only “true” sarcoma of the uterus and the worst histotype in terms of prognosis.

It is of paramount importance to ensure expertise management of the disease. Rarity and paucity of data are some reasons for a very poor prognosis and render them very good candidates for international multicentric studies.

References


Sentinel node biopsy in endometrial cancer: systematic review and meta-analysis of the literature

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Summary

Purpose: Sentinel lymph node biopsy is a fairly new approach for staging of gynecological malignancies. In the current study, the authors comprehensively reviewed the available reports on sentinel node biopsy of endometrial cancer. Materials and Methods: The authors searched Medline, SCOPUS, ISI web of knowledge, Science Direct, Springer, OVID SP, and Google Scholar with the following search terms: “endometrium OR endometrial OR uterine OR uterus AND sentinel”. The outcomes of interest were detection rate and sensitivity. Results: Overall, 35 studies had enough information for false negative rate evaluation and 51 studies (including the sub-groups of individual studies) for detection rate evaluation (2,071 patients overall). Pooled detection rate was 77.8% (95% CI: 73.5-81.5%) and pooled sensitivity was 89% (95% CI: 83-93%). Cervical injection, as well as using both blue dye and radiotracer, results in higher detection rate and sensitivity. New techniques such as fluorescent dye injection and robotic-assisted surgery showed high detection rate and sensitivity. Conclusion: Sentinel node mapping is feasible in endometrial cancer. Using both blue dye and radiotracer and cervical injection of the mapping material can optimize the sensitivity and detection rate of this technique. Larger studies are still needed to evaluate the false negative rate and the factors influencing the sensitivity before considering this method safe.

Key words: Endometrial cancer; Sentinel node biopsy; Meta-analysis; Systematic review; False negative rate; Detection rate.

Introduction

Endometrial cancer is one of the most common female malignancies, which is expected to increase in frequency due to the recent surge of obesity (one of the major risk factors of this cancer) around the world [1, 2]. Lymph node involvement is one of the most important prognostic factors in the treatment of endometrial cancer and since 1988, FIGO has included pelvic and para-aortic lymphadenectomy during surgical staging of this malignancy [3]. However, the incidence of nodal metastasis is very low (about 10%) in early stage of endometrial cancer and routine lymphadenectomy would not be required in many of these patients [4]. Furthermore, lymphadenectomy imposes significant morbidity for the patients [5], and due to this fact, many centers do not perform it but reserve it for high-risk patients [6].

Sentinel lymph node biopsy is a fairly new approach for staging of gynecological malignancies [2]. In this method, only patients with pathologically proven sentinel lymph nodes (detected by gamma probe and/or blue dye during surgery) would undergo complete lymph node dissection. This approach can decrease the morbidity of the patients, while the accuracy of the lymph node staging would not be compromised.

Recently, results of several groups on sentinel node mapping of endometrial cancer have been published [7, 8] and many studies have been published on this topic in 2012.

Materials and Methods

The authors searched Medline, SCOPUS, ISI web of knowledge, Science Direct, Springer, OVID SP, and Google Scholar with the following search terms: “endometrium OR endometrial OR uterine OR uterus AND sentinel". Last search was done in March 2012. No language or date limitation was used for the present search strategy. If meeting the following inclusion criteria, meeting abstracts were also included. The reference lists of the primary studies, as well as citing articles, were searched separately for any other possible relevant study. The authors contacted the corresponding authors for more information when necessary.

Inclusion criteria

For evaluating the sensitivity of sentinel lymph node biopsy, only studies with the following criteria were included: 1) Using at least pelvic lymph node dissection (preferably para-aortic lymphadenectomy in addition) as the gold standard of lymph node involvement. 2) Total number of patients with positive lymph nodes, as well as those with false negative results (positive lymph nodes despite negative sentinel node) were both reported. For evaluating the detection rate, only studies with the following criterion were included: 1) Total number of included patients, as well as those with detected sentinel nodes were both reported.

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Statistical analysis

Random effects model (Der-Simonian and Laird method [10]) was used for pooling detection and false negative rates across the studies having in mind the considerable heterogeneity of the included studies regarding the method and included patients. For statistical evaluation of heterogeneity, Cochrane Q test was used (p value less than 0.05 was considered statistically significant). I² index was used to quantify the extent of heterogeneity. This index shows the amount of the heterogeneity among the included studies which is not caused by sampling errors and is real [11]. Sub-group analysis was used for exploring the heterogeneity among studies regarding different variables such as injection site, injection material, etc.

Publication bias was graphically evaluated by funnel plots. Egger’s regression method was used to statistically evaluate the asymmetry of funnel plots which represents the publication bias [12].

All statistical analyses were done using Meta-Analyst [13], and Meta-Disc (version 1.4) [14].

Results

Figure 1 shows the summary of the search process of the present study. Forty-six studies were included in the review (2,071 patients) [8, 15-59]. Eight studies had two subgroups of patients and were included in the review separately [8, 20, 26, 35, 39, 41, 50, 52]. Another study had three different subgroups [25]. Overall 35 studies had enough information for false negative rate evaluation and 51 studies (including the sub-groups of the above-mentioned studies) for detection rate evaluation. Quality assessment and summary data of the included studies are shown in Tables 1 and 2, respectively.

Detection rate

Figure 2 shows the forest plot of the detection rate pooling. Pooled detection rate was 77.8% [95% CI: 73.5-81.5%]. Cochrane Q value was 132.3 (p < 0.0001) and I² index was 61.4%.

Funnel plot of the detection rate pooling is shown in Figure 3. Egger’s regression intercept was -0.08 (p = 0.84).

Sub-group analyses regarding injection site showed 84.9% [78.8-89.4%], 73.9% [63.9-81.9%], 69.7% [57.7-79.4%], 86.1% [66.3-95.1%], and 50% [9-91%] detection rates for cervical, sub-endometrial, subserosal, cervical/subserosal, and sub-endometrial/subserosal injections respectively.

Considering the method of sentinel node mapping, the following results were obtained: 71.1% [62.6-78.3%], 76.7% [67-84.2%], and 82.8% [76.4-87.8%] detection rates for blue dye, tracer, and blue dye/tracer techniques respectively. Rossi et al. [54] and Holloway et al. [56] used fluorescent dye and near infra-red imaging for detection of the sentinel nodes with pooled detection rate of 94.2% [64.7-99.3%].

Finally, the effect of surgery type was evaluated and the following detection rates were obtained: 73.8% [65.1-81%], 76% [68.5-82.2%], and 88.5% [72-95.8%] for laparoscopy, laparotomy, and robotic assisted surgeries, respectively.

Sensitivity

Figure 4 shows the forest plot of the sensitivity pooling. Pooled sensitivity was 89% [83-93%]. Cochrane Q value was 31.97 (p = 0.74 and I² = 0%).

Funnel plot of the sensitivity pooling is shown in Figure 5. Egger’s regression intercept was -0.02 (p = 0.95).

Subgroup analysis for injection site showed the following sensitivities: 89% [82-94%], 91% [79-98%], 84% [60-97%], and 100% [29-100%] for cervical, sub-endometrial, sub-serosal, and cervical/subserosal injections respectively.

Considering the method of sentinel node mapping, the following pooled sensitivities were obtained: 86% [75-93%], 85% [69-95%], 93% [85-97%] for blue dye, tracer, and blue dye/tracer methods, respectively.

Subgroup analysis for type of surgery showed the following pooled sensitivities: 87% [74-95%], and 93% [77-99%] for laparotomy and laparoscopic surgeries, respectively.
Table 1. — Quality assessment of the included studies.

<table>
<thead>
<tr>
<th>First author</th>
<th>Institution</th>
<th>Publication/Consecutive year</th>
<th>Prospective design</th>
<th>Gold standard</th>
<th>Enough explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burk et al.</td>
<td>US (Houston)</td>
<td>1996 N/A</td>
<td>Yes</td>
<td>Pelvic and para-aortic lymphadenectomy</td>
<td>Yes</td>
</tr>
<tr>
<td>Echt et al.</td>
<td>US (New Orleans)</td>
<td>1999 Yes Yes</td>
<td>Pelvic and para-aortic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Holub et al.</td>
<td>Czech Republic (Klando)</td>
<td>2001 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Holub et al.</td>
<td>Czech Republic (Klando)</td>
<td>2002 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Pelosi et al.</td>
<td>Italy (Torino)</td>
<td>2002 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Pitynski et al.</td>
<td>Poland (Krakow)</td>
<td>2003 N/A Yes</td>
<td>Pelvic lymphadenectomy and para-aortic in selected cases</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Pelosi et al.</td>
<td>Italy (Torino)</td>
<td>2003 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Holub et al.</td>
<td>Czech Republic (Klando)</td>
<td>2004 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Fersis et al.</td>
<td>Germany (Teubingen)</td>
<td>2004 Yes Yes</td>
<td>Pelvic lymphadenectomy (and para-aortic in selected cases)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Niikura et al.</td>
<td>Japan (Sendai)</td>
<td>2004 Yes Yes</td>
<td>Pelvic and paraaortic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Gien et al.</td>
<td>UK (London)</td>
<td>2005 No (only pelvic lymphadenectomy in patients with high risk of metastasis were included)</td>
<td>Yes Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dzvincuk et al.</td>
<td>Czech Republic (Olomouc)</td>
<td>2006 Yes Yes</td>
<td>Pelvic lymphadenectomy and para-aortic lymphadenectomy in 11 patients</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Niccoli A. et al.</td>
<td>Italy (Bari)</td>
<td>2006 N/A N/A</td>
<td>Pelvic and para-aortic lymphadenectomy up to the level of the renal veins</td>
<td>No</td>
<td></td>
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<tr>
<td>Lopes et al.</td>
<td>Brazil (Sao Paulo)</td>
<td>2007 Yes Yes</td>
<td>Pelvic lymph node dissection</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Yan et al.</td>
<td>China (Foshan)</td>
<td>2007 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Altgassen et al.</td>
<td>Germany (Leubeck)</td>
<td>2007 No Yes</td>
<td>Pelvic and para-aortic lymphadenectomy 15; pelvic 8</td>
<td>Yes</td>
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<tr>
<td>Delaloye et al.</td>
<td>Switzerland (Lausanne)</td>
<td>2007 N/A Yes</td>
<td>Pelvic and para-aortic lymphadenectomy</td>
<td>Yes</td>
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<tr>
<td>Fumovitz et al.</td>
<td>US (Houston)</td>
<td>2007 No Yes</td>
<td>Pelvic and para-aortic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Maccarato et al.</td>
<td>Italy (Milan)</td>
<td>2007 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Jiang et al.</td>
<td>China (Sun Yat-sen)</td>
<td>2008 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Bats et al.</td>
<td>France (Paris)</td>
<td>2008 Yes Yes</td>
<td>Pelvic lymphadenectomy/para-aortic in selected cases</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Clement et al.</td>
<td>France (Paris)</td>
<td>2008 No Yes</td>
<td>Pelvic lymphadenectomy/para-aortic in selected cases</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Ballester et al.</td>
<td>France (Paris)</td>
<td>2008 Yes Yes</td>
<td>Pelvic lymphadenectomy (40) para-aortic in selected cases (6) (patients with clear cell or serous cancers)</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Perrone et al.</td>
<td>Italy (Bologna)</td>
<td>2008 Yes Yes</td>
<td>Pelvic lymphadenectomy and lombo-aortic lymphadenectomy was performed in high grade EC and in cases of lombo-aortic capture of SLN</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Li et al.</td>
<td>China (Beijing)</td>
<td>2009 N/A Yes</td>
<td>Pelvic lymphadenectomy in 27 patients/pelvic node sampling in 4/7 of the 31 patients, a para-aortic lymph node sampling was performed</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Robova et al.</td>
<td>Czech Republic (Prague)</td>
<td>2009 N/A Yes</td>
<td>Pelvic and para-aortic lymphadenectomy up to the inferior mesenteric artery</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Vidal-Sicart et al.</td>
<td>Spain (Barcelona)</td>
<td>2009 N/A Yes</td>
<td>Selected lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Kara et al.</td>
<td>Turkey (Ankara)</td>
<td>2009 Yes Yes</td>
<td>Pelvic and para-aortic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Zenzola et al.</td>
<td>Venezuela (Caracas)</td>
<td>2009 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Gemignani et al.</td>
<td>US (New York)</td>
<td>2009 Yes Yes</td>
<td>Pelvic lymphadenectomy in all and pelvic</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Qu et al.</td>
<td>China (Shandong)</td>
<td>2010 Yes Yes</td>
<td>Pelvic and para-aortic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Ferandez et al.</td>
<td>Czech Republic (Brno)</td>
<td>2010 Yes Yes</td>
<td>Pelvic lymphadenectomy (para-aortic in high risk patients)</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Dittmann et al.</td>
<td>Germany (Teubingen)</td>
<td>2010 N/A Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Solà et al.</td>
<td>Spain (Barcelona)</td>
<td>2010 Yes Yes</td>
<td>Regional lymph node dissection</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Mais et al.</td>
<td>Italy (Cagliari)</td>
<td>2010 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Ballester et al.</td>
<td>France (Multicenter)</td>
<td>2011 Yes Yes</td>
<td>Pelvic lymphadenectomy and para-aortic lymphadenectomy in 15 patients</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Cordero Garcia et al.</td>
<td>Spain (Madrid)</td>
<td>2012 Yes Yes</td>
<td>Pelvic lymphadenectomy</td>
<td>Yes</td>
<td></td>
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<tr>
<td>Barlin et al.</td>
<td>US (New York)</td>
<td>2012 Yes Yes</td>
<td>Pelvic lymphadenectomy in all and pelvic as well as para-aortic in some at the surgeons discretion</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Leitao et al.</td>
<td>US (New York)</td>
<td>2011 Yes Yes</td>
<td>Pelvic lymphadenectomy in all and pelvic as well as para-aortic in some at the surgeons discretion</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Rossi et al.</td>
<td>US (Indiana)</td>
<td>2012 N/A Yes</td>
<td>Pelvic lymphadenectomy and para-aortic lymphadenectomy</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Holloway et al.</td>
<td>US (Orlando)</td>
<td>2012 N/A No</td>
<td>Pelvic lymphadenectomy in all and para-aortic lymphadenectomy in high risk patients</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Buda et al.</td>
<td>Italy (Monza)</td>
<td>2012 Yes Yes</td>
<td>Pelvic lymphadenectomy in all and para-aortic lymphadenectomy in selected patients</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>How et al.</td>
<td>Canada (Montreal)</td>
<td>2012 Yes Yes</td>
<td>Pelvic lymphadenectomy in all and para-aortic lymphadenectomy in high risk patients</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Solima et al.</td>
<td>Italy (Milan)</td>
<td>2012 Yes Yes</td>
<td>Pelvic lymphadenectomy in all and para-aortic lymphadenectomy in high risk patients</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
Rossi et al. [54], Holloway et al. [56], and How et al. [58] used robotic-assisted surgery with fluorescent dye with pooled sensitivity of 86% [64-97%]. Including only larger studies with more than five patients with positive lymph nodes showed pooled sensitivity of 89% [83-94%].

**Discussion**

Sentinel node biopsy is a novel method for regional lymph node staging of many solid tumors including breast cancer [60], urological malignancies [61], etc. This method can considerably decrease the morbidity of regional lymph node dissection by sparing many patients of this invasive procedure [62]. This concept had been used for endometrial cancer since 1996 [15] with different results. In the current systematic review, the authors comprehensively searched and pooled results of available studies. In should be mentioned that in 2011, similar systematic review was published by Kang et al. [63]. The present systematic review had considerable more complete search strategy as the authors could locate 46 relevant studies compared to Kang et al. This systematic review also included the most recent results of the most experienced.
group in sentinel node mapping of endometrial cancer (Barlin et al. study [53]) which makes it more valid. In addition, Kang et al. study had several errors in data extraction: for example they considered articles by Holub et al. [17, 18, 22] as duplicate and did not include two of them in the analysis. However these three studies are methodologically different and the authors included them in this study. Finally, sensitivities of Delaloye et al. [31] and Burk et al. [15] studies were mistakenly calculated by Kang et al.: 89% and 50%, despite being 100% and 67%.

**Detection rate**

Pooled detection rate was rather low (77.8%) compared to other malignancies such as breast cancer. However the included studies were highly heterogeneous (I² = 61.4%) and subgroup analysis showed that injection site, and mapping method could affect detection rate. For example Echt et al. reported 0% detection rate in their study, which could be attributed to the intramyometrial injection of the tracer as they mentioned themselves [16]. As shown above, cervical or combined cervical/subserosal injections of the tracer had the highest detection rates compared to other techniques such as sub-endometrial or subserosal. This can be due to ease of cervical injection compared to other sites [64].

Using both blue dye and radiotracer showed the highest detection rate compared to either of the methods alone. Fluorescent dye (using near infrared imaging intraoperatively) was used in two studies with very high detection rate and seems to be a promising method.

**Unilateral and bilateral mapping**

Endometrium as a midline organ has two different pathways of lymphatic drainage: right and left [8]. As shown in Table 2, the rate of bilateral drainage was not reported in many studies included in the current meta-analysis. This rate was between 97.1% in Holloway et al. study [56] and 12.5% in Frumovitz et al. study [32]. In the two largest series, this rate was 69% [8] and 63% [53], respectively. Failure to detect sentinel nodes bilaterally can be of importance regarding the need to perform lymphadenectomy on the failure side. Two of the largest studies thus far have evaluated this notion in detail. Ballester et al. reported 100% sensitivity while using hemipelves as the unit of calculations despite 84% using patients as the unit [8]. Barlin et al. also showed the same results as they reported 98.1% sensitivity using hemipelves as the unit of calculations despite 85.1% using the patients as the unit [53]. It is worth mentioning that for other midline organs such as the penis, this method has been used with fairly promising results [65].

**Distribution of sentinel nodes and para-aortic sentinel lymph nodes**

As shown in Table 2, distribution of sentinel nodes depends on the injection site of the mapping materials. The sub-serosal and sub-endometrial methods show para-aortic sentinel nodes more frequently (as high as 38% of all sentinel nodes in one study) [15]. Isolated para-aortic sentinel node detection was also reported in 1%-36% of patients in various studies [23, 24, 32, 40, 53, 58, 59].

Para-aortic lymph node dissection is under much debate in endometrial cancer. The incidence of para-aortic lymph node metastasis without pelvic lymph node involvement was reported to be 1% [66]. The incidence of isolated para-aortic recurrence was also reported to be only 6%, most of which were in grade 3 tumors. The present meta-analysis also supports these data since positive para-aortic sentinel nodes were only reported by limited studies: Lopes et al. (four out of five patients with positive nodes) [28], Feranec et al. (one patient which was the only positive patient) [47], Fersis et al. (one patient in both para-aortic and pelvic regions), Delaloye et al. (in 40% of positive sentinel nodes) [31], Maccaro et al. (one out of seven positive patients) [33], Niikura et al. (one out of six positive patients) [67], Holloway et al. (five out of ten positive patients) [56], and Solima et al. (one out of ten positive patients) [59].

Altogether, it seems that the incidence of para-aortic lymph node involvement is low and cervical injection (which is very easy to perform) of the mapping material would suffice for sentinel node mapping.

**Sensitivity**

Overall, the pooled sensitivity of sentinel node mapping was 89% [83-93], which is fairly acceptable compared to other known malignancies, such as breast cancer or melanoma [68]. The included studies were not that heterogeneous in this regard (Cochrane Q value = 31.9, p = 0.7 and I² = 0%).

Sub-group analysis showed that using both blue dye and tracer had the highest sensitivity compared to either technique alone. This was in accordance with other malignancies such as breast and urological cancers [61, 68].

Site of injection and type of surgery were both related to sensitivity: laparoscopic surgery and cervical/subserosal injection showed higher pooled sensitivities.

**Effect of immunohistochemistry (IHC)**

High frequency of lymph node micro-metastasis has been reported to occur in endometrial cancer, however detection of micro-metastases by routine IHC evaluation of all removed lymph nodes is not time or cost-effective [69]. However, using IHC on the removed sentinel nodes seems to be cost-effective and can decrease the false negative rate. This was supported by the results of the present meta-analysis. Lopes et al. [28], Bats et al. [36], Ballester et al. [38], Ballester et al. (multicenter study) [8], Altgassen et al. [30], Kara et al. [43], Perrone et al. [39], Niikura et al. [24], Barlin et al. [53], Holloway et al. [56], and Solima et al. [59] reported 5, 2, 4, 9, 3, 1, 5, 4, 9, 4, and six additional positive nodes using IHC compared to the conventional hematoxylin and eosin (H&E) staining.

Although using IHC can decrease the false negative rate, however the prognostic impact of micro-metastasis is not yet clear and needs further studies with high sample size and long follow up [69].
<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>No. of patients</th>
<th>No. of patients</th>
<th>No. of patients</th>
<th>Type</th>
<th>Injection site</th>
<th>Volume</th>
<th>Dose (in MBq)</th>
<th>Mean age</th>
<th>Grade</th>
<th>Stage</th>
<th>SLN distribution</th>
<th>No. of patients</th>
<th>Type of surgery</th>
<th>No. of positive node using IHC</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burke et al.</td>
<td>1996</td>
<td>15</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>IB</td>
<td>SS</td>
<td>3 ml</td>
<td>N/A</td>
<td>N/A</td>
<td>G2 7; G3 2</td>
<td>Para-aortic sites in 12, common iliac in 6, and pelvic in 13</td>
<td>N/A</td>
<td>LP</td>
<td>N/A</td>
<td>Only patients with high risk of metastasis were included (G2 or 3 or variant histology). The injection was intramyometrial</td>
</tr>
<tr>
<td>Echt et al.</td>
<td>1999</td>
<td>8</td>
<td>0</td>
<td>N/A</td>
<td>IB</td>
<td>SE</td>
<td>2 ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>G1 1; G2 5; G3 2</td>
<td>No detection</td>
<td>No SLN</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holub et al.</td>
<td>2001</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>PB</td>
<td>SE</td>
<td>2 ml</td>
<td>N/A</td>
<td>58.5</td>
<td>N/A</td>
<td>IB 7; IV 1</td>
<td>Stage: IA-1, Obturatar, internal and common iliac / No para-aortic</td>
<td>N/A</td>
<td>IB 1; Ib 6, Ic 4, Ile 1</td>
<td>N/A</td>
<td>The injection was intramyometrial</td>
</tr>
<tr>
<td>Holub et al.</td>
<td>2002</td>
<td>13</td>
<td>8</td>
<td>1</td>
<td>PB</td>
<td>SS</td>
<td>2 ml</td>
<td>N/A</td>
<td>59.8</td>
<td>N/A</td>
<td>IA 2; Ib 6; Ic 4; Ile 1</td>
<td>All internal iliac</td>
<td>6</td>
<td>LP</td>
<td>N/A</td>
<td>Only the subserosal group was included</td>
</tr>
<tr>
<td>Pelosi et al.</td>
<td>2002</td>
<td>11</td>
<td>11</td>
<td>N/A</td>
<td>PB</td>
<td>C</td>
<td>4 mL</td>
<td>4 in 4 deposits</td>
<td>37/ (0.4 mL) (from another study of the group)</td>
<td>N/A</td>
<td>IB 10; Ia 1</td>
<td>All internal iliac</td>
<td>N/A</td>
<td>Planar 2 hours post injection</td>
<td>Detection rate for the patients injected with tracer and blue dye both was 100% (10/10) and for those injected with blue dye only was 19/23 Lymphoscintigraphy showed SLNs in 15 patients</td>
<td></td>
</tr>
<tr>
<td>Holub et al.</td>
<td>2004</td>
<td>25</td>
<td>21</td>
<td>2</td>
<td>PB</td>
<td>SS</td>
<td>5 or 10 mL (4 or 9 mL in C) (four injections) and remaining in the midline SS</td>
<td>N/A</td>
<td>N/A</td>
<td>59.7</td>
<td>All Stage I</td>
<td>52.8% (28 of 53 lymph nodes) at the fossa obturatoria and internal iliac sites, in 13.2% (7 of 53 lymph nodes) at the division of the common iliac artery. In 34% in other pelvic areas. No para-aortic, Interiliac 2; anterior parametral tissue 2; para-aortic only 1; pelvic and para-aortic 2</td>
<td>17</td>
<td>LPS</td>
<td>N/A</td>
<td></td>
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<tr>
<td>Pelosi et al.</td>
<td>2003</td>
<td>16</td>
<td>15</td>
<td>3</td>
<td>PB</td>
<td>C</td>
<td>4 mL</td>
<td>4 in 4 deposits</td>
<td>37 (0.4 mL) (from another study of the group)</td>
<td>N/A</td>
<td>Ib 1</td>
<td>All Internal iliac</td>
<td>9</td>
<td>LPS</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Pirynski et al.</td>
<td>2003</td>
<td>33</td>
<td>29</td>
<td>2</td>
<td>PB</td>
<td>SS</td>
<td>4 mL</td>
<td>and 3 mL SS (in 10 cases)</td>
<td>100</td>
<td>N/A</td>
<td>N/A</td>
<td>All Stage I</td>
<td>N/A</td>
<td>Planar 2 hours post injection</td>
<td>Detection rate for the patients injected with tracer and blue dye both was 100% (10/10) and for those injected with blue dye only was 19/23 Lymphoscintigraphy showed SLNs in 15 patients</td>
<td></td>
</tr>
<tr>
<td>Pelosi et al.</td>
<td>2003</td>
<td>10</td>
<td>7</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>40-100</td>
<td>G1 2; G2 7; G3 1</td>
<td>Para-aortic only 1; pelvic and para-aortic 2</td>
<td>5</td>
<td>LP</td>
<td>N/A</td>
<td>Planar in 2</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. — Summary of the included studies information.**
<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>No. of patients</th>
<th>No. of patients with detected SN</th>
<th>No. of patients with positive pelvic SN</th>
<th>No. of patients with positive SN</th>
<th>No. of patients with bilateral drainage</th>
<th>Type of surgery</th>
<th>Imaging</th>
<th>No. of positive node using IHC</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niikura et al.</td>
<td>2004</td>
<td>28</td>
<td>23</td>
<td>6</td>
<td>5</td>
<td>N/A</td>
<td>P</td>
<td>SE</td>
<td>38-70/2 ml (median)</td>
<td>Pelvic 6; Para-aortic 8; Both basins 22</td>
</tr>
<tr>
<td>Gien et al.</td>
<td>2005</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>IB</td>
<td>SS in 9</td>
<td>SE</td>
<td>5-10 ml</td>
<td>Common iliac 3; External iliac 11; Obturator 2; Presacral 1</td>
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<tr>
<td>Dzvincuk et al.</td>
<td>2006</td>
<td>33</td>
<td>26</td>
<td>3</td>
<td>3</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>502.5 c in 4-6 portion</td>
<td>All pelvic and 11 para-aortic</td>
</tr>
<tr>
<td>Niccoli et al.</td>
<td>2006</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>111 / 4 ml (median)</td>
<td>3 external iliac nodes, 7 internal iliac nodes, 2 in the para-aortic area and 1 in common iliac site</td>
</tr>
<tr>
<td>Lopes et al.</td>
<td>2007</td>
<td>40</td>
<td>31</td>
<td>6</td>
<td>5</td>
<td>PB</td>
<td>SS in 8</td>
<td>N/A</td>
<td>3 ml</td>
<td>4 in the para-aortic, 3 in the pelvic region, and 4 in both regions.</td>
</tr>
<tr>
<td>Yan et al.</td>
<td>2007</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>MB c</td>
<td>4 ml in 4 points</td>
<td>N/A</td>
<td>N/A</td>
<td>No positive SLN</td>
</tr>
<tr>
<td>Altgassen et al.</td>
<td>2007</td>
<td>23</td>
<td>21</td>
<td>3</td>
<td>2</td>
<td>(5 with IHC)</td>
<td>SS in 8</td>
<td>N/A</td>
<td>4 ml/in 8</td>
<td>49 pelvic; 6 para-aortic; 1 presacral</td>
</tr>
<tr>
<td>Delaloye et al.</td>
<td>2007</td>
<td>60</td>
<td>49</td>
<td>8</td>
<td>8</td>
<td>PB SE</td>
<td>2 ml</td>
<td>NC</td>
<td>N/A</td>
<td>Pelvic 33; both pelvic and para-aortic 16</td>
</tr>
<tr>
<td>Frumovitz et al.</td>
<td>2007</td>
<td>18</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>IB</td>
<td>SS in 3 portions</td>
<td>N/A</td>
<td>18.5-37/ml (median) in 3 portions</td>
<td>4 only in the pelvis, 2 in the pelvis and above the bifurcation of the aorta; 2 patients above the bifurcation of the aorta only</td>
</tr>
</tbody>
</table>

Table 2. — Summary of the included studies information.
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<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>No. of patients</th>
<th>No. of patients with detected SN</th>
<th>No. of patients with positive SN</th>
<th>Type</th>
<th>Blue dye</th>
<th>Volume</th>
<th>Radiotracer</th>
<th>Stage</th>
<th>Grade</th>
<th>SLN detection</th>
<th>No. of patients with bilateral drainage</th>
<th>Type of surgery</th>
<th>No. of patients with positive node</th>
<th>Imaging</th>
<th>Imaging</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>Maccaro et al.</td>
<td>2007</td>
<td>45</td>
<td>45</td>
<td>7</td>
<td>PB</td>
<td>SE</td>
<td>8 ml/ portions</td>
<td>NC</td>
<td>SE</td>
<td>111</td>
<td>54</td>
<td>N/A</td>
<td>N/A</td>
<td>Common iliac 17; external iliac 16; internal iliac; obturator 12; para-aortic 14 (from another study of this group)</td>
<td>Obturator 11; External iliac 4; Internal iliac 2</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Jiang et al.</td>
<td>2008</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>MB</td>
<td>SS</td>
<td>4 ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>54.9</td>
<td>G1 11; G2 3</td>
<td>I:II 1; II: 1; III: 1</td>
<td>Interiliac 71; Common iliac 9; Promontory 6; No para-aortic Common iliac 2 external iliac 1 para-aortic 1</td>
<td>5</td>
<td>LPS 0</td>
<td>N/A</td>
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<tr>
<td>Jiang et al.</td>
<td>2008</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>MB</td>
<td>C</td>
<td>4 ml in 4 deposits</td>
<td>RSC</td>
<td>C</td>
<td>120 /in 4 aliquots</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>LPS 0</td>
<td>Cervical injection group</td>
<td></td>
<td></td>
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<tr>
<td>Bats et al.</td>
<td>2008</td>
<td>43</td>
<td>30</td>
<td>8</td>
<td>PB</td>
<td>C</td>
<td>2 ml/in 4 aliquots</td>
<td>RSC</td>
<td>C</td>
<td>120 /in 4 aliquots</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>LPS 2</td>
<td>In three patients sentinel node was detected by blue dye only</td>
<td></td>
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<tr>
<td>Clement et al.</td>
<td>2008</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>PB</td>
<td>SE in 4 and C in 1</td>
<td>2 ml/in 4 aliquots</td>
<td>RSC</td>
<td>SE</td>
<td>120 /in 4 aliquots</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>LPS</td>
<td>In 1 patient blue dye only and in another one radiotracer only detected the SLNs</td>
<td></td>
<td></td>
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<tr>
<td>Ballesta et al.</td>
<td>2008</td>
<td>46</td>
<td>40</td>
<td>10</td>
<td>PB</td>
<td>C</td>
<td>2 ml/in 2 aliquots</td>
<td>USC</td>
<td>C</td>
<td>80 /in 4 aliquots (0.2 ml each)</td>
<td>65</td>
<td>G1 27; G2 10; G3 3; G4 6</td>
<td>IA 7; IB 13; C 5; II 2; 1 IIIC 9</td>
<td>External iliac (lateral group), the iliac vessel bifurcation, the common iliac and the aortic bifurcation in 78 (78%), 16 (16%), 6 cases (6%) and 1 case (1%), No para-aortic Iliac region 15; obturator 1</td>
<td>25</td>
<td>LPS 4</td>
<td>LP4</td>
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<tr>
<td>Perrone et al.</td>
<td>2008</td>
<td>23</td>
<td>16</td>
<td>4</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>NC</td>
<td>C</td>
<td>55-74 /4 ml</td>
<td>63.4</td>
<td>G1 10; G2 7; G3 5</td>
<td>Stage I or II</td>
<td>Stage I or II</td>
<td>Dynamic and static imaging 15 and 30 minutes post injection</td>
<td>Dynamic and static imaging 15 and 30 minutes post injection</td>
<td>Cervical injection group/imaging showed SLN in 20 patients</td>
</tr>
<tr>
<td>Perrone et al.</td>
<td>2008</td>
<td>17</td>
<td>11</td>
<td>2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>110 /4 ml</td>
<td>68.6</td>
<td>G1 7; G2 6; G3 4</td>
<td>Stage I or II</td>
<td>Iliac region 10; obturator 1; aortic 2</td>
<td>3</td>
<td>LPS 1</td>
<td>1</td>
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</table>
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<table>
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<tr>
<th>First author</th>
<th>Year</th>
<th>No. of patients</th>
<th>No. of patients with detected SN</th>
<th>No. of patients with bilateral drainage</th>
<th>Blue dye</th>
<th>Radiotracer</th>
<th>Type</th>
<th>Injection site</th>
<th>Volume (in MBq)</th>
<th>Dose (in ml)</th>
<th>Mean age</th>
<th>Grade</th>
<th>Stage</th>
<th>SLN rate</th>
<th>No. of patients with positive SN</th>
<th>Tumor margin</th>
<th>No. of positive nodes</th>
<th>Surgery</th>
<th>No. of patients with metastasis</th>
<th>Imaging</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Li et al.</td>
<td>2009</td>
<td>27</td>
<td>23</td>
<td>2</td>
<td>MB</td>
<td>SS</td>
<td>N/A</td>
<td>N/A</td>
<td>4 ml in 3 (stage I) or 2 (stage II) aliquots</td>
<td>53 (median)</td>
<td>G1 in 13 and G2 in 12</td>
<td>IA 5; IB 8; IC 4; IIA 5; IIIB 3</td>
<td>1 para-aortic</td>
<td>LP</td>
<td>N/A</td>
<td>The first 4 patients were excluded due to defective injection</td>
<td></td>
<td></td>
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<tr>
<td>Robova et al.</td>
<td>2009</td>
<td>67</td>
<td>49</td>
<td>3</td>
<td>PB</td>
<td>SS</td>
<td>HSA</td>
<td>SS</td>
<td>2 ml / 2 ml</td>
<td>59.2 (median)</td>
<td>Grade 1 IC 26 3</td>
<td>IA 9 IB 48 IC 19 IIA 7 IIIB 3</td>
<td>11/134 SLNs in the para-aortic area</td>
<td>41</td>
<td>LP</td>
<td>N/A</td>
<td>No imaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robova et al.</td>
<td>2009</td>
<td>24</td>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>HSA</td>
<td>SE</td>
<td>40/2 ml</td>
<td>65 (median)</td>
<td>N/A</td>
<td>Stage I or II Pelvic 19</td>
<td>Parametrial (0%), obturator fossa (26%), external iliac region (32%), internal iliac (10%), primitive iliac (25%) and para-aortic zone (20%). Pelvic and external iliac in 5</td>
<td>39%</td>
<td>LPS</td>
<td>N/A</td>
<td>Planar 1 hour post injection</td>
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<td></td>
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<tr>
<td>Vidal-Sicart et al.</td>
<td>2009</td>
<td>35</td>
<td>22</td>
<td>3</td>
<td>N/A</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>148 /0.8 ml</td>
<td>(in 17 patients 111 /2-4 ml)</td>
<td>65</td>
<td>N/A</td>
<td>Stage I or II Pelvic 19</td>
<td>Parametrial (0%), obturator fossa (26%), external iliac region (32%), internal iliac (10%), primitive iliac (25%) and para-aortic zone (20%). Pelvic and external iliac in 5</td>
<td>39%</td>
<td>LPS</td>
<td>N/A</td>
<td>Planar 30 min post injection</td>
<td></td>
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<tr>
<td>Kara et al.</td>
<td>2009</td>
<td>24</td>
<td>24</td>
<td>3</td>
<td>N/A</td>
<td>N/A</td>
<td>NC</td>
<td>C</td>
<td>74 /4 doses</td>
<td>(median)</td>
<td>IA 4; IB 7; IC 6; IIA 7</td>
<td>Pelvic and para-aortic in 5</td>
<td>11</td>
<td>LP</td>
<td>1</td>
<td>Only patients with middle-high risk of metastasis were included</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Zenaola et al.</td>
<td>2009</td>
<td>14</td>
<td>10</td>
<td>1</td>
<td>PB</td>
<td>C</td>
<td>SC</td>
<td>0.5 ml in 4 deposits</td>
<td>37 in 2 deposits</td>
<td>65</td>
<td>G1 6; G2 5; G3 2</td>
<td>Ia 6; Ib 4; Ic 4</td>
<td>Right external iliac 6; Left external iliac 2; Right middle iliac 1; Left obturator 1</td>
<td>0</td>
<td>LP</td>
<td>N/A</td>
<td>Dynamic (40 frames of 20 s) and static (500,000 counts) for 4 hours in anterior projection. Planar 20-30 post-injection Dynamic imaging for 10 min (1 min per view) and static in 60 minutes/SPECT in 40 patients</td>
<td></td>
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<tr>
<td>Gemignani et al.</td>
<td>2009</td>
<td>73</td>
<td>62</td>
<td>8</td>
<td>IB</td>
<td>C or MB</td>
<td>FSC</td>
<td>C</td>
<td>4 ml (2 ml deep, 2 ml superficial)</td>
<td>37-148 /0.5-1 ml</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>All SLN were hot, 3 blue/four failures were in the first patients. Lymphoscintigraphy could detect sentinel nodes in 53 patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qu et al.</td>
<td>2010</td>
<td>18</td>
<td>13</td>
<td>2</td>
<td>MB</td>
<td>SS</td>
<td>N/A</td>
<td>N/A</td>
<td>4 ml in 4 deposits</td>
<td>56 (median)</td>
<td>G1 8; G2 4; G3 3</td>
<td>N/A</td>
<td>Internal iliac 11, external iliac 9, common iliac 7, obturator 18, abdominal aorta 2 (total 147)</td>
<td>N/A</td>
<td>LP</td>
<td>N/A</td>
<td>SPECT in 40 patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sentinel node biopsy in endometrial cancer: systematic review and meta-analysis of the literature
following table 2. — Summary of the included studies information.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>No. of patients</th>
<th>No. of patients</th>
<th>No. of patients</th>
<th>No. of patients</th>
<th>Type</th>
<th>Injection site</th>
<th>Volume</th>
<th>Type</th>
<th>Type</th>
<th>Age</th>
<th>Stage</th>
<th>SLN distribution</th>
<th>Blue dye</th>
<th>Radiotracer</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Feranec et al.</td>
<td>2010</td>
<td>21</td>
<td>17</td>
<td>1</td>
<td>PB</td>
<td>SE</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>100</td>
<td>61</td>
<td>G1 13; G2 3; G3 5</td>
<td>I A 4; IB 10; IIA 3; IIB 2; II A 1; II A 1</td>
<td>N/A</td>
<td>LP</td>
<td>0</td>
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<tr>
<td>Dittmann et al.</td>
<td>2010</td>
<td>62</td>
<td>48</td>
<td>2</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>300-350</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Sola et al.</td>
<td>2010</td>
<td>15</td>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>NC</td>
<td>SE</td>
<td>111/8 ml two deposits</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>LPS</td>
<td>N/A</td>
<td>SPECT/CT imaging 12/15 nodes 4 h post injection.</td>
</tr>
<tr>
<td>Mais et al.</td>
<td>2010</td>
<td>17</td>
<td>14</td>
<td>2</td>
<td>PB</td>
<td>C</td>
<td>4 ml/in 4 deposits</td>
<td>N/A</td>
<td>N/A</td>
<td>61</td>
<td>G1 11; G2 4; G3 2</td>
<td>Ib 14; 1c 1; IIa 2</td>
<td>Pelvic region mostly internal iliac</td>
<td>N/A</td>
<td>LP</td>
<td>N/A</td>
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<tr>
<td>Mais et al.</td>
<td>2010</td>
<td>17</td>
<td>7</td>
<td>1</td>
<td>PB</td>
<td>C</td>
<td>4 ml/in 4 deposits</td>
<td>N/A</td>
<td>N/A</td>
<td>65.9</td>
<td>G1 6; G2 7; G3 4</td>
<td>1a 1; 1e 1; IIa 2</td>
<td>Pelvic region mostly internal iliac</td>
<td>N/A</td>
<td>LP</td>
<td>N/A</td>
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<tr>
<td>Ballester et al.</td>
<td>2011</td>
<td>125</td>
<td>111</td>
<td>19</td>
<td>PB</td>
<td>C</td>
<td>2 ml/in 2 aliquots USC</td>
<td>C</td>
<td>80 /in 4 aliquots (0.2 ml each)</td>
<td>63</td>
<td>G1 12; 90; G3 10</td>
<td>IA 31; IB 45; IC 6; II A 6; IIB 1</td>
<td>All pelvic except 5 patients</td>
<td>LPS</td>
<td>77</td>
<td>9</td>
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<tr>
<td>Cordero Garcia et al.</td>
<td>2012</td>
<td>19</td>
<td>17</td>
<td>0</td>
<td>MB</td>
<td>C</td>
<td>2 ml/in 2 aliquots</td>
<td>NC</td>
<td>74 /2 doses 39-84</td>
<td>3</td>
<td>G1 12; G2 6; G3 1</td>
<td>1A 7; 1B 10; 1I 11; 1II 11</td>
<td>Pelvic in 15 and both pelvic and extrapelvic in 2</td>
<td>LP</td>
<td>N/A</td>
<td>3</td>
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</tbody>
</table>
following table 2. — Summary of the included studies information.

<table>
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<tr>
<th>First author</th>
<th>Year</th>
<th>No. of patients</th>
<th>No. of patients with detected SN</th>
<th>No. of patients with positive SN</th>
<th>Type</th>
<th>Injection</th>
<th>Radiotracer</th>
<th>Mean age</th>
<th>Grade</th>
<th>Stage</th>
<th>SLN distribution</th>
<th>No. of positive node using IHC</th>
<th>Type of surgery</th>
<th>No. of positive node using imaging</th>
<th>Imaging</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barlin et al.</td>
<td>2012</td>
<td>425</td>
<td>339</td>
<td>37</td>
<td>IB or MB</td>
<td>C (in 34 also SS)</td>
<td>4 ml (2 ml deep, 2 ml superficial)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Pelvis in 384; peri-aortic region in 2 (not known if they are in the group with fundal injection or not), and in both the pelvic and peri-aortic area in 15 (not known if they are in the group with fundal injection or not)</td>
<td>253/401</td>
<td>LPS 147; RA 189; LP 89</td>
<td>9 out of 32</td>
<td>N/A</td>
<td></td>
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<tr>
<td>Leitao et al.</td>
<td>2011</td>
<td>119</td>
<td>88</td>
<td>N/A</td>
<td>IB or MB</td>
<td>C (in 22 also SS)</td>
<td>4 ml (2 ml deep, 2 ml superficial)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>The RA group</td>
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<td></td>
<td></td>
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<tr>
<td>Leitao et al.</td>
<td>2011</td>
<td>151</td>
<td>136</td>
<td>N/A</td>
<td>IB or MB</td>
<td>C</td>
<td>4 ml (2 ml deep, 2 ml superficial)</td>
<td>FSC C</td>
<td>37-148 / 0.5-1 ml</td>
<td>N/A</td>
<td>The LPS group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rossi et al.</td>
<td>2012</td>
<td>16</td>
<td>14</td>
<td>2</td>
<td>ICG</td>
<td>C</td>
<td>1 ml in two aliquots</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Imaging was performed intraoperatively using near infrared imaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holloway et al.</td>
<td>2012</td>
<td>35</td>
<td>35</td>
<td>10</td>
<td>IB and ICG</td>
<td>C</td>
<td>1-2 ml IB and 0.5 ml ICG in four aliquots</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Detection rate for both methods were 100%. However bilateral detection rate was 34/35 for ICG and 27/35 for IB method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buda et al.</td>
<td>2012</td>
<td>25</td>
<td>22</td>
<td>3</td>
<td>MB</td>
<td>C</td>
<td>1 ml in two aliquots</td>
<td>NC C</td>
<td>4 injection of 5.7 ± 0.3</td>
<td>N/A</td>
<td>Identification for both planes and SPECT/CT imaging 3 hours post-injection N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How et al.</td>
<td>2012</td>
<td>100</td>
<td>92</td>
<td>9</td>
<td>PB</td>
<td>C</td>
<td>0.9 ml / 4 injections</td>
<td>FSC C</td>
<td>0.1 ml / dose is not available</td>
<td>63</td>
<td>Both planar and SPECT/CT imaging 3 hours post-injection N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solima et al.</td>
<td>2012</td>
<td>76</td>
<td>80</td>
<td>10</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>NC SE</td>
<td>57.7</td>
<td>I 39; II 5; III 14; IV 1</td>
<td>Pelvic only 26; para-aortic only 2; both locations 31</td>
<td>N/A</td>
<td>LP 49; 6 out of 10</td>
<td>Dynamic for 10 minutes and static every 5 minutes for 1 hour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. — Funnel plot of the detection pooling rate.

