EUROPEAN JOURNAL OF GYNAECOLOGICAL ONCOLOGY (ISSN 0392-2936) publishes original peer reviewed works in the fields of female genital cancers and related subjects and also proceedings of gynecologic oncology society meetings all over the world.

Allen H.H., London, Ontario (Canada)  
Anteby S.O., Jerusalem (Israel)  
Audet-Lapointe P., Montreal (Canada)  
Ayhan A., Ankara (Turkey)  
Balat O., Gaziantep (Turkey)  
Bânceanu G., București (Romania)  
Basta A., Krakòw (Poland)  
Bender H.C., Dusseldorf (Germany)  
Benedetti Panici P.L., Rome (Italy)  
Charkviani T., Tbilisi (Georgia)  
De Oliveira C.F., Coimbra (Portugal)  
Dexeus S. Jr., Barcelona (Spain)  
Di Paola G.R., Buenos Aires (Argentina)  
Di Re F., Milan (Italy)  
Di Saia P., Orange, CA (USA)  
Friedrich M., Hamburg (Germany)  
Fuller A.F. Jr., Boston, MA (USA)  
Geisler H.E., Indianapolis, IN (USA)  
Geraubet A., Villejuif (France)  
Gorins A., Paris (France)  
Heintz A.P.M., Utrecht (The Netherlands)  
Ioannidou-Mouzaka L., Athens (Greece)  
Jordan J.A., Birmingham, England (UK)  
Karseladze A.I., Moscow (Russia)  
Klasterisky J., Brussels (Belgium)  
Kubista E., Vienna (Austria)  
Kudelka A.P., Houston, TX (USA)  
Maggino T., Padua (Italy)  
Markowska J., Poznan (Poland)  
Marth C., Innsbruck (Austria)  
Massuger Leon F.A.G., Nijmegen (The Netherlands)  
Menczer J., Savyon (Israel)  
Monsonego J., Paris (France)  
Pálfalvi L., Budapest, (Hungary)  
Pinotti A., São Paulo (Brazil)  
Piura B., Beer Sheva (Israel)  
Piver S.M., Buffalo, NY (USA)  
Rakar S., Ljubljana (Slovenia)  
Shepherd J.H., London, England (UK)  
Stelmachów J., Warsaw (Poland)  
Tjalma W., Antwerpen (Belgium)  
Ungár L., Budapest (Hungary)  
Vermorken J.B., Edegem (Belgium)  
Wang P.-H., Taipei (Taiwan)  
Winter R., Graz (Austria)  
Yokoyama Y., Hirosaki (Japan)
### New concepts on risk factors of HPV and novel screening strategies for cervical cancer precursors

K. Syrjänen - Turku, FINLAND

Novel observations on risk factors that are still controversial (i.e., oral contraception, smoking) or not previously studied (drug addiction), reproductive factors as potential cofactors of HPV infections in cervical carcinogenesis (i.e., menarche, menopause), and finally on the performance of different optional screening strategies among younger and older women are discussed.

### Phase II study of radiation therapy combined with weekly nedaplatin in locally advanced uterine cervical carcinoma (LAUCC): Kitasato Gynecologic Radiation Oncology Group (KGROG 0501) - initial analysis


The phase II study of concurrent chemoradiotherapy using nedaplatin for LAUCC is reported.

### Resource use and cost analysis of managing abnormal Pap smears: a retrospective study in five countries


Resource use and costs associated with the management of abnormal pap smears vary between countries, depending on the screening programme and the histological type.

### Discovery of altered protein profiles in epithelial ovarian carcinogenesis by SELDI mass spectrometry


The combination of SELDI and laser capture microdissection (LCM) is effective in finding the key molecules in ovarian tumorigenesis.

### Sentinel lymph nodes in endometrial cancer: is hysteroscopic injection valid?


Hysteroscopic peritumoral radiotracer injection for detecting sentinel lymph nodes (SLNs) in patients with endometrial cancer is described together with the evaluation of the resulting SLN detection rate.

### Interval debulking in epithelial ovarian carcinomas: the past, present and the future

M. Gultekin, K. Diribas, E. Buru, P. Dursun, K. Yuce, A. Ayhan - Ankara, TURKEY

The approach to interval debulking surgery in ovarian cancer is discussed in relation to neo-adjuvant chemotherapy and compared with the literature data.

### Whole-body positron emission tomography with 18F-fluorodeoxyglucose is an effective method to detect extra-pelvic recurrence in uterine sarcomas


The use of F-18-fluorodeoxyglucose positron emission tomography (18-FDG PET) for post-therapy surveillance of uterine sarcoma is described.
Clinical implication of medroxyprogesterone acetate against advanced ovarian carcinoma: a pilot study
K. Niwa, K. Onogi, Y. Wu, H. Mori, R.C. Harrigan, T. Tamaya - Gifu-city, USA
MPA with chemotherapy might improve the prognosis of advanced epithelial ovarian cancers.

Synchronous ovarian and endometrial carcinoma: a strong link to endometriosis?
A clinicopathological study of synchronous ovarian and endometrial carcinomas reveals a strong link to endometriosis.

Prognostic factors in patients with carcinoma of the vulva - our own experience and literature review
Evaluation of prognostic factors and treatment outcomes in patients with vulvar cancer are examined.

KIT protein expression in uterine sarcomas: an immunohistochemical study and review of the literature
KIT expression was analyzed immunohistochemically in primary uterine sarcomas and was found to be very weak in the majority of tumors.

Cl-channel blockers inhibit cell proliferation and arrest the cell cycle of human ovarian cancer cells
M. Li, B. Wang, W. Lin - Jinan, CHINA
The role of chloride channels in cell proliferation and cell cycles of human ovarian cancer cell A2780 was investigated.

Cervical cancer associated with genital prolapse - a brief review of the literature and long-term results of successful treatment with radiochemotherapy and surgery in a very frail patient
D. Reimer, A. Sztankay, I. Steppan, E. Abfalter, H. Lunzer, C. Marth, A.G. Zeimet - Innsbruck, AUSTRIA
Successful treatment of cervical cancer associated with procidentia by external brachytherapy and extracorporeal HDR-AL with concomitant chemotherapy followed by surgery.

Stage 1B cervical cancer in a pregnant woman at 25 weeks of gestation
A case of a 28-year-old pregnant woman diagnosed with Stage 1B cervical cancer is described.

The role of ovarian transposition in patients with early stage cervical cancer - two case reports
The role of ovarian transposition in patients with early-stage cervical cancer is examined.

Squamous cell carcinoma arising in a mature cystic teratoma of the ovary in young patient with elevated carbohydrate antigen 19-9
A very rare case of squamous cell carcinoma arising in a mature cystic teratoma in a 31-year-old single woman with very high levels of CA19-9 and normal levels of CEA is presented.

Primary vaginal melanoma: a case report and literature review
M. Schmidt, A. Honig, M. Schwab, P. Adam, J. Dietl - Wuerzburg, GERMANY
Vaginal melanoma is a rare malignancy. Among different treatment options only wide local excision is associated with prolonged survival.

Malignant mixed müllerian tumor of primary mesenteric origin associated with a synchronous ovarian cancer: case report and literature review
Extragential MMMTs are rare and may be associated with synchronous gastrointestinal or gynecological malignancies.

Pelvic actinomyces mimicking ovarian malignancy: three cases
S.E. Akhan, Y. Dogan, S. Akhan, A.C. Iyibozkurt, S. Topuz, O. Yalcin - Istanbul, TURKEY
Three cases of pelvic actinomyces mimicking ovarian cancer are described. All cases had a history of IUD use.
A case of endometrial carcinoma arising in a 36-year-old woman with uterine atypical polypoid adenomyoma (APA)
K. Bakalianou, N. Salakos, C. Iavazzo, G. Paltoglou, K. Papadias, A. Kondi-Pafiti - Athens, Greece
A case of endometrial carcinoma arising in a 36-year-old woman with atypical polypoid adenomyoma is presented.

Immature teratoma in pregnancy: a case report and literature review
A. Daponte, E. Kostopoulou, A. Zavos, H. Skentou, A. Kallitsaris, G. Koukoulis, I.E. Messinis - Larissa, Greece
An immature Stage I teratoma was removed during pregnancy. Appropriate surgical staging enabled avoidance of chemotherapy despite the unexpected histological diagnosis.
New concepts on risk factors of HPV and novel screening strategies for cervical cancer precursors

K. Syrjänen, M.D., Ph.D., FIAC
Department of Oncology and Radiotherapy, Turku University Hospital, Turku (Finland)

Summary

During the past several years, this author has been engaged in coordinating two major multicentre trials testing optional screening tools for cervical cancer (CC) in low-resource settings both in East Europe and in Latin America. These international trials include the NIS (New Independent States of the former Soviet Union) cohort (n = 3,187 women) and the LAMS (Latin American Screening) study (n = 12,114 women). In both studies, a sizeable cohort of women (887 and 1,011, respectively) have been prospectively followed-up to assess the natural history of high-risk human papillomavirus (HR-HPV) infections and the role of implicated risk factors as potential predictors of disease outcome (acquisition, persistence and clearance).

In this communication some of the key observations recently reported from the NIS and LAMS studies will be discussed, with special emphasis on i) risk factors that are still controversial (i.e., oral contraception; OC, and smoking) or not previously studied (drug addiction), on ii) reproductive factors as potential cofactors of HPV infections in cervical carcinogenesis (i.e., age at menarche, menopause), and finally on iii) the performance of different screening strategies among young and older women. Although closely related to these topics, a detailed discussion on the dynamics of HPV infections (acquisition, persistence, clearance) and their predictive factors falls outside the scope of this communication, because they have been extensively discussed in a series of original reports and in a recent review of the author in this journal.

The NIS cohort failed to establish OC as a risk factor of CC. In all future studies, the strong confounding effects from the lifestyle and sexual factors must be taken into account, while interpreting the data on OCs as potential risk factors of CC. Similarly, it now seems that the increased risk (if any) of CC among smokers seems to be attributed to the increased acquisition of HR-HPV infections, of which the smoking status is an independent predictor in a multivariate model. The same seems to apply to drug addiction as a risk factor of CC as well. The recent LAMS data show that drug abuse itself is not a risk factor of i) contracting HR-HPV infection or ii) developing high-grade CIN. Instead, drug abuse seems to be closely associated with several of the indicators of risky sexual behaviour, which predisposes the women to oncogenic HPV infections and thus indirectly contributes to the development of CIN2+ lesions.

Data from the NIS cohort clearly implicate that menarche age is not associated with increased risk of HR-HPV infection, or development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of CIN lesions or HR-HPV infections in a longitudinal setting. Another special group are postmenopausal women, recently shown to have a second peak of HR-HPV prevalence in many populations. The NIS cohort data suggest that among women who fail to eradicate their HR-HPV infection by menopause, there is i) a transition from multiple infections to single-type infections, and ii) selection of an integrated viral clone has already taken place, driving the process towards an aggressively progressing cervical disease.

Finally, these special features of HR-HPV infections among younger and older women lead us to consider, whether different screening strategies are needed for younger and older women. Consonant with other recent reports, data from the LAMS study show that conventional Pap and HC2, but not LBC and VIA, perform significantly differently among younger and older women. However, the choice of an optimal screening test for young and older women depends on whether the highest positive predictive value (PPV) (Pap test) or the best balance between sensitivity and specificity (SE/SP) (HC2) is used as the selection criteria.

Both the NIS cohort and LAMS study have significantly contributed to solving several of the open issues in the natural history of HR-HPV infections, including their risk factors, covariates necessary in cervical carcinogenesis as well as in sorting out the optional screening strategies in low-resource settings and for women in different age groups. In the long run, it is most likely that the cost-effectiveness will be the decisive factor for which screening tests will be selected. Needless to reiterate that screening for cervical cancer precursors will be mandatory until the foreseeable future, even in this emerging era of prophylactic HPV vaccination.

Key words: Human papillomavirus; Risk factors; Covariates; Oral contraception; Smoking; Drug addiction; Reproductive factors; Menarche; Menopause; Screening strategies; CIN; Cervical cancer.

Introduction

Data from carefully controlled long-term prospective follow-up studies suggest that the natural history of clinical human papillomavirus (HPV) infections in the uterine cervix is identical to that of cervical cancer precursor (CIN = intraepithelial neoplasia) lesions, with a) progression, b) persistence, and c) regression as the three main outcomes [1-
3]. However, HPV infections have special features in their natural history that are related to the different risks of developing cervical cancer (CC) [4-6]. It seems obvious that virus type, viral load, acquisition of new (incident) infections as well as clearance of HPV or its remaining persistence, are salient features of the natural history of cervical HPV infections [2, 3, 7-11]. More light on these dynamic viral events has been provided only during the past few years, and their significance in cervical carcinogenesis is still incompletely understood. This applies equally well to the accumulation of incident HPV infections and their predictive factors [12-15] as well as to the relatively scanty data on the mechanisms of HPV clearance or persistence, reporting conflicting findings [16-21].

Since the recognition of HPV as the causal agent of CC and its precursor (CIN) lesions in the late 1970s [4, 5, 22], a substantial amount of epidemiological data has been accumulated on the potential risk factors of HPV infections, CIN, and CC [4, 5, 23-26]. It is generally accepted that oncogenic HPV types are the single most important etiological factors of CC, associated with this disease in nearly 100% of cases [4, 27, 28]. Since the first reports (in the mid 1980’s) on the risk factors predisposing women to genital HPV infections [29, 30], it has become increasingly clear that several cofactors are needed to complete the causal pathway from oncogenic HPV infection to high-grade CIN and eventually to invasive CC [4, 23-31]. The role of many such potential risk factors has been revisited in the recent literature, e.g., the role of cigarette smoking, drug addiction, oral contraception (OC), and reproductive factors (menarche, menopause).

Of the potential cofactors necessary for the development of high-grade CIN and CC, those associated with reproduction have attracted increasing attention only recently [4, 26, 32-38]. Apart from the role of OC [32-39], such reproductive factors of interest as potential co-factors of HPV infections in cervical carcinogenesis include parity [32, 34, 40-43], age at first intercourse [26, 33, 35, 39, 41-44], age at first full-term delivery [33, 35, 45, 46], menopause [47, 48], and age at menarche [42, 46, 49, 50]. The role of parity and age at first intercourse are well established risk factors for HPV, whereas data are more scanty and/or controversial concerning the other listed factors.

Since the general acceptance that HR-HPV types are the single most important etiological agents of CC, it has been widely recognized that testing for HPV might offer an alternative strategy to identify women at risk for CC; at the stage when conventional Pap smear cytology is still negative or inconclusive [51-54]. This has prompted launching of a variety of guidelines and recommendations for novel strategies in CC screening during the past few years [54-58]. Data from several recent trials suggest that screening strategies optimal for women below 30-35 years of age should be different from those used to target older women [59-61]. Until now, however, these strategies have been tested almost exclusively in well resourced Western countries, where a necessary infrastructure for CC screening exists [59-62], and little data [61] are available on the feasibility of these different screening strategies among non-privileged women in low-resource settings where CC burden is the highest.

During the past several years, this author has been engaged in coordinating two major multi-centre trials testing optional screening tools in low-resource settings in both East Europe and in Latin America. These international trials include the NIS (New Independent States of the former Soviet Union) cohort (n = 3,187 women) [63] and the LAMS (Latin American Screening) study (n = 12,114 women) [64]. In both studies, a sizeable cohort of women (887 and 1,011, respectively) have been prospectively followed-up to assess the natural course of HR-HPV infections and a wide variety of implicated risk factors as potential predictors of the disease outcome (acquisition, persistence and clearance).

In this communication, some of the key recent observations from the NIS and LAMS studies will be discussed, with special emphasis on i) risk factors that are still controversial (i.e., OC, smoking) or not previously studied (drug addiction), on ii) reproductive factors as potential cofactors of HPV infections in cervical carcinogenesis (i.e., menarche, menopause), and finally on iii) the performance of different optional screening strategies among young and older women. Although closely related to these topics, a detailed discussion on the events of HPV infections (acquisition, persistence, clearance) and their regulators falls outside the scope of this communication, because it has been extensively discussed in a series of original reports [21, 65-67] and in a recent review of the author in this journal [68].

**Oral contraceptives as a risk factor for HR-HPV infections and CIN**

Shortly after introduction into general use, OC was implicated as a risk factor with serious health impediments, including a variety of hormone-dependent cancers [69, 70]. The first reports on possibly increased risk of CC among OC users [69-72] were followed by a large number of epidemiological studies reporting contradictory results regarding OC use as a risk factor for CC. Up to today, there are many more reports that have failed to establish any increased risk for CC associated with OC use [73-87] than those implicating that OC use increases this risk [88-96].

