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DISTINGUISHED EXPERT SERIES

The ideal cervical cancer screening recommendation for Belgium, an industrialized country in Europe

W.A.A. Tjalma - Antwerp, Belgium

HPV screening at an interval of five years will have a 70% greater protection against cervical cancer than cytology. Together with vaccination, this will eradicate cervical cancer.

ORIGINAL ARTICLES

Accuracy of hysteroscopy made by young residents in detecting endometrial pathologies in postmenopausal women

F. De Marchi, A.M. Fabris, L. Tommasi, L. Nappi, C. Saccardi, P. Litta - Padova, Italy

More than 200 hysteroscopies are necessary for a resident to well recognize premalignant and malignant lesions.

Importance of platinum-free interval in second-line chemotherapy for advanced or recurrent endometrial cancer


An L1 HPV test can give a false negative result and subsequently may lead to a delay in cancer diagnosis.

Whole-body [18F]fluoro-2-deoxyglucose positron emission tomography scan as combined PET-CT staging prior to planned radical vulvectomy and inguinal/femoral lymphadenectomy for squamous vulvar cancer: a correlation with groin node metastasis

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BRMS1 inhibits expression of NF-kB subunit p65, uPA and OPN in ovarian cancer cells


A potential novel mechanism by which BRMS1 inhibits metastasis of ovarian cancer cell was investigated.

Carcinomas and sarcomas of Bartholin gland. A report of nine cases and review of the literature

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The management of carcinomas and sarcomas of Bartholin gland, based on the latest scientific knowledge, is discussed.

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Uterine leiomyosarcoma: report of three cases and review of the literature
A case report of three cases of unsuspected uterine leiomyosarcoma, diagnosed by pathologist after hysteroscopy resection, is discussed.
The ideal cervical cancer screening recommendation for Belgium, an industrialized country in Europe

W.A.A. Tjalma
Department of Obstetrics and Gynecology, University Multidisciplinary Breast and Gynecologic Oncology Clinic, Antwerp University Hospital - University of Antwerp, Antwerp (Belgium)

Summary
Cervical cancer should be a historical disease, why are we not succeeding! The prophylactic vaccination will reduce cervical cancer by almost 80% in Belgium. Cervical cancer screening should therefore remain in order to prevent the remaining 20%. The current used Pap cytology test misses 50% of all clinically significant precancers and cancers at the time of testing. The test should remain but the analysis should be altered. The screening should be modified based on our knowledge of human papillomavirus (HPV) as causal factor. Instead of looking for a cell abnormality, one should look for the presence of HPV. Then depending on the test, only two to ten percent of all relevant lesions are missed. The introduction of the vaccination should lead to the re-introduction of the screening based on HPV. This will not only lead to a considerable reduction in morbidity and mortality, allow longer screening intervals, but it will also be more cost-effective. More for less should be the driving force in cervical cancer screening if we want to be successful.

Key words: Cervical cancer screening; Cytology; HPV; Pap triage of HPV positive; Mortality; Sensitivity; History; Vaccination.

Introduction
The most recent cancer report in Belgium was published in 2011 and showed that in 2008, 643 women were diagnosed with cervical cancer at a mean age of 54 years and that 186 died of this disease. The crude incidence and mortality rates (n/100.000) are respectively 11.8 and 3.4 [1]. The five-year survival for the period 2004 - 2008 in our country regardless of stage was 70%. According to stage the survival was Stage 1: 92%, Stage 2: 64%, Stage 3: 55%, and Stage 4: 15%. These figures look good, but we have to keep in mind that 65% of the patient data is missing [2].

In our country cervical cancer is the eighth most frequent tumour in females (2.3%) and the third most frequently occurring gynecological tumour. The majority of cervical tumours are diagnosed in Stage 1, but no significant trend is observed for these tumours over the last ten years [1].

In Belgium the recommendation to screen is adapted from the European Guidelines and foresees one Pap smear or liquid-based cytology sample for women of 25 to 64 years at a three-year interval [3-6]. In the Flemish region, cervical cancer screening program began in 1994 and in Walloon region no formal screening program is in place [3,7,8].

Currently, the screening for cervical cancer is essentially still opportunistic, which means that a smear is taken on the initiative of the woman or her clinician (general practitioner or gynaecologist). The coverage has remained stable the last 15 years. For the period 1998 – 2000 and 2007 – 2010, the coverage rate was respectively, 59% and 62%. This is still well below the aim of 85%, which was set in 1994! Furthermore the screening is still based on cytology, with only reflex HPV testing in case of ASCUS.

Cervical cancer still exists due to the fact that still around 40% of the women in Belgium are not taking part in the screening and to the fact that cytology misses almost half of all abnormal smears. The reality is that half of the women with cervical cancer are never screened and that 20% of the women with cervical cancer did have a pap smear within the last five years, but the cytology was “normal” [9].

The cancers, which are often missed by conventional cytology, are the adeno- and adenosquamous carcinomas [10, 11]. In fact there is a rise in the absolute and relative incidence of cervical adenocarcinoma (ADC) in many countries [10, 11]. The decline of cervical cancer has to be attributed to a decrease in squamous cell carcinoma (SCC). ADC currently accounts for up to 25% of all cases of cervical cancer [12]. This may again be due to the limitations of detecting ADC at screening or it could be that the incidence of ADC is truly on the rise [13].
Human papillomavirus (HPV) has been identified as the causal factor of precancer and cancer of the cervix [14, 15]. Genital HPV is acquired through skin-to-skin contact. The best example of skin-to-skin contact is intimate genital or orogenital contact. The lifetime risk of getting infected with HPV is 80% [14, 15]. About 80 to 90% of all HPV infections are transient however and disappear within one to two years [11, 14-17]. If the HPV infection persists, then there is a significant risk of developing a precancerous lesion. The timeframe from initial infection to preinvasive and in the end invasive disease appears to be at least 10 to 15 years [11]. When one uses cytology for screening, one looks for the abnormality caused by a HPV infection. The cytology screening has a sensitivity of only 53% [18]. To be sure that everyone understands this, almost 50% of all precancers and cancers are missed; perhaps an underestimation but cytology has a high false negative rate. The latter is especially true for the adenocarcinomas. HPV is the causal factor for cervical (pre)cancer and it is therefore more logical to look if the HPV infection is present or not. Primary HPV screening would be a major improvement with a sensitivity of 93% [18].

The optimal screening strategy should identify those cervical cancer precursors likely to progress to invasive cancers (maximizing the benefits of screening) and avoid detection and unnecessary treatment of transient HPV infection and its associated benign lesions that are not destined to become cancerous (minimizing the potential harms associated with screening) [19].

Cervical cancer guidelines should be simple to use for the clinician and acceptable for women. Confusion among women and physicians leads to not attending the screening program, over- and under-screening, increased morbidity and mortality, and an increased cost for society without any benefits.

When to start and when to stop screening

The screening is recommended to start at 25 years and to stop at 65 years [3-8]. When you look at the distribution of smear according to age, then you notice that 10% is younger then 25 years, 82% of the women are from the target population (25-64 years) and 7% are older then 65 years [8]. The screening under 25 years and over 65 years cost the RIZIV/INAMI [20] about 12 million Euro per year. These costs are well-spent if lives are saved. In 2004, England raised the starting age of cervical screening from 20 to 25 years [21]. There is an increase of the incidence of cervical cancers in young women, but this increase in incidence is unrelated to the change in screening policy of 2004 [21]. The increase is likely to be associated with an increase in exposure to background risk including HPV [21]. The screening coverage in the women 25-29 years is declining. Efforts should be made to change this attempt.

A earlier large British study looked at odds of developing cervical cancer based on whether or not women had Pap in prior three-year interval [22]. Cervical screening in women ages 22-24 years had little or no impact on the rates of invasive cervical cancer up to age 30 years [22]. Due to the fact that women are getting older, in could be argued to increase the upper limit age to 70 years. The latter especially if you think that more women die of cervical cancer above 70 years than below 30 years. More research is needed before the upper age limit can be altered. Unfortunately this leads the screening of young women to unnecessary evaluation and potentially to treatment of pre-invasive cervical lesions that have a high probability of regressing spontaneously and that are on average many years from having significant potential for becoming invasive cancer [10, 11, 14, 19]. One of the greatest dangers of this over-treatment is premature birth [23].

Based on the first HPV vaccination trials, women below 25 years should be recommended to have a HPV vaccination [24]. The combination of HPV vaccination in adult and young adult women is expected to reduce substantially the cervical cancer disease burden in Belgium compared to screening alone [25]. Up to 40 years HPV vaccination is cost-effective in women [25].

The HPV vaccination coverage rate for a completed schedule in 12-13 year-olds in the Flemish region (Vlaanderen) is 84%, while in the Walloon region it hardly reaches 20% [26-28]. The Flemish region is the best-vaccinated region in the world. This is due to the school-based program, which started in September 2011. Instead of screening young women, they should be informed about the benefits of HPV vaccination, the risk of sexual transmitted infections, the use of condoms, and the methods of contraception. The high vaccination figures will reflect in 15 to 30 years in a substantial reduction of precancer (estimated > 50%) and cancer (estimated > 80%) [29]. Currently vaccines against more HPV types are in trials. Already the first data of a nine-valent vaccine have been presented [30]. This vaccine is directed against the high-risk HPV infections types 16, 18, 31, 33, 45, 52, and 58 and the low-risk HPV infections, types 6 and 11. With the high efficacy rates against precancer lesions, it is to expected that this vaccine can prevent approximately 90% of all cervical cancers. A biologies license application for this vaccine (V503) to the U.S. Food and Drug Administration (FDA) is to be expected at the end of 2013 or the beginning of 2014 [31].
What should be done: cytology, HPV, or HPV+, and cytology triage?

Multiple meta-analyses have shown that a Pap cytology test fails to detect on average 50% of clinically significant pre-cancers and cancers present at the time of testing [32]. The main reason why Pap smears miss almost 50% of all pre-cancers and cancers is the fact that it is performed by humans and therefore subjective. HPV screening is superior to cytology because it is objective, reproducible, and standardized. HPV testing has a sensitivity of 40 to 45% higher than cytology. This means automatically c.q. practically a strong reduction in false negatives.

The evidence for this can be found in 24 cross-sectional studies and 11 multi-country randomized controlled trials (RCT) [32-47]. In order to have a good comparison for Belgium, we will have to look at the four RCTs performed in industrialized European countries. The four countries were Sweden (Swedescreen), the Netherlands (POBASCAM), England (ARTISTIC), and Italy (NTCC) [37, 39, 41, 44, 47]. All studies had different screening protocols.

Precancer lesions and the 4 RCTs

The relative incidence of CIN3 or worse histological findings after the first screening round was similar in all studies (Table 1) with no evidence of heterogeneity ($p = 0.681$) [37, 39, 41, 44, 47, 48]. The fact that they are similar suggests that the efficacy in prevention depends primarily on the screening test and not on the different screening protocols [47].

All these results show clearly that HPV-based screening detects persistent precancer (high-grade) lesions before cytology. This early detection will allow treatment of these lesions before they can become invasive.

Cancer and the 4 RCTs

On an individual basis the 4 RCTs are not powered enough to measure the effect of HPV testing, as an alternative to regular cytological screening, on the incidence of invasive cancer [37, 39, 41, 44]. For this reason a follow-up study of the 4 RCTs was performed together with a pooling of the data [47].

The pooled analysis incorporated a total of 76,464 women aged 20–64 years followed up for a median of 6.5 years (1,214,415 person-years) [37, 39, 41, 44, 47]. The study-adjusted pooled relative detection rate for invasive cervical cancer among all women from recruitment to end of follow-up are shown in Table 2 [47].

The screening methods were not significantly different for the detection of invasive cancer during the first 2.5 years of follow-up after study entry (Table 3) [47]. After 2.5 years, the HPV screening arm became significantly lower then the standard cytology arm. The pooled rate ratio for invasive cervical carcinoma among all women from recruitment to end of follow-up were shown in Table 2 [47].

A random-effects model gave an almost identical estimate (0.61, 0.41–0.91) [47, 49]. The fact that the gain in reduction of cervical cancer started after 2.5 years, excludes prevalent cases and reflects to true gain of HPV based screening above cytological screening. The gain of HPV will only increase if the quality of cytology decreases.
In the introduction it was assumed that conventional cytology missed more often adenocarcinomas than squamous carcinoma. The key question is what will and can HPV screening achieve?

The assumption increased in validity when one looks at the pooled rate ratio for morphology. The rate ratios were lower for adenocarcinoma (0.31; 95% CI 0.14–0.69) than for squamous-cell carcinoma (0.78: 95% CI 0.49–1.25) [47]. Especially for the young women, the increased gain is high. Because the proportion of adenocarcinomas fell by age: 40% in women younger than 30 years, 35% in those aged 30–34 years, 30% in women age 35–49 years, and 23% in those 50 years or older [47]. The rate ratios did not differ for stage. HPV testing has an even higher gain for adenocarcinomas than for squamous cancers. It cannot be emphasized enough: adenocarcinomas are often missed in the classical screening. Adenocarcinomas are in 94% due to HPV 16, 18, and 45 [29]. The introduction of prophylactic HPV vaccination (primary prevention) and HPV screening (secondary prevention) will therefore have a major impact on the incidence of these cancers [14].

What to do when a woman is high-risk (HR) HPV positive

In the POBASCAM, Swedescreen, and ARTISITC a cytological triage was performed; the NTCC HPV positive women were directly referred for colposcopy with or without biopsy [37, 39, 41, 44]. The pooled ratio showed that in case of cytological triage, there was no increase in biopsies (1.02; CI 95% 0.97-1.07) [47]. In case of direct referral to colposcopy in case of HPV positivity (the NTCC trial), the number of biopsies were more then doubled (2.24; CI 95%, 2.09-2.39) [47]. As there is no difference in the detection rate of invasive cancer, it is to be recommended that all women who have a high-risk HPV infection should have a cytological triage. Practically this means that if a woman has a HR HPV infection a cytological analysis should be done (Figure 1). If cytology is normal the smear should be repeated after one year, and if cytology is abnormal, she should be referred for colposcopy with or without biopsy. The smear becomes a diagnostic smear instead of a screening smear.

Based on the HERACLES and SCALE trial, one can conclude that cervical cancer below 50 years is due to a HPV infection 16, 18 or 45 [50, 51]. This type-specific analysis could also be included in the triage of HR HPV positive women (Figure 2). Only women younger then 50 years of age and infected with one of these types should have reflex cytology; all the others should be rescreened after five years.

In the future this will probably alter when the current generation of vaccinated women reach the screening age. It is likely that this generation will only need a smear every 10, 15, or even 20 years. Attractive for the future is also the self-sampling in cervical screening. Women could take a sample by themselves and send it to the laboratory. If the results comes back as HPV positive, she should be invited to visit a doctor for the traditional type specific HPV screening and diagnostic cytology.

Additional staining of the smear and markers can do further fine-tuning, but current there is need for more evidence before in can be used in routine practice. It is important to do a type-specific analysis and not HR HPV positive or negative...
test; the reason why the majority of the HPV infections are transient and will disappear within two years. Only persistent infections should be treated from a clinical point of view. This will protect women from unnecessary harm and injury. If for instance a woman who has a HPV 16 infection today and two years later a HPV 18 infection, it is not a persistent infection. She should be informed that this is normal and that she does not need any treatment. If one would have done the analysis with a HR HPV positive or negative test, the clinician would not have known that she had a transient infection on the one hand and a new infection on the other hand. This would wrongly lead to the conclusion that it is a persistent infection that needs treatment.

An additional tool in the triage is the viral load of a HPV infection [52]. As most infections will disappear, you can follow the activity of the virus. The normal pattern will be an increase in the viral load and then a decrease back to zero. If this is happening, one can wait. If a viral load stabilizes regardless of the height because it is a logarithmic scale, the clinicians should be worried because this can be regarded as a persistent infection with a risk of progression of the lesion. When applying this rule of HPV screening and cytological triage in women between 25 and 30 years, one should be very cautious. The reason is that this could lead to anxiety among the women and their clinicians, leading increased additional investigations. There is a substantial risk for over-management in case of regressive precancer lesions caught by HPV screening. At the moment there is simply not enough data for HPV screening in this age group to draw firm conclusions. The evidence for using it in this age group is thin. There is however sufficient evidence for not screening below 25 years of age. If HPV screening is done in women between 25-30 years of age, then the algorithm of cytological triage in case of a HR HPV infection would lead to inappropriate high numbers of colposcopy and unnecessary biopsies. The number of unnecessary conisations with its associated morbidity and mortality, will undoubtedly also increase.

Especially in this age group it is necessary to perform a HPV type specific triage and an ASCUS or LSIL or more triage (Figure 3). This triage system takes in consideration the age together with the three possible HPV groups: 1) no HPV infection, 2) HPV positive not HPV types 16, 18, and 45 and 3) HPV 16,18, and 45 positive. It is complex in use, but the logically next step of acceptance. Alternatively one could also directly incorporate more rigorously all the available data in one figure (Figure 4) [50, 51]. The latter is logical and simple to use. It will however take time before everyone will accept this flow-chart and uses it.

Only in case of a persistent HPV infection of the same type for longer then two years a colposcopy should be performed. When for a LSIL an advice of colposcopy is given, this should be done very tactfully. If during a colposcopy the lesion(s) are clearly low grade or less, one should not biopsy these lesions. If there is doubt or if one does not feel comfortable, then one should take a biopsy. It is the opinion of the author that one should use common sense when reading a protocol. If the proto-

![Figure 3. Flow-chart of HPV screening for women between 25-30 years of age.](image3)

![Figure 4. Alternative flow-chart of HPV screening for women between 25-30 years of age.](image4)
col states to take a biopsy it should not automatically mean that the clinician should take a biopsy. It is hilarious if you always follow an opinion without thinking. It is not because your are equipped with… that you should have… with every one.

The current practice of serial monogamy leads to new HPV infections (at every age) without any direct significance. It is naïve to perform only cytology below 30 years if are screening. Women, men, and clinicians should accept that more then 80% of all HPV infections will disappear (at any age) spontaneously and do not need any additional treatment. The fact that some one has a HPV infection, is a reflection that she is sexual active. There is nothing wrong in consented sex. HPV in this regard can best be compared with the common cold. Reduce anxiety and explain this to your patient and their loved ones. This will of course cost more time, but it will also increase the satisfaction of avoiding unnecessary treatment. In the past when I did premature deliveries in the middle of the night, I often wondered how many of these deliveries could be avoided if we would have had the knowledge and talked to our patients. If your are still not convinced, please go to a Neonatal Intensive Care (NIC) Unit and check how many of the premature delivers were among women with a conisation in the passed.

Which HPV test should be used? [53]

This seems a strange topic because the clinicians are generally not involved in choosing the HPV test. The clinician should however be aware that there are multiple HPV tests available and that some earlier HPV tests will miss one out of ten cancers. This is important if screening is based on HPV testing. At the end of the day the clinician is held responsible for the missed cancer. Every clinician should therefore know what the differences are between an L1 test and an E6/E7 test [53].

A HPV infection can be present in a patient in a free form (episomal), in an integrated form in the host DNA or in a mixed form which means free and integrated. From a clinical point of view the integrated viruses are important because in this form the lesions are most likely to progress to a high-grade lesion or invasive cancer. It is therefore essential that HPV tests also look at the integrated HPV types [53]. For this reason HPV tests should not only type specific but also region specific. Crucial specific regions in the HPV genome are L1, E1/E2, and E6/E7. During integration of the HPV in the human genome sometimes L1 expression is lost, but E6/E7 expression remains always present. E6/E7 are pivotal in the development of cancer (L1 negative cancers exist, but not E6 or E7 negative cancers) [53]. In other words, if one were to use an E6/E7 test all cancers would be detected, including the ones where L1 was lost. A test looking only for L1 and not for E6/E7 will miss about 10% of all invasive cancers.

In our country the most frequent used HPV tests are probably Hybrid Capture II (Qiagen) and the Cobas 4800 HPV DNA Test. To cut a long story short, if you read their labels, then you notice that these are L1 only tests. For a more detailed description I would like to refer to a previous publication titled: “Cervical cancer screening: which HPV test should be used—L1 or E6/E7?” [53].

The introduction of the first HPV testing based on L1 increased the sensitivity for screening considerable (30 to 40%). With the current knowledge of integration and progression, it is time to fine-tune these HPV tests. Nowadays a HPV test should also look at E6/E7 in order not to miss the 10% integrated HPV cancers. It will cost money to alter the HPV testing system, but from a medical legal point it is difficult to defend an L1-only test. If the right test is used at the right place, unnecessary death due to cervical cancer in women can be avoided [54]. Let us remember cervical cancer affects mainly young women with young families and the qualities years lost after a wrong test will become uncountable.

At what frequency should screening be performed

In the pooled data of the 4 RTCs, the adjusted rate ratio after a negative test on entry was 0.30 (95% CI: 0.15–0.60), with no evidence of heterogeneity (p = 0.23) between the studies and with an almost identical random-effects model estimate (0.34, 0.14–0.86) [37, 39, 41, 44, 47, 49]. In Table 4 the cumulative incidence of invasive cervical cancer at entry after a negative cytology and after a HPV negative test are shown. The cumulative detection rate of invasive cervical cancer eight years after enrolment was more then doubled in the cytology screen women compared to the HPV screened women, respectively, 93.6 per 10^4 (95% CI 70.5–121.8) and 46.7 per 10^4 (95% CI 32.1–65.5) [47]. This is a clear indication how many relevant precancers and cervical cancers are missed by cytology.

The cumulative incidence of invasive cervical cancer after a negative cytology test at 3.5 years is almost doubled the figures after a negative HPV test at 5.5 years. In other words a five-year screening interval with HPV testing is far better and safer then a screenings interval of three years with cytology. The remarks: “ Larger screenings intervals when the current vaccinated population will enter the screening age is indicated” is without a shadow of a doubt, the understatement of the next century. Shorter screenings interval of less than five years with HPV testing will only lower the specificity as most HPV infections are transient [55].
The adagio less for more should be embraced. Compared to cytology screening, screening with HPV at a five-year interval will reduce the number of smears taken, the number of colposcopies, the number of biopsies, the number of coni-
sations, and most importantly will reduce the number of cervical cancers at a significantly lower cost. This win-win situation in an era of economical crisis should be used to speed up the integration of HPV screening.

In the vaccinated population, it seems reasonable to extend this interval to 10, 15 or even 20 years. However before this can only be introduced, the expected efficacy of the prophylactic HPV vaccination is confirmed in daily clinic. One should not rush and take too many steps at a time. This will only confuse people and make them unwilling to accept it. Every new step taken should be with sufficient evidence and pace, so that is becomes acceptable and understandable for everyone.

Current prophylactic HPV vaccination is expected to reduce cervical cancer with more then 80%. There is therefore still a need for screening. The introduction of the HPV based cervical screening will provide a 70% greater protection against invasive cervical cancer then the currently used cytology-based screening. Together HPV vaccination and screening will abandon cervical cancer to the history books. Every success comes with a price. Cervical cancer can only be eradicated if all stakeholders have the knowledge, work together and respect each other.

References


Address reprint requests to:
W.A.A. Tjalma, M.D., Ph.D.
Department of Gynecology and Gynecologic Oncology
Antwerp University Hospital, University of Antwerp,
Wilrijkstraat 10, 2650 Edegem (Belgium)
e-mail: Wiebren.Tjalma@ua.be
Introduction

Endometrial carcinoma (EC) is the sixth commonest female malignancy worldwide and it represents 5% of all women cancer; 288,000 new cases were registered in 2010 [1]. This carcinoma accounted for 4.8% of new cancer diagnosis among women between 2003 and 2005 in Italy; in the same country the mean annual number of endometrial cancer cases was 25.4 on 100,000 women [2].

Abnormal uterine bleeding (AUB) in menopause occurs in almost 90% of affected patients and is the most common first-presenting symptom. Ten percent of postmenopausal women presents AUB, [3] but only 10-15% of these women have endometrial cancer.

Actually no satisfactory screening method has been validated for EC; nevertheless this carcinoma is usually diagnosed at early stages because most women who experience AUB quickly consult with gynecologist.

Over the last few years many methods were developed for evaluating the uterine cavity. The first-line examination is worldwide considered transvaginal sonography (TVS) because of its simplicity and its good accuracy for most uterine abnormalities. However there are conflicting data about its diagnostic accuracy in case of endometrial cancer. Diagnostic hysteroscopy (DH) is considered second-line examination, but its advantage of allowing direct and dynamic visualization of the endometrium and the uterine cavity made it the gold standard in the management of AUB [4-6]. It can be performed as an office procedure without anesthesia and with minimal morbidity. Moreover, the possibility to perform focused biopsy improves its diagnostic accuracy [7].

Previous trials have evaluated the accuracy of hysteroscopy [8, 9] and have also compared it with other diagnostic techniques as transvaginal ultrasound, sonohysterography, and magnetic resonance imaging.

DH is a simple technically procedure, but the interpretation of anatomical features can be not so easy. To date there are no satisfactory data regarding the significance of this important diagnostic instrument in non-experienced hands.

The purpose of this study was to evaluate the diagnostic accuracy of hysteroscopy made by young residents in the assessment of uterine cavity in postmenopausal women, using the final histologic finding as reference.

Materials and Methods

Postmenopausal patients referred to the hysteroscopic service of the Department of Woman and Child Health, Obstetrics and Gynaecology Clinic, University of Padua, from January 2011 to December 2013 were enrolled in an observational longitudinal study. All women were referred to hysteroscopy for AUB or a suspected finding on ultrasonography (endometrial thickening, endometrial...
polyps or any other suspected irregularity) performed by the referring gynecologists.

For the study the authors selected the first 200 hysteroscopies performed by three young residents that had attended the hysteroscopic service at least three months prior to the beginning of the procedures.

All diagnostic outpatient hysteroscopies were performed in a dedicated room without sedation using a 2.9 mm-diameter hysteroscope with a continuous-flow and a 30° fore-oblique lens and a normal saline solution was used as the distention medium. Neither preoperative cervical ripening nor a cervical block was performed. No systemic drugs were given to women before the procedure. The hysteroscope was guided through the vagina, the endocervical canal, and the uterine cavity without using a speculum nor a tenaculum.

For the data analysis, the authors firstly considered endometrial features described by the young residents without considering the final diagnosis made by two experienced hysteroscopists that supervised all the procedures. Then they compared seniors' hysteroscopic results of the same patients with those of the residents.

Hysteroscopic findings were divided in negative (atrophy, nonmalignant lesions as endometrial polyps, and synechiae), hyperplasia without atypia (endometrium thickened diffusely or in polypoid appearance, that represents glandular-stromal growth with mucosal edema and increased vascularization), hyperplasia with atypia (dishomogeneous glandular-stromal growth with increased glandular-stromal ratio, and increased vascularization) or cancer (irregular friable polypoid formations with dilated and tortuous vessels, necrosis or bleeding).

The authors collected data regarding patient's age and body mass index. Menopause was defined as spontaneous cessation of menses for 12 consecutive months or more. AUB was considered any uterine bleeding thereafter.

For all patients, an office endometrial biopsy by stainless steel Novak curette or resectoscopy (i.e., polyp removal or focal endometrial resection) was performed, enabling histological diagnosis and appropriate therapy.

Histological diagnosis was considered the gold standard to define hysteroscopy efficacy in diagnosing all the endometrial lesions. Histological results were grouped into the same categories of DH findings: negative, hyperplasia without atypia, hyperplasia with atypia, and EC.

The authors calculated the sensitivity (SE), specificity (SP), positive and negative predictive values (PPV and NPV), and positive and negative likelihood ratios (LR + and LR -) for each hysteroscopic finding revealed by young residents and seniors.

Results

A total of 600 hysteroscopic findings were evaluated, 200 for each young resident. The mean age of women was 61.37 years and the mean body mass index was 25.97 kg.

No adverse effects such as vaso-vagal reactions or uterine perforations in DH neither in operative hysteroscopic procedures were reported.

The most common hysteroscopic descriptions, observed in 528 cases among residents and in 530 cases among seniors, were negative findings. The young residents registered 40 cases of hyperplasia without atypia, 11 cases of hyperplasia with atypia, and 27 cases of cancer.

The histological results revealed 522 negative findings, 40 cases of hyperplasia without atypia, 8 cases of hyperplasia with atypia, and 24 cases of EC at DH. On the other hand, the seniors described 35 cases of hyperplasia without atypia, nine cases of hyperplasia with atypia, and 26 cases of EC at the same DHs.

The histological results revealed 522 negative findings, 40 cases of hyperplasia without atypia, 11 cases of hyperplasia with atypia, and 27 cases of cancer.

SE, SP, PPV, and NPV and LR + and LR - for each hysteroscopic finding revealed by young residents and seniors.

Concordance between hysteroscopic features and histological diagnosis among residents.

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Negative</th>
<th>Hyperplasia without atypia</th>
<th>Hyperplasia with atypia</th>
<th>Cancer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R%</td>
<td>R%</td>
<td>R%</td>
<td>R%</td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97.32</td>
<td>97.14</td>
<td>9.09</td>
<td>96.30</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
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<td>99.82</td>
<td>99.81</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>96.21</td>
<td>97.14</td>
<td>88.89</td>
<td>99.13</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>80.56</td>
<td>99.49</td>
<td>99.13</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>LR +</td>
<td>3.80</td>
<td>21.00</td>
<td>7.65</td>
<td>80.64</td>
<td></td>
</tr>
<tr>
<td>LR -</td>
<td>0.04</td>
<td>0.41</td>
<td>0.92</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. — Sensitivity, specificity, positive, and negative predictive values (PPV and NPV) and positive and negative likelihood ratio (LR + and LR -) of hysteroscopies made by residents (R) and by seniors (S) with histology as reference.

<table>
<thead>
<tr>
<th></th>
<th>R%</th>
<th>S%</th>
<th>R%</th>
<th>S%</th>
<th>R%</th>
<th>S%</th>
<th>R%</th>
<th>S%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>97.32</td>
<td>100.00</td>
<td>60.00</td>
<td>85.0</td>
<td>9.09</td>
<td>72.73</td>
<td>70.37</td>
<td>96.30</td>
</tr>
<tr>
<td>Specificity</td>
<td>74.36</td>
<td>89.74</td>
<td>97.14</td>
<td>99.82</td>
<td>98.81</td>
<td>99.83</td>
<td>99.13</td>
<td>100.00</td>
</tr>
<tr>
<td>PPV</td>
<td>96.21</td>
<td>98.49</td>
<td>60.00</td>
<td>97.14</td>
<td>12.50</td>
<td>88.89</td>
<td>79.17</td>
<td>100.00</td>
</tr>
<tr>
<td>NPV</td>
<td>80.56</td>
<td>100.00</td>
<td>97.14</td>
<td>98.94</td>
<td>98.31</td>
<td>99.49</td>
<td>98.61</td>
<td>99.83</td>
</tr>
<tr>
<td>LR +</td>
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<td>9.75</td>
<td>21.00</td>
<td>476.0</td>
<td>7.65</td>
<td>428.36</td>
<td>80.64</td>
<td>-</td>
</tr>
<tr>
<td>LR -</td>
<td>0.04</td>
<td>0.00</td>
<td>0.41</td>
<td>0.15</td>
<td>0.92</td>
<td>0.27</td>
<td>0.30</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Hysteroscopy made by residents demonstrated a high SE and SP in recognising atrophy and benign lesion, such as polyps and synechiae.

Noteworthy is that hyperplasia without atypia was diagnosed in 40 cases, of which only 24 cases had been suspected by the young residents and the other 16 cases were not suspected during the procedures. Similarly, hyperplasia with atypia was confirmed in 11 cases, of which only one case had been suspected by the young residents and the other ten cases were not suspected (Table 2). These results demonstrated a low SE, PPV, and LR+ in suspecting atypical hyperplasia among young residents (Table 1). On the other hand, the residents hysteroscopic findings were not suspicious for malignancy in eight cases proved by histological examination, but they were diagnostic for cancer in 19 patients (Table 2). Therefore the authors found a good SP and VPN but low SE among residents in diagnosing cancer (Table 1).

Experienced hysteroscopists demonstrated a high SE and SP in recognising all hysteroscopic findings. In particular, hyperplasia without atypia was diagnosed in 40 cases, of which 34 cases had been suspected by the seniors and hyperplasia with atypia was suspected in eight out of 11 cases. Of 27 women with cancer, 26 of them were immediately diagnosed by seniors at DH (Table 3). Therefore these data revealed a SE, SP, PPV, and NPV higher than those of residents with and without atypia and cancer (Table 1).

### Discussion

The most common malignant carcinoma of the female genital tract is nowadays EC. AUB in menopause occurs in almost 90% of affected patients and is the most common first-presenting symptom [3]. Therefore differentiation of benign from malignant causes of postmenopausal bleeding is very important. Actually no satisfactory screening method has been validated for EC diagnosis.

In the past, the common practice to evaluate postmenopausal bleeding was dilatation and curettage. Over the last few years many other methods have been developed for evaluating the uterine cavity. Nowadays the first-line examination is worldwide considered TVS because of its simplicity and its good accuracy for most uterine abnormalities [4, 5].

However there is still discussion over the best cut-off value for endometrial thickness that should guarantee conservative management. After the menopause endometrium undergoes atrophic changes due to estrogentic lack and many cut-off values for pathological endometrial thickness have been proposed.

Almost every guideline refers to a meta-analysis performed in 1998 by Smith-Bindman et al. in which they suggested that cut-off value for endometrial thickness that recommended further investigations in women with postmenopausal bleeding is beyond five mm; below this range they proposed a conservative management [10].

Previous studies in fact have shown that the risk of malignancy decreases to one in 1,000 when endometrial thickness is < four mm in a postmenopausal women with bleeding [11].

Chandavarkar et al. underlined that type 2 EC may not always induce a thickened endometrium because it does not usually develop from hyperplasia, therefore postmenopausal bleeding requires endometrial direct evaluation despite endometrial thickness [12, 13]. They also underlined that type 2 tumors are more aggressive, because they metastasize far more rapidly. Hence they recommended that women be counselled that the risk of cancer in symptomatic women with endometrial thickness ≤ four mm is very low, but EC cannot be completely excluded, especially when there is persistent bleeding.

As a result of findings of Litta et al. in 220 postmenopausal women with AUB, transvaginal ultrasonography alone is inadequate to rule out EC and outpatient hysteroscopy with biopsy is mandatory in all of these [14].

The importance of incidentally detected thick endometrium in asymptomatic postmenopausal women is still controversial. A systematic review published in 2012 reported a mean endometrial thickness in menopause of 2.9 mm in 2,952 studied women and found out no valid cut-off to suggest histological examination in asymptomatic women [15].

DH is considered second-line examination, but its advantage of allowing direct and dynamic visualization of the endometrium and the uterine cavity made it the gold standard for evaluating them. Furthermore biopsy may improve its diagnostic accuracy [7, 16].

Both TVS and DH are accessible office procedures for the diagnosis of abnormal intrauterine pathologies. Direct
visualization of uterine cavity allows in deciding if further surgical procedures are needed and which technical approach is the most appropriate. After DH, to solve the problem, a resective hysteroscopy could be necessary in case of polyps or confined hyperplasia; a radical surgery in case of cancer or a radical endometrial resection by hysteroscopy as an alternative to hysterectomy in selected patients with atypical focal endometrial lesions [17].

Hysteroscopy can be performed as an office, safe procedure without anesthesia, because it is usually well-tolerated and has minimal morbidity. Cervical preparation before hysteroscopy is not used because there are not evidences of benefit in terms of pain reduction. Procedural pain is significantly reduced with the use of small-diameter hysteroscope, through a vaginoscopic approach, and with the use of sterile sodium chloride solution as the distention medium, because it seems to reduce incidence of vasovagal episode [18]. It was even demonstrated that office-based hysteroscopic polypectomy using a five mm-diameter hysteroscope could be a safe and a well-tolerated procedure [19]. In case of operative hysteroscopy, when a resectoscopic approach is necessary in high-risk patient, spinal anesthesia can be performed to reduce the risks associated to anesthesia [20].

In this study, hysteroscopic visualization of uterine cavity by young residents showed good accuracy in detecting overall atrophy and benign uterine cavity abnormalities, such as polyps and synechiae. The calculated SE and SP are comparable with senior and with others in literature [8, 16, 21].

Endometrial hyperplasia diagnosis may not be obvious especially in early stages of the disease. Other published studies evaluating endometrial hyperplasia reported a SE, SP, PPV, and NPV of 61.6%, 95.2%, 79.4%, and 89.3%, respectively [22], and 56.5%, 91.6%, 72.2%, and 84.6%, respectively [16]. These results are similar to seniors findings in this study. On the other hand, results of residents, especially for hyperplasia with atypia, are not in line with the aforementioned findings. In the latter, only about two-thirds of the cases of hyperplasia were correctly confirmed by histology, but in less than one-third of the cases, it was not well-recognized during DH. Lower SE and PPV of hysteroscopy made by residents in recognizing hyperplasia with or without atypia can be explained by the resident’s tendency to overestimate malignant lesion in order to avoid unrecognized cancer.

The main objective in postmenopausal women, especially if they presented with AUB, is to detect or rule out EC. Regarding the present findings, this cancer can be detected by residents with no high SE, but with very good SP. These results are consistent with others in literature. Rokita et al. reported a SE of 61% and a SP of 90% [23] and Lo et al. revealed a SE of 58.8% in detecting cancer at hysteroscopy not made by residents [7]. Elfayomy et al.’s data also showed a SE of 50% and SP of 94.2% [16].

All this data are concordant with opinion in the literature that reported a high accuracy in differentiating benign and malignant endometrial pathologies, but a limited role of DH in revealing hyperplasia, cancer or both [24, 25].

Conclusions

The results of the present study confirm the opinion that hysteroscopy has great accuracy in diagnosing focal pathology and especially in distinguishing benign and malignant endometrial pathologies.

According to the authors’ experience, outpatient hysteroscopy made by residents in their endoscopic practice beginning has good accuracy in detecting clear endometrial malignant lesions, but not as good an accuracy in detecting premalignant lesion as hyperplasia with atypia. DH accuracy in expert-hands is not comparable to that in residents-hands during their first procedures. This could signify that more than 200 hysteroscopies are necessary for a resident to well recognize premalignant and malignant lesions.

References

Accuracy of hysteroscopy made by young residents in detecting endometrial pathologies in postmenopausal women


Address reprint requests to:
F. DE MARCHI, M.D.,
Department of Women’s and Children’s Health,
Obstetrics and Gynecology Clinic
University of Padua, Italy
Via Giustiniani, 3 - 35128 Padua (Italy)
e-mail: francidema@hotmail.com
Introduction

Chemotherapy has held an important position in the treatment of recurrent and advanced endometrial cancer. Although most patients with isolated vaginal recurrence are treated by vaginal brachytherapy alone, patients with metastases at multiple sites or distant metastasis are usually treated by repeat chemotherapy. First-line chemotherapy for recurrent and advanced endometrial cancer has been well-documented in a series of Gynecologic Oncology Group (GOG) randomized trials [1-4], which yielded a strategy for a proper first-line chemotherapy regimen. However, an optimum strategy for second-line chemotherapy has not yet been determined.

With respect to ovarian cancer, the established strategy for selecting a chemotherapy regimen is based on the treatment-free interval (TFI) [5]. The response to platinum rechallenge increases with a TFI, which refers to a platinum-free interval (PFI) in most cases of recurrent ovarian cancer. In cases of advanced or recurrent endometrial cancer, however, the time to recurrence (TTR) after primary chemotherapy is considered to be predictive of survival after recurrence, as was shown in the ancillary data analysis of the GOG trials [6]. The analysis also pointed to the TFI as an important indicator when single agents are used as second-line chemotherapy for endometrial cancer. These findings raise the possibility that the TFI or PFI can be used in selecting a second-line chemotherapy regimen for patients with endometrial cancer. Thus, the authors investigated the effectiveness of platinum-based combination chemotherapy as second-line chemotherapy for patients with advanced or recurrent endometrial cancer who had been treated initially by platinum-based combination chemotherapy.

Materials and Methods

After obtaining approval from the institutional review board, the authors obtained clinical records of the Cancer Institute Hospital (Tokyo) to identify patients treated for recurrent endometrial cancer between January 1999 and December 2009. Because the aim of the study was to determine the effectiveness of second-line chemotherapy for recurrent endometrial cancer between January 1999 and December 2009. Because the aim of the study was to determine the effectiveness of second-line chemotherapy for recurrent endometrial cancer, clinical records of all patients who had received any second-line chemotherapy were reviewed. No patient in the series had been treated with radiotherapy. At the present institution, platinum-based combination chemotherapy is used for both first-line and second-line chemotherapy of endometrial cancer. If the PFI between first-line and second-line chemotherapy is six months or more, the same drug combination used for first-line chemotherapy is used for second-line chemotherapy. If the PFI is less than six months, a different drug combination is used for second-line chemotherapy. The platinum-based combinations include paclitaxel and carboplatin (TC), docetaxel and carboplatin (DC), adriamycin and cisplatin (AP), ifosfamide, epirubicin, and cisplatin (IEP), docetaxel and cisplatin (DP), paclitaxel and cisplatin (TP), and irinotecan and nedaplatin (CPT-11/NDP).

Patients were identified as falling in one of two categories (Figure 1). Category 1 comprised patients who received postoperative adjuvant chemotherapy as first-line chemotherapy in the apparent absence of residual disease and received second-line chemotherapy.
chemotherapy for recurrent disease. Category 2 comprised patients with either advanced or recurrent disease who received both first-line and second-line chemotherapy. For patients in this category, the first-line chemotherapy was performed not as adjuvant chemotherapy but for recurrent or residual disease. Responses to second-line chemotherapy were examined, and response rates were determined for each group. Response rates were also determined in relation to the PFI in both categories. Among Category 1 patients, overall survival (OS) after recurrence was determined in relation to the PFI, which was taken as the interval between the end of adjuvant chemotherapy and the start of second-line chemotherapy for disease recurrence, and among Category 2 patients, OS after the start of second-line chemotherapy was determined in relation to the PFI, which was taken as the time between the end of first-line chemotherapy and the start of second-line chemotherapy. Survival curves were drawn according to the Kaplan-Meier method.

Results
Seventy-seven patients with advanced or recurrent endometrial cancer were treated at the Cancer Institute Hospital during the period noted above. Fifty-six of these patients fell into Category 1, and 21 fell into Category 2. All had measurable disease.

Category 1
Clinicopathologic characteristics, including treatment details, of the 56 Category 1 patients are summarized in Table 1. Median age was 58 years. At the time of adjuvant chemotherapy, disease stages were as follows: Stage I (n=7), Stage II (n=2), Stage III (n=26), and Stage IV (n=11). Twenty-one patients had endometrioid adenocarcinoma grade 1-2,
Importance of platinum-free interval in second-line chemotherapy for advanced or recurrent endometrial cancer

Eight had endometrioid adenocarcinoma grade 3, and 15 had carcinosarcoma. There was no residual disease in these patients after surgery. The following adjuvant chemotherapy drug combinations were given, IEP (n=24), TC (n=30), and AP (n=2). Upon recurrence, these patients received IEP (n=12), TC or DC (n=35), AP (n=5), or DP (n=4).

The response to second-line chemotherapy in this group was 44.6%, with ten complete responses and 15 partial responses. Response rates differed markedly in relation to PFI (Figure 2). The response rate was 0% when PFI was less than six months, 38.4% when PFI was six to 11 months, and 64.7% when PFI was over 12 months. With a PFI of less than six months, median OS after recurrence was 5.4 months. With a PFI of six to 11 months, median OS was 5.6 months, and with a PFI of 12 months or more, median OS was 23.0 months (Table 2). Kaplan-Meier curves for survival of Category 1 patients after recurrence are shown per PFI in Figure 3.

Category 2
Clinicopathologic characteristics, including treatment details, of the 21 Category 2 patients are summarized in Table 3. Median age was 65 years. Initial disease stages in this group were as follows: Stage I (n=1), Stage III (n=4), and Stage IV (n=16). Four patients had endometrioid adenocarcinoma grade 1-2, 4 had grade 3 endometrioid adenocarcinoma, and nine had carcinosarcoma. The following first-line chemotherapy drug combinations were given: IEP (n=10) and TC or DC (n=11). Second-line chemotherapy drug combinations consisted of the following: IEP (n=4) and TC or DC (n=10).

Response to second-line chemotherapy in this group was 4.8% (with one partial response). Response rates differed markedly in relation to PFI (Figure 4). With a PFI of less than three months, the response rate was 0%, but with a PFI of three months or more, the response rate was 20.0%. Median OS after the start of second-line chemotherapy was nine months for patients with a PFI of less than three months, and 15.4 months for patients with a PFI of three months or more (Table 4). Kaplan-Meier curves for survival of Category 2 patients after the start of second-line chemotherapy are shown per PFI in Figure 5.
Discussion

The authors’ overall study goal was to determine which drug combination should be selected for second-line chemotherapy in patients with advanced or recurrent endometrial cancer. First-line chemotherapy regimens are already established. Results of the GOG randomized trials made it clear that platinum-based combination chemotherapy should be selected for first-line chemotherapy in such cases. In the GOG-77 trial, addition of cisplatin to doxorubicin for advanced endometrial cancer improved survival [2]. Currently, AP is the standard chemotherapy combination for recurrent and advanced
endometrial cancer. In the GOG-163 trial, AP was compared with AT (adriamycin and paclitaxel), and it was clarified that cisplatin combination chemotherapy was superior to non-platinum-containing chemotherapy. In the GOG-177 trial, TAP (paclitaxel, adriamycin, and cisplatin) was shown to significantly improve response rate, progression-free survival, and OS, but severe side-effects are associated with TAP [4]. Thus, TAP was not recommended in place of AP [4]. GOG-209 is underway to determine whether TC is therapeutically equivalent to TAP with respect to survival.