Figure 4. — Forrest plot of the pooling sensitivity.

Figure 5. — Funnel plot of the pooling sensitivity.
Learning curve effect

Introduction of sentinel node mapping into a surgical community needs certain time for the surgeons to gain enough experience. This concept has been shown in breast cancer surgery [60]. In the present meta-analysis, several studies reported learning curve effect on the mapping success. Khoury-Collado et al. reported that four out of five false negative cases in their study occurred when surgeons had less than ten patients experience, and detection rate during 2006 and 2007 was 37/46 while during 2008-2010 it was 82/86 [7]. Zenzola et al. reported that four detection failure in their study was in their first patients [44]. Delaloye et al. also reported that all 11 failures occurred during learning curve phase [31]. Dittmann et al. reported that most of the failures occurred during learning curve without giving any information [48]. Finally Li et al. reported that their first four patients had defective injection which were excluded from the analysis [40].

Overall, it seems that learning curve effect is also present in sentinel node mapping of endometrial cancer (high false negative and low detection rate) and surgeons should consider it before routine use.

Publication bias

Publication bias is an important issue which should be addressed in all systematic reviews. For minimizing this bias, the present authors searched several databases and extended no language limit in this search. They also included meeting abstracts to the systematic review. One study in Polish [20], two studies in Czech [26, 47], three studies in Chinese [29, 35, 46], and two studies in Spanish [44, 55] were included. Six of the included studies were meeting abstracts [27, 33, 45, 48, 49, 52], one was a proceeding paper [19], and one was a thesis [35].

Despite these efforts, funnel plots of detection rate and sensitivity pooling showed some asymmetry, although Egger’s test was not statistically significant in either one. This shows that publication bias can be a concern in the present meta-analysis as an important limitation.

Limitations

One of the major limitations of the present study is the quality of the included studies. Twenty-nine of the included studies did not recruit patients in a consecutive fashion. The spectrum of the included patients was not broad enough in some groups. For example Gien et al., Burke et al., and Frumovitz et al. only included patients with high risk of metastasis in their study [15, 25, 32]. Most importantly, the gold standard used by most studies was pelvic lymphadenectomy and para-aortic lymph node dissection that was performed in selected cases or not performed at all. Only ten studies included routine para-aortic lymphadenectomy in their study [15, 16, 24, 28, 31, 32, 41, 43, 46, 54]. This can influence the false negative rate of the present meta-analysis and is a major limitation this our study.

Another limitation is the low incidence of positive lymph nodes in endometrial carcinoma. Many studies included in this meta-analysis only had one patient with positive lymph nodes and overall 187 patients with positive nodes were included in the current systematic review. Although limiting the sensitivity pooling to the larger series did not affect the sensitivity (89% sensitivity when including only studies with more than five patients with involved nodes), it seems that larger studies with more positive lymph node patients are still required before considering sentinel node mapping a safe method in endometrial cancer.

Conclusion

Sentinel node mapping is feasible in endometrial cancer. Cervical injection, as well as using both blue dye and radiotracer, results in the highest detection rate and sensitivity. Larger studies are still needed to evaluate the false negative rate and the factors influencing the sensitivity in more detail.

Acknowledgements

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References

A comparison of outcomes between concurrent chemoradiotherapy and radiotherapy alone in cancer of the uterine cervix: a single institutional experience

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¹Departments of Radiation Oncology, ²Departments of Obstetrics and Gynecology, Dongsan Medical Center, Keimyung University School of Medicine, Daegu (Korea)

Summary

Purpose: To compare failure patterns and evaluate prognostic factors related to survival rates after concurrent chemoradiotherapy (CCRT) or radiotherapy (RT) alone in cervical cancer. Materials and Methods: From January 1996 to December 2006, 218 patients with cervical cancer (FIGO Stage IB₂ - III) treated with CCRT or RT alone as primary treatments were included, retrospectively. One-hundred eight patients were treated with CCRT and 110 with RT alone. Results: There was no significant difference in failure patterns between the treatment groups, but distant metastasis was the predominant pattern in both groups. The frequent metastatic sites were supraclavicular lymph node, lung, and brain. Treatment group, diabetes, and FIGO Stage were found to be significant for overall survival (OS) and disease-free survival (DFS), and initial hemoglobin level for DFS. Conclusion: Distant metastasis is the predominant failure pattern and diabetes is one of the independent prognostic factors to survival rates in this study.

Key words: Uterine cervical neoplasm; Concurrent chemoradiotherapy; Radiotherapy; Treatment outcome; Prognosis.

Introduction

Over the past few years, surgery and radiotherapy (RT) composed of external beam radiotherapy (EBRT) followed by intracavitary brachytherapy (ICBT), either alone or in combination, has been the standard treatment in cervical cancer [1-4]. There have been many studies, including proton therapy, and concurrent use of hyperthermia to improve loco-regional control and survival rates, but as currently used do not result in significant improvement [5, 6].

Several studies using concurrent administration of chemotherapy with RT for various cancer sites were carried out based on the concept that concurrent use of chemotherapy and RT may be beneficial to local control or survival rates, with favorable results for concurrent chemoradiotherapy (CCRT) [7-9]. Findings suggest that addition of chemotherapy to RT could have a synergistic effect, increasing the radio-sensitivity of tumors. In cervical cancer treatment, several randomized trials demonstrated the superiority of cisplatin-based CCRT, and the combined therapy has become a treatment of choice [10-14]. Based on those reports, in 1999 the US National Cancer Institute (NCI) recommended the use of cisplatin-based chemotherapy during RT for cervical cancer [15]. Nevertheless, to the best of the authors’ knowledge, there are few reports that compare patterns of failure between CCRT and RT alone in cervical cancer. A meta-analysis by Green et al. showed a reduction in local recurrence and distant metastases in CCRT compared to RT alone [16]. Therefore, this study was conducted retrospectively in cervical cancer patients to compare patterns of failure between these two treatment groups and to evaluate prognostic factors related to survival rates.

Materials and Methods

Patients characteristics

From January 1996 to December 2006, 271 women with biopsy-proven, International Federation of Gynecology and Obstetrics (FIGO) Stage IB₂-IIIB cervical cancer were treated with curative CCRT or RT alone at Keimyung University Dongsan Medical Center. Of the 271 patients, 53 with incomplete courses of RT or without regular follow-up after completion of RT were excluded and 218 patients were analyzed. This study was approved by the Institutional Review Board of Keimyung University Dongsan Medical Center, and informed consent was waived.

The patients underwent physical examinations, complete blood counts (CBC), liver function tests, urinalysis, chest X-ray, intravenous pyelography, sigmoidoscopy, cystoscopy, and pelvic magnetic resonance imaging (MRI) or computed tomography (CT) before treatments. All patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) Classification. The initial hemoglobin level for DFS was used to change the clinical FIGO staging [17]. Patient performance status was evaluated according to the guidelines of the Eastern Cooperative Oncology Group (ECOG) [18]. During the treatment, physical examinations and CBC were checked at least once a week. When the absolute neutrophil count was < 1,000/mm³ or the platelet count < 50,000/mm³, treatments were interrupted or delayed until the patient’s condition recovered. Red blood cell transfusion was given to patients with hemoglobin levels below 10.0 g/dl (Table 1).
EBRT was delivered using 6/10/15/20 mega-voltage photon beams to the whole pelvis with/without upper-extended fields including para-aortic area with a four-field box technique to a dose of 45 Gy in 25 fractions for five days per week within five weeks. For defining the pelvic fields in most of the patients, the superior border was the L4-L5 interspace and the inferior border was a transverse line below the obturator foramen. The L5-S1 interspace was considered the superior border in patients who were elderly, in poor general condition, or who had no lymph node involvement. The inferior border was moved to a lower position to create an adequate three-cm inferior margin from the gross tumor. The lateral border was two cm lateral to the true pelvis. On the lateral portal, the anterior border was placed anterior to the symphysis pubis and the posterior border was defined, including the entire sacrum or at least a three-cm margin posterior to areas of gross disease. A midline shield on the anteroposterior-posteroanterior portals was adapted after 36 - 45 Gy to the pelvis. A follow-up study using a CT scan was evaluated after 45 Gy EBRT and an additional boost radiation dose to the gross residual para-aortic or pelvic lymph node areas was administered at a dose of nine to 18 Gy.

After EBRT to the pelvis and upper-extended areas, patients received ICBT twice a week using a high-dose rate brachytherapy unit with 60Co or 192Ir sources. For ICBT, 60Co was used until October 1998 and 192Ir from November 1998. Most patients received 30 Gy administered as six fractions to point A, but some patients received 25 or 35 Gy according to the residual mass size or EBRT dose. Under local anesthesia, tandem and ovoids were inserted and the radioisotope was applied.

Chemotherapy was administered using platinum-based regimens. Of the 108 patients with CCRT, 53 received two cycles of 5-fluorouracil (5-FU) and cisplatin until 2002. Since 2003, the other 55 patients received three cycles of paclitaxel and carboplatin until 2006. In the 5-FU/cisplatin regimen, chemotherapy was administered every four weeks by intravenous infusion. Cisplatin (40 mg/m²) was given on the first day of the chemotherapy cycle (D1) within 16 hours after radiotherapy and 5-FU (4 g/m²) was given on the following four days (D2 to D5). In the paclitaxel/carboplatin regimen group, chemotherapy was administered every three weeks by intravenous infusion. Paclitaxel (135 mg/m²) and carboplatin (5 AUC/m²) were administered at the same time on the first day of each cycle (D1, D22, and D43).

Treatment details are listed in Table 2. One-hundred ten patients were treated with curative RT alone from January 1996 to December 2000 and 108 patients with CCRT according to treatment guidelines of this institution. There were 110 patients with RT alone from January 1996 to December 2000 and 108 patients with CCRT from January 2001 to December 2006. Patient ages were 31 - 84 years (median 58) in the CCRT group and 36 - 83 years (median 68) in the RT alone group. Vaginal bleeding and post-coital bleeding were the most common initial symptoms. Most patients showed good performance status, ECOG 0 or 1. On pathologic examination, 193 patients (88.5%) were squamous cell carcinoma and 25 (11.5%) showed adenocarcinoma. There was no significant difference in the distribution of patient characteristics between the two groups except nodal involvement. Fifty-four patients (50%) showed positive lymph nodes in the CCRT group, compared to 35 patients (31.8%) in the RT alone group (p = 0.006).

Chemotherapy was completed in 91 patients (84.3%) among the 108 patients, CCRT group. According to chemotherapeutic regimen, 51 patients (96.2%) completed in 5-FU/cisplatin regimen and 40 patients (72.7%) in paclitaxel/carboplatin regimen.

**Results**

**Patients characteristics and treatment**

The pretreatment characteristics of the 218 patients are listed in Table 1. Treatment groups were divided into RT alone and CCRT according to treatment guidelines of this institution. There were 110 patients with RT alone from January 1996 to December 2000 and 108 patients with CCRT from January 2001 to December 2006. Patient ages were 31 - 84 years (median 58) in the CCRT group and 36 - 83 years (median 68) in the RT alone group. Vaginal bleeding and post-coital bleeding were the most common initial symptoms. Most patients showed good performance status, ECOG 0 or 1. On pathologic examination, 193 patients (88.5%) were squamous cell carcinoma and 25 (11.5%) showed adenocarcinoma. There was no significant difference in the distribution of patient characteristics between the two groups except nodal involvement. Fifty-four patients (50%) showed positive lymph nodes in the CCRT group, compared to 35 patients (31.8%) in the RT alone group (p = 0.006).

Chemotherapy was completed in 91 patients (84.3%) among the 108 patients, CCRT group. According to chemotherapeutic regimen, 51 patients (96.2%) completed in 5-FU/cisplatin regimen and 40 patients (72.7%) in paclitaxel/carboplatin regimen.
Patterns of failure

Three months after completion of treatments, 103 patients showed CR and five patients showed PR in the CCRT group, whereas 106 patients showed CR, and four patients showed PR in the RT alone group. For patient status, there were 76, 0, 10, and 22 patients in the CCRT group, and 46, 1, 33, and 30 patients in the RT alone group who fell into the following categories (respectively): no evidence of disease, alive with disease, death from other causes, and death from cervical cancer.

There was no significant difference in patterns of failure between the two groups ($p = 0.617$). In the CCRT group, there were eight patients with loco-regional recurrence, 12 with distant metastases, and one with both, whereas in the RT alone group there were nine patients with loco-regional recurrence, 18 with distant metastases, and two with both (Table 3).

In the CCRT group, specific sites of loco-regional recurrence were cervix (two patients), vagina (three patients), both adnexa (one patient), and pelvic lymph nodes (two patients). Of the eight patients with loco-regional recurrence, two with local recurrence showed lesions confined to the cervix that were diagnosed 55 and 56 months after completion of treatment and that were proven to have no residual lesion after undergoing additional treatment. Of the 12 patients with distant metastases, six showed lymph node metastases in supraclavicular, para-aortic or aorto-caval areas, five in lung, brain or abdominal cavity, and one in pelvic bone. One patient with right supraclavicular lymph node metastasis only received chemotherapy, a combination of paclitaxel/cisplatin, and showed complete regression of the lesion with regular follow-up; however, the other five patients with para-aortic, aorto-caval or supraclavicular lymph node metastases died due to disease progression. The remaining patient with both loco-regional recurrence and distant metastases showed recurrence in the cervix one year after completion of CCRT and underwent chemotherapy with combination of paclitaxel/carboplatin. However, distant metastases in lung and para-aortic lymph nodes were found two years thereafter; she died due to pulmonary failure after irinotecan chemotherapy.

In the RT alone group, four patients showed loco-regional recurrence at the cervix only, four patients in the cervix, bladder or pelvic lymph nodes, and one patient at the cervix and vaginal stump. The four patients with recurrence in the cervix were diagnosed 8, 15, 18, and 20 months after RT and died from disease progression. Of the 18 patients with distant metastases, 11 had metastasis in lung, brain, liver, abdominal cavity, or transverse colon, four had metastasis in sacral bone or lumbar spine, and three had metastases in para-aortic or supraclavicular lymph nodes. One of the patients with distant metastasis in the lung showed complete regression of lung metastasis after five cycles of paclitaxel/cisplatin chemotherapy but died with intercurrent disease 27 months thereafter.

Of the five patients with PR in the CCRT group, four died with disease progression and one is alive with no evidence of disease after additional chemotherapy and extrafascial rectal dissection.

### Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>CCRT* (n = 108)</th>
<th>RT† alone (n = 110)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>31 - 84</td>
<td>36 - 83</td>
<td>0.45</td>
</tr>
<tr>
<td>Median</td>
<td>58</td>
<td>68</td>
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</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB2</td>
<td>12 (11.1%)</td>
<td>8 (7.3%)</td>
<td>0.176</td>
</tr>
<tr>
<td>IIA</td>
<td>17 (15.7%)</td>
<td>28 (25.5%)</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>52 (48.1%)</td>
<td>55 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>IIII</td>
<td>27 (25.0%)</td>
<td>19 (17.3%)</td>
<td></td>
</tr>
<tr>
<td>ECOG†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>87 (80.6%)</td>
<td>79 (71.8%)</td>
<td>0.306</td>
</tr>
<tr>
<td>1</td>
<td>20 (18.5%)</td>
<td>30 (27.3%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1 (0.9%)</td>
<td>1 (0.9%)</td>
<td></td>
</tr>
<tr>
<td>Nodal involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic or para-aortic</td>
<td>(-)</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>54 (50.0%)</td>
<td>75 (68.2%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>103 (95.4%)</td>
<td>99 (90.0%)</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>5 (4.6%)</td>
<td>11 (10.0%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>95 (88.0%)</td>
<td>93 (84.5%)</td>
<td>0.464</td>
</tr>
<tr>
<td></td>
<td>13 (12.0%)</td>
<td>17 (15.5%)</td>
<td></td>
</tr>
<tr>
<td>Pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous           ‡</td>
<td>93 (86.1%)</td>
<td>100 (90.9%)</td>
<td>0.365</td>
</tr>
<tr>
<td>Adenocarcinoma ‡</td>
<td>6 (5.6%)</td>
<td>7 (6.4%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>9 (8.3%)</td>
<td>3 (2.7%)</td>
<td></td>
</tr>
<tr>
<td>Initial hemoglobin</td>
<td>≤ 12 g/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 (58.3%)</td>
<td>55 (50.0%)</td>
<td>0.217</td>
</tr>
<tr>
<td>&gt;12 g/dl</td>
<td>45 (41.7%)</td>
<td>55 (50.0%)</td>
<td></td>
</tr>
</tbody>
</table>

*concurrent chemoradiotherapy, †radiotherapy, ‡International Federation of Gynecology and Obstetrics, §Eastern Cooperative Oncology Group, ‡squamous cell carcinoma.

### Table 2. — Treatments.

<table>
<thead>
<tr>
<th></th>
<th>All (n = 218)</th>
<th>CCRT* (n = 108)</th>
<th>RT† alone (n = 110)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT† duration</td>
<td>≤ 64</td>
<td>123 (53 (49.1%)</td>
<td>70 (63.6%)</td>
<td>0.03</td>
</tr>
<tr>
<td>(days)</td>
<td>&gt; 64</td>
<td>95 (55 (50.9%)</td>
<td>40 (36.4%)</td>
<td></td>
</tr>
<tr>
<td>EBR† fields</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvis only</td>
<td>126 (57 (52.8%)</td>
<td>69 (62.7%)</td>
<td>0.137</td>
<td></td>
</tr>
<tr>
<td>Extended field</td>
<td>92 (51 (47.2%)</td>
<td>41 (37.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBR† dose</td>
<td>Range</td>
<td>36 - 72</td>
<td>36 - 72</td>
<td>0.613</td>
</tr>
<tr>
<td>(Gy)</td>
<td>Median</td>
<td>54</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>ICBT‡ dose</td>
<td>Range</td>
<td>25 - 35</td>
<td>25 - 35</td>
<td>0.072</td>
</tr>
<tr>
<td>(Gy)</td>
<td>Median</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Bladder dose</td>
<td>Range</td>
<td>5.7 - 39.1</td>
<td>5.7 - 39.1</td>
<td>0.373</td>
</tr>
<tr>
<td>(Gy)</td>
<td>Median</td>
<td>16.1</td>
<td>16.3</td>
<td></td>
</tr>
<tr>
<td>Rectal dose</td>
<td>Range</td>
<td>6.3 - 46.8</td>
<td>8.3 - 46.8</td>
<td>0.5</td>
</tr>
<tr>
<td>(Gy)</td>
<td>Median</td>
<td>14.9</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-FU</td>
<td></td>
<td>/cisplatin</td>
<td>53 (53 (49.1%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Paclitaxel/carboplatin</td>
<td>55 (55 (50.9%)</td>
<td>0 (0.0%)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 (1.9%)</td>
<td>0 (0.0%)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>66 (61.1%)</td>
<td>0 (0.0%)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40 (37.0%)</td>
<td>0 (0.0%)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Extravesical splenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>199 (95 (88.0%))</td>
<td>104 (94.5%)</td>
<td>0.085</td>
<td></td>
</tr>
<tr>
<td>hysterectomy</td>
<td>(+)</td>
<td>19 (13 (12.0%))</td>
<td>6 (5.5%)</td>
<td></td>
</tr>
</tbody>
</table>

*concurrent chemoradiotherapy, †radiotherapy, ‡external beam radiotherapy, §intracavitary brachytherapy, 5-Fluorouracil.

### Table 3. — Patterns of failure.

<table>
<thead>
<tr>
<th></th>
<th>CCRT* (n = 108)</th>
<th>RT† alone (n = 110)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRR†</td>
<td>8 (7.4%)</td>
<td>9 (8.2%)</td>
<td></td>
</tr>
<tr>
<td>DM‡</td>
<td>12 (11.1%)</td>
<td>18 (16.4%)</td>
<td>0.617</td>
</tr>
<tr>
<td>LRR and DM‡</td>
<td>1 (0.9%)</td>
<td>2 (1.8%)</td>
<td></td>
</tr>
</tbody>
</table>

*concurrent chemoradiotherapy, †radiotherapy, ‡locoregional recurrence, ‡distant metastasis.
hysterectomy (EFH). However, of the four patients with PR in the RT alone group, three died due to disease progression after completion of primary treatment and the remaining one patient also died with liver metastasis that developed two years after completion of RT.

Distant metastasis was the predominant pattern of failure in both groups, and distant metastasis-free survival (DMFS) in CCRT group was significantly better (p = 0.044). Local DFS and regional DFS were not significantly different (p = 0.101, p = 0.064) between treatment groups.

Most of genitourinary or rectal complications were grade 1 or 2. There were four patients who showed genitourinary complications in the CCRT group and one patient in the RT alone group. There were also four patients with rectal complications in the CCRT group and eight patients in the RT alone group. There were also four patients with rectal complications in the CCRT group and one patient in the RT alone group.

Discussion

The present study was conducted to compare patterns of failure between CCRT and RT alone and to evaluate prognostic factors related to survival rates in cervical cancer, but there were several limitations to this retrospective study. There was no significant difference in the distribution of patient characteristics between the groups, including age, FIGO Stage, comorbidity conditions like hypertension and diabetes, pathology, and ECOG performance status, except nodal involvement. However, 50% of patients in the CCRT group had nodal involvement before treatment, whereas 31.8% in the RT alone group did (p = 0.006). Although it is thought that nodal involvement would result in poorer clinical outcomes, CCRT has shown better outcomes (including survival rates) and comparable patterns of failure compared with RT alone in this study.
For cervical cancer treatment, RT alone in combination with EBRT and ICBT was a standard treatment in this institution until the 1990s. Based on data from previous randomized clinical trials with concurrent use of cisplatin-based chemotherapy [10-14] and a meta-analysis [16], CCRT with a five-FU/cisplatin regimen at three-week intervals has been used since January 2001. The chemotherapeutic regimen was changed to paclitaxel/carboplatin at four-week intervals in January 2003 following several studies which demonstrated advantages of using paclitaxel and carboplatin [20-22].

Recently (since January 2007), a weekly cisplatin regimen for CCRT has been adopted based on results of the Gynecologic Oncologic Group (GOG) [12, 14]. However, no study has compared clinical outcomes after CCRT among the chemotherapeutic regimens in this institution. Although it was feasible to report satisfactory results for survival rates and several prognostic factors in this study, an additional study with a weekly cisplatin regimen that is recently used would be needed to verify the improvements of outcomes compared to other chemotherapeutic regimens.

In this study, the differences in patterns of failure between the two treatment groups were not significant. However, distant metastasis was a predominant pattern of failure in both groups. DMFS in the CCRT group was significantly better (p = 0.044). These results showed a different pattern of failure from the previously mentioned meta-analysis [16]. In the meta-analysis, using the methods of the Cochrane Collaboration, CCRT showed significant reductions in local recurrence (odds ratio = 0.61; 95% CI, 0.51 - 0.73) and distant metastasis (odds ratio = 0.57; 95% CI, 0.46 - 0.77). In most trials in the meta-analysis, patients with FIGO Stage I or II were dominant and adjuvant chemotherapy was not applied as it was in this study. Morris et al. [13] also showed different patterns of failure compared to this study, although it cannot be compared directly. They compared pelvic radiation plus concurrent chemotherapy with pelvic and para-aortic radiation for high-risk cervical cancer patients. Local recurrence rates were 19% in the CCRT group and 35% in the RT group (p < 0.001), and distant metastasis rates were 14% in the CCRT group and 33% in the RT group (p < 0.001). In the study by Morris et al. [13], approximately 70% of patients
had Stage IB–IIIB disease in each group. In the other study, by Parker et al. [23], they reported OS and toxicities after CCRT. The standard regimen consisted of 45 Gy of EBRT to the pelvis with concurrent weekly cisplatin (40 mg/m²), followed by 24 Gy in four fractions of ICBT. The predominant sites of relapse in their patients were distant, but specific sites of failure were not described.

Survival rates in this study showed satisfactory results, although those cannot be exactly compared with other trials which have shown the survival rates for various FIGO Stages and different chemotherapeutic regimens in patients after CCRT or RT alone [13, 24-26]. Also, the median follow-up period after treatment was longer than those of other studies, but survival rates were comparable in CCRT and RT alone groups. However, DFS after RT in Stage IB2 patients seemed to be lower in the present results, and it is thought that a relatively small patient number in the RT alone group (eight patients) caused the lack of a significant difference.

For the evaluation of prognostic factors to survival rates, FIGO Stage, diabetes, and treatment group (CCRT vs RT alone) were found to be related to OS and DFS. Pre-treatment hemoglobin level was related only to DFS. FIGO Stage is already known to be one of the most important prognostic factors related to patient prognosis [17]. Hemoglobin level as a prognostic factor for better outcomes in CCRT or RT was also noted in many studies [27-31]. They suggested that anemia may reflect tissue hypoxia related to radio-sensitivity. It has also been suggested that anemia might characterize biologically more aggressive tumors [32, 33].

Diabetes was another independent prognostic factor to survival rates in this study, but, to the best of the authors' knowledge, there has been no study that demonstrated diabetest as a prognostic factor after cervical cancer treatment with RT with/without chemotherapy. There have been a few reports about the impact of diabetes on survival rates or responses after treatments in other cancer patients [34-36]. Theoretically, it is believed that relatively worse survival outcomes in patients with diabetes are caused by problems in capillary vessels which may limit delivery of radio-sensitizing agents and poorer oxygen permeation, both of which can decrease tumor response to RT [37]. Therefore, it is believed that controlling hyperglycemia is needed for maintaining capillary vessel function in patients with diabetes. Also, further prospective study is needed to evaluate the impact of diabetes on clinical outcomes in cervical cancer with RT.

In comparisons of results after CCRT or RT alone, there was no difference in patterns of failure between the two groups in this study, which is not consistent with other randomized trials or a meta-analysis, but distant metastasis was a predominant pattern of failure. This study showed better outcomes in OS and DMFS with CCRT and satisfactory OS and DFS in each treatment group compared to previous studies. It was also confirmed in multivariate analysis that treatment group, FIGO Stage, and diabetes were independent prognostic factors for both OS and DFS, and initial hemoglobin level for DFS.

Conclusion

For treatments with CCRT or RT alone, pre-treatment evaluation of comorbidities including diabetes and anemia is necessary to improve survival rates and other treatment options to reduce distant metastasis, should also be evaluated. It is also suggested that further prospective or case-matched studies are necessary according to specific FIGO Stages or chemotherapeutic regimens.

References

Adjuvant treatment for uterine leiomyosarcoma

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Summary
The aims of this study were to evaluate outcomes in women diagnosed with uterine leiomyosarcoma (LMS). A retrospective chart review was conducted. Fifty-eight women with LMS were identified. Of the evaluable 52 patients (six patients were excluded), 73% had Stage I/II disease, and 27% had Stage III/IV disease. Sixty-three percent of patients received chemotherapy (97% doxorubicin-based therapy), eight percent received radiation alone, and 29% received no therapy. For patients with Stage I/II disease, no improvement in OS was demonstrated when adjuvant therapy was administered. There was a significant difference in OS (p = 0.0005) for patients with advanced Stage (III/IV) disease that received adjuvant chemotherapy. OS of the entire group, when adjusted for stage, failed to reveal a significant survival advantage for those receiving chemotherapy-based (p = 0.22). The present findings suggest further research into the role of chemotherapy in early stage disease is needed to better refine optimal treatment.

Key words: Leiomyosarcoma; Chemotherapy; Radiation.

Introduction

Uterine sarcomas are rare tumors and account for one to three percent of uterine neoplasms [1]. The most common histological types are leiomyosarcoma and endometrial stromal sarcoma. Uterine sarcomas are extremely aggressive and although the majority of patients present with early stage disease (I and II), five-year overall survival (OS) remains poor at 31% to 50% [2, 3]. Five-year OS for advanced stage is less than 20% [4-6]. The aggressive nature of uterine leiomyosarcomas (LMS) suggests the need for adjuvant therapy due to early metastatic disease and subsequent death. The five-year OS is 31% to 50% with a median survival of 16-52 months. Uterine leiomyosarcomas have a predilection for hematogenous dissemination resulting in distant metastasis signifying the need for adjuvant chemotherapy. The goal of adjuvant therapy is to eliminate micrometastases and prevent recurrent disease, arguing against the use of adjuvant radiation therapy. The cornerstone of treatment for early stage LMS is total hysterectomy with bilateral salpingo-oophorectomy. Unfortunately, most patients recur within two years of primary treatment [7]. Although some retrospective studies have suggested that adjuvant radiation therapy may decrease local recurrences [8-11], improvements in OS have not been demonstrated with this treatment modality. It is also noteworthy that many of the earlier studies included carcinosarcoma and endometrial stromal sarcoma along with LMS. There is a growing recognition that these histologic entities behave differently and should be evaluated independently. Furthermore, a recent randomized trial demonstrated no improvement in local recurrence, PFS or OS for 103 patients diagnosed with LMS. To date, this is the only randomized trial evaluating adjuvant RT in uterine sarcomas [12].

Since distant recurrences are more common, adjuvant chemotherapy is a rational approach. Chemotherapy regimens historically used in advanced uterine LMS have been similar to the ones used for other soft tissue sarcomas, with anthracyclines and ifosfamide being the most active drugs. More recently, Hensley et al. performed a phase II study evaluating the response rates of docetaxel and gemcitabine as first line therapy in patients with metastatic uterine leiomyosarcoma with response rates of 35% compared to historical response rates of 30% with doxorubicin and ifosfamide [13].

The use of adjuvant chemotherapy, in theory, should ideally eliminate any remaining micrometastasis and positively impact OS and progression free survival (PFS). Studies have shown that despite minimal residual disease after surgery, a significant number of patients demonstrate distant disease. With high recurrence rates after local treatment and adjuvant chemotherapy, the use of chemotherapy remains controversial. Several randomized trials of adjuvant chemotherapy have been completed in adult soft tissue sarcomas and the results are conflicting. In 1994, The European Organization for Research and Treatment of Cancer (EORTC) randomized patients with completely resected soft tissue sarcomas to receive either adjuvant cyclophosphamide, vincristine, doxorubicin, and decarbazine (CYVADIC) or no additional treatment [14]. Although recurrence free survival (RFS) was higher and the local recurrence rate lower in the chemotherapy arm, OS and rates of distant metastases were not affected by chemotherapy. Ravaud et al. demonstrated statistically significant differences in local recurrence, distant recurrences, and OS in 65 patients favoring the use of CYVADIC chemotherapy regimen versus no further therapy [15]. Currently there are no phase III trials demonstrating improvement in OS and PFS compared to no further adjuvant therapy in uterine leiomyosarcomas and no standard of care has been established.
Prior to 2006, CYVADIC was the most commonly used chemotherapy regimen used in the treatment of early stage uterine sarcomas at Roswell Park Cancer Institute. However due to its associated toxicities, platinum, doxorubicin, and cyclophosphamide (PAC) became the most commonly used regimen with the rationale that it would produce similar outcomes and decreased toxicity.

Materials and Methods

After obtaining Institutional Review Board approval, a retrospective chart review of all women seen at Roswell Park Cancer Institute with the diagnosis of LMS between 1990 and 2010 was conducted. Fifty-eight women diagnosed with LMS were identified; six patients were excluded because of incomplete data. Patient demographics, clinicopathologic data, and toxicities were extracted from patient charts. 2009 FIGO staging for uterine sarcoma was used to designate stage for the patients that were included in the review. Following completion of adjuvant therapy, surveillance was continued every three months for three years, every six months for an additional two years, and yearly thereafter. Only patients with a diagnosis of LMS were included.

Study outcomes included OS and time to progression, each measured from the time of definitive surgery. Progression was defined as objective evidence of recurrence since all therapy was given in the adjuvant setting. The duration of OS was the interval between definitive surgery and death. Observation time was the interval between definitive surgery and last contact (death or last follow-up). Data were censored at the last follow-up for patients with no evidence of recurrence, progression or death. PFS and OS were obtained using Kaplan-Meier curves and compared using the log-rank test statistic.

Results

Fifty-eight women with LMS were identified between 1990 and 2009 and six were excluded because of incomplete data. Of the evaluable 52 patients, 37 (71%) had Stage I, one (2%) had Stage II, eight (15%) had Stage III, and six (12%) had Stage IV disease. Thirty-three (63%) patients received chemotherapy, four (8%) received radiation alone, and 15 (29%) patients received no therapy (Table 1). Of the 33 receiving chemotherapy-based treatment, 32 received doxorubicin-based therapy. Doxorubicin based chemotherapy regimens included: mesna, doxorubicin, ifosfamide, and decarbazine (MAID) 35% CYVADIC 25%, PAC 16%, doxorubicin 9%, doxorubicin/ifosfamide 6%, doxorubicin/cisplatin 6%, doxorubicin/gemcitabine 3%. One patient received taxotere/gemcitabine. The specific chemotherapy regimens were selected at the discretion of the attending physician.

For patients with Stage I/II disease median OS was 53, 69 and 23 months for adjuvant chemotherapy, no adjuvant therapy or radiation alone respectively. No improvement in OS was demonstrated when adjuvant chemotherapy was administered (Figure 1). However there was an improvement in PFS for patients that received adjuvant chemotherapy (53 months) compared to the patients that received no further therapy (12 months) or radiation alone (12 months) (p = 0.006). There was a significant difference in OS (p = 0.0005) if patients with advanced stage disease received adjuvant chemotherapy compared to no additional therapy (Figure 2). For advanced stage disease, an improvement in PFS was demonstrated in patients that received chemotherapy with or without radiation (14 months) compared to patients that did not receive any additional therapy (one month) (p = 0.0005). OS of the entire group, when adjusted for stage, failed to reveal a significant survival advantage for those receiving chemotherapy-based treatment versus those receiving adjuvant radiation alone or no therapy (p = 0.22).

<table>
<thead>
<tr>
<th>Table 1. — Patient characteristics.</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>51 (34-76)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>45 (87%)</td>
</tr>
<tr>
<td>African-American</td>
<td>7 (13%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>38 (73%)</td>
</tr>
<tr>
<td>III/IV</td>
<td>14 (27%)</td>
</tr>
<tr>
<td>Residual disease</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (16)</td>
</tr>
<tr>
<td>No</td>
<td>41 (84%)</td>
</tr>
<tr>
<td>Radiation</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>44 (85%)</td>
</tr>
<tr>
<td>Brachytherapy</td>
<td>8 (15%)</td>
</tr>
<tr>
<td>Pelvic radiation</td>
<td>0</td>
</tr>
<tr>
<td>Brachytherapy &amp; pelvic radiation</td>
<td>0</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33 (63%)</td>
</tr>
<tr>
<td>No</td>
<td>19 (37%)</td>
</tr>
</tbody>
</table>

| Table 2. — Hematologic toxicities in triplet chemotherapy regimens compared to doublet chemotherapy regimens. |
|---------------------------------|--------|
| Doublet n = 6 | Triplet n = 23 | p value |
| Anemia |
| None | 1 | 4 | 0.2 |
| Grade 1/2 | 0 | 10 |
| Grade 3/4 | 1 | 2 |
| Unknown | 4 | 7 |
| Leukopenia |
| None | 1 | 3 | 0.08 |
| Grade 1/2 | 0 | 2 |
| Grade 3/4 | 0 | 11 |
| Unknown | 5 | 7 |
| Neutropenia |
| None | 1 | 3 | 0.08 |
| Grade 1/2 | 0 | 4 |
| Grade 3/4 | 0 | 2 |
| Unknown | 5 | 7 |
| Thrombocytopenia |
| None | 1 | 11 | 0.13 |
| Grade 1/2 | 0 | 3 |
| Grade 3/4 | 0 | 2 |
| Unknown | 5 | 7 |
| Cardiotoxicity |
| Yes | 1 | 14 | 0.06 |
| No | 0 | 2 |
| Unknown | 5 | 7 |
Adjuvant treatment for uterine leiomyosarcoma

Of patients receiving adjuvant chemotherapy, median OS was 48 months for the patients that received triplet combination-based chemotherapy compared to 14 months for the patients receiving doublet therapy ($p = 0.01$) with no demonstrable increase in toxicity ($p = 0.20$). Of the 37 patients with Stage I disease, 59% (22) patients developed recurrent disease. The mean time to recurrence for the no additional treatment group was 29 months compared to 22 months in the adjuvant chemotherapy.

Toxicity

As expected, the patients in the adjuvant chemotherapy groups experienced more adverse hematologic events; thrombocytopenia ($p < 0.001$), anemia ($p < 0.001$), leukopenia ($p < 0.001$), and neutropenia ($p < 0.001$) than the no additional treatment cohort. Table 2 depicts the hematologic toxicities experienced by the patients that received triplet chemotherapy regimens compared to doublet chemotherapy regimens. There was no increase in toxicity if patients received triplet compared to doublet therapy. When PAC, CYVADIC and MAID were compared, there was no difference in toxicity between the three regimens. However, patients receiving PAC chemotherapy were more likely to receive GCSF (granulocyte colony-stimulating factor) ($p = 0.03$). Two patients in the MAID group experienced cardiotoxicity, both requiring alterations in the chemotherapy.
Response data

Patients that received chemotherapy with or without radiation were less likely to have recurrent disease compared to those patients that received only radiation or no additional therapy ($p = 0.006$). Twelve (38%) patients in the chemotherapy group had recurrent disease compared to four (100%) in the radiation only group and eleven (79%) in the no additional therapy group. Eight (25%) patients in the chemotherapy group experienced progression while no patients in the radiation only or no additional therapy groups experienced progressive disease ($p = 0.07$).

The median OS in the no adjuvant treatment group was 69 months, 33 months in the adjuvant cytotoxic chemotherapy group, and 23 months in the radiation only group (Figure 3, $p = 0.62$). The median PFS in the no adjuvant treatment cohort was 12 months, 20 months in the adjuvant cytotoxic chemotherapy group, and 12 months in the radiation only group (Figure 4, $p = 0.03$). Patients that received adjuvant cytotoxic chemotherapy demonstrated a significant improvement in PFS. In a subset analysis of the doublet and triplet chemotherapy regimens, patients that had triplet regimen chemotherapy had an improvement in OS and PFS. Patients that received doublet regimen chemotherapy had an OS of 15 months compared to 48 months in the triplet regimen group ($p = 0.01$). Patients that received doublet regimen chemotherapy had a PFS of 12 months compared to 25 months in the triplet regimen chemotherapy group ($p = 0.02$). There was no improvement in OS ($p = 0.57$) or PFS ($p = 0.28$) in patients that received adjuvant radiation compared to the patients that did not receive adjuvant radiation. No difference in OS ($p = 0.31$) or PFS ($p = 0.39$) was detected in patients that received different triplet regimens.

A univariate analysis showed no statistically significant association with OS or PFS with adjuvant chemotherapy (yes/no), race or radiation. In the multivariate analysis, there was no statistically significant association with OS and adjuvant chemotherapy (yes/no) ($p = 0.22$), however there was an association with PFS ($p = 0.003$).

Discussion

In this retrospective data analysis, the authors report an objective response rate of 72% in patients with LMS treated with adjuvant doxorubicin-based chemotherapy. Only one of the 32 patients with Stage I/II disease had progression while eight of 12 patients with Stage III/IV disease had progression while receiving chemotherapy. Only two patients that were included received single agent chemotherapy with the remaining 32 patients receiving either doublet or triplet therapy in combination with doxorubicin. Overall, toxicity was well tolerated with no chemotherapy-related deaths.

There are several limitations to this study including a small sample size, the fact that the study was retrospective, and the heterogeneity of the chemotherapy regimens evaluated. While a meaningful subset analysis is limited by small numbers, this is the largest retrospective study evaluating the use of combination-based doxorubicin therapy in patients with LMS.

Pautier et al. evaluated the use of doxorubicin with cisplatin and ifosfamide, however only 13 patients that received chemotherapy had a diagnosis of LMS [16]. In 1973, the Gynecologic Oncology Group (GOG) performed a prospective trial of adjuvant doxorubicin in early-stage uterine sarcomas. Patients with a diagnosis of Stage I/II uterine sarcoma were randomly assigned to adjuvant chemotherapy with doxorubicin for six months versus no further treatment. Pelvic irradiation (external or intracavitary) was optional before randomization. Forty-eight of the 156 patients that were included had a diagnosis of LMS. Eleven of the 25 patients that received doxorubicin experienced a recurrence compared to 14 of 23 in the no additional therapy group. There was no significant difference between the two groups in response rates, progression-free interval (PFI) or PFS [17]. In this study, no difference in OS was detected for Stage I/II disease if patients received adjuvant chemotherapy ($p = 0.26$), however patients with Stage III/IV did have an improvement in OS if adjuvant chemotherapy was administered ($p = 0.0005$). When all stages were included adjuvant chemotherapy was not associated with an improvement in OS but was associated with an improvement in PFS ($p = 0.03$).

Bercuch et al. performed a retrospective study of Stage I and II uterine LMS which failed to demonstrate a decrease in recurrence rates in patients that received doxorubicin based adjuvant chemotherapy when compared to patients that did not receive any adjuvant therapy [18]. Kushner et al. reported on thirteen patients with completely resected uterine sarcoma that were treated with three cycles of adjuvant ifosfamide. The two- and three-year survival rates were 100 and 67%, respectively [19].

An outcome meta-analysis was performed by the Sarcoma Meta-Analysis Collaboration (SMAC) for over 1,900 patients from 18 randomized controlled trials (RCT) of adjuvant doxorubicin-based chemotherapy versus no further therapy. Thirteen trials evaluated doxorubicin alone, four trials evaluated doxorubicin plus ifosfamide and one evaluated doxorubicin in combination with both ifosfamide and dacarbazine [20]. The results of the meta-analysis showed improvements in local recurrence free interval (RFI), distant relapse-free interval, and overall RFS, with benefits for adjuvant chemotherapy of six, ten, and ten percent, respectively. The decrease in mortality with the use of doxorubicin chemotherapy did not reach statistical significance, while the addition of ifosfamide to doxorubicin chemotherapy did.

The most recent RCT on adjuvant chemotherapy for adult soft tissue sarcomas was conducted by an Italian cooperative group [21]. One hundred four patients were randomized after surgery to receive five cycles of high-dose epirubicin and ifosfamide versus no further therapy. The median RFI was 48 versus 16 months and median OS of 75 versus 46 months both in favor of the chemotherapy arm. These results can not be directly extrapolated to uterine sarcomas; however it does provide confirmatory
evidence for the potential benefit of adjuvant combination chemotherapy for the treatment of early stage sarcomas. In 1975, Gottlieb et al. reported a 49% response rate for 136 assessable patients with soft tissue sarcomas using the combination of cyclophosphamide, vincristine, doxorubicin, and dacarbazine (CYVADIC) [22]. This chemotherapy regimen has been evaluated in various adult soft tissue sarcomas and response rates have varied from 11% to 68%, but strong evidence of benefit in OS is lacking [20]. Piver et al. initiated a prospective trial of adjuvant chemotherapy with the CYVADIC regimen in patients with Stage I uterine sarcomas [23, 24]. The initial study on 11 patients followed for two to five years demonstrated an estimated five-year OS of 89% and a five-year RFS of 80% [23]. A follow-up report on 20 patients showed the five-year survival to be 68%, while the five-year PFS was 65% [24]. In 2004, Odusi et al. reported a follow-up of up to 17 years on 24 patients who received adjuvant CYVADIC chemotherapy for Stage I uterine sarcoma [25]. Eight patients developed recurrent disease with a median time to recurrence of 19 months. The estimated survival for the group was 88, 75, and 69% at two, five, and 15 years, respectively (compared to 42% five year survival of historical controls not receiving chemotherapy).

A recent phase II study from Hensley et al. evaluated the use of docetaxel and gemcitabine for front-line adjuvant therapy for uterine LMS with a reported response rate of 35%. The reported PFS and OS for all stages were 13 and 45 months, respectively [13]. The present authors report a PFS and OS of 20 and 33 months, respectively.