**Oral contraceptives and HPV infections**

Since the recognition of the causal link between HPV and CC [2-5, 28], increasing attention has been focused on interactions between HPV and OC use, raising the question as to whether OC is an independent risk factor of CC or whether such a reported risk is merely due to confounding effects by HR-HPV [73, 74, 77, 79, 80, 89, 90, 97-100]. In the first published report on risk factors for HPV transmission in 1984, use/non-use of contraception in general emerged...
among the most significant ones [29], but we subsequently failed to establish any increased risk for HPV among OC users [26]. Since the early 1990s, a large number of studies have been published, reporting either an increased risk of HPV infection among OC users [98-100, 106], no such risk at all [26, 34, 97, 107-113], or even a protective effect of OC use on the incidence of HPV infections [114-117].

**IARC multi-center case-control studies**

All these data have been repeatedly reviewed by IARC experts, resulting in two separate monographs [36, 118]. In the most recent one, these experts based their evaluation on the pooled data from eight IARC multi-centre case-control studies comprising 1,561 CC patients and 1,916 controls [118, 119]. Compared with never-users, women having used OC for less than five years did not show an increased risk of CC (OR = 0.73; 95% CI, 0.52-1.03) [119]. However, OR for CC was 2.82 (95% CI, 1.46-5.42) among OC users for five to nine years, and OR = 4.03 (95% CI, 2.09-8.02) for those having used OC for > 10 years, leading the authors to conclude that long-term use of OC could be a cofactor that increases the risk of CC in women who are positive for HPV DNA. In subsequent reviews, these data were interpreted with more caution, however [120, 121], and even the WHO does not recommend any changes in the practices of using oral contraceptives [122].

**Analysis of OC in the NIS cohort**

As mentioned above, a cohort study testing 3,187 women for optional screening tools was conducted in three NIS of the former Soviet Union; almost 900 of these women were followed-up to assess the natural history of HPV infections [21, 63, 65-67]. In this NIS cohort study, we also analysed sexual habits and other potential risk factors of CC [123]. Using A) women with no contraception and B) those with non-hormonal contraception as controls, we recently estimated the role of OC use i) in predisposing the women to HR-HPV infections, ii) as an independent risk factor for high-grade CIN or high-grade squamous intraepithelial neoplasia (HSIL) (intermediate endpoint markers in cervical carcinogenesis), and iii) as a predictor of HPV persistence during the follow-up [124]. The key observations are discussed in some detail in the following.

**Data from the NIS cohort**

Interestingly, the three groups of women with different modalities of contraception were practically identical with regard to their HR-HPV prevalence, Pap smear abnormalities and CIN grades [124]. In contrast, the three groups differed significantly (p = 0.0001) in several important characteristics of their obstetric and gynaecological history as well as their sexual preferences. In most respects, OC users and non-OC users were alike but differed from the group of non-users of contraception, e.g., the patient category, number of abortions, age at onset of sexual activity, number of partners during the previous 24 months, STD history, casual sex partners, and history of skin and/or genital warts. Although the significant differences represented a majority of the 66 items recorded by the questionnaire [123], they included all the key variables of sexual behaviour that are known risk factors for CC and CIN. These data indicate that women with different contraceptive modalities also have a significantly different sexual behaviour [124].

When the three groups were analysed for the predictors of high-grade CIN (CIN2+) in univariate analysis, HSIL Pap smear was the only significant predictor common to all three groups, while the number of deliveries predicted CIN2 in OC users and in women with no contraception [124]. All other predictors were different in the three groups, and the list of significant predictors was most extensive for women without any contraception. When analysed separately for HPV-positive and HPV-negative women, use of OC was not a significant predictor of CIN2/3 in either group; OR = 0.98 (95% CI, 0.53-1.82) and OR = 0.92 (95% CI, 0.10-8.85), respectively. This is another indicator that the factors explaining the detection of CIN2/3 in these three groups are different [124].

When the three groups were analysed for the predictors of HR-HPV infections, many more predictors were equally strong in all three groups. Accordingly, age below 35 years, being an STD or GYN patient, and HSIL Pap test were all highly significant predictors of HR-HPV, while previous pregnancy was a significant protective factor against HR-HPV. Several other variables seemed to be significant predictors in two of the three groups, and an additional few predicted HR-HPV in only one of the groups, implicating marked differences in the sexual habits and other recorded epidemiological variables among the three groups [124].

In the next step, whether the use of OC is of any significance to the outcomes of cervical disease and HPV infections was assessed as determined by repeated Pap tests and HPV-testing with HCII [21, 63, 65-67, 124]. Importantly, all three groups were practically identical in their baseline HPV/Pap status (p = 0.440), and no differences could be established among the three groups as to the outcome of their cervical disease or HR-HPV infections. This suggests that the mode of contraception (or non-use of any contraception) is not a significant determinant of the outcome of cervical disease or HR-HPV infections [124].

In the whole cohort, several of the variables were highly significant (p = 0.0001) predictors of HR-HPV in univari-
ate analysis, but importantly, neither the mode of contraception nor hormonal contraception (use/non-use) were of any predictive value [124]. When entered in a multivariate model, only four of these variables proved to be independent significant predictors: age < 35 yrs, patient category, HSIL (all with p = 0.0001), and being a current smoker (p = 0.001). Not unexpectedly, the mode of contraception or OC use were of no predictive value in this multivariate analysis. Finally, multivariate analysis was performed to disclose the independent predictors of high-grade CIN [124]. Only two of the almost 70 variables tested [123] proved to be significant in the final regression model: (i) Patient category (protective when STD category was used as reference), and (ii) HR-HPV detection. Importantly, the two variables recording contraception were not included among those five independent predictors of high-grade CIN [124].

**OC and the risk of HR-HPV and CIN: conclusions from the NIS cohort**

In analysing the role of OC as risk factor for CIN, three hypotheses were tested in the NIS cohort: i) to demonstrate that sexual behaviour is indeed different among OC users, non-OC users and non-users of contraception; ii) those different habits (irrespective of OC use) are the risk factors predisposing these women to HR-HPV, development of high-grade CIN or HSIL, and also influence on the outcome of their cervical disease/HR-HPV infection, and iii) that the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis.

On the basis of the results discussed above [124], it can be concluded that these observations fully confirm the three hypotheses, while demonstrating that i) sexual behaviour is different among OC users, non-OC users, and non-users of contraception; ii) these different risk factors predispose women to HR-HPV, development of high-grade CIN or HSIL, and also influence the outcome of their cervical disease/HR-HPV infection, which is similar irrespective of their OC status, and iii) the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis. The implications of these observations are straightforward: failure to record the epidemiological data on the sexual behaviour and gynaecological and obstetric history inevitably leads to erroneous conclusions on the role of OC as an independent risk factor of CC and its precursors [124].

**Smoking as a risk factor of high-risk HPV and CIN**

Of the other potential cofactors necessary for the development of high-grade CIN and CC, the role of cigarette smoking has attracted increasing attention since the early 1980s [125-130]. This early literature was reviewed in 1990 by Winkelstein, leaving the role of smoking as a risk factor for CC an open issue [131]. As emphasised, most of these early studies failed to control for the residual confounding from the sexual habits [29] as explanatory factors of this smoking-CC association [129, 131, 132]. Controlling for the confounding effect of HPV in studies assessing smoking as a risk factor for CC has been particularly problematic [92, 133-135].

Recent data on smoking as a risk factor are incomplete

During the past few years, smoking as a risk factor for CIN and CC has been examined in several studies using sensitive laboratory methods for HPV detection [84, 85, 97, 135-137]. Increasing evidence from these studies suggests that smoking increases the risk of HSIL, CIN and CC [93, 138-142]. Thus, in a recent pooled analysis of eight IARC case-control studies, there was an excess risk of CC among HPV+ women for both current smokers (OR = 2.30, 95% CI, 1.31-4.04) and exsmokers (OR = 1.80, 95% CI, 0.95-3.44) [143]. In the only prospective study published so far, smoking increased the progression from CIN1 to CIN3 [144]. Similarly, smoking was significantly associated with the failure of CIN treatment [145]. Data are emerging to suggest that smoking directly interferes with the natural history of HPV, i.e., by increasing the chance for virus persistence while prolonging its clearance [19].

Analysis of smoking in the NIS cohort

In the NIS cohort, the role of cigarette smoking as a potential predictor of two intermediate endpoint markers in cervical carcinogenesis was recently analysed: i) HR-HPV infection, and ii) development of high-grade CIN [146], as well as the effect of smoking on the outcome of cervical disease and HR-HPV infections. The original questions recording the patients’ smoking history included the following items: 1) Are you a regular smoker? 2) If yes, how long have you been a regular smoker? 3) How many cigarettes per day? 4) If not presently, have you ever smoked? 5) When did you stop smoking? 6) How long did you smoke regularly (yrs)? 7) How many cigarettes per day? 8) Does your sexual partner smoke? Based on these records, the cohort (n = 3,187) was stratified into three groups according to their smoking history: i) current smokers (n = 726), ii) past smokers (n = 365); and iii) never smokers (n = 2,096).

Data from the NIS cohort

Those three groups differed significantly in most of the key epidemiological variables implicated as risk factors of CIN/CC, including the prevalence of HR-HPV [146]. In fact, the list was very short for those variables which were not significantly different among the three groups. The most relevant of those non-significant variables include: a) the dis-
tribution of Pap smear abnormalities, b) detection of various CIN grades, c) mode of contraception, d) history of genital warts, and history of previous CIN. In smoking related items, the past smokers and current smokers seem to be clearly different as well. Having a sexual partner that was a smoker was significantly more frequent among current smokers.

As to the predictors of CIN2+ in univariate analysis there was no single risk factor in common for all three groups. Age below 35 years was most influential (protective) among never smokers and current smokers, but not significant in past smokers. The Hybrid Capture II (HC2) test was a significant predictor of CIN2+ only among never smokers. Not being nulliparous was a risk factor of CIN2+ only among current smokers. Of the other risk factors, previous Pap smears taken was of no significance among smokers. As compared with predictors of CIN2+, there are many more significant predictors of HR-HPV common to all three groups (or at least two of the three). The following were the risk factors common to all three groups: 1) age < 35 years, 2) patient category, and 3) HSIL in Pap tests.

Outcomes of cervical disease and HR-HPV infection in the prospective cohort of 854 women were determined by repeated Pap tests and HC2 assays. Importantly, all three groups were practically identical in their baseline HPV/Pap status ($p = 0.438$), and no differences could be established among the three groups as to the outcome of their cervical disease ($p = 0.147$) or HR-HPV infections ($p = 0.244$). These data clearly implicate that the outcome of cervical disease or HR-HPV infections are not related to the smoking status of these women [146].

In the whole cohort, several variables were highly significant ($p = 0.0001$) predictors of HR-HPV in univariate analysis. Importantly, being a current smoker and having a sexual partner as a current smoker were both significant predictors of HR-HPV. When all these highly significant predictors were entered to a in multivariate model, only five of these variables proved to be independent significant predictors: 1) age < 35 yrs, 2) patient category, 3) HSIL (all at $p = 0.0001$ level), 4) being a current smoker ($p = 0.014$), and 5) cervical erosion treated ($p = 0.035$, protective). Finally, multivariate analysis was performed to disclose the independent predictors of CIN2+ in the whole cohort. Only two of those proved to be significant in the final regression model: i) patient age < 35 years (protective) ($p = 0.0001$), and ii) HC2+ result ($p = 0.014$). Importantly, none of the smoking history/status variables were included among the independent predictors of high-grade CIN in this multivariate analysis [146].

Smoking and the risk of HR-HPV and CIN: conclusions from the NIS cohort

Taken together, this study does not provide evidence that cigarette smoking is an independent risk factor of CIN2+. Indeed, smoking status is strongly confounded by sexual habits, and few predictors of HR-HPV and CIN2+ are common to never smokers, past smokers and current smokers. There was no indication that the increase in the risk of CC is mediated by increased progression of the disease among smokers. Instead, the increased risk (if any) of CC among smokers is most likely attributed to the increased acquisition of HR-HPV infections, for which the smoking status was a significant independent predictor in a multivariate model.

Drug addiction as a potential risk factor of HR-HPV and CIN

Of the other potential cofactors contributing to the development of high-grade CIN and CC, the role of drug abuse or addiction has surprisingly attracted little attention [147-152]. Until now, all published studies have analysed only human immunodeficiency virus (HIV)-infected women who are drug addicts and/or intravenous drug abusers [147-158]. Data are unanimous in that HR-HPV infections and CIN or HSIL are significantly increased among these HIV+ intravenous drug abusers [159]. Only one recent study has addressed the risk factors of HPV infections in a cohort where drug abuse was the primary inclusion criteria [152]. In that study no controls were examined, however, of the 230 women, 24% were HIV-infected. Thus, we have no data whether drug addiction (abuse) is an independent risk factor of HR-HPV infections or CIN, when controlled for confounding effects from HIV infection or other indicators of high-risk sexual behaviour.

Analysis of drug addiction in the LAMS study

In the LAMS study [64], the role of drug addiction (abuse) was recently analysed as a potential predictor of two intermediate endpoint markers in cervical carcinogenesis: i) HR-HPV infection, and ii) development of high-grade CIN [160]. A nested case-control (1: 4) study was designed, with strict age-matching of the 109 cases (drug abusers) with 436 controls (non-abusers), and analysed using conditional logistic regression for covariates of drug abuse as well as for predictors of HR-HPV and CIN2+ in univariate and multivariate regression analyses.

Data from the LAMS study

In this age-matched case-control setting, the two groups were significantly different with regard to several known or implicated risk factors of HPV, CIN and CC [160]. Accordingly, HR-HPV infections were more prevalent (37.7%) among cases than in controls (21.9%) ($p = 0.019$). Age at first sexual intercourse was significantly ($p = 0.0001$) lower among cases. The case women were also more frequently pregnant at the time of the interview ($p = 0.022$), albeit not
different in any other pregnancy-related variables. There was a highly significant difference between the two groups in their number of lifetime sexual partners; 7.8 vs 2.8 among cases and controls, respectively. Case women had contracted with a partner who had a STD and also reported a personal history of STD significantly (p = 0.0001) more often than controls. Modes of contraception were dramatically different between the two groups, and use of oral contraception was more frequent among controls. The same was true with ever having a Pap smear taken (p = 0.021). Ever having been a smoker or being a current smoker was significantly more frequent among cases than in controls.

Conditional logistic regression analysis was conducted to assess the covariates (explanatory factors) associated with drug abuse/non-abuse (as a dependent variable) [160]. The following covariates were significantly associated with drug abuse in univariate analysis: 1) being single rather than married, 2) early onset of sexual activity, 3) being pregnant at the interview, 4) higher number of pregnancies, 5) higher number of abortions, 6) higher number of lifetime sexual partners, 7) contracted with sex partners who had a STD, 8) less frequently used OCs, 9) history of a STD, 10) more rarely a previous Pap smear taken, 11) being more often current or past smokers, with longer history of smoking and higher number of daily cigarettes smoked. When adjusted for all significant covariates in univariate analysis, only five of these variables proved to be independent covariates of drug abuse in multivariate conditional logistic regression: 1) more than five lifetime sexual partners (p = 0.0001), 2) ever having been an active smoker (p = 0.0001), 3) oral contraception (protective) (p = 0.013), 4) ever having had a Pap smear taken (protective) (p = 0.027), and 5) ever had a STD (p = 0.041) [160].

Not unexpectedly, several of these covariates of drug abuse were also risk factors of HR-HPV infections, when univariate and multivariate logistic regression were used to assess the predictors of HR-HPV infection. Of the multitude of factors associated with HR-HPV in univariate analysis, only three proved to be independent predictors in multivariate analysis: 1) age below 30 years (p = 0.045); 2) number of lifetime sexual partners > 5 (p = 0.046); and 3) being a current smoker (p = 0.005). Importantly, drug abuse itself was not a significant independent risk factor of HR-HPV infection (OR = 0.70, 95% CI, 0.31-1.58 for non-abusers), although it increased the risk in univariate analysis [160]. As anticipated, few variables predicted CIN2+ even in univariate analysis, and being a drug abuser was not one of those. Being HR-HPV positive was the only independent determinant of CIN2+ in multivariate analysis. Importantly, being a drug abuser did not increase the risk of CIN2+ in either univariate- or multivariate analysis.

**Drug addiction and the risk of CIN: conclusions from the LAMS study**

This was the first study addressing the importance of drug addiction as an independent risk factor of both oncogenic HPV infections and CIN2+ lesions [160]. These analyses confirm that drug abuse itself is not a risk factor of i) contracting HR-HPV infection or ii) developing CIN2+. In this matched case-control setting, being HR-HPV positive is not among the significant independent covariates of drug addiction, which is closely associated with several of the key indicators of risky sexual behaviour. The latter in turn are independent risk factors of HR-HPV infections, whereas drug addiction is clearly not. Being such a powerful surrogate marker of high-risk sexual behaviour, HR-HPV remains the only independent risk factor of CIN2+ in multivariate analyses. In simple terms, drug abuse seems to be closely associated with several of the key indicators of risk sexual behaviour, which predisposes women to oncogenic HPV infections, and thus indirectly contributes to the development of CIN2+ lesions.