The effectiveness of combination chemotherapy as second-line chemotherapy has been unclear. In the current study, patients who relapsed more than six months, especially more than 12 months, after adjuvant therapy and patients who relapsed more than three months after first-line chemotherapy for recurrent or advanced disease showed a good chance of response to rechallenge with platinum-based combination chemotherapy, which may translate to increased survival for similar patients. Conversely, in patients who relapse within six months after adjuvant therapy or within three months after first-line chemotherapy for recurrent or advanced disease, rechallenge with combination chemotherapy may be futile. In this situation, a different approach, such as single-agent chemotherapy, participation in a clinical trial, or hormonal therapy, may be recommended. Several phase II trials of single-agent regimens have been undertaken for second-line chemotherapy, but the response rates have been limited to 0-25% [7-15]. In addition, use of a single agent within three months after first-line chemotherapy is generally thought to be of little value [6].

Several molecular targeted agents have been recently investigated. Single agent VEGF inhibitor bevacizumab was tested in a GOG trial [16] in which more than half of the patients had been treated previously under one or two cytotoxic regimens. The response rate was 13.5% [16]. The reported response rate for mTOR inhibitor temsirolimus is 14% in chemotherapy-naive patients [17]. Because the response was not enough to select a single molecular targeted agent for second-line treatment, combination bevacizumab and temsirolimus was studied in a phase II trial, but severe toxicity was reported, possibly because the combination therapy was tested in patients who had received prior cytotoxic chemotherapy [18].

In summary, the present authors report the possibility of platinum-based combination chemotherapy as second-line treatment for recurrent and advanced endometrial cancer. The effectiveness clearly depends on the PFI between first-line and second-line chemotherapy. The PFI is a key to successful chemotherapy for endometrial cancer after failure of first-line chemotherapy.

References


Address reprint requests to:
M. MATODA, M.D.
Department of Gynecology Cancer Institute Hospital
Ariake 3-8-31, Koutou-ku, Tokyo, 135-8550 (Japan)
e-mail: maki.matsumura@jfcr.or.jp
Whole-body [18F]fluoro-2-deoxyglucose positron emission tomography scan as combined PET-CT staging prior to planned radical vulvectomy and inguinofemoral lymphadenectomy for squamous vulvar cancer: a correlation with groin node metastasis

M.W. Kamran, F. O’Toole, K. Meghen, A.N. Wahab, F.A. Saadeh, N. Gleeson

Division of Gynecological Oncology, Department of Obstetrics & Gynecology, Trinity College & St.James’ s Hospital, Dublin (Republic of Ireland)

Summary
Surgery is the mainstay of treatment for vulvar cancer. FIGO staging requires histopathological detail of the primary tumor and inguinofemoral lymph nodes but groin node dissection carries a substantial risk of short and long-term morbidity. The trend in current practice is towards sentinel lymphadenectomy for cancers with a low risk of metastases. Full lymphadenectomy is undertaken if the sentinel lymph node contains metastasis. The predictive value of 18F-FDG-PET in preoperative assessment of the groin in vulvar squamous cancer was assessed in retrospect at a single institution. A period of three years prior to the introduction of sentinel lymph node mapping was chosen in order to have full histopathological assessment of inguinal and femoral lymph nodes available as the gold standard for correlation with positron emission tomography-computerized tomography (PET-CT) to determine the accuracy of the enhanced radiological technique. In patients with histologically proven metastases to groin nodes, comparisons between PET-CT positive (True-positive/TP) and negative (False-negative/FN) groups vis-à-vis histology showed a tendency towards higher FDG avidity in the vulvar lesions, more bilateral nodes, multiple metastases, larger metastases and more extra-capsular extension in the TP group. Calculations per patient for PET-CT yielded a sensitivity of 50% and specificity at 100%. The positive predictive value (PPV) was 100% and the negative predictive value (NPV) was 57.1%. The test accuracy was 70% per patient. The high positive predictive value of PET-CT can be used to advance treatment planning prior to surgical staging of patients identified with Stage III disease. The poor sensitivity makes it unsuitable as a substitute for staging lymphadenectomy.

Key words: PET-CT; Vulvar Cancer; Specificity; Positive Predictive Value.

Introduction
Carcinoma of the vulva accounts for 5% of all gynecological malignancies [1]. Squamous is the predominant histological type accounting for 75% of these cancers [2]. FIGO staging for vulvar cancer (2010) includes tumor size, lymph node status, and the presence of local and distant metastases [3]. Lymph node status is the best predictor of survival and histological assessment of nodes is an integral part of the staging surgery [4]. Stage III disease is defined by lymphatic metastases. Clinical palpation is inaccurate [5]. Radiological detection on ultrasound or magnetic resonance imaging (MRI) of central necrosis is strongly suggestive of metastasis but improved radiological assessment is needed for the assessment of smaller or undetectable lymph nodes [6-12]. Positron emission tomography (PET) demonstrates metabolic activity in tumors and integration of the modality with computerized tomography (CT) accurately localizes that active tumor. This newer imaging modality has been shown to enhance the staging and management of malignancies such as malignant melanoma [13-15] and squamous cancers at other sites [16,17]. Cervical cancer is the most frequent squamous cancer of the genital tract and PET-CT has established a place in the pretreatment assessment of that disease [18-23]. The value of PET-CT in the preliminary assessment of squamous cancer of the vulva remains to be established. De Hullu et al. reported sensitivity of 75% and specificity of 62% per groin assessed using L-[1-11C]-tyrosine as a tracer in PET detection per groin assessed in twenty-three patients [24]. Cohn et al. reported sensitivity of 67% and specificity of 95% per groin and sensitivity of 80% and specificity at 90% per patient assessed with FDG-PET in 15 patients [25].

Radical vulvectomy or modification thereof with groin lymphadenectomy is the mainstay of treatment for squamous cancer that invades the vulvar stroma to > one mm in depth. Groin node dissection carries significant morbidity; infection, lymphedema, lymph cysts, cellulitis, and psychosexual dysfunction are frequent adverse outcomes [26-28]. Cellulitis, wound dehiscence and lymphocyst occur in the early to intermediate postoperative period. The interruption of lymph channels results in lower limb and vulvar edema. The vulvar lymphedema usually resolves but chronic lymphedema of the lower limb is not infrequent
Materials and Methods

All patients with squamous vulvar cancer and more than one mm of stromal invasion undergoing radical excision of their cancer and regional lymphadenectomy without prior treatment were identified from the gynecological oncology data base. Patients who had undergone chemotherapy and/or radiotherapy prior to surgery were excluded. The period between the introduction of PET-CT and commencement of sentinel node mapping was chosen to facilitate correlation between PET-CT and histopathological examination of the complete inguinofemoral lymph nodes. Clinical data were extracted from the database and patient records and included age, parity, body mass index (BMI), co-morbidities, smoking status, and details of the operative procedure. Patients without overt lymph node metastasis based on clinical exam (with/without additional ultrasound and MRI at the discretion of their attending clinician) who had undergone PET-CT preoperatively were identified. Their PET-CT findings including size and fluordeoxyglucose (FDG) avidity of vulvar tumor and lymph nodes were reviewed from radiological records. Other radiological abnormalities were noted. Comparison was made with histopathological outcomes to calculate the results.

18F-FDG-PET/CT image protocol

Fluoro-2-deoxyglucose positron emission tomography scans were performed on a VCT 64-slice PET/CT. After fasting for six hours the patient received an intravenous injection approximately 350 MBq of 18F-FDG. Pre-injection blood glucose was measured. Scans were performed approximately 60 minutes after injection of the radionuclide. Whole body PET imaging extended from the base of the skull to the mid-thighs. Low dose CT images were acquired over the same range for attenuation correction and anatomical localization. The PET images were reconstructed with iterative methods after correction for scatter, dead-time, decay, and random coincidences. The images were reformatted into axial data-sets and were reviewed on a advanced workstation using the PET VCAR module by radiologists experienced in PET/CT imaging. The diagnosis of pathologic lymph node on 18F-FDG-PET/CT images was based on the presence of focal increased tracer uptake on PET images, measured as maximum standardized uptake value (SUVmax) and corresponding to the lymph nodal chains on CT images, but independent of lymph node size on CT.

Clinical, surgical, and histopathological protocol

All patients had full clinical examination by a gynecological oncologist, full blood count, plasma glucose, renal and hepatic biochemistry. Surgery was radical excision of the vulvar lesion with two cm horizontal margin beyond the tumor and excision down to the deep fascia or periosseum. The groin incision was ellipitical and all adipose and lymph tissue was removed from the superficial inguinal and deep femoral spaces. All specimens were processed in a routine fashion and stained with H&E before microscopic examination by gynecological pathologists. The lymph nodes were reported as normal, reactive (follicular or sinusoidal hyperplasia) or malignant. The total number of lymph nodes harvested and size and number of metastases were recorded. Histopathological results were reviewed and decisions on adjuvant treatment were made at the multidisciplinary tumor board meetings.

Statistical analysis

For the purpose of statistical analysis, a true-positive (TP) was a patient with malignant lesion in a lymph node detected on PET-CT and found to be positive for metastasis at histological analysis. A false-positive (FP) was a patient with a lesion seen on PET-CT tissue but found to be negative for lymphatic metastasis at histologic analysis. A true-negative (TN) was indicated when no lesion was seen on PET-CT and the result of the histologic analysis of lymph nodes was negative for metastasis. A false-negative (FN) was a patient with histologically proven lymphatic metastasis that was not visible on PET-CT. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of PET-CT imaging in the diagnosis of groin node metastases were calculated.

Results

Twenty patients out of 58 cases of squamous vulvar cancer had a pre-op FDG PET-CT and full surgery comprising vulvectomy and groin node dissection without
Whole-body [18F]fluoro-2-deoxyglucose positron emission tomography scan as combined PET-CT staging prior to planned etc.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Outcome of PET vis-à-vis histopathology of groin nodes</th>
<th>Age at diagnosis (years)</th>
<th>BMI (Kg/m²)</th>
<th>Vular lesion(s) location and size of largest lesion</th>
<th>FDG avidity of vulvar lesion</th>
<th>Groin node FDG avidity (SUV max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>True Positive</td>
<td>72</td>
<td>24</td>
<td>Unifocal, posterior fourchette 3.5cm</td>
<td>12.6</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>True Positive</td>
<td>56</td>
<td>29</td>
<td>Multifocal, extending to perineum, lower vagina largest 2cm</td>
<td>4.5</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>True Positive</td>
<td>77</td>
<td>27</td>
<td>Unifocal, lateral 2.5cm</td>
<td>4.3</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>True Positive</td>
<td>69</td>
<td>31</td>
<td>Multifocal, bilateral anterior &amp; central labial</td>
<td>18.5</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td>True Positive</td>
<td>50</td>
<td>26</td>
<td>Unifocal, anterior central 1.5 cm</td>
<td>14.5</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>True Positive</td>
<td>61</td>
<td>35</td>
<td>Multifocal, unilateral 2cm</td>
<td>11.8</td>
<td>2.6</td>
</tr>
<tr>
<td>7</td>
<td>False Negative</td>
<td>41</td>
<td>21</td>
<td>Unifocal, central anterior, 1.5cm</td>
<td>7.8</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>False Negative</td>
<td>67</td>
<td>29</td>
<td>Multifocal, bilateral &amp; central anterior 3.5 cm</td>
<td>4.2</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>False Negative</td>
<td>63</td>
<td>26</td>
<td>Multifocal, lateral &amp; central anterior &lt;1cm</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>False Negative</td>
<td>83</td>
<td>26</td>
<td>Unifocal, lateral 1.5cm</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>False Negative</td>
<td>49</td>
<td>28</td>
<td>Multifocal, lateral 3.5 cm</td>
<td>14.7</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>False Negative</td>
<td>89</td>
<td>23</td>
<td>Multifocal, lateral 3.5 cm</td>
<td>9.8</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>True Negative</td>
<td>72</td>
<td>24</td>
<td>Unifocal, central anterior, 1.5 cm</td>
<td>4.4</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>True Negative</td>
<td>45</td>
<td>31</td>
<td>Multifocal, bilateral 2.5cm</td>
<td>2.5</td>
<td>Negative</td>
</tr>
<tr>
<td>15</td>
<td>True Negative</td>
<td>50</td>
<td>28</td>
<td>Unifocal, lateral 2cm</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>16</td>
<td>True Negative</td>
<td>57</td>
<td>26</td>
<td>Unifocal, lateral 1.5 cm</td>
<td>3.2</td>
<td>Negative</td>
</tr>
<tr>
<td>17</td>
<td>True Negative</td>
<td>43</td>
<td>23</td>
<td>Multifocal, lateral &amp; central posterior to perineum</td>
<td>9</td>
<td>Negative</td>
</tr>
<tr>
<td>18</td>
<td>True Negative</td>
<td>67</td>
<td>36</td>
<td>Unifocal, central anterior, 3.5 cm</td>
<td>8</td>
<td>Negative</td>
</tr>
<tr>
<td>19</td>
<td>True Negative</td>
<td>43</td>
<td>20</td>
<td>Unifocal, central extending to lower vagina</td>
<td>4.8</td>
<td>Negative</td>
</tr>
<tr>
<td>20</td>
<td>True Negative</td>
<td>38</td>
<td>35</td>
<td>Unifocal, central posterior scar only, no residual seen</td>
<td>0</td>
<td>Negative</td>
</tr>
</tbody>
</table>

(1) Rectal lesion on PET-CT; (2) Pulmonary lesion - primary adenocarcinoma of lung; (3) Pulmonary lesion - granuloma.

Table 2. — PET–CT in preoperative assessment of vulvar cancer: details of surgery and histo-pathological outcomes. Patients 1-6: true positive; 6-12: false negative; 12-20: true negative PET groin node assessment.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Surgery Grade</th>
<th>LVSI</th>
<th>Vulvar depth of invasion (mm)</th>
<th>Metastatic lymph nodes: number and laterality</th>
<th>Max diameter of nodal metastasis (mm)</th>
<th>Total nodes removed</th>
<th>Extracapsular extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 Positive</td>
<td>5</td>
<td>3, bilateral</td>
<td>24/26/18</td>
<td>13 R/11L</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 Negative</td>
<td>6</td>
<td>1, bilateral</td>
<td>&gt;5mm</td>
<td>9R/11L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2 Negative</td>
<td>7</td>
<td>5, bilateral</td>
<td>&gt;5mm</td>
<td>9R/7L</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3 Positive</td>
<td>15</td>
<td>2, bilateral</td>
<td>0.5 &amp; 3mm</td>
<td>4R/7L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3 Negative</td>
<td>6</td>
<td>1, bilateral</td>
<td>&gt;5mm</td>
<td>9R/10L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2 Positive</td>
<td>5</td>
<td>2 bilateral</td>
<td>22/16</td>
<td>13 R/9L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2 Negative</td>
<td>4.5</td>
<td>1, bilateral</td>
<td>3mm</td>
<td>13/9R</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2 Negative</td>
<td>4</td>
<td>1, bilateral</td>
<td>4mm</td>
<td>9R/10L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3 Positive</td>
<td>8</td>
<td>1, bilateral</td>
<td>4 &amp; 2mm in single node</td>
<td>6R/9L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2 Positive</td>
<td>12</td>
<td>9, bilateral</td>
<td>20mm</td>
<td>10R/6L</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2 Negative</td>
<td>3</td>
<td>1, bilateral</td>
<td>5mm</td>
<td>7R/6L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2 Negative</td>
<td>23</td>
<td>2, unilateral</td>
<td>5 &amp; 4 mm</td>
<td>9R/11L</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>1 Negative</td>
<td>3</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>7R/6L</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2 Negative</td>
<td>8</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>8R/9L</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1 Negative</td>
<td>4</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>10R</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1 Negative</td>
<td>7</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>6L</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>3 Negative</td>
<td>3</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>6R/5L</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1 Negative</td>
<td>7</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>12R/14L</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>2 Positive</td>
<td>18</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>5R/10L</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2 Negative</td>
<td>3</td>
<td>0, bilateral</td>
<td>N/A</td>
<td>16R/11 L</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

RVE: radical excision of vulvar lesion; UGND: unilateral groin node dissection; BGND: bilateral groin node dissection; URT: distal urethrectomy; VGTAC: partial vaginectomy/partial excision anal canal and/or sphincter; CBD: colostomy bowel diversion.
prior chemotherapy or radiotherapy between January 2010 and March 2012. A summary of the results is shown in Tables 1 and 2. Patients ranged in age from 38 to 83 (median 59) years. BMI ranged from 21-36 (median 26.5) kg/m². Twelve were current or past smokers. Two patients had unilateral groin node dissection (UGND). Eighteen patients had complete BGND: 16 at primary surgery and two after an interval following the detection of metastases in their first groin. The vulvar malignant lesions were unifocal in eleven. The lesions were unifocal and lateral in four, but two of these progressed to completion bilateral groin node dissection (BGND) on finding unilateral lymph node metastases. Twelve patients had lesions in or within one cm of the midline either anteriorly or posteriorly (central component). Some cancers encroached on the urethra (n = 1) or vagina (n = 2) and abutted the anal sphincter (n = 3). Radical excision of these clinical Stage II cancers necessitated distal urethrectomy (n = 1) and partial excision of anal sphincter with temporary bowel diversion (n = 3).

FDG avidity was measurable in 16 vulvas with $V_{\text{max}}$ range 2.5-14.7 (mean 8.4) and six groins with $V_{\text{max}}$ range 2.4-3.0 (mean 2.1). Fourteen patients had no FDG avid groin lymph nodes: 13 of these had no measurable disease on CT and one patient had an eight-mm lymph node with normal architecture (fatty hilum) deemed to be reactive/normal. Six women had FDG avid lymph nodes: four nodes were single, two multiple, and all measured less than two cm. PET-CT detected bilateral nodes in three patients. Extranodal extension was not detected on radiology. Three patients had unrelated lesions outside of the vulva/groins: one synchronous primary adenocarcinoma of lung, one granuloma of lung, and one rectal lesion with subsequent negative MRI and endoscopy.

Histopathological findings were of squamous grade 1 (n = 4), grade 2 (n = 12), and grade 3 (n = 4). All grade 1 cancers occurred in Stage I/II. Cancers with lymph node metastases were grade 2 (n = 8) and grade 3 (n = 4). Lymphovascular space invasion (LVSI) was described in six patients: one of eight Stage I/II cancers and five of 12 Stage III cancers. The maximum depth of stromal invasion ranged from three to 23 mm: 3-18 (median 5.5) mm in Stage I/II and 3-23 (median 5.5) mm in Stage III cancers. Metastasis to lymph nodes were single unilateral (n = 5), multiple in single node (n = 1), single in two nodes unilateral (n = 1), single in two nodes bilateral (n = 2), and multiple in bilateral nodes (n = 3). Extracapsular extension was present in three patients.

In patients with histologically proven metastases to groin nodes, comparisons between PET-CT positive (true-positive / TP) and negative (false-negative / FN) groups vis-à-vis histology yielded the following: the average vulvar SUV in TP was 11 (4.3 - 18.5) and 5.5 (0 - 14.7) in FN. Metastatic lymph nodes were bilateral in four of six (67%) TP and one of six (17%) FN, contained multiple metastases in four of six (67%) TP and two of six (33%) FN and largest metastases measured 11 (range 3 - 26) mm in TP compared to 6.6 (range 3-20) mm in FN group. Extracapsular extension was present in two of six (33%) TP and 1/6 (17%) FN. The average deepest invasion in the primary tumor was 7.3 (5 - 15) mm in TP and 9.1 (3 - 23) mm in the FN group.

The calculations per patient for PET-CT yielded a sensitivity of 50% and specifcity at 100%. The PPV was 100% and the NPV was 57.1%. The test accuracy was 70% per patient.

Discussion
Progress in the surgical management of many cancers is marked by less radical excision of tissues. Modifications in the radical surgical approach to vulvar cancers have been evolving since the 1980s [38, 39]. Complete vulvectomy is replaced by radical wide excision with maximal effort to preserve coital and orgasmic sexual function. The need for full groin lymphadenectomy is under review with lymph node sparing surgery being assessed in an international prospective trial (GroinSS-vii). Better radiological assessment of lymph node status has the potential to enhance the individualized approach to management of vulvar cancer surgery. A test with a sufficiently high PPV would allow patients with metastases to progress to full lymphadenectomy without recourse to sentinel node (SLN) sampling. A test with an excellent NPV could identify a lowest risk group that could be spared lymphadenectomy altogether.

This evaluation of CT-PET in preoperative assessment of vulvar squamous cancer in a single institution over three years was undertaken prior to the introduction of sentinel lymph node mapping into our clinical practice. This was a window of opportunity to compare CT-PET with the histopathological results of full inguinofermoral lymphadenectomy. Analysis was restricted to those without clinical suspicion of lymph node metastases who underwent PET-CT scanning prior to surgery. Other studies have reported figures calculated per lymph node or per groin [24,25]. The authors wanted to determine whether pre-treatment PET-CT could determine Stage III categorization prior to surgery and they analyzed the results per patient.

Three quarters of vulvar cancers are Stage I-II [40]. That only 34% of the present authors’ referred patients were eligible for this retrospective review reflects their unit’s referral pattern. Early-stage microinvasive disease is referred from their allied vulvar and colposcopy clinics and their tertiary referral status is skewed towards more advanced and metastatic cancers. Patients with microinvasive disease (<1 mm stromal invasion) and patients with locally advanced disease who underwent chemother-
apy or radiotherapy prior to surgery were excluded. Full groin histopathological status was regarded as the gold standard against which to compare PET-CT.

The pattern that emerged from this study was for PET detection of metastases when the primary tumor was more FDG-avid (higher SUVmax) and metastases were larger, multiple or bilateral. The low NPV and test accuracy rule out a potential role for PET-CT in identifying the lowest risk group who might be spared any sampling of lymph nodes and we are pursuing SLN based management in that group [41]. De Hullu et al. speculated that attenuation due to proximity of bony structures might contribute to the poorer performance of PET-CT in the pelvic region compared to other anatomical sites [24]. The high PPV is interpreted with caution because the group size is small but the authors consider it reasonable to progress to full bilateral lymphadenectomy when a groin is PET positive without preliminary recourse to SLN. That approach saves on hospital resources including nuclear medicine scanning, shortens operating time, and avoids the need for a second operation that arises when frozen section on SLN yields a false negative result. The treatment of patients with advanced-stage disease is a challenge and while chemotherapy and radiotherapy are used with increasing frequency in this group [40, 42, 43], there is insufficient evidence to eschew lymphadenectomy completely. Other series have found that high yield lymphadenectomy is beneficial in this group and higher rates of groin recurrence are observed when surgical removal is replaced by radiation alone [44, 45]. The present authors continue to offer a thorough inguinofemoral lymph node dissection for Stage III disease but treatment is individualized based on patient performance status and discussion by the multidisciplinary group.

Larger studies of PET-CT incorporating full groin node dissection are unlikely to emerge now that SLN based surgical management has become widespread. Detection of additional lesions adds value to PET-CT scanning as evidenced by the patient in this series with a synchronous lung cancer. 18F-FDG is taken up by inflammatory reactive cells as well and may lead to additional diagnostic testing for some patients. Other authors have recommended histological evaluation by biopsy of apparent metastases in other cancers including cervical malignancy [46]. Distant metastasis of vulvar cancer outside of the groin and pelvis is extremely rare at first presentation of the disease and only occurs in advanced/recurrent cancer in this (NG) author’s experience [47]. Low levels of FGD uptake on the vulvar surface can be due to urinary contamination by excreted 18F-FDG and may account for some surface false positivity in the vulva. However, radiological assessment of the vulvar lesion is not a priority as visual inspection will supersede the radiological findings in that organ.

Conclusion

The sensitivity and NPV of PET-CT are too low to identify women at lowest risk of groin node metastasis in vulvar cancer in order to avoid lymphadenectomy. A positive test predicts metastases, advancing the diagnosis of Stage III disease to the preoperative phase for some women and can be used to facilitate more robust therapeutic decision making prior to their surgery for vulvar cancer.

References

BRMS1 inhibits expression of NF-κB subunit p65, uPA and OPN in ovarian cancer cells

X.-J. Sheng¹, D.-M. Zhou¹, Q. Liu², S.-Y. Lou¹, Q.-Y. Song¹, Y.-Q. Zhou¹

¹Department of Obstetrics and Gynecology, the Third Affiliated Hospital of Guangzhou Medical University, Guangzhou
²Experimental Medical Research Center of Guangzhou Medical University, Guangzhou (China)

Summary

Background: Breast cancer metastasis suppressor 1 (BRMS1) is a potent metastasis suppressor of various types of malignancies, including melanoma and ovarian cancer. Unfortunately, the clinical data regarding its role as a true metastatic suppressor and its efficacy as a prognostic marker and therapeutic target remain controversial. This study was designed to investigate the effect of BRMS1 on the invasion and metastasis of human ovarian cancer cells and its potential underlying mechanisms. Materials and Methods: BRMS1 small interfering RNAs (siRNAs) or control siRNAs were transfected into the OVCAR3 human ovarian cancer cell line. Invasion and migration activities were assessed using the Transwell invasion and migration assay. Protein levels of nuclear factor-κB (NF-κB) subunit p65, osteopontin (OPN) and urokinase-type plasminogen activator (uPA) were evaluated by Western blot, immunofluorescence and immunocytochemistry methods. Results: Successful knockdown of BRMS1 was verified by quantitative real-time RT-PCR and Western blot. The invasion and migration capacities of OVCAR3 cells were significantly enhanced in the BRMS1-silenced group, compared to controls (p < 0.05). Silencing of BRMS1 significantly induced the expression of NF-κB subunit, p65, uPA, and OPN proteins. Conclusions: BRMS1 inhibits expression of p65, uPA and OPN protein. In turn, this leads to inhibition of ovarian cancer cell invasion and metastasis. This study unveils a potential novel mechanism by which BRMS1 inhibits metastasis of ovarian cancer cells.

Key words: BRMS1; Ovarian cancer cells; Metastasis; uPA; OPN; NF-κB/p65.

Introduction

Ovarian carcinoma is the leading cause of death from gynecologic malignancies worldwide, and has a dismal overall five-year survival rate (30%). The inhibition of invasion and metastasis of ovarian cancer cells is critical to improve the 5-year survival rate. Cancer metastasis is a multi-factor and multi-step process involving complex gene regulations and interactions. The molecular and biochemical mechanisms underlying cancer progression and metastasis remain poorly understood; although, metastasis suppressor genes, such as Non-metastatic 23 (Nm23), Kang ai 1 (KAI1), Raf kinase inhibitory protein (RKIP) and breast cancer metastasis suppressor 1 (BRMS1), have been defined as key modulators of the process [1-4]. As one of the metastasis suppressors, BRMS1 has the ability to inhibit the metastatic potential of cancer cells in vivo without affecting tumorigenicity [5-7]. Recent studies have shown that the nuclear factor-κB (NF-κB) transcription factor positively regulates urokinase-type plasminogen activator (uPA) and breast cancer metastasis suppressor 1 (BRMS1), have been defined as key modulators of the process [1-4]. As one of the metastasis suppressors, BRMS1 has the ability to inhibit the metastatic potential of cancer cells in vivo without affecting tumorigenicity [5-7]. Recent studies have shown that the nuclear factor-κB (NF-κB) transcription factor positively regulates urokinase-type plasminogen activator (uPA) expression, as well as several other genes implicated in angiogenesis and tumor metastasis [8-10]. One study found that BRMS1 suppressed osteopontin (OPN) gene expression, at least in part, through inhibition of the NF-κB signaling pathway in breast cancer cells [11]. In this study, we investigated whether BRMS1 is capable of suppressing ovarian cancer metastasis and the possible underlying mechanisms of this process. Our results indicate that BRMS1 inhibits the invasion and migration of human ovarian cancer cells through inhibiting expression of the NF-κB subunit p65, uPA and OPN.

Materials and Methods

Cell culture, chemicals, and reagents

The human ovarian cancer cell line, OVCAR3, was obtained from the American Type Culture Collection. Cells were cultured with Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin, in an atmosphere of 5% CO2 at 37°C. Lipofectamine 2000 and TRIzol reagent were used to conduct all transfections.

siRNAs transfection

Targeted small interfering RNAs (siRNAs) for BRMS1 (NM: 015399.3) were synthesized. Three BRMS1-siRNAs were designed to select the most efficient silencing. The three siRNAs were as follows: siRNA1: 5’-CCAUACACUGUGUAGCUUCGCUU-3’; siRNA2: 5’-GAAGCAGUUCUCGAGCUAAG-3’; siRNA3: 5’-UUUCGUAUUUCGGUACACACU-3’. Negative control siRNA was also synthesized: NC-siRNA: 5’-UUUUCGGCAGUGCCAGGTT3’. The cells were divided into three groups: experimental group, transfected with BRMS1-siRNA; negative control group with NC-siRNA; and blank control group. Mixtures of siRNA-liposome duplexes were transiently transfected into OVCAR3 cells using Lipofectamine 2000 according to the manufacturer’s instructions. FAM-siRNA was used to optimize transfection efficiency. A range of concentrations (0–100 nM) of the BRMS1-siRNAs were tested and inhibition of BRMS1 mRNA expression was confirmed by quantitative reverse transcription (qRT)-PCR after 48-hour transfection.
Results were calculated as means ± standard deviation (SD). Intergroup differences were analyzed by Student’s t-test and \( p < 0.05 \).
BRMS1 inhibits expression of NF-κB subunit p65, uPA and OPN in ovarian cancer cells

Values < 0.05 were considered statistically significant. SPSS v16.0 was used for all statistical procedures.

Results

Knockdown of BRMS1 by siRNAs

Cells were transfected with three different BRMS1 siRNAs for 48 hours followed by qRT-PCR examination of BRMS1 mRNA expression using BRMS1-specific primers. Only one of the BRMS1 siRNAs (siRNA3) remarkably inhibited BRMS1 mRNA expression (reduced by 82.3%; Figure 1a). The significant reduction of BRMS1 protein expression by siRNA3 was confirmed by Western blot (Figure 1b).

Silencing of BRMS1 enhances the invasion and migration of ovarian cancer cells

Using the matrigel invasion assay we examined the number of invading cells in the three groups (experimental, NC-siRNA, and blank control). BRMS1-siRNA3 cells had 190 ± 8.5 cells with invasive capacity, which was more than either NC-siRNA (144±7.8) or blank control (146 ± 6.8) (Figure 2a). Likewise, BRMS1-siRNA3 cells were more likely to migrate than the NC-siRNA or blank control cells (231 ± 8.9 vs. 177 ± 9.7 and 182 ± 7.9, respectively) (Figure 2b). These results revealed that BRMS1 silencing led to significantly increased cell invasion and migration (p < 0.05), suggesting BRMS1 plays a role as a suppressor of invasion and migration of ovarian cancer cells.

Silencing of BRMS1 up-regulates the expression of p65, uPA, and OPN

The authors next used the BRMS1 siRNA knockdown system to test whether BRMS1 could influence the expression of NF-κB signal and uPA and OPN gene expression. Real-time PCR analysis demonstrated that silencing of BRMS1 dramatically up-regulated the expression of NF-κB subunit p65 and uPA (p < 0.05) in a time-dependent manner; maximal induction occurred at 72 hours post-siRNA treatment (Figure 3).

The authors then examined the link between the expression level of OPN and BRMS1. They found that the expression of OPN in the BRMS1-silenced cells was dramatically higher than that in the NC-siRNA control cells, as evidenced by immunofluorescence (Figures 4a-b) and by immunocytochemistry (Figures 4d-e). The results indicated that BRMS1 inhibited expression of OPN in ovarian cancer cells.

Similar results were observed for the expression of uPA, by immunocytochemistry (Figures 5a-c); specifically, uPA expression was significantly higher in the BRMS1-silenced cells than that in the NC-siRNA control cells (Figures 5a-b). The results suggested that BRMS1 acts to suppress the expression levels of uPA in OVCAR3 cells.

Discussion

Metastasis is a complex process associated with tumor progression and is comprised of sequential advancement through several biological processes, including tumor an-
Figure 3. — BRMS1-siRNA3 silencing up-regulated uPA, OPN, and NF-κB/p65 in a time-dependent manner. Cellular proteins were collected from cells transfected with BRMS1-siRNA3 for up to three days. Western Blot detected expression of uPA, OPN, and p65 protein. GAPDH was used as the loading control. The densitometry results are listed at the bottom. * indicates statistically significant differences as compared to the control ($p < 0.05$).

Figure 4. — Silencing of BRMS1 induces OPN protein expression. Cells transfected with BRMS1-siRNA3 were incubated overnight with OPN antibody, followed by incubation with (a-c) fluorescent secondary FITC-goat anti-rabbit IgG or (d-f) HRP-conjugated goat anti-mouse/rabbit secondary antibody. Coloration was achieved through staining with DAB. BRMS1-siRNA3 transfected cells exhibited (a, d) high levels of OPN expression, whereas (b, e) low expression levels were observed in cells transfected with NC-control cells. No expression was detected in the blank control cells (c, f). The OPN was located mainly in the cytoplasm.
BRMS1 inhibits expression of NF-κB subunit p65, uPA and OPN in ovarian cancer cells

BRMS1, a tumor metastasis suppressor gene, has been shown to inhibit malignant tumor invasion and metastasis in several cancers, such as melanoma, bladder cancer, and ovarian cancer [14-16]. The anti-invasion and anti-migration properties of BRMS1 have been experimentally evidenced using ovarian cancer cells [14].

Invasion through BM to blood vessels and then to the secondary sites involves secretion of chemokines that enhance tumor cell motility in a certain direction, while proteolytic enzymes contribute to ECM degradation [17]. Degradation of ECM and BM further promotes invasion and metastasis of cancer cells. uPA is one of the serine-specific proteinases involved in ECM degradation. Elevated levels of uPA showed to be associated with cancer cell progression in many types of cancers, including ovarian cancer [18, 19]. The mechanism by this was, at least in part, involved in NF-κB signaling [9, 20, 21].

NF-κB is a family of transcription factors that exist as homo and heterodimers. Several members, including p65, p50, RelB, and c-Rel molecules, have been demonstrated to be involved in the regulation of multiple cellular processes, such as immune response, inflammation, cell proliferation,
and apoptosis [22]. NF-κB is recognized as an anti-apoptotic factor and protects cancer cells from apoptosis in order to facilitate tumor progression. Furthermore, NF-κB promotes transcription of genes that are associated with tumor metastasis, such as Interleukin-1 (IL-1), Interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), uPA [18, 23], and OPN [20, 24, 25].

Studies have shown that the NF-κB signaling pathway is also involved in tumor metastasis [20, 24, 25]. A recent study suggested that NF-κB could be an effective diagnostic biomarker of early-stage epithelial ovarian cancer (EOC) [26]. NF-κB is known to modulate the expression of genes involved in cancer cell invasion, metastasis, angiogenesis, and apoptosis, and constitutive activation of NF-κB has been clinically observed in several cancer cell types [21,27-29]. The authors demonstrated, here, that BRMS1 significantly reduces NF-κB subunit p65, suggesting a role of this transcription factor in the ovarian cancer malignant process. Consistent with this, another study found that blockade of NF-κB by BRMS1 leads to down-regulation of OPN expression in MDA-MB-435 melanoma cells [29]. Thus, compounds that block NF-κB signal may be useful as potential therapeutic agents to inhibit tumor invasion and migration.

As a member of the ECM family, OPN also regulates expression and activation of matrix metalloproteinases (MMPs), and plays a significant role in ECM degradation and in facilitating cell motility, tumor growth, and metastasis by interacting with integrin receptors. Induced cellular motility by OPN is a major step in cancer metastasis and involves the up-regulation of certain genes known to promote cell invasion [30]. Overexpression of OPN has been clinically detected in many human cancers [31]. Still other studies have indicated that BRMS1 can inhibit OPN expression in breast cancer and melanoma cells, suggesting that this functional interplay might contribute to suppression of cancer metastasis [15, 29, 32].

The present data showed that invasion and migration activities were enhanced dramatically in cells with silenced BRMS1. Meanwhile, these data were consistent with other studies demonstrating that BRMS1 can negatively regulate NF-κB binding activity [11, 29]. Using Western blot, immunofluorescence, and immunocytochemistry, the present authors demonstrated that BRMS1 does indeed act as a tumor suppressor in OVCAR3 cells, likely by inhibiting expression of p65, uPA and OPN. These data indicate a mechanistic profile by which BRMS1 might control ovarian cancer cell survival. Similarly, BRMS1 has been shown to inhibit OPN expression by modulating the activity of NF-κB signaling in breast cancer [29], and decreasing OPN has been suggested to contribute to inhibition of breast cancer metastasis [32]. Cicek et al. [21] reported that BRMS1 possibly regulated metastasis by influencing phosphoinositide signaling or recruiting of HDAC1 to NF-κB binding site of the uPA promoter.

In summary, the present findings suggest that BRMS1 inhibits human ovarian cancer cell invasion and metastasis by reducing the expression of NF-κB-p65, uPA and OPN proteins. It is possible the NF-κB mediates the effect of BRMS1 on reduction of uPA and OPN although this needs to be determined in our system. Further studies are expected to explore the detailed mechanism.

Acknowledgments

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References


BRMS1 inhibits expression of NF-κB subunit p65, uPA and OPN in ovarian cancer cells


Address reprint requests to:
X.-J. SHENG, M.D.
Department of Obstetrics and Gynecology
The Third Affiliated Hospital of
Guangzhou Medical University
No. 63, Duobao Road
Guangzhou 510150 (China)
e-mail: xjsheng2010@126.com
Carcinomas and sarcomas of Bartholin gland. 
A report of nine cases and review of the literature

B. Kozakiewicz1,2, E. Dmoch-Gajzlerska2, K. Roszkowska-Purska1
1 Cancer Center and Institute of Oncology in Warsaw; 2 Medical University of Warsaw, Warsaw (Poland)

Summary
The greater vestibular gland, also called Bartholin’s gland after the Danish anatomist Caspar Bartholin the Younger who first described it in the 17th century, is the site of tumours arising from different types of epithelium and characterized by a different clinical course. In the years 1980-2009, 1,296 patients with vulvar carcinoma were treated at the Oncology Centre in Warsaw, Poland and nine of them had carcinoma of Bartholin’s gland, including three patients with squamous cell carcinoma (SCC), three patients with adenoid cystic carcinoma (ACC) and three patients with sarcoma. In this paper the authors present the signs and symptoms, clinical course, treatment outcomes, and recurrence of these three malignant tumours of different histopathology. Own observations and evaluation of treatment results are compared with published reports from other centres. Interestingly, there is no consensus regarding diagnostic criteria or a uniform approach to management. Relatively poor knowledge of malignant tumours of Bartholin’s gland seems to be responsible for delays in proper diagnosis and hence optimal management. When instituted, the treatment is usually aggressive and involves adjuvant radio- and chemotherapy, while the chances of longer disease-free survival after treatment may be compromised. Conclusions: Bartholin sarcomas grow fast and invasive, SCC, and ACC infiltrate slowly and systematic. All types are curable at high interest rates if they are originally from the surgically removed lymph nodes on both sides and irradiated.

Key words: Squamous cell carcinoma of Bartholin’s gland; Malignant tumours of Bartholin’s gland; Vulvar carcinoma; Adenoid cystic carcinoma; Leiomyosarcoma of Bartholin’s gland.

Introduction
The greater vestibular gland was named Bartholin’s gland after the Dutch anatomist Caspar Bartholin the Younger who first described its anatomical position in 1675. Some 190 years later, in 1864, J.M. Klob was the first to report a case of a patient with a malignant tumour arising from the Bartholin’s gland. The structure of the gland was first described by T.S. Cullen in 1905.

The two Bartholin glands are the largest compound tubuloalveolar glands located on either side of the posterior part of the vestibule of the vagina below the bulbocavernous muscle inside the perineum. The secretory duct of the gland, which is two cm in length, runs diagonally and superiorly and opens below the urethra near the vaginal introitus. The duct is lined with the transitional epithelium and then the glandular epithelium with low columnar epithelium. In the alveoli of the gland, high columnar epithelium is found. Myoepithelial cells constitute the basal cell layer of the glandular epithelium and they are capable of multidirectional differentiation. Glandular cells, both low and high columnar cells, secrete mucus [1-4].

Malignant tumours of Bartholin’s gland are extremely rare as they are thought to account for less than one percent of all vulvar malignancies. These are predominantly primary cancers arising from the epithelium lining the opening of the gland duct. Like vulvar cancer, they are keratinizing or non-keratinizing squamous cell carcinomas (SCCs). To date, approximately 300 cases have been reported. The peak incidence of SCC is after menopause. The tumours develop along the duct of the gland and deeply infiltrate the adjacent tissue. They grow slowly and do not cause any pain or other symptoms. They may achieve a considerable size, are located in the orifice, and very rarely cause overlying skin ulceration [1-3, 5-7].

Adenocarcinomas originate in the cells of tubules and alveoli of Bartholin’s gland or in the glandular cells of the ducts and tend to grow deep into the perineal tissues along the nerves. Adenocarcinomas are rare and they include adenoid cystic carcinoma (ACC), papillary carcinoma originating in transitional epithelium, and mucous-secreting carcinomas like mucoepidermoid carcinoma or mucinous adenocarcinoma [1,4]. Adenocarcinoma presents as a firm solid tumour, which forms a bulge in the skin of the labium, is frequently cyst-like, and contains mucoid material.

ACC is a specific variant of adenocarcinoma and was first recognized in 1853. Initially it was described in salivary glands but subsequently other locations were identified such as breast, skin, and lung. In women, ACC is most commonly found in the cervix. To date, 64 cases of ACC found in Bartholin’s gland have been reported. This variant of carcinoma usually occurs in middle-aged women, the median age at presentation being 49 years. ACC often presents as pain or pruritus which may precede the actual tumour. ACC is a slow-growing tumour, which resembles inflammation or abscess and accordingly in most cases is
initially treated by incision and drainage of the “abscess”. The tumour invades deep tissues and spreads along the nerves causing pain, which becomes more severe as the tumour grows. Immunochemistry studies have demonstrated that the tumour cells produce keratins, carcinoembryonic antigen, lysozyme, and lactoferrin while steroid receptors are present in the tumour tissue [1, 3, 8, 9].

Sarcomas develop from the Bartholin gland’s mesoderm. Depending on their differentiation and morphology, they may form solid tumours, with well or poorly defined borders, e.g. leiomyosarcoma which is derived from smooth muscle. Another malignant neoplasm of Bartholin’s gland is rhabdomyosarcoma which is poorly differentiated and derived from striated muscle. It has features of a germ cell tumour of muscle origin with considerable invasiveness [2].

Epithelioid sarcomas with the location in Bartholin’s gland have also been reported. Sarcomas are diagnosed in young women and may develop during pregnancy [10-12]. Initially sarcomas are considered benign lesions but are rapidly growing tumours and may be soon over five cm in diameter with overlying skin ulceration and metastases to the inguinal, femoral, and pelvic lymph nodes. There are some slight differences between particular types of sarcoma concerning incidence but virtually no differences in their clinical course [2, 10-13].

Since malignant tumours of the Bartholin’s gland are so rare, it has been difficult to develop the criteria for their recognition, differential diagnosis, and management [9, 18-20].