Despite the many studies evaluating adjuvant therapy in LMS, the precise role of adjuvant treatment and the specific regimen after hysterectomy remains controversial and no standard of care has been established. To date, there are no prospective data proving a difference in outcomes in OS or PFS. The rarity of uterine LMS has made it difficult to prospectively enroll patients in trials to define the optimal adjuvant therapy. Doxorubicin alone or in combination with other cytotoxic chemotherapy has been used for the treatment of metastatic soft-tissue sarcomas and appears to be effective in some trials causing tumor regression [19, 24, 26-28]. The present study demonstrates that adjuvant triplet doxorubicin-based chemotherapy is well tolerated with minimal toxicity (compared to dooublet and single agent therapy) and improvement in OS (48 vs 15 months). Prospective studies comparing doxorubicin-based regimens with gemcitabine and docetaxel, as well as the role of biologic agents, such as bevacizumab, are warranted to optimize adjuvant therapy and to improve survival and quality of life for patients with this aggressive disease.

References


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Laparoscopic management of early stage ovarian cancer: is it feasible, safe, and adequate? A retrospective study

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Minimally Invasive Gynecological Surgery Unit, S. Orsola Hospital, University of Bologna, Bologna (Italy)

Summary

Introduction: Minimally invasive surgery to stage early ovarian cancer is still regarded as pioneering among gynecologic oncologists. Previous retrospective experiences demonstrated the safety and feasibility of laparoscopy in this field. Aims: To review the laparoscopic staging procedure in a series of patients with early ovarian cancer and compare results with the literature. Materials and Methods: From January 2004 to September 2011, 19 patients with apparent early stage ovarian/fallopian tube cancer Stage IA to IC underwent either primary treatment or completion staging by laparoscopy. Surgical, pathologic, and oncologic outcomes were analyzed. Results: The mean operative time was 212 ± 69 minutes. Three patients (16%) underwent fertility sparing surgery. The mean estimated blood loss was two ± two g/dl. The mean number of pelvic and para-aortic lymph nodes collected was 17 (range 7-27) and 14 (range 8-21), respectively. The mean volume of ovarian/tubal tumor was 119 cm³ (range 1.5-500). The disease was reclassified to a higher stage in ten women (52%). One major intraoperative complication (five percent) occurred which required the conversion to laparotomy. The mean follow up period was 30 ± 16 months (range 10-74). Overall survival and disease-free survival were 100% and 84%, respectively. Conclusions: Laparoscopic staging of early ovarian cancer appears to be feasible and comprehensive when performed by gynecologic oncologists experienced with advanced laparoscopy.

Key words: Early ovarian cancer; Laparoscopy; Staging; Minimally invasive treatment.

Introduction

Laparoscopic management of gynecological cancers is increasing. A national practice survey in France showed that in 2005, five percent of patients underwent laparoscopic treatment for gynecological cancer in a referral Center [1]. Many advantages have been evidenced compared to laparotomic approach when performing a minimally invasive technique for the management of early stage gynecological cancer: less intraoperative blood loss, shorter hospital stay, faster recovery, and less damage to body image [2]. Recently, laparoscopy has showed to be feasible and associated with less morbidity in obese women with early stage endometrial cancer [3] and in older patients with early stage gynecological malignancies [4].

While minimally invasive approach has been largely demonstrated as safe and feasible for early stage endometrial and cervical cancer, laparoscopic staging and management of early stage ovarian/tubal cancer is still under debate due to the concern about its feasibility and adequacy [2]. Nonetheless, early stage ovarian/tubal cancer is rare compared to advanced stage and it has numerous potential sites of occult metastatic disease [5]. Previous retrospective experiences of laparoscopic management of early ovarian cancer (EOC) demonstrated the safety and feasibility of laparoscopy in this field, however this approach is still not recommended by the guidelines [2].

Materials and Methods

From January 2004 to September 2011, 19 patients with apparent early stage ovarian/fallopian tube cancer Stage IA to IC underwent either primary treatment or completion staging by laparoscopy.

Laparoscopic staging protocol fulfilled oncological standards and adhered to FIGO recommendations. Gross evidence of spread of the disease beyond the ovaries was regarded as an exclusion criterion. A conservative approach was used when patient desired to maintain fertility and with apparent Stage IA, with preservation of the uterus and contralateral ovary. The study was approved by the ethical committees of Sant’Orsola Malpighi Hospital and patients gave informed consent to the chart review of their reports.

Laparoscopic technique

Patients were placed in lithotomic position. After pneumoperitoneum was created, a 10-mm 0° operative laparoscope was introduced at the umbilical site. Under direct vision, three ancillary trocars were inserted, one ten-mm laterally to the epigastric artery and two of five-mm placed suprapubically and laterally to the epigastric artery. First, sterile saline solution was instilled for peritoneal washing and the liquid aspirated was sent for cytologic examination. Parietal and visceral peritoneal surfaces were carefully inspected, including diaphragm, liver, gallbladder, small bowel and mesentery, recto-sigmoid colon, pouch of Douglas, paracolic gutters, and abdominal wall. Subsequently, in those women not referred for restaging, the ovary with the suspicious mass was removed and retrieved via an endobag to avoid contact with the port sites and it was submitted for frozen section assessment. The surgical specimens were removed through the trocar. In case of large cystic tumors, puncture of the mass and aspiration were performed within the retrieval bag. When
solid components were encountered, they were removed by morcellation (while in the bag) using Kocher clamps or curved Mayo clamps.

Once the mass was removed in its entirety along with the bag, endobag integrity was verified and surgeons’ gloves were changed. After the diagnosis of malignancy, multiple random peritoneal biopsies were performed. Patient underwent hysterectomy with adnexectomy. Bilateral pelvic lymphadenectomy was performed and in all patients, internal iliac, internal iliac, and obturator lymph nodes were removed. The peritoneum was opened over the common iliac arteries and the incision was extended cephalad over the underlying inferior vena cava and abdominal aorta, exposing the ureters, gonadal vessels, and inferior mesenteric artery. Under direct vision of the above mentioned structures, common iliac, precardal, and para-aortic nodal dissection were performed. In some cases an additional three-mm ancillary trocar was placed in the left hypochondrium in order to introduce a grasper to retract the visceral peritoneum, allowing an easier access to the retroperitoneal space. The upper limit of the nodal harvest was the insertion of the right ovarian vein in the vena cava on the right side and the left renal vein on the left side. In order to prevent the contamination of the abdominal wall with malignant cells, a specimen bag was used to retrieve the lymph nodes, separately from each sidewall. Total infracolic omentectomy was then performed using scissors and bipolar coagulation. Appendectomy was performed by coagulation of the mesoappendix, ligature of the appendix by endo-loops, and resection. The surgical specimens were extracted from the abdomen by individual endobags. In case of unilateral tumor, fertility-sparing surgery was offered to young patients who desired preservation of reproductive potential, after a biopsy of the contralateral ovary ruled-out the presence of malignant cells. In case of adhesions between the ovarian tumor and the pelvic peritoneum we performed pelvic peritoneectomy. In all cases, the peritoneal cavity was reinspected laparoscopically after closing the vaginal cuff to ensure adequate haemosta-
sis and abundant washing of the peritoneal cavity was then performed.

Results

A total of 19 patients underwent laparoscopic staging for presumed Stage I ovarian or fallopian tube cancer. Mean age of patients was 51 ± 14 years (range 20-74), mean body mass index was 22 ± 3 kg/m². Eleven patients had previous laparotomic surgery (eight appendectomies, one myomectomy, one hysterectomy, and three cesarean sections), and four patients had a history of previous laparoscopic surgery (one hysterectomy, one myomectomy, and three diagnostic laparoscopies). Thirteen (68%) patients presented with an adnexal mass and had their malignancies diagnosed on frozen-section analysis at the time of laparoscopic surgery at the present institution. One of them had received neoadjuvant chemotherapy before the end-staging operation. Six (32%) patients referred to the present Center for restaging after undergoing cystectomy or salpingo-oophorectomy after diagnosis on final pathology of an occult cancer. Histologic types and tumor grading are outlined in Table 1.

Three patients had fertility sparing treatment: one patient was 31-years-old and had granulose cells ovarian tumor grade 2. One patient was 20-years-old and had dysgerminoma tumor. The number and type of procedures performed in primary surgery and completion of staging are described in Table 2. The surgical outcomes are shown in Table 3.

A transaction of the right ureter during lumboaortic lymphadenectomy was the only intraoperative complication. The urologist attempted without success to perform ureteral reanastomosis through laparoscopy. The patient finally underwent laparotomic ureteral reanastomosis.

Regarding postoperative complications (within 30 days from surgery), there were three minor complications: one paralitic ileus, one vulvar edema, and one umbilical hematoma which were managed conservatively and resolved spontaneously. There was one major postopera-

### Table 1. — Histological characteristics and grading of tumor.

<table>
<thead>
<tr>
<th>Histologic type</th>
<th>N. patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (5,2%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>Granulosa Cell</td>
<td>2 (10,5%)</td>
</tr>
<tr>
<td>Dysegerminoma</td>
<td>1 (5,2%)</td>
</tr>
</tbody>
</table>

**Grade:**

<table>
<thead>
<tr>
<th>Grade</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>G2</td>
<td>2 (10.5%)</td>
</tr>
<tr>
<td>G3</td>
<td>11 (58%)</td>
</tr>
<tr>
<td>Not specified</td>
<td>2 (10.5%)</td>
</tr>
</tbody>
</table>

### Table 2. — Laparoscopic procedures performed.

<table>
<thead>
<tr>
<th>Procedures</th>
<th>N = 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic lymphadenectomy</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Lumboaortic lymphadenectomy</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>– transperitoneal</td>
<td>18 (95%)</td>
</tr>
<tr>
<td>– retroperitoneal</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Peritoneal biopsies</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Omentectomy</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Peritoneal cytology</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Pelvic peritonecetomcy</td>
<td>9 (47%)</td>
</tr>
<tr>
<td>Appendectomy</td>
<td>6 (31%)</td>
</tr>
<tr>
<td>Endobag used</td>
<td>15 (79%)</td>
</tr>
<tr>
<td>Intrapерitoneal tumor rupture</td>
<td>3 (16%)</td>
</tr>
</tbody>
</table>

### Table 3. — Surgical outcomes.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N = 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical time (min)</td>
<td>212 ± 69 (110-360)</td>
</tr>
<tr>
<td>Estimated blood loss (g/dl)</td>
<td>2 ± 2 (0,7-5)</td>
</tr>
<tr>
<td>Hospital stay (d)</td>
<td>6 ± 2 (2-13)</td>
</tr>
<tr>
<td>Volume of the ovarian/tubal tumor (cm³)</td>
<td>119 ± 148 (1.5-500)</td>
</tr>
<tr>
<td>Number of pelvic nodes</td>
<td>17 ± 7 (7-27)</td>
</tr>
<tr>
<td>Number of lumboaortic nodes</td>
<td>14 ± 5 (8-21)</td>
</tr>
<tr>
<td>Omental specimen (mm)</td>
<td>90 ± 30 (75-250)</td>
</tr>
<tr>
<td>Rate of upstaging</td>
<td>10/19 (52%)</td>
</tr>
<tr>
<td>Major intraoperative complicaions (%)</td>
<td>1/19 (5 %)</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD (range), where appropriate.
tive complication: a patient who had ureteral fistula diagnosed 24 days from surgery. The woman presented urine loss from the vagina. She underwent blind stenting with complete spontaneous resolution.

After a mean follow up of 30 ± 16 months (range ten-74), three recurrences occurred: two of them were patients previously treated in other Centers who underwent restaging surgery at the present Institution. One had pelvic recurrence at 30 months follow up and the other had a presacral lymph node recurrence at ten months follow up. One patient had pelvic and abdominal recurrences at 18 months follow up.

Discussion

From the results, some important questions emerged:

Is the laparoscopic staging of EOC feasible?

An important factor when a new approach is evaluated is the feasibility, defined as the state of being easily or conveniently performed. In the authors’ opinion, parameters which must be considered to assess the feasibility of laparoscopic technique are: operative time, rate of conversion to laparotomy, frequency of intraoperative mass rupture, and the possibilities to perform other surgical procedures. These parameters are strictly related with laparoscopic experience and its high learning curve [6]. In the present Minimally Invasive Surgery Center, the same surgeon and surgical team which have a consistent background in laparoscopic approach performed all the procedures. This may explain the relatively low operative time (around 212 minutes) compared to other studies [7-9]. The authors had one case of laparotomic conversion at the last step of the staging procedure due to intraoperative right ureteral resection. Mass rupture occurred in three patients: one patient was treated in another center and subsequently restaged in the present Center, two occurred in women with an apparent endometriotic ovarian cyst which was discovered to be malignant at the intraoperative frozen section examination. Tumor spillage into the peritoneal cavity is one of the major concerns of minimally invasive approach. However, the risk of tumor spillage is not specific to laparoscopy. Several studies were published on patients with EOC whose tumor was ruptured at the time of laparotomy [10-12]. Other surgical procedures performed by laparoscopic approach in the present series were appendectomy (31%) and pelvic peritoneectomy (47%). Fertility sparing surgery which was performed in three patients without recurrence during a mean follow up of 30 months, has demonstrated to be a reasonable alternative treatment for young women with Stage I epithelial ovarian cancer desiring fertility preservation [13, 14].

Is the laparoscopic staging of EOC adequate?

The frequency of intraoperative complications in the present study was 1/19 (5%): a case of ureteral injury during lumboaortic lymphadenectomy. The present results seem comparable with the ones of Colomer et al.: in the 20 patients identified in this retrospective review, one case of vascular injury was reported [15]. The study of Spirtos et al. evidenced a higher rate of intraoperative complications even if borderline ovarian tumors were considered, however it was a multicenter study, with different surgeons involved [16]. No intraoperative complications were noticed in other retrospective studies [9, 17].

The present results confirmed the well-known advantages of laparoscopy with a short hospital stay and reduced intraoperative blood loss.

Is the laparoscopic staging of EOC safe?

The etymology of the word “adequacy” is “to make equal”. Laparoscopy has to make equal results of laparotomy, which is the gold standard surgical approach to ovarian cancer [18], to be considered as an alternative approach to EOC. From the present results, the rate of upstaging, which may be considered as an index of adequacy of staging [17], was 10/19 (52%), higher compared to previous study [9, 15-17, 19] and to other studies using a laparotomic approach [5]. Five of them were upstaged to Stage IC due to positive peritoneal cytology, five were upstaged due to positive nodes or peritoneal biopsy. This data may be related with the high rate (58%) of undifferentiated tumor, G3 which affected the present patients.

In line with other studies [9, 15-17, 19] the mean number of nodes obtained in the present series of patients was 17 for pelvic nodes and 14 for para-aortic nodes. Three previous retrospective reviews evidenced no statistical difference in the number of pelvic and para-aortic lymph nodes retrieved with the two approaches [7, 8, 20]. Moreover, several studies with a larger number of patients have demonstrated the adequacy of laparoscopy for lymph node dissection [17].

The rate of recurrence in the present study was higher compared to other studies [4, 21], however it has to be underlined that their mean follow up evaluation was shorter and that the recurrences occurring in the present study involved patients who underwent a restaging procedure in this Center and not the primary treatment. This result may stress the importance of performing the tumor staging in tertiary care Center with a gynecologist oncologist expert.

This study is not without limitations. First limit is its retrospective design. Second, the fact that six patients were previously treated in another Center and restaged in the present Institution may bias the results. Third, the mid-term follow up evaluation: long-term survival results are important to compare minimally invasive surgical staging to the laparotomic approach.

In conclusion, when performed by appropriately skilled surgeons, laparoscopic comprehensive staging of EOC seems feasible and adequate with undoubted benefits for the women who may improve their functional status, lessen their suffering, and maintain their life’s dignity.
References


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Nationwide screening program for breast and cervical cancers in Hungary: special challenges, outcomes, and the role of the primary care provider

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²2nd Department of Obstetrics and Gynecology, Semmelweis University, Budapest (Hungary)

Summary

Introduction: Breast and cervical cancers are both common malignancies in Hungarian women. The aim of this study was to evaluate the impact of nationwide screening programs on the incidence and mortality of breast and cervical cancers and to assess the role of primary care providers in this context. Materials and Methods: Published records from 2000-2011 on breast and cervical cancer screening activities in Hungary were reviewed. Previously unpublished data from the Hungarian National Cancer Registry were also included in this review. Hungarian outcomes were compared to international results. Results: A nationwide screening program for breast cancer was established in Hungary in 2001. A similar program for cervical cancer was subsequently initiated in 2003. As of 2009, 50% of the population at risk took advantage of breast cancer screening, while the exact participation rate for cervical cancer screening could not be established due to deficiencies of reporting by private gynecologists. The Health Visitors Cervical Screening Program, a new initiative within the context of the nationwide cervical screening program, based on involvement of local primary care providers, had encouraging results which substantially raised participation rates. However, deficiencies were identified regarding flow of information between service providers, patients, and family physicians. There was a slight reduction in the incidence of breast cancer and a more pronounced reduction in the incidence of cervical cancer, as well as a reduction in mortality for both breast and cervical cancers associated with these screening initiatives. Conclusion: The inclusion of primary care providers may benefit nationwide screening programs by raising participation rates in the target population.

Key words: Breast cancer; Cervical cancer; Hungary; Screening program; Public health; Primary care.
smear technique, which is cheaper but yields a lower rate of satisfactory samples; and the more expensive liquid-based test [11]. As with breast cancer, the introduction of screening programs for cervical cancer was also associated with a decline in mortality. The reduction in mortality persisted decades after the introduction of screening. This phenomenon was observed in five Northern European countries including Denmark, Finland, Iceland, Norway, and Sweden where national screening programs have been present for decades [12].

The Hungarian College of Obstetrics and Gynecology guidelines recommend that cervical screening should always include a colposcopic examination simultaneously with the performance of a Pap smear. A Hungarian study from the 1990’s revealed that false negative results on Pap smears could be significantly reduced with a combined Pap smear and colposcopic exam [13].

**Materials and Methods**

**Institution of nationwide screening programs in Hungary**

The Hungarian Ministry of Welfare initiated nationwide screening programs for prevention and early detection of different chronic diseases in 1997. These screening programs assumed active participation by primary care providers. Included in these nationwide screening programs were cancer-screening initiatives for both breast and cervical cancers [14].

Both the original guidelines by the Ministry of Welfare and their subsequent modifications detailed the expectations regarding the particulars of implementation for the screening programs. These included the identification of acceptable screening methods and appropriate target populations. A modest financial compensation was also offered to participating family physicians. At the same time only very limited information was provided on the practical means to ensure adequate participation by the target population.

The nationwide public health program was subsequently launched in 2001 by the Ministry of Health. This initiative was also supported by the Hungarian government. Its official designation was “Public Health Program for a Healthy Nation, 2001-2010”. The main objectives set for this ambitious initiative included a decrease in mortality for almost all non-communicable chronic diseases. The target for mortality reduction in breast cancer was set at 20%; for cervical cancer it was 50% [15].

**Breast cancer screening**

A network of mammography stations, which also included mobile screening units, was established. This was accomplished by the National Public Health and Medical Officer Service (NPHMOS) which also received some aid from contracted private investors. The nationwide breast cancer screening program was primarily financed by the Health Insurance Fund organized by the Hungarian government.

The screening program was launched in 2002. All Hungarian women aged 45-64 years were eligible to participate in the screening program, which consisted of biannual mammography. The coordination division of the NPHMOS department of screening activities sent letters to all eligible women to invite them to participate in the screening program, each prospective participant in designated locations within her respective geographical area. In these letters, lists of participating mammography stations as well as participating family physicians were provided. The designated family physicians reported all participating subjects annually.

**Cervical cancer screening**

Cervical cancer screening at university clinics has been done since the 1950’s. It was an ad hoc screening activity performed locally, targeting small groups of women, primarily those employed in nearby factories or offices. During the early 1970’s, the Hungarian National Institute of Oncology organized mass cervical screenings for women involving both colposcopy and Pap smear. This so called “Cervixprogram” was started in 1981.

Subsequently in the 1990’s, cervical cancer screening as well as various other health initiatives benefited from financial support by the World Bank. Beginning in 2003, these World Bank-supported initiatives became part of the official public health program in Hungary.

With the initiation of the nationwide cervical screening program in 2003, all women aged 25-65 years became eligible for participation. The frequency of screening depended on the test result. If the initial result was negative, subsequent screening was performed every three years. In all cases, the screening method involved both Pap smear and colposcopic examination [14]. This combined screening method is unique to Hungary and differs from standard international guidelines in that colposcopic examination is not routinely included in the latter. It follows that the role of the gynecologist is markedly different in Hungary vs. most other countries; in Hungary the gynecologist is involved throughout the whole screening process, whereas his/her role is limited to the second phase of screening internationally and is mainly confined to the follow-up and treatment of positive cases [15].

Since the initial reports suggested an unacceptably low participation rate in the nationwide cervical cancer screening program, the Ministry of Health decided to launch a new model screening initiative in cooperation with NPHMOS. This new initiative under the designation “Health Visitors Cervical Screening Program” began in 2009 in the form of a pilot study [16, 17]. This latter initiative took advantage of the active participation of an existing community health nurse network in Hungary and required cooperation by community health nurses in reaching local residents in their designated areas.

**Results**

**Breast cancer screening**

Before the initiation of the nationwide screening program, only 27.4% of Hungarian women aged 45-64 years had received screening mammography. During the first phase of implementation (2002-2003), total percentage of women receiving mammograms increased to 61%. However, this percentage included all mammograms irrespective of whether they were actually performed as part of the nationwide program. It has been observed that the total number of mammograms performed outside the framework of the screening program rose substantially at this time period. At the same time, large local differences in mammography rates persisted among different Hungarian counties [18].

Compared to the first phase of screening (2002-2003), participation rates declined during the second phase (2004-2005). Total number of women screened as well as percentage participation during the second phase are presented
in Table 1 [19]. As it is demonstrated in Table 1, participation rates of follow-up cases after positive or suspicious cytological findings were substantially higher. The ratio of women undergoing surgery may also have been higher than reported due to under-reporting from non-participating health facilities. The nadir in participation rates (36.7%) was reached in 2005. Subsequently, participation rates approached half the population (Figure 1) [20].

Cervical cancer screening

Several Hungarian studies analyzed the changes in outcomes before vs. after the implementation of the nationwide cervical screening program [21-22]. These reports confirm that a total of 1,667,618 women were screened in the time period before implementation (2000-2002). This number increased to 1,749,498 after the implementation of nationwide screening (2003-2005). According to the Health Insurance Fund database, this amounts to 820,000-890,000 Pap smears performed annually, considering that some women required follow-up testing. These figures translate into a 48.9% participation rate within the target population before implementation of the program vs. 52.6% following implementation (an increase of 3.7%).

Table 1. — Outcomes of the second phase of the nationwide breast cancer screening program (2004-2005) [19].

<table>
<thead>
<tr>
<th>Number of women</th>
<th>Total number (in 1,000 subjects)</th>
<th>Participation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contacted</td>
<td>1,255</td>
<td></td>
</tr>
<tr>
<td>Participated</td>
<td>461</td>
<td>38.1</td>
</tr>
<tr>
<td>Contacted to follow-up</td>
<td>31.2</td>
<td></td>
</tr>
<tr>
<td>Attended follow-up exam</td>
<td>26.8</td>
<td>86.0</td>
</tr>
<tr>
<td>Surgical procedures offered</td>
<td>2.22</td>
<td></td>
</tr>
<tr>
<td>Surgeries performed</td>
<td>1.66</td>
<td>75.0</td>
</tr>
</tbody>
</table>

Table 2. — Participation rate in cervical screening before (2000-2002) and after (2003-2005) implementation of the nationwide cervical screening program, expressed as percentage of women receiving PAP smear testing using either the target population (age 43-64 years) or the entire Hungarian female population as reference [21].

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hungarian female population</td>
<td>16.2</td>
<td>15.5</td>
<td>15.4</td>
<td>31.2</td>
<td>16.3</td>
<td>16.4</td>
<td>16.8</td>
</tr>
<tr>
<td>Target population</td>
<td>23.3</td>
<td>22.1</td>
<td>22.0</td>
<td>48.9</td>
<td>23.4</td>
<td>23.6</td>
<td>24.3</td>
</tr>
</tbody>
</table>

Table 3. — Outcomes of the “Health visitors” screening program for cervical cancer (2009-2011) [16, 17].

<table>
<thead>
<tr>
<th>Year</th>
<th>Screening campaign season (months per year)</th>
<th>Health visitors contacted (n)</th>
<th>Subjects screened (n)</th>
<th>Participation rate (%)</th>
<th>Positive result on screening (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>4.5</td>
<td>110</td>
<td>30,717</td>
<td>4,764</td>
<td>15.8</td>
</tr>
<tr>
<td>2010</td>
<td>4</td>
<td>213</td>
<td>45,899</td>
<td>5,117</td>
<td>11.1</td>
</tr>
<tr>
<td>2011</td>
<td>1</td>
<td>181</td>
<td>25,258</td>
<td>3,771</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Figure 1. — Percent participation in the nationwide breast cancer screening program (2002-2009) [20].

Figure 2. — Incidence and mortality rates of female breast cancer in Hungary, between (2001-2010) [24-26].

Figure 3. — Incidence and mortality rates of female cervical cancer in Hungary, between (2001-2010) [24-26].

Table 3 shows the results of the Health Visitors Cervical Screening Program initiated in 2009. The success of this initiative is reflected by the fact that a two to three times greater number of women could be screened, as compared
to the results of the organized nationwide screening program.

Although the nationwide cervical cancer screening initiative specifically aimed at a higher participation rate within the framework of the program, most Pap smears were actually performed outside this network: in 2006 the ratio of Pap smears performed by non-participating private healthcare providers vs those involved in the nationwide screening program amounted to 20:1 [22].

Incidence and mortality of breast and cervical cancers

The increment in cumulative probability for both breast and cervical cancer deaths for women in Hungary aged 15-79 years declined between 1980 and 2010 [23]. Although these results are encouraging, Hungarian women still have a relatively high mortality risk for breast and cervical cancer. Recent reports indicate that in the year 2009, total mortality figures for Hungarian women were 2,169 for breast cancer and 396 for cervical cancer [24].

The incidence of breast cancer measured through the use of diagnostic code C50 of the International Classification of Diseases (ICD-10) WHO coding system [24-26] in Hungary increased in the time period between 2001-2010 from a total of 6,198 to 6,610 cases (a 6.6% increase) [25]. The number of new diagnoses peaked in 2003, and subsequently showed a small but steady decline. Annual mortality rates declined for both cervical and breast cancers in this time period. Breast cancer mortality decreased from 2,304 in 2001 to 2,169 in 2009 (a decline of 5.86%). The respective figures for cervical cancer were 539 in 2001 vs 396 in 2009 (a decline of 26.53%). Taking the decade as a whole, the average annual mortality rate for cervical cancer (approximately 400 deaths per year) remains relatively high [24].

Incidence and mortality rates for breast and cervical cancers in Hungary in the time period between 2001 and 2010 are shown in Figures 2 and 3.

Discussion

What lies behind the low effectiveness of screening programs? The answer is complex and involves financial, administrative, social, educational, medical, and epidemiological factors. Economically, the problem could be described as a consequence of diminishing financial resources put aside for national public health programs, partly through increased bureaucracy and a related increase in administrative expenses. There are also substantial regional differences in how effective local administrative structures are in ensuring reliable flow of information and effective communication between screening coordinators and family physicians.

An additional bias is introduced by the tendency for wealthier and more health-conscious Hungarian women to attend private gynecology clinics not associated with the nationwide screening program. Considering the fact that deficiencies in reporting screening activities have been identified in private clinics, precision of data regarding the number of women actually screened is not always reliable. It is reasonable to assume that the actual number of women screened for both breast and cervical cancers could be substantially higher than actually reported.

As far as outcomes of the screening tests, Pap smear results performed by private gynecologists are not always reported to the Health Insurance Fund. Although all gynecological examinations performed by State insurance funded gynecologists are reported to the Fund, it is not always clear whether the examination was associated with a referral from the nationwide screening program vs an acute complaint. Ambiguity is also introduced by the fact that follow-up is not restricted to a standard time frame. In addition, there were cases where follow-up involved more frequent testing than standard practice. [27].

One of the key problems with nationwide screening programs is that participation rates remain unacceptably low. Persistently high mortality rates for breast and cervical cancers may partially reflect this issue. Some experts suggest that the participation of primary health care providers in organized screening activities may improve local accessibility to these programs, thus increasing participation rates [22].

The health visitor network has an important role in the Hungarian primary care. The health visitors are community health nurses in Hungary. These nurses work in cooperation with family pediatricians and family physicians. Their primary role is to provide care for women before, during, and after pregnancy, as well as the follow-up of children through adolescence. In rural areas, the health visitor network assumes an extended role involving midwife services under certain circumstances. The present results indicate that participation rates for cervical screening were two to three times higher when the network of health visitors actively participated. This suggests that participation rates could be further improved if cervical screening was included among the primary tasks of these primary care providers, especially if they also would receive financial incentives for this activity.

Several Hungarian authors propose that primary care providers should have an essential role in raising oncological awareness [28-29]. Advanced cases of cervical cancer due to late diagnosis is a particular problem among low-educated women in poor, rural areas. Many of these women never take part in any organized cervical screening [16].

The health visitor network may be particularly helpful in reaching this rural, high-risk population of women. It is therefore especially unfortunate that an increasing number of health visitors have been lost to the nationwide screening program due to increasing workload and related burnout. In 2011, fewer health visitors were trained than in previous years and seasonal campaigns dedicated to cervical screening were also shortened due to lack of resources [17].

It is the authors’ impression that screening programs were not sufficiently covered in the media. Also, participation in organized screening events may not have been sufficiently promoted by family physicians, perhaps because of lack of financial incentives. Studies also suggest that patients may not have received adequate information and proper medical advice on screening activities. Lack of information may have adversely affected participation rates and may have
increased risk for adverse psychological events in those who participated [30]. While a national screening program would provide an optimal setting for the provision of medical advice, this mechanism is thwarted by the tendency for a majority of Hungarian women to ignore invitation to organized screening in favor of engaging the services of a private physician. Perhaps this tendency could be partially remedied through improved education regarding the nationwide program [31].

Hungarian guidelines regarding cervical cancer screening are uniquely different from those in similar programs in other countries. The primary difference lies in the Hungarian guidelines requiring a simultaneous colposcopic examination in addition to the standard Pap smear. Colposcopic exam without a Pap smear is not recommended for population screening, although having high sensitivity, its specificity is unacceptably low [32]. As already mentioned, Hungarian studies suggest a benefit from the combined use of the two diagnostic methods [13].

There are international differences in age recommendations for both breast and cervical cancer screening. For instance, the ability of mammography to save lives seems to depend on which age groups are included. Certain age groups may minimally benefit with only one to three weeks of average lifetime gain [33].

Given the relatively recent initiation of the nationwide programs, so far there is insufficient data for proper epidemiological analysis of screening-related outcomes. International data indicate that both incidence and mortality tend to decrease several years after the implementation of cancer screening programs. Clinical experience suggests that incidence for a disease may initially increase after initiation of a screening program due to improved detection of early-stage disease. Mortality however, provided that appropriate therapeutic options are employed, tends to gradually decline. In general, morbidity and mortality data may not run parallel because, unlike morbidity, mortality is reported later, usually not in the year the diagnosis was actually made.

Participation rates in the nationwide screening programs were low. In the first three years of implementation, only 52.6% of the population at risk was screened for cervical cancer and 53.4% for breast cancer [21]. These results are highly unsatisfactory, especially when compared to those of similar screening programs in Western and Northern European countries. For instance, 75% of women were screened for breast cancer, and 80% for cervical cancer in the United Kingdom around the same time period, while in Finland 87% were screened for breast cancer and 69% for cervical cancer in 2005 [34].

Conclusion

Nationwide cancer screening programs could benefit from a more pronounced involvement of primary care providers. Structural changes may be warranted within the Hungarian healthcare system to facilitate involvement of primary care providers in breast and cervical cancer screening. This may require more focused efforts by relevant policy makers.

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Comparison of adjuvant chemotherapy and radiotherapy in patients with cervical adenocarcinoma of the uterus after radical hysterectomy: SGSG/ TGCU Intergroup surveillance

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Summary

Purpose: The authors conducted this retrospective study to evaluate the efficacy of radiotherapy (RT) for high-risk patients with adenocarcinoma (AC) compared with chemotherapy (CT) after radical hysterectomy. Materials and Methods: There were 263 patients with AC and 58 with adenosquamous cell carcinoma (ASCC). Of these 321 patients, 151 received adjuvant treatment. Of these 151 patients, 69 received radiotherapy (RT) alone, including concurrent chemoradiotherapy (CCRT) with weekly cisdiaminodichloroplatinum (CDDP), 64 patients received CT alone, and 18 patients received concomitant RT and CT (RT + CT). Results: The five-year overall survival (OS) was 70.9% for patients receiving RT, 79.2% for CT, and 66.2% for RT + CT. Adjuvant treatment did not affect the incidence or the pattern of recurrence. The incidence of lymph node involvement was 9.0% in Stage Ib1, 23.9% in Stage Ib2, 30.8% in Stage IIa, and 41.2% in Stage IIb. Conclusions: Adjuvant CT may be effective for high-risk patients with cervical adenocarcinoma.

Key words: Adenocarcinoma; Uterine cervix; Radiotherapy; Chemotherapy.

Introduction

The standard treatment for patients with International Federation of Gynecology and Obstetrics (FIGO) Stage Ib to II cervical cancer is radical hysterectomy and/or radiotherapy (RT). National Comprehensive Cancer Network (NCCN) clinical practice guidelines recommend RT and radical hysterectomy as useful treatment having an equal effect for patients with Stage Ia2 – IIa cervical cancer having a non-bulky tumor [1]. In Japan, the majority of gynecologic oncologists choose radical hysterectomy for patients with Stage Ib–IIb cervical cancer.

Gynecologic Oncology Group (GOG) showed that adjuvant pelvic RT following radical hysterectomy reduced the number of recurrences in Stage Ib patients with intermediate risk factors [2]. In addition, another GOG study suggested that RT with concurrent cisdiaminodichloroplatinum (CDDP) containing chemotherapy (CT) was more useful for Stage Ia2–IIa patients with pelvic lymph node involvement, parametrial extension, or a compromised surgical margin than RT alone after radical hysterectomy [3]. Consequently, patients with pathologic risk factors, such as pelvic lymph node involvement and parametrial extension, should receive adjuvant RT, including concurrent chemoradiotherapy (CCRT) after radical hysterectomy.

Over the past 24 years, the incidence of adenocarcinoma (AC) of the uterine cervix has increased from approximately 12% to 24% of cervical cancers [4]. It is controversial whether the prognosis of patients with cervical cancer depends on the histologic type [5-7]. A GOG study of 813 patients with Stage Ib cervical cancer, 645 with squamous cell carcinomas (SCC), and 168 with AC, including adenosquamous cell carcinoma (ASCC), suggested that no statistically significant differences were seen in the recurrence-free interval among histological types [5]. In contrast, Park reported that the survival difference between AC and SCC was small but significant [6]. The present authors also reported that Stage II patients with AC showed a significantly worse prognosis than those with SCC; however, the survival in Stage Ib patients did not differ between AC and SCC [7]. Although a few studies have assessed specific treatment for AC, NCCN clinical practice guidelines recommend that AC can be effectively treated in a similar manner to SCC [1].
Radiotherapy may be important in the treatment of patients with pathologic risk factors after radical hysterectomy. Landoni suggested that RT was less effective for patients with AC than those with SCC [8]. Niibe also reported lower radiosensitivity for AC compared with SCC [9]. Consequently, adjuvant RT might be limited for high-risk patients with AC. The present authors conducted this large retrospective study to evaluate the efficacy of adjuvant RT for high-risk patients with AC compared with adjuvant CT after radical hysterectomy.

Materials and Methods

A total of 321 patients with FIGO Stage Ib to Iib cervical AC, who underwent type III radical hysterectomy in 13 institutes (Hyogo Cancer Center, Kagoshima City Hospital, National Hospital Organization Shikoku Cancer Center, National Hospital Organization Kure Medical Center, Miyagi Cancer Center, Fukushima Medical University, Yamagata University, Akita University, Tohoku University School of Medicine, Hiroaki University School of Medicine, Iwate Medical University, Saitama Medical University International Medical Center and Tottori University Hospital) between April 1997 and March 2003, were enrolled in this study. Data were collected from the patients’ medical records. Patients’ characteristics are shown in Table 1. There were 263 patients with AC and 58 with ASCC. The study protocol was approved by the institutional review board at each institution.

One hundred seventy patients (53.0%) underwent radical hysterectomy alone. The remaining 151 patients (47%) received adjuvant treatment after radical hysterectomy. Of these 151 patients, 69 patients received RT alone including CCRT with weekly CDDP, 64 patients received CT alone, and 18 patients received concomitant RT + CT. The indications for adjuvant treatment were as follows: pelvic lymph node involvement, parametrial extension, deep stromal invasion, lymphovascular invasion, and a compromised surgical margin, although the indications for adjuvant treatment were not identical among our 13 institutions. External irradiation with a parallel opposing portal technique using Lineac was used for the adjuvant RT. External irradiation consisted of 10-20 Gy whole pelvis and additional parametral dose with midline block to deliver a total of 45-50 Gy to the pelvic sidewall. Intensity-modulated whole radiation therapy was not used in all 13 institutions at 2003. Most of the adjuvant CT, including 31 subjects for mytomycin C, etoposide and cisplatin combination CT (MEP), 11 for taxane compound and platinum compound combination CT, nine for cyclophosphamide, doxorubicin HCl and cisplatin combination CT (PAF), three for etoposide and cisplatin combination CT (EP), two for doxorubicin HCl and cisplatin combination CT (AP), irinotecan HCl and cisplatin combination CT, was platinum-based CT. The chemotherapeutic regimens and number of cycles were also decided in each institution.

Patient survival distribution was calculated using the Kaplan-Meier method. The significance of the survival distribution in each group was tested by the log-rank test. The chi-square test was used to compare any associations of prognostic factors. Additionally, multivariate analysis was performed with Stat View Version J-5.0 to fit the Cox proportional hazards model. A p < 0.05 was considered significant.

Results

The five-year progression-free survival (PFS) rate and overall survival (OS) rate were 89.8% and 91.4% in Stage Ib1, 66.2% and 76.2% in Stage Ib2, 46.2% and 61.5% in Stage Iia, and 51.9% and 63.7% in Stage Iib, respectively. No significant difference in the outcome was shown between patients with AC and those with ASCC (five-year OS; 83.7% vs. 82.2%, p = 0.9725). The incidences of lymph node involvement and FIGO Stage are shown in Table 2.

The five-year OS for patients with and without adjuvant treatment was 86.5% and 95.6% in Stage Ib1, 78.6% and 66.7% in Stage Ib2, 42.9% and 75.0% in Stage Iia, and 57.3% and 64.8% in Stage Iib, respectively. These differences were significantly different in all FIGO Stages. Of 151 patients receiving adjuvant treatment after radical hysterectomy, there were 70 patients in Stage Ib1, 33 in Stage Ib2, 8 in Stage Iia, and 40 in Stage Iib. Table 3 shows full details of the risk factors and adjuvant treatments after radical hysterectomy. The five-year OS for patients receiving RT was 70.9%, 79.2% for those receiving CT, and 66.2% for those receiving RT + CT (Figure 1). CT showed a superior outcome to RT or RT+CT, but the difference was not statistically significant (p = 0.3701).

### Table 1. Patients’ characteristics.

| Age | 46.2 (18 – 84) |
| FIGO Stage | |
| Ib1 | 211 |
| Ib2 | 46 |
| Iia | 13 |
| Iib | 51 |
| Histological type | |
| Adenocarcinoma | 263 |
| Adenosquamous cell carcinoma | 58 |
| Adjuvant treatment | |
| Yes | 151 |
| No | 170 |

### Table 2. The incidence of lymph node involvement.

| FIGO Stage | LN involvement (%) |
| Ib1 | 9.5% (20 / 211) |
| Ib2 | 23.9% (11 / 46) |
| Iia | 30.8% (4 / 13) |
| Iib | 41.2% (21 / 51) |

### Table 3. Pathological risk factors and adjuvant treatment.

<table>
<thead>
<tr>
<th>Pathological risk factor</th>
<th>CT</th>
<th>RT</th>
<th>RT + CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN involvement</td>
<td>13</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Parametrial extension</td>
<td>12</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Deep stromal invasion</td>
<td>19</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Vessel permeation</td>
<td>26</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td>Compromised surgical margin</td>
<td>1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Bulky tumor</td>
<td>26</td>
<td>28</td>
<td>9</td>
</tr>
</tbody>
</table>
Univariate analysis revealed that pelvic lymph node involvement, parametrial extension, and tumor size affected the outcome of patients with adjuvant treatment, but deep stromal invasion, vessel permeation and a compromised surgical margin did not (Table 4). Adjuvant treatment did not affect the outcome of patients with lymph node involvement (five-year OS; 42.0% for RT, 51.4% for CT, 52.7% for RT + CT, \( p = 0.8214 \)). In patients with parametrial extension, adjuvant treatment also did not affect the outcome (five-year OS; 52.4% for RT, 44.4% for CT, 16.7% for RT + CT, \( p = 0.1475 \)). Multivariate analysis revealed that pelvic lymph node involvement and parametrial extension were independent prognostic factors, but tumor size was not (Table 5).

Eighteen patients recurred of those receiving adjuvant CT, 28 of those with adjuvant RT, and five of those with concomitant adjuvant RT and CT. The incidence of recurrence was not significantly different among adjuvant treatments (CT: 24.3% (18/74), RT: 40.0% (28/70), RT + CT: 29.4% (5/17), \( p = 0.1269 \)). Adjuvant treatment also did not affect the pattern of recurrence.

**Discussion**

Many authors have attempted to clarify whether the histological type affected the outcome of Stage I-II cervical cancer [5-7, 10-12]. Shingleton reported that the histological type had no significant effect on survival in patients with Stage Ib cervical cancer [10]. Kasamatsu also reported that FIGO Stage I-II patients with SCC and AC showed similar prognosis in their retrospective analysis [11]. In contrast, Chen reported that the outcome of 277 patients with AC was significantly worse than 2,917 patients with SCC in Stage I-II [12]. Lai also demonstrated that the prognosis of AC and ASC was slightly worse than SCC [13]. The present authors’ previous study also revealed that patients with AC showed significantly worse prognosis than those with SCC [7]. It has been controversial whether the prognosis of patients with cervical cancer is dependent on the histologic type.

Lymph node involvement is one of the most important prognostic factors in patients with cervical cancer. It remains unclear whether patients with AC had a higher incidence of lymph node involvement than those with SCC. Nakanishi showed no significant difference in the frequency of lymph node involvement between patients with AC and SCC [14]. Sakuragi also demonstrated that the histological type did not affect the incidence of lymph node involvement in patients with Stage Ib–IIb (20.0% (9/45) vs. 27.0% (44/163)) [15]. In contrast, the largest series of patients with cervical cancer suggested that the incidence of lymph node involvement was more frequent in patients with SCC than in those with AC and ASC (9.5% (51/538) vs. 12.6% (279/2,217), \( p = 0.0466 \)) [10]. To the authors’ knowledge, the present data on the incidence of lymph node involvement in each stage are one of the largest series of Stage Ib–II patients with cervical AC. In this series, the incidence of lymph node involvement was 9.0% (19/211) in Stage Ib1, 23.9% (9/38) in Stage Ib2, 30.8% (4/13) in Stage Ia, and 41.2% (21/51) in Stage Ib2. The incidence of lymph node involvement gradually rose in proportion to the FIGO Stage.