**Age at menarche as a risk factor of HR-HPV and CIN**

Increasing attention has been recently paid to characteristics associated with reproduction as potential cofactors of high-grade CIN and CC [4, 26, 32-38]. Apart from the role of OC [32-39, 124], such reproductive factors of interest include parity [32, 34, 40-43], age at first intercourse [26, 33, 35, 39, 41-44], age at the first full-term delivery [33, 35, 45, 46], menopause [47, 48], and age at menarche [42, 46, 49, 50]. The role of parity and age at first sexual intercourse are well established risk factors of HPV, whereas the data on the other listed factors are more scanty and/or controversial.

**Data on age at menarche are controversial**

Until now, only a few studies have specifically addressed the significance of age at menarche as a potential cofactor of HPV in the development of CIN [50, 161, 162], and in a few others, this topic has been assessed in addition to other potential reproductive risk factors [33, 34, 49, 163]. In most of these studies, only the role of age at menarche was analysed [33, 34, 49, 163], while three studies also assessed another menarche-age-derived variable, i.e., the time from menarche to the first sexual intercourse (TMI) [161-163]. Determined from these reports, the role of age at menarche (or TMI) as risk predictors of HPV infection or CIN seems to be highly controversial [33, 34, 42, 46, 49, 50, 161-163].

**Analysis of menarche age and derived variables in the NIS cohort**

The NIS cohort recently analysed age at menarche as potential predictor of two intermediate endpoint markers in cervical carcinogenesis: i) HR-HPV infection, and ii) development of high-grade CIN [164]. Apart from the menarche
age itself, the role of three menarche-derived variables were further evaluated: i) time from menarche to first sexual intercourse (TMI); ii) time to first pregnancy (TMP), and iii) time to the first full-term delivery (TMD), all variables being analysed both in univariate and multivariate models.

Data from the NIS cohort

Based on the patient records, women were stratified into three groups according to their menarche age: i) menarche age < 13 years; ii) those between 13-14 years; and iii) women with menarche age > 15 years [164]. Importantly, the three groups were shown to be identical with regard to HR-HPV prevalence (both HC2 and real-time PCR, TaqMan assay), detection of Pap smear abnormalities (ASCUS, LSIL, HSIL cutoff) and CIN lesions. There was an increasing trend of ever having been pregnant in parallel with older age at menarche (p = 0.024). Not unexpectedly, all variables calculated from menarche age (TMI, TMP, TMD) were significantly different in the three groups, being inversely related to the age at menarche. The other significant differences included: 1) number of deliveries (p = 0.001), 2) ever had abortions (p = 0.019), 3) age at first sexual intercourse (p = 0.004), 4) practice of oral sex (p = 0.001), and 5) time since the last Pap test (p = 0.046) [164].

When the three groups were analysed for the predictors of CIN2+ in univariate analysis, there was no single risk factor in common to all three patient groups. Age below 35 years was most influential (protective) among women who had their menarche at 13-14 years of age, less among those with late menarche (> 15 years), and of no significance in the youngest menarche age group. The HCII+ test was a significant predictor of CIN2+ only in the intermediate menarche age group, as was ever having been pregnant. Interestingly, for HSIL the Pap test failed to correlate with CIN2+ in the older menarche age group, but was a significant determinant in the two others [164].

As compared with predictors of CIN2+, the results were much more unanimous for predictors of HR-HPV. This applies particularly to the young menarche age group and intermediate age group, where practically all predictors were the same, with i) the age of sexual onset, and ii) being a current smoker as the only exceptions. On the other hand, in the older age group, fewer variables were significant predictors of HR-HPV, and the role of casual sexual contacts was a significant risk factor exclusively in this group. The following were the risk factors common to all three groups: 1) age < 35 years, 2) patient category, 3) HSIL in the Pap test, and 4) TMD. All three groups were practically identical in their baseline HPV/Pap status (p = 0.750), and no differences could be established between the three groups as to the outcome of their cervical disease (Pap smear abnormality) or HR-HPV infections. These data clearly implicate that the outcome of cervical disease or HR-HPV infections is not related to the menarche age of these women.

In the whole cohort, several variables were highly significant (p = 0.0001) predictors of HR-HPV in univariate analysis, but importantly, the age at menarche per se was not of any predictive value, irrespective of whether entered as a continuous or categorical variable. On the other hand, TMI, TMP and TMD were all highly significant at the p = 0.0001 level. Ever having been pregnant and number of deliveries (abortions, miscarriages) were all protective against HR-HPV [164]. When all highly significant predictors were entered in the multivariate model (including menarche age), only five of these variables proved to be independent significant predictors: 1) age < 35 yrs, 2) patient category, 3) HSIL (all at p = 0.0001 level), 4) being a current smoker (p = 0.014), and 5) cervical erosion treated (p = 0.035). Importantly, menarche age or any of the menarche-derived variables were not independent predictors in the multivariate model.

Finally, multivariate analysis was performed to disclose the independent predictors of high-grade CIN in the whole cohort. Only two of those proved to be significant in the final regression model: i) patient age < 35 years (protective) (p = 0.0001), and ii) HC2+ result (p = 0.014). Importantly, age at menarche or any of the menarche-derived variables were not included among the independent predictors of high-grade CIN in this multivariate analysis.

Menarche age and the risk of HR-HPV or CIN: conclusions from the NIS cohort

Data from this analysis indicate that menarche age is not associated with increased risk of HR-HPV infections, which are equally prevalent among women with early, intermediate and late menarche [164]. Instead, short intervals between menarche and onset of sexual activity (TMI), first pregnancy (TMP) and first delivery (TMD), are all significant predictors of HR-HPV infection. This impact disappears, however, in multivariate analysis, where the well established risk factors remain as only independent predictors. Similarly, menarche age or any of the three intervals do not predict the development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of cervical lesions or HR-HPV infections in a longitudinal setting over time [164].

Determinants of increased prevalence of HR-HPV infections among older women

Since the early reports on HPV and CIN, epidemiological data from different countries confirmed that the peak prevalence of cervical HPV infections (detected by Pap smear or DNA hybridisation techniques) occurs between 22-24 years of age, with constant decline in parallel with increasing age [22, 165-167]. This was neatly explained by the
early studies (based on Pap smear screening data) implicating a particularly high (8%) annual incidence of HPV infections among 22-year-old women [168, 169].

More recent studies on the natural history of HPV infections [1, 2] have further refined the dynamics of these viral events in different populations. Accordingly, incident HR-HPV infections are clearly age-dependent, the 3-year cumulative incidence exceeding 50% among women below 20 years of age, following the onset of their sexual activity [65, 170, 171]. On the other hand, clearance of the virus did not show such strict age-dependence [21], but continued at a constant rate among women over 30 years of age [165, 172]. These age-specific incidence and clearance rates used to estimate the age-specific prevalence of HR-HPV infections reproduce the true figures quite closely, except for a small gap in each of the 5-year age groups [67]. This gap between the true- and estimated age-specific prevalence rates is due to the fact that instead of clearance, some of the acquired infections remain persistent. These persistent HR-HPV infections are considered as a prerequisite for developing a progressive cervical disease and are currently the subject of intense study for their covariates [66].

**Second peak of HPV prevalence among postmenopausal women**

During the past few years, this dynamic model of HPV acquisition, clearance and persistence, explaining the linearly declining age-specific prevalence curve [22, 165-167] has been challenged by the data from several population-based studies, reporting a second peak in HPV prevalence among women > 55 years of age [43, 105, 173]. In some studies, a similar peak among older women has been reported for HPV incidence as well [174, 175]. Indeed, some recently published population-based studies report highly contradictory results from different geographic areas. There are populations, where the age-specific prevalence curve is clearly U-shaped, with a second peak among postmenopausal women [43, 105, 173-179]. In other studies, no such U-shaped prevalence curve was established, but the shape was that of a declining linear curve [29, 41, 180-183]. The IARC HPV Prevalence Survey data failed to give one single explanation for these differences, and several key questions still remained unanswered [48, 184].

**Analysis of age-specific HR-HPV prevalence in the NIS cohort**

In the NIS cohort, we wanted to clarify the reasons for the U-shaped HPV prevalence curve [185], previously reported in this cohort [63]. The whole cohort of 3,187 women was stratified into three age groups according to their different HR-HPV prevalence profile. These three age categories are: i) two youngest age groups (women below 20 years and those between 21 and 25 years; n = 1,103) with the peak HPV prevalence; ii) women between 26 and 55 years (n = 2,004) showing linearly declining HPV prevalence; and iii) women past 55 years (n = 80) with sharply increasing HR-HPV prevalence [63, 67]. To adjust for the differences in age distribution in the three NIS countries, the age-standardised HPV prevalence was calculated for 14 five-year age groups (15-84 years) of the European standard population [185]. Logistic regression modeling with the curve estimation procedure was used to assess the age profile in each of these countries, by fitting the logistic regression model with either i) linear, ii) quadratic or iii) cubic terms for those 14 five-year age groups. Curves with a significant (p < 0.05) cubic term were classified as non-linear (U-shaped), those with significant cubic term as non-linear (bi-phasic or S-shaped), to distinguish from those with only a linear age term. All curve fit procedures were controlled by scatter plots, where the fit parameters (= predicted parameters) were plotted against the residuals [185].

**Age-specific HR-HPV prevalence in the NIS cohort**

The age-standardised prevalence rate (ASPR) of HR-HPV infections was very similar in Russia (18.3/100 women; 95% CI, 16.6-19.9), and Belarus (17.2/100 women; 95% CI, 14.1-20.3), but in Latvia as high as 24.6/100 women (95% CI, 20.60-28.65) [185]. In the whole cohort, HPV prevalence curve was clearly U-shaped, steadily declining from 55.6% among women < 20 years of age, down to 10.1% among those aged 51-55 years, followed by a deep increase among women older than 55 years [185]. In the whole cohort, the F statistic for model fit was significant both in the linear and quadratic equation (p = 0.0001), but substantially higher (R² = 0.966) for the quadratic model (U-shape curve) than (R² = 0.809) for the linear model. In the curve of Russia, the results mimic those for the whole cohort; R² = 0.806 for the linear model and R² = 0.968 for the quadratic model (p = 0.0001 for both). In the curve of Belarus, there was not much difference between the linear and quadratic models; R² = 0.952 and R² = 0.995, respectively. The age-specific HPV curve of Latvia shows the least obvious linearity and the most accentuated second peak; R² = 0.647 for linear and R² = 0.915 for the quadratic model [185].

**Epidemiological, clinical and viral determinants of increased HPV prevalence in the older women**

The three age categories of women were shown to differ at the p = 0.0001 level with regard to the majority of the recorded epidemiological variables [185]. Many of these variables are directly explained by the age difference between the three categories. On the other hand, however, there are some interesting variables that do not show any difference between the three groups; e.g., history of skin and genital warts, time since last Pap smear, previous Pap smear normal, and ever having had cervical erosion [185].
Of the determinants of HR-HPV infection in the three groups, patient category was significant only in the two groups of younger women, but not among the older ones. HSIL Pap predicted HR-HPV only in the two older groups, whereas the CIN3 cutoff was a significant predictor only in women between 25 and 55 years of age. The same holds true with the number of deliveries, which had a protective effect among this age group (a surrogate of regular family life?). A history of previous CIN was significant only among the older women (OR = 5.62; 95% CI, 1.01-31.48) [185].

HPV prevalence was highest among the youngest age group, but not significantly different between the two older ones, irrespective of whether determined by the HC2 or TaqMan assay. The quantitative viral loads for HPV16, 18/45, 31 and 33 were markedly higher among the older women. The most interesting is the curve of HPV16 viral loads, showing the best fit with the cubic model (\(R^2 = 0.714\)) and resulting in a distinct biphasic S-shaped curve with a sharp second rise among women past 50 years of age.

The distribution of individual HPV types was significantly different among the three age categories [185]. As compared with the youngest age groups, there was a marked shift from multiple-type infections (from 30.7% to 6.3%) to the accumulation of HPV16 (37.5% of all HPV+ cases) and HPV31 (31.3%) among the older women. There was a transition from episomal to mixed and integrated state among the youngest age groups to those above 55 years of age, in whom, all HPV16 positive lesions showed viral integration (\(p = 0.009\)). Similarly, the viral load of integrated HPV16 was significantly higher (17.5) in the older women, as compared with the two other age categories, with practically identical loads of integrated HPV16 [185].

Finally, we noticed no difference in the clinical course of the cervical disease as determined by the repeated Pap tests [185]. In contrast, the outcome of HR-HPV infections was significantly different among the three groups. As compared with the age group 26-55, in which a sharp decline of HR-HPV prevalence was characteristic, women over 55 years of age showed a i) higher proportion of incident infections, ii) higher rate of viral persistence, and particularly iii) lower rate of HR-HPV clearance (\(p = 0.0001\)) [185].

**Determinants of age-specific prevalence of HR-HPV: conclusions from the NIS cohort**

This study sheds new light on most of the open issues related to the shape of the age-specific HPV prevalence curves [43, 105, 173-179], and in particular to the determinants of the second peak observed among women past 55 years of age. Taken together, these data feasibly explain what was suggested by our in vitro studies some years back [186-188].

The rapid acquisition of HR-HPV infections after onset of sexual activity [15, 67] leads to an early peak of both HR-HPV prevalence and viral loads between 20 and 25 years of age. This is followed by a constant clearance [21, 67] and reduced viral loads of the infections between 25 and 55 years of age. In women > 55, a sharp increase in both HPV prevalence and viral loads follows, shown by the U-shaped and S-shaped age-specific curves, respectively [185]. These data implicate that among women who fail to eradicate their HR-HPV infection by menopause, the selection of an integrated viral clone has probably taken place, driving the process towards an aggressively progressing disease. Consequent to this, most of the HR-HPV infections in women older than 55 years were associated with high-grade CIN or invasive carcinoma in the NIS cohort [185].

**Are different screening strategies needed for younger and older women?**

In the above, the special features of HR-HPV infections encountered in postmenopausal women have been discussed [185]. It sounds feasible to speculate that the optimal screening strategies for these older women might be different from those applied for younger age groups. Indeed, data from several recent trials suggest that screening strategies optimal for women below 30-35 years of age should be different from those used to target older women [59-62, 189-191]. In these trials, HPV testing has been shown to perform better among these older women [192-195], evidently due to the fact that HPV infections among younger women are extremely common, and in most cases resolve without inducing clinical lesions [3-5, 28, 196]. These data further implicate that adjunct HPV testing of these older women might enable extension of the screening interval to three to five years, leading to considerable cost savings in the screening programmes [59-62, 189-192]. Until now, however, these strategies have been tested almost exclusively in well resourced Western countries, where the necessary infrastructure for CC screening exists [59, 60, 62, 189-196], and little data [61] are available on the feasibility of these different screening strategies among non-privileged women in low-resource settings where CC burden is the highest.

**Testing optional screening strategies for younger and older women in the LAMS study**

In the LAMS study, eight optional screening tools have been compared in a population-based cohort of > 12,000 women in Brazil and Argentina [64, 197-200]. To address the effect of age on screening strategies, we recently compared the performance of conventional Pap smear cytology, liquid-based cytology (LBC), visual inspection with acetic acid (VIA) as well as HPV testing with HC2 assay in two sub-cohorts of these women; those younger than 35 years (\(n = 5,099\)), and those older than 35 years (\(n = 6,997\)) [201]. The aim was to test the concept as to whether optimal screening results could be obtained by different diagnostic tools among younger and older women, using the biopsy-confirmed CIN2+ as the gold standard with all test performance indicators being corrected for the verification bias.
Data from the LAMS study

Not unexpectedly, the two sub-cohorts analysed in this trial differ in the vast majority of the recorded variables, including the risk factors of HPV, CIN and CC. Although many of these differences are directly related to the different ages of these women, there are numerous indicators of life-style risk of sexual behaviour that are significantly different in these two cohorts [201]. Indeed, the list was very short for those variables that are not significantly different: i) prevalence of HSIL, ii) history of previous CIN or warts, and iii) being a current smoker. The prevalence of HR-HPV infections was almost twice as high (24%) among younger women than that (13.5%) among the older (p = 0.0001). The contrary was true with the prevalence of high-grade CIN lesions. Bearing that the performance of the diagnostic test is dependent on the prevalence of the target disease, it can be anticipated that tests detecting HPV and those detecting high-grade CIN will perform differently in these two sub-cohorts [201].