The diagnostic criteria for differentiating Bartholin’s gland carcinoma from vulvar carcinoma were established by Honan in 1897. In 1972, they were modified by D.L. Chamlian and H.B. Taylor from the Armed Forces Institute of Pathology and are currently in use worldwide. Microscopic evaluation of the specimen must demonstrate some normal gland tissue and tumour tissue. In patients with Bartholin’s gland tumour, it is necessary to exclude any other tumour with a similar microscopic structure. Frequently this is very difficult as distinguishing the Bartholin gland structure in

<table>
<thead>
<tr>
<th>Patient first treated in year</th>
<th>Patient's age (first treated age)</th>
<th>Side (Lesion observation)</th>
<th>Lesion size and appearance</th>
<th>Primary treatment</th>
<th>Histopathology</th>
<th>Stage</th>
<th>Adjuvant treatment</th>
<th>Treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BR** 1980</td>
<td>57</td>
<td>R+L (R)</td>
<td>4 cm; rectovaginal fistula, ulceration</td>
<td>RV+BGNDF</td>
<td>Infiltrating macrocellular carcinoma</td>
<td>T3N2M0</td>
<td><strong>Excision 1983</strong></td>
<td>NED 13 years</td>
<td></td>
</tr>
<tr>
<td>2. BE** 1988</td>
<td>75</td>
<td>R</td>
<td>5 cm; Ulceration, infiltration</td>
<td>RV</td>
<td>Squamous cell carcinoma</td>
<td>T4N2M0</td>
<td>**Excision DOD 3 years</td>
<td>Local recurrence</td>
<td></td>
</tr>
<tr>
<td>3. GU** 2000</td>
<td>42</td>
<td>L</td>
<td>Ca. 6 cm in diameter; nodule, no ulceration</td>
<td>EX</td>
<td>Squamous cell carcinoma</td>
<td>T3N0M0</td>
<td>RV+BGNDF</td>
<td>NED 6 years</td>
<td></td>
</tr>
<tr>
<td>4. EB 1991</td>
<td>20</td>
<td>R</td>
<td>15x8 cm; ulcerated lesion penetrating to bone</td>
<td>EX</td>
<td>Embryonal small-cell rhabdomyosarcoma</td>
<td>T3N2M0</td>
<td>**Chth+ RV+BGNDF RT 38 Gy</td>
<td>DOD 9 years</td>
<td>Lung metastases</td>
</tr>
<tr>
<td>5. GA 1991</td>
<td>32</td>
<td>R</td>
<td>3 cm; raised nodule, no ulceration</td>
<td>EX</td>
<td>Leiomyosarcoma G1</td>
<td>T2N0M0</td>
<td>RT 60 Gy + RV+BGNDF Lobectomy 1993 9th rib</td>
<td>DOD 4 years</td>
<td>Lung and bone metastases</td>
</tr>
<tr>
<td>6. SH 2000</td>
<td>43</td>
<td>R</td>
<td>3x4 cm; discoid, non-ulcerated tumour</td>
<td>EX</td>
<td>Leiomyosarcoma G2</td>
<td>T3N0M0</td>
<td>RV+BGNDF</td>
<td>NED 7 years</td>
<td></td>
</tr>
<tr>
<td>7. KW 1999</td>
<td>58</td>
<td>R</td>
<td>c. 10 cm; overlying skin ulceration</td>
<td>EX+BGNDF</td>
<td>Adenoid cystic carcinoma</td>
<td>T4N1M0</td>
<td>RT 50 Gy</td>
<td>NED 13 years</td>
<td></td>
</tr>
<tr>
<td>8. KL 2006</td>
<td>51</td>
<td>R+L (R+L)</td>
<td>c. 6 cm in diameter overlying skin</td>
<td>EX</td>
<td>Adenoid cystic carcinoma</td>
<td>T4N0M1</td>
<td>**Tumour resection + Chth CAP x 6 Lung metastasis</td>
<td>NED 32 months</td>
<td></td>
</tr>
<tr>
<td>9. PE 1995</td>
<td>50</td>
<td>R</td>
<td>c. 5 cm in diameter; ulcerated</td>
<td>RV+BGNDF</td>
<td>Adenoid cystic carcinoma</td>
<td>T2N2M0</td>
<td>RT 48 Gy *** 1999 Uterine Cancer IC/adenocarcinoma G2</td>
<td>DOD 11 years</td>
<td>Disseminated disease</td>
</tr>
</tbody>
</table>

EX – local excision; RV – radical vulvectomy; BGNDF – bilateral groin node dissection; Chth – chemotherapy; RT – radiotherapy; DOD – dead of disease; NED – no evidence of disease; Chth* CYVADIC; * - recurrence treatment, ** - earlier reported cases [5]; *** another malignancy treated with surgery and EBRT.
cases of diffuse invasion may be not feasible. Both sets of criteria rely on the subjective evaluation by the pathologist and gynaecologist. At present, a new classification could be established based on more objective molecular criteria, such as p53 protein expression, the presence of Ca 19-9 as a cancer marker or the use of immunohistochemistry methods to demonstrate desmin, actin or vimentin. Molecular methods could be used for precise and reliable differential diagnosis of vulvar malignancies [7, 18-23].

Materials and Methods

The study was based on the retrospective review of the medical records of patients treated at the present institution. All data concerning the patients are summarized in two tables. Table 1 presents such patient data as treatment duration, age, follow-up time, lesion size, type of tumour infiltration, histopathology findings, and primary treatment and its outcome. Table 2 summarizes the impact of risk factors on tumour recurrence and patient survival.

In the years 1980-2009, 1,296 patients were treated for vulvar carcinoma at the Oncology Centre in Warsaw. Three patients had SCC of Bartholin's gland [5], three had sarcoma (proximal-type epithelioid sarcoma (PES) in two patients, and malignant rhabdoid tumour (MRT) in one patient, and three had ACC.

Results

The patients' ages ranged from 20 to 75 years. The lesions were followed up for three to 24 months. In all patients, the primary treatment was surgical. Vulvectomy was performed in three patients and radical vulvectomy with bilateral inguinal lymph node dissection in two patients. The tumour size was described as T2 in two patients, as T3 in three patients, and as T4 in four patients. Primarily involved lymph nodes were found in five patients and in none was tumour dissemination identified. Of those five patients, four were subsequently treated with adjuvant radiotherapy and one underwent radical vulvectomy with lymph node dissection. During the follow-up, disease recurred in four patients within five months to three years of the primary treatment. In one patient, metastases to the
lungs were diagnosed two years after treatment and to the ribs after four years. Another patient suffered lung metastases and recurrence after eight years. Both patients with the disseminated disease were initially diagnosed with Bartholin gland sarcoma. In one patient, another malignant tumour, adenocarcinoma of the endometrium, was diagnosed four years after treatment for Bartholin gland carcinoma. The second malignancy was treated with surgery and adjuvant radiotherapy. Overall, the survival ranged from three to thirteen years.

**Discussion**

Malignant tumours of Bartholin’s gland are rare conditions. This paper presents cases of three different types of Bartholin gland malignancies i.e. SCC, adenocarcinoma, and sarcoma. SCC accounts for ca. 80% of all malignant tumours of the Bartholin gland, adenocarcinoma for ca. 15%, and sarcoma for ca. 2%. The reports of cases published in the literature are equally few. Up-to-date approximately 300 cases of SCC of Bartholin gland have been reported, 64 cases of ACC, and only single case of sarcoma [2, 3,5-7,21,22,24-26].

Slow development of the tumour and absence of characteristic symptoms of malignancy often delay the correct diagnosis. Not infrequently, gynaecologists do not suspect any malignancy and manage all lesions of Bartholin’s gland as inflammation or abscess.

It is a well-known fact that an early diagnosis makes early treatment possible with the relief of the troublesome symptoms and improved chance of enduring cure. When a malignant tumour of Bartholin gland is not differentiated from its abscess or vulvar carcinoma, treatment is delayed, which may affect its outcome.

The management of patients with vulvar carcinoma must be prompt and aggressive using very radical methods. A long period of observation before the treatment is instituted may produce a less successful outcome. Initially, malignant
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Tumours of the Bartholin gland — SCC, ACC or sarcoma — run a slower course than vulvar carcinoma, while their radical surgery and adjuvant radiotherapy may result in longer disease-free survival than in vulvar carcinoma.

The clinical courses of three types of malignant tumours of Bartholin’s gland described in this paper demonstrate their distinct clinical features and require to employ special treatment. Only small series of cases are reported in the literature and drawing definite conclusions is often difficult. As there are no universally recognized diagnostic and management standards, the present authors evaluated the cases with the aim of proposing appropriate management. The characteristic features which allow the differentiation of the three types of Bartholin’s gland malignancies are presented in Table 3 which summarises these differentiating features.

Initially, the tumour develops slowly and in the described patient group the duration of presenting symptoms, i.e. the interval from the reported onset of symptoms to the appropriate diagnosis was three to 24 months, which is also reported by other authors. The age at onset ranged from young adulthood in patients with a sarcoma of Bartholin’s gland to premenopausal women in their forties with ACC to postmenopausal patients with SCC [3, 5-7, 27].

SCCs often present as long and slowly growing tumours with features of inflammation. After reaching a larger size, the tumours become ulcerated with pain and metastases to the lymph nodes [5-7].

Symptoms such as itchiness and burning are reported by patients with ACC only. These symptoms are associated with the infiltration by the tumour spreading along the nerves from the onset of the disease and increase in intensity with the tumour development, although they may occur before the Bartholin gland begins to enlarge. The gland enlargement produces a continuous pain which becomes increasingly severe. Ulceration and deep penetration into the surrounding tissues are commonly observed. Initially, the pain is described as nagging and troublesome rather than severe and it is seldom relieved by analgesics. Patients with ACC require wide vulvectomy with bilateral dissection of the inguinal and femoral lymph nodes. Adjuvant radiotherapy is mandatory in all patients whether there are any cancer cells in the dissection line/resection margins or not. The patients should undergo a general evaluation as ACC may produce distant metastases, mostly to bones and lungs before the regional lymph nodes become involved. In such cases chemotherapy is recommended. A five-year progress-free interval is 43% and overall five-year survival rate is 71%, the corresponding rates being 38% and 50% at ten years and 13% and 51% at fifteen years [6-11, 28-30].

Bartholin gland sarcomas rapidly infiltrate the surrounding tissues and become ulcerated. The lesions are diagnosed as abscesses, not malignancies and repeated incisions are performed. Pain is reported only when the malignancy involves a large area of the surrounding tissues. The tumour size on diagnosis is an important predictive factor since some authors have reported worse treatment outcomes and more frequent distant metastases with lesions over five cm in diameter. Leiomyosarcoma occurred during pregnancy and steroid receptors for estrogens and progesterone were found in the tumour tissue and that is why its etiology was linked to hormone therapy [12, 24, 27]. In this study, only one patient used oral contraceptives.

To date, nine cases of epithelioid sarcoma have been reported in the international literature. The tumour presents as a painful abscess or ulceration with central necrosis or, at times, as multinodular ulceration. In eight of the nine patients, recurrence was observed after the excision of the primary lesion and in five distant metastases [12, 26, 27]. All types of sarcomas reported so far required radical vulvectomy combined with dissection of the inguinal and femoral nodes in patients with metastases to the lymph nodes. Prog-
nosis continues to be poor with recurrence in 77% of the patients and distant metastases in 55% [12, 31-33].

All of the patients, in whom malignancy was diagnosed in the excision line of the primary surgery underwent another surgery (Patients 2, 6 and 8) or adjuvant radiotherapy (Patients 7 and 9). One (Patient 2) died despite the treatment and the other patients survived for 16 months to 11 years.

Involvement of the lymph nodes is an important predictive factor in malignant tumours of Bartholin gland. Cases of contralateral lymph node involvement have been reported [3, 34]. According to many authors, metastases to lymph nodes are found in most patients (55%) [7]. In a series of 11 cases of Bartholin gland carcinoma, nine patients had bilateral metastases to the lymph nodes. The author advises bilateral dissection of the lymph nodes [30, 35]. However, there is no consensus concerning dissection of lymph nodes, especially in cases of early-diagnosed lesions. Monika Hampel, who analysed metastases of vulvar carcinoma to the sentinel nodes, found that bilateral involvement was associated with the location of the primary lesion on the vulva as midline tumours spread bilaterally. Considering the position of Bartholin’s gland, its malignant tumour is likely to spread bilaterally, especially as the vulvar region is characterized by a considerable anatomical variety in the course of the lymphatic channels [36, 37].

Based on the reports in the literature, the best treatment outcomes with the highest rates of recurrence-free survival in all types of Bartholin gland malignancies are observed with radical excision of the tumour (in cases of sarcoma the margin must be at least two cm) and bilateral dissection of the lymph nodes, followed by radio and chemotherapy. Chemotherapy is proposed as the treatment for metastases in cases of adenocarcinoma and sarcoma. Hormone therapy is justified solely when estrogen and progesterone receptors are found in the tumour tissue. Vulvar-conserving surgery may be advised in early stages of the disease only.

Reported survival rates in different types of Bartholin carcinomas are fairly satisfactory and in none of the groups death was reported within two years after primary treatment. Prognosis was adversely affected by recurrence or metasta
sis [38-40].

Proposed management of patients with malignant tumours of Bartholin’s gland:

1. Prompt microscopic evaluation of the affected Bartholin gland which enlarges in size, even in cases of diffuse edema in the area spreading towards the anal canal, without pain or ulceration;
2. Radical surgery of tumours of Bartholin’s gland with bilateral dissection of the lymph nodes;
3. When microscopic examination of the removed tumour tissue finds cancer cells in blood vessels, positive margins or metastases to the lymph nodes, radio and chemotherapy must be instituted and high-dose-rate brachytherapy considered;
4. The patient must be carefully monitored beyond the standard five years, including lymph node status;
5. Recurrence must be treated surgically or with brachytherapy. Distant metastases should be treated by chemotherapy when Hypertrehalosemic hormone (HTH) receptors are positive.

The proposed management based on the latest scientific and clinical knowledge offers a chance of cure for patients with Bartholin gland carcinoma.

**Acknowledgements**

The authors thank Professor Beata Śpiewankiewicz, head of the Department of Gynecology Oncology at the Oncology Centre in Warsaw, for his kind advice and suggestions for creating the article.

The authors also express their gratitude to the Archive workers, for their consistent and persistent collection of current data regarding the patients.

**References**

Rare ovarian tumors: a single center experience of 15 years

T. Gungor¹, S.O. Altinkaya², E. Baser¹, I. Guler¹, O. Uzunlar¹
¹Department of Gynecologic Oncology, Zekai Tahir Burak Women’s Health Education and Research Hospital, Ankara
²Department of Gynecology & Obstetrics, Adnan Menderes University Faculty of Medicine, Aydin (Turkey)

Summary
Objective: The present study aims to review cases of extremely rare primary ovarian tumors and thus, to evaluate the distribution of rare primary ovarian pathologies. Various aspects of the presentation, diagnosis, and treatment of these tumors are discussed. Materials and Methods: A retrospective review of women with final pathology of rare primary ovarian tumors, which were managed at the Gynecologic Oncology Department of Zekai Tahir Burak Women’s Health Education and Research Hospital, from 1995 to 2010 was undertaken. Results: Among the 2,210 women treated during the study period, extremely rare ovarian tumors accounted for 1.62% (36/2210) of all cases. Conclusion: It is important to be aware of these rare entities in the pathological diagnosis of ovarian tumors. Intraoperative frozen examination should be performed because rare benign conditions that mimic malignancy may not require radical surgery. The rarity of these tumors renders basic scientific advances more challenging.

Key words: Ovary; Tumor; Rare.

Introduction
Ovarian malignant disease accounts for four percent of all cancers in women and is still the leading cause of death from gynecologic malignancies [1]. Although serum Ca-125 levels, pelvic examination, and transvaginal ultrasonography are being used in the diagnosis of ovarian cancer, none of these methods has adequate sensitivity or specificity. Unfortunately, current screening methods for the detection of early stage ovarian cancer are inadequate [2].

Ovarian cancer is one of the most challenging diseases facing gynecologist oncologists. Surgery remains the cornerstone in the management of ovarian cancer. For a patient suspected of having ovarian cancer, primary surgery accomplishes firstly confirmation of the diagnosis of ovarian tumor type. Tumor should be classified histologically according to the World Health Organization (WHO) classification and nomenclature of ovarian tumors. The three common cell types are the epithelial, sex-cord stromal, and germ cell tumors. In addition, gonadoblastoma, mesothelial, and other uncommon ovarian tumors and secondary (metastatic) tumors are the other three groups in WHO histological classification of ovarian tumors [3]. Non-epithelial ovarian tumors represent approximately only five percent of all primary malignant tumors of the ovary [4].

Reports of rare primary ovarian tumors such as premenarchial borderline tumor [5], gynandroblastoma [6], pure Sertoli cell tumor [7-9], Sertoli-Leydig cell tumor [10-11], lipid (steroid) cell tumors (stromal luteoma, Leydig cell tumor, steroid cell tumor NOS) [12-19], sclerosing stromal tumor [20-22], malignant struma ovarii [23-26], non-gestational choriocarcinoma [27-32], primary ovarian leiomyoma [33], leiomyosarcoma [34-35], adenomyoma [36-37], lipoma [38], lipoleiomyoma [39-40], hemangioma [41-43], angiosarcoma [44-46], paraganglioma [47], small cell carcinoma [48-50], ovarian carcinoid [51-52], malignant Brenner tumor [53-54], and ovarian carcinomas [55] in the literature have been primarily limited to case series. Many series evaluated using current diagnostic tools for women with these rare tumors have yielded inconsistent results. The aim of the present study was to review cases of rare ovarian tumors with regard to clinical, laboratory, and diagnostic features and thus, to evaluate the distribution of rare primary ovarian pathologies.

Materials and Methods
The present retrospective study was approved by Institutional Review Board of Zekai Tahir Burak Women’s Health Education and Research Hospital. From 1995 to 2010, all patients who were suspected for ovarian cancer preoperatively and managed at the study center were retrospectively reviewed. Intraoperative frozen investigation was performed in all patients.

During the study period, 113 women (5.11%) with pelvic mass representing as ovarian malignancy but having several extra-ovarian diseases confirmed by final histopathological reports, from a total of 2,210 [56], of which 1,981 women (89.63%) were found to have malignant ovarian tumors, 116 (5.24%) women were diagnosed other benign adnexal masses including fibromatocystic group, and 1.62% (36/2210) of cases were diagnosed with rare (benign or malignant) ovarian tumors. Data were obtained from patients’ files and pathology reports.

Results
Among the 2,210 women treated during the study period, extremely rare ovarian tumors accounted for 1.62% (36/2210) of all cases. A total of 36 women in the present study were diagnosed with rare ovarian tumors. The patients ranged from 11 to 71 years of age (mean 40.97). Dis-
distribution of the primary rare ovarian pathology and baseline characteristics of patients are shown in Table 1.

Discussion

In the present retrospective study, cases of extremely rare primary ovarian tumors with regard to clinical, laboratory and diagnostic features were reviewed and thus, the distribution of primary rare ovarian pathology was evaluated.

Premenarchial borderline tumor

Unlike in adult women, ovarian epithelial tumors are uncommon in young girls and extremely rare prior to menarche. In girls under 20 years of age, epithelial ovarian tumors were found in 19.3%, with a malignancy rate of 15.9% [5]. In the case presented here the tumor was outlined by endocervical type epithelium, therefore it should be regarded as of Mullerian origin. Since experience with epithelial ovarian cancer in premenarchial girls is so limited, no definite conclusions regarding treatment of this case can be drawn. As the frozen investigation reported borderline mucinous tumor, cytoreductive staging surgery was done.

In adults with Stage 1A lesions, fertility sparing surgery with unilateral salpingo-oophorectomy and staging surgery was associated with a recurrence rate of 15% in serious lesions, however no recurrences were observed in Stage 1A mucinous tumors. Adjuvant therapy in Stage 1A and 1B lesions seems unjustified [5]. The girl remained clinically free of disease at seven years follow up.

Gynandroblastoma

Gynandroblastoma is an extremely rare subtype of sex cord stromal ovarian tumors. Not more than 40 cases have been reported in the literature up to now [6]. Morphologically it contains both ovarian (granulosa-teca cell) and testicular (Sertoli or Sertoli-Leydig cell) differentiation and may be functional. The patient presented in the present report had both androgenic and estrogenic symptoms such as hirsutism, postmenopausal bleeding, and endometrial hyperplasia. In frozen examination pathologists could not give a definite diagnosis as benign or malignant so that, lymphadenectomy was added in the surgery. Final pathology report revealed no malignant evidence. The surgical treatment is salpingo-oophorectomy and lymphadenectomy if malignancy is suspected. Although gynandroblastoma is very rare, it should be kept in mind in the differential diagnosis of ovarian tumors especially if the patient suffers from androgenic and/or estrogenic symptoms.

Pure Sertoli cell tumor

Pure Sertoli cell tumors of the ovary are rare, accounting for approximately 0.2% of all ovarian tumors and four percent of Sertoli stromal tumors. Pure Sertoli cell tumors are extremely rare, lack the Leydig component, and do not contain the immature neoplastic stroma found in the Sertoli-Leydig tumors [7-9]. Most cases are hormonally active [8]. However the case presented here had no estrogenic or androgenic symptoms. She complained of abdominal swelling and pain and Ca-125 level was elevated to 487 U/ml. Fertility sparing cytotereductive surgery was performed according to the frozen report. Final pathology revealed pure Sertoli cell tumor on the basis of histological findings. As tumor was in Stage 1A, adjuvant therapy was not given. No evidence of disease was found at five years follow-up. A Sertoli cell tumor is a rare occurrence; however it should be considered in the differential diagnosis for women with an adnexal mass.

Sertoli-Leydig tumor

Sertoli-Leydig cell tumors are gonadal tumors of the sex cord stromal type in which the histopathology recapitulates the cells of the testis at various stages of development. They account for less than one percent of ovarian tumors, occurring most commonly in young adults. A majority of the patients present with clinical features of virilization due to excessive secretion of testosterone by the tumor and more rarely estrogenization. However 50% of patients may have no endocrine symptoms [10-11]. Here the authors report two cases of Sertoli-Leydig cell tumor. Both of the patients were young and had androgenic complaints. On the basis of histology and immunohistochemistry, a diagnosis of Sertoli-Leydig cell tumor was given. Sertoli cells were positive for cytokeratin and negative for epithelial membrane antigen and carcinoembryonic antigen. All the lymph nodes received were reactive without metastasis. As the tumor Stage was 1A in both patients, fertility sparing approach without adjuvant therapy was chosen in both cases. Age of the patient, stage of the disease, and degree of tumor differentiation based on morphology are the most important factors to consider the management.

Lipid (Steroid) cell tumor

Ovarian steroid cell tumors are characterized by cells with abundant intracellular lipids that are similar to adrenocortical cells. They account for 0.1% - 0.2% of all ovarian tumors and the majority of them show virilization. Microscopically, steroid cell tumors are composed of large, round cells resembling Leydig cells or adrenal cortical cells. Mitosis of the cells, tumor diameter > 7 cm, necrosis, hemorrhage, and a high grade of nuclear atypia in the tumors are associated with increasing the chance of malignancy. There are three subtypes: stromal luteoma, Leydig cell tumor, and not otherwise specified (NOS). If crystals of Reinke are not identified in cytoplasm, the tumor is called a steroid cell tumor, NOS. Steroid cell tumor, NOS accounts for approximately 60% of steroid cell tumors, 25% to 45% of which are clinically malignant. Fifty percent of cases with the cases with this subtype are associated with androgenic symptoms. The majority of steroid cell tumors have a benign or low grade behavior. Interestingly,
Table 1 – Distribution of the primary rare ovarian pathology and baseline characteristics of patients.

<table>
<thead>
<tr>
<th>Case</th>
<th>Primary Ovarian Tumor</th>
<th>Age</th>
<th>Symptoms</th>
<th>Clinical/imaging findings</th>
<th>Ca-125</th>
<th>Stage</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Premenarchial borderline mucinous tumor</td>
<td>11</td>
<td>Abdominal swelling and discomfort</td>
<td>12 cm solid/cystic abdominopelvic mass</td>
<td>98</td>
<td>1A</td>
<td>Right USO, cytotherapeutic surgery</td>
</tr>
<tr>
<td>2</td>
<td>Gynandroblastoma</td>
<td>52</td>
<td>Hirsutism, postmenopausal bleeding, endometrial hyperplasia</td>
<td>Left adnexal solid mass 6 cm in diameter</td>
<td>13</td>
<td>1A</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA</td>
</tr>
<tr>
<td>3</td>
<td>Pure Sertoli</td>
<td>28</td>
<td>Abdominal swelling and pain</td>
<td>Right adnexal mass 9 cm in diameter</td>
<td>487</td>
<td>1A</td>
<td>Right USO, cytotherapeutic surgery</td>
</tr>
<tr>
<td>4</td>
<td>Sertoli-Leydig</td>
<td>17</td>
<td>Abdominal swelling, hirsutism</td>
<td>10 cm solid-cystic mass arising from left adnexa</td>
<td>45</td>
<td>1A</td>
<td>Left USO, ipsilateral LA, appendectomy, omentectomy</td>
</tr>
<tr>
<td>5</td>
<td>Sertoli-Leydig</td>
<td>19</td>
<td>Abdominal distension, hirsutism, irregular menses</td>
<td>12 cm solid-cystic mass arising from right adnexa</td>
<td>58</td>
<td>1A</td>
<td>Right USO, ipsilateral LA, appendectomy, omentectomy</td>
</tr>
<tr>
<td>6</td>
<td>Steroid cell tumor, NOS (lipid cell tumor)</td>
<td>24</td>
<td>Hirsutism, irregular menses</td>
<td>4 cm solid left adnexal mass</td>
<td>26</td>
<td>benign</td>
<td>Left USO</td>
</tr>
<tr>
<td>7</td>
<td>Stromal luteoma (lipid cell tumor)</td>
<td>58</td>
<td>Postmenopausal bleeding, endometrial hyperplasia</td>
<td>3 cm right ovarian solid mass, 8 mm endometrial thickness</td>
<td>31</td>
<td>benign</td>
<td>TAH+BSO</td>
</tr>
<tr>
<td>8</td>
<td>Leydig cell tumor (lipid cell tumor)</td>
<td>18</td>
<td>Amenorrhea, virilism</td>
<td>6 cm left ovarian solid mass</td>
<td>48</td>
<td>benign</td>
<td>Left USO</td>
</tr>
<tr>
<td>9</td>
<td>Sclerosing stromal tumor</td>
<td>38</td>
<td>Menorrhagia</td>
<td>7 cm right solid-cystic ovarian mass</td>
<td>21</td>
<td>benign</td>
<td>Tumoral excision</td>
</tr>
<tr>
<td>10</td>
<td>Sclerosing stromal tumor</td>
<td>28</td>
<td>Irregular menses</td>
<td>8 cm left solid-cystic ovarian mass</td>
<td>18</td>
<td>benign</td>
<td>Tumoral excision</td>
</tr>
<tr>
<td>11</td>
<td>Sclerosing stromal tumor</td>
<td>28</td>
<td>Pelvic pain</td>
<td>11 cm right solid-cystic ovarian mass</td>
<td>10</td>
<td>benign</td>
<td>Right USO</td>
</tr>
<tr>
<td>12</td>
<td>Sclerosing stromal tumor</td>
<td>27</td>
<td>Pelvic pain</td>
<td>12 cm right solid ovarian mass</td>
<td>72</td>
<td>benign</td>
<td>Right USO</td>
</tr>
<tr>
<td>13</td>
<td>Sclerosing stromal tumor</td>
<td>30</td>
<td>Asymptomatic mass</td>
<td>8 cm right solid-cystic ovarian mass</td>
<td>12</td>
<td>benign</td>
<td>Left USO</td>
</tr>
<tr>
<td>14</td>
<td>Malignant struma ovarii</td>
<td>53</td>
<td>Pelvic pain</td>
<td>6 cm left ovarian mass</td>
<td>16</td>
<td>1A</td>
<td>TAH + BSO, total thyroidectomy after final diagnosis</td>
</tr>
<tr>
<td>15</td>
<td>Malignant struma ovarii</td>
<td>41</td>
<td>Pelvic pain</td>
<td>13 cm right ovarian mass</td>
<td>186</td>
<td>1A</td>
<td>TAH + right USO, staging surgery after final diagnosis, total thyroidectomy offered</td>
</tr>
<tr>
<td>16</td>
<td>Malignant struma ovarii</td>
<td>47</td>
<td>Pelvic pain</td>
<td>10 cm left ovarian mass</td>
<td>28</td>
<td>1A</td>
<td>TAH+BSO, total thyroidectomy after final diagnosis</td>
</tr>
<tr>
<td>17</td>
<td>Choriocarcinoma</td>
<td>21</td>
<td>Abnormal uterine bleeding, elevated βHCG level</td>
<td>9 cm solid left adnexal mass</td>
<td>168</td>
<td>4B</td>
<td>(pulmonary metastasis) Left USO, cytotherapeutic surgery, BPPLA+chemotherapy</td>
</tr>
<tr>
<td>18</td>
<td>Ovarian leiomyoma</td>
<td>33</td>
<td>pelvic pain</td>
<td>8 cm solid right adnexal mass</td>
<td>25</td>
<td>benign</td>
<td>Right USO</td>
</tr>
<tr>
<td>19</td>
<td>Ovarian leiomyoma</td>
<td>38</td>
<td>asymptomatic</td>
<td>6 cm solid left adnexal mass</td>
<td>36</td>
<td>benign</td>
<td>Left USO</td>
</tr>
<tr>
<td>20</td>
<td>Ovarian leiomyosarcoma</td>
<td>52</td>
<td>pelvic pain</td>
<td>8 cm solid right adnexal mass</td>
<td>139</td>
<td>1A</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA+chemotherapy</td>
</tr>
<tr>
<td>21</td>
<td>Ovarian leiomyosarcoma</td>
<td>65</td>
<td>Abdominal distension</td>
<td>10 cm left adnexal mass</td>
<td>32</td>
<td>1A</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA+chemotherapy</td>
</tr>
<tr>
<td>22</td>
<td>Ovarian leiomyosarcoma</td>
<td>71</td>
<td>Abdominal distension</td>
<td>12 cm left adnexal mass</td>
<td>188</td>
<td>3C</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA+chemotherapy</td>
</tr>
<tr>
<td>23</td>
<td>Ovarian adenomyoma</td>
<td>44</td>
<td>Incidental diagnosis</td>
<td>6 cm right solid ovarian mass</td>
<td>29</td>
<td>benign</td>
<td>TAH + BSO</td>
</tr>
<tr>
<td>24</td>
<td>Ovarian lipoma</td>
<td>47</td>
<td>Incidental diagnosis</td>
<td>4 cm right solid ovarian mass</td>
<td>31</td>
<td>benign</td>
<td>TAH + BSO</td>
</tr>
<tr>
<td>25</td>
<td>Ovarian lipoleiomyoma</td>
<td>56</td>
<td>Incidental diagnosis</td>
<td>5 cm left solid ovarian mass</td>
<td>18</td>
<td>benign</td>
<td>TAH + BSO</td>
</tr>
<tr>
<td>26</td>
<td>Ovarian lipoleiomyoma</td>
<td>49</td>
<td>Abdominal distension</td>
<td>6 cm left solid ovarian mass</td>
<td>27</td>
<td>benign</td>
<td>TAH + BSO</td>
</tr>
<tr>
<td>27</td>
<td>Ovarian hemangiomia</td>
<td>45</td>
<td>Incidental diagnosis</td>
<td>5 cm left cystic ovarian mass</td>
<td>58</td>
<td>benign</td>
<td>TAH + BSO</td>
</tr>
<tr>
<td>28</td>
<td>Ovarian angiosarcoma</td>
<td>38</td>
<td>Abdominal distension</td>
<td>10 cm solid-cystic right adnexal mass</td>
<td>892</td>
<td>1C</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA+chemotherapy</td>
</tr>
<tr>
<td>29</td>
<td>Ovarian paraganglioma</td>
<td>47</td>
<td>Abdominal distension + hypertension</td>
<td>10 cm left solid ovarian mass, normal adrenal glands and other abdominal organs</td>
<td>1125</td>
<td>4B</td>
<td>(pulmonary metastasis) TAH + BSO, appendectomy, omentectomy, BPPLA, chemotherapy</td>
</tr>
<tr>
<td>30</td>
<td>Small cell carcinoma</td>
<td>26</td>
<td>Incidental diagnosis, constipation, hypercalcemia</td>
<td>8 cm right solid ovarian mass</td>
<td>1218</td>
<td>3C</td>
<td>Right USO, cytotherapeutic surgery, chemotherapy, radiotherapy</td>
</tr>
<tr>
<td>31</td>
<td>Ovarian carcinoid</td>
<td>47</td>
<td>Incidental diagnosis</td>
<td>4 cm right solid ovarian mass</td>
<td>18</td>
<td>1A</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA</td>
</tr>
</tbody>
</table>
32 Ovarian carcinoid 38 Incidental diagnosis 5 cm left solid ovarian mass 29 1A Left USO, appendectomy, omentectomy, BPPLA

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor Type</th>
<th>Presentation</th>
<th>Size</th>
<th>Stage</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>Malignant Brenner tumor</td>
<td>Abdominal pain and swelling</td>
<td>6 cm</td>
<td>solid</td>
<td>TAH + BSO, appendectomy, omentectomy, BPPLA</td>
</tr>
<tr>
<td>34</td>
<td>Ovarian carcinosarcoma</td>
<td>Abdominal distension</td>
<td>9 cm</td>
<td>solid-cystic left adnexal mass</td>
<td>TAH + BSO, cytoreductive surgery, chemotherapy</td>
</tr>
<tr>
<td>35</td>
<td>Ovarian carcinosarcoma</td>
<td>Abdominal pain and swelling, ascites</td>
<td>12 cm</td>
<td>solid-cystic left adnexal mass</td>
<td>TAH + BSO, cytoreductive surgery, chemotherapy</td>
</tr>
<tr>
<td>36</td>
<td>Ovarian carcinosarcoma</td>
<td>Pelvic pain, abdominal swelling, ascites</td>
<td>Bilateral, 9 cm left and 8 cm right adnexal masses</td>
<td>TAH+BSO, cytoreductive surgery, chemotherapy</td>
<td></td>
</tr>
</tbody>
</table>

TAH = total abdominal hysterectomy; BSO = bilateral salpingo-oophorectomy; USO = unilateral salpingo-oophorectomy; LA = lymphadenectomy
BPPLA = bilateral pelvic-para-aortic lymphadenectomy

logically benign tumors can behave in a clinically malignant fashion. They often present as unilateral solid tumors and occasionally as cystic tumors [12-19]. Steroid cell tumors which are composed of luteinized cells, located within ovarian stroma, especially when associated with hyperthecosis ovarii, indicate a stromal luteoma. Crystallloids of Reinke are conspicuously absent [18]. However, Leydig cell tumors include Reinke crystallloids. In the present study, three cases of steroid cell tumors are reported, one in each subtype. All of the cases were hormonally active and were benign. CT scan of the abdomen detected no adrenal gland enlargement or tumor. There was no significant necrosis, mitotic activity, or high grade nuclear atypia, consistent with a benign steroid cell tumor. Immunostaining was positive for inhibin and vimentin and negative for cytokeratin providing evidence in favor of a steroid cell tumor. Therapy should be individualized based on tumor histology, surgical staging, and the desire for future childbearing. Malignant tumors should be managed with surgical removal followed by combination chemotherapy.

Sclerosing stromal tumor

The vast majority of tumors in the thecoma-fibroma group are readily subclassified based on relatively distinct clinical and histologic characteristics. The major subcategories include thecoma, fibroma-fibrosarcoma, and sclerosing stromal tumor. Accounting for less than five percent of sex cord-stromal tumors, this relatively rare tumor characteristically differentiates itself histologically and clinically from both thecomas and fibromas. Histologically the presence of lobulation of cellular areas separated by edematous connective tissue, increased vascularity, and prominent areas of sclerosis are distinguishing features. Appearance of solid and very vascular features giving the impression of malignant tumors. They tend to occur in the second and third decades of life whereas other types of stromal tumors are most common in the fifth and sixth decades. To date, all sclerosing stromal tumors have been clinically benign [20-22]. Five cases of sclerosing stromal tumor, diagnosed in the present hospital were reported. The results of immunohistochemical stainings were positive for vimentin and smooth muscle actin. Sclerosing stromal tumor should be considered in young women with menstrual irregularity who have hypervascular solid and cystic adnexal masses. Though the tumor appears malignant, since it occurs in young women, care should be taken before embarking on radical surgery.

Malignant struma ovarii

Germ cell tumors account for 15% to 20% of all ovarian tumors and the majority of them are mature cystic teratomas. These tumors are composed of epithelial tissue and can include hair, skin, teeth, bone, and thyroid tissue. Fifteen percent of all teratomas contain thyroid tissue. Struma ovarii is diagnosed when the thyroid tissue is the predominant element (> 50%) and is usually a benign condition. Struma ovarii accounts for only two percent of all mature cystic teratomas and ~5% of struma ovarii show malignant transformation. Due to its rarity there has been controversy about the diagnosis and treatment [23-26]. In the present case series, all of the three patients complained about pelvic pain, one of them had elevated Ca-125 level. As the intraoperative frozen examination indicated a mature cystic teratoma with benign thyroid tissue in all three subjects, conservative surgery was performed according to the age of the cases. However, final pathology revealed a malignant struma ovarii with a focus of papillary thyroid cancer. One patient underwent staging surgery after final diagnosis. Immunohistochemically, the tumors were stained with thyroglobulin confirming the thyroid nature of the neoplasm, but there was no staining for chromogranin and calcitonin. To exclude the metastasis of thyroid carcinoma to the ovary, the absence of a primary tumor in the thyroid must be determined. After the review of the literature, patients were offered to have total thyroidectomy, two of them agreed, nodular hyperplasia of benign character in the thyroid gland was diagnosed. After thyroidectomy, I ¹³¹ thyroid scan was performed and no residual intra-abdominal disease was detected. As a result, it is difficult to conclude about the universal treatment and follow-up of patients with malignant struma ovarii due to its rarity. More data are needed to determine the management protocols and prognosis.
Non-gestational choriocarcinoma

Most cases of choriocarcinoma occur in uterine body and stem from chorionic villi following a normal or abnormal gestation. Most instances of choriocarcinoma of the ovary are gestational in origin. In contrast, non-gestational choriocarcinoma of the ovary is an extremely rare primary germ cell neoplasm that has a worse prognosis than gestational neoplasms, and most patients show metastasis to organ parenchyma at the time of the diagnosis. Non-gestational choriocarcinoma accounts for 0.6% or less of all ovarian neoplasms [29]. It is difficult to diagnose ovarian choriocarcinoma as gestational or non-gestational except in patients who are sexually immature, unable to conceive, or who have never had sexual intercourse. Histologically, non-gestational pure choriocarcinoma of the ovary presents the same appearance as the gestational choriocarcinoma metastatic to the ovaries. DNA analysis is a reliable method for distinguishing gestational tumors and non-gestational tumors [27-32]. The case reported here was a virgin girl who has never had sexual intercourse, with an intact hymen. Therefore the authors thought that the case was a non-gestational choriocarcinoma. Serum beta human chorionic gonadotropin (hCG) level was elevated to 12584 mIU/ml. Fertility sparing cytoreductive surgery was performed including lymphadenectomy. The surgical Stage was found to be 4B, because pulmonary metastases were detected in computed tomography. Four courses of chemotherapy (BEP: bleomycin, etoposide, cisplatin) were completed. Metastatic nodules decreased and beta hCG level was decreased to normal range. Follow up was available for two years, no signs of recurrence was reported. Although it is extremely rare, a high index of suspicion should be maintained and an estimation of serum beta hCG plays a key role in supporting the diagnosis.

Ovarian leiomyoma

Ovarian leiomyoma is a rare benign tumor that accounts for 0.5% to 1% of all benign ovarian tumors. It probably arises from smooth muscle cells in the ovarian hilar blood vessels but there are other possible origins including cells in the ovarian ligament, smooth muscle cells or multipotential cells in the ovarian stroma, undifferentiated germ cells, or cortical smooth muscle metaplasia [33]. Two cases of unilateral ovarian leiomyoma are reported here. They were treated with unilateral salpingo-oophorectomy. The tumor cells showed positive staining with desmin and smooth muscle actin, and negative staining with calretinin. Despite its rarity, ovarian leiomyoma should be considered in the differential diagnosis of ovarian spindle cell tumors. Appropriate diagnosis may require extensive tumor sampling and additional immunohistochemical analyses.

Ovarian leiomyosarcoma

Primary ovarian leiomyosarcoma constitutes a malignant subgroup of ovarian smooth muscle tumors which comprises only one percent of all ovarian tumors and < 0.1% of all ovarian malignancies [34-35]. Malignant behavior is almost always associated with any two of coagulative necrosis, cellular atypia, and mitotic index greater than 10. Less than 100 cases have been reported up to date. So that their origin, etiology, histologic features, clinical behavior, and optimal treatment are still obscure. Three patients are reported here with this rare ovarian malignancy. Immunohistochemical staining of the tumor cells was positive for smooth muscle actin and vimentin. Two of them died of disease in two years follow up. Precise decision or recommendations regarding treatment is difficult due to the limited number of reported cases and short follow-up period. The benefit of adjuvant therapy, both in early and late stages, is doubtful, and no adjuvant treatment modality has been shown to be superior. Stage 1 disease at the time of diagnosis seems to be an important prognostic factor [34].

Ovarian adenomyoma

Adenomyoma is an extremely rare, benign biphasic tumor of the uterus composed of smooth muscles and non-neoplastic endometrium. Extrauterin adenomyomas are very rare. Primary ovarian adenomyoma is an extremely rare tumor with less than ten cases reported in English literature [36-37]. Because of the very low prevalence of the tumor, the data regarding its clinical features and presentation is very limited. Herein the authors presented a case of unilateral ovarian adenomyoma in a 44-year-old woman who underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy with the preoperative diagnosis of a right solid adnexal mass defined in her routine gynecologic examination. Intraoperative frozen inspection revealed a benign ovarian tumor consisting of hyaline fibers. Although the tumor was firm and solid, it contained multiple cystic areas. Microscopically, the tumor was composed of smooth muscle bundles predominantly, and glands showing dispersed settlement lining endometrial epithelium without atypia, mitotic activity, and necrosis. No endometrial stroma was detected.

Ovarian lipoma

Lipomatous tumors of ovarian origin are rare and may arise from overgrowth of metaplastic fat cells in the ovarian stroma [38]. A 47-year-old woman with this extremely rare tumor is reported here, who was incidentally diagnosed with a solid adnexal mass at the time of ultrasonography for urolithiasis. The ovarian tumor was composed almost of benign adipocytes. The supplements of skin such as sweat glands were present in patches. No squamous epithelial cells, other skin structures, or other teratomatous elements were identified. This predominantly lipomatous ovarian tumor is thought to be representing a fat-rich solid teratoma.
**Ovarian lipoleiomyoma**

Lipoleiomyoma is an extremely rare benign tumor of the ovary. The tumor is identified by the presence of the mature adipose tissue intimately admixed with smooth muscle cells. It may mimic sarcoma clinically. Less than ten cases are reported up to date [39-40]. Two postmenopausal women are reported with this rare tumor in the present case series. One was diagnosed incidentally in her routine examination while the other one complained about abdominal distention. Tumor markers were in normal ranges. Microscopic features are similar to leiomyoma, but in addition lipoleiomyoma have mature adipose tissue. Immunohistochemically desmin showed diffuse staining. The theories on histogenesis of adipose tissue are unresolved even in the case of uterus where these tumors are relatively more common. The presence of adipose tissue is thought to represent metaplasia in both uterus and the ovary [40].

**Ovarian hemangioma**

Hemangiomas are benign vascular tumors that are rarely found in the ovaries. There are fewer than 50 reported cases in the English literature. Hemangiomas are benign lesions arising from a failure in vascular formation, forming abnormal vascular channels and are of two types: cavernous and capillary. Capillary hemangiomas are made up of vessels of the caliber of normal capillaries, while the cavernous type consists of larger channels. Unlike the rest of the body where capillary hemangiomas are more common, the ovaries usually develop cavernous hemangiomas [41-43]. In the present study, a case of ovarian hemangiomia diagnosed incidentally at the time of examining a patient for abnormal uterine bleeding. Uterine leiomyoma and a left ovarian mass was found in imaging studies. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed as the frozen investigation of the ovarian mass revealed a benign tumor. Microscopic examination revealed a hemangiomia, consisting of multiple thin-walled blood vessels. Mitotic activity, necrosis, and atypical cells were not seen. Although often an incidental finding at surgery, these lesions may rarely be associated with systemic manifestations. There are also reported cases with adnexal mass, elevated Ca-125 level, massive ascites, and even pseudo-Meigs syndrome in the literature [42-43].

**Ovarian angiosarcoma**

Primary ovarian angiosarcomas are an extremely rare malignancy. There are less than 35 reported cases in literature. Nearly six of these cases were reported as arising in mature cystic teratoma [44-46]. The current patient presented here, who complained of abdominal distention as the presenting symptom, underwent explorative surgery with frozen investigation. As the frozen examination revealed malignant spindle cell tumor, a full staging surgery was performed. Final pathology showed Stage 1C ovarian angiosarcoma with positive immunostaining with CD31 and vimentin. The origin of the angiosarcoma appears to occur from the rich ovarian vasculature. Most patients present with metastatic disease. Because of the rarity of this tumor, optimal management has not been defined. Surgery and radiotherapy have been the traditional treatment modalities. The precise role of adjuvant chemotherapy remains undefined [45]. However, given the extremely poor prognosis it is reasonable to prescribe chemotherapy regimen in an attempt to reduce the risk of progression and recurrence. Detection of Stage 1 disease appears to confer a better prognosis regardless of the utilization of adjuvant chemotherapy [46].

**Ovarian paraganglioma**

Paraganglioma is one of the rarest neoplasms to involve the ovary, whether primary or metastatic. Less than ten cases have been reported up to date [47]. In the present case series, a 47-year-old patient is described with complaints of hypertension and abdominal distention. As the imaging studies showed left ovarian mass with an elevated Ca-125 level, explorative laparotomy was performed. At laparotomy there was no evidence of extraovarian disease. Staging surgery was performed including lymphadenectomy due to enlarged lymph nodes and frozen investigation report. Final histologic diagnosis was paraganglioma with diffusely positive immunostaining with neuroendocrine markers and negative staining with cytokeratin. Tumor cells showed a nested arrangement resulting in a “zellballen” pattern with highly vascular stroma. Subsequent radiologic investigations showed several metastatic pulmonary nodules, but no tumor was seen in either adrenal gland or other organs. The tumor was accepted to be Stage 4. As a result, paraganglioma is an extremely rare primary ovarian neoplasm and should be considered in the differential diagnosis of any tumor with a predominantly a nested growth pattern. Possible theories of histogenesis of primary ovarian paraganglioma include an origin from extra-adrenal paraganglia in the region of the ovary or differentiation within a teratoma [47].