**Table 4. — Univariate analysis in patients receiving adjuvant treatment.**

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>Number</th>
<th>Five-year OS</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>51</td>
<td>32.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Negative</td>
<td>100</td>
<td>84.5%</td>
<td></td>
</tr>
<tr>
<td>Parametrial extension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>30</td>
<td>32.1%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Negative</td>
<td>121</td>
<td>75.5%</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 4cm</td>
<td>61</td>
<td>50.2%</td>
<td>0.0002</td>
</tr>
<tr>
<td>&lt; 4cm</td>
<td>90</td>
<td>78.3%</td>
<td></td>
</tr>
<tr>
<td>Deep stromal invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
<td>62.1%</td>
<td>0.0518</td>
</tr>
<tr>
<td>Negative</td>
<td>114</td>
<td>81.1%</td>
<td></td>
</tr>
<tr>
<td>Vessel permeation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>63</td>
<td>64.3%</td>
<td>0.7402</td>
</tr>
<tr>
<td>Negative</td>
<td>88</td>
<td>68.5%</td>
<td></td>
</tr>
<tr>
<td>Compromised surgical margin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>50.0%</td>
<td>0.2005</td>
</tr>
<tr>
<td>Negative</td>
<td>143</td>
<td>67.7%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5. — Multivariate analysis in patients receiving adjuvant treatment**

<table>
<thead>
<tr>
<th>Prognostic Factors</th>
<th>Risk ratio</th>
<th>95% CI</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node involvement</td>
<td>6.003</td>
<td>2.954 – 12.198</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parametrial extension</td>
<td>2.115</td>
<td>1.108 – 4.039</td>
<td>0.0232</td>
</tr>
<tr>
<td>Tumor size</td>
<td>1.597</td>
<td>0.844 – 3.024</td>
<td>0.1503</td>
</tr>
</tbody>
</table>
A randomized study of radical surgery and/or adjuvant RT versus RT for Stage Ib–II cervical cancer showed that RT was less effective than surgery in patients with AC [8], suggesting low radiosensitivity for AC of the uterine cervix. In addition, Eifel reported that AC had a worse prognosis than SCC in 1,767 patients with Stage I cervical cancer receiving initial RT because of the higher incidence of distant metastasis in patients with AC; however, there was no significant difference in the rate of pelvic disease recurrence between patients with AC and SCC [16]. In the present series, adjuvant treatment did not affect the site of recurrence in patients with AC. Furthermore, adjuvant RT might induce a relatively high incidence of serious complications, including small bowel obstruction and leg lymphedema [17, 18]. Consequently, it is suspected that RT including CCRT is the best treatment for high-risk patients with AC.

Adjuvant CT can avoid these serious complications after radical hysterectomy. Furthermore, RT can be selected as a useful strategy for possible pelvic recurrence. In the present retrospective study, adjuvant CT was equally effective for patients with AC as adjuvant RT. Takeshima et al. also indicated the potential role of adjuvant CT for patients with cervical cancer because of the equal effect and lower toxicity than with adjuvant RT [19, 20].

Conclusion

CT may be effective adjuvant treatment for high-risk patients with cervical AC. A phase II study is necessary to evaluate the efficacy and safety of adjuvant CT for cervical AC patients with pathological risk factors. Additionally, a randomized phase III trial to compare adjuvant CT and adjuvant RT for high-risk patients with AC is also necessary in the near future.

References


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RNA interference targeting extracellular matrix metalloproteinase inducer (CD147) inhibits growth and increases chemosensitivity in human cervical cancer cells

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¹School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou
²Cancer Hospital of Gansu Province, Lanzhou (China)

Summary
Overexpression of extracellular matrix metalloproteinase (MMP) inducer (EMMPRIN CD147) has been implicated in the growth and survival of malignant cells. However, its presence and role in cervical cancer cells has not been well-studied. In the present study, small interfering RNA (siRNA) was designed and synthesized to breakdown the expression of CD147. The present data demonstrated that 24 and 48 hours after transfecting CD147 siRNA, both the CD147 mRNA and protein expression were significantly inhibited as determined by quantitative real-time polymerase chain reaction (RT-PCR) and immunocytochemistry. Meanwhile, simultaneous silencing of CD147 resulted in distinctly increasing MMP-9, VEGF, and MDR-1. Further studies demonstrated decreased CD147 expression, resulted in G1/S phase transition with flow cytometry analysis, as well as the resistance of the cells to 5-FU. These findings provide further evidence that CD147 may become a promising therapeutic target for human cervical cancer and a potential chemotherapy-sensitizing agent.

Key words: Endometrial carcinoma; Clear cell carcinoma; Solitary bone metastasis; Treatment.

Introduction
Cervical cancer takes the lives of more than 250,000 women annually worldwide, particularly in under-resourced areas of low-, middle-, and high-income countries [1]. Despite improved understanding of the pathogenesis of this malignancy, patients with advanced cervical cancer still have a poor prognosis despite undergoing conventional therapy with significant side-effects, and the treatment outcome for cervical cancer remains poor. Only modest improvements in survival have been reported and these are attributed mainly to earlier diagnosis [2]. Thus, novel therapies for cervical cancer are greatly needed.

Extracellular matrix metalloproteinase (MMP) inducer (EMMPRIN CD147) is a highly-glycosylated transmembrane protein of 60 kDa with an ectodomain consisting of two regions exhibiting the characteristics of the immunoglobulin superfamily [3]. CD147 is enriched on the surface of tumor cells and stimulates adjacent stromal cells to produce several MMPs [4]. Tumor invasion and metastasis are complicated multi-step processes. Among the requirements is degradation or remodelling of extracellular matrix and basement membrane macromolecules by proteolytic enzymes, among which MMPs are particularly implicated [5]. Elevated CD147 stimulates MMP production in stromal fibroblasts and endothelial cells, leading to extracellular matrix degradation, tumor growth promotion, and metastasis [6]. CD147 is reported to be involved in the progression of malignancies by regulating expression of VEGFs in stromal cells, as well as in tumor cells themselves. CD147 also stimulates the expression of vascular endothelial growth factor (VEGF) and contributes to genesis, growth, and local invasion of malignant cells [7, 8]. The CD147 is overexpressed in multidrug resistant (MDR) cancer cell lines, suggesting that during the development of a multidrug resistance phenotype, the expression of CD147 stimulates MMP activity in MDR cells [9].

The discovery of interfering RNA (iRNA) has generated enthusiasm within the scientific community, not only because it has been used to rapidly identify key molecules involved in many disease processes including cancer, but also because iRNA has the potential to be translated into a technology with major therapeutic applications [10]. These tools have helped to delineate the roles of various cellular factors in oncogenesis and tumor suppression and lay the foundation for new approaches in gene discovery. Furthermore, successful inhibition of tumor cell growth by iRNA aimed at oncogenes in vitro and in vivo supports the enthusiasm for potential therapeutic applications of this technique [11].

Overexpressed biomarkers are of special interest because they may not only be used to predict patient outcome, but may also serve as potential targets in cancer therapy. Extracellular MMP inducer may be one of them [12]. All of this prompted the authors to investigate the putative role of CD147 as a target for anticancer therapy; therefore in this study, three pairs of small interfering RNA (siRNA) were designed directly to down-regulate the expression of CD147 according to its sequence in the Genebank (Accession NM_001728.2) and influences to cell proliferation and chemosensitivity with breakdown of CD147 expression by siRNA as detected in Hela cell line.
Materials and Methods

Materials and reagent

Bovine serum, methyl thiazolyl tetrazolium (MTT), and dimethyl sulfoxide (DMSO) were purchased. Polymerase chain reaction (PCR) primers were synthesized. Human breast cancer MCF-7 cell originated from Lanzhou University.

Design and synthesis of siRNA

According to human CD147 gene sequence, three small interference sequence BSG1, BSG2, and BSG3 and negative control non-silencing non-BSG were designed and chemically synthesized. All nucleotide sequences of siRNA are shown in Table 1.

Cell culture and siRNA transfection

Human cervical cancer cell lines Hela cells were investigated in this study and cultured in DMEM medium supplemented with 10% bovine serum, 2.05 mM of L-glutamine, 100 UI/ml of penicillin and 100 UI/ml of streptomycin at 37°C with 5% CO2. Cells were seeded in a six-well plate at a density of 5 × 104 cells/well and allowed overnight growth to reach 80%-90% confluency. Cells were then transfected with different small interference sequences BSG1, BSG2, BSG3, and non-BSG following the protocol set by the manufacturer, and the final concentration of siRNA was 100 nmol/l.

MTT assay

To quantitatively assess cell proliferation, cells were seeded in a 96-well culture plate at an optimal density of 5 × 103 cells per well in triplicate wells. Fifty µl per well with five mg/ml MTT solution was added at 48 hours post-transfection. Then, the medium was removed by centrifugation after four hours of incubation at 37°C, 5% CO2 incubator, then harvested and washed twice with PBS, fixed and 1.5% agarose gels and the gray scale ratio was calculated. Each well at 570 nm was read on enzyme-linked immunosorbent assay reader. The cell inhibition ratio calculated as [1-A490 (5-FU/BSG)/A490] × 100% [13].

Reverse transcription-PCR

Cells were collected and total cellular RNA was extracted using Trizol reagent kit according to manufacturer’s instructions. The purity and amount of total RNA were determined using ultraviolet (UV) spectrophotometry. The isolated RNA was converted into cDNA using a reverse transcription kit. The primers corresponding to human CD147, MMP-9, VEGF, MDR-1, and β-actin are shown in Table 2.

In this study, two µl of follow board, one µl of sense, and antisense primers, 2.5 µl of MgCl2 (25 mmol/l), one µl of oligonucleotides triphosphates, 2.5 µl of 10 × PCR buffer, five units of Taq DNA polymerase, and 14 µl of double-distilled water were used in each PCR reaction in a 20 µl reaction volume. RT-PCR for analysis CD147, MMP-9, VEGF, MDR-1, and β-actin are shown in Table 2.

Immunocytochemistry

Hela cells were seeded at a density of 1 × 105 cells/well into six-well plates and were transfected with siRNA sequence. Cells were allowed to attach to precoated glass coverslips and fixed the following day in 4% paraformaldehyde and then washed twice with PBS (phosphate-buffered saline), and incubated for 20 min with 0.5% Triton X-100. Film preparation was then employed to visualize the staining of the interesting proteins targeted.

Flow cytometric

For flow cytometer analysis, cells were seeded in a 24-well plate at a density of 5 × 103 cells/well and incubated at 37°C, 5% CO2 incubator, then harvested and washed twice with PBS, fixed with ice-cold 70% ethanol at -20°C overnight at 48h post-transfection. Cells were then washed with PBS for three times and stained with propidium iodide (PI) (20 µg/ml) in the dark at room temperature for 20 min. Cell cycle analysis was done with FAC station equipped with Cell Quest, and the cell cycle phase distribution was calculated from the resultant DNA histogram using Multicycle AV software. Each group detected in triplicate experiments and mean were calculated [14].

Drug sensitivity

To assess the effect on chemosensitivity to 5-FU of siRNA sequences, siRNA transfected cells (5 × 105/well) were cultured for six hours in 96-well plates, and the culture solution was replaced a fresh one. Then 5FU (20 µl/ml) were added and incubated for another 48 hours. Cells were treated with MTT as described earlier. Each group contained five wells. The cell survival inhibition ratio calculated as [1-A490 (5-FU/BSG)/A490 (5-FU)] × 100% [15].

Statistical analysis

Results are expressed as means ± standard deviation (SD). Statistical analyses were performed using SPSS statistical software (SPSS17.0). Student’s t-test was used for comparison between two groups. Significance was defined as p < 0.05.

Table 1. — Sequences of siRNA.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSG1 Sense siRNA strand</td>
<td>CGUGAAGCAGACAUAGGATT</td>
</tr>
<tr>
<td>BSG1 Antisense siRNA strand</td>
<td>UCGGUAACAGGAGUACAGT</td>
</tr>
<tr>
<td>BSG2 Sense siRNA strand</td>
<td>CCGGUAACAAAGACUGATT</td>
</tr>
<tr>
<td>BSG2 Antisense siRNA strand</td>
<td>UCAGUAUCUGUACAGGATT</td>
</tr>
<tr>
<td>BSG3 Sense siRNA strand</td>
<td>UCCAAAGUCACUCCUUATT</td>
</tr>
<tr>
<td>BSG3 Antisense siRNA strand</td>
<td>UAAGAAGUGAGACUUGATT</td>
</tr>
<tr>
<td>non-BSG Sense siRNA strand</td>
<td>CCAGGAACAAUAAGACCTT</td>
</tr>
<tr>
<td>non-BSG Antisense siRNA strand</td>
<td>GUGCCUAUAUGUGACCGGTT</td>
</tr>
</tbody>
</table>

Table 2. — Sequences of primer.

<table>
<thead>
<tr>
<th>Name</th>
<th>Primer</th>
<th>Sequence</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD147</td>
<td>Upper</td>
<td>5’-GAGTACTCTGCGTCCTTGCC-3’</td>
<td>692</td>
</tr>
<tr>
<td>Lower</td>
<td>5’-CTTCATCTGCGCTTGCTG-3’</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Upper</td>
<td>5’-GCTGCACCTTCCGCTTGCC-3’</td>
<td>192</td>
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<tr>
<td>Lower</td>
<td>5’-CTCGGACAGGCTCCTTGCG-3’</td>
<td>650</td>
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<tr>
<td>VEGF</td>
<td>Upper</td>
<td>5’-ACAGCAGCAGGCTGCTTGCC-3’</td>
<td>404</td>
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<tr>
<td>Lower</td>
<td>5’-GCTGCCGCTGCTTGCC-3’</td>
<td>130</td>
<td></td>
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<tr>
<td>MMP-9</td>
<td>Upper</td>
<td>5’-GGCCGCTGCTTGCC-3’</td>
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<tr>
<td>Lower</td>
<td>5’-GCTGCCGCTGCTTGCC-3’</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>MDR-1</td>
<td>Upper</td>
<td>5’-GGCCGCTGCTTGCC-3’</td>
<td>404</td>
</tr>
<tr>
<td>Lower</td>
<td>5’-GCTGCCGCTGCTTGCC-3’</td>
<td>130</td>
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</tr>
</tbody>
</table>
Results

Breakdown of CD147 significant inhibition of cell proliferation

Overexpression of CD147 is known to stimulate cell growth. To assess the effects of CD147 breakdown in cell proliferation, three siRNA BSG1-3 were chemically synthesized to examine proliferation rate of cells and screen better interference sequence. After 48 hours of transfection, compared with the negative group, the CD147-siRNA BSG1 significantly decreased the growth rate of cervical cancer Hela cells (\(p < 0.05\)). The cell inhibition rate of tumor growth achieved to 56%. In contrast, there was little effect for BSG2, BSG3 (Figure 1A). The effects on cell modality of transfected interference sequence for 48 hours were observed by an inverted microscope. The authors found that cells of control non-BSG groups grew well, while CD147 silenced BSG1 groups exhibited a worse growth state. Compared with controls, some distinctions, as shrinkage, megaly, and vacuole on cells surface, were distinguished in BSG1 group. The distinctions are shown in Figure 1B.

iRNA-mediated inhibition of CD147 mRNA and protein levels in cervical cancer cells

The authors adopted siRNA to break down the expression of CD147 in cervical cancer cells, and transfection of the CD147-siRNA into cervical cancer cells led to a remarkable inhibition of CD147 mRNA expression by quantitative RT-PCR. In terms of brightness of the bands with analysis with BANDSCAN 5.0 software, the blank control and \(\beta\)-actin groups exhibited stronger expression than did BSG1 groups (Figures 2A, B). The expression of CD147 protein in cervical cancer cells transfected with CD147-siRNA was strongly suppressed. Immunocytochemistry staining analysis demonstrated thatuffy masculine marker located on the endochylema and cell membrane and distributed dispersely after 72 hours transfection. Compared with the blank group cells, chromatosis extent of BSG1 group cells is more identifiable. In contrast, there were little changes for BSG2, BSG3 in the expression of CD147 protein. The said delineation demonstrated that CD147 protein is down-regulated in the transfection, with which BSG transfected (Figure 2C).

Breakdown of CD147 blocked the cervical cancer cell proliferation

To further confirm that the breakdown of CD147 on cell proliferation, cell cycle was also detected with flow cytometry. When the expression of CD147 gene was silenced, there was an obvious change of cell cycle distribute in cervical cancer Hela cells (shown in Figure 3), and the number of cells was increased significantly in G0/G1 phase from 53.73 ± 1.4% to 86.33 ± 2.3%, and those in S and G2/M phases were reduced sharply from 46.27 ± 1.9% to 13.67 ± 1.1%. In the silencing group, the proportion of G1 phase cells increased, however, the proportion of S phase cells reduced significantly, indicating the cell proliferation index was significantly reduced. There was no significant change in the fluorescence control group and in the blank group.

Down-regulation of CD147 reduced mRNA expression of MMP-9, VEGF and MDR-1 in cervical cancer cell

To verify whether the invasion, metastasis-related genes, and multi-drug resistance (MMP-9, VEGF, and MDR-1) was suppressed by siRNA against CD147(CD147-siRNA), the authors determined the mRNA levels of these genes in tumor tissues in cervical cancer cell by quantitative real-time RT-PCR, using specific primers and probes for MMP-9, VEGF, and MDR-1 with \(\beta\)-actin as internal control. The relative quantification results show that tumor cells transfected with CD147-siRNA-BSG1 significantly reduced the mRNA levels of
Figure 2. — Suppression of CD147 mRNA and protein levels in cervical cancer cells by siRNA. Figure 2A shows iRNA effects of siRNA-CD147 in human cervical cancer cells by analysis with RT-PCR. Figure 2B shows the signal intensity of all band analyzed with bioinformatics software. Figure 2C: iRNA effect on CD147 protein expression evaluated by immunocytochemistry. I: represents Hela/non-BSG; II: represents Hela/BSG1, III: represents Hela/BSG2; IV: represents Hela/BSG3. Experiments were designed with non-silencing siRNA as negative group (100 nmol/l non-BSG) and siRNA-CD147 groups (100 nmol/l BSG1, BSG2 and BSG3).

Figure 3. — The effects of down-regulation of CD147 on mRNA expression of MMP-9, VEGF, and MDR-1 in cervical cancer cell. Figure 3A: mRNA expression of MMP-9, VEGF and MDR-1 was analyzed with RT-PCR and the content of the band was evaluated with BANDSCAN 5.0 software. Figure 3B: The signal intensity of b-actin had no discernible change between the BSG1 group and negative control non-BSG group. Compared with non-BSG group, signals intensity of MMP-9, MDR-1, and VEGF were distinctly weakened.
MMP-9, VEGF, and MDR-1, compared to those in cervical cancer cell negative groups (shown in Figures 4A, 4B).

**Down-regulation of CD147 enhanced the chemosensitivity of cervical cancer cell**

Hela, Hela-negative iRNA, and Hela-CD147-iRNA cells were treated with 5-FU to determine chemosensitivity. As shown in Figure 5, 5-FU reduced the growth of all cell lines. Compared with blank and negative RNAi cells, BSG1-RNAi cells displayed increased sensitivity to 5-FU ($p < 0.05$), especially at 25 nmol/l, which inhibition ratio of siRNA BSG1 groups was achieved in 67%. The iRNA mediated CD147 down-regulation may synergistically enhance the cytotoxicity of 5-FU.

**Discussion**

As a tumor-derived MMP inducer, CD147 stimulates fibroblast and endothelial cells to facilitate tumor invasion, metastasis, and angiogenesis [16]. The interaction between CD147 and MMPs have been reported [17]. Expression of CD147 was enhanced in a variety of human carcinomas and correlated with tumor progression and invasion by inducing the production of MMPs by stromal cells [18]. Special attention was devoted to MMP-2 and MMP-9 enzymes. Host-derived MMP-9 contributes to tumor incidence and proliferation in a model of skin carcinogenesis [19]. The silencing of CD147 by siRNA resulted in the decreased proliferation and invasion of A375 cells and the expression of VEGF, constitutively elevated in these cells, were down-regulated in vitro [20]. Study of Yang [21] showed that breakdown of CD147 on viability of cells grown as attached monolayer or suspension culture core was different and breakdown of CD147 could inhibit cancer cell survival by regulating intercellular contacts and promote anoikis. The result about RNAi inhibited expression of CD147 in Hela cells for the first time was related here. Expression level of MMP-9 mRNA was down-regulated on account of silencing CD147 via RNA interference. Besides, the proliferation was inhibited remarkably in Hela cell line.

There are some essential features for malignant tumors, such as immortalized cellular proliferation, activated invasiveness into the surrounding stroma, distant metastasis, and angiogenesis via VEGF production [22]. Cancer cells acquire resistance to anti-cancer drugs to draw assistance from the MDR phenotype frequently. Distant metastasis and MDR, the major obstacles to the effective treatment of malignant tumors, remain to be overcome [23].

VEGF, a homodimeric lycoprotein of the platelet-derived growth factor family, plays a pivotal role in tumor angiogenesis and lymphangiogenesis which are crucial for tumor growth, invasion, metastasis [24] and VEGF production and affects the outcome in patients with tumors [25, 26]. Numerous clinical studies have demonstrated that the elevated expression of VEGF is strongly correlated with the density of tumor microvessels, the potential for malignancy, and a negative patient prognosis [27]. In this data, not only was the expression of the CD147 gene and protein suppressed in cervical cancer cells, but also the expression of VEGF. The deduction that expression of CD147 was positively correlated with the expression of VEGF in cervical cancer cells needs to be confirmed.

Chemotherapy is highly-effective in treating a number of gynecologic malignancies; however, its effectiveness often diminishes with repeated exposure due to the emergence of MDR [28]. Overexpression of CD147 promotes
tumor cells metastasis and confers the MDR to P-gp substrate drugs in breast cancer cells MCF-7 [29]. Analogical dependability was reported in multidrug resistant cancer cells MCF-7, ADR, KBV-1, and A2780Dx5 [30-33]. The fluoropyrimidine drug 5-fluorouracil (5-FU) is widely used in the treatment of gastrointestinal, breast, head, and cervical cancer [34, 35]. In fact, the combination of 5-FU with other anticancer agents, such as cisplatin as a neoadjuvant chemotherapy, has improved the response rate for cervical cancer [36]. However, some patients have a poor response to 5-FU-based chemotherapy. In the present study, down-regulation of CD147, which provoked MDR-1 expression depression, not only influenced proliferation and apoptosis, but also increased chemosensitivity to 5-FU in Hela cells. This suggests that CD147 can protect pancreatic cancer cells from chemotherapy-induced apoptosis and CD147-iRNA break down inhibit the protective effect of increasing chemosensitivity. This result showed that CD147 is an adjuvant chemotherapy target of tumor.

The FCM result shows that the cell proliferation index in CD147 siRNA transfected cervical cancer Hela cells was decreased significantly. However, there was no significant change in the fluorescence control and blank group, which indicated that the cell cycle of Hela cells was inhibited while cells in quiescent stage increased. That is, the proportion of G1 phase cells increased, however, the proportion of S phase cells reduced significantly in the silencing group [37].

In mammals, siRNA is expected to become a powerful tool, not only for large-scale gene silencing essential for functional genomics, but also for therapeutic purposes, including anti-cancer treatments [38, 39]. The successful employment of an iRNA-based gene breakdown technique depends on the proper design or selection of the siRNAs, and the adoption of an effective strategy to deliver the siRNAs to the target cells or tissues [40], since it was discovered that not all siRNAs are equally potent in their ability to silence the gene products [41]. Although design algorithms have been improved over the last few years, there is still a risk that not all siRNAs chosen will result in significant break down within the given experimental setting [42]. Therefore, performance of each siRNA must still be proven experimentally when selecting the most efficient siRNA for loss-of-function studies.

In conclusion, the down-regulation of CD147 using iRNA successfully reduced cervical cancer Hela cells proliferation in vitro. These findings provide further evidences of involvement of CD147 in a variety of cancer key cellular events as a versatile signaling orchestrator, and suggest that CD147 would be a promising gene-targeting therapy for cervical cancer.

Acknowledgments

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RNA interference targeting extracellular matrix metalloproteinase inducer (CD147) inhibits growth and increases etc.


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Effectiveness of radiotherapy in patients with primary invasive vaginal carcinoma

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4Oncological Surgery Department, 5Diagnostic Radiology Department
Centre of Oncology, Maria Sklodowska-Curie Memorial Institute, Krakow Branch, Krakow (Poland)

Summary

Introduction: The aim of the study was to present an institutional experience in radiation therapy of primary invasive vaginal carcinoma (PIVC) patients treated in the Krakow Branch of Centre of Oncology, with special regard to treatment effectiveness and failure causes. Materials and Methods: Between February 1967 and January 2007, 162 PIVC patients were treated with radical radiotherapy in the Krakow Branch of Centre of Oncology, Maria Sklodowska-Curie Memorial Institute. Twenty-seven (16.7%) patients in Stage I were treated with intracavitary brachytherapy alone; for 127 (78.4%) patients in Stage I - IV intracavitary brachytherapy was combined with external radiation therapy; and eight (4.9%) patients in Stage IV were given only external radiotherapy. Results: In the investigated group of 162 patients, five-year disease-free survival was observed in 46.3% of the cases. Patient age and FIGO Stage of neoplastic disease were independent favourable prognostic factors. Five-year disease-free survival was observed in 64.9% of the patients < 60 years of age and only in 30.7% ≥ 60 years of age; and in 62.3% of PIVC patients in Stages I and II as compared to 19.7% of Stages III and IV cases. Among 78 patients who died of PIVC, in 60 (76.9%) cases the cause of death was locoregional failure; in six (7.7%), locoregional failure and distant metastasis; and in 12 (15.4%), distant metastasis. Conclusions: Radiotherapy is effective treatment for PIVC patients. Age below 60 years and non-advanced neoplastic disease were independent favourable prognostic factors in the investigated group of patients. The primary cause of treatment failure was failure to achieve locoregional disease control.

Key words: PIVC; Radiotherapy; Effectiveness; Failure.

Introduction

Primary invasive vaginal carcinoma (PIVC) accounts for 0.1%-0.2% of all malignant neoplasms, hence it is a carcinoma of rare occurrence [1-6]. The treatment of choice for most PIVC patients is radiation therapy administered as intracavitary brachytherapy, interstitial brachytherapy, and external radiotherapy [1,5, 7-13]. The results of radiotherapy of PIVC patients are slow, however its prognosis has consistently improved; in the 1950s, five-year survival was running at around 25%, nowadays it amounts to 50%-60% for all Stages altogether [1, 3, 4, 12, 14-17]. However, treatment effectiveness reported in the literature varies significantly from one radiotherapy centre to another, which is mainly the result of rare occurrence of PIVC, and hence small number of patients in presented groups; differences in clinical profile of the groups; changes and development of therapeutic approaches, which were introduced to clinical practice of individual centres at different time, and finally, differences in evaluation criteria of treatment effectiveness and its presentation [3, 4, 8, 11-13, 16-24]. The literature also presents different views the researchers maintain regarding the causes of radiotherapy failures in PIVC patients. While all generally agree that the primary cause of treatment failures is locoregional failure of disease control, there is much dispute over the percentage of vaginal and/or pelvic failures, as well as incidence and location of distant metastases [3, 4, 8, 11, 13, 17-20, 25-27]. The purpose of this work is to present the 40-year experience of Centre of Oncology in Krakow (COOK) in radiation therapy of PIVC patients, with special regard to its effectiveness and failure causes.

Materials and Methods

Between January/February 1967 and January 31st, 2007, 162 PIVC patients in Stage I – IV were given radical radiotherapy in COOK. The very group of patients was the subject of further detailed analysis. The youngest patient was 26-years-old, the oldest 78-years-old; the median age of patients was 62 years. More than half (54.3%) of the patients in the investigated group was 60 years or older. Thirty (18.5%) patients were nulliparous, 58 (35.8%) had one or two children, and 74 (45.7%) had three or more children.

The most common histopathology type of PIVC in the investigated group was squamous cell carcinoma found in 137 (84.6%) cases; 22 (13.6%) patients had adenocarcinoma; and three (1.8%), undifferentiated cell carcinoma. Thirty-four (21.0%) patients had tumour of grade G1; 54 (33.3%) G2; and 74 (45.7%) G3.

In 85 (52.5%) cases, primary site of PIVC was the upper third of vagina; in 30 (18.5%), the middle third, and in 47 (29.0%), the lower third. In 98 (60.5%) patients, site of the original lesion was the posterior wall of vagina; in 40 (24.7%) the anterior wall; and in 24 (14.8%) the lateral walls. In total, in 79 (48.8%) cases, the site of primary tumour was the posterior wall of upper third of vagina.

Forty-two (26.0%) patients were in FIGO Stage I, 59 (36.4%) in Stage II, 37 (22.8%) in Stage III, and 24 (14.8%) in Stage IV. Nineteen (11.7%) patients of the investigated group

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underwent earlier hysterectomy indicated for uterine myomas (16 patients) or preinvasive cervix carcinoma (three patients); the latter, more than ten years before PIVC was diagnosed.

All patients in the investigated group were given radiation therapy. Radiotherapy treatment plans were customized according to tumour stage, its location in the vagina, patient age and condition, accompanying diseases, and performed earlier hysterectomy. One hundred fifty-five (95.7%) patients were given intracavitary low-dose-rate (LDR) brachytherapy performed with Ra-226 or Cs-137 sources. One hundred thirty-five (83.3%) patients underwent external radiotherapy, including 58 (43.0%) treated with Co-60 teletherapy and the remaining 77 (57.0%) with ten MV or six MV linear accelerator. Patients were treated with four external beams: anterior field, posterior field, and two opposite lateral fields (so called box technique). Entry field sizes were as following: 15 x 15 cm to 15 x 18 cm for AP-PA fields, and 15 x 8 cm to 15 x 10 cm for lateral fields. Pelvis minor area determined this way was irradiated with a daily dose of two Gy to total dose of 50 Gy in 25 fractions within five-week-period. Patients with primary tumour in the lower third of vagina were advised elective inguinal irradiation. Four patients with confirmed PIVC metastasis in inguinal lymph nodes were given additional 15-20 Gy dose (“boost”) to that area using smaller fields of 15 MeV electron beams.

For 27 (16.7%) patients in the investigated group, intracavitary brachytherapy was the only treatment advised; all of them had Stage I PIVC and the primary tumour did not exceed 0.5 cm in thickness and two cm in its largest dimension. The treatment was performed using vaginal colpostat (two applicators along vagina axis with two additional dome applicators in cases of upper vagina involvement). Total radiation dose to primary tumour calculated at 0.5 cm distance from vaginal mucosa was 65-70 Gy; vaginal mucosa received dose of 90-100 Gy. The remaining 15 (9.3%) patients in Stage I PIVC with primary tumour exceeding 0.5 cm thickness were additionally given external radiation therapy and received 50 Gy in 25 fractions within five-week-period.

All of the 96 patients (59.2%) with Stages II and III PIVC were treated with the combination of intracavitary brachytherapy and external radiotherapy. Brachytherapy dose to infiltration base was 65-70 Gy. Dose to Manchester A points varied from 44 to 62 Gy (median dose of 52 Gy) with the overall irradiation time from 72 to 132 hours, most commonly within 96-120 hour range. If the primary tumour was located in the upper third of vagina, vaginal colpostat was used together with intruterine applicator.

Sixteen (9.9%) of 22 IVA Stage PIVC patients were advised intracavitary brachytherapy (as in the case of Stage II and III patients) in combination with external radiotherapy; eight (4.9%) patients, for whom it was technically not possible to perform intracavitary brachytherapy due to the extent of neoplastic disease in vagina, were treated with external radiation therapy alone. The eight patients irradiated using four-field box technique to the total dose of 50 Gy received additional 15-20 Gy boost using “shrinking-field technique” up to total dose of 65-70 Gy.

Radiotherapy tolerance was good in the investigated group; 156 (96.3%) patients completed full-planned radiation therapy treatment. Six (3.7%) patients completed planned brachytherapy, but were not given full-planned external radiotherapy dose due to condition deterioration (two patients), exacerbation of accompanying disease symptoms (three patients), and further radiotherapy refusal (one patient).

Severe late radiotherapy complications of grade 3 according to the glossary by Chassagne et al. published in 1993 [28] were observed in six (3.7%) patients, and included five cases of rectovaginal fistula (G3a) and one case of vesico-vaginal fistula (G3d). Four of these patients died of locoregional failure, and one patient of simultaneous distant metastasis. One patient survived five years with no evidence of disease after surgical closure of fistula.

The criterion to assess radiotherapy effectiveness was five-year disease-free survival, counting from the day irradiation was begun. Survival probability was estimated using the Kaplan-Meier method [29]. Log-rank test by Peto et al. [30] was used to evaluate significance of the differences found in the research material. Influence of selected factors on patient survival times was assessed using Cox’s proportional hazard model [31].

Results

Of the 162 patients in the investigated group, 75 (46.3%) were disease-free for five years. The relationship between treatment outcome and demographic, clinical, and histopathological characteristics is presented in Table 1.

Single factor as well as multifactorial Cox analysis showed that age and FIGO Stage of PIVC were independent prognostic factors for five-year disease-free survival in the investigated group of patients; age 60 years or over, and Stages IIIb and IVA were of statistically significant unfavourable impact on therapy results. The fate of the patients in the investigated group is presented in Table 2.

Three (1.9%) patients died of second neoplasm - malignant glioma, and non-small cell lung cancer. Six (3.7%) patients died during five-year follow-up with no evidence of PIVC; three died of myocardial infarction, two of cerebral haemorrhage, and one of pulmonary infection combined with circulatory failure.

Causes of treatment failure in the group of 78 patients not cured of PIVC are shown in Table 3. Due to interpretation difficulty of the failure analysis, local and regional failures were taken as a whole and considered as locoregional failures.

In the investigated group of patients, the primary cause of radical radiotherapy failure was locoregional failure, which amounted to 84.6% of treatment failures; in six (7.7%) cases local recurrence was accompanied by distant metastasis. Distant metastasis was observed in 18 (23.1%) patients not cured of PIVC, and in 12 (15.4%) cases it was the only cause of radiotherapy treatment failure. Distant metastases were found in lungs (nine patients), liver (six patients), and bones (three patients). Average time to PIVC recurrence was nine months (one to 47-month range) with the median of eight months; 82.1% of treatment failures manifested within two years.

Of 78 patients not cured of PIVC, 22 (28.2%) died during the first year after the treatment; 48 (61.5%), during the second year; and 71 (91.0%), during the third year; none of the patients survived five years.

Discussion

Detailed analysis was performed for the group of 162 PIVC patients treated with radical radiotherapy. Comparison of the investigated group profile in terms of FIGO clin-
Table 1. — Relationship between treatment outcome and demographics, clinical, and histopathological characteristics in the group of 162 PIVC patients.

<table>
<thead>
<tr>
<th>Demographics, histopathological, and clinical characteristics</th>
<th>N. of patients treated</th>
<th>Five-year disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60-years-old</td>
<td>74</td>
<td>48</td>
</tr>
<tr>
<td>≥ 60-years-old</td>
<td>88</td>
<td>27</td>
</tr>
<tr>
<td>N. of births given:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>1 or 2</td>
<td>58</td>
<td>27</td>
</tr>
<tr>
<td>3 or more</td>
<td>74</td>
<td>34</td>
</tr>
<tr>
<td>Histopathology:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>squamous cell carcinoma</td>
<td>137</td>
<td>64</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>undifferentiated cell carcinoma</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tumour grade:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>34</td>
<td>17</td>
</tr>
<tr>
<td>G2</td>
<td>54</td>
<td>28</td>
</tr>
<tr>
<td>G3</td>
<td>74</td>
<td>30</td>
</tr>
<tr>
<td>Primary site of tumour in vagina:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>posterior wall of upper third of vagina</td>
<td>79</td>
<td>38</td>
</tr>
<tr>
<td>other locations</td>
<td>83</td>
<td>37</td>
</tr>
<tr>
<td>* FIGO Stage:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I0</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>II0</td>
<td>59</td>
<td>31</td>
</tr>
<tr>
<td>III0</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>IVA0</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Earlier hysterectomy:</td>
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<td></td>
</tr>
<tr>
<td>yes</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>no</td>
<td>143</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 2. — Fate of 162 patients of the investigated group.

<table>
<thead>
<tr>
<th>Fate of patients</th>
<th>N. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survived five-years disease-free</td>
<td>75</td>
<td>46.3</td>
</tr>
<tr>
<td>Died during five-year follow-up of other neoplasms</td>
<td>3</td>
<td>1.9</td>
</tr>
<tr>
<td>Died during five-year follow-up of other causes</td>
<td>6</td>
<td>3.7</td>
</tr>
<tr>
<td>Died during five-year follow-up of PIVC</td>
<td>78</td>
<td>48.1</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3. — Fate of 162 patients of the investigated group.

<table>
<thead>
<tr>
<th>Causes of radiotherapy treatment failure</th>
<th>N. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local failure</td>
<td>60</td>
<td>76.9</td>
</tr>
<tr>
<td>Local failure + distant metastasis</td>
<td>6</td>
<td>7.4</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>12</td>
<td>15.4</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4. — Clinical profile of patient groups presented in the literature in terms of FIGO Stage of PIVC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Publication date</th>
<th>FIGO Stage of carcinoma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiritos et al. [44]</td>
<td>1989</td>
<td>47.3% 13.2% 26.3% 13.2%</td>
</tr>
<tr>
<td>Malmström et al. [31]</td>
<td>1989</td>
<td>22.4% 41.4% 20.7% 15.5%</td>
</tr>
<tr>
<td>Eddy et al. [14]</td>
<td>1991</td>
<td>27.5% 42.8% 16.3% 13.2%</td>
</tr>
<tr>
<td>Reddy et al. [40]</td>
<td>1991</td>
<td>34.1% 50.0% 13.6% 2.3%</td>
</tr>
<tr>
<td>Stock et al. [45]</td>
<td>1992</td>
<td>12.2% 55.1% 20.4% 12.3%</td>
</tr>
<tr>
<td>Leung and Sexton [29]</td>
<td>1993</td>
<td>52.4% 10.7% 25.0% 11.9%</td>
</tr>
<tr>
<td>Dixit et al. [13]</td>
<td>1993</td>
<td>19.4% 14.3% 60.0% 14.3%</td>
</tr>
<tr>
<td>Lee et al. [27]</td>
<td>1994</td>
<td>28.8% 27.1% 33.9% 10.2%</td>
</tr>
<tr>
<td>Bouma et al. [4]</td>
<td>1994</td>
<td>43.7% 34.4% 6.3% 15.6%</td>
</tr>
<tr>
<td>Lemenen et al. [28]</td>
<td>1995</td>
<td>48.8% 23.3% 9.3% 18.6%</td>
</tr>
<tr>
<td>Chyle et al. [7]</td>
<td>1996</td>
<td>24.6% 46.2% 22.7% 6.5%</td>
</tr>
<tr>
<td>Ali et al. [1]</td>
<td>1996</td>
<td>33.0% 52.0% 10.0% 5.0%</td>
</tr>
<tr>
<td>Schäfer et al. [42]</td>
<td>1997</td>
<td>43.0% 24.0% 22.0% 11.0%</td>
</tr>
<tr>
<td>Perez et al. [36]</td>
<td>1999</td>
<td>30.7% 51.0% 10.5% 7.8%</td>
</tr>
<tr>
<td>Pingley et al. [38]</td>
<td>2000</td>
<td>6.7% 69.3% 18.7% 5.3%</td>
</tr>
<tr>
<td>Stryker [47]</td>
<td>2000</td>
<td>26.5% 47.1% 20.5% 5.9%</td>
</tr>
<tr>
<td>Tawari et al. [49]</td>
<td>2001</td>
<td>14.1% 54.9% 21.1% 9.9%</td>
</tr>
<tr>
<td>Tabata et al. [48]</td>
<td>2002</td>
<td>23.9% 50.0% 10.9% 15.2%</td>
</tr>
<tr>
<td>Mock et al. [32]</td>
<td>2003</td>
<td>21.2% 47.5% 25.0% 6.3%</td>
</tr>
<tr>
<td>Frank et al. [17]</td>
<td>2005</td>
<td>26.0% 50.0% 20.0% 4.0%</td>
</tr>
<tr>
<td>Samant et al. [41]</td>
<td>2007</td>
<td>14.3% 60.7% 17.8% 7.2%</td>
</tr>
<tr>
<td>Hellman et al. [21]</td>
<td>2006</td>
<td>33.4% 19.7% 26.8% 20.1%</td>
</tr>
<tr>
<td>de Crevoisier et al. [10]</td>
<td>2007</td>
<td>29.0% 38.0% 29.0% 4.0%</td>
</tr>
<tr>
<td>Tran et al. [51]</td>
<td>2007</td>
<td>42.0% 29.0% 17.0% 11.0%</td>
</tr>
<tr>
<td>Lian et al. [30]</td>
<td>2008</td>
<td>25.4% 50.9% 16.4% 7.3%</td>
</tr>
<tr>
<td>Hegeman et al. [20]</td>
<td>2009</td>
<td>17.1% 31.7% 31.7% 19.5%</td>
</tr>
<tr>
<td>Sinha et al. [43]</td>
<td>2009</td>
<td>29.5% 45.5% 22.7% 2.3%</td>
</tr>
<tr>
<td>Blecharz et al.</td>
<td>2012</td>
<td>26.0% 36.4% 22.8% 14.8%</td>
</tr>
</tbody>
</table>

The analysis of data in Table 4 shows great diversity of clinical profile in terms of FIGO stage of PIVC in patient groups presented in the literature. Stage I0 constitutes 6.7% to 52.4% of the cases; Stage II0, 10.7% to 69.3%; Stage III0, 6.3% to 60.0%; and Stage IV0, 2.3% to 20.1%. In 2004, Hacker [32] presented compilation of selected 13 reports on PIVC published in 1982 to 2001, and describing 1,501 patients in total; 395 (26.3%) were diagnosed Stage I0 PIVC; 562 (37.4%), Stage II0; 352 (23.5%), Stage III0; and 192 (12.8%), Stage IV0. The group of 162 patients discussed in this paper has a clinical profile similar to that presented by Hacker; Stage I0 – 26.0%, Stage II0- 36.4%, Stage III0 – 22.8%, and Stage IV0 – 14.8%.

Five-year PIVC-free survival was observed in 75 (46.3%) of the 162 patients in the investigated group. Table 5 presents comparison of treatment results achieved in COOK with literature data from the last 20 years. According to data in Table 5 and many other literature data published during the last 20 years, five-year survival for the whole investigated group amounted to 38%-66% with 40%-100% survival for Stage I0, 34%-90% for Stage II0, 0%-60% for Stage III0, and 0%-41% for Stage IV0. Five-year survival for Stage IVA0 ranges from 0 to even 41%, and is usually 0% for Stage IVB0. In comprehensive reports by Kosary (1994), Creasmam et al. (1998), and Hacker (2004), five-year survival for the whole group of PIVC patients were 51.0%, 52.2%, and 45.5%, respectively [2, 32, 33]. Treatment results achieved in the group of 152 patients given radiotherapy in COOK are comparable with data presented in the literature.

Multifactorial analysis of prognostic factors in the investigated group of 162 PIVC patients treated with radical radiotherapy showed that patient age and FIGO Stage of
carcinoma were of independent and statistically unfavourable impact on treatment results.

In the investigated group, five-year disease-free survival was observed in 64.9% of patients younger than 60 years, and 30.7% of patients aged 60 years or older. Straight majority of the authors agree that age is an independent prognostic factor in the group of PIVC patients treated with radiotherapy; the younger the age, the better the prognosis [6, 13, 18-20, 33-39]. Vavry et al. reported five-year survival for 50% and 34% of patients younger and older than 60 years, respectively; whereas Frank et al., 50% and 34.3% [19, 39]. In Hellman et al. research, multifactorial analysis showed that – apart from carcinoma stage and primary tumour size – age was the third independent prognostic factor [37]. Worse survival of patients > 65 years was also observed by Wu et al. during research in American population [6]. Malmströma et al. recorded five-year survival amounting to 43% in the group of patients younger than 70 years, and 21% for patients older than 70 years [38]. Some of the authors question independent prognostic significance of age and emphasize that its impact on treatment results is often shown in single factor analyses [2, 4, 10, 25, 40].

FIGO Stage of carcinoma is primary prognostic factor, never raising doubts in the literature [2-4, 8, 10-13, 15-17, 19, 20, 24-26, 35-39, 41-47].

Table 6 presents causes of radiotherapy failure in PIVC patients as reported by the literature. According to data in Table 6 as well as other literature data, the primary cause of treatment failure in PIVC patients is failure to achieve lo-

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### Table 6 — Radiotherapy treatment results in PIVC patients reported in the literature.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Publication date</th>
<th>N. of patients</th>
<th>Five-year survival (%)</th>
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<tr>
<td></td>
<td></td>
<td>N. in group total</td>
<td>I(0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I(0)</td>
<td>I(I)</td>
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<tr>
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<td>1992</td>
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<td>Reddy et al. (40)</td>
<td>1993</td>
<td>84</td>
<td>39.0</td>
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<tr>
<td>Davis et al. (12)</td>
<td>1991</td>
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<tr>
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<td>49</td>
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<td>75</td>
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<td>Stryker (47)</td>
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<tr>
<td>Kucera et al. (26)</td>
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<td>44</td>
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<td>Blecharz et al.</td>
<td>presented paper</td>
<td>2012</td>
<td>162</td>
</tr>
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</table>

Table 5 — Radiotherapy treatment results in PIVC patients reported in the literature.
coregional disease control; its risk increases in advanced stages of neoplastic disease [1, 8, 9, 12, 13, 18-20, 40, 43]. Chyle et al. observed failure in locoregional control in 15%, 18%, 35%, and 60% of PIVC patients in Stages I, II, III, and IV, respectively [8]; Tabata et al. recorded 36% of the failures in Stages 0-II; and 50%, in Stages III and IV [27]. Frank et al. found nine percent of locoregional failures in the group of 147 PIVC patients in Stages I and II; and 24%, in the group of 46 patients in Stages III and IV [19]. It should be emphasized that 66% of locoregional failures are vaginal [12, 43-45, 48, 49]. Data presented by Dixit et al. show that 68% of locoregional failures in PIVC patients in Stage III are cases of incomplete local regression of disease, despite radical radiation treatment given [44]. Research by Yeh et al. proved that 85% of locoregional failures occur within irradiated area [50].