Indeed, this was shown to be the case for all these diagnostic tests when separately analysed in these two sub-cohorts [201]. Starting from conventional Pap smear, we showed that among young women, the HSIL Pap smear is a highly specific test (98.6%) in detecting CIN2+, but suffers from low sensitivity (33.7%). Corrected for the verification bias increases the specificity close to 100% (99.7%), but further reduces the sensitivity. Using the LSIL cutoff slightly increases the sensitivity (SE) (45.5%) at the expense of reduced specificity (SP) and positive predictive value (PPV). Among women above 35 years, the HSIL Pap is an excellent test in detecting CIN2+, with AUC (area under ROC curve) 0.828 (95% CI, 0.779-0.876) and 67.4% SE, 98.2% SP, 87.3% PPV and 94.3% negative predictive value (NPV). Using LSIL cutoff increases SE but compromises SP and PPV, although AUC is practically identical (0.827), and also significantly different from that (p = 0.684) among younger women. Correcting for the verification bias makes the AUC values of HSIL and LSIL very similar in both sub-cohorts [201]. It was also shown that the performance indicators of liquid based cytology (LBC) were inferior to those of the conventional Pap smear in all aspects [201]. The results of LBC faithfully reproduce the changes in the indicator values of the Pap test, according to i) SIL cutoff, ii) correction for verification bias as well as iii) the difference between younger and older women. The best balance (AUC = 0.746, 95% CI, 0.560-0.932) is obtained for LSIL cutoff among the older cohort, but the difference between the sub-cohorts is not significant in this or any of the other test comparisons. When corrected for the verification bias, again LBC (HSIL) is an almost 100% specific test both among younger and older women.

Of the two optional screening tools (VIA, HC2), VIA with the regularly used “abnormal” as the cutoff seems to suffer from both low SE and low SP in both sub-cohorts [201], with no difference in AUC values: 0.535 and 0.541 for younger and older women, respectively. Correction for verification bias does not make the difference significant between the two sub-cohorts (p = 0.824). Using the “suggesting cancer” as the cutoff makes VIA an almost 100% specific (99.9%) test in detecting CIN2+ in both younger and older women, when corrected for the verification bias. However, because of the rarity of these lesions, particularly test sensitivity drops down to one percent in young women, and remains low (18.5%) even among the 35+ age groups [201]. This increase makes the difference between younger (AUC = 0.505) and older (AUC = 0.589) women significant (p = 0.0001), however.

In this comparison, the results on HPV testing as a screening tool are highly interesting. HC2 assay was tested with two thresholds; 1) the manufacturer recommended 1 pg/ml and 2) a higher, 10 pg/ml (RLU/CO) [201]. The results are straightforward; HC2 assay performs significantly better among older women, irrespective of the cutoff, or whether corrected for the verification bias or not. At the best balance, HC2 assay has 85.9% SE, 91.4% SP, 40% PPV and 99% NPV. This (AUC = 0.886) is significantly different from that (AUC = 0.722) for the younger sub-cohort, as are all other comparisons, except the one using the 10 pg/ml cutoff and correction for the verification bias (p = 0.174).

When similar figures were calculated for the combined use of conventional Pap and HC2 assay (with 1 pg/ml cutoff) in the two sub-cohorts, changing the Pap smear cutoff from HSIL to LSIL does not significantly affect the test performance. In all situations, this combination works significantly better in older women. The best balance (AUC = 0.919) is obtained when the values for HSIL/HC2 are corrected for the verification bias, and almost as good (AUC = 0.911) by using the LSIL/HC2 combination [201].

Screening strategies should be different for younger and older women: conclusions from the LAMS study

A notwithstanding conclusion from this analysis was that the conventional Pap and HC2, but not LBC and VIA, perform very differently among younger and older women (201). Similar to Western countries, also in this low-resource setting, both HC2 and conventional Pap tests perform significantly better among women older than 35 years. Correcting for the verification bias, however, will make this significance disappear for the Pap test but not for the HC2 assay. This further emphasises the important difference in HC2 performance among younger and older women, because correction for the verification bias is currently regarded as an essential measure to make the results comparable in studies where confirmation by the gold standard is imperfect [202]. The mechanistic explanation certainly includes the important events in HPV biology that emerge among older women. Instead of HPV clearance being the most common event among young women, there seems to be HPV persistence, transition from multiple-type infections to single-type infections, increase of viral load and higher probability for virus integration among the older age groups [185].
Of the single tests, the best performance is obtained for verification bias-corrected HC2 among the older women. If only the PPV is considered, there is no test superior to the conventional Pap test in both younger and older women. Among the latter, the best balance in SE and SP is obtained when HC2 is combined with the Pap test, whereas in younger women, such a combination does not give any added value to HC2 as the stand-alone test (AUC = 0.728 and 0.721, respectively), and adds very little to the conventional Pap test (AUC = 0.662 for HSIL and AUC = 0.684 for LSIL). The choice of an optimal screening test for younger and older women depends on whether the highest PPV (Pap test) or the best SE/SP balance (HC2) is used as the selection criteria [201].

Conclusions and future prospects

The NIS cohort and LAMS study have significantly contributed to solving several of the open issues in the natural history of HR-HPV infections, including the risk factors, covariates necessary in cervical carcinogenesis as well as in sorting out the optional screening strategies in low-resource settings and for women in different age groups. Many of these issues have been discussed in this communication. However, still several important issues remain to be elucidated before the complex natural history of HPV infections and their role in cervical carcinogenesis can be understood [203].

Accordingly, in light of the existing literature, the role of OC as a risk factor of CC still remains enigmatic. Data obtained from the NIS cohort [124], however, suggests that i) the sexual behaviour is different among OC users, non-OC users, and non-users of contraception; ii) these different risk factors predispose women to HR-HPV, development of high-grade CIN (HSIL), and also influence the outcome of their cervical disease/HR-HPV infection, which is similar irrespective of their OC status, and iii) the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis. Failure to record the epidemiological data on the sexual behaviour and gynaecological and obstetric history inevitably leads to erroneous conclusions on the role of OC as an independent risk factor of cervical cancer and its precursors [124]. In all future studies, the strong confounding effects from these lifestyle behavioural factors must be taken into account, while interpreting the data on OCs as potential risk factors of CC.

Similarly, the published data on the role of smoking as a risk factor of CC remains controversial. This is particularly due to the fact that too few population-based studies are available, where the evident strong confounding effects from the HR-HPV infections would have been adequately controlled. Our results from the NIS Cohort [146] failed to provide any evidence that cigarette smoking is an independent risk factor of CIN2+. Smoking status seems to be strongly confounded by sexual habits, and few predictors of HR-HPV and CIN2+ were common to never smokers, past- or current smokers. There was no indication whatsoever that increase in the risk of CC is mediated by increased progression of the disease among smokers. Instead, the increased risk (if any) of CC among smokers seems to be attributed to the increased acquisition of HR-HPV infections, of which smoking status was an independent predictor in the multivariate model (146). This fact must be adequately controlled in all future studies analysing the role of smoking as a covariate in cervical carcinogenesis.

The same seems to apply to drug addiction as a risk factor of CC as well. The analysis of the LAMS data was the first to address the importance of drug abuse as an independent risk factor of both HR-HPV infections and CIN2+ lesions, using a matched case-control setting [160]. These analyses confirm that drug abuse itself is not a risk factor of i) contracting oncogenic HPV infection or ii) developing CIN2+. In simple terms, drug abuse seems to be closely associated with several of the key indicators of risky sexual behaviour, which predisposes women to oncogenic HPV infections, and thus indirectly contribute to the development of CIN2+ lesions. Clearly, more studies are needed on this subject, based on large enough sample sizes to enable sub-group analysis of individual drugs, which was not possible in our study [160].

As to the role of reproductive factors as possible covariates, data from the NIS cohort clearly implicate that menarche age is not associated with increased risk of HR-HPV infection [164]. However, short intervals between menarche and onset of sexual activity, first pregnancy and first delivery, were all significant predictors of HR-HPV infection in univariate but not in multivariate analysis. Similarly, age at menarche or any of the three intervals (TMI, TMP, TMD) did not predict the development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of cervical lesions or HR-HPV infections during a prospective follow-up of this cohort [164]. This topic still merits further studies, however, to elucidate e.g., whether these results elaborated collectively for HR-HPV types also hold for individual HPV genotypes.

Another special group are postmenopausal women, now shown in several studies to have a second peak of HR-HPV prevalence among OC users, non-OC users, and non-users of contraception; ii) these different risk factors predispose women to HR-HPV, development of high-grade CIN (HSIL), and also influence the outcome of their cervical disease/HR-HPV infection, which is similar irrespective of their OC status, and iii) the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis. Failure to record the epidemiological data on the sexual behaviour and gynaecological and obstetric history inevitably leads to erroneous conclusions on the role of OC as an independent risk factor of cervical cancer and its precursors [124]. In all future studies, the strong confounding effects from these lifestyle behavioural factors must be taken into account, while interpreting the data on OCs as potential risk factors of CC.

Similarly, the published data on the role of smoking as a risk factor of CC remains controversial. This is particularly due to the fact that too few population-based studies are available, where the evident strong confounding effects from the HR-HPV infections would have been adequately controlled. Our results from the NIS Cohort [146] failed to provide any evidence that cigarette smoking is an independent risk factor of CIN2+. Smoking status seems to be strongly confounded by sexual habits, and few predictors of HR-HPV and CIN2+ were common to never smokers, past- or current smokers. There was no indication whatsoever that increase in the risk of CC is mediated by increased progression of the disease among smokers. Instead, the increased risk (if any) of CC among smokers seems to be attributed to the increased acquisition of HR-HPV infections, of which smoking status was an independent predictor in the multivariate model (146). This fact must be adequately controlled in all future studies analysing the role of smoking as a covariate in cervical carcinogenesis.

The same seems to apply to drug addiction as a risk factor of CC as well. The analysis of the LAMS data was the first to address the importance of drug abuse as an independent risk factor of both HR-HPV infections and CIN2+ lesions, using a matched case-control setting [160]. These analyses confirm that drug abuse itself is not a risk factor of i) contracting oncogenic HPV infection or ii) developing CIN2+. In simple terms, drug abuse seems to be closely associated with several of the key indicators of risky sexual behaviour, which predisposes women to oncogenic HPV infections, and thus indirectly contribute to the development of CIN2+ lesions. Clearly, more studies are needed on this subject, based on large enough sample sizes to enable sub-group analysis of individual drugs, which was not possible in our study [160].

As to the role of reproductive factors as possible covariates, data from the NIS cohort clearly implicate that menarche age is not associated with increased risk of HR-HPV infection [164]. However, short intervals between menarche and onset of sexual activity, first pregnancy and first delivery, were all significant predictors of HR-HPV infection in univariate but not in multivariate analysis. Similarly, age at menarche or any of the three intervals (TMI, TMP, TMD) did not predict the development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of cervical lesions or HR-HPV infections during a prospective follow-up of this cohort [164]. This topic still merits further studies, however, to elucidate e.g., whether these results elaborated collectively for HR-HPV types also hold for individual HPV genotypes.

Another special group are postmenopausal women, now shown in several studies to have a second peak of HR-HPV prevalence in many populations [43, 105, 173-179]. In the NIS cohort, rapid acquisition of HR-HPV infections after onset of sexual activity [15, 67] leads to an early peak of both HR-HPV prevalence and viral loads between 20 and 25 years of age. This is followed by a constant clearance [21, 67] and reduced viral loads of the infections between 25 and 55 years of age. In women > 55, a sharp increase in both HPV prevalence and viral loads follows, shown by the U-shaped and S-shaped age-specific curves, respectively [185]. These data implicate that in women who fail to eradicate their HR-HPV infection by menopause, there is a transition from multiple infections to single-type infections, most probably accompanied by selection of an integrated viral clone, driving the process towards an aggressively progressing disease [185]. This special group of women is currently under intense study in several population-based studies.
These peculiarities in the behaviour of HR-HPV infections among older women finally leads us to consider the issues, whether different screening strategies would be appropriate for younger and older women [201]. Consonant with other recent reports [59-62, 189-192], data from the LAMS study showed that conventional Pap and HC2, but not LBC and VIA, perform significantly differently among younger and older women [201]. However, the choice of an optimal screening test for younger and older women depends on whether the highest PPV (Pap test) or the best SE/SP balance (HC2) is used as the selection criteria [201]. It is generally acknowledged that an optimal screening test is the one with the highest PPV (204). A high PPV suggests that a reasonably high proportion of the programme costs are being spent for detection of true disease, while low PPV indicates that a high proportion of costs are being wasted for the evaluation of the false positives using other diagnostic tests. At the end of the day, it is most likely the cost-effectiveness that is the decisive factor of which screening test will be selected, at least in the low-resource setting. Needless to re-iterate that screening for cervical cancer precursors will be mandatory until the foreseeable future, even in this emerging era of prophylactic HPV vaccination.

Acknowledgements

The two multi-centre studies of the author extensively discussed in this communication have been supported by the European Commission. The grant for the NIS cohort study (INCO-Copernicus Program; Contract No. ERB IC15-CT98-0321) and the one for the LAMS study (INCO-DEV Program; Contract# ICA4-CT-2001-10013), are gratefully acknowledged.

References

New concepts on risk factors of HPV and novel screening strategies for cervical cancer precursors


New concepts on risk factors of HPV and novel screening strategies for cervical cancer precursors


Phase II study of radiation therapy combined with weekly nedaplatin in locally advanced uterine cervical carcinoma (LAUCC): Kitasato Gynecologic Radiation Oncology Group (KGROG 0501) - initial analysis

Y. Niibe1, M.D., Ph.D.; S. Tsunoda2, M.D., Ph.D.; T. Jobo2, M.D., Ph.D.; M. Imai2, M.D., Ph.D.; K. Matsuo3, M.D., M.S., Ph.D.; K. Matsunaga1, M.D.; N. Unno2, M.D., Ph.D.; K. Hayakawa1, M.D., Ph.D.

1Department of Radiology, Kitasato University School of Medicine, Sagamihara, 2Department of Gynecology, Kitasato University School of Medicine, Sagamihara, 3Division of Epidemiology and Prevention, Aichi Cancer Center, Nagoya (Japan)

Summary

Objective: Locally advanced uterine cervical carcinoma (LAUCC) treated with chemoradiotherapy is considered to be the standard treatment regimen. However, no evidence of its efficacy and safety has been obtained from the Japanese population. Furthermore, the total dose of Japanese radiation therapy protocol is less than that of the USA which indicated that chemoradiotherapy for LAUCC is better than radiation therapy alone by phase III clinical trials. Thus, the current phase II study was designed to evaluate chemoradiotherapy with a lower radiation dose for LAUCC using weekly nedaplatin effectively and safely in the Japanese population. Nedaplatin is a platinum drug and no hydration is required to infuse patients because it is less toxic on renal function. If this phase II trial is successful, chemoradiotherapy for LAUCC in out-patient clinics could be possible. Patients and Methods: Patients registered in the current study were found to have LAUCC based on the following criteria i) pathologically proven squamous cell carcinoma or adenocarcinoma, ii) FIGO clinical Stage Ib, IIa, IIb with bulky tumor (diameter > 40 mm assessed by pelvic magnetic resonance imaging) or pelvic lymph node swelling (diameter > 10 mm assessed by pelvic computed tomography); iii) FIGO clinical Stage IIIa, IIIb and IVa with no paraaortic lymph node swelling (diameter > 10 mm) observed by abdominal computed tomography; iv) age: 20-75 years; v) performance status: 0-2. The treatment protocol was as follows: Radiation therapy in a combination of external beam radiation therapy (total dose: 50 Gy-52 Gy/25-27 fractions with central shielding after 30-32 Gy) with high-dose rate intracavitary irradiation (24-30 Gy/4-6 fractions to point A). Chemotherapy applied in the current study was weekly nedaplatin infused intravenously (30 mg/mm²/time, once a week, total 150 mg/mm²/5 weeks). Sample size in the current study was 45 LAUCC patients recruited for three years at a single institution. This protocol was permitted by the ethics committee of Kitasato University Hospital.

Results: Ten patients were registered in this study between June 2005 and March 2006. The median age was 57.5 years (range 36-73). PS0 was five and PS1 was five. As for clinical stage, nine were IIIb and only one was IIb. Nine patients were proven to have squamous cell carcinoma and one adenocarcinoma. The median maximum tumor diameter was 62.5 mm (range 30-100 mm). As for initial response, eight had CR and two had PR (100% response rate). As for hematological acute morbidity, three were grade 2, six were grade 3, and one was grade 4.

Conclusions: This initial analysis of the phase II study confirmed that concurrent chemoradiotherapy using nedaplatin is safe and efficacious, thus we decided to undergo further studies.