**Ovarian small cell carcinoma**

Small cell carcinoma of the ovary, hypercalcemic type is a rare and aggressive malignancy found in reproductive age women. Large cell variant is less common [48-50]. Here, a case of 26-year-old woman who attended to internal medicine clinic with complaints of constipation and fatigue, was diagnosed with a pelvic mass and was referred to the gynecology clinic. At laparotomy, as the frozen section revealed a malignant tumor, right unilateral salpingo-oophorectomy with staging surgery was performed. Immunohistochemistry showed strong positivity for vimentin, and negative for antibodies for keratins. The tumor Stage was 3C. Subsequent imaging studies showed no tumor in other organs. Although the patient received neoadjuvant chemotherapy and radiotherapy, she died of disease 22 months from primary diagnosis. Small cell carcinoma
of the ovary, hypercalcemic type, is a very aggressive type of ovarian malignancy with a poor prognosis. Serum calcium levels should be evaluated as soon as the diagnosis is suspected or confirmed.

**Ovarian carcinosarcoma**

Carcinosarcoma of the ovary, also referred to as mixed Mullerian tumor of the ovary, is a rare and aggressive tumor. These tumors contain both malignant epithelial and sarcomatous elements. The epithelial component is often serous, endometrioid, or undifferentiated adenocarcinoma but may also be clear cell adenocarcinoma or squamous cell carcinoma. The sarcomatous element may be homologous tissue native to the ovary (endometrial stromal sarcoma, leiomyosarcoma, fibrosarcoma), or heterologous tissue not native to the ovary (rhabdomyosarcoma, chondrosarcoma, osteosarcoma) [55]. In the present case series, three patients with primary carcinosarcoma of the ovary are presented. They presented with symptoms similar to advanced ovarian cancer with abdominal symptoms and elevated Ca-125 levels. Two of them were diagnosed at Stage 3C, and the other who had both ovaries involved was diagnosed at Stage 4B. They died of disease in nearly two years follow up. Tumors showed malignant glandular elements and heterologous sarcomatous elements. Sarcomatous components showed positive staining with periodic acid Schiff. Due to the rarity of this tumor, optimal treatment for carcinosarcoma of the ovary has not been determined. Optimal surgical cytoreduction followed by platinum based chemotherapy appears to be the best treatment strategy. Patients present with symptoms similar to women with epithelial ovarian cancers, but stage for disease, outcomes are worse [55].

**References**


Address reprint requests to:
S.O. ALTINKAYA, M.D.
Department of Gynaecology & Obstetrics,
Adnan Menderes University, Faculty of Medicine
Kurtulus Mahallesi, 2018 Sokak Elib Apt. 39/9
Aydin (Turkey)
e-mail: altinkayaozlem@yahoo.com
Prognostic factors and treatment comparison in small cell neuroendocrine cervical carcinoma


Department of Gynecologic Oncology, The Zhejiang Cancer Hospital, Hangzhou (China)

Summary

Objective: To determine the clinicopathologic factors associated with survival in small cell neuroendocrine cervical cancer (SCNEC) patients. Materials and Methods: The study was approved by the ethics committee of the hospital. The records of 64 SCNEC patients from 9,474 Chinese patients with cervical cancer at the Zhejiang Cancer Hospital were reviewed. Kaplan-Meier and Cox regression methods were used for analyses. Results: Of 64 patients, 47 had Stages I-IIA, 12 had Stages IIB-IVA, and five had Stage IV-B disease. A total of 81.25% underwent surgery, 89.1% received chemotherapy, 62.5% received radiation, 34.4% received neoadjuvant chemotherapy (NACT), and 34.4% received concurrent chemoradiation (CCRT). The median follow-up for surviving patients was 35.7 months (range: 0.5-160), and 29 (50%) of the 58 patients with Stages I-III had either disease recurrence or progression. The median time to first relapse was 10.5 months (range: 0-88.2). The five-year overall survival of patients in Stages I-IIA and IIB-IVB disease was 54.4% and 9.8%, respectively (p = 0.001). Women with early-stage (Stages IB-IIA) disease had median survival rates of 94 months compared with 21.4 months in the advanced-stage (Stages IIB-IVB) group. In univariate analysis, advanced-stage (p = 0.001), without radical surgery (p = 0.002) and deep stromal invasion (DSI) (p = 0.000) were considered poor prognostic factors. In a multivariable analysis, tumor size > four cm (p = 0.048), postoperative radiation (p = 0.038) for early-stage patients and the FIGO stage(p = 0.040) of disease in the overall population remained as independent prognostic factor of survival. Conclusion: The FIGO stage was found to be an independent prognostic factor of SCNEC. In addition, tumor size > four cm and DSI was associated with poor survival. Postoperative radiation for early-stage patients may not improve survival. The role of primary and postoperative NACT or CCRT is unclear. Clinical trials are needed.

Key words: Neuroendocrine carcinoma; Prognosis; Small cell; Uterine cervix.

Introduction

Small cell neuroendocrine carcinoma of the uterine cervix (SCNEC) is a rare gynecologic malignancy that represents less than three percent of all cervical cancer [1-3]. The histology and biologic behaviors of the tumor are similar to that of small cell lung carcinoma (SCLC), which is highly aggressive. The tumor is characterized by a high incidence of early distant metastases, resulting in poorer prognosis than other subtypes of cervical cancer [3-5]. Due to its rarity studies exploring therapeutic efficacy in this setting generally require long enrolment period to obtain a sufficient number of cases. Therefore, to date most studies of neuroendocrine cervical cancer are comprised of a small series and case reports, making it difficult to draw conclusions on prognostic factors and optional treatment modalities.

Given the aggressive nature of neuroendocrine small cell cervical cancer, it is imperative to identify potential treatments that can improve the outcomes of these patients. As such, the authors carried out a retrospective review to determine the clinicopathologic factors associated with survival, patterns of relapse, and potential therapeutic modalities that may improve survival in neuroendocrine cervical cancer patients.

Materials and Methods

The study was approved by the ethics committee of the hospital. Due to the retrospective nature of the study, informed consent was waived. A total of 70 patients with SCNEC were identified from 9,474 Chinese patients with cervical cancer through registry databases at the Zhejiang Cancer Hospital from January 1997 to December 2010. All histopathologic review was carried out by two independent pathologists from the Pathology committee of the Zhejiang Cancer Hospital. Six patients were excluded because follow-up data were incomplete. Thus, the study population consisted of 64 patients.

Of the 64 patients with available paraffin blocks who were diagnosed as having small cell carcinoma on the basis of hematoxylin and eosin (H&E) staining, all had positive staining for one or more neuroendocrine markers. All tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) clinical staging system for cervical cancer based on physical examination, chest X-ray, intravenous paleography, cystoscopy, sigmoidoscopy, and abdomino-pelvic computed tomography (CT) or magnetic resonance imaging (MRI) scan. When there were suspicious findings on chest X-ray or the presence of signs and symptoms upon physical examination, a CT scan of the chest and/or brain was carried out.

For primary treatment, 52 patients underwent radical hysterectomy (RH), 57 patients received chemotherapy, 22 patients had neoadjuvant chemotherapy (NACT), and 19 patients received concurrent chemoradiation (CCRT). Among patients who received adjuvant chemotherapy or NACT, 38 received etoposide together with cisplatin (EP), five received etoposide together with Adriamycin and cisplatin (EAP), four received paclitaxel and cisplatin (TP), and two received bleomycin, vincristine, and cisplatin (BVP). In addition, cyclophosphamide, Adriamycin, and vin-
cristine (CAV), ifosfamide, adriamycin, and cisplatin (IAP), paclitaxel and carboplatin (TC), ifosfamide and etoposide and (IE), and ifosfamide together with etoposide and cisplatin (IEP) was given in one patient. Radiation was delivered using external beam radiation therapy and intracavitary brachytherapy. External-beam therapy was delivered using anterior-posterior fields, box fields, or conformal radiotherapy and ten MV photons. Intracavitary treatment was delivered using Fletcher-suit after loading high-dose-rate applicators.

The clinical and pathological variables analyzed included patient age, tumor size, stage, lymph node involvement (LNI), depth of stromal invasion (DSI), lymph vascular space invasion (LSI), and treatment modalities. The primary end point was any cancer-related death. All end points were calculated from the date of diagnosis to death, or censored at last follow-up. The date of death was obtained from the medical records, personal contact, or the National Registry of Death statistics of the China National Statistical Office.

All statistical analyses were performed using SPSS v.19 software. Survival curves were estimated using the Kaplan-Meier method, and \( p \) values were generated using the log-rank test. Cox regression analysis was used for multivariate analysis of significant variables. All tests were two-tailed with \( p \) values < 0.05 considered significant. All end points were updated in May 2012.

Results

The median age of the 64 patients was 37.5 years (range: 25-85). The mean gravidity of the 64 patients was three times (range: 0-6). Of the 64 patients, 28 had Stage IB1, six had Stage IB2, seven had Stage IIA1, six had Stage IIA2, six had Stage IIB, one had Stage IIIA, four had Stage IIIB, one had Stage IVA, and four had Stage IVB disease. Vaginal bleeding at presentation was noted in 59.4% of patients and 25% of patients had cervical bleeding. In addition, 10.9% of patients had vaginal drainage and 4.7% had other symptoms.

Of the 64 patients with FIGO Stages IB-IVB SCNEC, the estimated three- and five-year overall survival (OS) rates for all patients were 53.1% and 36.5%, respectively (Figure 1). Women with early-stage (Stages IB-IIA) disease had median survival rates of 94 months with 21.4 months in the advanced-stage (Stage IIB-IVB) group (Table 1). The five-year OS rates for all patients in Stages IB1-IIA and IIB-IVB diseases were 54.4% and 9.8%, respectively (\( p = 0.001; \) Figure 2).

The median survival was 35.7 months (range: 0.5-160) for all patients. The five-year survival for all patients who received a RH was 48.8% compared to 16.7% for those who did not undergo a RH (\( p = 0.002 \)). Women who received a RH had median survival rates of 54.4 months compared with 16.5 months for those who did not undergo a RH (Table 1). In univariate contrast, age (\( p = 0.666 \)), tumor size (\( p = 0.558 \)), chemotherapy (\( p = 0.712 \)), radiotherapy (\( p = 0.455 \)), CCRT (\( p = 0.242 \)), NACT (\( p = 0.338 \)), and menopause (\( p = 0.107 \)) were not found to be important prognostic factors. In a multivariate analysis, FIGO stage (HR, 2.83; 95%CI, 1.05-7.51; \( p = 0.040 \)) remained as a significant independent prognostic factor for survival (Table 1).

For 47 patients in FIGO Stages IB-IIA, in univariate contrast, age (\( p = 0.687 \)), menopause (\( p = 0.510 \)), stage (\( p = 0.532 \)), tumor size (\( p = 0.714 \)), primary RH (\( p = 0.132 \)), radiotherapy (\( p = 0.082 \)), chemotherapy (\( p = 0.631 \)), NACT (\( p = 0.109 \)), and CCRT (\( p = 0.778 \)) were not found to be important prognostic factors. Although not statistically significant, patients with Stages IB-IIA who received NACT tended to have a better prognosis, with a five-year survival of 76.9% compared to 46.7% for those who did not undergo NACT (\( p = 0.109 \)). Contrary to the authors’ experience, patients with Stages IB-IIA who received adjuvant radiation tended to show a worse prognosis compared to those who did not receive adjuvant radiation (five-year survival: 46.3% vs. 78%, respectively). To examine the variables identified as important in univariate analyses further, a multivariate analysis was performed. Tumor size > four cm (\( p = 0.048 \)), postoperative radiation (\( p = 0.038 \)) for early-stage patients as significant independent poor prognostic factors for survival in early-stage disease (Table 2).

For some of the patients who received a RH with surgical pathology, LNI, LSI, and DSI were assessed, and DSI (stromal invasion depth of cervix > 2/3) was found to be significantly associated with a worse prognosis compared to those patients without DSI (five-year survival rate: 22.4% vs. 82.5%, respectively \( p < 0.001 \)). Although not statistically significant, LNI and LSI tended to adversely affect survival (Table 3).

Forty (62.5%) of the 64 patients exhibited recurrence or uncontrolled tumor. Twenty-nine (50%) of the 58 patients with Stages I-III had a relapse. The median time to first relapse was 10.5 months (range: 0-88.2). Among 40 patients, 35 patients succumbed to the disease, and four patients were alive with disease. No patient had brain metastasis as the sole site of first recurrence. Among 64 patients, seven (10.9%) patients developed brain metastases; however, seven (100%) patients had brain metastases concurrently or after lung metastases. Among 11 patients with lung metastases, seven (63.6%) patients developed brain metastases.

Among the 64 analyzed patients, 28 patients were in Stages IB1, with 12 and 16 patients with and without recurrence, respectively. Based on the clinical and pathological factors for these two groups of patients, the treatment modality was similar, but the number of DSI, LSI, and LNI occurrences was higher in the recurrence group (Table 4).

Discussion

Based on reports from different hospitals, SCNEC is a rare disease [6]. That is associated with a poor prognosis. The present results found that the estimated three- and five-year survival rates for all patients were 53.1% and 36.5%, respectively. The five-year OS rates of patients with Stages
Table 1. — Demographic and treatment factors with associated five-year OS (n = 64).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median survival (mos)</th>
<th>Five-year OS %</th>
<th>Univariate p</th>
<th>HR (95%CI) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 40</td>
<td>36</td>
<td>54.5</td>
<td>46.2</td>
<td>0.666</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>28</td>
<td>35.7</td>
<td>39.1</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>Yes</td>
<td>15</td>
<td>27.8</td>
<td>0.107</td>
</tr>
<tr>
<td>No</td>
<td>49</td>
<td>54.5</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>IB-IIBA</td>
<td>47</td>
<td>94</td>
<td>0.001</td>
</tr>
<tr>
<td>IIB-IV</td>
<td>17</td>
<td>21.4</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td>≤ 4cm</td>
<td>45</td>
<td>39.7</td>
<td>0.558</td>
</tr>
<tr>
<td>&gt; 4cm</td>
<td>19</td>
<td>28.8</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>Yes</td>
<td>52</td>
<td>54.4</td>
<td>0.002</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>16.5</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>Yes</td>
<td>40</td>
<td>31.3</td>
<td>0.455</td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>54.5</td>
<td>39.5</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Yes</td>
<td>57</td>
<td>35.7</td>
<td>0.712</td>
</tr>
<tr>
<td>No</td>
<td>7</td>
<td>35.8</td>
<td>42.9</td>
<td></td>
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<td>NACT</td>
<td>Yes</td>
<td>22</td>
<td>54.5</td>
<td>0.338</td>
</tr>
<tr>
<td>No</td>
<td>42</td>
<td>34.9</td>
<td>40.2</td>
<td></td>
</tr>
<tr>
<td>CCRT</td>
<td>Yes</td>
<td>19</td>
<td>39.7</td>
<td>0.242</td>
</tr>
<tr>
<td>No</td>
<td>45</td>
<td>31.1</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

NACT: neoadjuvant chemotherapy; RT: radiation; CT: chemotherapy; NART: neoadjuvant radiation; CCRT: concurrent chemoradiation; RH: radical hysterectomy.

Table 2. — Demographic and treatment factors with associated five-year OS for IB1-IIBA (n = 47).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Five-year OS %</th>
<th>Univariate p</th>
<th>HR (95%CI) p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 40</td>
<td>28</td>
<td>60.3</td>
<td>0.687</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>19</td>
<td>46.6</td>
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</tr>
<tr>
<td>Menopause</td>
<td>Yes</td>
<td>39</td>
<td>57.3</td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>38.1</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>IB1</td>
<td>28</td>
<td>62.7</td>
</tr>
<tr>
<td>IB2-IIBA</td>
<td>19</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td>≤ 4cm</td>
<td>37</td>
<td>55.7</td>
</tr>
<tr>
<td>&gt; 4cm</td>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>Yes</td>
<td>44</td>
<td>33.3</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>RT</td>
<td>Yes</td>
<td>32</td>
<td>46.3</td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>Yes</td>
<td>43</td>
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<td>No</td>
<td>4</td>
<td>50</td>
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<tr>
<td>NACT</td>
<td>Yes</td>
<td>13</td>
<td>76.9</td>
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<tr>
<td>No</td>
<td>34</td>
<td>47.1</td>
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<tr>
<td>CCRT</td>
<td>Yes</td>
<td>18</td>
<td>55.6</td>
</tr>
<tr>
<td>No</td>
<td>29</td>
<td>53.4</td>
<td></td>
</tr>
</tbody>
</table>

NACT: neoadjuvant chemotherapy; RT: radiation; CT: chemotherapy; NART: neoadjuvant radiation; CCRT: concurrent chemoradiation; RH: radical hysterectomy.

Table 3. — Pathologies characteristic and associated five-year PFS and OS for postoperative patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Five-year PFS %</th>
<th>Univariate p</th>
<th>Five-year OS %</th>
<th>Univariate p</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNI</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>33.8</td>
<td>0.177</td>
<td>35.9</td>
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<td>No</td>
<td>35</td>
<td>51.1</td>
<td></td>
<td>51.9</td>
</tr>
<tr>
<td>LSI</td>
<td>Yes</td>
<td>25</td>
<td>27.8</td>
<td>0.052</td>
</tr>
<tr>
<td>No</td>
<td>21</td>
<td>56.7</td>
<td></td>
<td>55.6</td>
</tr>
<tr>
<td>DSI</td>
<td>Yes</td>
<td>26</td>
<td>13.9</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>84.4</td>
<td></td>
<td>82.5</td>
</tr>
</tbody>
</table>

LNI: lymph node involvement; LSI: lymphovascular space invasion; DSI: depth of stromal invasion.
I-IIA and IIB-IVB disease were 54.4% and 9.8%, respectively, which were consistent with previous reports (40-50% and 8.0%, respectively) [7-8].

Because SCNEC occurs infrequently, it is difficult to perform a randomized controlled clinical trial to determine optimal therapy. The current study analyzed a large series of patients diagnosed with SCNEC from a single institution experience, which included an update of a previous reported series [9]. The objective was to identify the clinical and pathologic factors that are responsible for survival of women with this aggressive tumor.

Stage, large tumor size, DSI, lymph node metastases, smoking, and a pure histologic type have been found to be possible poor prognostic factors in the literature [3-5, 10-13]. The present data showed that the FIGO stage is independent prognostic factors for all patients. Consistent with other studies [4, 5,12], according to the present data, the recurrence or progression rate increases as the stage of development increased. For patients with Stages I, II and III-IV, the recurrence or progression rate was 44% (15/34), 68% (13/19), and 100% (10/10), respectively, indicating that the FIGO stage was an important prognostic factor for survival.

In early-stage disease, patients with small (< four cm) tumors were found to have better survival rates than those with large (> four cm) tumor in multivariate analysis (p = 0.048). Similarly, Chan et al. Showed that in early-stage disease patients with tumor < two cm had significant better survival rates than patients with > two cm lesions in univariate analysis [5, 13].

Although there are few clinical data supporting the use of adjuvant multimodality treatment in early-stage SCNEC disease, most clinicians favor use of chemotherapy and/or radiation because of the strong evidence supporting CCRT in other subtypes of cervical cancer [4, 5, 10, 11, 14]. In early-stage disease, patients who received adjuvant radiation, however, had a poorer prognosis than those who did not; the five-year estimated survival rate were 46.3% and 78%, respectively, in multivariate analysis (p = 0.038). In the current study, adjuvant radiation did not improve outcome and this finding is consistent with other studies that adjuvant radiation did not alter the course of pelvic recurrence [8, 13]. However the present authors found that 32 patients with Stages IB-IIIA who received radiation had a mean of 1.437 risk factors (LNI, LSI, DSI, or large tumor size), but 15 patients who did not receive radiation had a mean of 0.733 risk factors. This suggests that the gynecologic oncologists at the present hospital tended to select patients with more risk factors for radiation therapy, similar to cervical cancer patients. This may partly explain why patients who had received radiation had a prognosis than patients who had not received radiation. However, the value of radiation in early-stage SCNEC patients will require further evaluation through addition clinical trials [9].

The authors also observed that DSI was a poor prognostic factor. The five-year survival rate for patients without DSI was 82.5% compared to 22.4% for patients with DSI (p < 0.001). These results were consistent with those of a previous study [15]. Although not statistically significant, LNI and LSI tended to adversely affect survival. Due to the small number of patients in the study, it is difficult to gain independent prognostic factors from DSI, LNI, and LSI. However, when the authors compared the clinical and pathological factors of 28 IB1 patients with and without recurrence, the treatment modality was similar, but the number of DSI, LSI, and LNI occurrences was completely different. In the recurrence group, there were nine (75%) patients with DSI, eight (66.7%) patients with LSI, and one (8.3%) patient with LNI, whereas there were three (18.8%) patients with DSI, three (18.8%) patients with LSI, and two (12.5%) patients with LNI in the group without recurrence. These results indicated that patients with more risk factors (DSI, LSI, or LNI) had a higher rate of recurrence and shorter survival time. Therefore, these factors may be prognostic indicators for patients, and a sufficient number of chemotheraphy courses is needed for those patients with these risk factors.

The current data also showed that RH (p = 0.002) is an important prognostic factor in all patients. RH may have been associated with better survival rates because most patients who had received the procedure were early-stage patients. However, for patients with Stages IB-IIIA, RH did not provide an obvious survival advantage, which was consistent with other studies showing that radical surgery was not associated with prolonged survival relative to definitive radiation for patients with SCNEC [3, 16]. Nevertheless, most gynecological oncologists and patients in China still favor RH as the treatment choice.

Table 4. — The clinic and pathologic factor compare for recurrence and no recurrence patients of Stage IB1 (n = 28).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Recurrence</th>
<th>No recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>38.2 (30-57)</td>
<td>41.6 (27-83)</td>
</tr>
<tr>
<td>Treatment modality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH</td>
<td>12 (100%)</td>
<td>15 (93.8%)</td>
</tr>
<tr>
<td>CT</td>
<td>10 (83.3%)</td>
<td>12 (75%)</td>
</tr>
<tr>
<td>RT</td>
<td>12 (100%)</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td>CCRT</td>
<td>5 (41.7%)</td>
<td>7 (43.8%)</td>
</tr>
<tr>
<td>NACT</td>
<td>1 (8.3%)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Period of CT (median)</td>
<td>3 (1-7)</td>
<td>4 (0-8)</td>
</tr>
<tr>
<td>DSI</td>
<td>9 (75%)</td>
<td>3 (18.8%)</td>
</tr>
<tr>
<td>LSI</td>
<td>8 (66.7%)</td>
<td>3 (18.8%)</td>
</tr>
<tr>
<td>LNI</td>
<td>1 (8.3%)</td>
<td>2 (12.5%)</td>
</tr>
</tbody>
</table>

NACT: neoadjuvant chemotherapy; RT: radiation; CT: chemotherapy; CCRT: concurrent chemoradiation. RH: radical hysterectomy; LNI: lymph node involvement; LSI: lymphovascular space invasion; DSI: depth of stromal invasion.
Adjuvant chemotherapy tended to favor survival, although the effect did not attain statistical significance. Many authors have recommended adjuvant chemotherapy due to the aggressive behavior of this disease [10, 11]. A recent study of 188 patients showed that chemotherapy and chemoradiation were independent prognostic factors for improving survival [7]. It is possible that the small sample size of the present study was not sufficient for showing a benefit associated with chemotherapy in the treatment of this aggressive cancer.

NACT has been recommended for patients with tumor size > four cm [5, 17]. However, another previous study that found that patients who received NACT tended to have a worse median OS that those who did not receive NACT [8]. Whether NACT can improve the prognosis for cervical cancer patients remains a matter of debate. The current data did show that the overall five-year survival for patients with early-stage disease who received NACT was higher compared to those who did not receive NACT (univariate: 76.9% vs. 47.1%, respectively). In the current study, NACT was found to have a marginal significance in univariate analysis (p = 0.109) or in multivariate analysis (p = 0.091). With more patients NACT may prove to be an prognostic for survival. The authors hypothesized that different chemotherapies, chemotherapy interval times, and chemotherapy periods may result in different results. Most gynecologic oncologists choose NACT for patients with tumors > four cm, and the use of NACT for patients with early-stage tumors may provide them with the opportunity for radical surgery. The present data showed that for 13 early-stage patients who received NACT, four (30.7%) patients achieved a complete response (CR) after one to two cycles of NACT. These four patients achieved long-term survival without recurrence, with a mean survival time of 77.1 months (range: 33.9-160). Therefore, NACT may be an approach for assessing response to treatment.

CCRT is recommended for small cell carcinoma of the lung, but the benefit of CCRT for SCNEC is unclear. Some studies have shown that chemoradiation is associated with higher survival in SCNEC [7, 17], but other studies have found that chemoradiation does not improve survival compared to adjuvant chemotherapy alone for early-stage patients [8]. Therefore, the value of CCRT for early-stage SCNEC patients will require further assessment through additional clinical trials.

The authors recognize some of the limitations of this study. This was a retrospective analysis of a single institutional experience with a small number of patients. Nevertheless, they hope that their experience contributes to the foundation of knowledge regarding this rare and aggressive tumor. Their data indicate that patients with early-stage tumors, tumor size < four cm, and without DSI or less risk factors (DSI, LSI, or LNI) are associated with improved survival.

References


Address reprint requests to: 
H. YU, M.D. 
Department of Gynecologic Oncology, Zhejiang Cancer Hospital, No 38, Guangji Road, Hangzhou 310022 (China) 
e-mail: ayuhua@126.com
Relationship between exposure to extremely low-frequency electromagnetic fields and breast cancer risk: a meta-analysis

G. Zhao¹, X. Lin², M. Zhou¹, J. Zhao¹

¹Department of Chest Surgery, Cancer Center of Guangzhou Medical University, Guangzhou
²Department of Radiotherapy, Cancer Center of Guangzhou Medical University, Guangzhou (China)

Summary

Objective: To comprehensively analyze the relationship between human exposure to extremely low frequency electromagnetic fields (ELF-EMFs) and breast cancer and to discuss the potential risk of ELF-EMFs to human breast cancer. Materials and Methods: Sixteen research reports of case-control studies which were published from 2000 to 2007 were collected. The fixed effect model (FEM) or the random effect model (REM) was chosen to calculate total ORs depending on the outcomes of the test of homogeneity (Q test): the subgroup was analyzed with the menopause and the non-menopause. Outcome: Sixteen research outcome was OR_DL = 1.10, 95% CI = (1.01, 1.20), the ORMH of the non-menopause status group was 1.25, 95% CI = (1.05, 1.49), the ORMH of the menopause status group was ORMH = 1.04, 95% CI = (0.93, 1.18). Conclusion: The authors found that ELF-EMFs may be increase the risk of human breast cancer. The women’s exposure to ELF-EMFs may be the risk factor of breast cancer when they are non-menopausal.

Key words: ELF-EMFs; Breast cancer; Meta-analysis.

Introduction

Extremely low frequency electromagnetic fields (ELF-EMFs) refer to 0-300 Hz electromagnetic fields generated by power transmission lines, power equipment (power dispatching office, electric lamp, electric wires, etc) or appliances (monitors, televisions, electric blanket, etc). Recent years studies have indicated that ELF-EMFs could give rise to various diseases in human beings, such as disorder of cardiovascular system, brain tumor, leukemia, endocrine disorder, and breast cancer [1]. Breast cancer is one of the serious diseases endangering human health, which occurs with increasing frequency in the recent years. Many factors, such as age of menarche, lactation, oral contraceptive, breast tumor, smoking, drinking, ELF-EMFs, etc, correlating with breast cancer have been found by scholars throughout the world. The outcomes of different researches on breast cancer are not identical because the occurrence of breast cancer is induced by multiple factors and each researcher possesses different information on it [2, 3]. As for ELF-EMFs, at the present time, it also fails to affirmatively have a same conclusion. This dissertation aimed to explore the hazard degree of breast cancer occurrence from exposure to ELF-EMFs, which will make a comprehensive review on domestic and international articles relating to ELF-EMFs and breast cancer occurrence by meta-analysis method. Through this study, it was expected to offer a basis on the study of exposure to ELF-EMFs causing breast cancer and render reasons on the precautionary measures correspondingly taken.

Materials and Methods

Identification and eligibility of studies

The authors include all the studies designed with a case-control study. The target sample size was more than 100 in each group. The research manipulated the main confounding factors, for instance, race, family history, age of menarche, menopause, and use of estrogen after menopause. The subjects of the cases were malignant breast cancer patients. There was no limitation on the pathological classification and clinical stages of the breast cancer. It is also without limitation on ages, races and areas of the subjects studied. The reports were published in English between 2000 and 2010. Measurement index was the related index OR of the risk of breast cancer from exposure to ELF-EMFs.

Paper extraction and analysis methods

Methods of report selection and the basic information

The researchers used Pub Med, CNKI, and Hirewire to search 40 papers creating a study of the relationship between ELF-EMFs and breast cancer. After carefully being read, these 40 papers were graded according to the quality evaluation standards published in 2004 by Oxford called CASP regarding papers applying case-control study (Table 1). There were 16 papers complying with the standards between 13 to 22 points. The papers were then analyzed by Review Manager 5.1.

Results

Basic information of the literature

In total, 40 articles addressing the relationship between ELF-EMFs and breast cancer were collected. There were ten articles compliant with the inclusive criteria. All were published in English from 2001 to 2007. One of the articles made a comparison between premenopause and post-menopause of four groups. There was also an article with such a comparison of two groups. The detailed information of the selected articles is shown in Table 2.
Table 1. — Appraisal checklist (Oxford CASP, 2004) of papers with a use of case-control study

<table>
<thead>
<tr>
<th>Evaluating items</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Does the study address a clearly focused question? Are the subjects selection of the study rationally selected? (study subjects, risk factors, potential benefits, and harm)</td>
<td></td>
</tr>
<tr>
<td>2. Are the ways to answer the studying questions appropriate?</td>
<td></td>
</tr>
<tr>
<td>3. Are the groups appropriately selected? (representativeness, span, sample size, power of test)</td>
<td></td>
</tr>
<tr>
<td>4. Are the ways to choose the control groups appropriate? (response rate, matching problem)</td>
<td></td>
</tr>
<tr>
<td>5. Is the assessment of exposure factors accurate enough to lower bias?</td>
<td></td>
</tr>
<tr>
<td>6. Do the authors consider the confounding factors? Are the potential confounding factors taken into consideration in the process of study and analysis? (for example: severity of disease, comorbidity, etc.)</td>
<td></td>
</tr>
<tr>
<td>7. What about the outcome of study? (Is the analytical method correct? What is the value of OR?)</td>
<td></td>
</tr>
<tr>
<td>8. What about the accuracy of the study? What about the accuracy of the estimated I and II mistakes? (p value, confidence interval)</td>
<td></td>
</tr>
<tr>
<td>9. Is the outcome credible?</td>
<td></td>
</tr>
<tr>
<td>10. Is the outcome applicable to the local people?</td>
<td></td>
</tr>
<tr>
<td>11. Does the outcome of study correspond to other evidence?</td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td></td>
</tr>
</tbody>
</table>

Note: the scoring method of each item: 0 to those non-compliant with requirements; 1 point to those without a detailed description; 2 points to those with a detailed, comprehensive, and correct description.

Table 2. — The detailed information of the selected articles.

<table>
<thead>
<tr>
<th>Lead author and year of publication</th>
<th>Grouping case/comparison</th>
<th>Study period</th>
<th>Main outcomes</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. F. Coogan, 1998 [5]</td>
<td>284 cases and 620 controls in Massachusetts</td>
<td>1983 - 1986</td>
<td>OR=0.88 (0.64, 1.21)</td>
<td>14</td>
</tr>
<tr>
<td>M. Feychting, 1998 [6]</td>
<td>699 cases and 699 controls in Sweden</td>
<td>1960 - 1985</td>
<td>OR=1.09 (0.82, 1.42)</td>
<td>18</td>
</tr>
<tr>
<td>P. P. Rosenbaum, 1994 [7]</td>
<td>63 cases and 253 controls in New York</td>
<td>1979 - 1988</td>
<td>OR=0.70 (0.28, 1.76)</td>
<td>15</td>
</tr>
<tr>
<td>D. P. Loomis, 1994 [8]</td>
<td>27,882 cases and 110,949 controls in USA</td>
<td>1985 - 1989</td>
<td>OR=1.36 (1.03, 1.49)</td>
<td>13</td>
</tr>
<tr>
<td>E. R. Schoenfeld, 2003 [9]</td>
<td>565 / 578 of EBCLIS group</td>
<td>1994 - 1998</td>
<td>OR=0.93 (0.70, 1.22)</td>
<td>16</td>
</tr>
<tr>
<td>J. Kliukiene, 2004 [10]</td>
<td>1,830 cases and 3,658 controls in Norway; age over 16 years</td>
<td>1986 - 1996</td>
<td>OR=1.53 (1.27, 1.85)</td>
<td>14</td>
</tr>
<tr>
<td>E. Van Wijngaarden, 2001 [11]</td>
<td>843 / 773 in eastern North Carolina, age 20–74 years</td>
<td>May 1, 1993 - September 30, 1995</td>
<td>Premenopausal women OR=1.06 (0.84, 1.34) Postmenopausal women OR=0.92 (0.71, 1.19) Total OR=0.93 (0.78, 1.20)</td>
<td>13</td>
</tr>
<tr>
<td>F. Labreche, 2003 [12]</td>
<td>536 / 600 in Canada, age 50-75 years</td>
<td>1996 - 1997</td>
<td>OR=1.16 (0.88, 1.53)</td>
<td>16</td>
</tr>
<tr>
<td>J. A. McElroy, 2007 [13]</td>
<td>6,213 / 7,390 in USA</td>
<td>1970 - 2002</td>
<td>OR=1.06 (0.86, 1.30)</td>
<td>15</td>
</tr>
<tr>
<td>J. A. McElroy, 2001 [14]</td>
<td>1,949 / 2,498 in Massachusetts, New Hampshire, and Wisconsin</td>
<td>June 1994 - July 1995</td>
<td>OR=0.97 (0.78, 1.20)</td>
<td>20</td>
</tr>
<tr>
<td>G. C. Kabat, 2003 [15]</td>
<td>1,354/ 1,426 of LIBCSP 576,585 of EBCLIS</td>
<td>Mid - 1996 - Mid - 1997</td>
<td>EBCLIS-postmenopausal women OR=0.89 (0.67, 1.17) EBCLIS-premenopausal women OR=1.32 (0.88, 2.00) LIBCSP-postmenopausal women OR=0.97 (0.76, 1.24) LIBCSP-premenopausal women OR=1.39 (1.05, 1.83) Total OR=1.07 (0.90, 1.27)</td>
<td>17</td>
</tr>
<tr>
<td>S. Davis, 2002 [17]</td>
<td>680 / 675 controls in Snohomish County</td>
<td>November 1992 - March 1995</td>
<td>OR=1.04 (0.80, 1.36)</td>
<td>16</td>
</tr>
<tr>
<td>U. M. Forssen, 2000 [19]</td>
<td>440 / 439 in Sweden</td>
<td>1960 - 1985</td>
<td>OR=1.02 (0.77, 1.35)</td>
<td>14</td>
</tr>
</tbody>
</table>
Table 3. — Basic information of the cases of each study group and exposure to ELF-EMFs.

<table>
<thead>
<tr>
<th>Reporters</th>
<th>Exposure to ELF-EMFs</th>
<th>Cases</th>
<th>Non-exposure to ELF-EMFs</th>
<th>Comparison Exposure to ELF-EMFs</th>
<th>Cases</th>
<th>Non-exposure to ELF-EMFs</th>
<th>SE</th>
<th>OR</th>
<th>Q</th>
<th>OR_{DL}</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.R.Schoenfeld</td>
<td>418</td>
<td>147</td>
<td>436</td>
<td>142</td>
<td>0.14</td>
<td>0.93</td>
<td>31.26</td>
<td>1.10</td>
<td>1.01</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>E.Van Wijngaarden</td>
<td>579</td>
<td>264</td>
<td>542</td>
<td>231</td>
<td>0.11</td>
<td>0.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Labreche</td>
<td>417</td>
<td>119</td>
<td>450</td>
<td>150</td>
<td>0.14</td>
<td>1.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.A. McElroy (2007)</td>
<td>3,430</td>
<td>2,783</td>
<td>3970</td>
<td>3,420</td>
<td>0.03</td>
<td>1.06</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.A. McElroy (2001)</td>
<td>834</td>
<td>1,115</td>
<td>1,090</td>
<td>1,408</td>
<td>0.06</td>
<td>0.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.C. Kabat</td>
<td>661</td>
<td>1,323</td>
<td>642</td>
<td>1,278</td>
<td>0.06</td>
<td>0.89</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>K. Zhu</td>
<td>73</td>
<td>205</td>
<td>51</td>
<td>213</td>
<td>0.21</td>
<td>1.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Davis</td>
<td>513</td>
<td>167</td>
<td>504</td>
<td>171</td>
<td>0.13</td>
<td>1.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. J. London</td>
<td>87</td>
<td>260</td>
<td>60</td>
<td>226</td>
<td>0.19</td>
<td>1.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.M. Forssen</td>
<td>284</td>
<td>156</td>
<td>281</td>
<td>158</td>
<td>0.14</td>
<td>1.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.A. Demers</td>
<td>33</td>
<td>194</td>
<td>26</td>
<td>274</td>
<td>0.28</td>
<td>1.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.F. Coogan</td>
<td>107</td>
<td>97</td>
<td>345</td>
<td>275</td>
<td>0.16</td>
<td>0.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Feychting</td>
<td>111</td>
<td>588</td>
<td>103</td>
<td>596</td>
<td>0.15</td>
<td>1.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.F. Rosenbaum</td>
<td>6</td>
<td>57</td>
<td>33</td>
<td>220</td>
<td>0.47</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.P. Loomis</td>
<td>68</td>
<td>27,814</td>
<td>199</td>
<td>110,750</td>
<td>0.14</td>
<td>1.36</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>J. Kluukiene</td>
<td>217</td>
<td>1,613</td>
<td>295</td>
<td>3363</td>
<td>0.09</td>
<td>1.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. — Forest stereogram of the meta-analysis on exposure to ELF-EMFs and breast cancer.

Figure 2. — Funnel plot analysis of the selected articles’ publication bias.
A combined analysis of comparative study on exposure to ELF-EMFs and breast cancer occurrence

Through test for heterogeneity, it turns out that Q value was 31.26 and degree of freedom was 15, $p < 0.05$. Therefore, random effect model was used to combine and the calculating outcome was ORDL = 1.10, 95% CI = (1.01, 1.20). The detailed information is given in Table 3. The combined analysis outcome is drawn in the forest stereogram as shown in Figure 1.

Offset estimation

Review Manager 5.1 software was used to draw a funnel plot and obtain a linear regression analysis. Figure 2 presents the data distribution, almost bilaterally symmetrical, which shows that the bias was small.

Subgroup analysis

A stratified analysis was performed to analyze the information of the subjects provided by the selected articles, such as the menopausal status, etc. M-H method was applied to calculate it. Table 3 shows the $\text{OR}_{MH} = 1.24$, 95% CI = (1.03, 1.49) of the relation between exposure to ELF-EMFs and breast cancer of premenopausal subgroup. The forest stereogram of combined analysis is shown in Figure 3. Table 4 shows the $\text{OR}_{MH} = 1.24$, 95% CI = (1.03, 1.49) of the relation between exposure to ELF-EMFs and breast cancer of postmenopausal subgroup female. The forest stereogram of combined analysis is shown in Figure 4.
Discussion

ELF-EMFs are widespread in people and the study on the relationship between ELF-EMFs and different cancers has become a hotspot in the recent years. It is however uncertain regarding the case and the control group in the study [20]. People’s exposure dose, exposure severity, and exposure way to ELF-EMFs is also difficult to determine [21]. Owing to these uncertainties, the study results at home and abroad regarding the relationship between ELF-EMFs and cancers are not identical. In recent years, it has been found that ELF-EMFs might cause the level of melatonin to change at night. Melatonin is useful for preventing breast cancer [22]; therefore the assumption is that ELF-EMFs cause the change of melatonin, which gives rise to the occurrence of breast cancer. It is very common for people to be exposed to ELF-EMFs. For example, it is at the peak for people to use electric blankets at night; moreover, melatonin is secreted more at night [21]. So it is of great necessity to study the relationship between exposure to ELF-EMFs at night and the occurrence of breast cancer.

With regards to the occurrence of breast cancer, Kliukiene [23] and other authors have performed a cohort study from 1961 to 1992. According to the information provided by the cancer registry in Norway, it was found that exposure to 50Hz ELF-EMFs may increase the risk of breast cancer. However, a distinct conclusion still cannot be made because of the lack of a direct access to the information [23, 24]. Many animal testing also indicate that exposure to ELF-EMFs can increase the risk of cancer. Loscher and others used DMBA to induce breast cancers on mice. They used 50Hz, 0.2 - 1μT, 10μT, 50μT, 100μT magnetic fields to irradiate 24 hours for 13 weeks. It was found that there was a dose-response relationship of cancer rate and magnetic fields [25, 26].

This article applied meta-analysis to comprehensively analyze ten items of case-control study abroad regarding the relationship between exposure to ELF-EMFs and breast cancer. Through homogeneity testing, $Q$ value was 6.53 and degree of freedom $9 (p < 0.05)$. The combined OR was $\text{ORDL} = 1.10$ through random effect model, 95% CI = (1.02, 1.20), which indicated that breast cancer occurrence may be related to exposure to ELF-EMFs. Through stratification analysis on literatures, the premenopausal subgroup was $\text{ORMH} = 1.25, 95\% \text{CI} = (1.05, 1.49)$ and in the menopausal group it was $\text{ORMH} = 1.04, 95\% \text{CI} = (0.93, 1.18)$. It indicated that, for the premenopausal group, the occurrence of breast cancer may be related to exposure to ELF-EMFs, but for the menopausal group it has no relationship. The specific mechanism still requires further study. It is difficult to clarify the occurrence regularity of ELF-EMFs to breast cancer only from population-based studies of various countries. This study drew a funnel plot to show that the publication bias of the selected article was small. From the result it is known that exposure to ELF-EMFs is one of the risk factors contributing to breast cancer, especially for premenopausal females. It is suggested that premenopausal female should minimize exposure to ELF-EMFs, (avoiding the use of bedding sets like electric blanket and so on as little as possible), which generate ELF-EMFs.

References


Address reprint requests to:
J. ZHAO, M.D.
Department of Chest Surgery,
Cancer Center of Guangzhou Medical University,
No.78 Heng Zhi Gang Luhe Road,
Guangzhou 510095 (China)
e-mail: zhaojian_gz@163.com
Cervical intraepithelial neoplasia based on array comparative genomic hybridization

Seung Do Choi¹, Tae-Hee Kim², Dong Han Bae¹

¹Department of Obstetrics and Gynaecology, College of Medicine, Soonchunhyang University, Cheonan
²Department of Obstetrics and Gynaecology, College of Medicine, Soonchunhyang University, Bucheon (Republic of Korea)

Summary

Uterine cervical cancer is one of the most frequently observed malignant gynaecologic tumors. Carcinoma in situ or invasive cervical carcinoma develops from a low-grade intraepithelial lesion of the cervix over time. Human papillomavirus (HPV) is known to be a major contributing factor. With improvements in molecular genetic technologies, the authors attempted to identify the genomic changes associated with cervical precancerous lesions. In this study, changes in gene copy numbers were evaluated in five cases of severe uterine cervical dysplasia (HPV negative, two cases; HPV 16 and 18 positive only, three cases) by array comparative genomic hybridization (array CGH), and genes with copy number changes were compared between the two groups. Between the HPV positive and negative groups, only one gene was found to be upregulated more than 1.5 fold (3q23-q24), and no downregulated genes were identified. In conclusion, it is useful to evaluate genomic aberrations in cervical cancer using array CGH.

Key words: Comparative genomic hybridization; Uterine cervical neoplasms; Human papillomavirus.

Introduction

Cervical cancer is the most prevalent gynaecologic malignancy in women worldwide. Microarray-based comparative genomic hybridization (CGH) has been used to investigate solid tumors, including cervical cancer. Genome-based aberrations induce precancerous oncogenes, oncosuppressor genes, and apoptosis. Specific areas of DNA amplification or deletion and loss are commonly observed. Further, specific gene mutations have been investigated regarding their relationship with tumor development, in addition to treatment and prognostic factors. Human papillomavirus (HPV) infection does not always progress to cervical cancer; however, HPV is a well-known risk factor. Although the precise pathogenic mechanisms underlying cervical cancer have not been elucidated, HPV is known to be a major contributing factor based on epidemiologic studies. However, the time span is extremely short in some cases and HPV negative cervical cancers have also been occasionally observed. Therefore, HPV infection alone cannot completely explain the process of carcinogenesis in the uterine cervix. With improvements in molecular genetic technologies, we can relatively easily evaluate genetic copy number changes and genetic alterations in solid tumors. The goal of the current experiment was to identify the cause of carcinogenesis in solid tumors by investigating genomic changes, as well as characterize the roles of internal or external influences that alter genomic stability. Evaluations focused on gene changes are very important, particularly in cervical precancerous lesions, for understanding the process of carcinogenesis, preventing and treating tumors, and for prognostic evaluation.

CGH was first introduced by Kallioniemi et al. in 1992. It is a useful method for identifying genetic changes relative to normal tissue DNA [1]. CGH is very fast and cost-effective compared to fluorescence Southern hybridization and loss of heterozygosity analyses. CGH is a very useful method for observing the presence or absence of genes, evaluating quantitative (copy number) changes, determining whether a mutation is a deletion, duplication, or translocation, and examining loss of heterozygosity mutations by single chip experiments with 40 Mb resolution [2].

Here, the authors report cases of uterine cervical intraepithelial neoplasia either with HPV infection 16 and 18 or without infection by evaluating CGH. They examined gene mutation sites in precancerous cervical lesions due to HPV infection and compared the relationship to cancer incidence by the numerical relationship of the mutation of a particular gene region.