Distant metastases in PIVC patients are observed in 8%-30% of the cases [11, 24, 26, 43-45]. Most of them develop in patients with PIVC in Stages III and IV [8, 11, 19, 20, 25, 27, 40, 43]. In material presented by Perez et al. in 1999, distant metastases were observed in 8%, 13%, 27%, and 20% of PIVC patients in Stages I, II, III, and IV respectively [40]. Tabata et al. recorded distant metastases in 42% of patients in Stages III and IV; in the group of patients in early stages (0- II), distant metastases were not observed [27]. In the group of patients presented by Davis et al., distant metastases developed in five percent of Stage I PIVC cases, and in 20% of Stage II [43]. In the material analyzed by Chyle et al., distant metastases were found in 7% of patients in Stage I; 18% in Stage II; 38% in Stage III; and also 38% in Stage IV [8]; whereas in the group presented by Mock et al. the numbers were as follows: 0% for Stage I, 10% for Stage II, and 20% for Stage III [19]. The most frequent location of distant metastasis include bones, lungs, liver, large intestine, brain, and mediastinal lymph nodes [17, 43-45, 49, 51].

In the investigated group of 162 PIVC patients, the primary cause of treatment failure was failure to achieve locoregional disease control, which was observed in 66 (40.7%) patients. Distant metastases developed in 18 (11.1%) patients and in 12 (7.4%) cases it was the only cause of treatment failure. Hence, causes of failure in the investigated group are similar to that reported in the literature.

Perez et al. analyzed a group of 100 PIVC patients with primary tumour site in upper or middle third of vagina. Despite the fact that inguinal and femoral lymph nodes were not irradiated, none of the patients developed metastasis in the lymph nodes during many years of follow-up. However, metastases in inguinal and femoral lymph nodes were observed in 10% (three of 29) of patients with primary tumour located in lower third of vagina. Of seven patients with confirmed metastasis in lymph nodes at the time of presentation and given radiation doses of around 60 Gy, the nodal failure was recorded in just one case [25, 40]. Similar observations were made by Stock et al. as well as Stryker et al. [12, 24]. In the investigated group, patients with primary tumour located in lower third of vagina were given elective irradiation of inguinal lymph nodes, and four patients with PIVC metastasis in these lymph nodes were given boost up to 60 Gy dose using electron fields covering the lymph node region. No failures in disease control in inguinal and femoral lymph nodes were observed.

**Conclusion**

Radiotherapy is effective management of PIVC patients providing the chance for five-year disease-free survival for around half of them. In early Stage (I, II) patients below 60 years of age, it is possible to achieve 70% of disease control. The primary cause of radiotherapy treatment failure in PIVC patients is failure to achieve locoregional disease control.

**References**


Comparing thermal welding instrument-assisted laparoscopic radical hysterectomy versus conventional radical hysterectomy in the management of FIGO IB1 squamous cell cervical carcinoma

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Summary

Purpose of investigation: The authors sought to evaluate the feasibility and acceptability of using the thermal welding technique with thermal ligating shear (TWT-TLS)-assisted laparoscopic radical hysterectomy (LRH) and systemic pelvic lymphadenectomy (SPL) in the management of Stage IB1 squamous cell carcinoma of the cervix. Materials and Methods: The authors compared operating time, blood loss, and other intra- and postoperative parameters and outcomes in 53 patients between May 2003 and April 2007. Results: Twenty-three patients were treated with TWT-TLS-assisted LRH and SPL (TWT-TLS group) and 30 patients with abdominal radical hysterectomy (ARH) and SPL (ARH group). The surgical time of the TWT-TLS group was significantly shorter than that of the ARH group (221.4 vs 264.6 min, \( p < 0.05 \)). The blood loss of the TWT-TLS group was less than that of the ARH group (195.7 vs 1,214.7 ml, \( p < 0.001 \)). The immediate postoperative recovery seemed to be rapid in the TWT-TLS group compared with the ARH group (1.4 vs 3.5 days for full diet, \( p < 0.001 \); 8.32 vs 12.14 days for hospital stay, \( p < 0.001 \)). The recurrence rate between the two groups was similar during the median four-year follow-up (8.7% vs 13.3%). Conclusions: TWT-TLS is a safe and efficient method for laparoscopic RH and SPL with reduction of morbidity for early-stage cervical cancer. A further study is needed to confirm the above observation.

Key words: Radical hysterectomy; Systemic pelvic lymphadenectomy; Thermal ligating shear; Thermal welding instrument; Squamous cell carcinoma of the cervix.

Introduction

The robotic-assisted laparoscopy system has become more popular in recent years, with the availability of more friendly instruments, easier techniques, and fewer limitations compared with conventional laparoscopic surgery [1], but only a few institutes can provide the robotic surgery service [2]. In addition, a recent review concluded that robot-assisted and total laparoscopic radical hysterectomy (LRH) appear to be equally adequate and feasible [1]. Therefore, conventional laparoscopic surgery is still widely used and is a most popular tool in the management of various kinds of diseases [3-5]. Laparoscopic surgery is not only applicable for benign tumors, but also for gynecologic oncology. A recent survey of members of the Society of Gynecologic Oncology showed an overall increase in the use of perceived indications for laparoscopic surgeries in gynecologic oncology [6]. Many gynecologic oncologists have already accepted that laparoscopic staging can be indicated in the management of endometrial cancers [7, 8]. Furthermore, other types of gynecologic malignancies, for example, cervical cancer, have also been evaluated, and favorable results were reported [9-12].

To successfully manage gynecologic malignancies some extensive surgeries, for example, lymph node dissection (lymphadenectomy), are needed [13], because these procedures provide information not only on the patient’s prognosis, but also on the needs of postoperative adjuvant therapy [14, 15]. However, these procedures are classified as advanced techniques. Besides surgical experience, a more powerful laparoscopic surgical system is of most importance, especially an efficacious and safe vessel sealing system.

Monopolar and bipolar coagulators are frequently used and function well during laparoscopic surgery. Without a doubt, monopolar and bipolar coagulation systems, either through laparoscopy or laparotomy, may induce complications, either directly or indirectly [16]. Therefore, new coagulation systems have been developed and are reported to be more secure and effective. Abu-Rustum’s group has used the argon-beam coagulation system during laparoscopic surgery to limit intraoperative blood loss [17]. Nezhat and colleagues performed total LRH and systemic pelvic lymphadenectomy (SPL) using harmonic shears, and concluded that this approach for cervical cancer is a technically feasible and safe procedure [18]. The Ligasure Vessel Sealing System (LVSS) was also successfully used to help conclude LRH and SPL for cervical cancer patients in the M. D. Anderson Cancer Center [19].

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Thermal welding technology (TWT) has been widely used in gynecologic surgery in this department, and consists of an electrosurgical generator and a hand-piece (thermal ligating shear-TLS); it has proved to be quite efficacious and safe in neck and head surgeries [20-22]. The TLS3 TLS can be used to simultaneously seal and divide, as well as to grasp and dissect soft tissue.

In this study, the feasibility and acceptability of the TLS3 in the management of patients with early-stage cervical cancers were evaluated. A control group was used for comparison. The parameters for comparison included: the surgical time, intraoperative blood loss, the number of pelvic lymph nodes dissected, and the postoperative recovery and tumor recurrence.

Materials and Methods

The medical records of all patients with FIGO Stage IB1 (tumour size ≤ two cm), squamous cell carcinoma of the cervix who underwent abdominal radical hysterectomy (ARH) and SPL between May 2006 and June 2007 were reviewed. Institutional Review Board approval was obtained. Data were obtained from medical and pathologic records. Enrolled patients had to fulfill the following criteria: (1) the initial pathologic diagnosis confirmed at this institution; (2) an absence of preoperative brachytherapy, and chemotherapy or conization; (3) an absence of major operations and medical illnesses, such as uncontrolled infection, unstable autoimmune disease, or others which might affect the evaluations, for example, pelvic adhesion.

All procedures were performed under general endotracheal anesthesia. Preoperative preparation included bowel cleaning and preoperative antibiotics. All patients underwent placement of a urinary Foley catheter and intraoperative lower extremity sequential compression devices for venous thrombosis prophylaxis.

In TWT using the TLS (TWT-TLS)-assisted LRH and SPL group (TWT-TLS RH group), the patient was placed in a steep Trendelenburg position. A ten-mm trocar that incorporates the 0° laparoscope was placed at the level of the umbilicus, and entrance into the abdominal cavity was made under direct visualization. Once the trocar had been safely introduced into the abdominal cavity, insufflation was performed. Intra-abdominal pressure was maintained at 15 mm Hg. Two additional five-mm trocars were placed in the right and left lower quadrants. One additional ten-mm trocar was inserted above the pubic symphysis. An incision was made in the peritoneum to assess the retroperitoneum over the psoas muscle immediately lateral to the infundibulo-pelvic ligament. The boundaries of the SPL were: the deep circumflex vein caudally, the common iliac artery bifurcation cephalad, the psoas muscle laterally, the ureter medially, and the obturator nerve posteriorly as described previously [23]. After completing SPL, total LRH was performed. At the same time, the patients treated with ARH and SPL acted as a control group.

Intraoperative parameters included: surgical time, blood loss, number of pelvic lymph nodes retrieved, percentage of patients receiving blood transfusion, and patient outcome. Intraoperative complications were defined as laceration of a great vessel, cystostomy or transection of the ureter. Infectious complications were defined as the presence of bacteria on culture or clinical evidence of infection, while non-infectious complications included wound dehiscence, deep venous thrombosis, fistula, prolonged ileus, bowel obstruction or lymphoedem formations. Bladder function was objectively assessed after removal of the urinary catheter by performing post-void residual catheterizations. If the residual urine was more than 100 ml (greater than ten days), it was defined urinary retention.

Data were entered into a computer-generated Excel spreadsheet and analyzed. The two-tailed Student t test was used to compare the variables between the two groups. A p value less than 0.05 was considered statistically significant. In terms of outcome, 83.3% of the control group and 95.7% of the TLS group had a free surgical margin, and 13.3% of the control group and 8.7% of the TLS group experienced recurrence.

Results

Twenty-three patients were treated with TWT-TLS-assisted LRH and SPL (TWT-TLS LRH group) and 30 with ARH and SPL (ARH group). The basic characteristics of the patients are shown in Table 1; no significant differences between the two groups were identified.

Table 2 lists the intraoperative factors; surgical time was significantly shorter in the TWT-TLS LRH group in than the ARH group (221.4 ± 55.0 min vs 264.6 ± 56.5 min, p < 0.005), and the volume of intraoperative blood loss in the TWT-TLS LRH group was significantly less than that in the ARH group (195.7 ± 104.3 ml vs 114.7 ± 540.3 ml, p < 0.001). Ninety percent (27 of 30) of the patients in the ARH group received blood transfer, while none of the patients in the TWT-TLS LRH group did. The number of pelvic lymph nodes dissected in the TWT-TLS LRH group was 29.5 ± 15.8, and the number in the ARH group was 28.0 ± 12.3; however, no significant difference was found between the two groups (p = 0.7).

Postoperative evaluation factors are also listed in Table 2. Compared with the ARH group, the time to resumption of a
full diet was significantly shorter in the TWT-TLS LRH group (1.4 ± 0.5 days vs 3.5 ± 1.9 days, \( p < 0.001 \)), and the duration of hospitalization in the TWT-TLS LRH group was significantly shorter than that in the ARH group (8.3 ± 2.8 days vs 12.1 ± 3.7 days, \( p < 0.001 \)). To evaluate the thermal effect of the TLS instrument on tissue, a dissected sample was examined histologically. The sections showed that the thermal-damaged tissue affected by the TLS was about two-mm in depth (Figure 1).

**Discussion**

In 1990, Querleu’s group in Europe and Gershman’s group in the United States pioneered the completion of SPL by laparoscopy [24, 25]. Nezhat’s group further extended the pelvic lymph nodes to the para-aortic lymph nodes and completed laparoscopic para-aortic lymphadenectomy [26]. In fact, laparoscopic lymphadenectomy has become a popular and standard procedure for gynecological or urological malignancies, if these patients are undergoing laparoscopic surgery. The main advantages of laparoscopy in the management of many kinds of benign gynecological diseases, such as ectopic pregnancy, myoma, and hysterectomy, are less pain, rapid recovery (shortened hospital stay), and similar therapeutic outcomes [27-30].

However, there are more concerns involved in the management of cancer; SPL is an important procedure during the surgical treatment of gynecological or urological cancers. Parra et al. compared laparoscopic SPL with open SPL in prostate cancer patients, and concluded that laparoscopic SPL offered a reliable and minimally invasive alternative to open SPL in selected patients, because of the similar number of removed lymph nodes [31]. In addition to this report from urology [30], gynecologists have also supported the feasibility and safety of using laparoscopy to complete SPL [32-34]. In this study, the authors substantiated previous findings that laparoscopic SPL was a safe and feasible procedure.

Differing from previous studies [3, 10-13, 17-19, 24-26, 32-34], the authors pioneered the use of TWTc-TLSs – an inexpensive instrument that can perform cutting and coagulating at the same time during laparoscopic SPL and LRH, although the same instruments have been used with various kinds of other diseases, such as tonsillectomy [20-22] and hemmoroidectomy [35]. According to Wang et al., TWTc-TLSs could significantly decrease pain after hemmoroidectomy, which was superior to LVSS [35]. TWTc-TLSs has been widely used in clinical practice in this Institute. Burning or electric injuries due to the unstable electricity of the monopolar coagulator were extremely rare in this Institute when using TWTc-TLSs during laparoscopic surgery (unpublished). Similar to TWTc-TLSs, an argon-beam coagulator or LVSS might be alternative instruments, based on previous reports [16, 18]. All evidence supported the observations that laparoscopic SPL and LRT provided good results with the assistance of effective coagulator systems.

Consistent with nearly all previous reports [3, 10-13, 17-19, 24-26, 32-36], the total number of SPL through laparoscopy procedures did not differ significantly from those through open laparotomy in this study (29.5 ± 15.8 in the TWT-TLS LRH group vs 28.0 ± 12.3 in the ARH group, \( p = 0.7 \)). However, surgical time in the TWT-TLS LRH group was significantly shorter than that in the ARH group. Although there may be many reasons for this observation, one of the most important was thought to be the shorter coagulation time with simultaneous cutting and coagulation. Besides, a decreased blood loss was clearly shown in the TWT-TLS LRH group due to the special coagulating system (TWT-TLS). The lower volume of blood loss and clear operative field rendered lymph node dissection easier and safer. Another advantage of this technique is the minor amount of damage it causes. Figure 1 indicates the thermal effects of TLS on the dissected tissue, which were about two-mm thick. As compared to conventional bipolar coagulation, TLS results in less damage to the tissue, and avoids damaging large vessels and the ureters during surgery. Surgeons are facilitated when dissecting peri-vessel lymph nodes.

In this study, no intraoperative complications, such as neural and vascular injury occurred in the TWT-TLS LRH group. By contrast, in the laparotomy group, there was one case of iliac vein rupture, supporting the importance of the use of a more secure and safer coagulation system for surgery, not only for laparoscopy, but also for open laparotomy, TWT-TLS could fit the aforementioned indications.

In conclusion, in terms of surgical parameters, the surgical time and the volume of blood loss in the TWT-TLS group was less than that in the ARH group. Furthermore, the TWT-TLS LRH approach also provided a clearer operative field, and less of a need of blood transfer than open ARH. In terms of postoperative parameters, the TWT-TLS LRH group attained a full diet in less time, had a shorter hospitalization time, and lower recurrence rate than the ARH group. These outcomes indicated that the use of the TWT-TLS-assisted laparoscopic system could be successfully applied to LRH and SPL in the management of women with early FIGO IB1 Stage squamous cell carcinoma of the cervix.
High pathologic misdiagnosis of cervical adenocarcinoma in situ

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Summary

Objective: The objectives of this research were to evaluate cases of adenocarcinoma in situ (AIS) and early invasive adenocarcinoma (AC) of the uterine cervix in order to: (1) calculate the pathologic discordance between initial biopsies and final surgical excision specimens and (2) describe the clinical and pathologic factors associated with discordance. Materials and Methods: The University of California, Irvine and Long Beach Memorial tumor registries were used to identify 105 women with AIS and early AC treated between 1990 and 2008. The primary endpoint measured was change in diagnosis when comparing pathology from the initial biopsy to specimens from a large loop excision of the transformation zone (LLETZ), cold knife cone (CKC), or hysterectomy. The variables studied were: age, endocervical curettage (ECC), co-existing cervical intraepithelial neoplasia (CIN), race, and insurance type, as surrogates for socioeconomic status. Results: Initial biopsies were diagnosed as AIS and AC in 44% and 56% of patients, respectively. Of the patients with a biopsy diagnosis of AIS, 29% had a final diagnosis of AC after excisional procedure, and this discordance was not associated with any of the factors studied. Conclusions: A concerning high rate of discordance between colposcopic-guided punch biopsy and final pathology reinforces the current guidelines to always perform an excisional biopsy following diagnosis of AIS on punch biopsy.

Key words: Cervical cancer; Adenocarcinoma in situ; Adenocarcinoma; Pathologic discrepancy.
and glandular cells, (2) the difficulty to sample the glandular lesion due to small size or location within the endocervical canal, (3) the relative infrequency of diagnosing a glandular lesion compared to a squamous lesion, and (4) the overlap in cytologic features between benign reactive endocervical cells and the cells from AC. In addition to the cytologic challenges, histologic diagnosis is similarly complex. Specific histologic features unique to adenocarcinoma in situ (AIS) and invasive adenocarcinoma have been described to aid in differentiating the two disease processes, however in reality, the distinguishing factors are debatable [7]. In addition, AIS frequently co-exists with AC.

Due to this diagnostic dilemma, glandular lesions of the cervix are frequently diagnosed at a later stage than squamous lesions [8, 9]. This may explain the perceived worse prognosis of adenocarcinoma (AC) compared to SCC, although when matched stage to stage, the prognosis for AC is equivalent to SCC. HPV is associated with 95% of SCC and 71% of AC [10], but HPV type 18 is more associated with AC than SCC [8] and HPV 18-associated cervical cancers have a higher incidence of pelvic lymph node metastasis, and have been associated with deeper cervical stromal invasion [10-12].

Accurate initial diagnosis has important implications related to appropriate triage of high-risk patients, counseling of patients regarding fertility preservation, timely referral to a gynecologic oncologist, and decision to perform pelvic lymph node dissection. Initial pathologic biopsies are critical as to not over- or under-treat patients. A young patient with adenocarcinoma in situ (AIS) or early invasive AC may be offered fertility-conserving surgery such as an excisional biopsy or trachelectomy alone [13], whereas a postmenopausal woman with the same diagnosis may be treated with an extrafascial hysterectomy. Treatment of larger invasive lesions, however, requires more radical surgery, including pelvic lymph node dissection. Therefore the accuracy of the diagnosis is critical in regards to offering appropriate treatment.

The authors hypothesized a high rate of discrepancy, specifically under-diagnosis, between initial biopsy and final post-excisional pathology. In an attempt to clarify the diagnosis of invasive AC, the objectives of this research were: (1) to calculate the pathologic discordance between the initial biopsies and final surgical excision specimens and (2) to describe the clinical and pathologic factors associated with discordance.

Materials and Methods

Prior to initiating a retrospective review of AIS and early AC, a complete protocol was approved by the Institutional Review Board at both the University of California, Irvine (UCI) and Long Beach (LB) Memorial medical centers. The UCI and LB tumor registries were used to identify women with AIS and early AC treated between 1990 and 2008. All patients who had a diagnosis of either AIS or AC on the tumor board registries and who had available pathology reports from their initial cervical biopsy and subsequent large loop excision of the transformation zone (LLETZ) or cold knife cone (CKC) were included in the study. Upon chart review, patients without an initial diagnosis of AIS or AC were excluded from the statistical analysis.

In reviewing these cases, the primary endpoint evaluated was change in diagnosis when comparing initial pathology to final pathology after LLETZ, CKC, or subsequent hysterectomy. Standard of care requires LLETZ or CKC prior to hysterectomy to evaluate extent of invasion if present. In most cases, the final diagnosis was based on the LLETZ or CKC. If a hysterectomy was performed as follow-up to the excisional biopsy, the diagnosis was evaluated and if upstaged from the previous pathology, this was used as the final pathology. This study was not designed to evaluate the development of invasive disease after conservative treatment; therefore hysterectomy specimens were reported only if done as follow-up to the excisional biopsy, not if done years later. If the hysterectomy diagnosis was the same or less concerning than the excisional biopsy diagnosis, it was not reported since it was assumed that the LLETZ or CKC likely removed the lesion and this would not accurately count as a pathologic discordance.

Pathologic reports, although not histologic slides, of the initial biopsies and final excisional specimens were reviewed. The discordance rate was calculated. The factors potentially related to discordance that were studied included: age, endocervical curettage (ECC) results, co-existing cervical intraepithelial neoplasia (CIN), race, and insurance type. Groups were compared using two-group t-tests with a 0.05 significance level for continuous variables and Pearson chi-square tests for categorical variables.
Results

The tumor registries identified 105 patients between 1990-2008 that had a diagnosis of either AIS or AC (Figure 1) of the usual type on either initial or final pathology. Of those 105 patients, nine did not have an initial biopsy diagnosis AIS or AC and eight of the remaining 96 patients did not have a final diagnosis of AIS or AC. These other diagnoses included: SCC, squamous carcinoma in situ, glandular hyperplasia, and clear cell carcinoma. Patient demographic data is listed in Table 1.

Of the 96 patients with initial diagnosis of AIS or AC, 44% had AIS and 56% had AC on initial pathology. Pre-excisional biopsy of AIS was upstaged to AC in 12/42 patients (29%) on final pathology after LLETZ, CKC, or hysterectomy. Of these 12 patients, three patients (25%) had International Federation of Gynecology and Obstetrics (FIGO) Stage IB lesions defined as a clinically visible lesion confined to the cervix which is larger than seven mm horizontal spread or greater than five mm in depth [14]. A high concordance rate between initial and final pathology was calculated for initial biopsy of AC compared to initial AIS, as only one patient (2%) had only residual AIS after initial diagnosis of AC (Figure 1).

Factors associated with discordance are listed in Table 2. Co-existing CIN ($p = 0.10$) had a trend towards increased discordance although no factors were significant. Discordance was also assessed according to the type of procedure performed. There was essentially no difference in discordance rate whether a LLETZ or CKC was performed or whether a hysterectomy was performed.

Discordance was found in 17% versus 14% of patients treated with conservative and definitive treatment, respectively ($p = 0.72$).

Discussion

As hypothesized, a high rate of discrepancy, specifically under-diagnosis, was found between initial biopsy and final post-excisional pathology. Nearly one-third (29%) of patients were upstaged from AIS to AC and one-quarter of these patients had FIGO Stage IB AC. The factor most closely associated with increased discordance was co-existing CIN, but this was merely a trend without statistical significance.

Patients with subsequent definitive surgery were significantly older and also more likely to have a positive ECC. Those treated with conservative surgery were significantly more likely to have co-existing CIN. This is likely due to a higher prevalence of CIN among younger patients. In a Kaiser Permanente Northwest study of over 150,000 females, the incidence of CIN1 peaked among women ages 20 to 24 years (5.1 per 100,000) and CIN2-3 peaked among those 25 to 29 years (8.1 per 100,000) [15].

The diagnostic discrepancy between colposcopic guided biopsy and excisional biopsy has been evaluated in several other studies in the literature, most of which however report about squamous rather than glandular lesions. There are very few studies that address the discrepancy between biopsy and CKC for glandular lesions of the cervix. A study of 346 cases of CIN concluded that CIN was underdiagnosed in 15% of cases and microinvasive SCC was missed on guided biopsy and detected on cone biopsy alone in 2.9% of cases [16]. In a study of 104 patients with CIN on punch biopsy, 11.5% had cone diagnosis consistent with microinvasive SCC [17]. A 2011 paper from the Gardasil clinical trials evaluated 737 cases and concluded that the overall agreement between the same day biopsy and definitive therapy specimen was 56% (95% CI: 0.36-0.47), and the underestimation of CIN2-3 and AIS was 57% [18]. This study, however, did not specifically address the underdiagnosis of microinvasive AC. The present study is important because it is one of few papers reporting the high discrepancy between biopsy and definitive excision for AIS and AC of the cervix. It also reports a concerning high rate of under-diagnosis of AC and confirms the necessity of performing an excisional procedure after a biopsy diagnosis of AIS in order to exclude invasion.

Conclusion

This study further emphasizes the need for excisional biopsy after an initial biopsy diagnosis of AIS due to the high rate of discordance between punch biopsy and excisional biopsy histology. In this study, 29% of AIS on initial biopsy was upstaged to AC and 25% of these upstaged cases were FIGO Stage IB.

Other studies have evaluated discrepancy between excisional biopsy and hysterectomy and found that AIS associated with positive margins on excisional biopsy signifi-
cantly increases the risk of AC on hysterectomy. Salani et
al. performed a meta-analysis of 33 studies that included
1,278 patients with an initial diagnosis of AIS who were
treated conservatively [19]. Invasive AC was diagnosed in
5.2% of patients with positive margins and only 0.1% of
patients with negative margins. Young et al. did a retro-
spective chart review of 74 patients between 1988-2006
with an initial biopsy of AIS [20]. Thirty percent (22/74)
of patients with AIS and positive margins had AC on hys-
terectomy pathology, whereas only four percent (2/46) of
patients with AIS and negative margins had AC on hys-
terectomy. Therefore, a repeat CKC is recommended prior to hysterectomy for any CKC with AIS or AC with posi-
tive margins in the unlikely event that a lesion greater than
a FIGO Stage IA1 is found necessitating more extensive
therapy.

According to standard management, a biopsy consistent
with AIS or AC in the absence of a gross lesion mandates
a CKC prior to treatment planning [21]. While a gynecol-
ogist would likely manage AIS, referral to a gynecologic
oncologist is warranted for a diagnosis of AC. A repeat
CKC is recommended prior to hysterectomy for any CKC
with AIS or AC with positive margins in the unlikely event that a lesion greater than a FIGO Stage IA1 is found necessitating more extensive therapy.

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DNA damage in peripheral blood lymphocytes of ovarian cancer patients after radiotherapy

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Summary

Objective: Radiotherapy is a common mode of treatment for many types of cancer, particularly cancers that are not detected until late stages, as is common with ovarian cancer. Although radiotherapy is effective in preferentially killing tumor cells, DNA damage induced by ionizing radiation can also have toxic effects on non-tumor cells. The aim of this study was to investigate the extent of toxicity on non-tumor cells following radiotherapy for ovarian cancer. Materials and Methods: The authors used the comet assay to assess DNA damage in peripheral blood lymphocytes of 60 ovarian cancer patients undergoing radiotherapy. Venous blood samples were collected from patients before radiotherapy and after accumulated doses of 10, 20, 30, 40, and 50 Gy of radiotherapy. Results: Comet frequencies, reflecting the proportion of damaged cells, were significantly higher after radiotherapy than before radiotherapy \( f = 69.66, p < 0.05 \) and demonstrated a linear relationship with accumulated dose \( (y = 9.87 + 0.2987x, r = 0.9497, p < 0.05) \). Additionally, the comet tail length, reflecting the relationship between undamaged and damaged DNA, was significantly longer after radiotherapy \( (f = 175.13, p < 0.05) \). Conclusions: These results demonstrate that radiotherapy induces DNA damage in lymphocytes of ovarian cancer patients and suggest that radiotherapy doses should be limited during clinical treatment to reduce toxic side-effects.

Key words: Ovarian cancer; Radiotherapy; Lymphocyte; Radiation-induced damage.

Introduction

Ovarian cancer is the third most common gynecologic cancer worldwide, behind only cervical and uterine cancers; however, ovarian cancer has the highest mortality rate among all gynecologic cancers [1]. This is primarily due to a misdiagnosis of early-stage symptoms [2], as abdominal swelling and compression symptoms are not observed until later stages of the disease [3]. Early-stage ovarian cancer is generally treated by surgery, while late-stage disease is treated more comprehensively through radiotherapy and chemotherapy [4].

Radiotherapy relies on direct or indirect effects of high-energy rays of ionizing radiation that damage DNA within irradiated cells [5]. Radiation preferentially affects tumor cells, but it can also damage DNA of non-tumor cells, causing toxic side-effects. Radiotherapy is a primary treatment for malignant tumors [6]: 60%-70% of patients with malignant tumors receive radiotherapy [7]. Some tumors, such as those of the nasopharynx, can be eliminated entirely through radiotherapy [8], albeit with side-effects resulting from DNA damage of non-tumor cells. Because of the prevalence of radiotherapy in cancer treatment, limiting radiotherapy dosage to minimize aberrant DNA damage is a valid concern.

This study investigated whether DNA damage was detectable in peripheral blood lymphocytes of ovarian cancer patients after radiotherapy. The comet assay was used to measure DNA damage levels in lymphocytes before radiotherapy and after different accumulated doses of radiotherapy. The results suggest that although radiotherapy is an effective treatment for ovarian cancer [9], radiation can induce DNA damage in non-tumor cells.

Materials and Methods

Clinical data

Sixty cases of ovarian cancer were diagnosed and confirmed by pathology and imaging analysis in the Obstetrics and Gynecology Department of The Fourth Affiliated Hospital of Harbin Medical University from June 2008 to June 2011. Patients were aged 43-69 years, with an average age of 56.13 years. All patients had no radiotherapy contraindication and received no other treatment before radiotherapy. A peripheral blood sample was taken from each patient before radiotherapy (0 Gy) as a background sample. Radiotherapy was administered at 50 Gy and peripheral blood samples were collected at doses of 10, 20, 30, 40, and 50 Gy. The study was approved by the ethics committee of The Fourth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, China.

Comet assay

One ml of venous blood was collected in a centrifuge tube and heparin was added to prevent coagulation. One ml of lymphocyte separation media (produced by Chinese Academy of Medical Sciences) was added along the wall of the centrifuge tube before centrifugation at 2,000 rpm for ten minutes. Lymphocytes were removed with a pipette and placed in a ten ml centrifuge tube. Phosphate buffered saline (PBS) was added to a total volume of five ml. Lymphocytes were centrifuged at 1,500 rpm for five minutes and washed twice with PBS. Washed lymphocytes were suspended in PBS, and cell density was adjusted to \( 1 \times 10^5 \) cells/ml. Cells were stored at 4°C.
Regular glass microscope slides were cleaned and stored at 4°C for pre-cooling. Normal melting point agarose was used to prepare a 0.5% solution with PBS. Solution was cooled to 50°C, dropped onto the pre-cooled slides, and covered with cover glass. Slides were cooled at 4°C. Low melting point agarose was used to prepare a 0.5% solution with PBS. Eighty μl of the solution was mixed evenly with 20 μl of lymphocytes in PBS. The coverslip was removed from the slide and cell mixture was dropped onto the surface of pre-poured normal melting point agarose gel. The coverslip was replaced and agarose was cooled. After cooling, the coverslip was again removed and additional low melting point agarose was dropped on top of cells.

Slides were placed in lysis buffer for one hour, washed with triple-distilled water twice, and air-dried. Dry slides were then placed at the anode of a horizontal tank and electrophoresed at 20 V for 20 minutes in buffer. After electrophoresis, slides were fixed in methanol for ten minutes and washed with triple-distilled water twice.

Slides were stained with freshly prepared silver nitrate solution for 30 minutes and washed with triple-distilled water twice. Slides were then developed in coloration solution for five minutes and washed with methanol for five minutes. Slides were analyzed by light microscopy.

**Slide analysis**

One hundred cells were observed per sample. Five tailing cells were randomly selected and analyzed per sample and DNA tail lengths were measured by image analysis software.

**Statistical analysis**

Statistical data were analyzed using SPSS13.0 for Windows. Measurements are reported as mean ± standard deviation. The background and multi-sample average were compared by single-factor analysis of variance. The relationship between dose and effect was analyzed by linear regression. A $p < 0.05$ was considered statistically significant.

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**Results**

**Dose-dependent increase in comet frequency after radiotherapy**

Comet frequencies before and after radiotherapy of different accumulated doses were measured, and results are summarized in Table 1. Comet frequency after radiotherapy was significantly higher for all doses compared to those pre-radiotherapy ($f = 69.66, p < 0.05$). Linear regression analysis demonstrates a linear relationship between comet frequency and accumulated radiotherapy dose ($y = 9.87 + 0.2987x, r = 0.9497, p < 0.05$), indicating a dose-dependent response (Figure 1).

**Dose-independent increase in comet tail length after radiotherapy**

Comet tail lengths before and after radiotherapy of different accumulated doses were measured, and results are summarized in Table 2. Comet tail lengths after radiotherapy were significantly longer than those pre-radiotherapy ($f = 175.13, p < 0.05$). Linear regression analysis indicated no correlation between comet tail length and radiotherapy dose ($r = 0.6217, p > 0.05$), Figure 2).

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**Discussion**

The comet assay provides a single-cell assessment of DNA damage by measuring DNA strand breaks [10]. Cells embedded in an agarose gel are exposed to lysis buffer to destroy the cell membrane, allowing cellular components to diffuse into the gel. Intact DNA remains attached to the nu-

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**Table 1. — Comet frequencies in lymphocytes of ovarian cancer patients before and after radiotherapy.**

<table>
<thead>
<tr>
<th>Accumulated dose (Gy)</th>
<th>n</th>
<th>Comet frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>60</td>
<td>7.20 ± 1.93</td>
</tr>
<tr>
<td>10 Gy</td>
<td>60</td>
<td>14.32 ± 3.93</td>
</tr>
<tr>
<td>20 Gy</td>
<td>60</td>
<td>17.40 ± 4.58</td>
</tr>
<tr>
<td>30 Gy</td>
<td>60</td>
<td>19.58 ± 5.34</td>
</tr>
<tr>
<td>40 Gy</td>
<td>60</td>
<td>21.36 ± 5.78</td>
</tr>
<tr>
<td>50 Gy</td>
<td>60</td>
<td>22.82 ± 8.14</td>
</tr>
</tbody>
</table>

* $f = 69.66, p < 0.05.$

**Table 2. — Comet tail lengths in lymphocytes of ovarian cancer patients before and after radiotherapy.**

<table>
<thead>
<tr>
<th>Accumulated dose (Gy)</th>
<th>n</th>
<th>Cell number</th>
<th>Tail length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Gy</td>
<td>60</td>
<td>300</td>
<td>16.32 ± 2.65</td>
</tr>
<tr>
<td>10 Gy</td>
<td>60</td>
<td>300</td>
<td>21.32 ± 3.50</td>
</tr>
<tr>
<td>20 Gy</td>
<td>60</td>
<td>300</td>
<td>22.60 ± 3.97</td>
</tr>
<tr>
<td>30 Gy</td>
<td>60</td>
<td>300</td>
<td>24.49 ± 3.98</td>
</tr>
<tr>
<td>40 Gy</td>
<td>60</td>
<td>300</td>
<td>21.53 ± 3.53</td>
</tr>
<tr>
<td>50 Gy</td>
<td>60</td>
<td>300</td>
<td>22.18 ± 3.64</td>
</tr>
</tbody>
</table>

* $f = 175.13, p < 0.05.$

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Figure 1. — Relationship between comet frequency and accumulated radiotherapy dose ($y = 9.87 + 0.2987x, r = 0.9497, p < 0.05$).

Figure 2. — Relationship between comet tail length and accumulated radiotherapy dose ($r = 0.6217, p > 0.05$).
clear matrix during electrophoresis, while damaged DNA uncoils in the alkaline electrophoresis buffer (pH > 13). These single-stranded fragments migrate toward the anode during electrophoresis, forming the comet tail. An increase in DNA damage results in an increase in liberated fragments, producing a longer and brighter comet tail, correlating comet tail intensity with amount of DNA damage [11].

In this study, the comet assay was used to measure DNA damage in peripheral blood lymphocytes of ovarian cancer patients after radiotherapy and to investigate the relationship between DNA damage level and accumulated radiotherapy dose. Analysis indicated that comet frequencies of peripheral blood lymphocytes were significantly higher after radiotherapy, as compared to pre-radiotherapy ($f = 69.66, p < 0.05$) and demonstrated a dose-dependent increase with radiotherapy. Previous studies have also demonstrated a dose-dependent increase in comet frequency of peripheral lymphocytes post-radiotherapy [12], consistent with the results of this study.

Radiotherapy-induced cell damage can occur in two ways: high-energy X-rays can produce direct effects on the double-stranded DNA, such as cross-linking; or high energy X-rays can induce water molecules to produce large amounts of free radicals, which can indirectly damage double-stranded DNA [13]. However, normal cells have the capacity to repair DNA adducts. For example, X-ray repair cross-complementing gene 1 (XRCC1) can repair X-ray-induced DNA damage [14-17]. In addition, intracellular antioxidants can clear free radicals and include enzymes such as superoxide dismutase, hydrogen peroxidase, and glutathione peroxidase [14-17]. In this study, comet tail lengths of post-radiotherapy lymphocytes were significantly longer than those pre-radiotherapy; however, the present analysis found no significant correlation between comet tail lengths of post-radiotherapy lymphocytes and accumulated radiotherapy dose. Comet tails reached a maximum length at an accumulated dose of 30 Gy, but lengths decreased with further increases in accumulated dose. Within a cell, DNA repair systems such as XRCC1 and antioxidants may increase with increasing radiotherapy dose to attempt to repair the DNA damage. This could explain why a dose-dependent relationship between comet tail length and accumulated dose was not observed.

### Conclusions

These results demonstrate that radiotherapy can induce DNA damage in lymphocytes of ovarian cancer patients. The comet assay showed that comet frequency and tail length significantly increased post-radiotherapy, and comet frequency showed a dose-dependent response to accumulated radiotherapy. These results suggest that radiotherapy doses should be limited during clinical treatment to reduce toxic side-effects.

### References


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Role of surgical staging and adjuvant treatment in uterine serous carcinoma

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Summary

Purpose of investigation: This study evaluates the association of clinical and pathologic characteristics of patients with uterine serous carcinoma (USC) with disease recurrence. Materials and Methods: Surgically-staged patients with USC at a single institution were identified and clinical and pathologic variables were compared. Results: Of the 51 patients included in this analysis, 75% percent received adjuvant chemotherapy, 51% received radiation therapy, and 47% received both. After a median follow-up of 33 months, 42% of patients had disease recurrence. On multivariable analysis, positive pelvic lymph nodes were associated with a shorter interval between surgery and recurrence: 13.6 months progression-free survival (PFS) with positive vs 17.2 months with negative lymph nodes (p = 0.05). Patients with early-stage disease who did not receive any adjuvant treatments had a significantly greater risk of disease recurrence. Of the 51 patients included in this analysis, 75% percent received adjuvant chemotherapy, 51% received radiation therapy, and 47% received both. After a median follow-up of 33 months, 42% of patients had disease recurrence. On multivariable analysis, positive pelvic lymph nodes were associated with a shorter interval between surgery and recurrence: 13.6 months progression-free survival (PFS) with positive vs 17.2 months with negative lymph nodes (p = 0.05). Patients with early-stage disease who did not receive any adjuvant treatments had a significantly greater risk of disease recurrence (44.4% vs 7.70%, p = 0.043). Conclusion: In this population of surgically-staged patients with USC, pelvic lymph node metastases were predictive of a shorter PFS.

Key words: Uterine serous carcinoma; Surgical staging; Adjuvant therapy.

Introduction

Endometrial cancer is the most common gynecologic malignancy in the United States with approximately 40,000 new cases diagnosed each year [1]. Endometrial tumors are divided into type I and type II malignancies, a dualistic classification based upon distinct molecular features, pathogenesis, and clinical outcomes. Type I malignancies are endometrioid tumors that are driven by estrogen and generally diagnosed at an early stage, while type II malignancies have high-grade serous or clear cell features, often present at advanced stages at the time of diagnosis, and are associated with poor prognosis [2].

Uterine serous carcinoma (USC), the most common of the type II endometrial cancers, was described as a distinct entity from endometrioid endometrial cancer (EEC) in 1982 [3]. USC is histologically similar to serous epithelial ovarian cancer with a propensity for peritoneal spread and approximately 40% chance of being diagnosed at Stage III or IV disease. Stage for stage, USC is associated with worse prognosis as compared to EEC [4]. While representing less than ten percent of all endometrial cancer cases, USC accounts for 40% of all endometrial cancer-related deaths [5, 6]. Comprehensive surgical staging as per the International Federation of Gynecology and Obstetrics (FIGO) guidelines is indicated in patients with USC [7]. Many gynecologic oncologists have adopted staging and debulking procedures similar to ovarian cancer and routinely perform peritoneal biopsies and omentectomy. Given the relatively high risk of both local and distant relapse in USC, the National Comprehensive Cancer Network treatment guidelines advocate a combined modality approach with chemotherapy and radiation therapy [8].

In USC in contrast to EEC, the risk of extraperitoneal spread remains high despite the absence of traditional risk factors such as deep myometrial invasion or lymphovascular space invasion [9]. Elevated serum CA125 has been shown to correlate with metastatic disease, but its use in USC as a biomarker has not been validated in prospective studies [10, 11]. It remains unclear whether prognostic variables for recurrence in type I endometrial tumors, such as age, myometrial invasion, lymphovascular space invasion (LVS1), tumor size, disease stage, and type of therapy are relevant in type II endometrial disease [12]. This study examines the demographic and clinical variables of USC patients to determine if any significantly impact disease recurrence or progression-free survival (PFS).

Materials and Methods

A retrospective clinical review was conducted of patients undergoing surgery for endometrial cancer between 2002 and 2008 at a single, urban, university hospital. Patients with Stage I-IV USC who had undergone complete surgical staging at the present institution were included. Patients who had undergone neoadjuvant chemotherapy and those diagnosed with synchronous gynecologic malignancies were excluded. Approval for this study was obtained from the Institutional Review Board. Clinical and pathologic variables were abstracted from hospital medical records and the pathology database. The following clinical information was extracted for all patients undergoing surgical staging for USC: age at diagnosis, gravidity, parity, body mass index (BMI), FIGO disease Stage, adjuvant...
chemotherapy, radiation therapy, and time to progression. Pathology outcomes collected included depth of myometrial invasion, LVSI, positive pelvic cytology, extension to the cervix, ovaries, fallopian tubes, omentum, appendix, and pelvic and para-aortic lymph nodes, and number of pelvic and para-aortic lymph nodes harvested.

Data were analyzed using IBM SPSS Statistics 19. Kolmogorov-Smirnov test was used to examine outcome variable distributions for normality. Clinical and pathologic variables were compared using independent sample t-tests for normally-distributed continuous data and Mann-Whitney U tests for non-normally-distributed continuous variables. Chi-squared and Fisher’s exact test were used for categorical variables. Multiple logistic regression was used to assess disease recurrence with the odds ratio, 95% confidence interval, and p value reported for each variable. Multiple linear regression was used to assess PFS, which was defined as the time interval from the date of surgery to the date of the documented first recurrence or progression of disease. For linear regression analysis unstandardized B, p value and 95% confidence interval are reported. The unstandardized B represents the effect of an independent variable on the dependent variable, statistically controlling for the effects of the other independent variables. Type I error threshold was set at a p value of less than 0.05 for all tests.