Key words: Concurrent chemoradiotherapy; High-dose rate intracavitary brachytherapy (HDR-ICBT); Nedaplatin; Locally advanced uterine cervical carcinoma.

Introduction

Locally advanced uterine cervical carcinoma (LAUCC) treated with chemoradiotherapy is considered to be the standard treatment regimen. However, no evidence of its efficacy and safety has been obtained from the Japanese population. Furthermore, the total dose of the Japanese radiation therapy protocol is less than that of the USA which indicated that concurrent chemoradiotherapy achieved better treatment outcomes than radiation therapy alone by phase III trials [1-3]. Nakano et al. reported a more than 20-year retrospective analysis of LAUCC of the Japanese population treated with radiation therapy alone using the standard Japanese radiation therapy protocol [4]. The method is the combination of external beam radiation therapy with concomitant use of high-dose-rate intracavitary brachytherapy (HDR-ICBT). The external beam radiation therapy protocol was the following: the fractionation was 1.8-2 Gy per fraction, 5 fractions per week, totaling 20-40 Gy using the entire pelvic irradiation field without central shielding, followed by 2 Gy per fraction, 5 fractions per week totaling 15-30 Gy using the entire pelvic field with central shielding. Thus, the total dose was 45-60 Gy. The HDR-ICBT protocol

This study was presented at the 100th Annual Meeting of American Association for Cancer Research, April 13-18, 2007, Los Angeles, CA, USA.

Revised manuscript accepted for publication July 25, 2008
was the following: fractionation was 5-6 Gy per fraction to point A, once per week totaling 15-24 Gy per 3-4 fractions, which was performed concomitantly with entire pelvic irradiation using central shielding. Then, the total BED was about 62-86 Gy10 [4]. These are less than the ABS guideline dose of 100-108 Gy10 [5, 6].

Thus, the current phase II study was designed to evaluate chemoradiotherapy with this lower radiation dose of the Japanese HDR-ICBT protocol for LAUCC using weekly nedaplatin effectively and safely in the Japanese population. Nedaplatin is a platinum drug produced by Shionogi Pharmaceutical Co., Japan and requires no hydration to infuse patients because it is less toxic for renal function. If this phase II trial is successful, chemoradiotherapy for LAUCC in out-patient clinics could be possible.

The purpose of the current initial analysis was to evaluate the safety and efficacy by evaluating acute toxicities and initial response.

Patients and Methods

Patients registered in the current study were found to have LAUCC based on the following criteria (Table 1): i) pathologically proven squamous cell carcinoma or adenocarcinoma; ii) FIGO clinical Stage Ib, IIA, IIb with bulky tumor (diameter > 40 mm assessed by pelvic magnetic resonance imaging) or pelvic lymph node swelling (diameter > 10 mm assessed by pelvic computed tomography); iii) FIGO clinical Stage IIIa, IIIb and IVa with no paraaortic lymph node swelling (diameter > 10 mm) observed by abdominal computed tomography; iv) age 20-75 years; v) performance status 0-2. The treatment protocol was the following: radiation therapy with a combination of external beam radiation therapy (total dose 50 Gy-52 Gy/25-27 fractions with central shielding after 30-32 Gy) with high-dose rate intracavitary radiation therapy (total dose 50 Gy-52 Gy/25-27 fractions with central shielding after 30-32 Gy) and only one was IIb. Nine patients were proven to be squamous cell carcinoma and one adenocarcinoma. Median maximum tumor diameter was 62.5 mm (range 30-100 mm). As for initial response, eight were CR and two were PR (100% response rate) (Table 3). Hematological acute morbidity in white blood cells resulted in four grade 3 and one grade 4, in neutrophils there were five grade 3, in hemoglobin there was one grade 3 and in platelet cells one grade 3 (Table 4). No patient experienced grade 3 or greater acute non-hematological morbidity.

Table 2. — Patient characteristics.

<table>
<thead>
<tr>
<th>Age</th>
<th>36-73 years (median: 57.5 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performance status:</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Clinical FIGO stage:</td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>1</td>
</tr>
<tr>
<td>IIIb</td>
<td>9</td>
</tr>
<tr>
<td>Histopathology:</td>
<td></td>
</tr>
<tr>
<td>squamous cell carcinoma</td>
<td>9</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Maximum tumor diameter</td>
<td>30-100 mm (median: 62.5 mm)</td>
</tr>
</tbody>
</table>

Table 3. — Initial Response.

| CR | 8 |
| PR | 2 |

Table 4. — Hematological acute morbidity.

| White blood cell | 0 | 2 | 3 | 4 | 1 |
| Neutrophils | 2 | 2 | 1 | 5 | 0 |
| Hemoglobin | 4 | 4 | 1 | 1 | 0 |
| Platelet cells | 5 | 3 | 1 | 1 | 0 |

Discussion

Concurrent chemoradiotherapy treatment (CCRT) for LAUCC has been established as the standard treatment since three large phase III trials and one meta-analysis comparing RT with CCRT for LAUCC revealed its superiority to RT alone [1-3, 7]. The standard regimens of chemotherapy contain cisplatin (CDDP). CDDP is a widely used chemotherapeutic platinum drug for various malignancies [8-11]. However, CDDP has severe renal toxicity, and then heavy hydration is required to undergo treatment using CDDP. Thus, CCRT using CDDP in out-patient clinics is difficult. On the other hand, nedaplatin is also a platinum drug which lessens renal toxicity and requires no hydration. Kodaira et al. reported that a phase II study of a combination of nedaplatin and 5-FU for locally advanced esophageal cancer with radiation therapy achieved better outcomes of 45.9% for 2-year

---

<table>
<thead>
<tr>
<th>Table 1. — Eligibility criteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td>i) Pathologically proven squamous cell carcinoma or adenocarcinoma</td>
</tr>
<tr>
<td>ii) No paraaortic lymph node swelling (≥ 10 mm) by abdominal CT</td>
</tr>
<tr>
<td>iii) Clinical FIGO Stage Ib, IIA, IIb with bulky tumor (≥ 40 mm assessed by pelvic MRI or pelvic CT)</td>
</tr>
<tr>
<td>iv) Clinical FIGO Stage IIIa, IIIb and IVa with no paraaortic lymph node swelling (≥ 10 mm) observed by abdominal computed tomography</td>
</tr>
<tr>
<td>v) Age: 20-75 years</td>
</tr>
<tr>
<td>vi) Performance status (Eastern Cooperative Oncology Group): 0-2</td>
</tr>
<tr>
<td>vii) No prior radiation therapy for abdomen or pelvis</td>
</tr>
<tr>
<td>viii) Adequate function of bone marrow, kidney and liver</td>
</tr>
<tr>
<td>white blood cell count ≥ 2500 mm$^3$</td>
</tr>
<tr>
<td>neutrophils ≥ 1000 mm$^3$</td>
</tr>
<tr>
<td>hemoglobin ≥ 8.0g/dl</td>
</tr>
<tr>
<td>platelet count ≥ 75000 mm$^3$</td>
</tr>
<tr>
<td>creatinin ≤ 2.0 mg/dl</td>
</tr>
<tr>
<td>24 h-Cr ≥ 60 ml/min</td>
</tr>
<tr>
<td>GOT and GPT ≤ 2 times of upper limit of normal at our institution</td>
</tr>
<tr>
<td>T.Bil ≤ 2 times or the upper limit of normal at our institution</td>
</tr>
<tr>
<td>ix) Written informed consent</td>
</tr>
</tbody>
</table>

| MRT: magnetic resonance imaging; CT: computed tomography; GOT: glutamic-oxalacetate transaminase; GPT: glutamic-pyruvate transaminase; T.Bil: T. bilirubin. |
survival [9]. Nemoto et al. also reported that a retrospective analysis of a combination of nedaplatin and 5-FU for recurrent esophageal cancer with radiation therapy achieved better outcomes of 69.0% for 2-year survival [10]. A phase I trial of CCRT for LAUCC using nedaplatin has been completed in a Japanese protocol of the HDR-ICBT schedule [11]. Thus, we conducted the current phase II trial. The details of the current trial have been previously reported [12]. CCRT for LAUCC in outpatient clinics could be possible if the current phase II study is successful.

In the current study, all ten patients had CR or PR. Thus, the response rate was 100% and CR rate was 80% (Table 3).

There is a weak point of nedaplatin use which is more severe hematological toxicities than CDDP [8]. In the current study, only one patient experienced grade 4 hematological toxicity (neutropenia). G-CSF has been found to overcome neutropenia in recent years. Thus, this hematological toxicity is acceptable. Grade 3 or greater hematological toxicity occurred in five patients (Table 4). This rate is also acceptable.

Ohno et al. reported that their retrospective analysis of 43 LAUCC patients treated with CCRT using CDDP in a Japanese protocol radiation treatment schedule achieved 14 grade ≥ 3 leucopenia (58%), and two grade ≥ 3 anorexia (8%) [13]. These results including the current phase II study suggest that CCRT using nedaplatin is not more severely toxic for hematological events than CDDP. Moreover, no grade 3 or greater anorexia occurred in the current phase II study, which suggests that CCRT using nedaplatin could be less toxic than CDDP.

Toita et al. also reported a retrospective analysis of CCRT for LAUCC using CDDP in a Japanese-style radiation therapy protocol [14]. There were 33 out of 40 grade ≥ 3 leucopenia (83%), which indicated more toxicity than the current phase II study.

In conclusion, the initial analysis of the current phase II study suggests that CCRT using nedaplatin for LAUCC in a Japanese protocol of radiation therapy schedule (HDR-ICBT, lower dose of BED10) could be effective and safe. Further studies on a mature population are strongly recommended.

Acknowledgments
This study was supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Address reprint requests to:
Y. NIIBE, M.D., Ph.D.
Department of Radiology,
Kitasato University School of Medicine,
1-15-1, Sagamihara, Kanagawa
28-8555 (Japan)
e-mail: jce-n@hkg.odn.ne.jp
Resource use and cost analysis of managing abnormal Pap smears: a retrospective study in five countries


1Health Market International Ann Arbor, MI (USA); 2Lancashire teaching Hospitals, Royal Preston Hospital, Preston (UK);
3Charité, Campus Benjamin Franklin, Berlin (Germany); 4European Institute of Oncology, Milan (Italy);
5Royal Women’s Hospital, Carlton, Melbourne (Australia); 6Hospital Clinic Barcelona (Spain);
7Health Economics, GSKbio, Rixensart (Belgium)

Summary

Objective: To evaluate and compare treatment patterns and related resource use and costs in women with abnormal cervical smears in five countries. Methods: Data from patient charts were collected for a minimum of 24 months, starting from the first recorded abnormal cervical smear. Costs, from the public health perspective, were calculated based on country-specific unit costs per procedure and expressed in euros. Results: A total of 3,380 patient charts were reviewed. Subjects with suspected or detectable cervical cancer were excluded from the analysis (n = 380). A significant age difference of 1.8-2.6 years was observed between the lowest and highest severity of cytological and histological types (p < 0.05). The correlation between cytology and histology results was weak overall (35.8%) and varied widely between countries (ranging from 48% for Australia to 29.7% for the UK). As expected, countries with an organised screening programme (UK, Australia) diagnosed and initiated treatment at earlier disease stages. These countries demonstrated a much lower and narrower cost band for more advanced histological types. In contrast, other countries (Germany, Italy, Spain) followed an opportunistic screening programme in which advanced disease was diagnosed and treated at much higher and more varied costs. Histological, not cytological, results were the main factor underlying the cost differences per type. Conclusion: Costs and treatment patterns in women with abnormal cervical smears differ among countries due to the type of screening programme (organised versus opportunistic) and, consequently, the histological type. These results need to be taken into consideration when designing cost-effectiveness studies which include cervical cancer screening data.

Key words: Cervical cytology; Screening; HPV; Cervical cancer; Cost analysis; Pap smear.

Introduction

A proven way to effectively reduce the public health burden caused by cervical cancer is through the implementation of a regular screening programme designed for the early detection of abnormal cytology of the cervix [1]. The screening is carried out with pap smears taken from the cervix in which abnormal cervical squamous cells are searched with a microscope [2]. Several cytological classifications of squamous cells have been developed to grade cell abnormality [3, 4]. Depending on the level of abnormality further examination of the cervix, which may include a biopsy of the cervix, is undertaken followed by appropriate treatment if necessary. The whole process is perceived as cumbersome by the individual undergoing the screening [5-7].

An organised screening programme requires an extensive infrastructure and a large labour force for taking the pap smears, analysing the cytology, setting up a follow-up, and implementing an appropriate and consistent treatment scheme. Countries with the most sophisticated programmes have observed the highest reduction in cancer incidence such as the Netherlands, the UK and the Nordic region in Europe since their implementation [8]. Other countries with a less aggressive systematic screening programme, also termed “opportunistic” screening, have seen changes in cancer incidence but to a lesser extent. To maintain or enhance an efficient screening programme with a high sensitivity and specificity rate, a quality assurance programme needs to be established at each level of the screening process which again requires an increase in manpower for controlling and adjusting procedures where necessary [9].

Different screening management models have been developed to define the best screening frequency, the best start- and end-age for screening in order to achieve the most efficient reduction in cervical cancer incidence and mortality over time [10, 11]. Subsequent to these initial models, country-specific screening and treatment guidelines have been developed taking into account the characteristics of the country [12].

The cost of developing and organising screening as well as the cost of treating cervical cancer has been well documented [13]. However, the cost implications for the management of abnormal pap smears, representing about 5% of all the Pap smears taken, are a poorly explored domain [14-16].

Because different countries apply different methods of cytological analysis and follow-up, an evaluation of several countries would be helpful to better highlight the
Methods

Study design

A retrospective study was conducted in five countries (UK, Germany, Spain, Italy and Australia) from June 2005 through May 2006 of clinical records of women who had been referred to a gynaecology or a colposcopy clinic by the patient’s general practitioner (GP) following a confirmed abnormal smear two to three years before (2002-2003). Between six and eight specialised centres were selected per country with the aim of gathering an adequate number of patients and ensuring a reasonable geographic spread in each selected country. Each centre contributed approximately 100 randomly selected patients to the study, producing a total of 600 to 800 patients per country or approximately 3,400 subjects in total. In most of the countries studied the primary cervical screening is carried out by the GP, while further evaluation and follow-up treatment is carried out in specialised clinics. Referral from the GP might occur immediately after an abnormal smear or the GP might choose to repeat the test to first confirm the results.

The requirement of ethics approval for the study was reviewed in each of the countries selected. However, as the study was retrospective in nature and therefore no direct contact with patients was required. Out of the five countries, two countries (UK & Australia) asked for ethics approval. All data was collected and maintained anonymously by patient and by centre. This formulation was approved by all the authors.

Selection of study centres

Recruitment of a random sample of the centres at country level was not considered feasible, due to the low response and study acceptance rates with such a procedure. Specific centres with a published service level of follow-up and treatment of women with cervical abnormalities detected by a smear test were identified and contacted by mail or phone to determine their interest to participate in the study. At the same time, data were collected about the centre and its physicians (e.g., number of women treated each year). From this information, those centres were selected to be representative of the management of women with abnormal smear tests in each country. Additionally, consideration was given in each country to ensure that appropriate regional representation was given to identify any potential management variances that might occur.

Data collection

Within each centre, the objective was to identify an adequate number of patients to ensure a sufficient number of patients from each cytological diagnostic group: mild (approximately equivalent to ASCUS and atypia), moderate (equivalent to low-grade squamous intraepithelial lesions [LSIL]), and severe dyskaryosis (equivalent to high-grade squamous intraepithelial lesions [HSIL]), and cancer. Each selected country has its own cytological classification system of pap smears: the Bethesda (Spain & Italy) [18], the British Society of Clinical Cytology (BSCC) (UK) [19], the Munich II (Germany) [20], or the AMBS (Australia) [21] classification system. The various classifications were compared and standardised to allow an overall evaluation as well as a cross-country comparison.

Clinical data of all the selected women were reviewed for a minimum of 24 months from the date of the first confirmed and recorded abnormal cervical smear which resulted in the referral to the gynaecological clinic. This first screen was designated as the ‘referral smear’. Details of the clinic visits, tests and procedures during that period were collected. If a woman returned to a ‘normal’ pap smear within the 2-year period, no further medical activities were recorded. If the patient continued to receive treatment after the minimum 2-year review period, all further medical activities were recorded until the most recent one or until a ‘normal’ result was reported.

Data collection forms were developed, which defined the minimum data set to be collected for each subject. These forms served as the basis for the review of the patient charts. Data from these forms were then transferred to a central database in MS Access. Three separate databases were extracted from the central database, all linked by patient identifiers: a master database which included demographic data for all subjects; a procedure database which recorded details of each test/procedure and outcomes for all subjects; and a cancer database which included only those patients with a confirmed histological diagnosis of cervical cancer.