Materials and Methods

Samples of human uterine cervix were collected between May 2001 and October 2003 by conization from women diagnosed with high-grade squamous intraepithelial lesions in situ based on the Papanicolaou test. Exclusion criteria included necrotic tissue and severe inflammation. Approval was given by the Human Ethics Committee at SCH Medical Center (Cheonan, Korea). The authors selected three cases with HPV 16, which were coincidentally positive for HPV 18, and two cases without any HPV infection.
Each paraffin-embedded section was cut to a thickness of 15 μm and heated at 58°C for 30 minutes in an oven to melt the paraffin. The sections were washed in xylene three times, each for five minutes; washed in 100% ethanol two times, each for one minute; and then dehydrated using an ethanol gradient (95%, 90%, 80%, 70%, and 50% ethanol) for one minute; and then dehydrated using an ethanol gradient. After dehydrating, the sections were washed in xylene three times, each for one minute; and then dehydrated using an ethanol gradient. Each paraffin-embedded section was cut to a thickness of 15 μm and heated at 58°C for 30 minutes in an oven to melt the paraffin. The sections were washed in xylene three times, each for five minutes; washed in 100% ethanol two times, each for one minute; and then dehydrated using an ethanol gradient (95%, 90%, 80%, 70%, and 50% ethanol) for one minute in each concentration after treatment. The section was then placed in sterile D/W for ten minutes and stained with haematoxylin and then dehydrated using an ethanol gradient (95%, 90%, 80%, 70%, and 50% ethanol) for one minute; and then dehydrated using an ethanol gradient. Each CGH served as a control for the reaction of the same sample. Normal DNA and 100 ng each of genomic DNA were treated with polymerase Ψ 29 and amplified. Alul (20 units) and Rsal (20 units) were added to all of the amplified samples for two hours at 37°C. After the reaction, case and control DNA samples were purified with a QIAQuick PCR clean-up kit and dissolved. Samples were dried in an array, scanned on an image plate, and then analyzed with GenePix Pro 6.0. The fluorescence intensity of each gene was quantified and the average fluorescence intensity was calculated at each point. The default value was calculated and each gene was quantified and the average fluorescence intensity was obtained by removing the default value. A total of 10,290 genes were selected from samples infected with HPV and 10,127 genes were selected from uninfected samples. The average normalized ratio was calculated by dividing the average of normalized signal channel intensity by the average of control channel intensity. Based on the knowledge that changes for commonly observed mutations are 1.5 fold or higher, the HPV DNA Chip test subtypes included three cases of cervical intraepithelial neoplasia that were positive for HPV 16 and 18 and two HPV negative cases. Table 1. — Genes that showed changes of over 1.5-fold (eight genes) in human papillomavirus-negative specimens.

<table>
<thead>
<tr>
<th>Bank</th>
<th>Gain/ Loss</th>
<th>Gene</th>
<th>Map</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_182943</td>
<td>gain</td>
<td>PLOD2 (LH2;TLH)</td>
<td>3q23-q24</td>
<td>procollagen-lysine, 2-oxidoglutarate 5-dioxygenase 2</td>
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<tr>
<td>BX537559</td>
<td>gain</td>
<td>UGP2</td>
<td>2p14-p13</td>
<td>UDP-glucose pyrophosphorylase 2</td>
</tr>
<tr>
<td>BC065192</td>
<td>gain</td>
<td>C2orf12(HCC-4 DKFZp564 H0764)</td>
<td>2q24.3</td>
<td>open reading frame 12</td>
</tr>
<tr>
<td>AK092622</td>
<td>gain</td>
<td>HNRPLL</td>
<td>2p22.1</td>
<td>Heterogeneous nuclear ribonucleoprotein L-like</td>
</tr>
<tr>
<td>BC050347</td>
<td>gain</td>
<td>FAM19A2</td>
<td>12q14.1</td>
<td>Family with sequence similarity 19 (chemokine (C-C motif)-like), member A2</td>
</tr>
<tr>
<td>BC043502</td>
<td>gain</td>
<td>NEK2(NLK1; TAF2; MGC42403; TAF2)</td>
<td>1q32.2-q41</td>
<td>NIMA (never in mitosis gene a)-related kinase 2</td>
</tr>
<tr>
<td>AK095750</td>
<td>gain</td>
<td>RER1</td>
<td>1pter-q24</td>
<td>RER1 retention in endoplasmic reticulum 1 homolog (S. cerevisiae)</td>
</tr>
<tr>
<td>AK098071</td>
<td>loss</td>
<td>C10orf32</td>
<td>10q24.3</td>
<td>procollagen-lysine, 2-oxidoglutarate 5-dioxygenase 2</td>
</tr>
</tbody>
</table>

Table 2. — Genes that showed changes of over 1.5-fold (16 genes) in human papillomavirus-positive specimens.

<table>
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<th>Gene</th>
<th>Map</th>
<th>Function</th>
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</thead>
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<tr>
<td>XM_376171</td>
<td>gain</td>
<td>unknown</td>
<td>unknown</td>
<td>procollagen-lysine, 2-oxidoglutarate 5-dioxygenase 2</td>
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<tr>
<td>BC035514</td>
<td>gain</td>
<td>TEK(TIE2; VMCM; TIE-2; VMCM1; CD202B)</td>
<td>9q21</td>
<td>TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)</td>
</tr>
<tr>
<td>AN758762</td>
<td>gain</td>
<td>S100A12</td>
<td>1q21</td>
<td>S100 calcium binding protein A12 (calgranulin C)</td>
</tr>
<tr>
<td>BX649193</td>
<td>gain</td>
<td>TKT(TKT1)</td>
<td>3p14.3</td>
<td>Chromosome 15 open reading frame 35</td>
</tr>
<tr>
<td>AW966841</td>
<td>gain</td>
<td>FLJ32800</td>
<td>15q11-12</td>
<td>Chromosome 15 open reading frame 35</td>
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<tr>
<td>AK124747</td>
<td>gain</td>
<td>PDE5A</td>
<td>4q25-q27</td>
<td>Phosphodiesterase 5A, cGMP-specific</td>
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<tr>
<td>AJ672441</td>
<td>gain</td>
<td>unknown</td>
<td>unknown</td>
<td>Transcribed locus</td>
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<tr>
<td>NM_014730</td>
<td>loss</td>
<td>KIAA0152</td>
<td>12q24.3</td>
<td>NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75KDa (NADH-coenzyme Q reductase)</td>
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<tr>
<td>BC030833</td>
<td>loss</td>
<td>NDUF51</td>
<td>9q33-q34</td>
<td>NDUFA5 (CI-75Kd; (ubiquinone) Fe-S protein 1, 75KDa (NADH-coenzyme Q reductase)</td>
</tr>
<tr>
<td>AF360549</td>
<td>loss</td>
<td>BRIP1(OF; BACH1; FANCI; FLJ90232; MGC126521; MGC126523)</td>
<td>17q22-q24</td>
<td>BRCA1 interacting protein C-terminal helicase 1</td>
</tr>
<tr>
<td>BX538024</td>
<td>loss</td>
<td>USP37</td>
<td>2q35</td>
<td>ubiquitin specific peptidase 37</td>
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<tr>
<td>BG250953</td>
<td>loss</td>
<td>GAGE2</td>
<td>Xp11.23</td>
<td>G antigen 1</td>
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<tr>
<td>BM926633</td>
<td>loss</td>
<td>WDR18</td>
<td>19p11.3</td>
<td>WD repeat domain 18</td>
</tr>
<tr>
<td>AJ141579</td>
<td>loss</td>
<td>unknown</td>
<td>unknown</td>
<td>Transcribed locus</td>
</tr>
<tr>
<td>BC043435</td>
<td>loss</td>
<td>BMS1L</td>
<td>10q11.21</td>
<td>BMS1-like, ribosome assembly protein (yeast)</td>
</tr>
</tbody>
</table>

Results

The HPV DNA Chip test subtypes included three cases of cervical intraepithelial neoplasia that were positive for HPV 16 and 18 and two HPV negative cases. Eight samples of HPV negative DNA showed an increase or decrease of 1.5 times or more. Seven regions (3q23-q24,
Cervical intraepithelial neoplasia based on array comparative genomic hybridization

2p14-p13, 2q24.3, 2p22.1, 1q32.2-q41, and 1pter-q24) were increased and one region (10q24.32) was decreased. Changes in the degree of color change were expressed as an increase or decrease of HPV (Figure 1A, B).

DNA mutations in HPV positive samples were observed in 16 regions. Eight regions were increased: (3q23-q24, 9p21, 1q21, 3p14.3, 15q21.1-q21.2, 4q25-q27, and two unknown cases). The remaining eight regions (12q24.31, 2q33-q34, 17q22-q24, 2q35, Xp11.23, 19p13.3, 10q11.21, and one unknown case) were decreased. Changes in the degree of color change were expressed as an increase or decrease of HPV (Figure 2A, B).

One gene that was common to the two groups (3q23-q24) showed an increase, and there was no reduction in area. HPV negative genes are described in Table 1 and HPV positive genes are described in Table 2.

Discussion

Based on the present results, it is hypothesized that some tumor suppressor genes or cell cycle regulators can exist in the downregulated genomic copy number regions, and some genes related to carcinogenesis can exist in upregulated genomic copy number regions. DNA copy number changed by more than 1.5-fold, and changes were found
two-fold more frequently in HPV infected specimens. Therefore, it is thought that oncogene upregulation is enhanced by HPV. In contrast, tumor suppressor genes would be downregulated by HPV. Because the 3q23-24 region was increased in both groups and the degree of change did not differ; these regions may contain cancer-related genes unrelated to HPV infection.

The genetic features of this area included enzyme functions such as procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2, oxidoreductase activity, protein metabolism and transformation, L-ascorbic acid combined functions, iron ion binding functions, and endoplasmic reticulum-related genes.

Increases in q25-q26, 3q26-q27, and 3q26-qter have been reported in cervical cancer, endometrial cancer, ovarian cancer, lung cancer, and head and neck cancer. These results indicate that these regions include genes important in cancer development [3].

Another interesting result was the decrease in 2q33-q34, which may be correlated with HPV infection and ubiquitin-specific peptidase. Decreases in the 2q33-q34 region of tumor suppressor genes are recognized in precancerous lesions, and a reduction in this area for cancer lesions has been reported, making it an important marker [4]. Another study found a low level of 20q in 63% of cervical cancer cases [5].

Large-cell neuroendocrine carcinoma of the uterine cervix is a rare cervical cancer. Amplification of 3q in cervical cancer was demonstrated by CGH [6]. HPV integration can induce alterations in genomic structure through the amplification, deletion, and rearrangement of DNA [7]. Thirty-three (33%) were associated with copy number alterations, including the regions of amplification/gain at 11q22 and 8q24.21. Furthermore, these alterations were associated with HPV-16 and HPV-18 integration in Ca Ski and HeLa cells, respectively [7].

Future studies should evaluate the genomic changes in uterine cervical precancerous lesions and the internal and external factors that influence genomic stability independent of HPV infection. These data will provide useful tools for evaluating the process of carcinogenesis, preventing tumor progression, treating solid tumors, and forecasting tumor prognosis.

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Address reprint requests to:
TAE-HEE KIM, M.D.
Department of Obstetrics and Gynaecology
Soonchunhyang University Bucheon Hospital
1174 Jung-l-dong, Wonmi-gu, Bucheon-si, Gyunggi-do, 424-767 (Republic of Korea)
e-mail: heeobgy@schmc.ac.kr; heeobgy@naver.com
The texture quantitative analysis of the normal mammary parenchyma and in breast lesions: acoustic radiation force impulse (ARFI) technology

Y. Li¹, C. Liu¹, J. Geng¹, X. Zheng², B. Chen², Z. Lu³, X. Wang¹

¹ Department of Ultrasound Diagnosis, the First Affiliated Hospital, China Medical University, Shenyang
² Department of Breast Surgery, the First Affiliated Hospital, China Medical University, Shenyang
³ Department of Clinical Application and Education, Chindex Medical Limited, Beijing (China)

Summary

Objective: The purpose of this work is to investigate the feasibility of acoustic radiation force impulse (ARFI) technology in the normal mammary parenchyma and in breast lesions. Materials and Methods: The virtual touch tissue quantification (VTQ) value was measured on a total of 150 cases in the normal mammary parenchyma and a total of 69 cases in breast lesions (19 cases of nodules, 28 cases of fibroadenoma, and 22 cases of cancer). Then the statistic analysis was carried out on the VTQ value combined with mammographic density, ages, menstrual stages, and pathological result. Results: The VTQ value of mammary parenchyma rose with the increase of the mammographic density, and the value of VTQ had statistical differences in the comparison of group C with group B and in the comparison of group D with group C. The comparison of the VTQ value of the mammary parenchyma in patients with breast cancer and the nodule had statistical difference. The comparison of the VTQ value of the mammary parenchyma in patients with breast cancer, and the fibroadenoma had statistical difference. The value of VTQ in masses gradually increased in the groups of nodule, fibroadenoma, and breast cancer. There was significant difference in the comparison of VTQ value of the nodule group and the fibroadenoma group with breast cancer group respectively. Conclusion: ARFI-VTQ technology has some reference value in assessing mammographic density. ARFI-VTQ can be used as the quantitative indicator for differentially diagnosing the breast lesions.

Key words: ARFI technology; Quantitative analysis; Mammary parenchyma; Breast lesions.

Introduction

Breast cancer is one of the most common malignant tumors for women. According to WHO, the new cases of breast cancer are more than one million each year and morbidity as well as mortality occupy the top three in female malignancy [1, 2]. A number of studies have identified the relationship between the visual appearance of high breast density with mammography and an increased risk of breast cancer [3, 4]. Because Chinese women have high breast density, the false negative rate was high in reconnaissance survey for breast cancer [5]. Though the dense mammary gland is an important influencing factor for the interpretation of the mammographic report and a very important reason for missed diagnosis, until now the preferred means of detecting breast cancer has been the mammography, which is also used to indicate mammographic density.

Mammography is the earliest breast imaging technology in grading the breast density and exploring the relationship between mammographic density and the incidence of breast cancer. However, ultrasound imaging caused by the different acoustic velocity and acoustic impedance difference in different media, so there is no correlation between ultrasound and the breast density, not to mention the quantification of breast density. In recent years, ultrasound has gradually been adopted in some countries’ breast cancer screening guide or taken as a supplement to the mammography, especially the dense mammary gland. Therefore, if the ultrasonic test can acquire the information of mammographic density and can quantitatively diagnose breast lesions, it will be very significant for the development of imaging examination.

The ultrasonic elastography, one of the newest imaging methods [6, 7], has become the research hotspot in ultrasonic imaging as it can non-invasively assess the elasticity of biological tissue. The strain ratio is different when tissues are under compression. With this characteristic the transient elastography can reveal the location, characteristic, and size of focal lesions by color coding. The current study suggests that this method is helpful to diagnose breast lesions [8, 9]. Real-time elastography, which can have real-time and rapid diagnosis on the diseased tissues, has already been used in clinical medicine. The problem, however, of this traditional elastography technology uses “pressure” assistant force pattern, it has large handlers’ individual difference, and cannot have quantitative analysis on the absolute value of hardness of the different tissues. The authors found that the false positive and negative rates unavoidably exist due to the different hardness of breast glandular parenchyma of different population, which confused the ultrasound doctors.

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Acoustic radiation force impulse (ARFI) imaging technology is a newly developed quantitative elastography, which uses short-term and focal acoustic impulse acting on the regions of interest of the tissue to produce transient micror-grade displacement, and meanwhile sends acoustic impulse sequence to detect the displacement that depends on the elasticity of the tissue. With these small changes, the machine records and calculates its speed, which is in proportion to the square root of the elasticity [10]. According to this principle, ARFI technology can detect the tissues of different biological characteristics. ARFI technology includes virtual touch tissue imaging (VTI) and virtual touch tissue quantification (VTQ). ARFI causes the tissues to produce longitudinal compression and lateral vibration. The longitudinal displacement is to some extent related to the elasticity of the tissue, and the elastography based on the longitudinal displacement is called VTI, which can intuitively reflect the elasticity of the tissue with black color indicating the relative hardness of the tissue. The lateral vibration spreads around in the way of shear wave, and the shear wave velocity (SWV) can be calculated with the time difference of successive wave crests and the wave length of the shear wave. VTQ refers to the elastography based on the lateral vibration and it can quantitatively reflect the elasticity of the tissues.

Nowadays, there have been many international studies on ARFI technology in liver diffused disease, and the technology is relatively mature [11, 12]. However, studies on the role of ARFI in breast are limited. Glied et al. [13] studied the feasibility of acoustic velocity detecting the mammary density. Their result laid a foundation for further examination in density of breast by ultrasound. In recent years, Tozaki M et al. [14-16] reported the research result of differentiating malignant and benign breast lesions, and believed that the value of VTQ was helpful in diagnosing breast lesions. It is not clear whether ARFI can reflect the density of mammary parenchyma by using VTQ value, whether VTQ value is related to the age, and whether VTQ value can be used as an indicator for quantitatively diagnosing breast lesions. Therefore, the study examined the VTQ values of both the normal mammary parenchyma and the breast lesions, combined with mammographic and pathological results to facilitate an analysis and explore the value of ARFI technology in measuring the mammographic density and differentially diagnosing breast lesions.

Materials and Methods

Patients
The subjects in this study were patients who were treated in outpatient and inpatient departments, including 150 cases with normal mammary parenchyma and 69 cases with breast lesions. A total of 69 cases of breast lesions with pathological results were all surgically removed or treated by mammotome minimal invasive system, including 19 cases of nodules, 28 cases of fibroadenoma, and 22 cases of breast cancer (all were the invasive ductal carcinoma). Ultrasonographic device with a 4-9 MHz linear array transducer was used. This study was conducted in accordance with the declaration of Helsinki. This study was also conducted with approval from the Ethics Committee of China Medical University. Written informed consent was obtained from all participants.

Criteria of normal mammary parenchyma
The criteria for choosing the cases of normal mammary parenchyma were as follows: 1) the glands without local or diffused ductal ectasia and the glands without fibrocystic change or dysplasia, according to the BI-RADS [17]. Mammography BI-RADS in Category 1 were classified into Group A: fatty (<25%), Group B: scattered (25~50%), Group C: heterogeneously dense (50~75%), and Group D: dense amounts of fibroglandular tissue (>75%); 2) according to the ages, the cases were classified into Group A: <30 years, Group B: 30-40 years, Group C: 41-50 years, Group D: 51-60 years, and Group E: >60 years (except the glands in pregnancy and lactation); 3) according to the menstrual stages, the cases were classified into Group A: sexual maturity stage and Group B: gerontic stage; 4) there were no nodules observed by two-dimension in the skin, subcutaneous fat, and ectopectoralis in the mammary and there were no abnormally large lymph nodes in the glands and armpits.

The measurements in the normal breast glandular tissue
Patients were placed in a supine position with the upper limbs on the side of the head. Firstly, two-dimension ultrasound was used to scan the mammary parenchyma. The value of VTQ was measured in the normal mammary parenchyma in a 12 o’clock direction and two cm from the nipple by the doctor who had more than ten years experience. The patients were asked to relax, to remain still, and hold their breath. The probe was gently placed above the breast. The region of interest in the center of mammary gland was chosen and the depth did not exceed four cm which was calibrated by the instrument. The same position was measured five times and the highest and lowest values were removed in order to exclude offset results from surgery. The remaining three values were calculated to obtain the average.

Measurements of the breast lesions
First, the breast lesions were determined by B-mode ultrasound; then the probe with radial direction was gently placed above the breast lesions. The patients were asked to relax, to remain still, and hold their breath. The value of VTQ was measured in the breast lesions by the doctor who had more than ten years experience. The region of interest in the center of breast lesions was chosen and the depth did not exceed four cm which was calibrated by the instrument. The same position was measured five times and the highest and lowest values were removed in order to exclude offset results from surgery. The remaining three values were calculated to obtain the average.

Statistical analysis
Statistical analysis was performed by using SPSS 17.0 software. Quantitative data were expressed as mean ± SD. T-test was used for intergroup comparison of averages. A p < 0.05 was considered statistically significant and p < 0.01 was considered statistically highly significant.

Results
The thickness and the VTQ value of the normal breast glandular parenchyma in different mammographic density
There was no case of fatty group in this study. The breast glandular thickness had no statistical differences between
the groups ($p > 0.05$). However, the value of VTQ rose with the increase of the mammographic density (Figure 1) and the value of VTQ had statistical differences in the comparison of group C with group B ($p < 0.01$, respectively) and in the comparison of group D with group C ($p < 0.05$, respectively) (Table 1).

### The thickness and the VTQ value of the normal mammary parenchyma in different ages and menstrual stages

The breast glandular thickness and the value of VTQ had no statistical differences between different age groups ($p > 0.05$) (Table 2). Both the breast glandular thickness and the VTQ value had statistical differences in the comparison of the sexual maturity stage with the gerontic stage ($p < 0.05$, $p < 0.01$), respectively (Table 3).

### The VTQ value in the breast parenchyma with masses and in the breast lesions

The comparison of the VTQ value of the mammary parenchyma in patients with nodules and fibroadenoma had no statistical difference ($p > 0.05$). The comparison of the VTQ value of the mammary parenchyma in patients with breast cancer and nodule had statistical difference ($p < 0.01$). The comparison of the VTQ value of the mammary parenchyma in patients with breast cancer and the fibroadenoma had statistical difference ($p < 0.01$).

The value of VTQ in masses gradually increased in the nodule groups, fibroadenoma, and breast cancer (Figure 2). The comparison of VTQ value of the nodule group with the fibroadenoma group had statistical difference ($p > 0.05$). There was significant difference in the comparison of VTQ value of the nodule group and the fibroadenoma group with breast cancer group, respectively ($p < 0.01$) (Table 4).

### Discussion

The density of the mammary gland is closely related to the fiber, glandular tissue, and fat of the breast. The more epithelial cells, cell matrix, and collagen the breast has, the higher the glandular density is [18]. In this study, the breast glandular thickness had no statistical differences between the groups with different mammographic density. This is perhaps one of the reasons that the ultrasound cannot acquire the information of the breast density. At present, the studies on the value of ARFI-VTQ in mammary parenchyma are limited and there is also no study comparing mammographic density. Tozaki et al. [15] measured the value of VTQ in subcutaneous fat and mammary parenchyma, with the average 2.66 m/s and 3.03 m/s, respectively. Their result is higher than the value of VTQ in mammary parenchyma that the present authors measured. It may be related with the different study population and the skill of different ultrasound operators.

Approximately 45% of the mammographic density is the type of heterogeneously dense and the mammary glandular

---

**Table 1. — The thickness and the VTQ value of the normal breast glandular parenchyma in different mammographic density.**

<table>
<thead>
<tr>
<th>Mammography</th>
<th>Thickness (cm)</th>
<th>VTQ value (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(case)</td>
<td>R</td>
</tr>
<tr>
<td>A: (0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B: (58)</td>
<td>1.12±0.13</td>
<td>1.20±0.07</td>
</tr>
<tr>
<td>C: (59)</td>
<td>1.25±0.06*</td>
<td>1.15±0.09*</td>
</tr>
<tr>
<td>D: (33)</td>
<td>1.22±0.15*</td>
<td>1.19±0.14*</td>
</tr>
</tbody>
</table>

Note: the comparison of group C with group B; the comparison of group D with group C

---

**Table 2. — The thickness and the VTQ value of the normal breast glandular parenchyma in different ages.**

<table>
<thead>
<tr>
<th>Age (case)</th>
<th>Thickness (cm)</th>
<th>VTQ value (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: (19)</td>
<td>1.52±0.02</td>
<td>1.47±0.07</td>
</tr>
<tr>
<td>B: (27)</td>
<td>1.49±0.04</td>
<td>1.51±0.05</td>
</tr>
<tr>
<td>C: (41)</td>
<td>1.59±0.11</td>
<td>1.54±0.08</td>
</tr>
<tr>
<td>D: (42)</td>
<td>1.51±0.08</td>
<td>1.48±0.13</td>
</tr>
</tbody>
</table>

Note: the comparison among groups, $p > 0.05$.

---

**Table 3. — The thickness and the VTQ value of the normal mammary parenchyma in different menstrual stages.**

<table>
<thead>
<tr>
<th>Menstrual stages (case)</th>
<th>The thickness (cm)</th>
<th>VTQ value (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: (53)</td>
<td>1.44±0.13</td>
<td>1.52±0.15</td>
</tr>
<tr>
<td>B: (47)</td>
<td>1.19±0.05*</td>
<td>1.15±0.08*</td>
</tr>
</tbody>
</table>

Note: the comparison of group B with group A; $* = p < 0.05; ** = p < 0.01$

---

**Table 4. — The VTQ value in the breast parenchyma with masses in the breast lesions.**

<table>
<thead>
<tr>
<th>Breast lesion (case)</th>
<th>VTQ value in the breast parenchyma (m/s)</th>
<th>VTQ value in the breast lesions (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>Nodule (19)</td>
<td>1.75±0.05</td>
<td>1.81±0.03</td>
</tr>
<tr>
<td>Fibroadenoma (28)</td>
<td>1.91±0.07*</td>
<td>1.79±0.04*</td>
</tr>
<tr>
<td>Breast cancer (9)</td>
<td>2.35±0.55***</td>
<td>2.44±0.38***</td>
</tr>
</tbody>
</table>

Note: the comparison of group B with group A; the comparison of group C with group A; the comparison of group C with group B

$* = p > 0.05; ** = p < 0.05; *** = p < 0.01$
density will directly affect the interpretation of the mammographic report. The higher the mammographic density is, the less the detection rate of breast cancer by mammography [19, 20]. This is the most important result showed in this study: that the value of VTQ increased as the mammographic density rose and the value of VTQ had statistical differences between the groups. This result indicates that the VTQ value in normal glandular parenchyma is in correlation with the mammographic density. So far, mammography has been used as the golden standard in judging the breast density. Therefore the result proves the correlation between the value of VTQ and mammographic density grading, further confirms ARFI as a quantitative means of breast texture, and compensates for the defect of the ultrasound regarding mammographic density grading.

The mammary parenchyma spreads between the shallow and deep layers of superficial fascia, including mammary duct, body, and matrix (some are fibrous tissue), which is also called fibroglandular tissue (FGT). The present results showed that the breast glandular thickness had no statistical differences between different age groups. However, Dong et al. [21] studied women in Taiwan and their results indicated that the breast glandular thickness was in correlation to age. Chen et al. [22] also indicated that the compressed breast glandular thickness was related to women’s age, which is different from the present results. The reason for this perhaps lies in the different study population and the breast glandular thickness of women in the present country who are largely different in the breast glandular development. The comparison of breast glandular thickness in different physiological stages has statistical difference, which indicates that glandular thickness is affected by estrogen.

Many studies have demonstrated that there is a complex relationship between age and breast density [23-25]. Young women have more abundant mammary parenchyma, less mesenchyme, and higher mammary glandular density; as the age increases, the proportion of fat, fibrous mes-
ence, and glandular epithelium tissue will change, and the density also begins to decrease. There is an accepted fact that the glandular thickness and density are decreased as the age is increased in each woman. The present results showed that the VTQ value had no statistical differences between different age groups. The VTQ also related to breast density; therefore this study suggests that the breast density has no correlation to age. It also lies in the different study population and the breast glandular thickness of women in the present country who are largely different in the breast glandular development. As a result, the statistical differences of glandular density in different physiological stages imply that the glandular thickness, the value of VTQ, and the glandular density are all affected by estrogen.

This aforementioned studies have limitations. The present authors did not take into account the influence of the menstrual period on the breast glandular thickness and the density of breast. Furthermore this study did not analyze the differences of the glandular thickness and the breast density in different individuals of the same age group and the same physiological stage group which will require further study.

In the present study, the VTQ value of 13 cases of breast cancer was XXX m/s (beyond the measuring range), the VTQ value of nine cases of breast cancer remained at the average of 5.68 ± 0.78 m/s. The results showed that there was significant difference in the average VTQ value of nodules and fibroadenoma group compared with breast cancer group, which was consistent with the conclusion reached by Postnova et al. [26, 27]. Therefore this ARFI technology can be used to quantitatively diagnose the breast lesions and can be used to differential diagnosis malignant and benign lesions. It can avoid the false positive and negative rates caused by the different hardness of mammary parenchyma. Because of the small sample, this result maybe have bias; it also requires further study with larger sample to approve this result and others.

Real-time elastography can diagnose breast lesions quantitatively by the different scores [28]. Therefore it is not specific for benign lesions and cannot distinguish from the nodules and fibroadenomata. Interestingly, however, is that the present results showed that the average VTQ value of nodule group had statistical difference with the fibroadenoma group. Therefore the average VTQ value can be used to distinguish the nodules and fibroadenoma. In the authors’ opinion, further study is required to evaluate whether it can distinguish other benign lesions, such as the intraductal papilloma and solid inflammatory mass.

There is a correlation between mammographic density and the incidence of breast cancer. Mammography is of no benefit in assessing compact mammary gland, but ultrasound has advantages, and is more valuable if combined with texture density of the VTQ. Therefore, the present results compared the VTQ values of the patients with breast lesion. The VTQ value of breast cancer has statistical differences in the comparison of nodule and fibroadenoma, respectively. This indicates the relationship between the value of VTQ of the mammary parenchyma and the incidence of breast cancer.

Conclusion
In conclusion, ARFI-VTQ technology has some reference value in assessing the mammographic density and it compensates analyzing breast density by ultrasound. ARFI-VTQ can be used as the quantitative indicator for differentially diagnosing breast lesions.

References


Address reprint requests to:
X. WANG, M.D.
Department of Ultrasound Diagnosis
The First Affiliated Hospital of
China Medical University
Shenyang, 110001 (China)
e-mail: xuemeiwangcn@126.com
The relationship between ovarian volume and serum CA-125 levels

U.K. Gulec¹, S. Paydas², A.B. Guzel¹, M.A. Vardar¹, I.F. Urunsak¹, M.T. Cetin¹

¹Department of Obstetrics and Gynecology, Faculty of Medicine, University of Cukurova, Adana
²Department of Medical Oncology, Faculty of Medicine, University of Cukurova, Adana (Turkey)

Summary

Purpose: The aim of this study was to investigate the relationship between ovarian volume and serum CA-125 levels. Materials and Methods: Serum CA-125 levels and ovarian volume were compared among the cases with benign ovarian neoplasms, primary epithelial ovarian cancer (EOC), controlled ovarian hyperstimulation, and ovarian hyperstimulation syndrome (OHSS). Also, the correlation between CA-125 levels and ovarian volume were evaluated in the presence of peritoneal fluid and/or peritoneal carcinomatosis. Results: Although ovarian volume was not different among the groups, CA-125 levels were higher in the cases with EOC than with benign ovarian tumors (p = 0.001). Baseline CA-125 levels were not found to have increased while ovarian volume went up with controlled hyperstimulation in the infertile group (p = 0.555). However, uncontrolled hyperstimulation of the ovaries and the presence of peritoneal fluid caused an increase in the levels of CA-125 (p = 0.001). There was no correlation between ovarian volume and CA-125 levels in the cases with malignant ovarian tumors (r = 0.083). Conclusions: The results of this study have confirmed that CA-125 is a peritoneal marker and increased ovarian volume with benign ovarian neoplasms or controlled hyperstimulation does not increase CA-125 levels in the same way. The presence of peritoneal carcinomatosis and/or peritoneal fluid seems to be an important factor for high CA-125 levels in patients with epithelial ovarian cancer (EOC).

Key words: CA-125; Epithelial ovarian cancer; Peritoneal carcinomatosis; Peritoneal fluid; Ovarian volume.

Introduction

CA-125 is the most commonly used tumor marker for malignant ovarian tumors. It is not only used in the process of diagnosis but also for the follow up of the epithelial ovarian cancer (EOC). Low specificity is the weakest point of this marker. It is very well known that irritation of mesothelial cells of peritoneal, pleural or pericardial surfaces due to inflammatory or malignant conditions is the main cause of increased serum CA-125 levels. However the source of CA-125 is still an open question and its origin may be ovarian, endometrial, peritoneal, or amniotic cells [1]. CA-125 shedding in human peritoneal mesothelial cells has been found to be fivefold higher than ovarian cancer cell lines [2]. So far the majority of the studies have investigated the association between the presence of peritoneal/pleural fluid and CA-125 levels. Although CA-125 is the most commonly used marker in ovarian tumors, there are limited data on the association between the ovarian volume and/or peritoneal fluid on serum CA-125 levels. There are only a few studies evaluating the correlation between CA-125 levels and ovarian volume [3-5]. This study was designed to clarify the relationship between ovarian volume and serum CA-125 levels. For this purpose, serum CA-125 levels were compared among the cases with benign ovarian neoplasm without peritoneal fluid, EOC with or without peritoneal fluid, and large hyperstimulated ovaries with or without peritoneal fluid.

Materials and Methods

This study consisted of 122 patients followed by the Department of Obstetrics and Gynecology, Faculty of Medicine, Cukurova University between June 2010 and March 2012. It was approved by local ethics committee and informed consent was obtained from all patients for participating in the study. Exclusion criteria included a) patients with endometriosis, endometrioma, pelvic inflammatory infection, pregnancy, chronic renal disease, cardiac failure, acute lower respiratory system infection, pleurisy, tuberculosis, history of non-gynecologic malignancies, autoimmune disease including systemic lupus erythematosus, rheumatoid arthritis, which are known to increase CA125 level and b) patients with premature ovarian failure, ovarian-paraovarian cysts or neoplasms in the case of infertility, and those who were older than 40 years. Furthermore, the patients with non-epithelioid, mucinous, endometrioid, and pure clear cell type ovarian cancers were excluded.

Patient groups

There were four groups based on the diagnosis. Group 1 consisted of 38 patients with benign ovarian neoplasms, who were evaluated surgically and histopathologically. Of these patients, seven patients had serous cystadenoma, six had mucinous cystadenoma, 15 patients had dermoid cyst, five patients had ovarian fibroma, and five had simple ovarian cyst. Group 2 consisted of 39 patients with EOC, who were categorized according to the FIGO classification system. Six patients were at the early stage (1 and 2) and 33 patients were at the advanced stage (3 and 4) of the disease. The amount of peritoneal fluid was determined during operation and classified as mild (less than one litre), moderate (one to three litres) and massive (≥ four litres). Only one patient had low grade serous carcinoma, and the others had high grade serous (n=33) and serous plus clear cell carcinoma (n=5). Thirty-three patients had peritoneal carcinomatosis and 32 patients had...
peritoneal fluid. Group 3 consisted of 34 primary infertile patients who were admitted to infertility clinic and underwent controlled ovarian hyperstimulation (COH) for in-vitro fertilization (IVF). Ovarian volume for these cases was evaluated with transvaginal sonography. The patients in Group 3 received gonadotropins with long luteal protocol. Ovarian volume and CA-125 levels were determined before COH (Group 3-A) and after the hyperstimulation, on the day of human chorionic gonadotropin (hCG) (Group 3-B). Group 4 consisted of 11 patients with moderate to severe ovarian hyperstimulation syndrome (OHSS) classified according to Golan [6]. Group 4 was used as a model for increased ovarian volume and the presence of the peritoneal fluid. Ovarian volume and serum CA-125 levels were determined at admission to the hospital. Ovarian volume was calculated using the prolate ellipsoidal formula (L*H*W*0.523) and histomorphometric evaluation methods for Groups 1 and 2. Bilateral ovarian volume was evaluated with two-dimensional ultrasonography and the mean of the bilateral ovarian volume measurements was used in the ovarian volume data in Groups 3 and 4. Serum CA-125 concentrations were analyzed at the central laboratory in the present hospital with chemiluminescent immunoassay kits (OV monitor ref no: 386357).

Statistical Package for Social Sciences (SPSS version 16.0) was used for statistical analysis. Kruskal Wallis and Mann Whitney U tests for non-parametric data were preferred to determine the differences in terms of single and plural comparisons. The comparisons were made using Wilcoxon Signed Ranks test for Group 3 before and after COH. Correlations were assessed through the Spearman Correlation Coefficient test. Data were shown as mean ± SD, median, and min-max value. A significance level of 0.05 (two-sided $p$ values < 0.05) was used in all tests.

Results

There were 122 cases in this study. Mean and median age, ovarian volume, and CA-125 levels and comparison of these variables among the groups are shown in Table 1. Figure 1 shows scatters of CA-125 and ovarian volume for the groups. Serum CA-125 levels were found to be significantly higher in Group 2 than in Group 1 ($p = 0.001$) although ovarian volume was similar in both groups ($p = 0.151$). Increased ovarian volume by COH did not change the serum CA-125 levels in the infertile group ($p = 0.555$). However uncontrolled hyperstimulation of the ovaries and the presence of peritoneal fluid (Group 4) caused an increase in the levels of CA-125 ($p = 0.001$). Comparisons of serum CA-125 levels and ovarian volume in terms of the presence and amount of peritoneal fluids and peritoneal carcinomatosis are shown in Table 2. The presence of peritoneal fluids and peritoneal carcinomatosis were associated with high levels of CA-125 but ovarian volume was not different among the subgroups.

The correlations of ovarian volume and CA-125 levels in the groups are shown in Table 3. There was no relationship was observed in the group with ovarian cancer and COH group ($r = 0.083$ and 0.056, respectively).

### Table 1. — The comparison of ovarian volume and serum CA-125 levels among the study groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=38)</th>
<th>Group 2 (n=39)</th>
<th>Group 3A (n=34)</th>
<th>Group 3B (n=34)</th>
<th>Group 4 (n=11)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.0 ± 17</td>
<td>58.3 ± 13.4</td>
<td>30.2 ± 5.4</td>
<td>25.4 ± 2.1</td>
<td>25 (22-28)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Ovarian volume (cm³)</td>
<td>164.7 ± 221</td>
<td>193.1 ± 339</td>
<td>7.9 ± 4.5</td>
<td>39.4 ± 23</td>
<td>169.1 ± 92.1</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>92.5 (1-988)</td>
<td>35 (1-1569)</td>
<td>7.7 (1.8-23)</td>
<td>33 (13-115)</td>
<td>140 (60-374)</td>
<td>0.151**</td>
</tr>
<tr>
<td></td>
<td>24.5 ± 17.8</td>
<td>945 ± 1327</td>
<td>21.0 ± 17.2</td>
<td>21.4 ± 13.4</td>
<td>780 ± 494.5</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>14 (3-64)</td>
<td>414 (8-4509)</td>
<td>14 (3-64)</td>
<td>19.3 (6-71)</td>
<td>547 (256-1632)</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

*: Kruskal-Wallis Test, **: Mann Whitney U Test between Groups 1 and 2, ***: Wilcoxon Signed Ranks test between Groups 3A and 3B, ****: Man Whithney U Test between Groups 3B and 4.
The relationship between ovarian volume and serum CA-125 levels

Discussion

This study was designed to investigate the association between serum CA-125 levels and ovarian volume in different patient populations with ovarian enlargement. The study groups included both large neoplastic and non-neoplastic ovaries with or without peritoneal fluid. In the literature review, the authors found only a few studies evaluating the association between CA-125 levels and ovarian volume. Van Altena et al., [3] investigated CA-125 level in the patients undergoing prophylactic bilateral salpingo-oophorectomy (BSO) and found that ovarian volume did not contribute to the levels of CA-125. Granberg et al. [4] did not demonstrate a correlation between CA-125 levels and ovarian volume in 106 women with different cycle of phase and in postmenopausal status. The present authors did not find a correlation between ovarian volume and serum CA-125 levels in the patients with EOC. Additionally there was no association between CA-125 and enlarged ovaries by COH. High CA-125 levels were found to be related to EOC and the presence of peritoneal fluids and peritoneal carcinomatosis. These findings suggest that CA-125 is a peritoneal marker.

In the literature review, there is a controversy regarding the relationship between CA-125 and COH. However, in this study the authors did not find an association between serum CA-125 levels and COH. This result shows concordance with the previous report by Vujisic et al. [7]. The relationship between CA-125 level and OHSS had been discussed in the literature [8]. For the OHSS group, high levels of CA-125 were found in this study. The present results suggest that the presence of peritoneal fluid may be responsible for elevated CA-125 levels in OHSS.

It is very well known that the presence and amount of the peritoneal fluid and peritoneal carcinomatosis affect the levels of serum CA-125 [9,10]. Topalak et al., [11] reported that high serum CA-125 levels were closely related to the presence of serosal fluids and serosal involvement. In another study, five to six fold higher serum CA-125 levels were found in the ovarian cancer patients with peritoneal fluids as compared with the patients without peritoneal fluids [5]. Considering the results of this study, serum CA-125 levels in the ovarian cancer patients with peritoneal carcinomatosis were approximately six fold higher than in those without peritoneal carcinomatosis and high serum CA-125 levels were closely correlated with the peritoneal extension of the disease. In another study, it was reported that peritoneal carcinomatosis did not play an important role on serum CA-125 levels [10]. The present authors found higher serum CA-125 levels for the patients with peritoneal fluids and peritoneal carcinomatosis than for the patients without peritoneal fluids and/or peritoneal carcinomatosis. This finding suggests that serum CA-125 levels are associated with the presence of the peritoneal fluids and peritoneal carcinomatosis rather than ovarian volume.

Table 2. — The comparison of serum CA-125 levels and ovarian volume according to the presence and amount of peritoneal fluids and peritoneal carcinomatosis in ovarian cancer patients.

<table>
<thead>
<tr>
<th>Group 2</th>
<th>Ovarian volume (cm³)</th>
<th>CA-125 (IU/l)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascite absent (n=7)</td>
<td>268 ± 223</td>
<td>442.6 ± 851.1</td>
<td>0.368*</td>
</tr>
<tr>
<td>Ascite present-mild (n=12)</td>
<td>280.6 ± 455.3</td>
<td>471.7 ± 583.5</td>
<td>0.022**</td>
</tr>
<tr>
<td>Ascite present-moderate (n=9)</td>
<td>221.5 ± 406.9</td>
<td>1821 ± 1835.1</td>
<td>0.028**</td>
</tr>
<tr>
<td>Ascite present-massive (n=11)</td>
<td>26.9 ± 44.4</td>
<td>1064.8 ± 1427</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. — The correlations of ovarian volume and CA-125 levels in the study groups.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3A-B</th>
<th>Group 4</th>
<th>All groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign ovarian neoplasm (n=38)</td>
<td>0.548**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malign ovarian neoplasm (n=39)</td>
<td>0.083</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-post induction COH (n=34)</td>
<td>0.056</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHSS (n=11)</td>
<td>0.619*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All groups (n=122)</td>
<td>0.264**</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Comparison of the ovarian volume, **: Comparison of the CA-125 levels.

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). OHSS: Ovarian hyperstimulation syndrome.
It has been shown many times that the levels of CA-125 are related to the stage of the ovarian cancer and some type of histologic subtypes [12]. Peritoneal fluids and peritoneal involvement are more frequent in the cases with advanced stage disease and higher CA-125 levels are found in the cases with serous tumors as compared to other epithelioid or non-epithelioid ovarian cancers. For this reason, the authors included the patients with serous ovarian cancer and excluded the patients with mucinous or other non-epithelioid ovarian cancers. Almost all of our ovarian cancer patients had advance stage disease (33/39), peritoneal carcinomatosis (33/39) and peritoneal fluid (32/39). These results have supposed that the presence and amount of peritoneal fluid and peritoneal carcinomatosis cause high levels of CA-125 in the patients with EOC.

The evaluation of ovarian cancer mass volume has distinct and serious limitations especially for the management of the ovarian cancer patients [13]. There are controversies in the literature on monitoring the ovarian cancer patients with serum CA-125 levels [14-16]. The present study results may contribute to the routine for monitoring serum CA125 levels in patients with ovarian carcinoma”. Int. J. Gynecol. Cancer, 2002, 12, 438.

In conclusion, the presence and amount of not ovarian volume but of peritoneal fluids and peritoneal carcinomatosis are related to serum CA-125 levels in EOC. This finding supports the discordance between CA-125 levels and ovarian volume shown by imaging methods.

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References


Address reprint requests to: Ü.K. GÜLEÇ, M.D.
Çukurova Üniversitesi Tıp Fakültesi
Kadın Hastalıkları ve Doğum A.D.
01330 Adana (Turkey)
e-mail: ukucukgoz@yahoo.com
Introduction

Carcinomas of the uterine cervix are frequently preceded by precancerous lesions denominated cervical intraepithelial neoplasias (CIN). These lesions may remain in a non-invasive phase for a long period of time, releasing abnormal cells that are detected by cytologic examinations [1].

GTPases Rho regulate a large variety of signal transduction pathways in eukaryotic cells [2, 3]. Twenty different GTPases Rho have already been identified in mammals [4]. Among the most widely studied members of this family are: RhoA (Ras homologous member A), RhoB (Ras homologous member B), Rac1 (Ras-related C3 botulinum toxin substrate 1) and Cdc42 (cell division cycle 42) [5].

Several studies have shown the involvement of GTPases Rho in most malignant neoplasias onset and progression, including acquisition of unlimited proliferative potential, survival, tissue invasion, establishment of metastasis, and stimulation of angiogenesis [4, 6].

Through the characterization of the expression pattern of GTPases RhoA, RhoB, and Cdc42, possible diagnostic biomarkers or target molecules to new modalities of treatment of uterine cervix neoplasias may be identified.