Results

Sixty-five patients with USC were identified during the study period from hospital databases. Fourteen patients had incomplete medical records or primary surgery performed at an outside institution and were excluded from further analysis. The remaining 51 patients are included in this analysis. Disease distribution was as follows: 22-Stage I, 5-Stage II, 16-Stage III, and 8-Stage IV. The mean age at diagnosis was 67 years. Patient demographic variables are presented in Table 1. Thirty-eight (75%) patients received adjuvant chemotherapy, 82% of which received a regimen of combined carboplatin and paclitaxel. Twenty-six (51%) patients received postoperative radiation therapy (10% external beam pelvic radiotherapy, 18% vaginal brachytherapy, and 24% both). Twenty-four patients (47%) received combination chemotherapy and radiation therapy. Three patients (6%) received neither adjuvant chemotherapy or radiation therapy. After a median follow-up of 33 months, 42% of patients had disease recurrence. The median time to recurrence was 14 months (range 1.6 - 53.8).

Stage of disease was found to significantly affect disease recurrence. Seventy-three percent of patients with Stage III or Stage IV disease recurred (median 15.3 months) vs 21% for patients with Stage I or Stage II disease (median 26.6 months) (p < 0.001). Univariate analysis demonstrated multiple pathologic features that were predictive of disease recurrence, including LVSI (p = 0.03), positive pelvic cytology (p = 0.05), disease extension to serosa (p = 0.004), adnexe (p = 0.001), omentum (p < 0.001), and appendix (p = 0.03). Furthermore, three pathologic features were significantly associated with a shorter PFS, the presence of LVSI (17.9 vs 18.2 months, p = 0.03), disease extension to the serosa (12.4 vs 15.2 months, p = 0.04), and positive cytology (12.0 vs 23.5 months, p = 0.05).

On multivariable analysis, there were no independent significant predictors of disease recurrence; however the presence of LVSI approached significance (p = 0.066). Patients with LVSI were approximately 11 times more likely to recur than those without LVSI (Table 2). On multivariable analysis of PFS, only positive pelvic lymph nodes were associated with a shorter interval between surgery and recurrence: 13.6 months PFS with positive lymph nodes vs 17.2 months PFS with negative lymph nodes (p = 0.046, Table 3).

Finally, the role of adjuvant treatment in this cohort of patients was examined. Among patients with both early Stage (Stages I/II) and advanced disease (Stages III/IV), the authors found no difference in disease recurrence or PFS for patients who received chemotherapy or radiation therapy. The only significant finding among the adjuvant therapy data was that patients with early-stage disease who received neither radiation therapy nor chemotherapy had a significantly greater risk of disease recurrence (44.4% vs 7.70%, p = 0.043).

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Patient demographics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>67 (13)</td>
</tr>
<tr>
<td>(mean, standard deviation)</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>28 (7)</td>
</tr>
<tr>
<td>(mean, standard deviation)</td>
<td></td>
</tr>
<tr>
<td>Stage no. (%)</td>
<td>22 (43)</td>
</tr>
<tr>
<td>I</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5 (10)</td>
</tr>
<tr>
<td>III</td>
<td>16 (31)</td>
</tr>
<tr>
<td>IV</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Depth of invasion (mm)</td>
<td>44 (35)</td>
</tr>
<tr>
<td>(mean, standard deviation)</td>
<td></td>
</tr>
<tr>
<td>Sites of extrauterine disease, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td>42 (84)</td>
</tr>
<tr>
<td>Lymphovascular space invasion</td>
<td>30 (59)</td>
</tr>
<tr>
<td>Cervix</td>
<td>18 (35)</td>
</tr>
<tr>
<td>Ovaries</td>
<td>13 (26)</td>
</tr>
<tr>
<td>Fallopian tubes</td>
<td>13 (26)</td>
</tr>
<tr>
<td>Omentum</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Appendix</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Pelvic lymph nodes</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Para-aortic lymph nodes</td>
<td>2 (4)</td>
</tr>
<tr>
<td># Total lymph nodes</td>
<td>12.7 (14.2)</td>
</tr>
<tr>
<td># Pelvic lymph nodes (mean, SD)</td>
<td>11.5 (12.6)</td>
</tr>
<tr>
<td># Para-aortic lymph nodes (median, range)</td>
<td>1.2 (0-10)</td>
</tr>
<tr>
<td>Adjuvant chemotherapy, no. (%)</td>
<td>38 (75)</td>
</tr>
<tr>
<td>Paclitaxel/carboplatin</td>
<td>31 (61)</td>
</tr>
<tr>
<td>Paclitaxel/cisplatin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Doxorubicin/cisplatin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Cyclophosphamide/cisplatin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Adjuvant radiation therapy, no. (%)</td>
<td>26 (51)</td>
</tr>
<tr>
<td>External beam</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Vaginal brachytherapy</td>
<td>9 (18)</td>
</tr>
<tr>
<td>External beam and vaginal Brachytherapy</td>
<td>12 (24)</td>
</tr>
</tbody>
</table>
that the variable is associated with a shortening of the progression-free survival. An unstandardized B less than 0 (negative number) indicates a negative association with the dependent variable, statistically controlling for the effects of the other independent variables. The unstandardized B represents the effect of an independent variable on the dependent variable, while the p-value demonstrates significance. The 95% confidence interval provides an interval estimate for the true effect size.

Table 2. — Multivariable analysis of disease recurrence.

<table>
<thead>
<tr>
<th>Disease Extension</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of invasion</td>
<td>1.0 (0.8 - 1.1) 0.8</td>
</tr>
<tr>
<td>Lymphovascular space</td>
<td>11.3 (0.9 - 150.2) 0.1</td>
</tr>
<tr>
<td>Serosa</td>
<td>229.5 (0.4 - 1446(9.2) 0.1</td>
</tr>
<tr>
<td>Cervix</td>
<td>1.0 (0.1 - 9.3) 1.0</td>
</tr>
<tr>
<td>Adnexa</td>
<td>0.9 (0.0 - 1.76) 0.9</td>
</tr>
<tr>
<td>Pelvic lymph nodes</td>
<td>32.9 (0.5 - 2303.4) 0.1</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>0.4 (0.02 - 6.4) 0.5</td>
</tr>
<tr>
<td>Adjuvant radiation therapy</td>
<td>0.4 (0.0 - 5.0) 0.5</td>
</tr>
</tbody>
</table>

Multiple logistic regression analysis of disease recurrence using adjusted odds ratio, 95% confidence interval, and p value to demonstrate significance.

Table 3. — Multivariable analysis of progression-free survival (months).

<table>
<thead>
<tr>
<th>Disease Extension</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of invasion</td>
<td>4.0 (-26.4 - 34.4) 0.7</td>
</tr>
<tr>
<td>Lymphovascular space</td>
<td>1.7 (-35.3 - 38.7) 0.9</td>
</tr>
<tr>
<td>Serosa</td>
<td>-6.5 (-36.8 - 23.7) 0.5</td>
</tr>
<tr>
<td>Cervix</td>
<td>-11.9 (-58.5 - 34.8) 0.5</td>
</tr>
<tr>
<td>Adnexa</td>
<td>-28.8 (-56.7 - -0.9) &lt; 0.05</td>
</tr>
<tr>
<td>Pelvic lymph nodes</td>
<td>-6.5 (-38.8 - 25.9) 0.6</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>7.0 (-17.5 - 31.4) 0.4</td>
</tr>
<tr>
<td>Adjuvant radiation therapy</td>
<td>11.3 (0.9 - 150.2) 0.1</td>
</tr>
</tbody>
</table>

Multivariable linear regression analysis of months of progression-free survival using unstandardized B. 95% confidence interval and p value to demonstrate significance. The unstandardized B represents the effect of an independent variable on the dependent variable, statistically controlling for the effects of the other independent variables. An unstandardized B less than 0 (negative number) indicates that the variable is associated with a shortening of the progression-free survival.

Discussion

USC is an aggressive subtype of endometrial cancer with a propensity for intra-abdominal spread and distant metastases [4]. Despite multiple proposed treatment strategies, including pelvic radiation therapy, whole abdominal pelvic radiation therapy, single agent or combination chemotherapy, or combination chemo-radiation, the optimal management after surgery remains unclear [13]. Due to the high-risk of metastatic disease even in the absence of deep myometrial invasion, comprehensive surgical staging is recommended. Christman et al. discovered that 50% of USC cases were upstaged after surgical staging was performed [14].

Despite the trend towards comprehensive staging in USC, there is currently inadequate data to use pathologic information to guide adjuvant treatment or to develop a prognostic prediction model. It is well-established from retrospective studies that patients with advanced-stage disease have higher risks of recurrence and disease-related mortality [4]. However, even patients with early-stage disease or disease limited to the uterus have significant risks of recurrence. In the present study, 21% of patients with Stage I or II disease recurred in a median time of 26.6 months. The established risk-assessment models used in type I endometrial cancers to guide adjuvant chemotherapy and/or radiation therapy are less effective in USC. Prior studies suggest that increasing age, Stage of disease, depth of myometrial invasion, and LVS may be pathologic determinants of poor prognosis in USC [15, 16].

The present study confirmed some of these risk factors in addition to positive pelvic cytology. On multivariable analysis, positive pelvic lymph nodes were independently associated with a shorter PFS. This highlights the importance of complete surgical staging in this disease subtype even in the absence of high-risk uterine features.

Adjuvant therapy remains a controversial topic in USC without adequate prospective data to guide practice patterns. Due to the poor survival outcomes and the high-risk of extrapelvic recurrence [8], many gynecologic oncologists recommend adjuvant treatment after surgery. Chemotherapy with radiotherapy (external beam and/or vaginal brachytherapy) is routinely offered in the adjuvant setting to patients with newly diagnosed USC at the present institution. In this study, the authors were unable to demonstrate a benefit in the rate of recurrence or PFS with chemotherapy or radiation therapy alone or in combination. It is interesting to note that patients with Stage I/II disease who did not undergo adjuvant treatment had a significantly higher risk of disease recurrence.

The optimal treatment of USC remains unclear as currently there are no randomized studies, and the existing retrospective studies are limited by a heterogeneous patient population and diverse adjuvant therapy protocols [13]. Some groups have demonstrated a response to platinum-based chemotherapy in USC [15, 17, 18]. Whole abdominal radiation therapy was first proposed in the 1980s but resulted in severe toxicities with minimal evidence of response [19-22]. Pelvic external-beam radiation therapy with or without vaginal brachytherapy has shown to decrease pelvic recurrences in single-institution studies [23]. More recently, a single-institution, Phase II study of multimodality treatment in USC patients with no visible residual disease after surgery showed a significantly increased three-year survival in low (Stage I/II) and high (Stage III/IV) stage patients as compared to historical controls. “Sandwich” therapy comprising of carboplatin and paclitaxel for three cycles followed by radiation followed by another three chemotherapy cycles was overall well-tolerated in this patient population [24].

Conclusion

The authors acknowledge the limitations of the current study, including the retrospective study design and small number of patients. Both limit the ability to draw definitive conclusions about the prognostic variables and the role of adjuvant treatments. However, despite the limited sample size, this study further highlights the aggressive clinical course of patients with USC. Known prognostic variables for EEC have limited validity in patients with USC and are not as helpful in guiding treatment decisions or discussions of prognosis with patients. More scientific studies are needed to identify the biological mechanisms that portend
a more aggressive course for this disease. In addition, multi-center prospective studies that include only patients with USC are urgently needed to identify chemotherapy and biologic agents that will affect recurrence and overall survival in this patient population.

Acknowledgement

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References


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Analyses of atypical glandular cells re-defined by the 2006 Bethesda System: histologic outcomes and clinical implication of follow-up management.

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Summary

Background: To evaluate the histopathology and the long-term follow-up outcome of women who had atypical glandular cells on Pap smears. Materials and Methods: All women with atypical glandular cells (AGC) who underwent colposcopic and histopathologic evaluation between January 2005 and October 2010 were reviewed. Patient data were examined up to October 2012, allowing for at least two years of follow-up for all patients. Results: Forty-four women with AGC Pap test underwent histologic follow-up during the study period. Overall, upon reclassification of smears, 35 (79.5%) cases were diagnosed with AGC “not otherwise specified” (NOS) and nine (20.5%) with AGC “favour neoplasia”. Seven out of nine patients (77.7%) with AGC “favour neoplasia” had significant pathology. On the other hand, only 11 out of 35 cases (31.4%) with AGC “NOS” had significant pathology. Significant correlation was found between AGC “favour neoplasia” smears and a significant pathology ($p$: 0.01). Of the 44 patients, 18 (40.9%) had significant pathology. Eight patients (18.2%) had low grade cervical intraepithelial neoplasia (CIN 1), four (9%) had high-grade cervical intraepithelial neoplasia (CIN 2 / 3), one (2.2%) had microinvasive squamous cell carcinoma of uterine cervix, one (2.2%) had cervical adenocarcinoma in situ, one (2.2%) had cervical adenocarcinoma, one (2.2%) had endometrial adenocarcinoma, and two (4.5%) had endometrial hyperplasia. Conclusion: Reporting AGC in the population is clinically significant due to the high prevalence of underlying preinvasive and invasive diseases (40.9%). The subtypes of the AGC category are significant predictor of such lesions.

Key words: Atypical glandular cells; AGC; AGUS; Favour neoplasia; Not otherwise specified; NOS.

Introduction

The diagnostic category of atypical glandular cells of undetermined significance (AGUS) was introduced by the Bethesda System in 1988 [1]. In 2001, the category was renamed ‘atypical glandular cells’ (AGC) to avoid confusion with atypical squamous cells of undetermined significance (ASCUS) [2]. The Bethesda system classifies AGC as glandular cells that demonstrate changes beyond those typical of benign reactive processes but that lack unequivocal features of adenocarcinoma. These abnormal glandular cells may be endocervical or endometrial in origin. The Bethesda definition (2001) further divides the glandular cell abnormalities less severe than adenocarcinoma in situ (AIS) and invasive adenocarcinoma into two categories; AGC “not otherwise specified” (NOS) and atypical glandular cells (AGC) “favour neoplasia” because the risk of neoplasia associated with the latter is substantially higher [3].

The diagnosis of AGC occurs relatively infrequently (0.05-0.74%) compared with other cytological abnormalities [4,5]. Besides its low frequency, the rate of biopsy-proven clinically significant lesions ranges from 9% to 38%, including high-grade squamous cervical intraepithelial neoplasia (CIN2/ 3) and endocervical AIS and 3% - 17% of women with AGC have invasive cervical carcinoma or non-cervical uterine / adnexal carcinoma [6]. Because of the spectrum of neoplasia linked to AGC, initial evaluation must include multiple testing modalities. The 2006 consensus guidelines of American Society of Colposcopy and Cervical Pathology (ASCCP), as well as National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology-V.2.2012, indicates the immediate colposcopic evaluation with endocervical sampling for all women with AGC. Endometrial biopsy is recommended for all women 35 years or older and on younger women with risk factors for endometrial neoplasia (unexplained uterine bleeding or chronic anovulation). As well, testing for high-risk human papillomavirus (HPV) is especially useful as an adjunctive screen in patients with AGC-NOS cytology [6,7].

The objective of this study was to determine the association between atypical glandular cells on Pap smear and clinically significant histology. In this research, the authors also evaluated the long-term (minimum of two years) follow-up outcome of women with an AGC diagnosis in a referral colposcopy-gynecological oncology clinic at a tertiary-care health center.
Materials and Methods
This retrospective study was approved by the Institutional Review Board at the Kanuni Sultan Suleyman Training and Research Hospital (KSS-TRH). The computerized cytopathology archives and tumor registry databases of KSS-TRH were searched from January 2005 to October 2010 for patients with AGC or AGUS Pap results. The interpretations of AGC were made by staff cytopathologists, based on categories defined by the 2001/2006 Bethesda System. In this study, the authors reviewed the archival smears reported as AGUS and these smears were reclassified by a cytopathologist experienced in cervical cytology, according to the Bethesda 2001/2006 System, reporting the AGC as NOS or “favor neoplasia”. The patients’ clinical information and final diagnoses were not known to the cytopathologist during the review process. For the purpose of this study, patient data were examined up to October 2012, allowing for at least two years of follow-up for all patients.

Women with AGC results were managed according to the American Society for Colposcopy and Cervical Pathology (ASCCP) recommendations [6]. The colposcopic examination, postcolposcopy management including surgical procedures, and pathologic interpretation were performed by gynecologic oncologists and pathologists, respectively. The pathologic results were obtained from one or more of the following sources: tissue biopsy of suspected lesions under colposcopy, endocervical and/or endometrial curettage, loop electrosurgical excision procedure (LEEP) or cold knife conization, and surgical specimens (cervix and uterus). For patients undergoing two or more procedures during the initial evaluation, only the most abnormal histologic diagnoses were recorded. Significant histopathologic findings included CIN 1, CIN2, 3, AIS, endometrial hyperplasia, and cancer of any primary site. In this report CIN terminology is used exclusively for histologic diagnoses. Benign lesions, including endometrial polyp, chronic endocervicitis, endocervical polyp, endocervical tubal metaplasia, and unintentional sampling of the lower uterine segment were also recorded. The postcolposcopy follow-up of women with AGC NOS who do not have CIN or glandular neoplasia histologically identified was to repeat cytologic testing at six-month intervals. After four consecutive “negative for intraepithelial lesion or malignancy” results were obtained, the patients were advised to return routine cytological tests. In all patients with AGC favor neoplasia, if the initial biopsies were nondiagnostic or negative, then diagnostic excision procedure was performed because of the high risk for underlying premalignant or malignant conditions. Since HPV status has only been investigated in a limited number of patients, the results of this test were not taken into consideration. Cases with cytologic interpretations of suspicious for AIS or adenocarcinoma were excluded from the study as well as women with a history of gynecologic malignancy or endometrial hyperplasia. During the study period, approximately 61% of Pap tests were obtained using liquid-based cytology (LBC). Statistical analyses were performed using the Statistical Package for the Social Sciences software version 13.0 (SPSS). Pearson’s chi-square or Fisher’s exact tests were used to analyze the categorical variables. The results were considered statistically significant if the p-value was < 0.05.

Results
Over the six-year study period, 42,027 Pap tests were reported. The number of records with AGC / AGUS abnormalities during this period was 49 (0.11%), of which five records were excluded from the study for the following reasons: one record because the further analysis of Pap smears with an initial cytological classification diagnosed as AGUS were considered compatible with benign reactive changes; one record owing to a previous history of endometrial hyperplasia; and one record because further investigations after the AGC result were lost. In addition, two records were excluded owing to management that was against the ASCCP recommendations or because there were no histologic reports although guidelines had been followed. As a result, 44 records were included for analyses. Among the 44 smears from patients with follow-up data, 27 were done by LBC and 17 were conventional smears.

The mean age of the study population was 39.2 ± 7.5 years (range 23 to 51) with a median of 40 years. The average follow-up period was 40.7 months (range 26 to 60). In addition to cervical biopsies and endocervical / endometrial sampling, tissue follow-up specimens included 14 LEEP / cold knife cone biopsies, five hysterectomies, and one radical hysterectomy. Of the 44 patients, 18 (40.9%) had significant pathology. Eight patients (18.2%) had low grade CIN 1, 4 (9%) had high-grade CIN 2 / 3, one (2.2%) had microinvasive squamous cell carcinoma of uterine cervix, one (2.2%) had cervical AIS, one (2.2%) had adenocarcinoma of cervix, one (2.2%) had endometrial adenocarcinoma, and two (4.5%) had endometrial hyperplasia. Both of the AIS and invasive cervical adenocarcinoma patients had concurrent CIN 1, while one microinvasive squamous cell carcinoma patient had synchronous simple hyperplasia. Additionally, two patients (4.5%) with AGC “NOS” had vulvar condyloma. Diagnoses of cervical squamous neoplasia after AGC Pap results were significantly more likely than diagnoses of endometrial neoplasia (P: 0.005). The distribution of clinically significant lesions is shown in Table 1. Overall, upon reclassification of smears, 35 (79.5%) cases were diagnosed AGC “NOS” and nine (20.5%) were diagnosed AGC “favor neoplasia”. Seven of the nine patients (77.7%) who had AGC “favor neoplasia” had significant pathology. On the other hand, only 11 of the 35 cases with AGC “NOS” had significant pathology (31.4%). Significant correlation was found between AGC “favor neoplasia” smears and a significant pathology (P: 0.01). Results were analyzed with respect to the initial Pap smear subclassification (Table 2). Of the 18 patients who had significant pathologies, 16 had the diagnoses made during their initial investigations. The remaining two patients had diagnoses made during follow-up visits. Both of them had AGC “NOS” smears. One of them had colposcopic examination with endocervical curettage only as the initial investigation. Endometrial sampling was performed after they had abnormally thickened endometrium on pelvic sonography during her infertility work-up. They were finally diagnosed as having simple endometrial hyperplasia. The remaining patient had cervical biopsy with endocervical curettage as the initial investigation and no abnormality had been detected.
Cervical smear was repeated six months later and it was suggestive of presence of high-grade CIN. Colposcopy with endocervical curettage was repeated and the diagnosis of CIN 2 was made. Of those 26 patients who did not have any significant pathology, two had hysterectomies for non-neoplastic gynecological conditions and defaulted on follow-up after the postoperative histopathologic examination. The remaining 24 patients and the other 18 patients who had significant pathology were followed-up with Pap smears with or without colposcopy or endometrial biopsy for a mean of 40.7 months.

Significant benign biopsy diagnoses from 26 women with AGC Pap test results who had non-neoplastic histologic outcomes were also documented. Non-specific chronic cervicitis and reactive squamous metaplasia were the most common benign histologic findings reported. Endocervical polyps, endocervical tubal metaplasia, and endometrial polyps were the first, second, and third most common benign histologic outcomes (15.3%, 11.5%, and 7.6%, respectively).

Results were also analyzed with respect to patient age. When stratified according to age, it was found that AGC results in patients younger than the age of 40 were more significantly associated with CIN 2/CxSqCa histologic outcomes than with either tissue findings of endocervical or endometrial glandular neoplasia \( (p: 0.009) \). Five women over the age 40 years or older, adenocarcinoma / glandular hyperplasia were the most common significant histologic outcomes, including two cases of adenocarcinoma of the uterine cervix and one case of atypical endometrial hyperplasia. On the other hand, a clinically significant lesion was noted in 52.1% of women younger than 40 years and in 28.5% of women aged 40 years or more \( (p: 0.06) \) (Table 3).

**Discussion**

Cervical cancer screening has proved to be a valuable method that reduces both the incidence of and mortality from cervical cancer. In contrast to the decreased incidence of squamous cell carcinoma, the prevalence of glandular cell neoplasia of the cervix has increased in countries with screening. Although many studies have addressed AGC cytology, few were based on LBC or carried out the analysis under the subclassification of AGC cytology [8]. In addition, a significant number of patients with AGC in previous study did not receive appropriate investigations and histologic follow-up.

The incidence of AGCs in the present study (0.11%) is relatively low compared with that found in previous studies in other countries (0.2% - 0.8%) [9-11]. The differences between the results from the Western series and the present experiences may be attributed to the fact of the low prevalence of cervical cancer in Turkey. The prevalence of preinvasive cervical neoplasia and invasive cervical neoplasia in Turkey has been reported as 1.7% and 0.06%, respectively, by a Turkish cervical cancer and cervical cytology research group [12]. The prevalence of abnormal cervical cytology based on the data from 33 healthcare centers in this study was two to five times lower than in Europe and North America. Therefore, authors concluded that, this might be due to socio-cultural differences and lower HPV prevalence rate in Turkey.

<table>
<thead>
<tr>
<th>Table 1. — <em>Histological correlation of initial Pap tests with AGC “NOS” and AGC “favour neoplasia”.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>AGC “NOS” no. (%)</td>
</tr>
<tr>
<td>Low-grade CIN</td>
</tr>
<tr>
<td>High-grade CIN</td>
</tr>
<tr>
<td>Squamous carcinoma of the cervix</td>
</tr>
<tr>
<td>AIS</td>
</tr>
<tr>
<td>Adenocarcinoma of the cervix</td>
</tr>
<tr>
<td>Endometrial hyperplasia (simple/atypical)</td>
</tr>
<tr>
<td>Endometrial adenocarcinoma</td>
</tr>
<tr>
<td>AGC: atypical glandular cell; NOS: not otherwise specified; CIN: cervical intraepithelial neoplasia; AIS: adenocarcinoma in situ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. — <em>Analysis of histological diagnosis with respect to the initial Pap smear subclassification.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>AGC “NOS”</td>
</tr>
<tr>
<td>Significant lesion no. (%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3. — <em>Histological follow-up results according to patient age.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Significant pathology</td>
</tr>
<tr>
<td>Squamous vs. glandular lesions</td>
</tr>
</tbody>
</table>

The incidence of AGCs in the present study (0.11%) is not significantly different from that found in previous studies in other countries (0.2% - 0.8%) [9-11]. The differences between the results from the Western series and the present experiences may be attributed to the fact of the low prevalence of cervical cancer in Turkey.
The finding of AGCs is important clinically because the percentage of cases associated with underlying high-grade disease is higher than for ASC-US. Various studies have found that 9% to 54% of women with AGCs have biopsy-confirmed CIN, 0% to 8% have biopsy confirmed AIS, and less than 1% to 9% have invasive carcinoma [13]. The present experience with AGC patients is very similar to that reported in the literature and the incidence of clinically significant uterine lesions for AGC patients with a histologic follow-up was 40.9%. According to results of the present study, the subtypes of AGC reflected the incidence of significant pathology. Among the cases of AGC “favour neoplasia” and AGC “NOS”, significant disease was found 77.7% and 31.4% of women, respectively. These findings support the Bethesda 2001 subclassification of AGC cytology and ASCCP 2006 recommendations, although interpretation of AGC is poorly reproducible among cytologists primarily due to subjective interpretation and subcategorization. Thus, women with AGCs should undergo extensive examination by colposcopy, endocervical curettage, endometrial sampling, and further investigation may include hysteroscopy with strong consideration given the likelihood of the involvement of organ sites.

Several factors contribute to the difficulty in studying the natural history and progression of glandular atypia’s in gynecologic cytology. First is the relatively rare occurrence of AGCs. There are also no well-defined preneoplastic endocervical lesions compared with squamous counterparts. The next factor is that AGC is a heterogeneous entity caused by a wide variety of squamous, endocervical, and endometrial lesions. Finally, the frequency of clinically significant lesions found on subsequent follow-up varies not only with the population’s risk of development of cervical cancer, but also with the age distribution among different patient populations [14,15]. In the present study, the mean age of the women with AGCs was 39.2 ± 7.5 years (range 23 to 51) with a median of 40 years. It is interesting to note that although younger patients (< 40 years) were more likely to have a clinically significant uterine lesion (54.5%) on follow-up compared with older patients (> 40 years) (27.2%), the proportion of high-grade squamous or glandular lesions was higher in older than in younger patients.

In the present analysis, all glandular neoplasms including: two case of adenocarcinoma of the uterine cervix, one case of endometrial adenocarcinoma, and one case of atypical endometrial hyperplasia occurred in women older than 40 years of age. In one retrospective study, the rate of malignancy was highest in women 50 years or older (15% including: endometrial 12.7%; ovarian cancer 1.4%; cervical adenocarcinoma 0.9%) compared with those ages 40 to 49 years (2.8%) or less than 40 years (2.0%) [16].

The present study confirms that squamous lesions are very common diagnoses (29.5%) in women with a Pap smear result suggesting AGC [4]. Among these women, 18.2% had low grade CIN, 9% had high-grade CIN, and 2.2% had squamous cell carcinoma. On the other hand, adenocarcinomas (6.8%) were the most common malignancies identified in women with AGC (75% of all diagnosed malignancy); 2.2% had endometrial adenocarcinoma, 2.2% had cervical AIS, and 2.2% had adenocarcinoma of the cervix. Based on data from largest published meta-analysis [17], endometrial adenocarcinoma is still the most commonly diagnosed malignancy in women with AGC (57.6%), followed by cervical adenocarcinoma (23.6%), and cervical squamous cell carcinoma (5.4%). The present authors found approximately a 2.27% incidence of endometrial adenocarcinoma among patients with AGCs in Pap smears. This is consistent with reports in two previous publications [18,19], and is lower than the 6% reported by Zweizig et al [20]. In addition, AGC has been associated with ovarian, fallopian tube, or vaginal cancer, and some cases of colon or breast cancer have also been reported [17,21]. Ovarian cancer has been reported in 0.1% to 0.6% of women with AGC [16,22].

It is noteworthy that in the present series, two patients with previously undiagnosed squamous and endometrial premalignant lesions were found during follow-up visits by repeat cytological testing at six-month intervals. One patient had a simple endometrial hyperplasia, while the other one had a high grade squamous intraepithelial lesion. Some data suggest that women with persistent AGC-NOS (two or more cytology results) are at especially high risk of significant glandular disease (three of five women had endometrial adenocarcinoma in one study) [15]. Women with negative findings on initial evaluation require further assessment, including repeat assessment of the cervix or endometrium. Further evaluation may consist of repeat cervical cytology, colposcopy, HPV testing, endometrial biopsy, or cervical conization.

It has been established that neither HPV-DNA testing nor repeat cervical cytology is sensitive enough to be used alone as an initial screening test for women with AGC. Studies have shown that only 24% to 45% of AGC cytology test positive for high-risk HPV-DNA [23,24]. However, patients with AGC cytology who also test positive for high-risk HPV-DNA have an increased risk for cervical pathology. One study demonstrated that 96% of women with biopsy-confirmed CIN 2-3 and 85% with AIS or invasive cervical adenocarcinoma had AGC cytology and were positive for high-risk HPV-DNA [23]. As well, Zeféron et al. reported that the probability of detecting a significant cervical lesion, either squamous or glandular, in women with AGC “NOS” and negative HPV-DNA test is low [3]. HPV testing has also limitations although it was considered to have high sensitivity for the detection of cervical lesions. Of 251 women with AGC and a negative HPV test, Chen and Yang [25] found one case of CIN 2, and three cases of adenocarcinoma in situ. On the other hand, HPV-DNA testing does not appear to add any significant clinical information in cases of AGC “favour neo-
plasia”, since the likelihood of a glandular lesion, especially of glandular origin, is higher in these cases, and is not dependent on the result of the HPV-DNA test [3].

As a consequence, the incidence of AGC on Pap smear in a large population is low, but the risk associated with such a diagnosis necessitates follow-up and investigation. On the basis of the present results, for women with AGC “favour neoplasia”, the risk is substantial and a thorough evaluation is indicated. In addition, in a small proportion of patients, these clinically significant lesions were diagnosed during follow-up visits within at least one year of evaluation is indicated. In addition, in a small proportion of patients, these clinically significant lesions were diagnosed during follow-up visits within at least one year of the initial cytological diagnosis of AGCs. Until a better understanding of the natural history of AGCs and the relationship of this finding to high-grade squamous or glandular lesions are obtained, patients with AGCs should be followed-up for a substantial period despite initial negative findings.

References

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Correlation of subclinical HPV infection with genital warts and cervical erosion

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Summary

Objective: To investigate the correlation of subclinical human papillomavirus (HPV) infection (SPI) with genital warts and cervical erosion. Materials and Methods: The questionnaire was firstly conducted in experimental groups (genital warts + cervical erosion), and then cervical liquid-based cytology was performed, followed by colposcopy and pathological diagnosis. In the control group, cervical liquid-based cytology and pathological diagnosis were performed. Hybrid Capture 2 assay (HC2) was conducted to detect the cervical high-risk HPV DNA. Results: The positive rate of cervical SPI in experimental groups were significantly higher than control group (p < 0.01), and in the genital warts group it was significantly higher than cervical erosion group (p < 0.05). There was no significant difference of SPI positive rate for cervical erosion with different area and degree (p > 0.05). Compared to control group, the detection rates of cervical high-risk HPV DNA in the experimental groups significantly increased (p < 0.01), and the difference between vulvar condyloma and cervical erosion groups was not statistically significant (p < 0.05). The detection rate of high-risk HPV DNA in positive SPI cases was significantly higher than negative SPI cases. Conclusions: Women with genital warts and cervical erosion are high-risk individuals for cervical cancer, and deserve a focused initial and follow-up management.

Key words: HPV infection; Genital warts; Cervical erosion; Cervical cancer.

Introduction

Cervical cancer is a common malignant tumor threatening the health of women [1]. There are about 500,000 new cases of cervical cancer annually in the world, and 80% cases occur in developing countries, in which it is one of the leading causes of death for women [2]. Furthermore, this disease is at a younger age trend in recent years [3]. HPV, a sexually-transmitted virus, is the most minimal DNA virus discovered so far. The correlation of HPV with cervical cancer has been confirmed in previous studies [4-6].

The cervical HPV infection is divided into clinical, subclinical, and latent infections [7]. The clinical HPV infection exists in all cases with cervical fig wart, and 80%-95% of cases are low-risk type 6 and 11 HPV infections. There is no clear correlation between clinical HPV infection and cervical cancer. For subclinical HPV infection (SPI), there is no clinical symptom or macroscopic change in the cervix. It can be confirmed only by acetic acid colposcopy. For latent HPV infection, there is only cervical HPV infection, with no clinical symptoms or morphological changes. It is negative in acetic acid colposcopy, and no tissue signature of HPV infection is found by pathological examination. This infection is confirmed only by detection of HPV DNA, which is different with SPI.

In this study, the SPI in patients with genital warts (without macroscopic cervical warts) and cervical erosion were observed. The objective is to seek the high-risk individuals for cervical cancer by detection of HPV DNA, thus conducting a targeted screening for cervical cancer.

Materials and Methods

Research objects

129 patients aged 40-years and over in First Clinical College of China Medical University (China) from December 2002 to August 2003 were enrolled in this study. They were divided into genital warts group (32 cases, without macroscopic cervical warts) and cervical erosion group (97 cases). Forty women with hysterectomy due to benign diseases (normal cervical appearance) were selected as control.

Research methods

The questionnaire was firstly conducted in experimental groups (genital warts + cervical erosion), and then the cervical liquid-based cytology and pathological diagnosis were performed. Hybrid Capture 2 assay (HC2) was conducted in 12 cases in genital warts group, 94 cases in cervical erosion group, and 32 cases in control group to detect high-risk HPV DNA.

Collection and treatment of cytologic specimens

The exfoliated cells in cervical transitional zone were collected using a special sampler, and kept in a flask containing AutoCyte preservative fluid. In Clinical Pathology Diagnostic Center, First Clinical College of China Medical University, the samples were further treated in AutoCyte Prep system to obtain the cell monolayer smears (diameter, 13 mm), followed by fixation with 95% alcohol and papanicolaou staining.

The HPV DNA detection was performed in First Affiliated Hospital of Guangzhou Medical College and Digene Corporation (USA). After denaturation treatment on the collected exfoliated...
cells, HC2 assay was conducted to detect 13 types of high-risk HPV DNA (16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59, and 68).

**Determination of SPI**

SPI was determined using pathological diagnosis as gold criteria. The cases in which there was no macroscopic cervical warts, and the liquid-based cytology, colposcopy, and pathological diagnosis findings consistent with the corresponding criteria were considered as SPI positive.

Diagnostic criteria of HPV by liquid-based cytology included the following: (1) koilocytosis: slightly enlarged squamous cells; slightly larger single or dual nucleus; mild-dark staining; perinuclear acupoint like vacuoles; uneven edge thickness; blue, red or double color cytoplasm; scattered or grouping dispersion; size disunity; (2) dyskeratosis or atypical parakeratosis: scatter or grouping dispersion; red-stained cytoplasm; slightly larger nucleus with pyknosis; (3) bottom cells outside squamous epithelium: normal or slightly larger cells; filthy chromatin; perinuclear narrow space halo.

Diagnostic criteria of SPI by colposcopy included the following: (1) acetic acid white test. The cervical mucus was wiped, and then three percent acetic acid was smeared. After 15-20 seconds, the epithelial tissue swelled and turned white. The main features of observation are as follows: a) regional distribution: the change often occurs in conversion region, or on squamous epithelium outside; b) color: the acetic acid white epithelium was slightly shiny, with uneven transparency. There were occasionally bright white spots, white plaque or satellite like-lesions; c) surface configuration: there were flat, rough or small papillary lesions, with tiny gyrus shapes and different mosaic pattern; d) vascular structure: the wart-characteristic vessels existed, including hair-pin-like punctuation with bad configuration, mosaic and central vascular area; (2) iodine test: after compound iodine was smeared, the lesion parts presented stippling or similar mosaic state.

Diagnostic criteria of HPV by pathological diagnosis included the following: (1) excessive dyskeratosis or atypical parakeratosis of superficial cells; (2) spinous layer cell hyperplasia; (3) epidermal basal cell hyperplasia, with no pathological mitosis; (4) increased interstitial nipples, elongating to the surface; (5) characteristic koilocytes (nuclear-enlarged perinuclear acupoint like vacuoles; scattering or clustering in mesexine; large volume; nuclear atypia; dual and multiple nuclei. ribbon cytoplasm in cell edge.

**Statistical analysis**

Statistical analysis was performed using SPSS 11.0 statistical software. A chi-square test was performed for comparisons among groups. A $p < 0.05$ was considered as statistically significant.

**Results**

**Questionnaire results in experimental groups**

The questionnaire results in genital warts and cervical erosion groups are shown in Table 1.

**Detection rates of cervical SPI in three groups**

As shown in Table 2, the positive rate of cervical SPI in genital warts and cervical erosion groups was 53.1% and 22.7%, respectively, which were significantly higher than control group (no SPI) ($p < 0.01$). In addition, the positive rate in genital warts group was significantly higher than in cervical erosion group ($p < 0.05$).

**Detection rates of cervical SPI for cervical erosion with different type and degree**

The detection rates of cervical SPI for cervical erosion group with different type and degree are shown in Table 3. The positive rate of SPI for degree I, II, and III cervical erosion were 17.4%, 23.1%, and 27.3%, respectively, with a ascend trend. There was no significant difference among them ($p > 0.05$). The positive rate of SPI for pure, granule, and papillary type cervical erosion were 20%, 23.4%, 22.5%, respectively. The positive rates in granule and papillary type cervical erosion were slightly higher than pure type, with no significant difference among them ($p > 0.05$).

**Detection rates of cervical high-risk HPV DNA in three groups**

As shown in Table 4, the detection rates of cervical high-risk HPV DNA in genital warts, cervical erosion, and control groups were 41.7%, 28.7%, and 8.3%, respectively. Compared to the control group, the detection rates in experimental groups significantly increased ($p < 0.01$). The difference of detection rate between genital warts and cervical erosion groups was not statistically significant ($p < 0.05$).

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**Table 1. — Questionnaire results in the experimental groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Average age at first coitus (years)</th>
<th>Proportion of patients (%)</th>
<th>Proportion of patients without fixed work (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital warts</td>
<td>17-35</td>
<td>21.8 (7/32)</td>
<td>56.3 (18/32)</td>
</tr>
<tr>
<td>Cervical erosion</td>
<td>21-24</td>
<td>7.2 (7/97)</td>
<td>20.6 (20/97)</td>
</tr>
</tbody>
</table>

**Table 2. — Detection rates of cervical SPI in the three groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Total</th>
<th>Positive SPI</th>
<th>Negative SPI</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital warts</td>
<td>32</td>
<td>64</td>
<td>17</td>
<td>22</td>
<td>53</td>
</tr>
<tr>
<td>Cervical erosion</td>
<td>97</td>
<td>101</td>
<td>75</td>
<td>26</td>
<td>22.7</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>169</td>
<td>130</td>
<td>39</td>
<td>23.1</td>
</tr>
</tbody>
</table>

**Table 3. — Detection rates of SPI in the cervical erosion group with different type and degree.**

<table>
<thead>
<tr>
<th>Degree</th>
<th>Cases</th>
<th>Total</th>
<th>Positive SPI</th>
<th>Negative SPI</th>
<th>Positive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>23</td>
<td>48</td>
<td>19</td>
<td>29</td>
<td>39.5</td>
</tr>
<tr>
<td>II</td>
<td>52</td>
<td>104</td>
<td>40</td>
<td>64</td>
<td>38.5</td>
</tr>
<tr>
<td>III</td>
<td>22</td>
<td>44</td>
<td>16</td>
<td>28</td>
<td>36.4</td>
</tr>
<tr>
<td>Pure</td>
<td>10</td>
<td>20</td>
<td>8</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>Granule</td>
<td>47</td>
<td>94</td>
<td>36</td>
<td>58</td>
<td>38.3</td>
</tr>
<tr>
<td>Papillary</td>
<td>40</td>
<td>80</td>
<td>31</td>
<td>49</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>194</td>
<td>75</td>
<td>119</td>
<td>38.6</td>
</tr>
</tbody>
</table>

**Table 4. — Detection rates of cervical high-risk HPV DNA in three groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Average age (years)</th>
<th>Average age at first coitus (years)</th>
<th>Proportion of patients (%)</th>
<th>Proportion of patients without fixed work (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital warts</td>
<td>26</td>
<td>17-35</td>
<td>7.2 (7/32)</td>
<td>51 (18/32)</td>
</tr>
<tr>
<td>Cervical erosion</td>
<td>35</td>
<td>21-40</td>
<td>7.2 (7/97)</td>
<td>20.6 (20/97)</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>21-24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>169</td>
<td>130</td>
<td>23.1</td>
</tr>
</tbody>
</table>
Correlation between existence of high-risk HPV DNA and cervical SPI

The correlation between existence of high-risk HPV DNA and cervical SPI is shown in Table 5. The positive rate of high-risk HPV DNA in positive SPI cases was significantly higher than that in negative SPI cases (69.2% and 7.8%, respectively; \( p < 0.01 \)).

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Positive HPV DNA</th>
<th>Negative HPV DNA</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital warts</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>41.7</td>
</tr>
<tr>
<td>Cervical erosion</td>
<td>94</td>
<td>27</td>
<td>67</td>
<td>28.7</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>3</td>
<td>33</td>
<td>8.3</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>35</td>
<td>107</td>
<td>24.6</td>
</tr>
</tbody>
</table>

Discussion

Cervical cancer is an infectious disease. The role of HPV infection, especially high-risk HPV infection, in cervical cancer and precancerous lesions, has been basically recognized. Fig wart is mainly a low-risk HPV infection, with no correlation to cervical cancer. The HPV detected in cervical SPI includes almost all HPV subtypes, especially high-risk types 16 and 18 [8].

As found by Jamison et al. [9], in 634 women with cervical SPI positive rate of 36%, HPV 16 and 18 are the main subtypes (7.3%), followed by HPV 31, 33, 35 (4.7%), and HPV 6, 11 (3.5%). Existence of high-risk HPV DNA accounts for 12% of total cases, which is four times more than low-risk type. As shown in Table 4, the high-risk HPV DNA can be detected in genital warts, cervical erosion and control groups. This indicates that, the high susceptibility of high-risk HPV DNA exists in cervical epithelium. Table 5 shows that, the detection rate of high-risk HPV DNA in positive SPI cases is 69.2%, which is significantly higher than negative SPI cases. This indicates that, the cervical SPI has relevance with existence of high-risk HPV DNA. The repeated incidence of SPI will eventually lead to occurrence of cervical cancer [10]. The HPV infection in genital tract often coexists with other sexually-transmitted diseases including gonorrhea, syphilis, chlamydia, trichomoniasis, and candidiasis infections [11, 12]. In recent years, the incidence of cervical cancer is at a younger trend. It is found that, the increased HPV infection rate in sexually-transmitted diseases is parallel to mortality of cervical cancer [13]. Therefore, the early diagnosis and treatment for high-risk HPV infection is extraordinarily important. However, as SPI has no obvious clinical symptom, it is often easily ignored, thus delaying the diagnosis and treatment. The screening and management for persons with high-risk HPV infection become a new topic of clinical research.

Diagnosis of SPI is often conducted by cytologic, colposcopic, and pathology. The cytology examination has a high-false negative rate due to many factors. Colposcopy (acetatic acid white test) is often used for diagnosis of cervical SPI, with a predicted positive rate of 76.7%, in which the coincidence rate with biopsy pathology is 69.6%. It is reported that, colposcopy is better than cytology and pathology in diagnosing cervical SPI [14, 15]. In this study, the diagnosis of cervical SPI is performed by the combination of three methods.

Muckerman et al. [16] studied 249 young women with sexual experience and found that the detection rate of cervical SPI was 10.4%. In this study, the detection rate of cervical SPI in 169 cases was 23.1%. Patients with genital warts and cervical erosion are high-risk, with SPI positive rate of 22.7% and 53.1%, respectively (average, 37.9%). This is at a similar trend with study of Jamison et al. [11]. No SPI is detected in cases with normal cervical appearance. Therefore, more clinical attention should be paid to patients with genital warts and cervical erosion. For patients with genital warts, the cervical checks including smear cytology should not be omitted, and the colposcopy and biopsy under colposcopy should be conducted as far as possible.