Calculation of costs

All costs were applied from the payer perspective (i.e., the national health authorities) and only patients that were treated under the national health system were included (i.e., no privately insured patients). Unit costs for all resource use were determined from national country specific references on reimbursed prices, and in the absence of having a well-defined reference cost, from discussions with the purchasing departments of the participating sites. These unit costs were assigned to each procedure in the database (Table 1). They were converted into the national currency of each country and adjusted by country with the health specific purchasing power parity exchange rates from the OECD, 2005 to allow cross-country comparison [22]. For those patients with a cytological or histological diagnosis of cancer it was difficult to collect complete information on costs related to cancer treatment (both acute and long-term). Resource use and costs associated with treating cervical cancer are in a different order of magnitude than those associated with the management of abnormal cytology and vary widely depending upon the cancer grade. A separate study, with more cancer cases and of longer duration would be required, therefore for this study patients with cervical cancer were excluded.

The detailed database was used to create a summary file which contained the cytology result of the referral smear, the time of first and last intervention post referral smear expressed in days, the first and the maximum histological diagnoses during the observation period, and the related total cost for each patient excluding the cost of cancer treatment. This file allowed for the calculation per country of the average cost associated with the management of women per cytology or histology stage of precancerous lesions.
Table 1. — Unit cost for the different interventions occurring after a positive pap smear expressed in 2005 euros and adjusted using PPP exchange rates (2005).

<table>
<thead>
<tr>
<th>Procedure</th>
<th>UK</th>
<th>Aus</th>
<th>G</th>
<th>It</th>
<th>Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap smear</td>
<td>20.0€</td>
<td>48.3€</td>
<td>10.9€</td>
<td>26.3€</td>
<td>98.6€</td>
</tr>
<tr>
<td>HPV test</td>
<td>n/a</td>
<td>n/a</td>
<td>66.6€</td>
<td>71.4€</td>
<td>36.7€</td>
</tr>
<tr>
<td>Cervical Biopsy</td>
<td>30.7€</td>
<td>85.5€</td>
<td>13.7€</td>
<td>79.0€</td>
<td>51.2€</td>
</tr>
<tr>
<td>Colposcopy</td>
<td>119.1€</td>
<td>273.2€</td>
<td>27.3€</td>
<td>47.6€</td>
<td>118.5€</td>
</tr>
<tr>
<td>Conisation</td>
<td>300.9€</td>
<td>307.7€</td>
<td>599.8€</td>
<td>678.3€</td>
<td>429.9€</td>
</tr>
<tr>
<td>LEEP/LLETZ</td>
<td>300.9€</td>
<td>317.4€</td>
<td>599.8€</td>
<td>657.9€</td>
<td>405.4€</td>
</tr>
<tr>
<td>Hysterectomy</td>
<td>2,121.7€</td>
<td>4,581.6€</td>
<td>3,616.3€</td>
<td>3,257.2€</td>
<td>3,020.2€</td>
</tr>
<tr>
<td>PPP: exchange rate; UK: 0.627; Aus: 1.38; G: 0.913; It: 0.85; Sp: 0.765.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

UK: United Kingdom; Aus: Australia; G: Germany; It: Italy; Sp: Spain.

Resource use and associated cost results were analysed from the perspective of both baseline cytology and the histology result. Both perspectives are important as many of the cytology results that suggested the presence of a precancerous lesion appeared to be negative by histology and/or observation of the cervix. The histology result therefore became the starting point for new assessments and treatments that may be different from those indicated by the initial cytology.

Statistical analyses

All statistical analyses were undertaken using SPSS software version 14.0 (Chicago, IL, USA) and BestFit® 4.5 from Palisade (Ithaca, NY, USA) to estimate the appropriate distributions. First analyses were descriptive in nature, including mean, median, standard deviation (SD), minimum and maximum value for continuous variables and absolute numbers and percentages for categorical variables. Where comparisons were made, the statistical significance of any differences was evaluated using rank tests or parametric tests depending on the variables selected and their spread in values, with p < 0.05 indicating statistical significance. The strength of association between the cytological and histological findings was measured using Kappa statistics expressing mainly the level of agreement.

Results

Study population

A total of 3,380 women with the first abnormal pap smear were initially enrolled in the study. Three hundred and eighty women suspected by cytology or with confirmed histological invasive cancer were excluded from further analysis.

Table 2. — Number of subjects enrolled per country with age structure (years).

<table>
<thead>
<tr>
<th>Country</th>
<th>UK</th>
<th>Aus</th>
<th>G</th>
<th>It</th>
<th>Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>3,000</td>
<td>34.9</td>
<td>11.1</td>
<td>16</td>
<td>90</td>
</tr>
<tr>
<td>CIN-1</td>
<td>118</td>
<td>244</td>
<td>617</td>
<td>979</td>
<td></td>
</tr>
<tr>
<td>CIN-2</td>
<td>191</td>
<td>248</td>
<td>181</td>
<td>620</td>
<td></td>
</tr>
<tr>
<td>CIN-3</td>
<td>118</td>
<td>244</td>
<td>617</td>
<td>979</td>
<td></td>
</tr>
<tr>
<td>CIN-0</td>
<td>942</td>
<td>1,095</td>
<td>963</td>
<td>3,000</td>
<td></td>
</tr>
</tbody>
</table>

UK: United Kingdom; Aus: Australia; G: Germany; It: Italy; Sp: Spain.

An analysis by country, by cytology and histology result of the remaining women indicates that the UK and Australia enrolled significantly more early subjects (mild/CIN-1 disease) than Germany, Spain or Italy (Table 2 and Figures 1A and B).

The age distribution was skewed according to the baseline cytology result as well as according to the histology grade, as illustrated in Table 3 and Figures 2A and B. There was a significant difference of a few years for women with a more advanced disease stage (suspected by cytology and confirmed by histology) (ANOVA-testing, p
< 0.05). As a consequence of the above, the age distribution of the 3,000 remaining women in the study was not only slightly skewed to the right overall but was significantly different between the countries (Table 4 and Figure 3).

Time before taking action

An analysis by cytology type indicates that the time from the referral smear to first intervention expressed in days was significantly different between the three groups as illustrated in Figure 4A. The difference is mainly observed between the severe cases (50% seen within 36 days) versus the other cases (mild and moderate, 50% seen within 57 days). This is also reflected in an analysis by country where not much difference is seen between the first 50% of the subjects in whom a first intervention occurred within 42-44 days, but subsequent subjects are seen at a different rate by country depending on how many mild and moderate subjects were in each country (Figure 4B).

Correlation between cytology and histology results

Cytology classifications were regrouped into a uniformed classification, as reported in Table 5, allowing comparison across countries. Cytological classes were regrouped into three and the histological grades into four categories. The overall level of agreement between cytological and histological findings was 35.8%. This result
is not overwhelming and two elements highlight the poor association: 1) false-positive data (814 negative results on 3,000 subjects (27%)); and 2) under- or overvaluing the histological disease stage (25% in any direction (553 subjects undervalued and 560 overvalued)).

Resource use and cost estimates

Resource use and cost data are reported by cytology and histology type and by country in Table 6. An overall result has not been constructed because the resource use and cost is very country specific as well as the number of subjects per category per country. The former is demonstrated for the negative histological results in Figure 5 as an example with quite different cost distributions per country.

From Table 6, two trends from the above analysis can be highlighted. First, those countries with a programmed screening such as the UK and Australia have a much lower cost per advanced cytology and histology type compared to the countries with an opportunistic screening process such as Spain and Italy. This is well illustrated in Figures 6A and B comparing the UK and Italy in their average cost distribution per histology type as an example. Related to that, the standard deviations in the UK figures are much smaller compared to the results for Italy.

Second, the histology results demonstrate a gradual increase in cost from CIN-1 to CIN-3 which is less the case for the cytological data. The histological data therefore better determine the cost figures than the cytological results as seen in the example for the UK and Italy (Figures 7A and B).

Discussion

It is clearly demonstrated that countries with an organised screening programme are doing better regarding cost outcome. It can be hypothesised that they are less expensive as they detect earlier cases on which less costly interventions are applied and they act under more comfortable conditions [23]. There is less urgency to intervene because of the very early disease stages detected. Hence the longer period to start any intervention observed in the UK compared to other countries (Figure 4b).

A second observation is the low performance of the screening results expressed by the poor level of agreement found between the cytology and histology data independent as to whether an organised or an opportunistic screening has been put into place [24-27].

Using the same study protocol to investigate resource use and cost data for a medical intervention in different countries, it will eventually lead to the observation of dramatic cost differences as we have shown here. Many reasons explain the differences such as treatment guidelines that are country specific, as well as the health care system with its reimbursement processes for interven-
Table 6. — Average cost per histology and cytology type and by country.

<table>
<thead>
<tr>
<th>Histology</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>SE</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>199</td>
<td>249.0</td>
<td>199.7</td>
<td>14.0</td>
<td>50.4</td>
<td>2138.1</td>
</tr>
<tr>
<td>Aus</td>
<td>83</td>
<td>375.7</td>
<td>276.3</td>
<td>24.8</td>
<td>46.6</td>
<td>1514.2</td>
</tr>
<tr>
<td>G</td>
<td>200</td>
<td>200.7</td>
<td>106.3</td>
<td>37.6</td>
<td>11.0</td>
<td>4243.4</td>
</tr>
<tr>
<td>It</td>
<td>136</td>
<td>265.9</td>
<td>140.0</td>
<td>24.6</td>
<td>21.8</td>
<td>1163.9</td>
</tr>
<tr>
<td>Sp</td>
<td>196</td>
<td>392.9</td>
<td>319.9</td>
<td>33.5</td>
<td>66.2</td>
<td>5927.2</td>
</tr>
<tr>
<td>Total</td>
<td>587</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>87</td>
<td>381.3</td>
<td>329.8</td>
<td>22.7</td>
<td>19.7</td>
<td>7741</td>
</tr>
<tr>
<td>Aus</td>
<td>107</td>
<td>606.4</td>
<td>649.1</td>
<td>24.5</td>
<td>266.3</td>
<td>1191.6</td>
</tr>
<tr>
<td>G</td>
<td>90</td>
<td>353.4</td>
<td>170.5</td>
<td>66.6</td>
<td>11.0</td>
<td>4257.0</td>
</tr>
<tr>
<td>It</td>
<td>144</td>
<td>550.7</td>
<td>459.2</td>
<td>26.0</td>
<td>48.2</td>
<td>1632.8</td>
</tr>
<tr>
<td>Sp</td>
<td>159</td>
<td>1090.4</td>
<td>1052.4</td>
<td>59.4</td>
<td>159.1</td>
<td>6168.2</td>
</tr>
<tr>
<td>Total</td>
<td>1095</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>130</td>
<td>520.3</td>
<td>529.5</td>
<td>14.8</td>
<td>148.9</td>
<td>964.6</td>
</tr>
<tr>
<td>Aus</td>
<td>146</td>
<td>760.4</td>
<td>718.1</td>
<td>22.1</td>
<td>266.3</td>
<td>1702.6</td>
</tr>
<tr>
<td>G</td>
<td>93</td>
<td>510.8</td>
<td>613.4</td>
<td>28.9</td>
<td>41.0</td>
<td>1308.8</td>
</tr>
<tr>
<td>It</td>
<td>115</td>
<td>1007.0</td>
<td>970.0</td>
<td>24.2</td>
<td>246.7</td>
<td>2214.4</td>
</tr>
<tr>
<td>Sp</td>
<td>118</td>
<td>1570.9</td>
<td>1309.5</td>
<td>96.6</td>
<td>318.1</td>
<td>6729.4</td>
</tr>
<tr>
<td>Total</td>
<td>620</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIN-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>122</td>
<td>550.9</td>
<td>529.5</td>
<td>19.3</td>
<td>148.9</td>
<td>2188.5</td>
</tr>
<tr>
<td>Aus</td>
<td>146</td>
<td>766.5</td>
<td>695.7</td>
<td>22.2</td>
<td>382.8</td>
<td>1503.7</td>
</tr>
<tr>
<td>G</td>
<td>200</td>
<td>1215.9</td>
<td>691.0</td>
<td>94.4</td>
<td>54.6</td>
<td>7859.8</td>
</tr>
<tr>
<td>It</td>
<td>229</td>
<td>1092.0</td>
<td>982.9</td>
<td>45.2</td>
<td>279.1</td>
<td>2214.4</td>
</tr>
<tr>
<td>Sp</td>
<td>282</td>
<td>1640.6</td>
<td>1254.5</td>
<td>83.5</td>
<td>439.7</td>
<td>6729.4</td>
</tr>
<tr>
<td>Total</td>
<td>979</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>275</td>
<td>371.8</td>
<td>318.8</td>
<td>13.6</td>
<td>19.7</td>
<td>1006.3</td>
</tr>
<tr>
<td>Aus</td>
<td>244</td>
<td>630.1</td>
<td>671.5</td>
<td>19.2</td>
<td>46.6</td>
<td>1680.9</td>
</tr>
<tr>
<td>G</td>
<td>153</td>
<td>177.5</td>
<td>107.6</td>
<td>17.8</td>
<td>41.0</td>
<td>1290.9</td>
</tr>
<tr>
<td>It</td>
<td>123</td>
<td>736.7</td>
<td>863.3</td>
<td>37.7</td>
<td>21.8</td>
<td>2063.4</td>
</tr>
<tr>
<td>Sp</td>
<td>147</td>
<td>1023.3</td>
<td>576.2</td>
<td>123.0</td>
<td>66.2</td>
<td>13137.1</td>
</tr>
<tr>
<td>Total</td>
<td>942</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>155</td>
<td>416.9</td>
<td>448.0</td>
<td>19.5</td>
<td>50.4</td>
<td>2138.1</td>
</tr>
<tr>
<td>Aus</td>
<td>136</td>
<td>646.0</td>
<td>649.1</td>
<td>22.5</td>
<td>139.0</td>
<td>1446.3</td>
</tr>
<tr>
<td>G</td>
<td>291</td>
<td>561.6</td>
<td>278.2</td>
<td>46.6</td>
<td>11.0</td>
<td>4310.1</td>
</tr>
<tr>
<td>It</td>
<td>249</td>
<td>621.5</td>
<td>472.8</td>
<td>39.3</td>
<td>21.8</td>
<td>6796.3</td>
</tr>
<tr>
<td>Sp</td>
<td>264</td>
<td>1003.2</td>
<td>986.2</td>
<td>63.4</td>
<td>66.2</td>
<td>6795.6</td>
</tr>
<tr>
<td>Total</td>
<td>1095</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>108</td>
<td>469.4</td>
<td>529.5</td>
<td>23.0</td>
<td>148.9</td>
<td>2188.5</td>
</tr>
<tr>
<td>Aus</td>
<td>120</td>
<td>759.0</td>
<td>695.7</td>
<td>29.3</td>
<td>229.7</td>
<td>1702.6</td>
</tr>
<tr>
<td>G</td>
<td>139</td>
<td>1237.8</td>
<td>662.5</td>
<td>123.6</td>
<td>41.0</td>
<td>7859.8</td>
</tr>
<tr>
<td>It</td>
<td>252</td>
<td>936.4</td>
<td>898.6</td>
<td>35.9</td>
<td>21.8</td>
<td>7295.2</td>
</tr>
<tr>
<td>Sp</td>
<td>344</td>
<td>1404.4</td>
<td>1229.4</td>
<td>56.8</td>
<td>159.1</td>
<td>7549.1</td>
</tr>
<tr>
<td>Total</td>
<td>963</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

UK: United Kingdom; Aus: Australia; G: Germany; It: Italy; Sp: Spain.

tions, the unit costs, the incentives to intervene, and finally the culture of practicing medicine. It is therefore risky to pool and analyse resource use and cost data from different countries and to report an overall cost result and an overall statement about the management of the disease. This was avoided by not reporting any overall cost result per cytology or histology class as selection bias at country and/or disease stage level heavily impacted those cost results. Moreover there is no demand for that kind of information as cost decision makers in health care remain at the level of a country.

Based on the results, a number of important observations were made. Firstly, cytology results do not drive differences in intervention type or costs. One could classify the cytology result into essentially two outcomes: suspected and non-suspected subjects. Additional subclassification leads to a different urgency of intervention as seen and reported in Figure 4a, but will not lead to a different use of diagnostics and treatment. The correlation with the biopsy result is therefore too weak. Secondly, a negative histology result or a false-positive cytology result leads to a much lower cost than subsequent histology stages as reported in Table 6 and Figures 6A and B. Thirdly, the cost distribution per histology type is not normal but skewed with a tail to the right for every histology type (Figures 6 and 7). In addition, the cost distribution per histology type has a wider spread in early versus late histological stages for obvious reasons (more exploration at start, better defined intervention pattern at later stages). Furthermore, countries such as the UK and Australia with their organised screening programmes show a more distinct cost difference by histology type. They are cheaper in each histology type excepted for the false-positive results compared to the other countries like Spain and Italy. The standard deviations of their cost distributions are smaller indicating a narrower spread of their cost figures (Figure 6 as an example). A reason for that could be that the treatment guidelines are better defined and followed by histological type for both countries. Lastly, hysterectomy is quite often done in women independent from the disease stage of cervical abnormality as there are other reasons within a certain age-group to do hysterectomies. A financial bias in the cost analysis by histology type could therefore appear so it was excluded from our analysis up to CIN-3.