Materials and Methods

This study was approved by the Triângulo Mineiro Federal University (UFTM) Research Ethics Committee. Samples of neoplastic lesions of the uterine cervix of 44 patients subjected to biopsy in the Clinical Hospital of UFTM were used in this study. The cases were revised by a pathologist and classified as: CIN I (n=10), CIN II (n=10), CIN III (n=9), and invasive carcinoma (n=15).

For the immunostaining of GTPases RhoA, RhoB, and Cdc42, antibodies anti-RhoA, anti-RhoB, and anti-Cdc42 1 : 50 PB 0.1M/triton 0.2% were respectively used and incubated for 16 hours at room temperature. Non-specific sites were previously blocked with non-immune goat serum 1 : 10 PB 0.1M/triton 0.2% for an hour. The secondary antibody Goat anti-rabbit biotinylated IgG was incubated in 1 : 200 PB 0.1M/triton 0.2% concentration for 90 minutes. Avidin-biotin-peroxidase complex was used and the reaction was evidenced with 3,3'-diaminobenzidine - DAB.

The immunostained cells were assessed in three random fields by two independent observers. Staining was quantified according to the intensity of impregnation of the chromogenic substance and was considered negative (-), mild positive (+), moderate positive (++), and intense positive (+++). The assessment of immunohistochemical reactions was performed with microscope and figures were created with Photoshop 7.0.1.

The results were analysed using Graphpad Prism 5 program. Variance analysis (ANOVA) and Tukey’s post test were used for the expression pattern of GTPases Rho in the groups CIN I, II, and III; and t-test to compare the CIN dysplastic cells with the invasive carcinoma cells. The significance level determined was \( p < 0.05 \).

Results

The patients’ mean age in the different groups was: CIN I (32.80 ± 13.47 years), CIN II (24.70 ± 6.21 years), CIN III (34.67 ± 8.84 years), and invasive carcinoma (47.20 ± 16.56 years).
The mean staining intensity for RhoA was not different between dysplastic and superficial cells in the groups CIN I ($p = 0.0541$), CIN II ($p = 0.7730$), and CIN III (moderate marked); nevertheless, the staining of dysplastic cells appeared to gradually decrease. Also no difference ($p = 0.1166$) was observed between in the groups CIN I, II, and III. Interestingly, invasive carcinoma cells expressed significantly less RhoA ($p = 0.0002$), when compared with CIN dysplastic cells, and were seen moderately stained (Figure 1).

Although the mean staining intensity for RhoB in dysplastic and superficial cells were strongly marked in CIN I, a statistical difference was observed ($p = 0.0018$), whereas no statistical difference between the staining of dysplastic and superficial cells in CIN II ($p = 0.4486$) and CIN III ($p = 1.0000$) was observed. Also no difference ($p = 0.1659$) was observed between CIN I, II, and III. Cells of CIN dysplastic were moderately or strongly stained and a statistically significant difference ($p = 0.0046$) was seen when compared to invasive carcinoma cells, observed as weakly stained (Figure 2).

Dysplastic cells showed strong staining for Cdc42 in the groups CIN I, II, and III, however only in group CIN I ($p = 0.0225$) was a statistical difference observed, when compared to superficial cells. No significant difference ($p = 0.3756$) was observed between the mean staining intensity in CIN I, II, and III. Also no difference ($p = 0.0564$) was observed between CIN dysplastic cells and invasive carcinoma cells (Figure 3).

**Discussion**

This study showed that cells from CIN grades I, II, and III, and from invasive cervical cancer express GTPases RhoA, RhoB, and Cdc42. Interestingly, invasive cancer cells expressed less RhoA and Rho B than dysplastic cells from CIN grades I, II and III. Some studies have previously demonstrated the involvement of these proteins in altered signaling pathways in cell lines derived from cervical neoplasias [7-10]. Previously, RhoA was found overexpressed in high-grade squamous intraepithelial when compared to cervical epithelium without squamous intraepithelial lesions, and Cdc42 as not associated with low-grade or high-grade squamous intraepithelia [11].

The immunohistochemical analysis of lesions of the uterine cervix showed RhoA protein expression in all the groups observed. However, the cells from invasive lesions presented less intense staining than CIN I, II, and
GTPases Rho distribution in intraepithelial and invasive neoplasias of the uterine cervix

Figure 2. — Expression pattern of GTPase RhoB in intraepithelial and invasive carcinoma of the uterine cervix. Positivity of immunohistochemical reaction in brown and nuclei stained with hematoxylin. (A) CIN I; (B) CIN II; (C) CIN III; (D) invasive carcinoma; (E) negative control. Bar = 25 μm.

Figure 3. — Expression pattern of GTPase Cdc42 in intraepithelial and invasive carcinoma of the uterine cervix. Positivity of immunohistochemical reaction in brown and nuclei stained with hematoxylin. (A) CIN I; (B) CIN II; (C) CIN III; (D) invasive carcinoma; (E) negative control. Bar = 25 μm.
III cells. These results suggest that the expression of RhoA decreases as the lesions progress. Therefore, the action of GTPase RhoA in carcinomas of the uterine cervix and precursor lesions may be related to maintenance of cell differentiation. RhoA is involved in all stages of cancer progression, including transformation, survival, and proliferation of tumor cells [12]. The formation of stress fibers mediated by GTPases Rho in a cell line derived from cervical adenocarcinoma (HeLa) was demonstrated [7].

As for protein distribution in the different cell compartments, both cytoplasmic and nuclear immunostaining for GTPases RhoA were observed in this study. In the cytoplasm, RhoA regulates the signaling pathways involved in actin cytoskeleton remodeling. Nuclear expression may be associated with the activation of transcription factors. Localization of RhoA protein in the nucleus has already been demonstrated in cell lines derived from cervical adenocarcinoma (HeLa) [13]. A study using subcellular fractionation technique with HEK293 and HeLa cell lines showed that the necessary signals for RhoA activation originate in the nucleus [13].

The immunohistochemical analysis for RhoB protein in CIN I, II, and III groups and invasive carcinoma showed GTPase expression in all cases. The variation in staining intensity was low amongst CIN I, II, and III groups. However, in the invasive carcinoma group, the staining intensity was lower. RhoB protein has been regarded as a tumor suppressor. This GTPase is activated in response to several stress stimuli, such as damage to DNA or hypoxia and may inhibit tumor growth, cell migration and invasion, besides having proapoptotic functions [4].

The nuclear staining for RhoB was more intense in cells of undifferentiated aspect in the deeper layers of the epithelium in CIN I, II, and III cases. In the invasive carcinoma group the nuclear staining was lighter. In the present study there was staining for RhoB in the cytoplasm, nucleus, and plasmatic membrane of dysplastic cells, and predominantly cytoplasmatic in normal cells. RhoB seems to protect cells from malignant cervical neoplasia.

Depending on subcellular localization of the GTPases, different signaling pathways may be activated [14]. The GTPase RhoB is predominantly located in the plasmatic and endosomal membranes [15, 26], indicating that this GTPase plays a role in the endocytic signaling pathways, which favors the transport of signaling molecules to the nucleus, lysosomes, and cell surface [17, 18]. It was demonstrated that RhoB nuclear expression may be associated with DB1 transcription factor [19]. Despite being expressed in all eukaryotic cells, the participation of the Rho family proteins in biological processes may vary according to cellular type and extracellular matrix composition (ECM) [20]. Studies of neoplasias in other sites also demonstrated different results from the ones obtained in this paper.

In this study, the authors observed through immunohistochemical analysis that Cdc42 protein is more widely expressed than the other GTPases Rho analysed, either in precursor lesions or in invasive carcinoma of the uterine cervix. Moreover, the staining was intense in all the CIN I, II, and III groups studied, as well as in invasive carcinoma, suggesting that Cdc42 protein appears to be involved in the regulation of cell proliferation in intraepithelial and invasive cervical cancer.

One of the best known cellular functions of Cdc42 is to regulate cellular proliferation [3, 21]. It is known that Cdc42 protein may stimulate the transformation induced by Ras oncogene in vitro, probably due to its effect in the traffic and degradation of receptors [22]. In cell cycle, the authors observed that Cdc42 and its effector mDia3 are involved in the biorientation and stabilization of the attachment of spindle microtubules to kinetochores and also regulate chromosome alignment in metaphase [23]. Cdc42 expression is increased in breast tumors [24]. Therefore, Cdc42 contribution for cancer progression may be tissue-specific.

The results in the present study demonstrated that the majority of dysplastic and superficial cells presented cytoplasmatic staining for Cdc42, whereas only some cells showed nuclear staining for this protein. In consequence of the stimulus of some signaling molecules, such as the platelet-derived growth factor (PDGF), Cdc42 may migrate from the perinuclear region to cell periphery [25]. In this study there was cytoplasmic and nuclear staining in dysplastic cells for RhoB and Cdc42, which possibly indicates an association between both GTPases. Studies suggest that RhoB takes part in the activation of Cdc42 and may influence its localization, since the endosomal RhoB contributes to the redistribution of Cdc42 and for actin remodeling, which is an important event in cell migration [25].

Conclusions

The present results suggest that GTPases Rho participate in signal transduction pathways that may be involved with the regulation of biological processes, important to the progression of cervical neoplasias. RhoA is most likely important for maintenance of cell differentiation and RhoB protects cells from malignant cervical neoplasia. Cdc42 protein appears to be involved in the regulation of cell proliferation in intraepithelial and invasive cervical cancer.

Acknowledgments

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References


Address reprint requests to:
V.O. CREMA, M.D., D.D.S.
Structural Biology Department
Biological and Natural Science Institute
Federal University of Triângulo Mineiro
Praca Manoel Terra, 330
380150-050 Uberaba-MG (Brazil)
e-mail: virginiacrema@icbn.uftm.edu.br
Prospective study of hTERC gene detection by fluorescence in situ hybridization (FISH) in cervical intraepithelial neoplasia 1 natural prognosis

L. Li, W. Jiang, S.Y. Zeng, L.Y. Li
Maternal and Child Health Hospital of Jiangxi Province, Nanchang (China)

Summary

Objective: The aim of this study was to evaluate the prognostic significance of human chromosome telomerase gene (hTERC) overexpression in cervical intraepithelial neoplasia grade 1 (CIN1) natural prognosis. Materials and Methods: A total number of 2,499 women aged 30-49 years were screened in a population-based cervical cancer screening study from Jiangxi province rural sites. Pathology as the gold standard, 74 CIN1 patients first diagnosed by pathological examination were studied. They were observed by carrying the hybrid capture 2 (HC2) and hTERC genetic testing to understand the baseline. All observed women accepted voluntary follow-up. Follow-up for the first time in the first 12 months after screening included hr-HPV HC-2 testing. The second follow-up after screening the first 24 months, included hr-HPV HC-2, colposcopy + pathological examinations. Results: Of the 74 CIN1 cases that were followed-up for 24 months, seven cases (9.5%) progressed; 25 cases (33.8%) persisted, and 42 patients (56.7%) regressed. There was significant difference between hTERC amplification positive and negative group ($\chi^2 = 21.07, p < 0.001$). The risk of CIN1 persistence and progression in positive group was 3.24 (1.96-5.37) times higher than that in negative group. There was significant difference between hr-HPV persist positive and turn to negative or persistent negative group ($\chi^2 = 7.645, p = 0.006$). There was significant difference between hTERC gene and the initial test of hr-HPV both positive and both negative group ($\chi^2 = 4.544, p = 0.033$). Conclusion: There was a strong association between prevalence of hTERC gene overexpression and CIN1 natural prognosis. The follow-up results indicated that hr-HPV required repeat testing and that there was significant difference between hr-HPV persistent positive and turn to negative/ persistent negative group ($\chi^2 = 7.645, p = 0.006$). hTERC gene overexpression could prognoses cervical intraepithelial neoplasia 1 natural prognosis individually.

Key words: Fluorescence in situ hybridization; Cervical intraepithelial neoplasia 1; High risk human papilomavirus; Natural prognosis.
Table 1. — Predictive validity of hTERC amplification in CIN1 prognosis.

<table>
<thead>
<tr>
<th>hTERC</th>
<th>Follow-up result of CIN1</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progression/Regression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19/4</td>
<td>3.24</td>
<td>1.96-5.37</td>
</tr>
<tr>
<td>Negative</td>
<td>13/38</td>
<td>0.23</td>
<td>0.09-0.58</td>
</tr>
<tr>
<td>Total</td>
<td>32/42</td>
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<td></td>
</tr>
</tbody>
</table>

Table 2. — Predictive validity of initial test of hr-HPV in CIN1 prognosis.

<table>
<thead>
<tr>
<th>HR-HPV</th>
<th>Follow-up result of CIN1</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Progression/Regression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29/37</td>
<td>1.17</td>
<td>0.46-2.99</td>
</tr>
<tr>
<td>Negative</td>
<td>3/5</td>
<td>0.90</td>
<td>0.50-1.60</td>
</tr>
<tr>
<td>Total</td>
<td>32/42</td>
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<td></td>
</tr>
</tbody>
</table>

Table 3. — Predictive validity of test of hr-HPV in CIN1 prognosis.

<table>
<thead>
<tr>
<th>HR-HPV</th>
<th>Follow-up result of CIN1</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent</td>
<td>24/18</td>
<td>2.29</td>
<td>1.19-4.40</td>
</tr>
<tr>
<td>Turn to Negative</td>
<td>5/19</td>
<td>0.57</td>
<td>0.38-0.86</td>
</tr>
<tr>
<td>Persisted</td>
<td>3/5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>32/42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. — Predictive validity of hTERC amplification combined with initial test of hr-HPV in CIN1 prognosis.

<table>
<thead>
<tr>
<th>hTERC HR-HPV</th>
<th>Follow-up result of CIN1</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>18/3</td>
<td>2.29</td>
<td>0.92-5.69</td>
</tr>
<tr>
<td>Either Positive</td>
<td>11/34</td>
<td>0.65</td>
<td>0.23-1.83</td>
</tr>
<tr>
<td>Negative</td>
<td>3/5</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>32/42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3 (3p11.1-q11.1) would show a green fluorescence signal. For each specimen, 100 cells were evaluated. In a normal cell, the signal ratio of CSP3 to hTERC is 2:2, whereas in abnormal cells the ratio was 2:3, 2:4, 2:5, 3:3, 4:4, and so on. Therefore, a cell with three or more hTERC signals, regardless of the signal numbers of CSP3, would be considered as having an abnormal signal pattern. For a positive result with hTERC amplification, the percentage of cells with normal signal patterns should be more than the threshold value. Cervical cells from additional 20 normal individuals, with both normal cytopathologic test and negative HPV test, were used to establish the threshold value. The threshold value was mean and three times standard deviation (SD) of the percentage of cells with abnormal signal patterns of these 20 specimens. In this study, the threshold value was 5.84%. Therefore greater than or equal to 6% by hTERC gene abnormal amplification was considered positive.

Statistical Analysis
For statistical analysis, the row × column chi square test (R × C ) test (SPSS, Version 13) was used to compare the positive rates between groups. The results were considered to be statistically significant at a p-value less than 0.05. All the p-values presented in the present study are two-sided.

Results
CIN1 natural prognosis
Of the 74 CIN1 cases followed-up for 24 months, seven cases (9.5%) progressed; 25 cases (33.8%) persisted, 42 patients (56.7%) regressed, five patients developed CIN2, and two patients developed CIN3.

hTERC amplification in association with CIN1 prognosis evaluations (Table 1):
In 74 CIN1 cases, hTERC amplification positive was observed in 23 (31.1%), in positive group progression or persistence cases were 19 (82.6%), regression cases were four (17.4%) after 24 months. In hTERC amplification negative group, progression or persistence cases were 13 (25.5%), and regression cases were 38 (74.5%). There was significant difference between positive and negative group (χ² =21.07, p < 0.001). The risk of CIN1 persistence and progression in hTERC positive group was 3.24(1.96-5.37) times higher than that in negative group.

hr-HPV in association with CIN1 prognosis evaluations (Table 2, 3)
In 74 CIN1 cases, hr-HPV first test positive was observed in 66 (89.2%), in persistent positive group progression or persistence cases were 29 (43.9%), and regression cases were 37 (56.1%) after 24 months. There was no significant difference between positive and negative groups (p = 0.976).

HR-HPV test were examined again in all 74 cases after 12 and 24 months, in persistent positive group progression or persistent cases were 24 of 42 (57.1%), and regression cases were 18 of 42 (42.9%) after 24 months. There was significant difference between hr-HPV persistent positive and turn to negative/persistent negative group (χ² = 7.645, p = 0.006). The risk of CIN1 persistence and progression in persistent positive group was 2.29 (1.19-4.40) times higher than that in turn to negative/persistent negative group.

hTERC amplification combined with initial test of hr-HPV in association with CIN1 prognosis evaluations (Table 4):
Analysis of 74 cases case hTERC gene and the initial test of hr-HPV, in both positive group progression or persistent cases were 18 of 21 (85.7%) and regression cases were 3 (14.3%). There was significant difference between both positive and both negative group (χ² = 7.645, p = 0.006). The risk of CIN1 persistence and progression in both positive group was 2.29 (0.92-5.69) times higher than both negative groups.
Discussion

In this study, hTERC amplification positive rate was 31.1% in 74 cases of CIN1, higher than other studies [5, 6]. It may be related to the fact that the study population was concentrated in cervical cancer high incidence areas of Jiangxi province. Many scholars have studied the hTERC gene amplification in association with cervical lesions [7, 8], but it has not been reported to be applied to predict the progression of cervical precancerous lesions in prospective study. In this study, hTERC gene was tested once and hr-HPV was tested three times; 56.1% of cases regressed, 43.9% of cases progressed or persisted after 24 months non-intervention follow-up. There was significant difference between hTERC amplification positive and negative groups in one-time test. However there was no significant difference between positive and negative groups in hr-HPV first test. Hr-HPV test were examined again after 12 and 24 months and there was significant difference between persistent positive and turn to negative/persistent negative group. Therefore hr-HPV as a means of detecting cervical precancerous lesions progress, requires longer-term follow-up and monitoring, but it faces many problems: lost at follow-up, high cost, and technique updates [9]. In this study there were 19 cases (19/23) in positive group; the RR value of hTERC gene positive predictive CIN1 was 3.24 (1.96-5.37) better than that of hr-HPV persistent positive 2.29 (1.19-4.40). From the results, it can be concluded that hTERC genetic testing as an individual test to predict disease progression is superior to the continuous test of hr-HPV and its specificity is worthy of recognition. hTERC gene overexpression could predict CIN1 natural prognosis individually.

Some studies indicated genomic integration of oncogenic hr-HPV and gain of the human telomerase gene TERC appear to be important associated genetic events in the progression of uterine cervical dysplasia to invasive cancer [10, 11]. The present findings demonstrated that 85.7% patients of hTERC combined with initial test of hr-HPV that both positively progressed or persisted, had a significant difference in both negative groups. Although oncogenic hr-HPV and gain of hTERC maybe associated with the progression of cervical dysplasia to invasive cancer, detection of hTERC amplification may be a useful test to predict CIN1 natural prognosis individually.

References


Address reprint requests to: L.Y. LI, M.D.
Gynecologic Oncology Department of Jiangxi Maternity and Child Hygiene Hospital 318# Bayi Road Nanchang 330006 (China) e-mail: lilingxnc@hotmail.com
Comparison of diagnostic methods for evaluation of postmenopausal bleeding

S. Nergiz, S. Demircan-Sezer, M. Küçük, H. Yüksel, A.R. Odabaşı, S.Ö. Altinkaya

Department of Gynecology and Obstetrics, Adnan Menderes University, School of Medicine, Aydın (Turkey)

Summary

Objective: To determine and compare diagnostic accuracy parameters of saline infusion sonohysterography (SIS), transvaginal ultrasonography (TVUSG), and hysteroscopy (H/S) based on histopathologic results which are accepted to be the gold standard in patients with postmenopausal bleeding (PMB). Materials and Methods: Forty-seven patients who applied to Gynecology clinic of Adnan Menderes University, School of Medicine with PMB complaint aged between 43-76 years were included to the study. Fractioned curettage (F/C) and H/S guided biopsy were used for endometrial sampling. Diagnostic accuracy parameters (sensitivity, specificity, and positive and negative predictive values) of different methods; TVUSG, SIS, and H/S based on histopathologic findings were investigated. Results: Specificity and sensitivity values calculated based on histopathologic results for all endometrial cavity lesions were found, respectively: 44.4% and 25% for TVUSG, 88.8% and 60.7% for SIS, and 100% and 77.7% for H/S. Conclusion: SIS is superior to TVUSG and as effective as H/S for assessment of endometrial cavity lesions in patients with PMB.

Key words: Postmenopausal bleeding; Transvaginal ultrasonography; Saline infusion sonohysteroscopy; Hysteroscopy.

Introduction

Postmenopausal bleeding (PMB) is vaginal bleeding after minimum one year without any menstrual bleeding. As endometrial malignancy may be responsible for PMB, identification etiology is important in evaluation of diagnosis. The most common reason of PMB (60-80%) is endometrial atrophy [1]. The other reasons are hormone replacement treatment (15-25%), endometrial polyps (2-12%), endometrial hyperplasia (5-10%) and endometrial cancer (5-15%) [1-2]. Although risk of endometrial malignancy is 1% under the age of 50, this risk reaches 15% after 50 years of age [3].

Transvaginal ultrasonography (TVUSG) is the first step examination in the evaluation of patients with PMB. The risk of malignancy increases with increase in endometrial thickness measured by TVUSG. Nearly 80% of patients with PMB, and also those with an endometrial thickness > 5 mm were found to have an endometrial pathology [4-6]. Saline infusion sonohysterography (SIS) is performed by administering serum physiologic to endometrial cavity. SIS increases the diagnostic sensitivity of TVUSG. Definitive diagnosis and golden standard in PMB is pathologic evaluation. Biopsies can be performed by directly imaging with hysteroscopy (H/S) or blind fractioned curettage (F/C) or office biopsy with pipelle [5-6].

In the postmenopausal period, although diffuse endometrial pathologies are mostly seen, focal lesions can also be detected. Focal lesions are: endometrial polyp, submucosal fibroids, and focal endometrial hyperplasia. Diffuse lesions are: endometrial malignancy and diffuse endometrial hyperplasia. The conventional method for endometrial sampling is F/C. All endometrial sampling techniques performed blind, including F/C, were reported to be insufficient for the diagnosis of focal endometrial lesions [7]. As F/C is a blinded procedure, histologic evaluation of 50 hysterectomy specimens after curettage showed that in 60% less than half of endometrial lining was reached with the curette and in 16% less than one-fourth [8]. The diagnostic accuracy value of F/C to recognize intrauterine pathologies was found in 60% [9].

The aim of the present study was to evaluate diagnostic accuracy of TVUSG, SIS, and comparing them with invasive procedure such as H/S, in the evaluation of PMB. Additionally, the authors examined the effectiveness of SIS, which is a minimally invasive procedure that can be performed in office conditions with an affordable cost, provides high patient comfort and satisfaction, and with minimal complication rate and does not require general anesthesia.

Materials and Methods

The present study was approved by local ethics committee of Adnan Menderes University School of Medicine, where the study was conducted. Informed consent was obtained from all participants.

Forty-seven patients who applied to the Gynecology Clinic with PMB complaint aged between 43-76 years were included in the study. The bleeding a year after the complete cessation of menses was evaluated as PMB. Patients with endometrial cancer, history of atypical endometrial hyperplasia or abnormal cervical cytology results were excluded from the study.
Before endometrial sampling, all the cases were recorded for demographics (age, body mass index, etc.), gynecological, and obstetric histories. TVUSG and SIS were performed in the same session by the same researcher. The ultrasonographic examinations were performed by an ultrasound machine with 6.5 mHz transvaginal probe.

The uterus was visualized longitudinally and axially, and measurement of myometrial and endometrial thickness and echogenicity were noted. Endometrial thickness was recorded in millimeters by measuring double layer of endometrium at the widest point. In the case endometrial thickness measured > 5 mm, it was suspected as endometrial hyperplasia. All the pathologies were recorded for size and localization. The lesions that were totally within the endometrial cavity and observed as hyperechoic, were considered as polyp, those which were associated with myometrium, scrolled endometrium, and reached the cavity and observed as isoechoic or hypoechoic than myometrium were considered as fibroids.

The cases evaluated with TVUSG were administered SIS procedure by the same researcher. Patients with vaginal infection detected prior to the process were given an appropriate treatment. After recovery from the infection, SIS were performed. The patient was instructed to empty the bladder. An appropriate size speculum was placed in lithotomy position, in sterile conditions. After vaginal disinfection with povidon iodine, cervix upper lip was held with a tenaculum, the catheter was inserted into the uterine cavity. To be sure that the catheter was within endometrial cavity, its balloon was inflated with one to two ml of saline. Tenaculum and the speculum were removed. After placing the TVUSG probe, appropriate section was found. Endometrial cavity was evaluated by giving approximately ten ml sterile serum physiologic to the endometrial cavity by the catheter. The endometrial area which is likely to be hidden by balloon was evaluated after deflation of the balloon and holding the catheter at the level of internal cervical os. SIS images were recorded. Catheter system was removed when the field of whole endometrial cavity was adequately assessed. The procedure took an average of three to ten minutes.

In SIS process, longitudinal and axial views of the uterus were obtained by TVUSG during saline solution instillation. Deformations of the central echo line, variability of endometrial echogenicity or circumscribed changes in the echogenicity of the uterine wall that impinged on the cavity were noted. Symmetrical and smooth endometrial shape was considered as normal. The lesions that were observed totally within the endometrial cavity, surrounded by serum physiologic except for endometrial roots were thought to be endometrial polyps. They were seen generally more hyperechoic than endometrium. Also lesions that were in the depths of endometrium, with same or less echogenicity with myometrium and partially filling the endometrial cavity when serum physiologic was applied were considered as submucous fibroids.

When endometrium surrounding anechoic saline was asymmetrical, thick, irregular, and the margin between endometrium—myometrium was intact, it was considered as endometrial pathology (hyperplasia or cancer).

After the women were evaluated by TVUS and SIS, surgical procedures were performed. The prediagnosis achieved with TVUS and SIS was compared with the pathology results of the specimens obtained with F/C or H/S which were accepted to be the gold standard for the definitive diagnosis. When a focal lesion was seen within the endometrial cavity H/S was performed, whereas in diffuse lesions F/C was applied. All the interventions were performed in operating room under general anesthesia. After application of an appropriate size speculum, following the vaginal disinfection by povidone-iodine; firstly endocervical canal was curetted and tissue sample was taken. Cervical os was dilated by Hegar’s dilators until rigid hysteroscope could pass through and be placed into endometrial cavity. Endometrial cavity was expanded with 1.5% glycine. Hysteroscopic-directed endometrial sampling was performed in women having any pathology. All of the specimens were examined by the same pathologist.

For statistical analysis, Statistical Package for Social Sciences (SPSS) Windows 11.5 version program was used. Descriptive characteristics were demonstrated as mean ± standard deviation (SD) or as a percentage where appropriate. In dependent group’s Student T-test, chi-square test and One Way ANOVA tests were used. A p value < 0.05 was accepted to be statistically significant.

Results

Demographic characteristics of the cases are shown in Table 1. In 17 cases, endometrial biopsies were performed with hysteroscopic observation and in 29 cases biopsies were performed with direct F/C. Only in one case SIS and H/S could not be performed due to severe cervical stenosis. In two cases mechanic dilatation during SIS was required due to cervical stenosis. The incidence of endometrial polyps and cancer was significantly increased in patients after 55 years and who had no menstruation greater than five years. No complication was observed during or after the interventions. None of the patients required termination of the procedure due to pain.

Distribution of endometrial lesions (endometrial polyp, cancer, hyperplasia) and diagnostic accuracy parameters of TVUSG, SIS, and H/S for endometrial pathologies is shown in Table 2. In TVUSG, nine patients (19.56%) were evaluated as normal, six patients (13.4%) were pre-diagnosed with endometrial polyp, two patients (4.34%) with submucosal fibroid, 26 patients (56.52%) with endometrial hyperplasia, and three patients (6.52%) with cancer. With SIS, 19 patients (41.30%) were evaluated normal, 14 patients (30.43%) were diagnosed with endometrial polyp, four patients (8.6%) with submucosal fibroid, four patients (8.6%) with endometrial hyperplasia, and five patients (10.8%) were diagnosed with cancer (Table 2). In this research, H/S was used in 17 patients. In H/S, endometrial cavity was observed to be normal in seven patients (41.17%). Of the endometrial polyps detected in eight patients (47.05%), two patients (11.7%) were diagnosed with

Table 1. — Demographic characteristics of cases.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number (n=46)</th>
<th>Mean ± SD and %</th>
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<td>Age (years)</td>
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</tr>
<tr>
<td>Menopause duration (years)</td>
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<tr>
<td>Parity</td>
<td>2.9 ± 1.7</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>Hypertension</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Other systemic diseases</td>
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</table>

Body mass index

S. Nergiz, S. Demircan-Sezer, M. Küçük, H. Yüksel, A.R. Odabaşı, S.Ö. Altınkaya
Comparison of diagnostic methods for evaluation of postmenopausal bleeding

Endometrial hyperplasia and two of them acancer was detected on the basis of endometrial polyp. Distributions of pathologic findings according to imaging methods are seen in Table 2. According to pathology results, endometrial cancer was seen in six (13.4%), polyp in 12 (26.08%), and hyperplasia in three (6.5%) patients. In 28 patients (60.86%) pathology results were reported to be normal (Table 2). Cancer was observed on the basis of endometrial polyp in two patients by H/S and the sensitivity of H/S for endometrial polyp was 100%.

Discussion

The diagnosis of the correct etiology of PMB is very important because bleeding is an important finding of endometrial malignancy in menopause. Endometrial cavity sampling should absolutely be performed in patients with PMB. TVUSG is the first step examination before endometrial cavity sampling to evaluate the uterine cavity. Irregularity of endometrial cavity and increase in thickness detected by TVUSG require further examination [10]. Approximately 80% of patients with PMB and also those with an endometrial thickness > 5 mm have an endometrial pathology and endometrial cancer is observed 3.7% to 17.9% of cases [4].

In the present study, 47 patients that applied with complaint of PMB were evaluated. SIS was applied in 46 of the cases, but it could not be performed in one patient because of severe cervical stenosis. These findings are compatible with literature [6,11]. It is suggested that frequency of cervical stenosis increased with age and menopause. This disorder caused the failure of the process [12].

Four different diagnosis were reported in the pathology reports of the study; normal endometrium, endometrial hyperplasia, endometrial polyp, and endometrial cancer. According to the pathology reports, the incidence of endometrial hyperplasia and cancer was similar, whereas the incidence of endometrial polyp was higher than reported in the published studies [13-15]. This discrepancy might be due to the mean age of patients included in the present study was higher than the aforementioned studies. In addition, the prevalence of endometrial polyp and cancer increased with age in the present study similar to the findings of the aforementioned studies.

TVUSG and SIS were performed in the same session to patients that applied with PMB. Although TVUSG provides a close examination of uterus and ovaries, it has a limited value for the evaluation of uterine cavity. For diagnostic accuracy parameters of TVUSG for intracavitary lesions, there are various published results. Sensitivity of TVUSG for intracavitary lesions varies between 48-96% and specificity between 68-95% [16-18]. In the present study, different from the published data, sensitivity of TVUSG was found to be 44.4% and specificity 25%. This discrepancy may be attributed to the over-diagnoses of endometrial hyperplasia by TVUSG. It may be suggested that differential

| Table 2. — Distribution of endometrial lesions (endometrial polyp, cancer, hyperplasia) and diagnostic accuracy parameters of TVUSG, SIS, and H/S for endometrial pathologies |
|---------------------------------|------------|------------|--------------|----------------|
| Polyp                          | Endometrial hyperplasia | Endometrial cancer | All pathologies |
| TVUSG1                          | 6          | 26         | 3            | 37             |
| Sensitivity                     | 80         | 66.6       | 50           | 44.4           |
| Specificity                     | 94.2       | 55.5       | 97.5         | 25             |
| PPV4                            | 66.6       | 11.1       | 75           | 59.2           |
| NPV5                            | 96.9       | 95.2       | 92.8         | 25.9           |
| SIS2                            | 14         | 4          | 5            | 29             |
| Sensitivity                     | 90.9       | 66.6       | 60           | 88.8           |
| Specificity                     | 88.2       | 95.3       | 97.5         | 60.7           |
| PPV4                            | 71.4       | 50         | 75           | 59.2           |
| NPV5                            | 96.7       | 97.6       | 95.1         | 89.4           |
| H/S3                            | 10         | 2          | 0            | 12             |
| Sensitivity                     | 100        | 0          | 100          | 100            |
| Specificity                     | 100        | 88.2       | 100          | 77.7           |
| PPV4                            | 100        | 0          | 100          | 80             |
| NPV5                            | 100        | 100        | 82           | 100            |
| Pathology                      | (26.08%)   | (6.5%)     | (13.4%)      | (39.14%)       |
| Results (%)                     | (12)       | (3)        | (6)          | (21)           |

1 USG: ultrasonography, 2 SIS: saline infusion sonohysterography, 3 H/S: hysteroscopy, 4 PPV: positive predictive value, 5 NPV: negative predictive value.

| Table 3. — Likelihood ratios of TVUSG, SIS, and H/S for uterine cavity pathologies |
|---------------------------------|------------|------------|-------------|
| Endometrial polyp               |            |            |             |
| LR+                             | 13.3       | 7.58       | 100         |
| LR-                             | 0.21       | 0.11       | 0           |
| Endometrial hyperplasia         |            |            |             |
| LR+                             | 1.46       | 13.2       | 0           |
| LR-                             | 0.61       | 0.35       | 1.13        |
| Endometrial cancer              |            |            |             |
| LR+                             | 16.66      | 20         | 1           |
| LR-                             | 0.51       | 0.41       | 1           |
| All intracavitary lesions       |            |            |             |
| LR+                             | 0.58       | 2.2        | 4.34        |
| LR-                             | 2.24       | 0.20       | 0           |

LR: likelihood ratio
diagnosis of hyperplasia, polyp, and submucous myoma is not possible with TVUSG. SIS is observed to be very effective in distinguishing these three items. [19-21]. Especially endometrial polyp located at cornual and cervical portion may be omitted by TVUSG. Doppler USG is recommended for differential diagnosis [22].

In this study as 5 mm was taken as the cut-off point in TVUSG, sensitivity and specificity for endometrial hyperplasia and cancer were found to be 66.6%, 55.5%, and 50%, 97.5% respectively. However, Karlsson et al. [4] reported that sensitivity and specificity for endometrial cancer was 96% and 68%, respectively. The endometrial thickness measured by TVUSG may not be a highly reliable parameter. Endometrial hyperplasia should be also evaluated by SIS as diagnostic accuracy parameters of this method were better. Since the use of TVUSG causes an increase in rate of surgical treatments and complications, it leads to psychological stress in patients and inappropriate use of healthcare costs, in other words will cause overtreatment. As a result, in order to avoid over diagnosis further examination with SIS is recommended for endometrial hyperplasia.

In the present study, sensitivity and specificity for endometrial polyp with TVUSG was found to be 80% and 94.2%, respectively. Sensitivity and specificity values of TVUSG for endometrial polyps were reported to be 21.9% to 93.8% [23-27]. In this study, some endometrial polyps were misdiagnosed as normal endometrium with TVUSG. Compatible with the previous published studies, TVUSG was found insufficient to evaluate the uterine cavity in patients with PMB since it had a low sensitivity, because endometrial polyps, particularly those small in size, were commonly hidden in the endometrium and observed as normal endometrium or endometrial hyperplasia. So they may have been compressed in the endometrium. To enlarge the endometrial cavity, giving fluid inside cavity in SIS, causes mobilization of the polyps and therefore could be more accurately observed. For this reason, as demonstrated in the present study, SIS was able to diagnose the endometrial polyps with a higher sensitivity and specificity rate. TVUSG, SIS should be performed if endometrial polyp is suspected. Consequently, SIS improves the sensitivity of TVUSG, however can miss very tiny polyps and endometrial sampling is recommended.

To evaluate the endometrial cavity lesions in patients with PMB, H/S was also performed in addition to TVUSG and SIS. Diagnostic availability parameters of H/S were found to be better than other diagnostic methods in the evaluation of all endometrial cavity lesions. Diagnostic accuracy parameters of H/S were low for endometrial hyperplasia and endometrial cancer. It can be explained that hyperplasia and cancer could not be diagnosed via imaging as were all histopathologic diagnoses. Besides, all endometrial cancers observed with H/S were found on the base of endometrial polyp.

In the present study, endometrial sampling was performed with H/S and F/C. Four endometrial polyps observed in this study with SIS were reported as normal epithelium in the pathology results. This discrepancy can be attributed to the fact that, endometrial sampling was performed by blinded F/C in three of the patients. It is suggested that sensitivity of F/C for diagnosis of intacavitary lesions was low as whole endometrium could not be sampled by this method. In histologic evaluation of 50 hysterectomy specimens after curettage showed that in 60%, less than half of endometrial lining was reached with the curette and in 16% less than one-fourth [8]. Additionally, Epstein et al. reported that the diagnostic value of F/C in focal lesions was quite low (59%) [28]. Lower sensitivity of F/C for diagnosis originated from that particularly pedicle and mobile polyps could not be removed during the procedure [29-30]. In the present study, one of the four cases had bleeding during the procedure and in this patient, it was supposed that clots, endometrial debris, and folds that could be in the uterine cavity were interpreted as polyp. Also in this study an endometrial hyperplasia case was missed with SIS. Similarly, Kroon et al. [31] reported that seven percent of the endometrial pathologies were skipped with SIS and this rate was particularly high in post menopausal women. In the present study, in 12 patients, endometrial polyp was detected and three patients had endometrial cancer which was determined on the base of endometrial polyp. All endometrial polyps were diagnosed with H/S. In none of the three patients, cancer was suspected in images of TVUSG, SIS, and H/S. As a result, the authors concluded that, SIS and H/S both could not differentiate endometrial polyp and malignancy, so the differential diagnosis of malign and benign lesions may not be done by these imaging methods. When a focal endometrial lesion is suspected with TVUSG, SIS should be performed and endometrial sampling should be added to the procedure [32].

In this study, endometrial malignancy was detected in six patients. Among the six patients diagnosed with cancer, particularly two patients with an intracavitary mass greater than four cm, it was observed that the uterine cavity was dilated quite difficultly. Similarly, Liifer-Narin et al. [33] indicated that the difficulty of enlarging uterine cavity during SIS, was observed in 71% in advanced stage endometrial cancer patients and 12% in early stage endometrial cancer patients.

There is limited data regarding performing SIS in suspicion of endometrial malignancy. Alcazar et al. [34] performed SIS, during laparotomy of 14 Stage-1 endometrial cancer patients and observed only in one case (7%) that the malign cells had spread through tubal fluid. This was observed when an excess of 10-20 ml fluid was given for SIS and it was suggested that if lesser amount of fluid was given, spreading would be less. In contrast, intra-abdominal irrigation fluid obtained during laparotomy in four of
endometrial cancer patients was determined benign for all cases. Also there was no tubal spreading detected. These results demonstrate that, SIS might also be used for PMB patients with suspicion of malignancy, however further researches are needed.

As a consequence; SIS is superior to TVUSG and is as effective as H/S for evaluating endometrial cavity lesions in PMB. It has been demonstrated that for detecting the intracavitary lesions in PMB, SIS is a safe method that improves the diagnostic accuracy of TVUSG. SIS is also an effective method in the diagnosis of intracavitary lesions, particularly endometrial polyps. SIS is a technique which is non-invasive, performed without the need of anesthesia, with high patient compliance and comfort, which causes complications very rarely, less time-consuming, quick-to-learn, not effecting endometrium quality and histology, provides the evaluation of whole pelvis, and has sensitivity levels close to H/S. For this reason, mainly during SIS instead of H/S, a biopsy method that can sample the endometrial cavity and can be used in office conditions is needed. If such a biopsy method and equipment together with SIS can be developed, it can be an alternative to H/S which is invasive, may require anesthesia, have a certain rate of complication, and risk. Besides, utilization of SIS is predicted to be cost-effective and expected to reduce government healthcare expenditure in the evaluation of PMB.

References


Address reprint requests to:
S. NERGİZ, M.D.
Adnan Menderes Üniversitesi Tıp Fakültesi
Kadın Hastalıkları ve Doğum Anabilim Dalı
Aydın (Turkey)
e-mail: sumeyranergiz80@gmail.com
Significance of combined detection of p53 and FHIT in cervical carcinoma diagnosis

C.X. Du1, S.Q. Li2, A.H. Wang1, Y. Wang1

1Department of Obstetrics and Gynecology, The First Affiliated Hospital of He'nan University of Science and Technology, Luoyang
2Department of Obstetrics and Gynecology, The People’s Hospital of Luoshan Xian, He’nan Province (China)

Summary

Purpose: To explore the significance of combined detection of p53 genes and fragile histidine triad (FHIT) genes in cervical carcinoma. Materials and Methods: Specimens taken from 161 cases invasive carcinoma, 23 cases carcinoma in situ or cervical intraepithelial neoplasia III (CIN III), 74 cases CIN I - II, 25 cases normal cervical tissue, and 32 cases tumor–adjacent tissues were processed by immunohistochemistry to determine the expression of p53 and FHIT genes. The results of the combined detection were compared for clinical diagnostic value of cervical carcinoma diagnosis. Results: The p53 gene, FHIT gene and the two genes combined examination of cervical carcinoma diagnostic sensitivity were: 65.8% (121/184), 66.3% (122/184), 90.2% (166/184), respectively. There were no significant differences between the p53 gene and the FHIT gene detected (p > 0.05). Combined detection of the two gene were more sensitivity than single detection, the difference was significant (p < 0.001). Although diagnosis specificity had dropped somewhat, no significant statistical appeared (χ² = 0.022, p > 0.05). Conclusion: Combined detection of p53 genes and FHIT genes can increase the sensitivity diagnosis and specificity diagnosis for early cervical carcinoma and precancerous lesions has a positive meaning.

Key words: Cervical carcinoma; CIN; p53 genes; FHIT gene.

Introduction

Cervical carcinoma is the second most common cancer in woman worldwide, with some 500,000 new cases and 250,000 death each year [1]. There is now a general consensus that cervical carcinoma occurrence with the results of multi-factor and multi-gene interaction. The p53 play a role as a tumor suppressor gene, which has been considered. The p53 can induce apoptosis in some cells. Cells expressing mutant p53 have lost the ability to arrest the cycle and show enhanced genomic instability [2, 3]. Recent studies have shown that the fragile histidine triad gene (FHIT) as a tumor suppressor gene is closely related with the cervical carcinoma [4-6]. Deletions in the FHIT gene have been observed in several types of tumor, particularly those resulting from exposure to environmental carcinogens such as lung, kidney, esophageal, head and neck, stomach, and cervical cancer [7]. In this study, p53 and FHIT proteins were detected by immunohistochemistry in patients with cervical cancer, in order to explore the clinical significance of early diagnosis of cervical carcinoma with combined detection application.

Materials and Methods

Tissue specimens

Paraffin blocks (cases) were obtained from conization and hysterectomy specimens in this study. Specimens were collected from September 2008 through to July 2010 at the First Affiliated Hospital of He’nan University of Science and Technology. According to the WHO grading histopathological classification, there were 161 cases invasive carcinoma, 23 cases carcinoma in situ/cervical intraepithelial neoplasia III (CIN III), 74 cases CIN I - II, 25 cases normal cervical tissue, and 32 cases tumor–adjacent tissues. All the patients were not treated by radiotherapy, chemotherapy or any other therapy. The mean age of the patients was 36.26 years with a range from 26 to 73 years. The study was approved by the local ethical board and patient confidentiality was protected. The specimens were fixed in 10% formalin dehydrated and embedded in paraffin. Main reagents included rabbit anti-human FHIT monoclonal antibody, rabbit anti-human P53 monoclonal antibody, streptavidin-biotin – peroxidase hypersensitivity (SP) kit, concentrated DAB kit.

Immunohistochemistry

Four μm thick sections were cut onto silanized glass slides and air-dried overnight at room temperature. Then sections were dewaxed in xylene and rehydrated through graded alcohol and incubating the slides for 15 min in three percent hydrogen peroxide quenched endogenous peroxidase activity. Sections for microwave antigen retrieval pretreatment (FHIT, p53 antibodies) were immersed in citrate buffer. They were irradiated twice in a domestic microwave oven (800 W) at full power for four minutes and then left to cool for 15 minutes in the hot buffer at room temperature. Added primary antibody working solution (FHIT antibody, P53 antibody) was refrigerated overnight at 4°C. PBS, plus two anti-biotin working solution, 37 °C incubator for 30 minutes. PBS wash. Plus three anti-horseradish peroxidase-labeled streptavidin working solution, 37 °C incubator for 30 minutes. PBS wash. DAB color 8 minutes, terminate the color reaction. Hematoxylin, n-butanol dehydration. Mounted with neutral gum after dried film. Experimental section Pieces of strict consensus. With PBS buffer instead of primary antibody as negative control. FHIT positive for FHIT expression was a known photograph of normal breast tissue. All sections were performed with the universal labeled streptavidin-biotin
method according to the manufacturer’s instructions. Positive staining was detected with diaminobenzidine substrate solution, and nuclei were counterstained with hematoxylin. Immunostaining of all sections was evaluated for positive or negative staining as follows: −, 0; +, weak with <10% positive cells; ++, >10% but <50% strongly positive cells; and +++, >50% strongly positive cells. Negative immunoreactivity was scored as either − or +, and positive immunostaining as ++ or +++ [8].