In this study, the detection rates of cervical SPI in cervical erosion with different area and degree are analyzed. This has not been reported before. Results show that, the detection rate of SPI increases with the increased erosion area, and that in erosion group is significantly higher than that in control group. This indicates that, the incompleteness of cervical epithelium causes the decline of resistance to invasion of pathogenic microorganism. So the clinicians should pay more attention to treatment of cervical erosion, which has important significance for preventing the occurrence of cervical cancer and precancerous lesions.

It is found that, for genital warts, more patients are young and unmarried, with no fixed job. They often have multiple sexual partners, with young age of sexual initiation. These are consistent with the high-risk factors for HPV infection [17-19], and are associated with the younger trend of cervical cancer in recent years.

As reported by Bekkers et al. [20], the attributable risk of genital tract HPV infection is 80%, but only a few cases with HPV infection develop into cervical cancer in these patients. Many studies have confirmed that, the persistent HPV infection is specially-correlated with development process of cervical cancer. Therefore, strengthening management of high-risk persons with HPV infection before and after therapy, and reinforcing propaganda and education to obtain patient’s recognition and cooperation, are very important for preventing the occurrence of cervical cancer.
References


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Introduction

Several studies have shown that spontaneous regression or progression of the low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL) is dependent on the patient’s immune system and it is known that the T-helper cells (CD4+) play a key role in this process. These cells, according to the microenvironment have the capacity to differentiate into different subpopulations (Th1, Th2, Treg, Th9, Th22 or Th17) that will secrete cytokines and thus mediate different actions on the immune system and inflammatory response [1-6].

As a result of this activation through antigen presenting cells (APCs), T helper cells (CD4+) are able to differentiate between subpopulations of effector cells (Th cells), activating secretion of cytokines, and mediating immunological activities [7].

Among the subpopulations of CD4+ T cells, Th17 cells are distinct because of their regulatory role in chronic inflammatory processes, clarifying previously unexplained facts about the interaction of the Th1/Th2 axis [8]. The cytokines produced by Th17 profile cells, such as interleukin (IL)-17 and IL-22, have important functions in the immune system. IL-17 is mainly involved in the pathogenesis of inflammatory diseases [9]. Moreover, these cytokines appear to be involved in the antitumor response, however, its action is still unclear because some studies describe it as contributing to the reduction of tumor growth and inhibition of metastasis [10], while the majority describes the IL-17 as having an important role in tumor proliferation through the regulation of the production of various pro-angiogenic factors [11]. A study performed on nude mice transplanted with human cervical cancer cells showed that the IL-17 cytokine increased tumor development, possibly due to induction of IL-16 expression and recruitment of macrophages to the neoplastic site [12]. IL-22, a member of the IL-10 cytokine family [13, 14], generally does not inhibit pro-inflammatory cytokine activities [13]. Expression of IL-22 has been documented in dermatitis and psoriasis [15, 16].

The role of IL-17 and IL-22 on SIL is unknown and the presence of the same could be a potential biomarker, indicative of lesion progression or regression, thus the authors analyzed the presence of these cytokines in serum from patients with SIL and cervical cancer.

Materials and Methods

Patients

Patients were recruited from the Gynecology Clinic (IPON) of the Clinical Hospital of Uberaba. All participating patients provided written informed consent after the study was explained to them. This study enrolled 81 female subjects, including 23 controls (patients without a history of infection or lesions), 11 patients with LSIL, 36 patients with HSIL, and 11 patients with invasive carcinoma confirmed by anatomopathological exam. Ethical Committee of UFTM approved the experiments.
Cytokine levels

Serum concentrations of IL-17 and IL-22 in the women in each of the four groups were assessed by enzyme-linked immunosorbent assay (ELISA), in accordance with the manufacturer’s instructions. Briefly, the authors used flat-bottomed 96-well plates, coated with monoclonal antibodies targeting IL-17 or IL-22 cytokines. Negative control wells (shown in white) did not contain cytokine antibodies. To rows one and two of each plate, they added 100 µl of pattern recombinant cytokines following seriated dilutions (1:2) in PBS containing 5% FCS based on the initial diluted concentrations. They added 100 µl/well of blood from each patient, obtained by centrifugation of a blood sample, to the remaining rows. The plates were incubated at 37°C for one hour and washed six times with a solution containing PBS-Tween 20 (PBS-T). Next, they added 100 µl/well of biotin-conjugated antibody directed against the cytokine to be measured, diluted 1:1000 in PBS-T. The plates were incubated for one hour at room temperature and again washed six times in PBS-T. After this step, they added 100 µl/well of avidin conjugated with alkaline phosphatase, diluted 1:1000 in PBS-T. The plates were incubated for one hour, washed six times with PBS-T, and then 100 µl of DNP substrate was added to each well.

The results were obtained based on the difference between the 450 nm and 490 nm (Abs 450-Abs490) absorptions measured by an automatic ELISA reader. The blood concentrations of the cytokines were determined in pg/ml by comparing the absorptions obtained on a pattern curve of the respective recombinant cytokine.

Data analysis

For the statistical analysis, the non-parametric Mann-Whitney test using specific software, was applied. The authors thereby obtained scatter plots, which were expressed in terms of the means of the results; all the results described in this study are reported based on these means. For all the analyses, a $p$ value less than or equal to 0.05 was considered significant.

Results

The median IL-17 level observed for the control group (10.75 pg/ml) was much lower than those observed for the other groups. Patients with LSILs had a significant more IL-17 than control group (22.50 pg/ml, $p = 0.0089$). Meanwhile, patients who had developed HSIL had a mean cytokine concentration that was significantly reduced compared to the LSIL group (12.20 pg/ml, $p = 0.047$). The mean IL-17 concentration for the group of patients with invasive carcinoma was near that for the control group, but did not differ significantly from that of any of the other groups (Figure 1).

As shown in Figure 2, the cytokine IL-22 showed behavior similar to IL-17. The control group had a median concentration of 36.91 pg/ml, while the LSIL group showed a much higher median concentration (168.2 pg/ml). Patients in the HSIL group had an intermediate value (61.48 pg/ml), that was significantly reduced when compared with LSIL group ($p < 0.05$). Patients with invasive carcinoma had a median IL-22 concentration that was similar to that of the control group.

Discussion

The Th17 profile is known for its regulatory action over chronic inflammatory [3]. It is also active in autoimmune diseases and allergic responses, as well as in some viral, fungal, and bacterial infections, and in responses to certain neoplasias, clarifying some previously unexplained observations related to interactions of the Th1/Th2 axis [17-21]. Activated in the presence of IL-6 and IL-23 cytokines (or TGF-β), Th17 cells produce IL-17, IL-21, and IL-22 cytokines, demonstrating a phenomenon immunologists have called tissue immunity, an innate immune response in epithelial tis-
sues [22-24]. Upon binding their receptors, these ILs act to increase tissue integrity through innate immunity. When they are present in excess, however, they can contribute to the pathogenesis of diseases such as rheumatoid arthritis, psoriasis, uveitis, and may also play a role in anti-tumor immunity [22, 24]. The present study evaluated the expression of Th-17 profile cytokines in patients with SILs, including invasive cervical carcinoma, vs healthy controls.

It is known that only around 22% of patients with LSILs develop HSILs, and among these only 12% develop invasive cancer [25]. The low proportion (< 1%) of human papillomavirus (HPV) - infected patients who progress to invasive carcinoma suggests that in general, with the correct activation of the immune system, are able to eliminate the virus and induce a spontaneous regression of lesions. However, the cells and mediators involved in these mechanisms are unknown.

The authors observed that the concentration of both cytokines in the LSIL patients increased significantly when compared with HSIL patients. Although, based on their knowledge, no studies in the literature analyze the role of IL-17 and IL-22 in cervical uterine neoplasia; they were detected in situ in other types of cancer, such as mammary neoplasias [26] and hepatocarcinomas, and their possible relation to tumor growth and metastasis has been suggested [27].

The low IL-17 and IL-22 concentrations in the present in-vasive cancer group could indicate that low concentrations of these cytokines are related to an inability on the part of the Th17 profile to convert itself, leading to progressive tumor growth, or that the tumor is able to block the action of cytokines of the Th17 profile, thereby enabling its growth. Indeed, the majority of patients in the invasive cancer group had an IL-17 concentration that was much lower than the average, which may suggest a close relationship between the development/progression of neoplasias and a low concentration of IL-17.

Hence, lower blood concentrations of IL-17 or IL-22 may be associated with a higher probability of developing high-grade lesions and/or invasive cancer. Based on the results of this study, the authors conclude that patients with LSILs show increased blood concentrations of IL-17 and IL-22 compared to HSIL patients.

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References


Immunoreactivity for Ca 125 and INI 1 loss of expression are useful markers in the diagnosis of vulvar proximal-type epithelioid sarcomas: report of two cases

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Summary
Epithelioid sarcomas (ES) are rare soft tissue tumours of obscure histogenesis. Diagnosis is often difficult as specific morphological and immunohistochemical patterns do not exist. Two distinct clinico-pathological entities have been identified: the classic or distal type and the proximal type. Recently, immunohistochemical detection of Ca 125 was described in ES, as well as loss of INI 1 expression. The authors describe in this paper the morphological and immunohistochemical features of two cases of proximal ES of the vulva. Immunoreactivity for Ca 125 and loss of INI 1 expression were present in both cases. These results confirm previous observations in Asian reports showing that these markers can be used as immunohistochemical markers for the diagnostic assessment of ES.

Key words: Cancer; Vulva; Epithelioid sarcoma; Ca 125; Immunohistochemistry.

Introduction
Epithelioid sarcomas (ES) are uncommon soft tissue tumours of unknown histogenesis, first described by Enzinger in 1970 [1, 2]. Two distinct clinico-pathological entities have been described: the classic or distal type and the proximal type. The former occurs generally in the distal extremities of adolescents and young adults, but rarely in children. It is a slow-growing neoplasm that can be found either in solitary or multiple forms, more commonly in males than in females. Typically, it presents an indolent clinical course, characterized by frequent recurrences and regional lymph node or pulmonary metastases [2, 3-6]. The latter generally occurs in middle-aged or older patients and involves more proximal anatomic districts, such as the pelvis, perineum, and external genitalia and shows a more aggressive neoplastic behaviour [2, 3].

As for other soft tissue tumours, diagnosis of ES is generally challenging, given the small number of cases reported in literature and the lack of precise morphological and immunohistochemical diagnostic criteria. Several histological and immunohistochemical parameters must be taken into account for differential diagnosis with other mesenchymal neoplasias, but none of them is to be considered pathognomonic [2, 4-5, 7-12].

Recently, some Asian Authors reported Ca 125 immunoreactivity in neoplastic cells of epithelioid sarcomas [13-16]. This observation suggests that Ca 125 immunoreactivity could be a useful immunohistochemical tumour marker for the diagnostic assessment of ES. To the authors’ knowledge, no data from Western countries exist on the topic. Furthermore, loss of INI 1 expression have been described as a useful marker in the diagnosis of epithelioid sarcomas [17,18].

The authors report two cases of vulvar ES, in order to describe the morphological and immunohistochemical characteristics of proximal epithelioid sarcomas. Furthermore, they studied immunoreactivity for Ca 125 and INI 1 expression loss with the aim to compare their results with those observed in literature.

Case Report
Case 1
A 85-year-old Caucasian woman, gravida 4, para 4, was referred to gynaecologists for a small nodular mass of the vulva. The patient was affected by osteoarthritis and hypertensive heart disease and had a history of several previous surgical interventions: appendectomy at the age of 16 years, total abdominal hysterectomy for uterine fibromatosis, cholecystectomy for gallstones, and bowel resection for carcinoma of the colon. This last operation was performed twelve years prior and subsequently the patient had six cycles of adjuvant chemo-radiation therapy.

The patient reported that she noticed a nodular mass of about 1.5 cm in diameter at the level of the right labia majora, eight months ago. A biopsy was performed and the pathological examination showed an infiltration of the middle and deep dermis by an atypical cell population, arranged in a solid pattern (Figure 1). The neoplastic cells appeared bulky epithelioid and showed a large eosinophilic cytoplasm. Immunohistochemistry showed positivity for cytokeratin, vimentin, and weak positivity for CD34.

*The authors contributed equally to this work.
Preoperative staging was performed with chest X-ray and magnetic resonance imaging (MRI) of the pelvis. The latter demonstrated a skin ulcer of approximately two cm at the site of the previous biopsy; there was a modest enhancement after contrast medium injection near to the muscular structures of the anterolateral profile of the anal canal, without a defined cleavage plane. It was not evidenced any lymphadenopathy. All the serum tumor markers tested (Ca 19-9, Ca 15-3, CEA, Ca 125) were within normal ranges.

Once completed the preoperative evaluations the patient underwent tumorectomy. Pathological examination of the specimen demonstrated a nodular lesion of 1.5 cm in maximum diameter, surrounded by a pseudo-capsule and with a central necrotic – haemorrhagic area. The atypical epithelial neoplastic cells were characterized by a rich cytoplasm and large nuclei with small nucleoli. Rhabdoid cells were also detected. These cells were organized in irregular fasciae or solid star-like areas with central ischemic necrosis. Numerous apoptotic cells were recognized with a high mitotic index (more than 20 mitoses per 10 high power fields). The pseudo-capsule appeared invaded by the tumor in more areas, as well as the peri-lesional adipose tissue. Furthermore, several neoplastic emboli were evidenced. The margins of resection were very close to the tumor, especially in the deeper part.

Immunohistochemistry demonstrated intense reactivity for cytokeratins, vimentin, and Ca 125 (Figure 2), and focal reactivity for desmin, actin, and HMB 45. Immunoreactivity for protein S-100, CD 34, caldesmin, HPL, estrogen receptors, and Melan-A was absent. MIB 1 index was positive in 70% of the nuclei. INI 1 gene expression, studied with immunohistochemistry, was absent. Furthermore, analysis of the INI 1 gene with fluorescence in situ hybridization (FISH) demonstrated the absence of molecular alterations.

Adjuvant chemotherapy was not performed due to the age of the patient and the margins of excision free of disease. Three years after surgery the patient is alive and free from disease.

**Case 2**

A 36-year-old unwed Caucasian woman was referred for surgery for a subcutaneous nodule at the external genitalia. The patient did not have any history of relevant comorbidities. She complained the appearance of a progressively growing small nodule at the external genitalia, three months prior. Clinical evaluation revealed a firm, painful nodule of about four cm in maximum diameter and hard consistency involving the right labia majora.

The lesion was clinically interpreted as a granuloma and was surgically excised. Pathological examination of the specimen evidenced morphological and immunohistochemical findings identical to those describe in case 1. Loss of expression and absence of molecular alterations of the gene INI 1 were evidenced with immunohistochemistry (Figure 3) and FISH also in this case.

A total body computed tomography was subsequently performed which evidenced an extensive abdominal lymphadenopathy, involving mainly the iliac, femoral, and mesenteric axis. The patient refused further adjuvant treatments and died ten months after tumorectomy.

**Discussion**

Epithelioid sarcomas are rare soft tissue tumours of unknown histogenesis. The first description of an ES was made by Enzinger in 1970 [1, 2]. Since then, two distinct clinic-pathological entities have been recognized: the clas-
The morphological characteristics of the distal form consist in a nodular proliferation of round-to-spindle-shaped cells with abundant eosinophilic cytoplasm surrounding areas of necrosis, and resulting in a pattern resembling a benign necrobiotic granulomatous process. Less commonly, ES can exhibit a collagen-rich storiform pattern or a pseudo-vascular growth pattern [2, 3, 6]. In the proximal type of ES, a tendency to form epithelioid cells was observed, as well as marked nuclear atypias, frequent occurrence of rhabdoid elements, and absence of granulomatous appearance [2, 3, 6, 19].

Differential diagnosis of ES with other soft tissue lesions is generally demanding. ES can be misinterpreted pathologically as non-neoplastic granuloma (e.g. rheumatoid nodule, necrobiosis lipidica) and can be confused with other malignancies [2, 5]. In particular, neoplasias like synovial sarcomas, clear cell sarcomas, leiomyosarcomas, rhabdomyosarcomas, malignant peripheral nerve sheath tumors, malignant fibrous histiocytomas, desmoid tumors, liposarcomas, squamous cell carcinomas and epithelioid angiosarcomas must be excluded. Histology together with immunohistochemical findings can allow diagnosis, also in difficult cases [4, 5, 7-9, 12].

Differential diagnosis is generally based on co-expression of vimentin and cytokeratin, positivity for epithelial membrane antigen (EMA), and in about 60% of cases for CD 34. However, this immunohistochemical pattern is not pathognomonic, since it can be shared with other sarcomas of both epithelioid and non-epithelioid appearance. Negativity for other tumour markers can be useful to exclude several types of neoplasms [2, 4, 5, 7-9, 12]. Both cancers in the presented cases were located in the vulva, they were morphologically consistent with the proximal type, and presented similar immunohistochemical profiles including expression of vimentin and cytokeratin, positivity for CK 8, CAM 5.2, 19, and EMA. Only one case showed focal immunoreactivity for CD 34, while both were negative for S-100 protein, desmin, CK5.6, CK 7, CK 20, CD 68, and CD 31. Intense immunoreactivity for Ca 125 was observed in both cases.

Two cases of ES with an elevated serum Ca 125 level in 2003, and subsequently other nine additional cases in 2004 have been reported by Kato et al. [13, 14]. The authors observed that ten of the eleven epithelioid sarcomas studied were positive for Ca 125 immunostaining, while immunoreactivity lacked in other tumours analysed for differential diagnosis. One additional case reported on 2006 and a recent series of seven cases confirmed these observations [15, 16]. Among the seven cases of the later group, six were immunohistochemically positive for Ca 125, and only one case of ES of the leg was negative. All these cases are reported in East Asia; to the authors’ knowledge, no cases of immunoreactivity for Ca 125 in ES have been reported in Western countries.

In most of the cases reporting high serum levels of Ca 125 were observed both at clinical presentation and during disease recurrences, suggesting the usefulness of this marker, not only for diagnosis, but also for monitoring the neoplasia [13-16]. Ca 125 is a high molecular mass glycoprotein. It is an antigen associated with coelomic epithelium used as a tumour marker in the diagnosis and follow up of epithelial ovarian carcinoma [20-21]. Elevated serum Ca 125 levels have been found in patients with other malignancies, like pancreatic, biliary, and liver carcinoma [22, 23]. Furthermore, Ca 125 has been reported to be present in various normal tissues, such as esophageal epithelium, pancreatic exocrine glands, and hepatocytes [24].

Immunoreactivity for Ca 125 has been sporadically reported in mesenchymal tumors, like uterine leiomyoma, alveolar rhabdomyosarcoma desmoplastic small round cell tumor; also normal mesenchymal tissues (e.g. smooth and striated muscle cells and chondrocytes) present some reactivity for this marker [25-27]. Therefore, immunoreactivity for Ca 125 does not necessarily indicate an epithelial or ovarian origin of the neoplasia. In the presented cases, the positivity for Ca 125 contributed substantially in differential diagnosis with other neoplasias of similar histology.

Another useful marker in differential diagnosis with other neoplasms is the expression status of the gene INI 1. This gene is a member of the SWI/SNF chromatin remodelling complex located on the long arm of the chromosome 22 (22q11.2). In normal cells it is possible to evidence the nuclear expression of the protein produced by INI 1 using immunohistochemistry. It was observed that such expression lacks in several types of malignant tumors with rhabdoid component, including ES of the vulva [17, 18]. This observation was confirmed also in our cases, as immunohistochemistry and FISH demonstrated the loss of expression and the absence of molecular alterations of the gene INI 1.

Vulvar epithelioid sarcomas have an aggressive neoplastic behaviour and the prognosis is generally poor. Surgical excision is the better approach when possible, but often it is only apparently radical, as the local recurrence rates are high (30-50%) [28]. In one of the presented cases the complete surgical excision of the tumour was demonstrated curative and no recurrence was observed within the first three years from surgery and without any adjuvant treatment. In the other case there was an advanced stage disease, with an unexpectedly rapid lymphatic diffusion, that led the patient to death a few months after diagnosis.

In conclusion, immunoreactivity for Ca 125 and INI 1 loss of expression are useful markers in the diagnosis of vulvar proximal-type epithelioid sarcomas.
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All vertebral body metastases of breast cancer: a case report and literature review

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Summary

Objective: Investigating the clinical and imaging features of bone metastasis in breast cancer, in order to raise early diagnosis level and to avoid misdiagnosis. Materials and Methods: An analysis of imaging of breast cancer bone metastasis by consulting relevant literatures. The authors also performed bone biopsy in the patient presented. Results: Biopsy results show that ductal carcinoma can be seen in bone marrow and in immune markers CK (+) and CD68 (–). Conclusion: Multiple systemic metastases are common for breast cancer, but it is rare that one patient has metastasis in all vertebrae. The positive rate of ordinary X-ray is lower than magnetic resonance imaging (MRI) or positron emission tomography/computed tomography (PET/CT) for detecting bone metastasis, but if an accurate diagnosis is to be made, all the imaging and clinical data should be combined.

Key words: Breast cancer; Bone Metastasis; Imaging.

Introduction

Breast cancer is one of the major malignant neoplasms which threatens the health of women. In China, the incidence of breast cancer has been increasing. Early diagnosis and treatment is significant for improving the quality of life and decreasing the death rate of the patients with breast cancer. The route of metastasis of breast cancer includes direct infiltration, lymphatic metastasis, and hematogenous metastasis. Lung, bone, and liver are usually the organs invaded in turn by the third route. Bone metastasis is often concealed, and many micrometastases cannot be found at an early period, which has become the main cause of therapy failure. Bone metastasis for breast cancer patients occurs in 1% and 2.8% per capita annually, which seriously affects the quality of life of these patients. Diphosphonate therapy for early bone metastasis can obviously delay the time of the occurrence of fractures and refractory pain caused by the destruction of bone [1]. Therefore the authors attempt to provide some clues regarding early detection and early therapy of bone metastasis through the imaging features of a case and the related literature review.

Case Report

A 55-year-old woman presented with lumbodorsal pain for more than four months, and was admitted on January 8, 2008. On admission, the patient showed a chronic disease face and emaciated, and appeared to suffer intolerable pain. The Eastern Cooperative Oncology Group performance status (ECOG PS) score was 3. The patient had been suffering from hypertension and was taking nifedipine orally to control it. Since September 2007, the patient began to feel persistent lumbodorsal pain, and a radiograph showed osteoporosis and hyperostosis of L2 vertebrae on November 6, 2007. Magnetic resonance imaging (MRI) on thoracic spine showed extensive degeneration and slight compression fracture of T4 on November 29, 2007 (Figure 1). Therefore, she received some symptomatic treatment including pain-relieving therapy, but her pain was not completely alleviated. She was then admitted to a tertiary hospital, when a physical examination revealed a lump in her left breast. On December 25, 2007, ultrasonic inspection showed a mass measuring two by three cm. On January 2, 2008, an excision biopsy of the lump was carried out and the results suggested an infiltrating ductal carcinoma (left breast, grade 2 according to WHO classification). Immunohistochemistry examination showed ER (-), PR ( ), HER2 (-), and CK (+). To identify the causes of lumbodorsal pain, she was admitted to the present hospital on January 8, 2008. A fludeoxyglucose positron emission tomography (18F-FDG PET) image showed increased activity at all vertebral bodies (Figure 2), several appendix, bilateral flank bone, bilateral ilium wing, bilateral ischiadic branch, several ribs, left side of breast bone, and manubrium sterni, and the largest standard uptake value (SUV) was 9.9, of which the average was 6.7. Therefore, the authors suspected that the cause of osseous metastasis may be breast cancer or other diseases, such as multiple myeloma and metabolic bone disease. They performed a bone marrow biopsy on the right anterior superior iliac spine on January 9, 2008, and found a ductal carcinoma in bone marrow. Immunohistochemistry examination showed CK (+) and CD68 (-) and the final pathological diagnosis was metastatic adenocarcinoma in bone marrow. After the final diagnosis of metastasis to all vertebral bodies due to breast cancer, the patient received treatment to relieve her lumbodorsal pain after two cycles of TAC (docetaxel 75 mg/m², epirubicin 50 mg/m², and cyclophosphamide 600 mg/m²) chemotherapy and pamidronate disodium was administered at an equal dose. Then, the general conditions of the patient worsened and regardless of the best supporting therapy administered, she finally died in October 2009.

Discussion

The skeleton is a common site of cancer metastasis and the most frequent primary tumor is breast cancer, lung cancer, prostate cancer, kidney cancer, and thyroid carci-
noma. It was reported that osseous metastasis was found by autopsy in up to 70% of the patients with breast cancer, and which was associated with the interaction between the cancer cells with the microenvironment of bone marrow mesenchyme [2]. The most frequent osseous metastasis sites are vertebral bodies, pelvis, and ribs, then humerus and femur. However, breast cancer metastasizing to all vertebral bodies is rare [3]. Osseous metastasis is the major reason of pain caused by cancer, and the pain is obstinate and difficult to relieve. Early diagnosis and treatment of osseous metastasis is significant for delaying the progression of osteolytic destruction and improving the quality of life. Besides clinical symptoms, the diagnosis of osseous metastasis mainly depends on diagnostic imaging. The specificity of X-ray in diagnosing osseous metastasis is 94.4%, but its sensitivity is only 48.1%. The destruction of bone cannot be detected by X-ray until more than 50% of bone is destructed. Computed tomography (CT) is more sensitive, by 63%, in the diagnosis of cortical bone metastasis compared to X-ray, but it is not sensitive in diagnosing bone marrow metastasis, especially when patients are elderly and suffer from osteoporosis and/or degeneration [4].

Usually, when tumor metastasizes to vertebral body, tumor cells first infiltrate into the fat tissue in the bone marrow, and then into the cortical bone and appendicular bone. Therefore, metastasis (hypointensity) can be distinguished from the normal fat cells (hyperintensity) in T1-weighted images. SPIR (spectral presaturation with inversion recovery) can find the pathological changes in fat tissue before the bone of vertebral bodies is destroyed. It is reported that the sensitivity of SPIR in diagnosing osseous metastasis is about 96.5%, and its specificity is about 100% [5, 6]. SPIR was not used in this patient, therefore, the authors did not detect any changes of vertebral body through MRI.

PET-CT is an equipment combining PET and CT together, which can reveal pathophysiological change, metabolic alteration, and morphologic changes. This technology is more sensitive. Therefore, it assists in making a more accurate diagnosis, especially with tumor diagnosis. It was reported that if the results of PET and CT were both positive, the positive accuracy of identifying osseous metastasis could reach 98%; but if the result of PET was negative and the result of CT was positive, the positive accuracy was only 17% [7]. Edmunds et al inspected 25 patients with osseous metastasis from breast cancer with PET, Tc-99m-MDP single-photon emission computed tomography (SPECT), and PET/CT separately, and revealed that the sensitivity and specificity of PET imaging were 89% and 73%, respectively, of SPECT imaging they were 71% and
87%, respectively, and of PET/CT imaging they were 100% and 96%, respectively. Moreover, PET/CT imaging could show osteolytic and osteoblastic lesions [8]. It was also reported that the detection rate was 98% by PET/CT and 76% by diffusion-weighted imaging (DWI) [9]. All these data reveal that PET/CT can detect osseous metastasis more sensitively and accurately.

For this patient, ""F-FDG PET images showed increased activity in many bones, especially in vertebral bodies. The largest SUV in the patient was 9.9 and the usual average of SUV is 6.7. However, MRI did not show any changes except for the slight compression fracture on T4. This particular case was rarely reported in the literature before the present.

Conclusion

Multiple metastasis derived from breast cancer is common, but the osseous metastasis to all vertebral bodies is rare. The detection rate of osseous metastasis through X-ray is very low. Although the accurate rate of MRI or PET/CT in diagnosing osseous metastasis is high, it is still necessary to integrate all the clinical data in order to make a more accurate diagnosis.

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Pure primary osteosarcoma of the breast: a case report


Introduction: Mammary sarcomas are relatively uncommon and they represent less than one percent of all primary breast malignancies. Osteosarcoma of the breast, unassociated with other tumors, is distinctly rare, with published references generally limited to case reports and occasional cases in several series encompassing a heterogeneous group of mammary sarcomas and extraosseous osteosarcomas at various sites. The authors present a patient with pure osteosarcoma of the breast, osteoblastic type, with biologically aggressive pattern. Case Report: A 79-year-old lady became aware of a rapidly enlarging lump in the lateral part of the right breast. Clinical examination revealed a firm to hard, mobile, irregular, and painful breast lump measuring about six by four cm. On examination there was no axillary or supraclavicular lymphadenopathy. After initial diagnosis, excisional biopsy without dissection of the axillary lymph nodes was performed. Therefore, the histological and immunohistochemical findings established the diagnosis of pure primary osteosarcoma of the breast. Conclusion: Pure osteosarcoma of the breast is extremely rare and needs to be distinguished from a variety of benign and malignant breast lesions producing metaplastic bone. Less than a hundred cases of pure osteosarcoma of the breast were reported, but diagnostic confirmation with immunohistochemistry has been performed in relatively few of these cases.

Key words: Breast; Osteosarcoma; Immunohistochemistry.

Case Report

A 79-year-old lady became aware of a rapidly enlarging lump in the lateral part of the right breast. She noticed the mass and did not seek medical attention until six months after. In this period she did not have any pain or discharge from a nipple. She had no significant past medical history and no history of familial breast cancer. Clinical examination revealed a firm to hard, mobile, irregular and painful breast lump measuring approximately six by four cm. The tumor was not fixed to the skin or underlying ribs. Nipple, areola, and skin were unremarkable. On examination there was no axillary or supraclavicular lymphadenopathy. Ultrasonography (US) demonstrated an irregular hypoechoic oval region in interface over quadrants with central on echogenic space that probably represented necrosis. (Figure 1a). The mammographic appearance showed a macro lobulated, well-circumscribed radiodense lesion with extensive coarse calcifications. (Figure1b). Clinical findings of the right breast and imaging features were highly suspect for malignancy. Conciliar team staged the tumor as T3 N0 M0 and recommended the surgical treatment. Excisional biopsy without dissection of the axillary lymph nodes was performed. Frozen section analysis did not confirm malignancy tumor in the resected specimen.

Macroscopic examination of the specimen revealed a grayish white, solid and firm tumoral mass with bony consistency, a maximum diameter of six cm and well-defined margins. Microscopically (Figure 2a), an encapsulated tumour composed of a pleomorphic population of round, polygonal to spindle cells, intimately associated with deposits of neoplastic osteoid or rarely bone. The cytoplasm was pale to eosinophilic with indistinct cell border. More than 20 mitoses per ten high-power fields were observed, and these included atypical forms. The tumour infiltrated the breast capsule and extended very close to the resection margins. Immunohistochemically, neoplastic cells were positive for vimentin, but negative for cytokeratin...
(CK), epithelial membrane antigen (EMA), estrogen (ER) and progesterone (PR) receptors. Ki-67 proliferation index was intermediate (30%) and more than 80% of cells were positive for p53. (Figure 2b–f). Therefore, the histological and immunohistochemical findings established the diagnosis of pure primary osteosarcoma of the breast.

Further postoperative examination included CT of thorax and abdomen, and no evidence of metastatic disease was observed. Laboratory parameters were within normal limits. No complications were observed in the postoperative period and after seven days the patient was discharged from hospital in good condition. Doctors prescribed adjuvant chemotherapy and radiotherapy which the patient refused. Two years after the surgery, the patient died with multiple lung metastases.

**Discussion**

EOS account for less than one percent of soft tissue sarcomas. Mammary sarcomas are also relatively uncommon, representing less than one percent of all primary breast malignancies and 12.5% of all mammary sarcoma, with published references generally limited to case reports [4,5,8]. The precise frequency of primary osteosarcoma of breast is difficult to assess because of its infrequent occurrence and due to variation in nomenclature [4]. The largest series reported is a clinicopathological analysis of 50 cases, seen over a 38-year period at the Armed Forces Institute of Pathology, Washington [6]. Due to the low incidence of OS of the breast, it is difficult to point to a specific survival rate.

OS of the breast is a disease of middle and old age (mean age, 64.5 years), as contrasted with the younger age of patients with skeletal osteosarcoma [6]. The most frequent histological subtype of breast sarcoma is malignant fibrous histiocytoma, followed by fibrosarcoma, angiosarcoma and liposarcoma, but nearly all subtypes of sarcomas have been observed in breast [6,7]. The etiological factors and histogenesis of this type of tumor are unknown, although it has rarely been reported arising in breast tissue, due to prior local irradiation, trauma or foreign body [9]. The presented patient had no history of trauma, local irradiation or a biphasic tumor.

The typical clinical appearance of breast OS is a mobile, hard, irregular lump with no axillary lymphadenopathy [9,10]. The present patient also had similar clinical findings. The preoperative diagnosis of osteosarcoma of the breast is difficult to make. Mammographic finding and targeted breast US are important but often not enough and can be insufficient for diagnosis. They usually show well-circumscribed, dense lesion with prominent calcifications within the mass and confirm the presence of a well-defined mass, hypoechoic with an echogenic centre, due to the existence of calcifications [9,10].

Many OS of the breast have been described in association with a biphasic tumor, which suggests that a biphasic tumor is a possible precursor, or has a de novo origin from the breast tissue. Tumors fulfilling the following criteria are called “pure osteosarcoma”, exclusion of origin in the bone, presence of neoplastic osteoid or bone, absence of an epithelial component, and no association with a benign tumor, and these are the basic requirements for the diagnosis of a primary osteosarcoma of the breast [6,11]. The presented case fulfilled these criteria. Immunohistochemistry is performed to exclude epithelial differentiation or metaplastic carcinoma. Histologically, the tumours are divided into fibroblastic, osteoelastic, and osteoblastic variety [12]. In the present patient, the osteoblastic cells were the prevailing neoplastic element of the lesion, thus subcategorising the tumour under the osteoblastic subtype. The main pathological differential diagnosis of primary mammary OS is metaplastic carcinoma containing osteoid and bone. This differentiation is of prime importance as the two entities have different histological behavior and require different treatment [4]. Metaplastic carcinoma is immunoreactive for CK, whereas OS is negative. The cells of OS also display negativity for EMA, ER, PR, and Her2, and are vimentin-positive [4]. Although immunohistochemistry plays a major role in differentiating OS from sarcomatoid carcinoma, some sarcomas may display abundant CK expression.
Therefore, extensive sampling of the specimen is still considered a crucial step [6].

The therapy for primary osteogenic sarcoma of the breast is surgery. The gold standard in the treatment should include total wide excision of the primary tumour with clear resection margins, in order to reduce the possibility of tumour recurrence [4]. Current treatment recommendations for extraskeletal OS do not include lymphadenectomy [13]. The present case confirms this opinion, since the patient had no metastases to axillary nodes. There are no clear guidelines for adjuvant treatment after primary surgery, but most centers will advocate use of chemother-
apeutic in the treatment of soft-tissue sarcomas in fit patients with large high grade tumors. However, extraosseous OS seems less responsive to chemotherapy than osseous OS.

The present patient died with multiple lung metastases, two years after surgery. Generally, long-term prognosis is uncertain because of the small number of reported cases in the literature [14]. OSs of the breast are biologically aggressive neoplasms characterised by early recurrences (43% at a median of one year) and haematogeneous metastatic spread, frequently to the lungs, bone, liver, and soft tissues. A large tumour size significantly predicts a worse therapeutic outcome, while the five-year survival rate is dismal (approximately 38%) [6,15].

Conclusion

Pure OS of the breast is extremely rare and needs to be distinguished from a variety of benign and malignant breast lesions producing metaplastic bone. Less than a hundred cases of pure OS of the breast were reported, but diagnostic confirmation with immunohistohemistry has been performed in relatively few of these cases.

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Ovarian cancer presenting as a metastasis to a trocar tract used for a gasless lift-laparoscopy to resect a benign ovarian cyst: an unusual case report

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Summary

Background: Metastasis to a trocar tract (port-site metastasis, PSM) is an uncommon but serious complication that possibly compromises the prognosis of cancer patients treated laparoscopically. Case: A 42-year-old Japanese woman had a 20-cm benign right ovarian cyst resected using gasless lift-laparoscopy. Five years and eight months postoperatively, she noticed a three-cm subcutaneous tumor involving the trocar tract. She was also found to have a pelvic mass and an exploratory laparotomy revealed left ovarian cancer. Based on the histopathological findings, the subcutaneous tumor was diagnosed as a metastasis from the ovarian cancer. Conclusions: This case suggested that PSM could occur without direct or indirect wound contamination during laparoscopic surgery.

Key words: Laparoscopic surgery; Ovarian cancer; Port-site metastasis.

Introduction

Laparoscopic surgery offers patients many benefits, including minimal pain after surgery, faster recovery, shorter hospital stay, superior cosmetic results compared to open surgery, and has recently been increasingly applied to malignant diseases. However, the seeding of malignancies at laparoscopic port sites (port-site metastasis, PSM) has become a major concern for surgeons. PSM is an uncommon complication after laparoscopic surgery for cancer patients, with an estimated incidence of one to two percent in the setting of gynecology [1]. Laparoscopic surgeries for malignant conditions are usually applied to early-stage diseases. However, once complicated, PSM may change potentially curable conditions into incurable conditions and may predispose patients to poor prognoses [2].

Herein, the authors report an unusual case of PSM in which a metastasis to the trocar tract of laparoscopic surgery for a benign ovarian cyst led to a diagnosis of ovarian cancer five years and eight months postoperatively.

Case Report

A 42-year-old, gravida 3, para 3 Japanese woman noticed an increased abdominal girth and was then diagnosed with a right ovarian cyst measuring over 20 cm in size. The cyst was multiloculated but lacked solid components or thickened septations and was interpreted as a benign ovarian cyst. A gasless lift-laparoscopy was performed with a two-cm subumbilical incision, and a ten-mm trocar was placed in the right iliac fossa. The cyst was punctured with the SAND balloon needle to prevent spillage [3], and a total of 3,450 ml of fluid was aspirated when the cyst had totally collapsed. The trocar was removed, and the incision was extended to four cm in length to remove the right ovary from the abdominal cavity. An extra-corporeal cystectomy was performed and the ovary was reconstituted and returned to the peritoneal cavity. The left ovary appeared normal. No drain was brought out through the port site after surgery. Microscopically, the cyst was diagnosed as a mucinous cystadenoma (Figure 1A) and ascites cytology was negative.

Five years and eight months postoperatively at the age of 48, the patient noticed a subcutaneous tumor involving the trocar tract. She also developed lower abdominal pain and noticed her abdominal girth increasing in size over time. Pelvic examination, ultrasonography (USG), and computed tomography (CT) revealed a left ovarian mass with solid components measuring over ten cm in diameter. A CT scan also demonstrated a well-circumscribed three-cm subcutaneous tumor (Figure 2) and an extrahepatic solitary mass extending into the subdiaphragmatic space. Her serum CA125 and CEA were within normal limits, but CA19-9 was elevated to 374.8 U/ml. These findings led the authors to presume that she had ovarian cancer with disseminated lesions. An exploratory laparotomy was performed, and she was found to have a left ovarian tumor with 100 ml of ascites. She underwent a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and sampling of the pelvic lymph nodes. The subcutaneous tumor and the solitary disseminated mass located at the subdiaphragmatic space were also resected. A histopathological examination of the excised subcutaneous (Figure 1B) and subdiaphragmatic masses revealed mucinous cystadenocarcinoma, which was compatible with the histology of the left ovarian tumor (Figure 1C). Ascites cytology was positive, but no cancer tissue was found in the right ovary that had previously been cystectomized laparoscopically. A histological re-examination of the right ovarian cyst failed to detect any cancerous lesions on the archival slides. These findings suggest that the subcutaneous tumor was a metastasis to the trocar tract of the previous laparoscopic surgery for the benign right ovarian cyst, and she was diagnosed with FIGO Stage IV ovarian cancer. Postoperatively she was given six cycles of chemotherapy, consisting of paclitaxel and carboplatin. The patient tolerated chemotherapy and has survived without evidence of disease for five years after the second surgery.
Discussion

The definition of PSM is not yet established, but Rey mond et al. [4] proposed that it should be defined as early tumor recurrences developing locally within the scar tissue of trocar sites or an incision wound after laparoscopy and that these should not be associated with peritoneal carcinomatosis. Although this patient might not fulfill the definition because the metastasis developed over five years post-laparoscopic surgery for the benign ovarian cyst, this unusual case might provide clues for understanding the etiology of PSM.

The pathogenesis of PSM is not fully understood. It is considered to be multifactorial, and three possible factors, including mechanical, metabolic/immunological, and hematogenous, have been proposed [5]. The mechanical factors include direct and indirect wound contamination. Direct contamination is considered to be the most plausible mechanism, and cancer cells may be seeded during tumor extraction through a small wound or by contact with instruments contaminated with tumor cells. However, it is intriguing that 15 cases, summarized in Table 1, of PSM without tumor manipulation during surgery, have been reported to date [6-19]. Although cancer tissues had not been manipulated in those cases, a malignancy had already been detected at other sites (one uterine cervical squamous; two pancreatic) at the time of surgery in three cases [6, 9, 12] or between surgery and PSM manifestation (one colonic; one uterine; one ovarian) in three cases [10, 15, 17]. Neuhaus et al. [5] reported that so-called PSM tends to occur most commonly between three and nine months after malignancy resection. In addition, seven of nine (77.8%) remaining cases manifested a metastasis to the trocar tract within one year of the surgery (Table 1). These patients might have had microscopic or undetected disease at the time of laparoscopy and the metastases might have been caused by
Ovarian cancer presenting as a metastasis to a trocar tract used for a gasless lift-laparoscopy to resect a benign ovarian cyst: etc.

In summary, the authors report an unusual case of ovarian cancer presenting as a metastasis to a trocar tract used for a gasless lift-laparoscopy to resect a benign ovarian cyst. This case suggested that PSM could occur without direct or indirect wound contamination. Further investigation is required to help us better understand the etiology of PSM.