There are limitations with the retrospective design and methodology used in this study. The retrospective design of the study may lead to an underestimation of the resource use and cost. The observational period, limited to two years post-referral screen, was difficult to extend over a longer period due to local, logistic problems as well as to the potential bias of change in management of a same case definition over a longer observation period. In addition, due to time and financial constraints, the selection of countries enrolled in the study as well as the site selection per country was not performed following a rigorous, scientific randomisation process to avoid selection bias.

Previous studies that have published cost results on the management of abnormal pap smears indicated a clear cost increase per higher histology as well as per higher cytology type [16]. The study methods used by these studies used different data sources for resource use and costs and may therefore claim to report ‘best estimates’. Our analysis has tried to be as close to the data sources as possible and may therefore better reflect reality. The
results show a level of consistency in the data analysis given the premises that countries having a different screening programme, may have different treatment guidelines and have a poor correlation between cytology and histology results. Meanwhile there is an urgency to reassess the exact value of the cytology and to redefine a precise algorithm for screening given that HPV-testing is now available as well as vaccination against cervical cancer [28-30].

Conclusion

In conclusion, resource use and cost estimates for managing different cytology and histology types in abnormal cervical lesions should be reported by country. There are limitations in presenting a pooled cost analysis because of the expected big differences in types and costs per type between countries. Moreover it is likely that using treatment guidelines as a reference to resource use and cost estimates for the management of cervical precancer lesions are not appropriate given the cost distribution observed that could be heavily skewed. Finally it is more important to consider the management cost per histology type of precancerous lesions instead of per cytology type given the poor correlation observed between both results.

Acknowledgement

GlaxoSmithKline Biologicals provided financial support for this study.

References


[22] Purchasing power parities. OECD 2007 [cited 2007 Jan]; Available from: URL: http://www.oecd.org/document/47/0,2340,36202863_1_1_1,00.html


Address reprint requests to:
B. STANDAERT, M.D.
Health Economics
GlaxoSmithKline Biologicals
B43 N366
Rue de l’Institut 89
1330 Rixensart (Belgium)
e-mail: baudouin.a.standaert@gskbio.com
Discovery of altered protein profiles in epithelial ovarian carcinogenesis by SELDI mass spectrometry

J. Luo¹, J.H. Qian¹, J.K. Yu², S. Zheng², X. Xie¹, W.G. Lu¹

¹Department of Gynecologic Oncology, Women’s Hospital, School of Medicine, Zhejiang University, Hangzhou (China)
²Department of Oncology, Cancer Institute, Clinical Laboratory, Second Affiliated Hospital of Zhejiang University College of Medicine, Hangzhou (China)

Summary

Objective: Identification of proteomic alterations in epithelial ovarian tumorigenesis may facilitate the understanding of progression of this disease. Methods: Specific protein peak patterns were identified in 20 microdissected epithelial ovarian tumors (13 epithelial ovarian cancers (EOCs) and 7 low malignant potential (LMP) tumors), as well as in the matched normal cells. Protein profiles were generated by surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS) from all the different types of cells. Results: Among seven protein peaks from EOC cells, six were significantly increased while one was decreased compared with normal cells, and three peaks from LMP cells were markedly increased while one was decreased compared with normal cells. Conclusions: The combination of SELDI and laser capture microdissection (LCM) is effective in finding the key molecules in ovarian tumorigenesis. Further identification of these protein peaks is important and these malignant protein signatures lend themselves to identification of populations at high-risk for EOC and for monitoring response to EOC chemopreventive agents.

Key words: Epithelial ovarian cancer; Surface-enhanced laser desorption/ionization mass spectrometry (SELDI-MS); Laser capture microdissection (LCM).

Introduction

Epithelial ovarian cancer (EOC) remains a major cause of gynecologic cancer mortality in women, with an estimated 15,310 deaths in 2006 in the United States [1]. Given our knowledge about the steep survival gradient relative to the stage at which then disease is diagnosed, it is reasonable to suggest that early detection remains the most promising approach to improve the long-term survival of ovarian cancer patients. The key molecular events in the pathogenesis of EOC are not well defined or understood. It is becoming increasingly clear that because of the inherent molecular heterogeneity and multifocal nature of EOC [2], additional improvement in early detection, diagnosis, and prognosis will likely require the measurement of a panel of biomarkers. These cancers are embedded in a heterogeneous tissue. This has also blocked the study of molecular mechanisms in cancerous pathways, but now the advent of LCM can isolated separate pure cell populations [3]. There are many techniques in the research of molecular mechanisms in ovarian carcinogenesis, including patterns of single nucleotide polymorphisms, DNA methylation or changes in mRNA/protein expression [4-6]. It has been demonstrated, however, that there is often no predictive correlation between mRNA abundance and the quantity of the corresponding functional protein present within a cell. Hence, since proteins represent the preponderance of the biologically active molecules responsible for most cellular functions, it is believed that the direct measurement of protein expression can more accurately indicate cellular dysfunction underlying the development of disease.

The proteome is the full complement of proteins that regulate the physiological and pathophysiological phenotype of a cell. Because proteins initiate all cell functions and pathways, identifying differentially expressed proteins between normal and pathological states can lead to a better understanding of the cellular mechanisms involved in cancer. The identification of changes in protein expression and modifications that occur in the early stages of a developing cancer could lead to the discovery of protein biomarkers and novel strategies for the improvement of early detection, diagnosis, and therapy of cancer. SELDI-TOF-MS can provide a rapid protein expression profile from a variety of biological samples. This technology has been used effectively in several cancers for analyzing protein expression [7-9]. The advantage of the SELDI is the ability to simultaneously detect multiple protein changes with a high degree of sensitivity in a rapid high throughput process. The precision makes it possible to delineate very small proteins and peptides.

The first study that utilized SELDI-TOF-MS in cancer research was for the detection of ovarian cancer utilizing serum samples [10, 11]. In this report we have used SELDI-TOF-MS to take comparative analyses of proteins in LMP, EOC and each matched normal cells procured by LCM. The objectives of our research were to discover potential biomarkers that could be used to differentiate malignant from the nonmalignant cell populations, and to understand the key molecular events in the pathogenesis of ovarian cancers.

Revised manuscript accepted for publication August 30, 2007
Materials and Methods

Patients and specimens

We investigated 20 cases of specimens from patients who were surgically treated at the Women's Hospital School of Medicine, Zhejiang University from 2003-2006. Specimens included 13 EOCs and seven LMPs and the matched normal ovarian tissues in the same patients. None of the patients had received chemotherapy or radiotherapy before surgical resection. All cases were reevaluated and classified according to the classification recently accepted by the World Health Organization (WHO). The degree of histological grade was also classified according to WHO criteria. After surgical removal, all tissues were immediately frozen in liquid nitrogen and stored at -70°C until analysis. Ethical approval was obtained before this study was undertaken.

Laser capture microdissection

Frozen samples were embedded in OCT medium, cut in a cryostat at 5 μm thickness, and mounted on membrane-based slides. LCM was performed on the ovarian tissue specimens to enrich cell populations (Figure 1). To assure visual discrimination of specific cell populations procured by LCM, slides were fixed in 70% alcohol for 30 sec and stained with hematoxylin, and one section from each of the frozen samples was stained with H&E, and examined by a pathologist. Frozen sections were dehydrated for 5 sec in 70, 90, and 100% ethanol with a final 5 min dehydration step in xylene. Air-dried sections were laser capture microdissected by a Leica LCM system (Germany). The objective cells were identified and targeted through a microscope, and a 15 μm laser beam pulse activated the film on a CapSure LCM Cap (Arcturus Engineering). In each case, 1×10^5 cells were captured. LCM cells were pooled from multiple caps, which were stored on dry ice until dissection was complete. On the basis of careful review of the histological sections, each microdissection was estimated to contain 95% of desired cells. The time of microdissection was less than 30 min for each slide to avoid proteolysis.

Cell lysates

Microdissected cells for SELDI studies were lysed in 6 μl lysis buffer (7 mol/l urea, 4% CHAPS, 2 mol/l Thiourea, 50 mmol/l DTT and 0.2% protease inhibitor mixture) directly on the LCM cap and incubated for 20 min at 4°C, and then centrifuged briefly to remove cellular debris. The lysates were frozen immediately and stored at -70°C.

SELDI analysis

Ciphergen SELDI-TOF-MS (PBS-II plus) and CM10 ProteinChip (weak cation exchanger) were purchased from Ciphergen Biosystems. Sinapinic acid (SPA) was purchased from Fluka (USA). All other reagents were purchased from Sigma (USA). The samples were thawed in ice and 20 μl of sodium acetate (50 mM, pH4) was added and was further agitated on a platform shaker at 4°C for 2 min. CM10 chips were activated by adding 200 μl of sodium acetate and agitated for 5 min twice. Diluted samples (20 μl) were applied to each spot of the bioprocessor (Ciphergen Biosystems) that contains the ProteinChip arrays. The bioprocessor was then sealed and agitated on a platform shaker for 60 min at 4°C. The excess of serum mixtures was discarded. The chips were then washed three times with 200 μl of sodium acetate and another two times with deionized water. Finally, the chips were removed from the bioprocessor and air-dried. Prior to the SELDI-TOF-MS analysis, 1 μl of a saturated solution of SPA in 0.5 ml/L CAN and 5 ml/L trifluoroacetic acid was applied onto each chip twice and the chips were again air-dried. Chips were detected by the PBS-II plus mass spectrometer reader. Data were obtained by averaging of 140 laser shots with an intensity of 200, a detector sensitivity of 8, a high mass of 100,000 Da and an optimized range of 2000-20,000 Da. Mass accuracy was calibrated by the all-in-one peptide molecular mass standard (Ciphergen Biosystems).

Experiment data analysis by ZUCIPDAS

The total experimental data was handled by the Zhejiang University Cancer Institute ProteinChip Data Analysis System (ZUCIPDAS, www.zlzx.net) which was designed by Yu Jiekai including preprocessed and model construction data. Firstly, the original data were handled by using an undedicated discrete wavelet transform method and denoising the signals. Secondly, according to three labeled peaks (M/Z 4096, 6637 and 1 3764Da) which appeared in all the selected spectra, we adjusted the intensity scale. The spectra were subjected to baseline corrections by aligning with a monotone local minimum curve and mass calibration. The proteomic peaks were detected and quantified by an algorithm that takes the maximal height of every denoised, baseline-corrected, and calibrated mass spectrum into account. Thirdly, we filtered out the peaks with signal-to-noise (S/N) of more than three. Finally, to match peaks across spectra, we pooled the detected peaks if the relative difference in their mass sizes was no more than 0.3%. The minimal percentage of each peak, appearing in all the spectra, is specified to ten. The matched peak across spectra is defined as a peak cluster. If a spectrum does not have a peak within a given cluster, the maximal height within the cluster will be assigned to its peak value. The identified peak clusters were normalized together.

The preprocessed data were used to establish models. In this experiment, we used a nonlinear SVM classifier with a radial based function kernel, and with the parameter gamma of 0.6, being the cost of the constrain violation of 19 to discriminate the different groups. The diagnostic model was evaluated and validated by leaving one cross validation. The principle of validation is that the approach takes out one sample each time as the test set and keeps the remaining samples as the training set, and then the test is repeated until each sample has been taken once as a test sample. Each peak in experiment data was estimated by the p value of the Wilcoxon T-test. The top ten peaks with the smallest p value were selected for further analysis. Combinations with the highest accuracy in distinguishing different groups of data were selected as potential biomarkers. The SVM model with the highest Youden’s index was selected as the model for detecting esophageal carcinoma and precancerous lesions.

Results

Sample harvest and SELDI array

The pure populations of ovarian malignant epithelial, low malignant potential epithelial cells and organ-matched normal or benign epithelial cells from seven LMP and 13 EOC specimens were selectively microdissected. In two of the EOC specimens, benign, low malignant potential and malignant cell types were identified and harvested. An average of 5,000 cells was microdissected in duplicate for each cell type. Afterwards filtrat-
ing noise by Ciphergen ProteinChip Software 3.1, 200 peaks were detected. The peaks were 2 kDa to 30 kDa. Peaks with m/z < 2 kDa were mainly ion noise from the matrix and therefore excluded.

**Protein profiles of malignant and the matched normal cells in the same patients from LCM**

We found that intensities for seven peaks detected in the mass spectra were significantly different between tumor cells and normal or benign cells. These peaks were m/z of 11659 Da, 4697 Da, 10143 Da, 5077 Da, 10182 Da, 9970 Da and 10107 Da. Among them, six peaks from tumor cells showed marked increases when compared with normal or benign cells, but one peak (4697 Da) was overexpressed in normal or benign cells than in tumor cell lysates (p < 0.05). Figure 2 and Table 1 give the descriptive statistics of the seven peaks.

**Protein profiles of LMP and the matched normal or benign ovarian cells in the same patients from LCM**

The peaks with m/z of 4951 Da, 4481 Da, 10852 Da, 4433 Da were significantly different between LMP and normal or benign ovarian cells (p < 0.05). Among them, three peaks from LMP cells were overexpressed when compared with normal or benign cells, but one peak (4951 Da) was decreased (p < 0.05). Figure 2 and Table 2 give the descriptive statistics of the four peaks.

**Comparison of protein profiles of malignant and LMP cells**

In our samples, two of the EOC specimens held the benign, LMP and malignant cell types. We found that the peaks at 10181Da and 10852Da were elevated both in malignant and LMP cells compared with normal or benign cells, and that the peaks were more overexpressed in malignant cells than in LMP cells, as shown in Figure 2 (C-F).
Figure 2. — Representative examples of SELDI-MS spectra on EOC, LMP and matched normal or benign cells. (Group 0) normal or benign cells. (Group 1) tumor cells. (A.C.D) EOC. (B.E.F) LMP. (A) m/z,4697Da. (B) m/z,4951Da. (C.E) m/z,10181Da. (D.F) m/z 10852Da.
Comparison of protein profiles of different histological type of EOCs

We did not find that different histological types of EOC cells shared any of the same peaks.

Discussion

EOC is a highly heterogeneous group of cancers. It is the fourth most common cause of death from cancer among women in the United States [1]. The 5-year survival rate is less than 40% [12] because of the presentation of the majority of cases at an advanced stage, and the etiology and precursor lesions of EOC are poorly understood. The development of EOC is a multistep process encompassing multiple events involving oncogenic and tumor suppressor gene products. These events can occur pre- or post-translationally and will be reflected in differential changes in a lot of proteins [13]. Analyzing the proteomic changes that occur in EOC progression is very difficult because of the biological heterogeneity of it. To compare protein expression in EOC, sufficient numbers of these cells are needed. One solution is microdissection of pure cell populations. SELDI has provided an approach for the sensitive and direct analysis of proteins in complex biological samples. The principle of this technique is very simple. A few microliters of an interesting sample are deposited on the chromatographic surface. The protein chip arrays are incubated and then washed with an appropriate buffer. The proteins of interest are captured on the chromatographic surface by adsorption, partition, electrostatic interaction or affinity chromatography depending on their properties, and analyzed by TOF-MS. The result is a mass spectrum comprised of the mass-to-charge (m/z) values and intensities of the bound proteins/peptides [14]. A unique strength of SELDI is its ability to analyze proteins from a variety of crude sample types, with minimal sample consumption. The number of publications describing the use of this technology has increased significantly in the past few years. In particular, the technology has been applied extensively in cancer research. Previous studies have demonstrated the successful application of this technology in the serum of EOC patients [10]. In this study, we first combined SELDI with LCM to get the protein profiles from microdissected epithelial ovarian tumors. Our findings indicate specific protein peaks that show a marked change in expression associated with EOC or LMP. The patterns of increases and decreases in protein levels were observed when protein profiles of tumor cells were compared with normal or benign cells. Seven peaks detected in the mass spectra were significantly different between tumor cells and normal or benign cells. Among them, six peaks from tumor cells showed marked increases when compared with normal or benign cells, but one peak (4697 Da) was more overexpressed in normal or benign cells than in tumor cell lysates. The four peaks were significantly different between LMP and normal or benign ovarian cells. Among them, three peaks from LMP cells were overexpressed when compared with normal or benign cells, but one peak (4951Da) was decreased. Interestingly, some of these peaks appeared in serum from some of the same patients in this study (data not shown). This approach could also lead to a several new possibilities in EOC detection including evaluation of “malignant” peptide expression patterns in tissue and serum samples on high throughput platforms, serving as screening tools for populations at high risk for EOC or LMP, and for monitoring response to EOC chemopreventive agents, therefore identity of the peaks and the examined serum samples will need to be done to confirm these results.