Statistical analysis

Data of p53 gene mutation and FHIT gene loss statistical analysis was performed using SPSS, version 17.0. \( \chi^2 \) was used to calculate statistical significance; a \( p \) value less than 0.05 (\( p < 0.05 \)) was considered statistically significant.

Results

Table 1 shows each group of patients’ p53 protein and FHIT protein union examinations. The positive expression of p53 was mainly located in the nucleus, with diffuse pattern, and with no expression in normal cervical tissue. Immunostaining for FHIT protein showed cytoplasm localization brown granules of diffuse or focal distribution. All normal cervical tissue was strongly positive for FHIT expression, and no expression was reduced or absent. Of the specimen joint detection of p53 gene mutation and FHIT gene deletion, while the positive rate of two genetic testing in the normal cervix group, CIN I - II, carcinoma in situ/CIN III, invasive carcinoma, tumor–adjacent tissues were: 0%, 25.8%, 30.4%, 37.3%, 0%, respectively. Positive rate of any one of the two genes were 0%, 56.8%, 86.9%, 90.7%, 9.4%. With the degree of malignant cells increasing, the positivity of the two genes protein were increased in cell-layer level of immunostaining. The positive rate of combined detection of two genes were significant difference between normal tissue and CIN I - II (\( \chi^2 = 21.594, p < 0.001 \)); were significant different between tumor–adjacent tissues and CIN I - II (\( \chi^2 = 14.715, p < 0.001 \)); were significant different between CIN I - II and carcinoma in situ / CIN III (\( \chi^2 = 11.002, p < 0.001 \)); were no significant difference between carcinoma in situ/ CIN III and invasive carcinoma (\( \chi^2 = 0.022, p > 0.05 \)).

Combined detection of two genes were both positive to cervical carcinoma diagnosis sensitivity which was 36.4% (67/184) and specificity was 77.9% (67/86); while to cervical carcinoma and cancer precursor lesion diagnosis sensitivity was 33.3% (86/258) and specificity was 100% (86/86). Detection of p53 gene and/or FHIT gene were positive to cervical carcinoma and precancerous lesions of the sensitivity of 80.6% (208/258), specificity 98.6% (208/211) were 80.6% (208/258) and specificity were 98.6% (208/211). The p53 genes mutations, FHIT genes deletions, p53 genes mutations or FHIT genes deletions, p53 genes mutations and FHIT genes deletions 86 67 19 77.9%.
FHIT genes deletions, p53 gene mutations and FHIT genes deletions for cervical cancer diagnostic specificity were as follows: 85.5% (121/141), 73.5% (122/166), 78.7% (166/211), and 77.9% (67/86). The difference was not statistically significant ($\chi^2 = 6.997, p > 0.05$) (Tables 2 and 3).

Therefore combined detection of p53 genes mutations and FHIT genes deletions can increase the sensitivity diagnosis and specificity diagnosis to early cervical carcinoma and pre-cancerous lesions.

Discussion

Many methods currently exist to detect cervical carcinoma, such as Pap test, tests for human papillomavirus (HPV), colposcopy, and biopsy, in addition to tumor microvessel density (MVD), serum markers and telomerase detection, etc. As molecular biology research advances, the understanding of pathogenesis of cervical cancer gradually increases. High-risk HPV infection appears to be the leading cause of carcinoma of the uterine cervix [9], but the HPV-positive woman does not necessarily develop cervical carcinoma, suggesting that there may also be the presence of other risk factors involved in cervical cancer. It has been demonstrated that tumor suppressor gene (TSG) inactivation is the most important molecular basis of tumors. p53 genes and FHIT gene were TSG closely related with the cervical carcinoma. The p53 gene located on chromosome 17 encodes a $M_t$ 53,000 protein that is involved in cell growth regulation frequently found to be mutated in human cancers. The wild form of p53 protein half-life is very short, and it cannot be detected by immunohistochemistry [10, 11]. However, overexpressed or mutated p53 proteins have a longer half-life and can be recognized by immunohistochemistry [12]. Chromosome arm 3p is re-arranged in many tumor types, including cervical carcinomas. Putative tumor-suppressor genes on 3p have been proposed, including the FHIT gene, which maps to chromosome band 3p14.2 [5]. Several studies found that loss of FHIT expression in HSILs could serve as a useful marker of high-grade preinvasive lesions that have an increased likelihood of progression to invasive carcinoma [12,13].

In this study combined detection of the two gene were more sensitivity than single detection and the difference was significant ($p < 0.001$). Although diagnosis specificity had dropped somewhat, no statistical significance appeared ($\chi^2 = 0.022, p > 0.05$).

Combined examination of the p53 gene mutation and the FHIT gene deletion may enhance cervical carcinomas’ earlier diagnosis, sensitivity, and specificity. Combined examination can help to reduce the probability of cervical carcinoma missed diagnosis and benefit outpatient screening.

References


Address reprint requests to:
CHEN XIANG DU, M.D.
Department of Obstetrics and Gynecology
The First Affiliated Hospital of He’nan University
of Science and Technology
Jianxiqiu Jinghua Road 24#
Luoyang 471003 (China)
e-mail: dxc5668@126.com
Clinical usefulness of concentrated ascites reinfusion therapy (CART) for gynecological cancer patients with refractory massive ascites due to cancerous peritonitis

S. Togami, S. Hori, M. Kamio, T. Matsuo, M. Yoshinaga, T. Douchi

Department of Obstetrics and Gynecology, Faculty of Medicine, Kagoshima University, Kagoshima (Japan)

Summary

Purpose: Cell-free and concentrated ascites reinfusion therapy (CART) is intended to treat patients by ultrafiltration and reinfusion of their refractory ascites. In the CART system, bacteria and cancer cells in removed massive ascites are filtrated. Then, water is removed in the condenser, resulting in a higher protein concentration. The purpose of this study was to assess the clinical usefulness of CART in the treatment of refractory massive ascites in patients with cancerous peritonitis.

Materials and Methods: CART was performed 13 times in four patients with ovarian and endometrial cancer. Results: Autologous protein with a higher concentration was intravenously administered. The amount of aspirated and condensed ascites was 3,190 ± 1,086 ml (975 - 4,500 ml) and 538 ± 249 ml (100 - 860 ml), respectively. Condensed albumin, albumin concentration, and concentration time were 43.2 ± 25.8 g, 8.2 ± 3.3 g/dl, and 73.3 ± 24.8 min (28 - 122 min), respectively. CART was effective in maintaining serum albumin concentrations, and it is possible to repeat infusion. During CART, patients performance status was 1-2 and vital signs were stable except for mild elevations in body temperature. Daily life was maintained without serious side-effects.

Conclusions: The use of CART for gynecological cancer patients with refractory massive ascites due to cancerous peritonitis contributes to improvements in quality of life and relief of symptoms. With autologous infusion of condensed ascites, patients can avoid infection, allergic reactions, and administration of expensive blood products.

Key words: Cell-free and concentrated ascites reinfusion therapy; Gynecological cancer; Refractory massive ascites.

Introduction

Refractory ascites associated with cirrhosis and cancerous peritonitis causes a strong sense of fullness in the abdomen, respiratory discomfort, and significant decreases in quality of life (QOL). Abdominal drainage is valid, but its effect is transient and may also worsen nutritional status and immune status due to the loss of albumin and globulin, making it easier for patient to be readmitted due to the re-accumulation of ascites.

Cell-free and concentrated ascites reinfusion therapy (CART) is a technique which recovers protein components (albumin and globulin) from ascites after removal of cancer cells and bacteria using a filter. Recovered protein components can be reused in the blood vessels of the patient. CART has a long history in Japan. In 1977, Inoue et al. [1] reported a filtration concentration method with two kinds of hollow fibers for cancerous ascites. However, this system required complicated circuit and membrane clogging tending to occur in cancerous ascites. In 2011, a simpler and higher safety procedure was improved by Matsushita et al. [2] Their system includes a membrane cleaning function and enabled the processing of more ascites in a shorter period.

Advanced ovarian cancer is often accompanied by refractory ascites, which is closely associated with delays in commencing chemotherapy and the loss of QOL. In the present study, the authors investigated the clinical usefulness of CART for gynecological cancer patients with refractory massive ascites due to cancerous peritonitis.

Materials and Methods

Four patients with refractory ascites (two with ovarian cancer and two with endometrial cancer) were enrolled in this study. All subjects underwent 12 CART at the Department of Obstetrics and Gynecology, Kagoshima University Hospital between December 2011 and April 2012.

Before abdominal paracentesis, a safe and effective puncture site was determined by abdominal ultrasound tomography, and paracentesis was then performed with a 16-G needle.

As much ascitic fluid as possible was collected in a collection bag by gravity flow. For ascites filtration and concentration, AHF-MOW and AHF-UP were used. CART in the present hospital was connected to the coupler in the membrane of the AHC-UP. This system has a concentrated 4,000 ml/hour water removal speed set to sink the dialysate. The concentrated fluid was intravenously administered with drip infusion at a speed of 100–150 ml/h. Patients with large amounts of endotoxin in their ascites were excluded from this study.

Results were expressed as the mean ± standard deviation. The significance of changes due to the treatment was evaluated by the t-test. A p < 0.05 was considered significant.

Results

In this study, four patients with refractory ascites underwent 12 treatments of CART. Patient characteristics...
are summarized in Table 1. A total of 3,190 ml (range 975 - 4,500) of ascites was filtrated and concentrated to 538 ml (range 100 - 860). The concentration of albumin in the concentrated fluid was 8.2 g/dl (range 2.5 - 12.6) and the average time to process the fluid collection was 73.3 min (range 28 - 122). Significant increases in plasma albumin concentrations were evident after CART ($p = 0.02$).

All patients complained of strong abdominal distension due to massive ascites. After CART, they could undergo anti-cancer chemotherapy without any abdominal distension and QOL was improved.

### Discussion

Progression and recurrent gynecological cancer is accompanied by abdominal distention due to a large amount of ascites. Patients with massive ascites often face problems such as reduced QOL and treatment discontinuation in clinical practice. Abdominal paracentesis is chosen for the treatment of refractory ascites, but the effect has been transient. Therefore, nutritional status and immune status due to the loss of albumin are worsened. Alternatively, a peritoneo-venous shunt was reported for the treatment of refractory ascites[3, 4]. However, this treatment may bring about many serious complications including coagulation abnormalities, sepsis, and volume overload [5, 6].

Using cell free and concentrated ascites reinfusion therapy as key words, the authors conducted a Medline search of articles on CART in the English literature and found only eight reports. Reinfusion of ascites has been studied primarily as a therapeutic method for refractory ascites in liver cirrhosis [7]. CART can be performed safely in patients with ascites due to malignant tumors. After cell components such as bacteria, blood cells, and cancer cells are removed, concentrated ascites can be reinfused. The current CART system became available from 1997, and its application was permitted by the Japanese National Insurance Scheme in 1981.

In the present study, the authors found that plasma albumin concentrations after CART therapy were significantly higher than pre-treatment concentrations ($p = 0.02$). CART was effective in maintaining serum albumin concentrations, and it is possible to repeat infusion. Autologous protein with a higher concentration was intravenously administered. All patients were relieved of strong abdominal distension and could undergo chemotherapy without discomfort. CART has the advantage of saving blood products through the use of a patient’s own plasma protein, such that there is no risk of infection from an unknown pathogen.

In the current CART for refractory ascites associated with cancerous peritonitis, there were no serious side effects, with only one case of a slight fever. Some reports have shown the mechanism of the febrile reaction on reinfusion of ascites and its possible prevention. Katoh et al. [8] reported that in order to prevent fever occurring on reinfusion of ascites, a screen filter and depth filter were used in combination. Bernardi et al. [9] reported filters with a molecular weight cut-off of 300,000 decreased the incidence and grade of fever. Recently, it has been demonstrated that IL-6 exists in ascites [10], which may be considered as a cause of fever. The CART system used in this study is characterized to connect the coupler to the membrane of AHF-UP and flow the dialysate, and make the concentration such that the water removal rate of a 4,000 ml/h, monitoring dialysis pressure to a range that does not exceed 200 ml/h.

In conclusion, the authors performed CART for massive ascites in cancerous peritonitis with gynecologic cancer. They found that CART is safe and is expected to improve the symptoms, nutritional status, and QOL of

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**Table 1. — Outcome of CART in four cases of cancerous ascites**

<table>
<thead>
<tr>
<th>Patient (age) (diagnosis)</th>
<th>Ascites (ml)</th>
<th>Ascites concentration (ml)</th>
<th>Albumin concentration (g/dl)</th>
<th>Processing time (min)</th>
<th>Serum albumin (g/dl) (pre-reinfusion/after reinfusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 (52) - Ovarian cancer</td>
<td>3,475</td>
<td>390</td>
<td>6.1</td>
<td>78</td>
<td>1.8 / 2.1</td>
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<tr>
<td>Case 2 (63) - Endometrial cancer</td>
<td>975</td>
<td>110</td>
<td>3.0</td>
<td>30</td>
<td>2.0 / 2.4</td>
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<tr>
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<td>1,265</td>
<td>100</td>
<td>6.9</td>
<td>28</td>
<td>2.0 / 2.1</td>
</tr>
<tr>
<td>Case 3 (60) - Endometrial cancer</td>
<td>3,375</td>
<td>545</td>
<td>9.3</td>
<td>80</td>
<td>3.6 / 3.6</td>
</tr>
<tr>
<td>#2</td>
<td>2,275</td>
<td>370</td>
<td>12.6</td>
<td>60</td>
<td>3.3 / 3.3</td>
</tr>
<tr>
<td>Case 4 (63) - Ovarian cancer</td>
<td>3,400</td>
<td>550</td>
<td>-</td>
<td>65</td>
<td>2.3 / 2.6</td>
</tr>
<tr>
<td>#2</td>
<td>4,500</td>
<td>860</td>
<td>-</td>
<td>75</td>
<td>2.6 / 3.2</td>
</tr>
<tr>
<td>#3</td>
<td>4,400</td>
<td>860</td>
<td>2.5</td>
<td>97</td>
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<td>3,700</td>
<td>755</td>
<td>9.3</td>
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<td>780</td>
<td>9.9</td>
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<tr>
<td>#6</td>
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<td>520</td>
<td>9.6</td>
<td>80</td>
<td>3.2 / 3.2</td>
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<tr>
<td>#7</td>
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<td>620</td>
<td>12.6</td>
<td>80</td>
<td>3.2 / 3.2</td>
</tr>
<tr>
<td>Mean ± SD (standard deviation)</td>
<td>3,190 ± 1,086</td>
<td>538 ± 249</td>
<td>8.18 ± 3.3</td>
<td>73 ± 25</td>
<td>2.8 ± 0.6 / 3.0 ± 0.5*</td>
</tr>
</tbody>
</table>

* significance of the t-test: $p = 0.02$
patients. They recommend that CART be more actively used as a supplementary treatment for cancerous peritonitis and palliation.

References


Address reprint requests to:
S. TOGAMI, M.D., Ph.D.
Department of Obstetrics and Gynecology,
Faculty of Medicine, Kagoshima University,
8-35-1 Sakuragaoka,
Kagoshima 890-8520 (Japan)
e-mail: togami@m3.kufm.kagoshima-u.ac.jp
Serum and tissue level of YKL-40 in endometrial cancer

J.T. Fan, M.J. Li, P. Shen, H. Xu, D.H. Li, H.Q. Yan

Department of Gynecology and Obstetrics, The First Affiliated Hospital, Guangxi Medical University, Nanning (China)

Summary

Objective: Serum YKL-40 level is elevated in patients with several malignancies. This study was designed to assess the correlation between serum YKL-40 and the corresponding tissue expression in endometrial cancer (EC). Materials and Methods: Preoperative serum levels of YKL-40 were measured by enzyme-linked immunosorbent assay (ELISA) from 41 patients with EC, 27 patients with uterine myoma, and 30 healthy women. YKL-40 protein expression in tissue was determined by immunohistochemistry for patients with EC and patients with uterine myoma. Results: Median preoperative serum YKL-40 level was 157.2 μg/l (range 76.0 - 301.2) in EC compared with 86.6 μg/l (range 69.3 - 191.1) in uterine myoma, and 86.2 μg/l (range 52.1 - 201.1) in healthy women (p < 0.05). Of 41 patients with EC, 26 patients with elevated serum YKL-40 level statistically differed from the remaining 15 patients with normal serum YKL-40 level with respect to FIGO Stage, tumor grade, washing cytology, and serum CA125 (p < 0.05). The elevated preoperative serum YKL-40 significantly correlated with FIGO stage (p < 0.05) and tumor grade (p < 0.01). The percentage of positive YKL-40 tissue staining was higher in EC patients (34.1%, 14/41) than in uterine myoma patients (11.1%, 3/27) (p < 0.05) and was lower than that of elevated serum levels in EC (26/41, 63.4%) (p < 0.05). Conclusions: The elevated preoperative serum YKL-40 is related to stage and histologic grade of EC. The discordance between serum and tissue level of YKL-40 in EC indicates intrauterine tumor may not be the only source of serum YKL-40.

Key words: Serum YKL-40; Tissue YKL-40; Endometrial cancer.

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in the United States [1]. It has a better prognosis than cervical and ovarian cancers. However, patients who are diagnosed with advanced-stage disease (III or IV) have a poor prognosis, with a five-year survival rate of approximately 16%, compared with 95.8% in early-stage endometrial cancer [2]. Therefore it is important to detect the disease in its early stage so that the prognosis of EC is improved.

YKL-40, a secreted glycoprotein of the chitinase family, is a potential biomarker and has been previously described in many types of cancer cells. Elevated serum levels of YKL-40 are predictive of poor prognosis in patients with 13 different types of cancer [3]. In all of these different types of cancer, a higher serum YKL-40 is related to poorer prognosis. These studies suggest that this protein may play a fundamental role in the neoplastic process. Furthermore, serum YKL-40 has provided independent information on prognosis over clinical characteristics and biomarkers, such as serum CA125, LDH, PSA, CEA, and HER2 [4-7]. One hypothesis is that YKL-40 secreted by cancer cells and inflammatory cells surrounding and/or infiltrating the tumor may play a role in proliferation, activation, and differentiation of the fibroblasts/myofibroblasts surrounding the tumor [8]. Published studies have shown that elevated serum YKL-40 in EC may represent the shorter survival and a higher risk for disease progression [9]. The results from studies on YKL-40 tissue expression and the correlation to clinical-pathological parameters in EC [10] and ovarian cancer [11] showed that high immunoreactivity of YKL-40 protein was associated with advanced stage and histological grade. However data from the study of Hogdall et al. [12] demonstrated that tissue expression of YKL-40 was not related to the survival of ovarian cancer. To obtain the better understanding of the role of YKL-40 in EC, the relationship of tissue and serum YKL-40 in EC need to be defined. To the best of the authors’ knowledge, there are no published studies on the correlation between serum level and tissue immunohistochemical staining of YKL-40 in EC.

The aim of this study was to assess the correlation between elevated serum YKL-40 levels and the expression of YKL-40 in EC tissue, as well as its possible correlation to clinical pathological parameters in EC.

Materials and Methods

Study population

Serum levels of YKL-40 were examined in 50 patients with endometrial cancer, 27 patients with uterine myoma prior to definitive surgery and 30 healthy individuals at The First Affiliated Hospital of Guangxi Medical University from March 2009 to March 2011. Five patients with prior history of arthritis or other malignancy were excluded because rare histologic types may differ largely in biological behaviours, four patients with rare histologic type (two squamous cell carcinoma, one clear cell carcinoma, and one serous carcinoma) were excluded from this study. The remaining 41 patients with EC were surgically staged according to the FIGO 2009 surgical staging system. The corresponding tumor tissue samples (41 patients with EC and 27 patients with uterine myoma) were obtained from the pathology department of The First Affiliated Hospital of Guangxi Medical...
University. The median age of the healthy individuals were 40.9 years (range 35-51). The clinicopathologic profile of patients with EC are shown in Table 1. Patients undergoing gynecologic surgery at the present hospital had their tumor specimens and serum samples banked under the Medical Ethics Committee of GuangXi Medical University-approved tissue-acquisition protocol after signing informed consent.

**Blood collection and serum YKL-40 and CA125 analysis**

The preoperative blood samples from patients and from the healthy population were left to clot at room temperature for at least 30 minutes and were then centrifuged at 4°C for ten min at 3,000 rpm. The serum was stored at -80°C until tested. All samples were retested for CA125 in immunoradiometric assay (RIA). YKL-40 levels were determined in triplicate for all serum samples using the commercially available YKL-40 enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s protocol. The authors defined an abnormal YKL-40 value based on the mean value (99.2 μg/l) obtained from 30 healthy subjects, plus two standard deviations (95% CI), to be >106.6 μg/l. CA125 serum testing was performed in the clinical chemistry laboratory of The First Affiliated Hospital of Guangxi Medical University and the cut-off value was defined as 35 U/ml.

**Tissue preparation and immunohistochemistry of YKL-40**

Formalin-fixed hematoxylin and eosin (HE) stained four-μm slides from the tumor tissue of the same patients were performed and revised by two senior pathologists. Additional four-μm unstained tissue slides were dewaxed and rehydrated in a descending series of ethanol to water. The slides were incubated in three percent hydrogen peroxide for ten minutes to quench endogenous peroxidase activity. High pressure mediated epitope retrieval was performed for ten minutes in 0.01%M citrate buffer, pH 6.0. The sections were incubated overnight at 4°C with primary antibody against YKL-40 (1:100 dilution, mouse NO.35135). Immunoperoxidase stains were performed using a polymer detection system and a commercially available two-step (non-biotinylated) detection kit. For colour development, sections were incubated with 3’d-diaminobenzidine tetrachloride (DAB kit ZLI-9032) for five minutes and counterstained with haematoxylin and eosin. Section of glioblastoma multiforme was used as the positive control for YKL-40 staining. Phosphate buffered saline (PBS) replaced the primary antibody as negative control. Immunoreactivity to YKL-40 protein was localized in the cytoplasm of tumour and normal cells but not in the stroma. Scoring for YKL-40 protein expression was based on the proportion of cells in a given tumor specimen exhibiting distinct cytoplasmic immunopositivity, as well as intensity of staining. Percentage of positive cells examined was scored as 1 (<20%), 2 (21-70%), and 3 (> 71%). The staining intensity was graded as 1, 2, and 3. The two scores were multiplied and the immunoreactive score was determined to calculate the final staining score (0-9) for YKL-40 and immunoreactive score was determined as follows: 0 as negative; 1 to 3 as weak; 4 to 6 as positive; 7 to 9 as strongly positive. In case of discrepancies, the sections were re-examined by two of the authors (J.T. Fan and P. Shen) individually.

**Statistical analysis**

Before relative comparison, the serum YKL-40 and CA125 levels were stratified into two groups respectively: ≤106.6 μg/l vs > 106.6 μg/l for YKL-40, ≤35 U/ml vs > 35 U/ml for CA125. Pelvic lymph node metastasis and positive washing cytology were dichotomized based on the presence or absence of each factor. Tumor grade (well vs moderately to poorly differentiated), FIGO Stage (Stage I, II vs III, IV), depth of myometrial invasion (none or less than one-half of the myometrium vs one-half or greater), and YKL-40 tissue staining (the score ≤ 3 vs > 3) were divided into two groups. Comparisons between groups were done using student’s t-test. The relationship of the serum YKL-40 levels to YKL-40 tissue staining and other clinicopathologic profiles was established using the Chi-square test or the Fisher’s exact test. To identify statistically independent factors responsible for high preoperative serum YKL-40 level (> 106.6 μg/l), significant vari-

| Table 1. — Clinical characteristics from patients diagnosed with EC |
|------------------|------------------|------------------|------------------|
| Age (years) | No. of patients (n=41) |
| median | 54.8 |
| range | 33-72 |
| FIGO stage |  |
| I+II | 26 |
| III+IV | 15 |
| Histologic grade |  |
| G1 | 23 |
| G2+G3 | 18 |
| Myometrial invasion |  |
| ≤1/2 | 27 |
| >1/2 | 14 |
| Lymph node metastasis |  |
| Positive | 9 |
| Negative | 32 |
| Peritoneal cytology |  |
| Positive | 18 |
| Negative | 23 |

| Table 2. — Relationship between the serum YKL-40 level and clinicopathologic profile of patients with EC |
|------------------|------------------|------------------|
| Clinicopathologic factors | YKL-40≤106.6μg/l | YKL-40>106.6μg/l |
| Total | 26 | 15 |
| FIGO stage |  |
| I+II | 13 | 50 | 13 | 50 | <0.05 |
| III+IV | 13 | 86.7 | 2 | 13.3 |
| Histologic grade |  |
| G1 | 9 | 39.1 | 14 | 60.9 |
| G2+G3 | 17 | 94.4 | 1 | 6.6 |
| Myometrial invasion |  |
| ≤1/2 | 16 | 59.3 | 11 | 40.7 |
| >1/2 | 10 | 71.4 | 4 | 28.6 |
| Lymph node metastasis | 1.00 |
| Positive | 6 | 66.7 | 3 | 33.3 |
| Negative | 20 | 62.5 | 12 | 37.5 |
| Peritoneal cytology | <0.01 |
| Positive | 16 | 88.9 | 2 | 11.1 |
| Negative | 10 | 43.4 | 13 | 56.6 |
| Serum CA125 | <0.05 |
| ≥35U/ml | 14 | 82.4 | 3 | 17.6 |
| <35U/ml | 12 | 50 | 12 | 50 |

* Chi-square test or the Fisher’s exact test

FIGO, International Federation of Gynecology and Obstetrics
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Variables were included in the logistic regression analysis and forward stepwise method were performed. A p-value < 0.05 was considered significant. All statistical analyses were performed using the SPSS statistical package, version 13.0 for windows.

Results

Serum YKL-40 in relation to clinical profiles of EC

The median preoperative serum YKL-40 value in the patients with EC was 157.2 μg/l (range 76.0-301.2). Serum YKL-40 levels in EC patients were significantly higher than that of uterine myoma (median 86.6; range 69.3-191.1) and healthy subjects (median, 86.2; range, 52.1-201.1) (p < 0.05). The authors defined the cut-off value of preoperative serum YKL-40 level at 106.6 μg/l. Table 2 shows the correlation between elevated preoperative serum YKL-40 level and each clinicopathologic profile. The median age of 41 EC patients was 54.8 years (range, 33-72). For all EC patients, elevated level of YKL-40 was seen in 9/23 (39.1%) patients in histologic grade 1 (G1) vs 17/18 (94.4%) patients in histologic grade 2-3 (G2-3), 13/26 (50%) patients with Stage I-II vs 13/15 (86.7%) with Stage III-IV, 6/9 (66.7%) patients with positive lymph node metastasis vs 20/32 (62.5%) with no metastasis, 18/29 (62.1%) patients who have less than one-half of the myometrium invasion vs 10/14 (71.4%) patients with one-half or greater myometrium invasion, 16/18 (88.9%) patients with positive washing cytology vs 10/23 (43.4%) with negative washing cytology, and 14/17 (82.4%) patients with elevated serum CA125 level vs 12/24 (50%) with CA125 < 35 U/ml. Twenty-six patients with elevated serum YKL-40 levels statistically differed from the remaining 15 patients with normal serum YKL-40 level with respect to FIGO Stage (p < 0.05), histologic grade (p < 0.01), positive washing cytology (p < 0.01), and serum CA125 level (p < 0.01). Significant variables were included in the logistic regression analysis and forward stepwise method were performed in a multivariate analysis, elevated serum YKL-40 levels only closely correlated with FIGO Stage (p < 0.05) and histologic grade (p < 0.01) and all other factors including positive washing cytology, and serum CA125, which had a significant effect on high serum YKL-40 level in univariate analysis, were no longer significant in multivariate analysis.

Immunohistochemistry of YKL-40

Immunoreactivity to YKL-40 was recognized as brown staining within cells, as shown in Figure 1, and was localized in the cytoplasm of tumor cells. The positive rate of YKL-40 tissue staining was higher in EC (34.1%, 14/41) than in uterine myoma (11.1%, 3/27) (p < 0.05). YKL-40 staining was negative in 27 of 41 patients (65.9%), weakly positive in four (9.8%), positive in three (7.3%), and strongly positive in seven (17.1%) of EC patients. Thus, 14 of 41 (34.1%) EC tissues tested immunohistochemically were demonstrated to contain YKL-40 protein.

Serum YKL-40 levels and immunohistochemistry of YKL-40

Table 3 shows the correlation between serum and tissue YKL-40 in patients with EC. Elevated serum YKL-40 level in patients with negative tissue staining was found in 14 of 27 (51.9%) EC patients. Meanwhile, normal serum YKL-40 in patients with positive or strongly positive tissue staining was found in 1 of 10 (10.0%). In all patients, the total frequency of EC with positive tissue staining (14/41, 34.1%) tended to be lower than that of elevated serum levels (26/41, 63.4%), and the difference reached statistical significance (p < 0.05).

Discussion

Patients diagnosed with early-stage endometrial cancer have a good prognosis with a five-year survival rate of 84% [2]. Conversely patients who are diagnosed with advanced-stage disease have a poor prognosis. As we all know, there

![Figure 1. — The staining results of EC and uterine myoma. A: EC (positive immunostaining ×400); B: EC (positive immunostaining ×100); C: EC (HE ×400); D: uterine myoma (no immunostaining ×400)
is no specific tumor marker in detecting endometrial cancer recently. The elevated serum CA125 had been reported in some endometrial cancer patients but only in about ten to 20% of patient with Stage I disease [13,14]. Due to the small lesions confined to the uterine cavity of early-stage endometrial cancer, there is less serum CA125 in circulation. Thus, mostly early-stage diseases have a low serum CA125 level. YKL-40 (chitinase-3-like-1), a member of ‘mammalian chitinase-like proteins’, is produced by tumor-associated inflammatory cells and cancer cells and has a role in inflammatory cell proliferation, and differentiation, thus protecting the cells from undergoing apoptosis, stimulating angiogenesis and remodeling extracellular tissue [3-7]. The aim of the present study was to determine the expression of YKL-40 in tumor tissue and serum in patients with EC, and to investigate clinical value of this marker.

Many studies have demonstrated that elevated YKL-40 protein expression was closely associated with the shorter survival in several types of cancers [15-18], poor radiation response, early disease progression, and death in glioblastoma [18]. The present preliminary data in a forward step-wise analysis showed that elevation of preoperative serum YKL-40 in EC was closely correlated with FIGO Stage and tumor grade, indicating that serum YKL-40 may represent some worse biologic characteristic of EC patients. FIGO Stage refers to the range and spread of the degree of tumor growth, reflecting the deterioration of the disease. Results from the present study suggest that the protein YKL-40 may have a role in tissue remodeling, contributing to endometrial cancer growth, and metastasis. Tumor grade identification is based on the degree of differentiation of malignant tumors, atypia, and mitotic figures. The higher grade means the worse prognosis. This suggests that YKL-40 testing can be performed to reflect histologic characteristics of tumor. Preoperative serum YKL-40 levels were elevated in 63.4% (26/41) of EC patients. By contrast 41.5% (17/41) of the patients had elevated serum CA125. It can be inferred that YKL-40 may be a better predictor of EC than CA125 (p = 0.007).

Peng et al. [10] had examined the tissue expression of YKI-40 in EC and noted that high YKI-40 immunoreactivity in EC might be associated with poor prognosis, but they did not correlate the serum YKL-40 level with tissue expression. To the best of the authors' knowledge, the present study is the first one to assess the correlation between tissue expression of YKL-40 with the corresponding serum level in endometrial cancer patients. Data from this study showed a discrepancy between tissue staining and serum level of YKL-40. The present study indicated that only 34.1% of EC tissues contain YKL-40, which was significantly lower than that of elevated serum YKL-40 level (63.4%). A similar study has previously been reported in ovarian carcinoma, in which no correlation was found between plasma YKL-40 and the YKL-40 tissue expression percentage score [12]. The phenomenon of the discordance between protein expression in cancer tissue and circulating levels of the protein has been reported in other studies with respect to CA125 [20,21], in which they found the percentage of tissue staining was higher than that of serum level of CA125, assuming that there might be some mechanism that prevents the access of intrauterine CA125 into circulation, and inferred that the main source of elevated serum CA125 levels was closely related to the presence of disseminated cancer cells in the peritoneal cavity in the patients with endometrial cancer, rather than intrauterine tumor cells. The observation in the present study is possibly due to the contribution to the circulation of YKL-40 from the many tumor-associated inflammatory cells surrounding the tumor tissue, such as macrophages [22-24], mast cells [25,26] and neutrophils, which can also produce YKL-40 protein. Serum YKL-40 level alone cannot sufficiently reflect the real biologic characteristics of endometrial cancer. Due to the small sample size of this study of both study and controlled groups, larger prospective studies are still needed to investigate the exact function of YKL-40 protein in endometrial cancer.

In summary, this study has shown that YKL-40 protein can be expressed in EC cells. Elevated preoperative serum YKL-40 level is related to FIGO Stage and tumor grade. There is no correlation between tissue staining and serum level of YKL-40, indicating that the tumor tissue of EC may be not the only source of YKL-40 in circulation. The tumor-associated inflammatory cells can also contribute to the level of serum YKL-40.

References

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Address reprint requests to:
J.T. FAN, M.D.
#6 Shuangyong Road,
Nanning city, Guangxi Province (China)
E-mail: jiangtao_fan1969@163.com
Introduction

The term perivascular epithelioid cells was first proposed in 1992 by Bonetti et al. to describe the presence in two different lung neoplasms of a morphologically and immunohistochemically unusual cell type, with perivascular distribution [1]. In particular, the Authors describe these unusual cells as “immunoreactive with melanocytic markers, and exhibiting an epithelioid appearance, a clear-acidophilic cytoplasm, and a perivascular distribution” [1]. The lung lesions in which these cells were found were an angiomyolipoma (AML) and a clear cell sugar tumor (CCST); these lesions were originally described in the 1960s [2, 3].

Over the following years a continuously growing number of tumors in several anatomic locations were found to comprise the cells described by Bonetti et al. A variety of appellations has been proposed to describe these tumors, but the term perivascular epithelioid cell neoplasms (PEComas), proposed by Zamboni et al. [4] in 1996, is currently the most diffusely used. The progressive enlargement of the PEComa family led the World Health Organization to provide a definition of these tumors: “mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells” [5]. Some of the lesions included in this family, like AML, CCST, and lymphangiomatosis (LAM) of the lung were previously pathologically described. Other tumors of multiple anatomical locations are less characterized and the term PEComa not otherwise specified (PEComa-NOS) has been proposed to define them [6]. Excepting the lung, PEComa-NOS have been described in numerous anatomical organs and districts, including breast, bone, hepatobiliary system, pancreas, cervix and uterus, urinary bladder, colon, soft tissues and others [7].

The present authors describe in this article the clinical management and the pathological, immunohistochemical, and molecular features of two PEComas of the uterus.

Cases Report

Case report 1

A 53-year-old woman suffered from menometrorrhagia and uterine fibromatosis. Her past medical history was otherwise unremarkable. She had anemia unresponsive to medical therapy and the authors decided to perform total hysterectomy and bilateral aneoplastic surgery.

Pathology review of the surgical specimens revealed an endometrial polyp mass arising from the superior aspect of the uterine corpus and infiltrating the subserosa and >50% of the myometrium. On gross examination, the mass was described as having hemorrhagic, tan/yellow, and softened cut surfaces with multiple finger like projections into the myometrium (Figure 1). Microscopic review revealed predominantly epithelioid cells with a clear to lightly eosinophilic cytoplasm; in certain areas the neoplastic cells showed granular cell change. Extensive areas of hemorrhage, coagulative necrosis, and prominent vascular invasion were also noted.

The neoplastic cells were immunoreactive for HMB-45, Melan A, smooth muscle actin (SMA), desmin, and estrogen receptor, while they were negative for S100, pankeratin, inhibin alpha, and tyrosinase. Given the myomelanocytic phenotype, a diagnosis of PEComa was made. Given the size of the primary mass and the presence of multiple high risk features, this was considered to represent a malignant PEComa.

The patient made a full recovery, and CT of the chest, abdomen, and pelvis at five weeks following surgery revealed no evidence of recurrence.
of metastases. After a lengthy conversation regarding the potential utility of systemic therapy, the patient opted not to pursue adjuvant therapy, and she died of relapse of the disease two years later.

Case report 2

A 52-year-old woman was referred to the present clinic for respiratory distress, uterine fibromatosis, and menometrorrhagia. A biopsy of a polypoid lesion of the uterus was performed which evidenced a scarcely differentiated neoplasia, comprised of spindle cells with dismorphic voluminous or vesicular nucleous, and with numerous marked atypias. Preoperative staging did not revealed other peripheral lesions, and the patient underwent a total abdominal hysterectomy and bilateral anneesectomy. Histopathological analysis of the specimen evidenced a microscopic pattern predominantly comprised of epithelioid cells with a clear to lightly eosinophilic cytoplasm and, occasionally, granular cell change. Areas of hemorrhage, coagulative necrosis, and prominent vascular invasion were also noted. Immunoreactivity for HMB-45, Melan A, SMA, desmin, and estrogen receptor were evidenced, while no reactivity was shown for S100, pankeratin, inhibin alpha, and tyrosinase, and diagnosis of primary uterine PEComa was made.

Three years later the patient developed multiple liver and lung nodules. A surgical biopsy of a lung lesion was performed because less invasive biopsies found no malignant tissue, and diagnosis of metastatic PEComa was obtained, as the lesion presented identical pattern to that previously described (Figure 2). The woman refused adjuvant chemotherapy and died 24 months later.

Discussion

Gynecological PEComas involve the uterus (more commonly), the vagina, the ovary, and other pelvic organs, and can be benign or malignant. They represent approximately one-fourth of the overall PEComa cases described in literature [8]. The peak of incidence occurs generally within the fourth decade of life. Most of the uterine tumors arise in the corpus, while cervix is less frequently involved [9]. The clinical manifestations of uterine PEComas vary in relation to the dimensions, location, and diffusion of the tumor. Generally, small non-symptomatic tumors are casually discovered. The most common signs and symptoms of clinically evident lesions include: abnormal vaginal bleeding, pelvic pain, rupture of the uterus, and hemoperitoneum. Menometrorrhagia was the primary symptom in both the presented patients.

Similarly to clinical manifestations, also the radiological appearance of uterine PEComas is extremely variable, in relation to their consistence, dimensions, and local or distant diffusion. They can appear either as small benign smooth cell neoplasms, and as large, heterogeneous masses [10]. Uterine fibromatosis may further complicate radiological detection of PEComas, as occurred in the presented cases. The lack of specific clinical and radiological findings, makes the diagnosis and the management of PEComas challenging, causing in some cases delay in commencing treatment.

An association between uterine PEComas and tuberus sclerosis complex (TSC) has been demonstrated in approximately 10% of cases; this must be kept in mind by the clinician, who has always to search for TSC clinical manifestations in patients with a suspected or ascertained uterine PEComa [11].

Most of the morphological features of PEComas of the uterus are common to PEComas of other anatomical sites. They are comprised of epithelioid and/or spindled cells with abundant clear to eosinophilic cytoplasm. Cells with bland nuclei or clear anaplasia may be found, as well as multinucleated giant cells [12]. The epithelioid component
seems to be predominant in most cases, generally characterized by a nested growth pattern or, more rarely, by a fascicular or diffuse evolution [13, 14]. Irrespective of the growth pattern, a quote of uterine PEComas shows a variable quote of stromal hyalinization, which in some cases is so relevant, making the epithelioid cells to seem immersed in a hyalinized – fibrotic background [11, 13-14]. The tumors can be well- or partially circumscribed, or can diffusely infiltrate the myometrium with a “tonguelike” pattern [9, 11]. Finally, the vascularization of PEComas often present characteristic features, being composed generally by a network of small vessels distributed in the entire context of the tumor [11].

Concerning immunohistochemistry, immunoreactivity for HMB-45 and some other melanocytic markers has been widely demonstrated; the most relevant ones are microphthalmia transcription factor (MTF), Melan A, and Mart-1, HMSA-1. In approximately 70% of cases immune reaction for SMA has been reported, while in about half of cases immuno-positivity for vimentin and/or desmin was described, as occurred in the presented cases [9, 11]. Cathepsin K expressions was also reported to be useful in the diagnosis of PEComas [15].

The presence of constant morphological and immunohistochemical features, the variety of the anatomical distribution and the absence of a normal counterpart of PEComas, induced pathologists, and molecular biologists to research the origin of these tumors. Most efforts were mainly directed towards multipotent primitive cells, in particular those prevalently located in perivascular areas. Ardeleanu et al. [16] recently hypothesized that telocytes may represent the cell of origin of different stromal tumors, including PEComas and gastrointestinal stromal tumors (GISTs). In order to investigate this hypothesis, the present authors performed c-kit molecular analysis in both their cases, without evidencing any mutation.

Surgery is the cornerstone of the treatment of benign uterine PEComas, while not a unanimously accepted approach has been established for lesions with high-risk features and a variety of treatments and approaches are employed in clinical practice throughout the world. The lack of consensus for the treatment of PEComas depends on several factors, like the small number of cases described in literature, the non-existence of randomized data, and the poor results obtained with the various therapeutic strategies proposed, especially in non-surgical patients. Furthermore, in a quote of patients, diagnosis is delayed until surgical resection is performed, consequently delaying therapy initiation and effectiveness. The vast majority of patients with uterine PEComa in literature received surgical resection (at least hysterectomy) [11].

Neoadjuvant treatments have been employed in a limited number of cases in literature without relevant benefits in arresting progression and tumoral growth, sporadic cases apart. The adjuvant treatments used are numerous and reflect generally the rationale to contrast a soft tissue sarcoma. Also these treatments have demonstrated to be poorly effective; in some series the patients who underwent adjuvant therapy presented a higher incidence of recurrences in comparison with those who were not submitted to this treatment [17]. Finally, in the setting of metastatic disease, several protocols of systemic chemotherapy have been used with little efficacy. Surgery seem to represent an optimal option in the oligometastatic patient; unexpectedly, good survival rates for up to one year from diagnosis, without any treatment, have been reported [18]. Targeted therapies, especially with mTOR inhibitors, seem to provide encouraging results, but further data are necessary to better understand their possible role in the treatment of PEComas [19-21]. Unfortunately, both the presented patients refused any adjuvant treatment and they died 24 months after diagnosis of metastatic disease.

Irrespective of the postoperative treatment employed, in patients with malignant uterine PEComa, a long-term accurate follow up must be programmed, given the high rates of recurrences registered even five years or more after initial treatment and the relatively good results by surgical excision of early detected metastasis [7].

In conclusion, uterine PEComas account for about one-fourth of all PEComas. Diagnosis and management may be challenging, given the lack of specific clinical and radiological manifestations and the non-existence of unanimously accepted therapeutic strategies. As a consequence, prognosis in malignant forms, especially those in advanced stage, is poor. There do not seem to be any useful treatment relation between PEComas and GISTs; the most promising approach, currently, is based on the employment of mTOR inhibitors.

References


Address reprint requests to:
G. CAPOBIANCO, M.D., PhD
Gynecologic and Obstetric Clinic
Department of Surgical, Microsurgical and Medical Sciences,
University of Sassari
Viale San Pietro 12, 07100 Sassari (Italy)
e-mail: capobia@uniss.it
A very rare case of vaginal angiokeratoma

A.A. Mendivil¹, J.P. Micha¹, J.M. Stallman², B.H. Goldstein¹
¹ Gynecologic Oncology Associates, Hoag Memorial Hospital, Newport Beach, CA
² Hoag Memorial Hospital Presbyterian, Department of Pathology, Newport Beach, CA (USA)

Summary
Angiokeratomas are benign, vascular lesions that are very rarely identified in the vagina. A patient originally presented with endometrial cancer in 1993 and was cured following surgery and adjuvant radiotherapy. However, in 2007, she developed multiple, erythematous, vaginal nodules that were eventually diagnosed as angiokeratomas of the vagina. The diagnosis of vaginal angiokeratoma may not be initially suspected. Therefore, physicians should perform a histologic examination to verify the condition and accordingly, provide relevant clinical management.

Key words: Angiokeratoma; Vagina; Gynecologic oncology; Patient management.

Introduction
Angiokeratoma is a benign vascular lesion, seldom identified on the external genitalia. They are typified by distinct, ectatic blood vessels within the papillary dermis [1]. Microscopically, angiokeratomas are further characterized by epidermal hyperkeratosis, papillomatosis, and acanthosis [2,3]. The pathogenesis of angiokeratoma, albeit poorly understood, is imputed to dilated blood vessels and congested capillaries within the sub-dermal layer [4]; the vessel changes may be attributed to a primary or secondary degenerative process, such as previous radiotherapy or chronic inflammation [2,3].

Clinically, angiokeratomas of the female genital tract afflict middle-aged women without any racial predilection [5]; the disease often coincides with certain lysosomal storage disorders (e.g., Fabry Disease and fucosidosis) [6]. Patients may present with bleeding, pruritus, and dyspareunia [7]; the lesions are frequently comprised of purple, verrucous papules, measuring two to three mm in size [7].

Angiokeratoma of the vulva has been documented in the literature, although the condition is very uncommon [1, 8]. Moreover, clitoral angiokeratoma is quite unusual, with few reported studies [9, 10]. To the best of the authors’ knowledge, it is believed that this is the first reported case involving a patient treated for vaginal angiokeratoma.