Table 1. — Pathologic findings.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Age</th>
<th>Sex</th>
<th>Laparoscopic procedure</th>
<th>Pathological diagnosis of the laparoscopic surgery</th>
<th>Time interval</th>
<th>Tumor that caused port-site metastasis</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patner and Damien [6]</td>
<td>1992</td>
<td>42</td>
<td>F</td>
<td>Diagnostic laparoscopy</td>
<td>Endometriosis (detected uterine cervical squamous carcinoma)</td>
<td>4 mo</td>
<td>Uterine cervical squamous cell carcinoma Stage 1B</td>
<td>DOD (9 mo)</td>
</tr>
<tr>
<td>Siriwardena and Samaroo [7]</td>
<td>1993</td>
<td>71</td>
<td>M</td>
<td>Laparoscopic cholecystectomy</td>
<td>Chronic inflammation and fibrosis</td>
<td>4 mo</td>
<td>Pancreatic adenocarcinoma</td>
<td>DOC (1 wk)</td>
</tr>
<tr>
<td>Watson [8]</td>
<td>1995</td>
<td>63</td>
<td>F</td>
<td>Laparoscopic gastroenterotomy</td>
<td>N/A</td>
<td>15 wk</td>
<td>Pancreatic adenocarcinoma</td>
<td>AWD (?)</td>
</tr>
<tr>
<td>Jorgensen et al. [9]</td>
<td>1995</td>
<td>63</td>
<td>M</td>
<td>Diagnostic laparoscopy</td>
<td>N/A (detected pancreatic adenocarcinoma)</td>
<td>7 wk</td>
<td>Pancreatic adenocarcinoma</td>
<td>DOD (?)</td>
</tr>
<tr>
<td>Ugarie [10]</td>
<td>1985</td>
<td>79</td>
<td>F</td>
<td>Laparoscopic cholecystectomy</td>
<td>Unknown (transverse colon carcinoma)</td>
<td>9 mo</td>
<td>Transverse colon carcinoma</td>
<td>DOD (9 mo)</td>
</tr>
<tr>
<td>Lane and Pfau [11]</td>
<td>1996</td>
<td>32</td>
<td>F</td>
<td>Diagnostic laparoscopy</td>
<td>N/A</td>
<td>24 mo</td>
<td>Ovarian serous papillary adenocarcinoma</td>
<td>AWD (?)</td>
</tr>
<tr>
<td>Naumann and Spencer [12]</td>
<td>1997</td>
<td>41</td>
<td>F</td>
<td>Laparoscopic-assisted Syed needle placement</td>
<td>N/A (detected uterine cervical squamous carcinoma)</td>
<td>5 mo</td>
<td>Uterine cervical squamous cell carcinoma Stage 3B</td>
<td>DOD (3 wk)</td>
</tr>
<tr>
<td>Rieger and McIntosh [13]</td>
<td>1998</td>
<td>74</td>
<td>M</td>
<td>Laparoscopic cholecystectomy</td>
<td>Chronic cholecystis with incidental gallbladder cancer</td>
<td>6 mo</td>
<td>Ovarian moderately differentiated adenocarcinoma</td>
<td>DOD (3 mo)</td>
</tr>
<tr>
<td>Lane and Cook [14]</td>
<td>1999</td>
<td>69</td>
<td>F</td>
<td>Laparoscopic cholecystectomy</td>
<td>Chronic inflammation and fibrosis</td>
<td>12 mo</td>
<td>Pancreatic adenocarcinoma</td>
<td>AWD (?)</td>
</tr>
<tr>
<td>Carlson et al. [15]</td>
<td>2001</td>
<td>77</td>
<td>F</td>
<td>Laparoscopic cholecystectomy</td>
<td>Gallstones (ovarian serous papillary adenocarcinoma resected 6 mo after cholecystectomy)</td>
<td>27 mo</td>
<td>Ovarian serous papillary adenocarcinoma</td>
<td>NED (?)</td>
</tr>
<tr>
<td>Nauhaus S et al. [16]</td>
<td>2001</td>
<td>66</td>
<td>F</td>
<td>Laparoscopic cholecystectomy</td>
<td>Chronic cholelithiasis (N/A (ovarian metastasis resected 6 wk after ovarian transposition))</td>
<td>3 mo</td>
<td>Colonic adenocarcinoma</td>
<td>AWD (3 mo)</td>
</tr>
<tr>
<td>Piconc et al. [17]</td>
<td>2003</td>
<td>37</td>
<td>F</td>
<td>Laparoscopic ovarian transposition</td>
<td>N/A (ovarian metastasis resected 6 wk after ovarian transposition)</td>
<td>6.5 mo</td>
<td>Uterine cervical adenocarcinoma Stage 2B</td>
<td>DOD (2 mo)</td>
</tr>
<tr>
<td>Yildirim et al. [18]</td>
<td>2006</td>
<td>52</td>
<td>F</td>
<td>Laparoscopic cholecystectomy</td>
<td>Chronic cholecystis</td>
<td>6 mo</td>
<td>Adenocarcinoma of unknown origin</td>
<td>DOD (18 mo)</td>
</tr>
<tr>
<td>Piekart et al. [19] (13 mo)</td>
<td>2008</td>
<td>57</td>
<td>F</td>
<td>Laparoscopic cholecystectomy</td>
<td>Chronic cholecystis</td>
<td>22 mo</td>
<td>Unknown origin (cholangiocellular carcino</td>
<td>NED</td>
</tr>
<tr>
<td>This case</td>
<td></td>
<td></td>
<td></td>
<td>Laparoscopic ovarian cystectomy</td>
<td>Ovarian mucinous cystadenoma</td>
<td>68 mo</td>
<td>Ovarian mucinous cystadenocarcinoma</td>
<td>NED (60 mo)</td>
</tr>
</tbody>
</table>

1Comments in the parentheses indicate a malignancy that had been detected at the time of the laparoscopy or detected soon after the laparoscopy.
2Time interval between laparoscopy and the diagnosis of port-site metastasis.
3Prognosis after the confirmation of port-site metastasis.
N/A: not applicable; DOD: death due to disease; DOC: death due to other causes; AWD: alive with disease; NED: no evidence of disease; mo: month; wk: week.

References


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Laparoscopic para-aortic and pelvic lymphadenectomy and radical hysterectomy in a patient with cervical cancer, six months after primary chemoradiation

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Summary

Treatment of Stage IB-IIA cervical carcinoma is controversial. The choice to perform surgery or chemoradiation depends on the FIGO Stage, which does not include evaluation of lymph node involvement, although the prognosis of the patients depends on this evaluation. There is no method however, to safely evaluate preoperative lymph nodes metastases, as both magnetic resonance imaging (MRI) and computed tomography (CT) have poor sensitivity and high specificity. As a result, inaccurate preoperative lymph node assessment can lead to suboptimal treatment. The authors report the case of a 42-year-old patient with cervical cancer Stage IB2, who was primary treated with chemoradiation. Although at the time of diagnosis no lymph node metastasis was detected, six months after treatment, an enlarged five-cm lymph node was found in the area of left iliac vein. The patient underwent laparoscopic pelvic and para-aortic lymphadenectomy and nerve sparing radical hysterectomy. Pathologic examination revealed one positive lymph node out of the 41 removed and no cancer cells in the uetal structures. There are cases of cervical cancer in which chemoradiation seems to be insufficient. Laparoscopic nerve-sparing radical hysterectomy can be the treatment in patients with lymph node metastasis after primary chemoradiation. It offers oncological safety combining the advantages of laparoscopy and the nerve-sparing technique. Furthermore, adjuvant chemotheraphy or radiation can be initiated immediately, offering the best therapeutic choice in the authors’ opinion.

Key words: Laparoscopic nerve-sparing radical hysterectomy; Cervical cancer; Recurrence; Lymph node metastasis.

Introduction

Cervical carcinoma is the second most common female malignancy worldwide [1], accounting for six percent of all malignancies. Despite the introduction and widespread of Papanicolaou (Pap) smears in the screening of cervical cancer, many cases are first diagnosed in an advanced stage.

Treatment of Stage IB-IIA cervical carcinoma is controversial. The choice to perform surgery (radical hysterectomy) or chemoradiation depends on the FIGO Stage, which does not include evaluation of lymph node involvement, although the prognosis of the patients depends on this involvement [2]. The used method depends on the center’s experience and possibilities.

Lymph node metastasis has been proven as one of the most important risk factors for relapse and poor prognosis [3, 4], and lymph node resection before radiotherapy results in improved survival in patients with positive lymph nodes; however, there is no method to safely evaluate preoperative lymph nodes metastasis, as both magnetic resonance imaging (MRI) and computed tomography (CT) have poor sensitivity and high specificity [5]. As a result, inaccurate preoperative lymph node assessment can lead to suboptimal treatment [6, 7].

In this paper the authors report a case of a 42-year-old, who underwent laparoscopic para-aortic and pelvic lymphadenectomy and radical hysterectomy six months after primary chemoradiation, for a lymph node recurrence.

Case Report

The patient, a 42-year-old Greek Caucasian woman, gravid 3 - para 2 was referred to this department for an enlarged five-cm lymph node in the area of left iliac vein. The patient was diagnosed nine months prior with cervical cancer, after a colposcopic-guided punch biopsy. The histopathological examination revealed the presence of squamous cell cervical carcinoma, grade 1 and clinical evaluation indicated the presence of a Stage IB2 cancer. A concomitant cisplatin-based chemoradiation was decided. The patient received a total radiotherapy of 4,500 cGy (a daily front field of 90 cGy and a rear field of 90 cGy) with linear accelerator, combined with a weekly infusion of cis-diamminedichloroplatinum [II] (CDDP-70 mg/m²), and brachytherapy of 2,500 cGy.

The patient was followed-up regularly, with blood test, Pap-test and CT or MRI scan, every three months. The first follow-up control at three months post-chemoradiation therapy indicated no recurrence. Six months after treatment, the woman underwent her second follow up. An MRI of both upper and lower abdomen was performed. Although no pathologic magnetic signal from the cervix was found, a 2.5 cm. enlarged internal iliac vein lymph node was seen (Figure 1). Considering all possible treatment approaches, a total laparoscopic nerve-sparing radical hysterectomy with bilateral pelvic and para-aortic lymphadenectomy was decided and successfully performed (Figure 2). The operating time was 232 min and the estimated blood loss was 90 ml. The third postoperative day, the

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urethral catheter was removed and the residual urine volume was less than 50 ml. The patient recovered uneventfully and was discharged on the fifth postoperative day.

Pathologic examination showed a lymph node metastasis out of the 41 removed lymph nodes and there were no cancer cells found in the uterine structures. The last gynecological and MRI examination was 12 months after the operation and showed no evidence of recurrence.

Discussion

The treatment of Stage IB-IIA cervical carcinomas is still controversial. The prognosis of cervical cancer is determined primarily not only by the FIGO Stage, but also by nodal status, tumor volume, depth of invasion, and lymphovascular space invasion. The presence of metastatic lymph nodes modifies the prognosis of patients with cervical cancer [7].

The selected treatment approach usually depends on the center’s experience and internal guidelines and protocols. In the present case, the choice of primary chemoradiation, was based on the hospital’s experience and the used protocol.

The non-invasive methods that are used most for detection of lymph node metastasis are MRI and positron emission tomography computed tomography (PET/CT). Both do not have satisfactory results, as the accuracy and sensitivity for MRI are 76% – 100% and 36% – 71%, respectively [8-10] and for PET/CT 67% – 100% and 92% – 99%, respectively [11-13]. These limitations are due to the size-based characterization of lymph nodes defined as positive in cases with a diameter > one cm [12, 14, 15].

Moreover in gynecological cancers, there is not yet a clear definition of micro-metastasis. There are studies however that have shown that the rate of detection of lymph node micro-metastasis in patients with cervical cancer is very low [16, 17]. As a result, there still is no efficient method to preoperatively evaluate the status of lymph nodes. The authors believe that the best approach for patients with cervical cancer is pelvic lymphadenectomy, even in cases where there is no obvious lymph node involvement.

In the present study, the patient received and completed concurrent chemoradiation as it was planned from the beginning, without any major side-effects. Although at the beginning of treatment there was no indication of lymph node metastases, after the end of chemoradiation and within six months, lymph node metastasis was found. The authors believe that these lymph nodes might have undergone micro-metastases from the time of diagnosis, which were not detected with non-invasive methods.

Chemoradiation proved to be an insufficient treatment for cases with pelvic lymph node involvement and could not prevent the recurrence of the disease. Only with lymphadenectomy there is certainty that the affected lymph nodes have been removed, offering an optimal treatment. Of course, it is necessary with this surgical procedure, to avoid increasing morbidity and mortality.

Over the last ten years, many studies evaluated laparoscopy as an alternative approach for cervical cancer [18-20]. Laparoscopic lymphadenectomy performed by experienced surgeons gives the opportunity for the best oncological outcome while minimizing postoperative complications. Furthermore adjuvant chemotherapy or radiation can be initiated earlier [21].

The authors’ choice for lymphadenectomy was through a laparoscopic approach. By laparoscopy anatomic structures are magnified and lymph nodes can be easily identified and removed. They removed the enlarged lymph nodes en bloc with the other pelvic lymph nodes, without any major complication, or excessive blood loss. Moreover the authors were able to continue performing nerve-sparing radical hysterectomy. This technique maintains the radicality of typical Piver III hysterectomy and has no complications concerning bladder urination, or bowel dysfunctions, be-
cause the inferior hypogastric nerve and inferior hypogastric plexus are preserved, resulting in a high quality of the patient’s postoperative life [22, 23].

Conclusion

There are cases of cervical cancer in which chemoradiation seems to be insufficient. Laparoscopic nerve-sparing radical hysterectomy can be the treatment in patients with lymph node metastasis after primary chemoradiation. It offers oncological safety combining the advantages of laparoscopy and nerve-sparing technique. Furthermore, adjuvant chemotherapy or radiation can be initiated immediately while offering the best therapeutic choice in the authors’ opinion.

References


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Adenoid cystic carcinoma of Bartholin’s gland receiving adjuvant radiation therapy: case report

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Institute of Radiation Oncology, Sant’Andrea Hospital, “Sapienza” University, Rome (Italy)

Summary

Adenoid cystic carcinoma (ACC) of Bartholin’s gland is an extremely rare tumor of the female genital tract, representing about 5%-15% of Bartholin’s gland malignancies. Approximately 80 cases have been reported in the literature. The authors describe the case of a 54-year-old woman with locally advanced ACC of Bartholin’s gland treated with surgery and adjuvant radiotherapy (RT). She underwent radical hemivulvectomy associated with ipsilateral inguinal and femoral lymph node dissection. Subsequently, she received postoperative three-dimensional conformal RT. Total dose prescribed was 56 Gy in 28 fractions of two Gy each. After 20 months of follow up, there was no evidence of local failure or distant progression.

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

Introduction

Adenoid cystic carcinoma (ACC) is an epithelial tumor that predominantly involves the salivary glands, upper respiratory tract, breast, skin, and uterine cervix [1]. ACC of Bartholin’s gland is an extremely rare neoplasm of the female genital tract representing about 5%-15% of Bartholin’s gland malignancies [2, 3]. To date, approximately 80 cases have been reported.

ACC are slow-growing tumors which commonly exhibit perineural space infiltration and local invasion [4]. Signs and symptoms are non-specific and diagnosis is often difficult.

Distant metastases may occur after a long period of time [3], but local recurrences are frequent. There are some controversies regarding the optimal treatment. Surgery is generally recommended as primary treatment. Therefore, adjuvant radiotherapy (RT) could be necessary to improve loco-regional control. There are no large series due to the rarity of this tumor and only reported cases are available in the literature.

The authors describe the case of a 54-year-old woman with locally-advanced ACC of Bartholin’s gland treated with primary surgery and adjuvant RT.

Case Report

A 54-year-old Italian woman presented with palpable vulvar mass and gradually increasing pain over a two-year period. Suspecting a cystic disease, the patient was submitted to excision of the nodule. Microscopic examination revealed an ACC of Bartholin’s gland that extensively infiltrated the subepithelial and deep connective tissue, the striated muscles, and the perineal space. The lateral resection margins and the deep margin were positive. Computed tomography (CT) of abdomen and pelvis showed inguinal lymph nodes with a maximum diameter of 13 mm.

A radical hemivulvectomy with right inguinal-femoral node dissection and multiple biopsies of the labia majora and perineum were performed. Pathological examination confirmed ACC diagnosis with extensive invasion of the adjacent muscle tissue and with perineural space infiltration. Neoplastic emboli were not observed. The tumor was completely excised and the resection margins were negative. The inguinal lymph nodes or labia majora were not affected.

Postoperatively, the patient came to the attention of the Institute of Radiation Oncology. It was decided to treat the woman with external beam radiotherapy (EBRT) based on definitive histology examination.

Pre-treatment CT was performed to three-dimensional conformal planning. She received adjuvant RT to the pelvis and inguinal lymph nodes bilaterally, up to a total dose 56 Gy in 28 fractions of two Gy each, five times a week for about six weeks. EBRT was delivered by a 16 MV linear accelerator utilizing a four-field box technique.

The treatment was well to moderately tolerated. Toxicities were recorded according to Radiation Therapy Oncology Group (RTOG) acute and late toxicity scale criteria. She presented grade 2 acute erythema at the perianal skin and external genitalia and also grade 3 symptomatic vaginal mucositis requiring narcotics. No late toxicities occurred but grade 1 fibrosis (slight hardening) of subcutaneous tissue. After a follow up period of 20 months, the patient is currently healthy without evidence of local relapse or systemic disease.

Discussion

Adenocarcinomas and squamous cell carcinomas constitute approximately 90% of primary Bartholin’s gland neoplasms [5]. ACC of Bartholin’s gland constitutes only 0.001% of all female genital tract and approximately 0.1%-0.5% of all vulvar malignancies. The diagnosis is often difficult. Signs and symptoms include pain, bleeding, burning sensation, palpable mass, dyspareunia, or itching that may be non-specific. Frequently, patients are treated by drainage and marsupialization because the tumor may resemble a Bartholin duct cyst or an infection resulting in a delay of diagnosis [6].
Histologically, ACCs demonstrate a cribriform arrangement of tubules and gland-like elements [7]. The cells are small and darkly-stained with scanty cytoplasms [8]. Infiltration of the perineural space is a very characteristic microscopic feature of adenoid cystic carcinoma and this may explain the propensity of causing pain, itching, and local recurrence [9].

The described patient presented with a palpable vulvar mass and pain and was submitted to excision of the nodule. Microscopic examination revealed an ACC of Bartholin’s gland with perineural space infiltration and positive margins.

Currently, there is no consensus regarding the most favorable therapy for patients with ACC of Bartholin’s gland; surgical treatment is primarily recommended. The surgical extension varies from wide local excision of the tumor to radical vulvectomy with or without lymph node dissection [10]. The surgical procedure should be as conservative as possible, based on the tumor’s dimensions and extension, as suggested by De Pasquale et al. [6]. Radical vulvectomy is recommended instead of wide local excision for extensive lesions or tumors close to the anterior or posterior midline.

The current patient underwent a radical hemivulvectomy with right inguinal-femoral node dissection and ACC diagnosis was confirmed through pathological examination. Resection margins were free of disease, but there was extensive invasion of the tumor into the adjacent muscle tissue and with perineural space infiltration.

This tumor is characterized by a marked tendency for local invasion [11] and this may lead to local recurrence even after radical surgery. The five- and ten-year disease-free survivals are 47%-83% and 33%-38%, respectively [7]. Local recurrences generally precede distant metastases by several months or years. Adjuvant RT has been recommended for patients with perineural space infiltration or positive surgical margins at the histological examination. Rosenberg et al. [12] and Copeland et al. [2] showed that adjuvant RT resulted in good local control in patients who developed recurrent disease.

The authors examined the current case and decided to perform adjuvant RT to improve local control. The patient received a total dose of 56 Gy in 28 fractions of two Gy/each to the pelvis and inguinal lymph nodes bilaterally.

A review by Yang et al. [8] reported that no local recurrence occurred in 15 patients who underwent adjuvant RT. Another recent review by Alsan Cetin et al. [13] analyzed 79 cases of ACC of Bartholin’s gland reported in the literature. They concluded that local and distant recurrence rates are about 30% and 31%, respectively. Recurrence rates were lower in patients who received adjuvant RT compared to those who underwent surgery alone (2/21 patients vs. 21/56 patients; \( p = 0.01 \)).

After 20 months of follow up, the present patient is in good clinical condition without evidence of local or distant disease.

Metastatic spread of this neoplasm seems to be hematological. The first pathway could result in lung, bone, and less often in liver, kidney, and brain [14]. Several chemotherapy regimens such as methotrexate, cyclophosphamide, adriamycin, and cisplatin have been reported, but with no actual demonstrated benefits [15].

In the present case, the patient was successfully treated, but recurrence may occur over a long period of time; therefore, long-term follow-up is needed for such a patient.

In conclusion, ACCs of Bartholin’s gland are slow-growing tumors with marked tendency for local invasion and with perineural space infiltration. At present, no consensus exists regarding the optimal treatment; surgery is a common option as an initial treatment. Adjuvant RT seems to improve regional control and disease-free survival. The most favorable chemotherapy regimen is still unknown.

References


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Pelvic pain, free fluid in pelvis, and human chorionic gonadotropin serum elevation: recurrence of malignant ovarian germ-cell tumor or early pregnancy?

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Summary

Conservative treatment of metastatic germ-cell tumor of the ovary does not exclude the possibility of pregnancy in the future. Serum beta-human chorionic gonadotropin (beta-hCG) serves as pregnancy test, and has also been proven to be a useful marker for ovarian germ-cell tumors. This paper is a case report of a 19-year-old patient who was admitted to a district hospital in emergency due to pelvic pain, amenorrhoea, and free fluid in the pelvis. Laboratory tests demonstrated slight increase in beta-hCG serum concentration and transvaginal ultrasound (TVUS) showed no evidence of gestational sac in the uterus. At the age of 14, the patient was diagnosed with malignant germ-cell tumor of the left ovary in FIGO Stage IV and was treated with four courses of chemotherapy according to TGM-95 protocol with etoposide, ifosfamide, and cisplatin, followed by conservative surgery and adjuvant two courses of cytostatics. The initial diagnosis was recurrence of ovarian malignancy and the patient was referred to an oncology center. Wait-and-see approach and repeated ultrasound examination confirmed a normal intrauterine pregnancy which concluded with the delivery of a healthy newborn through cesarean section.

Key words: Germ-cell ovarian tumor; Conservative treatment; Pregnancy.

Introduction

Malignant ovarian germ-cell tumors are heterogenic and relatively rare malignancies that typically present in adolescents and young women up to 30 years of age. For patients with early-stage disease, cured rates approach 100%, while for those with advanced-stage disease are at least 75% [1]. Typical symptoms include lower abdominal pain and/or incidentally diagnosed abdominal/pelvic mass, sometimes easily palpable during routine physical examination. Initial diagnosis is based on physical examination, imaging examinations (pelvic/abdominal ultrasoundography, computed abdominal/pelvic tomography, chest radiography) and concentration of tumor markers which typically include alpha-fetoprotein (AFP), beta-human chorionic gonadotropin (beta-hCG), cancer antigen 125 (CA-125) and lactate dehydrogenase (LDH). Serum marker concentration facilitates to establish a diagnosis and is useful in monitoring treatment, as well as post-treatment surveillance. Increasing tumor marker concentration usually and reliably predicts clinical recurrence and is sometimes used to initiate cytotoxic systemic therapy for recurrence in the absence of clinically demonstrable disease.

Conservative treatment of metastatic germ-cell tumor of the ovary does not exclude the possibility of pregnancy in the future. Appropriate modern surgical approach to malignant germ-cell tumors even in an advanced stage include preservation of reproductive function, particularly if the contralateral ovary is normal [1, 2]. Recommended adjuvant treatment for most cases of malignant germ-cell tumors in adults is combined chemotherapy with bleomycin, etoposide, and cisplatin (PEB) [3, 4]. However, some national pediatric oncology groups presented specific protocols for malignant germ-cell tumors treatment in children and adolescents, i.e. bleomycin, etoposide, and carboplatin (JEB) in United Kingdom [5]; PEB in Germany and USA [6, 7] or vinblastine, bleomycin, and cisplatin (VBP) for standard-risk patients and ifosfamide, etoposide, and cisplatin (VBP) for high-risk patients in Poland and France (TGM-95 protocol) [8]. According to new data, the five-year survival rate for germ-cell tumors is 89.3% in girls aged zero to nine years, and 97.7% in girls aged ten to 19 years, with more favorable indices for gonadal compared with extragonadal tumors [9].

Case Report

The 19-year-old nulliparous female was admitted to a district hospital as emergency admission with severe pelvic pain and amenorrhoea. Her last menstrual period was five weeks prior. She had regular menstrual cycles, with periods lasting for an average of four to five days and showed no complaints of any genital tract symptoms over the last three to four years. She reported that the pelvic pain appeared two weeks prior and became more severe over the last 48 hours. Physical gynecological examination showed normal cervix without any discharge and moderate tenderness of the uterus. Vital signs as well as haematology and biochemistry blood tests showed no deviation from the norm. CA-125 was 23.1 U/ml and beta-hCG was 124.2
U/ml. Abdominal ultrasound examination of the liver, pancreas, spleen, kidneys, and para-aortic area was normal. Transvaginal ultrasound (TVUS) scan showed normal anteflexed uterus with regular hyperechogenic endometrium of 13.0 mm thickness, with no evidence of a gestational sac (Figure 1). Right ovary was 36.7 x 22.7 mm with dominant follicle of 20.0 mm in diameter, and left ovary was not visualized. Large amount of free fluid in pouch of Douglas was detected (Figure 2).

Past medical history of the subject included management of malignant germ-cell tumor of the left ovary, which was diagnosed five years prior, when the 14-year-old girl was admitted to the children’s hospital with abdominal pain and pelvic mass. Abdominal ultrasound revealed a solid-cystic non-homogenous mass with regular margins and poor vascularization, originating from the left ovary. Additionally, ultrasound scan showed single metastatic hypoechogenic lesion in left lobe of the liver, measuring 40 x 40 mm, normal pancreas, spleen, kidneys, and para-aortic area. Computed tomography (CT) of the abdomen/pelvis confirmed a solid-cystic ovarian tumor measuring 181 x 160 x 151 mm with contrast-enhanced septa and solid part of the lesion (Figure 3). Liver lesion was a hypodense lesion (52 jH peripherally and 32 jH centrally), characteristic for liver metastasis (Figure 4). Blood markers included: beta-hCG: 2.27 U/ml, CA125: 86.10 U/ml, LDH: 377.0 U/l, and AFP > 3,5350.0 ng/ml. The diagnosis of metastatic germ-cell tumor of ovary FIGO Stage IV was made and the patient qualified for initial chemotherapy according to protocol TGM-95, and received four courses of ifosfamide 5.6 g (3 g/m²), etoposide 140 mg (75 mg/m²), and cisplatin 37 mg (20 mg/m²), every three weeks. Markers concentration after a fourth course of chemotherapy decreased to normal values (beta-hCG: 0.0 U/ml, LDH 255.0 U/l, and AFP: 5.14 ng/ml), with only slight increase in CA125 concentration, to 125.13 U/ml. After induction therapy, the patient underwent conservative surgical treatment which included unilateral adnexectomy, selective iliac lymphadenectomy, and radical resection of left lobe hepatic metastasis. Histopathological postoperative examination revealed features of benign adult teratoma with extensive fibrosis, hyaline degeneration, and necrosis, with no signs of malignancy. The patient received an additional two courses of adjuvant VIP combined chemotherapy. All blood tumor markers were within the norm, with CA125 level of 32.1 U/ml, and control CT of the chest, abdomen, and pelvis showed...
no abnormalities. Complete remission was diagnosed and the girl was referred to standard scheduled follow-up visits, which were performed every four months for the first two years, and every six months during the third and fourth year. The next visit was routinely planned in 12 months time.

Based on past medical history, physical examination, and the results of additional tests, the patient was initially diagnosed with malignant germ-cell tumor recurrence five years after oncologic treatment. She was treated with intravenous crystalloids and anti-spasmodic drugs, that provided adequate control of pain symptoms and was finally referred to oncology centre. Repeated ultrasound examination three weeks later showed regular gestational sac in the uterine cavity with diagnosis of normal intrauterine pregnancy. The actual fetal age was not equal with age of the pregnancy from the last menstrual period and the difference was four weeks. The woman had prenatal first-trimester screening test (ultrasound scan, PAPP-A, total hCG) which showed no increased risk in fetal chromosomal abnormalities. The pregnancy lasted 39 weeks without serious complications and finished with delivery of healthy newborn infant weighing 4,100 g by cesarean section. The surgery completed without complications and there were no signs of anatomical changes and adhesions in the abdomen/pelvis.

**Discussion**

The initial diagnosis of malignant ovarian germ-cell tumors should be based on physical examination, imaging examinations, and tumor markers concentration. However, the final diagnosis of malignancy should always be histologically confirmed. Even in patients with advanced ovarian neoplasms, oncologic standards require histopathological malignancy confirmation before cytotoxic chemotherapy administration. Paediatric oncology groups (including French and Polish Paediatric Oncology Societies) divide malignant non-seminomatous ovarian germ-cell tumors into two groups: secreting and non-secreting tumors. In case of secreting tumors, final diagnosis may be based on the results of high AFP and/or beta-hCG serum concentrations. Recommended treatment of advanced stage malignant secreting ovarian germ-cell tumor (FIGO Stages III-IV) in children and adolescents includes initial chemotherapy until negativization of tumor markers and debulking surgery (TGM-95). Primary resection of secreting tumors at all sites may be indicated only in those tumors, in which the diagnostic imaging suggests a high-risk of a primary complete tumor resection [6]. Complete removal of all post-chemotherapeutic residual masses in non-seminomatous germ-cell tumors remains the standard of care and allows for improved prognostication of the long-term outcome [10]. All residual masses should be excised to exclude active residual tumor, and in case of malignant teratomas, prevent a mature growing teratoma syndrome, or subsequent dedifferentiation of teratoma back into active cancer [11]. Although histological findings may show only fibrosis, necrosis, or mature teratoma components, tumor tissue status is essential in planning further management [11]. Growing teratoma syndrome has to be taken into consideration, especially in younger patients [12, 13]. Although the phenomenon of malignant transformation of teratoma is rare, it is always to be expected if a tumor is not completely resected [14].

The number of initial chemotherapeutic courses depends on the presence of risk factors. Patients with AFP serum concentration < 15,000 ng/ml and lack of distant metastases are stratified as a standard-risk group, whereas patients with AFP serum concentration ≥ 15,000 ng/ml and/or distant metastases are stratified as a high-risk group and have to be treated more intensively (TGM-95). Additionally high AFP concentration (> 1,000 kU/l) at diagnosis may be associated with higher rates of recurrence [15, 16].

At the time of initial tumor diagnosis, the present patient had elevated blood markers, with extremely elevated AFP, average LDH/CA 125, and very slight increase in beta-hCG. These results referred her into the group of high-risk patients and she was administered four courses of VIP chemotherapy followed by conservative surgery and two courses of adjuvant chemotherapy until negativization of all tumor markers occurred. Despite successful treatment and good germ-cell tumors prognosis, still 26.8% of women with an advanced-stage of the disease relapse [17], with median time to recurrence of eight to nine months [17, 18]. Because serum tumor markers are very sensitive for the presence of relapse, and CT scans may lead to significant radiation exposure over time, CT imaging is not indicated without clinical symptoms or evidence of serum markers elevation, especially in children and adolescents [19].

Based on the present symptoms, physical examination, past medical history, beta-hCG serum concentration, and the results of ultrasound scans, the initial diagnosis of malignancy recurrence was made; especially TVUS examination was very suggestive for relapse, with signs of large amount of free fluid in pelvis, dominant follicle in the ovary, and no gestational sac in uterine cavity. Certainly, differential diagnosis had to exclude other causes of pelvic pain, amenorrhoea, and free fluid in pelvis, including normal and extrauterine pregnancies. More than 90% of relapses of malignant ovarian germ-cell tumors occur at the primary site of the tumor [6]. Relapse chemotherapy in these cases must be accompanied by an intensive local therapy, preferably complete resection of the recurrent tumor after tumor-reduction by preoperative neoadjuvant chemotherapy [6]. In order to maximize chances of survival, rapid diagnosis and referral to oncology centre are essential.

In the case of the present patent, wait-and-see approach and TVUS examination performed three weeks later allowed to diagnose normal intrauterine pregnancy which concluded with the delivery of healthy newborn through cesarean section. Having undergone complex treatment of metastatic malignant ovarian germ-cell tumor does not exclude the possibility of normal pregnancy in the future [20, 21]. However, in specific clinical circumstances, oncologic decision-making process requires repeated examinations and, as in this case, final diagnosis has to be postponed for several weeks.
References


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Endometrial stromal sarcoma with coexistent endometrioid adenocarcinoma in a woman with previous breast cancer: a preliminary case report

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Summary

Endometrial stromal sarcoma (ESS) is a rare neoplasm of the uterus. The authors report a case of low-grade ESS coexistent with endometrioid adenocarcinoma of the uterus in a woman with a previous history of breast cancer. To the best of their knowledge, such a case has not been reported to date.

Key words: Endometrial stromal carcinoma; Adenocarcinoma; Breast cancer.

Introduction

Endometrial stromal sarcomas (ESSs) are rare neoplasms that account for 0.2% – 1% of all uterine malignancies [1]. The annual incidence of ESS in Europe is one to two per million women [2]. Currently, ESSs are classified into low-grade endometrial stromal sarcomas and undifferentiated stromal sarcomas [3]. Low-grade ESSs are low malignant potential tumours composed of endometrial stromal cells in the proliferative phase that invade the myometrium or vascular spaces and have a fairly indolent course. High-grade ESSs are less common than low-grade ESSs and are undifferentiated or poorly differentiated uterine sarcomas. They are highly malignant tumours that lack overt stromal differentiation and have aggressive clinical course (4).

To date, no study about the coexistence of ESS with endometrial adenocarcinoma has been reported in the English literature. Here, the authors report a case of low-grade ESS coexistent with endometrioid adenocarcinoma in the uterus of a woman with a previous history of breast cancer.

Case Report

A 77-year-old multiparous woman complained of vaginal bleeding for one month. The patient had hypertension but did not have diabetes. She had undergone right mastectomy for breast cancer five years prior, and she was given four cycles of chemotherapy after the operation. She had been using tamoxifen for the subsequent five years. Her gynaecological examination indicated an eight- to ten-week pregnancy-sized uterus with non-palpable ovaries. Ultrasonography examination showed that the uterus (diameter, 10 × 10 × 7 cm) had multiple fibroids and an endometrial thickness of 38 mm, while the ovaries had normal size and appearance.

She underwent endometrial biopsy, and histopathological examination showed a well-differentiated endometrioid adenocarcinoma. The patient was scheduled for exploratory laparotomy. The laparotomy indicated that the uterus was enlarged with multiple fibroids but the ovaries seemed normal. Peritoneal washing cytology was performed. Furthermore, total abdominal hysterectomy and bilateral salpingo-oophorectomy and pelvic and para-aortic lymphadenectomy were performed.

After routine procedures, specimens were embedded in paraffin and cut into five µm sections. The sections were stained with haematoxylin and eosin (H&E) for routine evaluation. Moreover, they were immunohistochemically stained with smooth muscle actin (SMA), cytokeratin 7, vimentin, and CD10 to check for the differentiation of epithelial/endometrial and mesenchymal/endometrial stromal cells.

Histopathological examination showed crowded, complex branching glands with cribriform architecture, and back-to-back glands without intervening stroma. Tumour cells showed loss of polarity and cytological atypia. They had large, round nuclei with prominent nucleoli, and were positive for cytokeratin 7 and were negative for CD10 and SMA (adenocarcinoma). Moreover, diffuse infiltrative spindle tumour cells were observed in the myometrial bundles in different areas. They resembled endometrial stromal cells superficially, and were CD10 and vimentin positive, cytokeratin 7 and SMA negative. Mitotic cell rate was more than ten mitotic figures/ten high-power fields (ESS). Immunohistochemical analysis indicated that the tumor cells in the ESS and endometrial adenocarcinoma components were negative for estrogen and progesterone receptors. (Figures 1-A and B–D). The final histopathological diagnosis indicated a well-differentiated endometrial adenocarcinoma and low-grade ESS.

Discussion

Low-grade ESS typically occurs in perimenopausal women (age, 40–55 years) [4]. Patients usually present with abnormal uterine bleeding, pelvic pain, and uterine enlargement. At presentation, approximately 30% of the patients with low-grade ESS have extraterine diseases [5]. These tumours are often misdiagnosed as leiomyomas.
Most patients with ESS undergo surgery because of the diagnosis of myoma uteri or the presence of pelvic mass. Because most of the ESSs grow in the myometrium rather than intracavitary, accurate diagnosis using curettage may also be difficult. Therefore, most often the tumour is finally diagnosed only after surgery via pathological examination [4, 6]. The neoplastic cells in low-grade ESS are morphologically the same as the cells in endometrial stromal nodules, which is a benign endometrial stromal neoplasm that has non-infiltrative behaviour. The main distinction between them is that low-grade ESS shows myometrial and vascular invasion. Although low-grade ESSs have an indolent course, they often invade extraterine veins and lymphatic tissues. Although low-grade ESS has a good prognosis, extraterine metastasis is reported in up to one-third of women at diagnosis, and ovaries are the main site in extraterine metastasis [4]. ESSs are commonly positive for estrogen and progesterone receptors [6]. The negativity of oestrogen and progesterone receptors in the present case might be because of long-term tamoxifen use. It has been known that unopposed estrogen is the main risk factor for the development of endometrial carcinoma. The pathogenesis of stromal sarcoma is still unknown; however, the relationship between unopposed estrogen and exposure to tamoxifen with ESS has been implicated in some cases [7, 8].

For the first time in the literature, a case of endometrial adenocarcinoma coexistent with low-grade ESS has been reported. Interestingly, the coexistence of these two different tumours in a patient with a history of breast cancer and tamoxifen use for five years is also a remarkable finding. The present case suggests that long-term tamoxifen use might be among many etiological factors in ESS development.
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Vulvar squamous cell carcinoma developing in a young black African HIV woman

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Summary

Vulvar cancers are uncommon, represented in 90% of cases by squamous cell carcinoma (SCC). The reduction of the frequency and the severity proceed by recognition of precancerous or beginning lesions. They occur most often in the third age in postmenopausal women. The diagnosis is almost difficult and often late and therefore prognosis is severe. Conditions for diagnosis and treatment are difficult in underdeveloped countries due to the inaccessibility of proper equipment in the healthcare system. The authors report a case of SCC diagnosed late in a young human immunodeficiency virus (HIV) women who have been treated with neoadjuvant chemotherapy and radical surgery of the vulva.

Key words: Carcinoma; Vulvar cancer; Diagnosis; Chemotherapy; Radiotherapy.

Introduction

Cancers of the vulva are uncommon and represent four to five percent of female genital cancers. Squamous cell carcinoma (SCC) is the most frequent of them and is estimated to occur in 90% of vulvar cancers [1]. In the present authors’ daily practice, no cases have been reported, occurring especially in the elderly. They have a poor prognosis in the poor countries mainly due to late diagnosis and the inaccessibility of the diagnosis means. The scarcity of the frequency and clinical polymorphism is also the source of misdiagnosis and the poor prognosis.

The authors report this observation to show its uniqueness, maturity of onset (young woman), and difficulties of the diagnostic in the present under medicalized country.

Case Report

A 25-year-old female was admitted for a large painless swelling extended to the entire vulva which began three months prior. Her medical history, revealed external suppuration of the vulvar lips. She received treatment for Bartholinitis of left vulvar lips which included local care and oral antibiotics. The evolution was brief, marked by appearance of indurated vulvar bud. Vulvar examination subsequently found an indurated swelling with ulcerative necrotic areas, extended to all areas of the vulva (Figure 1). The vaginal wall and cervix were not affected. The authors also found large bilateral inguinal lymphadenopathy which was painless but with significant weight loss. They discussed the diagnosis of SCC of the vulva as Stage III (FIGO). Biopsy of lesion was performed for histological examination which was in favor of a mature differentiated SCC.

Neoadjuvant chemotherapy was undertaken involving 5FU, mitomycin C. A second time surgery included a partial vulvec-

tomy with bilateral inguinal lymphadenectomy. Monitoring is currently being evaluated as we are at six months post-surgery and chemotherapy.

Discussion

Epidemiological factors

SCC is the most common vulvar cancers of the vulva. It is most often preceded by precancerous lesions or intraepithelial neoplasia (IN) or vulvar intraepithelial neoplasia (VIN) for the Anglo-Saxons. The association with human papillomavirus (HPV) is common and affects females under 50 years of age with also an early appearance in girls, such as Bowenoid papulosis type [1]. This could partly explain the fairly early occurrence of this vulvar carcinoma in the case the authors report. In some studies, the existence of other risk factors for occurrence of invasive vulvar which involve HPV have been reported [2]. Some of them include multiple sexual partners or a history of condyloma concept [3-5]. Similarly, tobacco intoxication also seems to be associated with risk of developing vulvar SCC. Furthermore, other contributing factors such as immunosuppression in human immunodeficiency virus (HIV) and drug addiction can allow the emergence of this disease as was the case in the present patient. The association of HIV and pregnancy is very common especially in black Africa sub-Saharan context. In fact, HIV infection aggravates physiological immunosuppression of pregnancy and causes the emergence of many opportunistic infections as well as some “opportunistic cancers”. Indeed, many cases of cervical cancer were mainly described in the literature and very few works allude to vulvar cancers in pregnant women. Immunosuppression promotes the appearance of invasive vulvar SCC especially in young women infected with HIV [5]. Most of these predisposing factors were found in the reported case.
Clinical aspects

In the reported case, the vulvar lesions were mainly localized at the entire vulva (Figure 1). It is indeed actually their preferred seat observed in 70% of cases [1]. However in rare cases (5%) lesions may be multifocal and in 10% while they are too large and difficult to be recognized [6]. It seems similar with the case the authors report, particularly because of the delay in diagnosis of the disease that does not allow the assessment of initial injury.

The circumstances of diagnosis of the disease is variable. Most often these carcinomas are revealed by vulvar pain, bleeding, and signs of urinary compression. Sometimes they are preceded by a chronic vulvar pruritus evoking an underlying lychen sclerosis [1]. At other times, as in the present patient, the tumor may present with a budding, bleeding on contact, or performing invasive lesion aspect, which can be more or less ulcerated [6].

The physical examination can also, as in the present case, reveal the existence of numerous bilateral inguinal lymphadenopathy, with a median spread (clitoris). [1] Indeed, the mode of dissemination of SCC is primarily vulvar lymphatic and rarely hematogenous. In most cases, the initial damage is superficial inguinal and ipsilateral. The presence of distant metastases is rarely observed at initial stage [7], as illustrated by the present observation. However, the most common site of visceral metastases is the lung [7]. Extension of the initial assessment consisted of an ultrasoundinguinal ligament soft tissue, abdominal-pelvic ultrasound, computed tomography (CT) thoraco-abdominal-pelvic, cystoscopy and/or proctoscopy.

In all cases, the diagnosis is made by histological examination of the tumor after a biopsy performed under general anesthesia. It should, however, be emphasized that in the present limited context, it is important to consider any tumor of the vulva as a cancer until proven otherwise, and histologically treat it as such.

Histological aspects

In the case of vulvar invasive SCC, histology is crucial and is the only one exam that allows the diagnosis. She shows at first the macroscopic aspects of the SCC of vulva (dimensions of the specimen fixed size and uni-or multicentric and its exophytic or infiltrative appearance) [1]. The microscopic characteristics are equally important (histological type, degree of differentiation and maturation, degree of stromal invasion, resection margin) and must also be sought with priority. SCC histologically mature differentiation is the most common type. The present authors found the same in this case which showed an atypical proliferation of squamous and invasive cells, consisting of clusters of varying sizes. These microscopic lesions are rounded or stretched more or less confluent (Figure 2). They are mainly constituted by squamous cells with eosinophilic cytoplasm, sometimes dyskeratotic with large nuclei, and irregular mitosis [1]. Vulvar mucosa adjacent to carcinomatous lesions is often the seat of a lychen sclerosis with or without epithelial hyperplasia regular [1]. (Figure 2).

Prognosis

Vulvar cancer prognosis is bleak in African poor countries because of late diagnosis, limited diagnostic capabilities and inefficient support. It is in all cases correlated with the size of the primary tumor, the degree of stromal invasion, and lymph node involvement [8,9]. FIGO and TNM classifications indeed take account of all these factors (Table 1).
The main prognostic factor remains the lymph nodes and the number of affected lymph nodes [10-12]. The five-year survival is between 25% and 91% for many Western authors [9-11]. The five-year survival is low (25%) when the inguinal region affected is bilateral [13] as in the presented case. Involvement of pelvic lymph nodes (FIGO Stage IV) is a very poor factor of prognosis (23% survival at three years). Capsular rupture is also considered as a factor of worse prognosis [2, 3]. In the present context with low income, illiteracy, and less medicalization, five-year survival is difficult to assess. Overall survival at five years of endometrial carcinoma with all stages combined is approximately 70%. Two-thirds of patients will initially present with Stage I and II (FIGO) and have a five-year survival of approximately 80% to 90% [10,14], but this rate is only 60% to 50% and from 18% to 15% for Stages III and IV (FIGO), respectively [10, 15].

Regarding recurrence, most (60%-70%) occur within the first two years, and particularly in the case of lymphadenopathy [12, 16] and earlier when they are more often poor prognosis and lymph nodes. However, 20%-35% of recurrences occur within five years and over [16, 17].

In the reported observation, the authors could not predict survival at five years. The patient was lost at follow-up and therefore the authors can not accurately assess the risk of recurrence. However, they believe it is around 35%, given their limited means of support.

Treatment
It is based mainly on the combination of radiotherapy pre-postoperative surgery (vulvectomy and lymphadenectomy) and chemotherapy. The lack of equipment of health structures leads to limit the association with neoadjuvant chemotherapy and surgery. Indeed radiotherapy is not available in this country, which greatly reduces the efficiency of the management of cancers. It would allow a reduction in volume and a better control of nodal disease or improve survival [1].

In the absence of radiotherapy, neoadjuvant chemotherapy is widely used in the fight against cancers in poor countries (5FU, vincristine, mitomycin C, cisplatin) [17, 18]. Overall, the five-year survival remains low (5%) of patients treated by chemotherapy [7].

Sentinel nodes research is promising as it limits illegitimate and systematic lymphadenectomy - a recourse for extended carcinoma. It also allows the identification of patients that have micrometastases [1]. The treatment is based on measures that take into account the treatment of HIV infection, as the fight against other predisposing factors for the occurrence of extended carcinoma.

Monitoring
Several authors propose monitoring three times per year for two years, then twice a year for three years, with a chest radiograph and abdominopelvic imaging if there are lymph nodes [12, 19-22]. Transabdominal and transvaginal ultrasound may be sufficient in asymptomatic forms, complemented by a CT scan or magnetic resonance imaging in abdominopelvic symptomatic forms [21].

Conclusion
Vulvar cancers are rare, making it difficult for their diagnosis and management. Screening by regular gynecological examinations and biopsy of any suspicious lesion, allows early treatment thus improved prognosis.

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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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