Case Report
A 63-year-old (gravida 1, para 1) woman was originally referred to the present clinic with a poorly differentiated, Stage IA endometrial cancer in July 1993. She underwent a laparotomy, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and bilateral pelvic and para-aortic lymphadenectomy. In August, the patient began six weeks of pelvic radiotherapy (50.4 Gy), from which she did not presumably develop any delayed radiation-induced complications. The patient’s medical history was significant for hypertension, arthritis, and a salpingectomy in response to an ectopic pregnancy in 1960.

In March 2007, she developed vaginal bleeding that coincided with five, bilateral three-mm erythematous, polypoid nodules on the distal, posterior vagina proximal to the hymenal ring (Figure 1); the vaginal lesions were removed via punch biopsy and submitted for pathologic evaluation. The hemorrhagic nodules were encircled by benign squamous epithelium. Moreover, some of the fragments contained successive, engorged blood vessels, consistent with angiokeratomas (Figures 2A-D).

The patient’s vaginal bleeding was intermittent and thus, her status was closely monitored. She was evaluated again in November 2011, whereupon four nodules, approximately two to three mm in size, were identified on the left vaginal sidewall, adjacent to the introitus. The surrounding tissue was inflamed and hemorrhagic. A punch biopsy was performed in three of the lesions and they were submitted to pathology; they resembled a hemangioma and were consistent with angiokeratomas (Figures 2A-D).

The patient’s nodules were subsequently incised circumferentially with approximately two-mm margins; the surgical procedure successfully stanched the bleeding.

Discussion
Angiokeratomas are very uncommon, cutaneous, vascular lesions that presumably result from degenerative changes in perivascular, elastic tissue. The condition is presumably related to radiotherapy changes in the vascular elastic tissue, which results in ectasia and secondary epidermal hyperkeratosis [3].

Angiokeratomas are benign, although they can clinically mimic a malignant process (e.g., melanoma) [2, 8]. The diagnosis of angiokeratoma may be further confounded since the disease often coincides with lysosomal storage disorders and can resemble an infection or inflammation [5, 6]. Even with non-cancerous lesions, the reappearance of a vaginal lump in a patient who has presumably been cured of her disease is concerning; thus, all patients should
be initially evaluated via biopsy and histologic examination [9, 10].

There have been documented cases of vulvar angiokeratoma and albeit unusual, studies involving clitoral angiokeratoma [1, 8-10]. Terzakis et al. reported on two vulvar angiokeratoma patients who initially complained of pruritus [1]. Following surgical excision of their respective lesions, the patients remained disease-free after 48 and 32 months, respectively. Kontogianni-Katsaros et al. described four vulvar angiokeratoma patients whom they evaluated over a ten-year period [8]. A histologic evaluation revealed enlarged vascular channels underlying an acanthotic epidermis, without evidence of degenerative changes.

In the current study, the authors present what is possibly the first reported case of vaginal angiokeratoma. There was no association with Fabry disease or fucosidosis; however, the patient was treated with radiotherapy, of which angiokeratoma may be a long-term sequela [3, 6]. Therefore, despite any evidence of radiation therapy related complications, one could conjecture that the current study patient’s previous radiation exposure induced blood vessel dilation near the skin’s surface or mucous membranes, effectuating the development of the angiokeratomas [3]. Additionally, since the patient had pelvic radiotherapy, the authors do not preclude the likelihood of further lesions manifesting themselves.

Figure 1. — Gross photograph of the angiokeratoma.

Figure 2. — A: Polypoid angiokeratoma with surface epidermis attenuation, and underlying blood filled spaces. Select aspects of the angiokeratoma is hyalinized (i.e., becoming scarred). B: The flat epidermis is in the lower left. There is a polypoid angiokeratoma extending above the level of the epidermis, demonstrating surface epithelial erosion and blood filled spaces. C: Polypoid angiokeratoma with very thin epidermis. D: Angiokeratoma demonstrating thinning of the epidermis and underlying blood filled spaces.
A very rare case of vaginal angiokeratoma

Since angiokeratomas are predominantly benign, superficial lesions, observational management may be indicated. Conversely, when a patient’s symptoms are intractable, the angiokeratomas can be resected via local excision or electro-surgery [8]; laser surgery may be preferable because the pain is mitigated, blood loss is minimal, and cosmesis is preserved [8]. A physician should consider the diagnosis of angiokeratoma when examining a patient with pruritic, painful, or bleeding lesions in the genital region. Further study of the pathologic and biologic characteristics of an angiokeratoma may confer a better appreciation of the disease, potentially effectuating improved clinical diagnosis and patient management.

Acknowledgement
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References

Address reprint requests to:
B.H. GOLDSTEIN, PhD
Gynecologic Oncology Associates
Hoag Memorial Hospital
351 Hospital Road, Suite 507,
Newport Beach, CA 92663 (USA)
e-mail: Bram.Goldstein@gynoncology.com
Introduction
The presence of primitive abdominal extrauterine leiomyomas is anecdotal [1]. The primitive leiomyomas of the abdominal cavity arise in the retroperitoneum or in the abdominal cavity, are more frequently found in women than in men, and seem to express hormone receptors. Myomas of the retroperitoneum or of the abdominal cavity are at times synchronous with leiomyomas of the uterus and may be multiple.

In the framework of this type of tumour, primitive omental location is quite difficult to determine, as the possible presence of “parasite” myomas and metastasizing myomas in the omentum have been described [2,3]. Specifically, “parasite” myomas are those which have lost their vascular connection with the uterus, and are replenished by vessels of the omentum [2].

Given the rarity of primitive omental myomas, it is deemed useful to report a new case of primitive omental leiomyoma.

Case Report
A 35-year-old multiparous woman was subjected to laparotomy and multiple myomectomy. Her past medical history was uneventful. The patient had two massive uterine leiomyomas, which had been diagnosed clinically as well as by ultrasound scanning and nuclear magnetic resonance. There was an anterior subserosal fibroid measuring about eight cm wide, with emergence towards the vesico-uterine fold and consequent compression of the bladder, and a posterior leiomyoma, more voluminous, about ten cm wide, with emergence towards the Douglas-pouch.

At the end of the operation, while repositioning the omental apron, a hard, isolated swelling of about three cm was palpated in its thickness, located at a short distance from the transverse mesocolon without any vascular connection with neighbouring structures. This swelling was removed with a portion of the surrounding omentum of seven by three cm. The postoperative course was uncomplicated. The patient is currently well.

The histological examination of the omental swelling provided evidence of a morphological picture compatible with benign leiomyoma and with sclerohyalinosis and regressive aspects and the presence of peripheral coarse calcifications (Figure 1), surrounded by adipose tissue. The histological examination of the swellings also confirmed the presence of leiomyomas, with low mitotic index and devoid of morphological abnormalities.

Discussion
In this case, the intraoperative and anatomo-pathologic findings supported the diagnosis of a primitive omental myoma. The likelihood of a “parasite” myoma should be excluded because vascular connection between omental myoma and uterus were not found intraoperatively. Moreover, histological finding of benignity and low index activity do not support the hypothesis of a metastasis. However it has been reported that the diagnosis of primitive omental myomas remains merely speculative [4], and only a few cases of uterine primitive omental myomas have been described. It is possible that these leiomyomas may be hormone-sensitive and grow, as occurs with more commonly found uterine tumours [1]; however, due to the rarity of the disease, it is doubtful whether it is useful to remove them in the absence of specific indications.

Conclusion
Given the possible localization of malignant primitive omental diseases [5], however, it is deemed prudent, before closing the abdominal wall at the end of surgery performed for any pelvis-abdominal pathology, and in particular for...
removal of uterine leiomyomas, to perform an accurate control of the omentum as a possible site of disease. In the very unlikely event that this is present, it seems appropriate to remove it with abundant surrounding omental tissue in order to rule out its malignancy and facilitate the diagnosis of primitive leiomyoma. Moreover, given the possibility of recurrence, it is advisable to provide outpatient surveillance.

References


Figure 1. — The lesion appears mostly consisting of a central sclerohyaline and calcified area, with a thin peripheral band of smooth muscle cells. The lesion is surrounded by a thin layer of loose connective tissue (Hematoxylin and Eosin (40X)).

Address reprint requests to:
U. INDRACCOLO, M.D., PhD
Via Montagnano, 16
62032 Camerino (MC) (Italy).
e-mail: ugo.indraccolo@libero.it
Complete response after MAID treatment for advanced primary ovarian angiosarcoma: case report and literature review

P.C. Wu1, C.T. Yue2-5, S.C. Huang4,5

1 Department of Chinese Medicine, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taipei
2 Department of Pathology, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taipei
3 Division of Laboratory Medicine, School of Medicine, Tzu Chi University, Hualien
4 Department of Obstetrics and Gynecology, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taipei
5 School of Medicine, Tzu Chi University, Hualien (Taiwan)

Summary:
The patient presented in this case report was a 45-year-old female, with a Stage IIIA ovarian angiosarcoma combined with mature teratoma, that underwent debulking surgery and achieved complete remission for 11 months after six cycles of MAID chemotherapy (mesna, adriamycin/doxorubicin, ifosfamide, and dacarbazine). Thereafter, she had tumor recurrence with peritoneal seeding and massive pleural effusion; hence she received chemotherapy again. Although she had been undergoing a series of chemotherapies, the tumor continued to progress. Hence, she refused further chemotherapy since September 2012. Unfortunately, she passed away in January 2013 due to severe dyspnea with wide spread tumor progression. She had the longest survival period (31 months) and complete remission period than the other advanced primary ovarian angiosarcoma cases ever reported in the literature.

Key words: Ovarian malignancy; Angiosarcoma; MAID.

Introduction

Sarcomas of the female reproductive tract are rare, especially those from the ovary [1, 2]. Primary ovarian angiosarcoma is even more rare. The authors present such a case treated in our hospital and also reviewed the literature.

Case Report

This previously healthy 45-year-old female patient was found to have ovarian tumors incidentally on June 24, 2010 and her initial transvaginal ultrasonographic examination revealed right and left ovarian tumors measuring 2.1 x 1.4 cm and 7.1 x 4.7 cm in size, respectively. Components of teratoma were found at left ovary. She underwent laparoscopic left oophorectomy on July 26, 2010. The left ovarian teratoma contained hair components. The uterus, right ovary, and bilateral tubes were grossly normal. The routine pathological examination revealed incidental presence of malignant components in the left ovarian tumor. Postoperative abdominal computer tomography (CT) work up revealed left para-aortic lymph nodes enlargement on August 3, 2010. She underwent debulking surgery (transabdominal hysterectomy, right salpingo-oophorectomy, pelvic lymph node dissection, para-aortic lymph node dissection, omentectomy, and appendectomy) on August 4, 2010. Postoperative levels of tumor markers in August 2010 showed elevated level of CA-125 (300.5 U/ml), but normal levels of AFP, CEA, SCC, β-hCG, and LDH. Microscopically, ovarian angiosarcoma was found along with teratoma. Malignant cells were present in ascites, omentum, and appendix. The Federation Internationale de Gynecologie et d’Obstetrique classification (FIGO) Stage was IIIA. The angiosarcoma showed irregular vascular channels lined by plump neoplastic endothelial cells (Figures 1 and 2), which were positive for endothelial markers with CD34 and CD31 immunohistochemical stain. She received six cycles of chemotherapy with MAID (mesna, adriamycin/doxorubicin, ifosfamide, and dacarbazine) over the following five and a half months. The initial follow-up pelvic CT examinations in January and April 2011 both showed no tumor recurrence. In August 2011, mesentery fat became mildly enhanced on pelvic CT scan, but levels of tumor markers of AFP, CEA, β-hCG, and CA-125 (18.978 U/ml) were not elevated. Three month later, in November 2011, the level of CA-125 was significantly elevated to 182.3 U/ml and CT scan showed peritoneal tumor seeding and pleural effusion. Laparoscopic omental biopsy with pathological examination confirmed recurrence of angiosarcoma. Due to tumor recurrence with peritoneal seeding and massive pleural effusion, she received a series of chemotherapy: one cycle of chemotherapy with weekly paclitaxel, three more cycles of palliative chemotherapy with MAID, and six-month oral chemotherapy with vinorelbine. Although she had been undergoing the chemotherapy, the tumor continued to progress; hence, she refused further chemotherapy since September 2012. She passed away in January 2013 due to severe dyspnea with massive peritoneal tumor seeding and pleural effusion.

Discussion

In the literature, a total of 40 cases of primary ovarian angiosarcoma were reported. After a detailed review, only 26 cases had well-documented survival periods and treatment (surgery or chemotherapy) [1-20]. The other 14 cases lacked either or both of the aforementioned elements. In the former 26 cases, there were nine early-stage patients (Stages I and II) and 17 advanced-stage patients (Stages III and IV). Furthermore, the authors organized these 17 advanced-stage patients into Table 1 [4, 6-9, 11-
Two of these nine early-stage patients, who did not receive adjuvant chemotherapy, were still alive with no evidence of disease in their 66th and 108th months after diagnosis [13]. Other four cases of these nine early-stage patients, who underwent adjuvant chemotherapy, were still alive (survival period: 5+, 6+, 10+, and 10+ months, respectively), and two of these four cases had complete response [1-3, 10]. Other two cases of these nine early-stage patients were also still alive (survival period: 2+ and 14+ months, respectively), but their adjuvant chemotherapies were unknown [5, 14]. The last one of these nine early-stage patients died of septicemia on the 18th postoperative day [16]. Thus, early detection of ovarian angiosarcoma is a better prognostic factor regardless of utilization of adjuvant chemotherapy [2]. In Table 1, all these advanced stage patients were alive for less than 30 months, but the presented case was alive for 31 months. Eight of these advanced-stage patients and our patient, who underwent surgery and chemotherapy, had 12 months median survival. The other eight cases, which

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**Table 1.** *The pathology, treatment, and outcome of advanced ovarian angiosarcoma.*

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yrs)</th>
<th>Pathology</th>
<th>Stage</th>
<th>OP</th>
<th>C/T</th>
<th>Survival (months)</th>
<th>Reference</th>
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<td>1</td>
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<td>AS</td>
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<td>2</td>
<td>[12]</td>
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<td>1</td>
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<td>3</td>
<td>77</td>
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</tr>
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<td>4</td>
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<td>[13]</td>
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<td>5</td>
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<td>15</td>
<td>[13]</td>
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<td>24</td>
<td>[14]</td>
</tr>
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<td>20-32*</td>
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<td>30</td>
<td>[13]</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
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<td>IV</td>
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<td>none</td>
<td>1</td>
<td>[14]</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>AS+MC</td>
<td>IV</td>
<td>yes</td>
<td>none</td>
<td>3</td>
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</tr>
<tr>
<td>10</td>
<td>19</td>
<td>AS</td>
<td>III</td>
<td>yes</td>
<td>ADM &amp; IFO X 6, then RT against enlarged mediastinal lymph nodes</td>
<td>12</td>
<td>[8]</td>
</tr>
<tr>
<td>11</td>
<td>19</td>
<td>AS</td>
<td>IV</td>
<td>yes</td>
<td>ADM &amp; IFO X 5, cisplatin &amp; etoposide X 1</td>
<td>7</td>
<td>[7]</td>
</tr>
<tr>
<td>12</td>
<td>38</td>
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<td>IV</td>
<td>yes</td>
<td>ADM &amp; IFO X 8</td>
<td>7</td>
<td>[17]</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>AS+MCT</td>
<td>III</td>
<td>yes</td>
<td>4 courses C/T, type not specified, s/p staging, followed by debulking surgery</td>
<td>9</td>
<td>[9]</td>
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<tr>
<td>15</td>
<td>32</td>
<td>AS+MCT</td>
<td>IV</td>
<td>yes</td>
<td>high-dose ADM &amp; IFO X 5, IFO X 4</td>
<td>29</td>
<td>[6]</td>
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<td>16</td>
<td>40</td>
<td>AS</td>
<td>III</td>
<td>yes</td>
<td>ADM &amp; IFO X 6 (CR for 5 months), weekly paclitaxel</td>
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<td>[19]</td>
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<td>23</td>
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<td>12+</td>
<td>[20]</td>
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<td>18</td>
<td>45</td>
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<td>yes</td>
<td>MAID X 6 (CR for 11 months), Taxol X1, MAID X 3, VNR X 6</td>
<td>31</td>
<td>this case</td>
</tr>
</tbody>
</table>

* ages not individually reported; # autopsy; &: and; OP: operation; s/p: status post; C/T: chemotherapy; AS: angiosarcoma; RT: radiotherapy; CR: complete response; MCT: mature cystic teratoma; ADM: adriamycin (doxorubicin); IFO: ifosfamide; VNR: vinorelbine.; MC: mucinous cystadenoma.
underwent surgery alone, had 2.75 months median survival. The last one (Stage IV) who did not undergo surgery (just only autopsy) and chemotherapy remained alive for two months.

According to the retrospective analysis from Penel et al., doxorubicin-based regimens or weekly paclitaxel as first-line regimen may significantly improve the outcome of metastatic angiosarcomas [21]. Because tumor sensitivity to paclitaxel becomes lower if the primary site arises below the clavicle [22] and no visceral angiosarcoma achieved any response in the phase II trial in which paclitaxel was administered, a doxorubicin-based regimen still remains the first choice for those angiosarcomas that arise below the clavicle (including ovary). Thus, current recommended chemotherapy for ovarian angiosarcoma are MAID and ifosfamide/doxorubicin [23-25].

To date, including our case, only three advanced stage ovarian angiosarcoma cases received the MAID treatment and they also had complete response after MAID treatment [20, 26, and the present case]. Among these three advanced cases, first, Platt et al. described a case of complete resolution of Stage IV ovarian angiosarcoma with mature teratoma after five cycles of MAID chemotherapy, but no definitive sustained remission period was mentioned [26]. Second, Serrano et al. described a case of Stage IIIC ovarian angiosarcoma with complete response for eight months after six cycles of MAID chemotherapy [20]. Third, the present case, Stage IIIA ovarian angiosarcoma with mature teratoma, remained disease-free for 11 months after six cycles of MAID chemotherapy. Besides, the authors also found six advanced-stage ovarian angiosarcoma patients that had received doxorubicin and ifosfamide chemotherapy [6-8, 11, 17, 19], but only one case with Stage IIIC ovarian angiosarcoma, described by Guseh et al., had complete remission for five months after six cycles of doxorubicin and ifosfamide chemotherapy [19].

Although the significant morbidities of this MAID treatment were bone marrow toxicity and nadir sepsis with 5% treatment-related death rate [23, 25, 27], the above three advanced cases (including the present case) with MAID treatment were well-tolerated to this regimen. Therefore, taking into account the reported results and the present case, the MAID regimen may have better complete response (definitive sustained remission period: eight to 11 months) for advanced primary ovarian angiosarcoma with or without mature teratoma.

References


Address reprint requests to:
S.C. HUANG, M.D.
Department of Obstetrics and Gynecology,
Taipei Tzu Chi Hospital,
289, Jianguo Road, Xindian District,
New Taipei City (Taiwan)
e-mail: tcmoftc@gmail.com
Myxoid leiomyosarcoma of the uterus: a case report

A. Barone¹, M.R. Ambrosio⁵*, B.J. Rocca¹, M.G. Mastrogiulio¹, A. Ambrosio²*, R. Santopietro¹
¹Department of Medical Biotechnologies, Section of Pathology, University of Siena, Siena
²University “Magna Graecia” of Catanzaro, Catanzaro (Italy)

Summary

Only 30 cases of myxoid leiomyosarcomas (MLMS) have been reported to date. The authors describe a further case in a 66-year-old woman. The main differential diagnoses include: myxoid inflammatory myofibroblastic tumours, mixoid leiomyoma, and endometrial stromal tumours. Surgery remains the appropriate treatment. However, in spite of an aggressive surgical approach and local and systemic control, recurrences and metastasis are frequent.

Key words: Myxoid leiomyosarcoma; Uterine sarcomas; Prognosis.

Introduction

Uterine sarcomas are rare tumours that account for only 3% of all uterine neoplasms and less than 1% of all female genital tract cancers [1]. The three most common types of uterine sarcomas are malignant mixed mesodermal sarcomas, leiomyosarcomas, and endometrial stromal sarcomas. One third of uterine sarcomas are leiomyosarcomas, of which myxoid leiomyosarcomas (MLMS) are an extremely rare variant. Firstly described by King et al. in 1982 [2], it represents a diagnostic challenge, because of the relatively bland appearance of the cells usually displaying a low mitotic count, and the hypocellular nature of the proliferation. The usual diagnostic criteria for classification of uterine smooth muscle tumours, such as prominent nuclear pleomorphism and high mitotic index (>10 mitotic figures per 10 high-power fields-HPF), are often absent in MLMS, and the presence of tumour cell necrosis is quite difficult to ascertain. In the case of typical leiomyosarcoma, the presence of two of these three features is sufficient for the diagnosis [3]. In epithelioid and myxoid variants, the usual clues for malignancy are often absent. Only 30 cases of MLMS have been reported to date [4]. The authors here describe a further case, adding a brief review of the literature and underlying the importance of the differential diagnosis for management and prognosis.

Case Report

A 66-year-old woman who had reached the menopausal status 15 years before, presented at the Gynaecologic Unit of Siena University Hospital with a four-month history of progressive abdominal enlargement, dyspepsia, abdominal pain, and uterine bleeding. Thirty years before, the patient had undergone right ovariectomy for cystic endometriosis. At pelvic examination, a massive, ill-defined, symmetric, soft and slightly tender mass was detected extending from the lower abdomen to the xiphoid. The uterus and left adnexa were not palpable. Ultrasonography (US) and computer tomography (CT) revealed an enlarged uterus with a huge abdominal mass and a hypoechoic lesion of 40 mm of the left ovary. Laboratory values were within reference range. The clinical diagnosis was uterine leiomyoma. The patient underwent total hysterectomy and left salpingo-oophorectomy.

Macroscopically, the uterus was deformed by a large mass growing from the fundus, with a gelatinous consistency, infiltrating the myometrium and the serous covering; the mass measured 11.5 x 7 x 3cm and was gelatinous. The cut surface was variegated, ranging from brownish to amber yellow. Only small solid areas were present. Microscopically, the tumor was strikingly myxomatous, with large necrotic and hemorrhagic areas and an invasive growth pattern (Figures 1, 2). Spindle-shaped cells were seen in a copious mucoid matrix alcian blue positive, and showed nuclear atypia and pleomorphism (Figures 3, 4). The tumor invaded endothelium-lined vascular spaces; the infiltrating borders were jagged and irregular with projections into the myometrium (Figure 5). The mitotic count was 20 mitoses per 10 HPFs (Figure 6). The tumor invaded surrounding tissues and the uterine cervix. Immunohistochemically, the neoplasm was positive for antibodies against smooth muscle actin (SMA) (Figure 7), epithelial membrane antigen (EMA), vimentin, and caldesmon. p53 was strongly positive. Desmin, CD10, progesterone and estrogen receptors, and ALK-1 were negative. Proliferative index (Ki-67) was about 80% (Figure 8). On the basis of these features, a diagnosis of MLMs was made.

Conclusions

MLMs of the uterus is an extremely uncommon and aggressive neoplasm, with a poor prognosis despite its bland cytology and histology [5]. It presents problems in diagnosis and management to be faced by pathologists, gynaecologists, and oncologists. It may show little atypia, well-circumscribed borders, absence of necrosis and relatively low mitotic index which are opposite findings to those characterizing the usual uterine leiomyosarcomas [6]. As the usual clues for malignancy for smooth muscle tumours, such as prominent nuclear pleomorphism and high mitotic rate index, are often absent and tumour...
Figure 1-6. — Histologic aspect [1-6: Haematoxylin and Eosin; Original Magnification (OM), 1-2: x2.5; 3-6: x10].
Figures 7. — SMA stain, OM x10. Figure 8. — Proliferation index (Ki-67), OM x10.
necrosis may be absent or difficult to recognize in MLMS, other features may be used to help to identify those with a likelihood of aggressive behaviour, particularly invasive margins and vascular invasion [7-9]. To date, about 30 cases of MLMS have been described. The mean age was 54.1 years (reference range 20-86 years), the mean tumor size was 92.78 mm (reference range 10-300 mm), two cases presented no atypia and the mean number of mitoses for 10 HPF was 10.8 (reference range 0-100/10 HPF). The majority of patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by chemotherapy and/or radiation therapy. In all cases, the prognosis was poor and the 13 patients alive presented with disease [3]. Burch et al. [3] identified infiltrative margins, intravascular extension, large size (> 80 mm), and p53 positivity as the most important features associated with aggressive behaviour. MLMS must be differentiated from other myxoid tumors of the uterus as myxoid inflammatory myofibroblastic tumour (IMT), myxoid leiomyoma, and endometrial stromal tumour. IMT is the most important lesion to be excluded in the differential diagnosis as it may display significant myxoid change and high mitotic activity. The relatively young age of many patients with IMT, the presence of an inflammatory (lymphoplasmacytic) infiltrate and immunoreactivity with ALK1 are useful in distinguishing this entity from myxoid leiomyosarcoma [10, 11]. In fact, the myofibroblastic cells may show positivity to caldesmon and rarely to SMA. Leiomyomas with edematous, hydropic change could be confused with myxoid change, particularly when myxoid change is found in large areas. However, hydropic change is patchy and appears eosinophilic, and alcin blue negative, whereas myxoid areas in MLMS are basophilic and alcin blue positive [7]. Also endometrial stromal tumours may be characterized by myxoid changes [12]. However, typical endometrial stromal cells are CD10 positive and prominent vascularity are evident somewhere in the lesion [3].

In spite of the bland appearance, radical surgery remains the established treatment for MLMS. Nonetheless, recurrence and metastasis are frequent and adjuvant radiation therapy as well as chemotherapy do not seem to improve long-term survival. The present patient was treated with surgery and is well after a follow-up of 15 months.
Solid neuroendocrine carcinoma of the breast: a rare tumor

A. Zizi-Sermpetzoglou1, V. Kontostolis2, E. Moustou1, K. Koulia1, E. Kouroumpas2,
D. Myoteri1, E. Arkoumani1

1Department of Surgical Pathology, 22nd Department of Surgery, Tzaneio General Hospital, Piraeus (Greece)

Summary

Solid Neuroendocrine carcinoma of the breast (SNECB) is a subtype of primary neuroendocrine carcinoma (NEC) of the breast with several distinctive features. In the present study, the authors report a case of 84-year old woman who was admitted in the hospital with a lump in her right breast. Mammography revealed a well-defined nodule in the outer lower quadrant of her right breast. She underwent lumpectomy and sentinel lymph node biopsy, which showed no metastasis. The histological diagnosis was solid neuroendocrine carcinoma of the breast. Microscopically, the tumor is formed from cells arranged in nests or trabeculae and separated by scant connective tissue. Immunohistochemical staining demonstrates strong positivity for NSE, chromogranin, synaptophysin, ER, and PR. The patient is still alive 14 months after diagnosis. Because of the rarity of this disease, there is no standard treatment protocol and a large variety of chemotherapy protocols have been employed in treating this disease.

Key words: Neuroendocrine carcinoma; Invasive carcinoma; Solid neuroendocrine carcinoma; Immunohistochemistry; Breast.

Introduction

Primary neuroendocrine carcinoma (NEC) of the breast is rare, accounting for less than two percent of all breast cancers and less than one percent of all neuroendocrine tumors [1]. The first case was described in 1963 by Feyrter et al. [2]. The formal criteria for NEC of the breast were not established until 2003, when the World Health Organization (WHO) classification of Tumors of the Breast and Genital Organs proposed the following diagnostic criteria for breast NEC: I) presence of morphological features similar to those of NEC of both gastrointestinal tract and lung; II) expression of neuroendocrine markers in more than 50% of cell population [3]. According to the WHO, solid neuroendocrine carcinoma of the breast (SNECB) is one type of NEC, the other types are: small cell/oat cell carcinoma and large neuroendocrine carcinoma. SNECB is considered to be well-differentiated tumor and has a better prognosis than those with small cell and large cell NEC.

The authors report a case of primary neuroendocrine carcinoma of the breast and discuss the clinical and pathological features, treatment, and prognosis of this entity.

Case Report

A 84-year-old woman presented with a palpable nodule in her right breast. The patient noticed it one month before and it was rapidly growing. According to her medical history ten months prior (in November 2011), she was operated with tumor in colon, histological examination of which showed tubular adenocarcinoma of the colon Stage IIA (T3N0M0) TNM 2010, but neither chemotherapy nor radiotherapy was given.

On physical examination at the time of presentation, a relatively well-circumscribed nodule was revealed. The left breast examination and other clinical examination were within normal limits.

Mammography (Figure 1) showed a 3.5 cm well-defined nodule in the outer lower quadrant of her right breast. She underwent lumpectomy and sentinel lymph node biopsy. Her sentinel lymph node biopsy was negative.

Figure 1. — The mammography shows a well-defined high density mass.
On gross examination, the resected tumor was 3.5 cm in maximum diameter. It was yellow-white with a relatively smooth border.

Microscopically, the tumor was organized in solid and trabecular arrangements separated by delicate fibrous stroma (Figure 2).

The neoplastic cells were monomorphic with fine granular eosinophilic cytoplasm and hyperchromatic nuclei. Mitosis were rare and necrosis was absent.

Immunohistochemical staining revealed diffuse cytoplasmic positivity for neuron specific enolase (NSE), chromogranin A (Figure 3) and negative for CK20, Her2/neu, TTF1.

Estrogen receptor (ER) were strongly positive in 90% of the neoplastic cells (Figure 4); progesterone receptor (PR) were positive in 60%, Ki67 < 10%.

After that the diagnosis of a SNECB was established. The patient underwent further diagnostic procedure consisting of computed tomography (CT) scan of the chest, abdominal CT scan, brain CT scan, and bone scintigraphy and all of them were negative for both metastatic and other primary diseases. The patient received adjuvant chemotherapy.

Discussion

Neuroendocrine tumors are slow-growing tumors and may arise anywhere in the body producing well-defined clinical outcomes.

Primary neuroendocrine tumors of the breast are rare, accounting for less than one of all cancers arising from the breast.

Although NEC of the breast was first described in 1963 by Feyrter et al. [2], the formal criteria for this entity was first clearly defined in the most recent WHO classification of tumors in 2003.

The new criteria of WHO were based on the study of Sapino et al. [4] and thus defined as NEC the tumor which express neuroendocrine (NE) markers in more than 50% of the cell population [3]. This criterion distinguishes NEC of the breast from other mammary carcinomas that show only NE morphological features or local NE differentiation. The latter had no prognostic significance as compared with breast carcinoma NOS [5, 6].

Many theories have been proposed regarding the histogenesis of these tumors. The most common is that mammary NEC derives from progressive NE differentiation of a breast carcinoma rather than from pre-existing endocrine cells in the breast [7].

NECB are more common in elderly women and most patients are in the sixth to seventh decades of life [8].
these tumors have been reported in male patients [9, 10]. NECB have no specific clinical or imaging feature and may mimic those of breast carcinoma [8].

Morphologically, NEC of the breast include solid NEC, small cell/oat cell carcinoma and large cell neuroendocrine carcinoma. The histological features of the presented case corresponded to a solid NEC of the breast. These tumors consist of solid nests, sheets or trabeculae of cells and separated by delicate fibrovascular stroma. The cells are relatively uniform with eosinophilic granular cytoplasm, but may have a spindle, polygonal or plasmacytoid appearance. Some of these originate from solitary, solid papillary intraductal carcinoma [3]. Other form multiple, solid nests separated by a dense collagenous stroma resembling the alveolar pattern of invasive lobular carcinoma. Mitotic rate is low.

Immunohistochemically, ER and PR are positive and Her2/neu score is negative (0) as in the present case. The expression pattern of neuroendocrine markers is variable [4, 8, 11].

Lopez-Bonet et al. reported that all cases demonstrated positive immunoreactivity for synaptophysin (>50% tumor cells) but chromogranin A expression was observed only locally in five of seven cases. [8]

In contrast, Sapino et al. reported that 53% of NEC expressed chromogranin A (>50% of tumor cells) [4] as in the present case where the percentage of positive cells was about 65%.

Histological grading is one of the most important prognostic parameters. Solid neuroendocrine carcinoma is considered to be well-differentiated tumor unlike small/oat cell and large cell neuroendocrine carcinoma which are poorly differentiated. Also mucinous differentiation is a favorable prognostic factor [3] and has better prognosis.

It is noteworthy that the prognosis of solid NECB is thought to be better than invasive ductal carcinoma [12].

Righi et al. in their study reported that none of the investigated solid NECB (35 cases) had distant metastasis [13].

Lopez-Bonet et al. reported that only one of seven cases of solid NECB demonstrated metastasis (soft tissue of the cheek; this patient is still alive seven years later) [8].

Most patients NEC’s are treated like adenocarcinoma of the breast. There is no standard treatment or chemotherapy protocol because the series that have been studied are small.

Conclusion

Although the follow-up period of the present patient was only 18 months, the authors believe that she should have a good prognosis because the tumor was well-differentiated with a low nuclear grade, negative lymph nodes, and estrogen receptor positivity.

References

Uterine leiomyosarcoma: report of three cases and review of the literature

L. Nappi¹, G. Mele¹, S. Angioni², A. Di Spiezo Sardo³, E. Cicinelli⁴, P. Greco¹

¹Department of Medical and Surgical Sciences, Institute of Obstetrics and Gynaecology, University of Foggia, Foggia
²Department of Surgical Sciences, Division of Obstetrics and Gynaecology, University of Cagliari, Cagliari
³Department of Gynaecology and Obstetrics, and Pathophysiology of Human Reproduction, University of Naples "Federico II", Naples
⁴Department of Obstetrics and Gynaecology, University of Bari, Bari (Italy)

Summary
This is the report of three cases of unsuspected uterine leiomyosarcoma diagnosed by pathologist after hysteroscopic resection. The literature on this issue has been reviewed. Mesenchymal uterine tumors are rare malignancies, occurring in only 17 per one million women annually. The three most common variants of uterine sarcoma are endometrial stromal sarcoma, leiomyosarcoma, and malignant mixed Müllerian tumour. Less than one percent of women believed to have a leiomyma actually have a sarcoma at hysterectomy. According to the authors’ experience and the available literature reviewed, the removal of the whole myomatosus lesion, even if its appearance suggests a typical submucosal myoma, represents the only method to definitively rule out the presence of sarcomatous tissue.

Key words: Operative hysteroscopy; Myoma; Leiomyosarcoma; Diagnosis.

Introduction
Uterine sarcomas are uncommon tumours accounting between three and seven percent of all malignant diseases of the uterine corpus with higher incidence among Black compared with White women. Usually, they can be classified into leiomyosarcomas, which arise from the smooth muscle of the myometrium, and endometrial stromal tumours, which originate from the endometrial stroma. Mixed mesodermal tumours or carcinosarcomas have both epithelial and mesenchymal components and are now thought to be metaplastic carcinomas, rather than a subgroup of sarcomas. Leiomyosarcomas are the most common, accounting for about 25% to 36% of uterine sarcomas, and they are known for their aggressive nature and poor prognosis with an overall survival of less than 50% at two years, even when diagnosed at an early stage. This may be due to their location into the vascular myometrium of the uterus, which allows for early invasion and widespread metastases, particularly to the lung (40%). In a large aetiological study of uterine sarcomas, was found significant association of obesity and history of diabetes as risk factors for uterine sarcoma. Older age at menarche was inversely associated with uterine sarcoma risk, instead. BMI was significantly, but less strongly related to uterine sarcomas compared to endometrioid endometrial carcinomas or mixed Müllerian tumours. Unfortunately, there are no specific signs or symptoms of leiomyosarcomas. Abnormal uterine bleeding (56%), palpable pelvic mass (54%), and pelvic or abdominal pain (22%) are the most common signs and symptoms, but the similarity to the presentation of benign leiomyomas further compounds the difficulty in diagnosis. At the present time, the lack of ability of imaging techniques to detect these tumours, especially in differentiating malignant from benign disease, is disappointing. However, in recent years, the introduction of minimal invasive techniques such as hysteroscopy [1-15] enables to diagnose earlier these rare tumours. The standard of care of the uterine sarcomas is total abdominal hysterectomy and bilateral salpingo-oophorectomy. Removal of the ovaries and lymph node dissection remain controversial as metastases to these organs occur in a small percentage of cases and are frequently associated with intraabdominal disease. Ovarian preservation may be considered in premenopausal patients with early-stage leiomyosarcomas. The influence of adjuvant therapy on survival is uncertain, depending on histological grade as prognostic factor. Radiotherapy may be useful in controlling local recurrences and systemic chemotherapy (i.e. doxorubicin, ifosfamide, gemcitabine, gemcitabine plus docetaxel, or trabeptatin) is now used for advanced or recurrent disease, with response rates ranging from 25% to 53%. The optimal management of leiomyosarcomas of broad ligament is controversial. In most cases the same management as in leiomyosarcoma of the uterus is followed.

The present study describes the cases of three patients with suspect myomas, that resulted to be leiomyosarcomas after pathological analyses.
Case 1
A caucasian 35-year-old woman, gravida 2 para 2, was referred to the present department. She had pelvic pain and menorrhagia in the last two months. Her personal history was positive for cervical polyp that had been removed two years before but negative for genital neoplasia and medical diseases. Transvaginal sonography showed a retroverted uterus with disomogeneous structure; in the fundus of the uterus was found an intramural-submucous myoma of 47 x 51 mm which deformed the uterine cavity. A diagnostic hysteroscopy was performed by using a five-mm continuous-flow operative office hystroscope with a 2.9 mm rod lens (Betocchi size 5), showing a G1 myoma of nearly five cm commencing from the fundus of the uterus. Before hysteroscopic myomectomy, the patient underwent a treatment with gonadotropin releasing hormone (GnRH) analog for three months. During this period although there were no changes in the extent of myoma, the patient reported excessive fatigue. Hysteroscopic resection of the myoma was carried out by using a continuous flow nine-mm bipolar resectoscope and saline solution was used for the distention and the irrigation of the uterine cavity. No intraoperative or postoperative complications occurred. The excised tissue was examined by the pathologist who diagnosed a leiomysarcoma. A total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. Pathology showed a high-grade stromal sarcoma of the uterus with full-thickness penetration of the myometrium and into, but not through, the serosa. Lymphovascular invasion was present. Under high-power view, the large spindle and polygonal neoplastic cells showed a high nuclear-cytoplasmic ratio with numerous mitoses, up to 30 per ten high power fields. Magnetic resonance revealed widespread metastases in the lung, liver, and pelvis. The patient came to the present department and was subsequently treated with six cycles of therapy with doxorubicin and ifosfamide (with mesna). Unfortunately, she did not respond to treatment and died 11 months later.

Case 2
A Caucasian woman, 42-year-old, in full health, nulliparous, and with two previous abortions, was referred to the present department because of menorrhagia. Her personal genital history was negative for neoplasia and other diseases. Transvaginal sonography showed an antverted uterus with disomogeneous structure. In the cervical part of the uterus was found a defined mass of 35 x 45 mm. It was suspected a myoma. Then the patient underwent an operative hysteroscopy. Hysteroscopic resection of the myoma was carried out by using a continuous flow nine-mm bipolar resectoscope and saline solution was used for the distention and the irrigation of the uterine cavity. No intraoperative or postoperative complications occurred. The excised tissue was examined by the pathologist who diagnosed a leiomysarcoma. Then she delayed the staging surgery of one month for personal reasons. Computed tomography scan before the staging surgery failed detecting an abdominal mass. The patient underwent a laparotomic total hysterectomy with bilateral salpingo-oophrectomy, revealing a 95-mm, regular, solid, fibrotic mass of tumour. Peritoneal washings for cytology, together with omental and peritoneal biopsies were performed. The tumour was located in the right part of the uterine body. The tubes and the ovaries appeared normal and seemed completely independent of the mass. The patient was discharged five days later after an uneventful postoperative course. Histological examination of the tumour revealed a malignancy with more than ten mitosis per ten high power fields, associated with severe cytornuclear atypia. Subsequent immunohistological examination showed smooth muscle myosin staining. Ovaries, tubes, peritoneal biopsies, and peritoneal washings were free of malignancy. Adjuvant chemotherapy was planned considering high grade of the tumour along with presence of the lung metastases. The patient underwent six cycles of therapy with doxorubicin and ifosfamide (with mesna) and after ten months she remained symptom-free with no recurrence.

Case 3
A 55-year-old, gravida 4 para 4, postmenopausal, Caucasian woman was referred to the present department, with two months history of pain in lower abdomen and abnormal uterine bleeding in the last ten days. She had neither associated systemic diseases nor other signs, nor other symptoms. The physical examination revealed lower abdomen distension and a palpable mass on the right side of the uterus, confirmed with bimanual pelvic examination. Ultrasonography revealed an uterus with disomogeneous structure and a 88 x 42 mm mass filling the pelvis by the right edge of the uterus. Computing tomography scan showed multiples lung metastases and the serum CA125 was 35 U/ml. Patient underwent a laparotomic total hysterectomy with bilateral salpingo-oophrectomy, revealing a 95-mm, regular, solid, fibrotic mass of tumour. Peritoneal washings for cytology, together with omental and peritoneal biopsies were performed. The tumour was located in the right part of the uterine body. The tubes and the ovaries appeared normal and seemed completely independent of the mass. The patient was discharged five days later after an uneventful postoperative course. Histological examination of the tumour revealed a malignancy with more than ten mitosis per ten high power fields, associated with severe cytonuclear atypia. Subsequent immunohistological examination showed smooth muscle myosin staining. Ovaries, tubes, peritoneal biopsies, and peritoneal washings were free of malignancy. Adjuvant chemotherapy was planned considering high grade of the tumour along with presence of the lung metastases. The patient underwent six cycles of therapy with doxorubicin and ifosfamide (with mesna) and after ten months she remained symptom-free with no recurrence.

Discussion
Mesenchymal uterine tumours are rare malignancies, occurring in only 17 per one million women annually, thus representing fewer than five percent of all uterine malignancies. The three most common variants of uterine sarcoma are endometrial stromal sarcoma, leiomyosarcoma, and malignant mixed Müllerian tumour. Less than one percent of women believed to have a leiomyoma actually have a sarcoma at hysterectomy. The incidence of leiomyosarcoma arising on a leiomyoma is less than one percent.

The diagnosis of such tumours is often made postoperatively for a presumed benign condition. A vaginal digital and speculum examination should be performed, together with a smear. A transvaginal ultrasound examination, hysteroscopy, and endometrial biopsy are usually performed as part of the general investigative procedure but are seldom useful in the diagnosis of leiomyosarcoma. Blind endometrial biopsy misses the diagnosis of leiomyosarcoma in 40% to 80%. Doppler flow study may not assist in differentiating between leiomyomas and leiomyosarcomas. Hysteroscopic reports of uterine sarcomatous tumors are not frequent, even if hysteroscopy plays an important role in the evaluation and evolution of both recurrent and de novo disease [16].

The total incidence of uterine sarcoma (leiomyosarcoma, endometrial stromal sarcoma, and mixed mesoder-
mal tumor) among patients surgically treated for uterine leiomyoma is extremely low (0.23%) and does not substantiate the concept of increased risk of sarcoma in these women. Still, the malignant transformation of a uterine leiomyoma is debated and very rare.

There are no specific hysteroscopic characteristics to identify uterine sarcomas before tissue being sent for pathological review. The biopsy of myometrial lesions by means of conventional instruments (i.e., grasping forceps, scissors) might pose notable difficulties because of the high consistency of these lesions. Furthermore, a target eye biopsy could miss a focal area of malignancy. The removal of the whole myomatous lesion represents the only method to definitively rule out the presence of sarcomatous tissue. Resectoscopic slicing should be preferred to laser or electrosurgical vaporization as it has the great advantage of offering the pathologist the possibility to analyze the tumour entirely. The main disadvantage of vaporizing electrodes is the lack of tissue sample for pathology. When vaporizing electrodes are used, it is mandatory that no myoma be vaporized in its entirety but substantial portions should be retrieved for microscopic examination.

Conclusions

In the presented patients, because of the notable size of the lesions [17] and the severity of their symptoms, the complete resectoscopic removal of the lesion was scheduled right after the outpatient diagnostic hysteroscopy. The endoscopic approach to uterine lesions hiding a sarcoma may pose other practical issues besides the need for a histologic specimen to reach a definitive diagnosis. The use of preoperative GnRh agonist before the removal of a normal-appearing submucous myoma may delay the final tissue diagnosis, as it occurred in the presented patients. Taking into account the aggressive nature of mesenchymal tumors, a particularly careful attitude to avoid the spread of mesenchymal cells during hysteroscopy should be recommended. An electronically controlled system for irrigation and aspiration can be helpful to maintain a correct low intrauterine pressure (50 mmHg) in case of suspicion of neoplastic lesion avoiding or at least reducing the spread of malignant cells through the fallopian tubes. In the presented patients, neither abdominal metastasis nor positive peritoneal washing were found. According to the authors’ experience and the available literature reviewed, the removal of the whole myomatous lesion, even if its appearance suggests a typical submucous myoma, represents the only method to definitively rule out the presence of sarcomatous tissue. A preoperative target biopsy under hysteroscopic view could be useful, but it could miss focal areas of malignancy. Whether a uterine sarcoma is diagnosed, total abdominal hysterectomy and bilateral salpingo-oophorectomy are considered the standard therapy [18-20].

References


Address reprint requests to:
L. NAPPI, M.D.
Department of Medical and Surgical Sciences
Institute of Obstetrics and Gynaecology
University of Foggia
Viale L. Pinto, 71100, Foggia (Italy)
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Foreword

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

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

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

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

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

M. MARCHETTI

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

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

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

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

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

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

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

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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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