EOC appears to arise via one of at least two pathways; spontaneously and aggressively, with no precursor lesion (Type 2), or by slower development from an inclusion cyst to a benign adenoma or cystadenoma of LMP, through to metastatic adenocarcinoma (Type 1) [15]. Previous studies have shown a cytogenetic link between LMP and low-grade EOC strengthening its role as a precursor lesion [16, 17]. Therefore, the identification of proteins specifically associated with LMP would have a tremendous impact as markers for the early detection of EOC. In our study, two of the EOC specimens held the benign, LMP and malignant cell types. We found that the peaks at 10181Da and 10852Da were elevated both in malignant and LMP cells compared with normal or benign cells, and the peaks were more overexpressed in malignant cells than in LMP cells. EOC and LMP cells exhibited similar SELDI protein profiles underscoring the phenotypic similarity of these two disease states.

A recent profiling study compared serous, mucinous, endometrioid and clear cell EOC with normal OSE brushings, as well as the fallopian tube, endometrium and colon [18]. When compared with normal OSE, the changes in gene expression noted in serous EOC correlated with those found in the fallopian tube, but not in other normal tissues. Similarly, differences between mucinous EOC and normal OSE correlated with those in normal colon, and differences between both endometrioid and clear cell cancers and OSE correlated with those in normal endometrium. In this research, we did not find different histological types of EOC cells sharing any of the same peaks. All these studies identify the specific molecular alterations and pathways that lead to the different EOC histotypes, as well as identifying important histotype-specific biomarkers.

Conclusion

The application of molecular biology techniques to tumor biology has produced information about the cellular processes that regulate proliferation, differentiation, and apoptosis in normal cells, and disrupting these normal functions in cancer initiation and progression. It is clear that a new approach is needed to accurately distinguish between normal cells, early precancerous lesions, and malignant diseases. A variety of mass spectrometry-based platforms are currently available for providing information on both protein patterns and protein
identity. In our research, differential SELDI protein profiles were observed for cell lysates prepared from microdissected LMP, EOC and normal cells. Additional studies are under way to identify and characterize these potential peptide/protein biomarkers. Once identified, characterization of their function and biological role in ovarian tumorigenesis may lead to their potential use as diagnostic and prognostic biomarkers as well as conceivable therapeutic targets.

References


Address reprint requests to:
J.H. QIAN, M.D.
Department of Gynecologic Oncology
Women’s Hospital
School of Medicine
Zhejiang University, Xue-Shi Road 2#
310006 Hangzhou (China)
e-mail: qianjianhua1110@126.com
Sentinel lymph nodes in endometrial cancer: is hysteroscopic injection valid?

D. Clement1, 4, A.S. Bats1, 4, N. Ghazzar-Pierquet2, M.A. Le Frere Belda3, 4, F. Larousserie1, 4, C. Nos1, 4, F. Lecuru1, 4

1Service de Chirurgie Gynécologique et Cancérologique, Hôpital Européen Georges Pompidou
2Département de Médecine Nucléaire, Hôpital Européen Georges Pompidou
3Service d’Anatomie Pathologique, Hôpital Européen Georges Pompidou
4Université Paris-Descartes, Faculté de Médecine, Paris (France)

Summary

We aimed to describe hysteroscopic peritumoral tracer injection for detecting sentinel lymph nodes (SLNs) in patients with endometrial cancer and to evaluate tolerance of the procedure, detection rate and location of SLNs. Five patients with early endometrial cancer underwent hysteroscopic radiotracer injection followed by lymphoscintigraphy, then by surgery with hysteroscopic peritumoral blue dye injection, and radioactivity measurement using an endoscopic handheld gamma probe. SLNs and other nodes were sent separately to the pathology laboratory. SLNs were evaluated by hematoxylin-eosin-saffron staining and, when negative, by immunohistochemistry. Tolerance of the injection by the patients was poor (mean visual analog scale score, 8/10). SLNs were detected in only two patients (external iliac and common iliac-paraaortic, respectively). Detection rates were 1/5 by radiotracer, 1/5 by dye, and 2/5 by the combined method. One SLN was involved in a patient whose other nodes were negative. In three patients no SLNs were found by radiotracer or blue dye. Of the 83 non sentinel nodes removed from these patients, none was involved. Hysteroscopic peritumoral injection may be more difficult than cervical injection and, in our experience, carries a lower SLN detection rate.

Key words: Endometrial cancer; Sentinel lymph node; Hysteroscopy; Paraaortic chain.

Introduction

Sentinel lymph node (SLN) detection in patients with endometrial cancer was first evaluated by Burke et al. in 1996 [1] with the goal of improving staging, most notably via better detection of paraaortic nodes. SLN detection also ensures identification of micrometastases. Most of the subsequent studies used intracervical radiotracer injection and blue dye, which proved effective in identifying pelvic SLNs [2-4]. However, paraaortic SLNs were rarely identified with this technique [2-4]. Injection around the tumor via hysteroscopy was used in a few studies to evaluate drainage of the tumor, as opposed to drainage of the cervix [5-8]. This method may be more appropriate for evaluating drainage toward the paraaortic nodes.

The objective of this study was to describe our preliminary experience with SLN detection by hysteroscopic radiotracer injection in patients with early endometrial cancer and to evaluate the SLN detection rate.

Materials and Methods

Between July 2005 and February 2006, five non-consecutive patients with early-stage endometrial cancer underwent SLN detection after hysteroscopic radiotracer injection. All patients gave their informed consent to the study before inclusion. In all patients, the work-up included a physical examination, pelvic ultrasonography, magnetic resonance imaging, and endometrial biopsy or curettage. The results showed endometrial cancer with no evidence of spread beyond the uterus. The main patient characteristics are reported in Table 1.

On the day before surgery, 120 MBq of technetium-99m colloidal rhenium sulfide (Nanocis, Schering, CIS BIO International, Gif-sur-Yvette, France) was injected via a VERSA-POINT 5Fr hystroscope (Gynecare, Issy les Moulineaux, France). Saline at hydrostatic pressure was used to dilate the uterus. The radiotracer was injected under the endometrium at four sites (anterior wall x 2, posterior wall x 2) around the tumor using a 17-gauge oocyte aspiration needle (Laboratoire CCD, Paris, France) (Figure 1). Although the injections induced no major complications, they were poorly tolerated by the patients (mean visual analog scale for pain score of 8/10). Lymphoscintigraphy was performed 12 hours after radiotracer injection, using a dual-head camera (Axis 2000°, Philips Medical Systems, Cleveland, OH). During surgery, 2 ml of patent blue dye (2.5% patent blue V dye, sodium salt, Guerbet, Roissy, France) was injected into the uterine cavity in the first four patients, using the same technique as for the radiotracer. In the fifth patient, blue dye was injected into the cervix, at 0, 3, 6, and 9 o’clock.

SLNs were looked for during laparoscopy. First, blue lymphatics were sought in the broad ligaments and in the pelvic and paraaortic node areas, with the peritoneum closed. A handheld gamma probe (CdTe probe, Eurorad, Constellation Technology, Largo, FL) was then used to detect radioactivity, using the lymphoscintigram as a roadmap. The peritoneum was opened at the level of the iliac vessels, and the pelvic nodes were dissected to look for blue and/or radioactive nodes. Paraaortic SLNs were sought using the same procedure, with inspection for blue nodes followed by gamma probe detection based on lymphoscintigraphy findings. Then, the pelvic nodes were dissected routinely. Paraaortic dissection was to be performed in patients with paraaortic SLNs, adnexal involvement, pelvic node metastasis, Stage Ic disease, papillary serous or clear cell carcinoma. SLNs and other nodes were removed in a bag. All operative specimens were sent to the pathology laboratory.

Revised manuscript accepted for publication June 18, 2007

EUR. J. Gynaec. Oncol. - ISSN: 0392-2936
XXIX, n. 3, 2007
SLNs were embedded in paraffin. Five sections were obtained at 250-μ intervals. Four sections were stained with hematoxylin-eosin-saffron (HES) and examined by light microscopy. When these sections were negative, the fifth section was used for immunohistochemistry with the broad-spectrum monoclonal antikeratin antibody KL1 (Immunotec, Marseille, France). Non-sentinel nodes were evaluated by light microscopy examination of a single HES-stained section after paraffin embedding.

Results

Although the injections induced no major complications, they were poorly tolerated by the patients (mean visual analog scale for pain score of 8/10).

SLNs were detected in only two of the five patients (Table 2). One patient (#2) had three SLNs, two in the common left iliac territory and one in the paraaortic territory (preaortic and inframesenteric). The SLNs were identified by lymphoscintigraphy and gamma probe detection. All three SLNs were negative for cancer cells. The six pelvic nodes and nine paraaortic nodes removed during routine node detection were also negative. In the other patient (#5), the radiotracer failed to detect SLNs but intracervical blue-dye injection identified an SLN in the right external iliac chain. This SLN contained a metastasis, whereas the other 15 iliac nodes and the 11 paraaortic nodes were negative. Neither radiotracer injection nor blue dye showed SLNs in the other three patients. Thus, the radiotracer detection rate was 1/5, the dye detection rate was 1/5, and the combined detection rate was 2/5. In all, 83 nodes were removed from the five patients. No nodal metastases were found in non sentinel nodes. Peritoneal cytology was negative in all five patients. No intra- or post-operative complications were recorded.

Discussion

Evaluation of the lymph nodes is a crucial step in the management of endometrial cancer [9], which can metastasize to the pelvic and/or paraaortic chains. The staging procedure recommended by the FIGO involves routine assessment of pelvic and paraaortic nodes [9]. However, routine removal of the paraaortic nodes remains controversial, and the relative merits of full dissection versus selective dissection are unclear [10]. SLN detection may offer a solution by determining which nodes must be removed.

Intracervical injection of a radiotracer with or without blue dye was used in the studies of SLN detection in patients with endometrial cancer. This method is generally described as easy to perform, well tolerated by the patients, and effective in identifying SLNs in over 80% of cases [2-4]. However, the SLNs detected using this technique are usually located in the iliac and obturator chains. In our experience, SLNs may be found in the paraaortic chains, although pelvic SLNs are usually detected also [4], in keeping with the fact that cervical injection produces a drainage map of the cervix, as opposed to the uterine body or tumor.

Burke et al. [1] used blue-dye injection into the subserosal myometrium during surgery. Results were judged poor with this approach [11]. Subsequently, other groups used hysteroscopic peritumoral injections, and reported good acceptability, high detection rates and low false-negative rates [5-7].

In contrast, tolerance and detection rates were low in our study. The reasons for this failure are unclear. First we used a similar technique to previous reports, so that the lymphatic drainage should have been similar [5-7]. Second, we recorded no detection in 3/5 patients, whatever the technique used (radiotracer, blue dye). It appears that the use of blue dye alone by hysteroscopy limited the

Table 1. — Main characteristics of the five patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>BMI</th>
<th>Final stage</th>
<th>Histology</th>
<th>Grade</th>
<th>Surgical approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>24</td>
<td>Ib</td>
<td>Endometroid</td>
<td>II</td>
<td>Laparoscopy</td>
</tr>
<tr>
<td>2</td>
<td>87</td>
<td>22</td>
<td>Ic</td>
<td>Endometroid</td>
<td>II</td>
<td>Laparoscopy</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>37</td>
<td>Ic</td>
<td>Endometroid</td>
<td>I</td>
<td>Laparoscopy</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>38</td>
<td>Ib</td>
<td>Endometroid</td>
<td>I</td>
<td>Laparoscopy</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>30</td>
<td>IIIc</td>
<td>Endometroid</td>
<td>I</td>
<td>Laparoscopy</td>
</tr>
</tbody>
</table>

BMI: body mass index.

Table 2. — Detection of sentinel lymph nodes after hysteroscopic injection of radiotracer and blue dye.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Depth invaded by preoperative MRI</th>
<th>Tumor size</th>
<th>Hysteroscopic injection of 99m Tc</th>
<th>SLNs by lymphoscintigraphy</th>
<th>SLNs by blue dye</th>
<th>Location of SLNs (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 1/2 Focal</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 1/2 Focal</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>3</td>
<td>&lt; 1/2 Focal</td>
<td>Yes</td>
<td>2</td>
<td>0</td>
<td>Left common iliac (2) and paraaortic (1)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&lt; 1/2 Entire cavity</td>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td>None detected</td>
<td>None detected</td>
</tr>
<tr>
<td>5</td>
<td>&lt; 1/2 Entire cavity</td>
<td>Yes</td>
<td>0</td>
<td>1*</td>
<td>Right external iliac (1)</td>
<td></td>
</tr>
</tbody>
</table>

MRI: magnetic resonance imaging; SLN: sentinel lymph node; *: blue dye was injected in the cervix.

Figure 1. — Peritumoral injection of isotope in the anterior wall.
Table 3. — Main results of studies of hysteroscopic injection for detecting sentinel lymph nodes in patients with endometrial cancer.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Tolerance</th>
<th>Lymphoscintigraphic SLN detection rate</th>
<th>Intraoperative SLN detection rate</th>
<th>SLN location (n)</th>
<th>SLN metastases (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raspagliesi (18)</td>
<td>Vag malaise: 2 Failure: 1 Bleeding: 1</td>
<td>100% 100%</td>
<td>23 1, 2 + 10 CI, 1 +</td>
<td>12 PA, 1 +</td>
<td></td>
</tr>
<tr>
<td>Niikura (28)</td>
<td>Not recorded</td>
<td></td>
<td>37 1, 1 + 4 CI, 0 +</td>
<td>30 PA, 0+</td>
<td></td>
</tr>
</tbody>
</table>

SLN: sentinel lymph node; PA: paraaortic; CI: common iliac; I: iliac (external iliac, internal iliac, and/or obturator); FN: false negative; (n): number of patients in the study.

SLN detection rate [5, 7, 8] (Table 3). This does not explain the failure with the radiotracer. Third, although there may be a learning curve, we have extensive experience with outpatient hysteroscopy, operative hysteroscopy, and cervical radiotracer and dye injection for endometrial or cervical cancer. Furthermore, no learning curve effect was reported for hysteroscopic injection by other groups [5-7]. Fourth, time interval between the injection and the detection (lymphoscintigraphy and peroperative detection) as well as the radiotracer size do not appear as determinant since different approaches provided similar results [5-7]. Fifth, depth of myometrial invasion could decrease the detection rate [6].

Radiotracer injection on the day before surgery was poorly tolerated by the patients. We did not use general or local anesthesia. In contrast, Niikura et al. (28 patients) reported good tolerance and Maccauro et al. (26 patients) mentioned only transient vaginal symptoms in two patients [5, 6]. Raspagliesi et al. (18 patients) reported one failure and one case of procedure-limiting intrauterine bleeding [7]. In fact, patient tolerance was not evaluated in previous studies, whereas we evaluated the pain due to the hysteroscopy with a visual analog scale. We found that hysteroscopic injection considerably complicated the SLN-detection procedure in patients with early-stage endometrial cancer, whose management is now simple and well standardized [12].

The main goal of hysteroscopic injection is detection of both pelvic and paraaortic SLNs and metastases. Results in our patient #2 show that this goal can be achieved. Maccauro et al. reported 21% of SLNs in the paraaortic area; one being metastatic without pelvic involvement [7]. Similarly, Nikura et al. reported SLNs in the paraaortic chain in 72% of patients (SLNs were distributed in all paraaortic areas) [6]. SLNs were exclusively paraaortic in 13% of patients [6]. However, neither report provides information on tumor stage, most notably on whether pelvic node disease or cervical spread was found. These factors could influence the risk of paraaortic involvement [13, 14].

Another group reported negative results with the hysteroscopic approach [8]. In this series of 16 patients, only the first three injections were performed by hysteroscopic, the subsequent having a combination of hysteroscopic and subserosal or simply subserosal injection of blue dye. Detection rate was of 0% after hysteroscopy alone (0/3) [8].

**Conclusion**

We suggest that the feasibility and relevance of the present hysteroscopic tracer injection for SLN detection in endometrial cancer may be in doubt. This approach has to be compared to intracervical injections, in terms of detection rate and node areas explored.

**References**


Interval debulking in epithelial ovarian carcinomas: the past, present and the future

M. Gultekin¹, M.D.; K. Diribas¹, M.D.; E. Buru¹, M.D.; P. Dursun², M.D.; K. Yuce³, M.D.; A. Ayhan¹, M.D.

¹Department of Obstetrics and Gynecology, Cankiri Government Hospital, Cankiri
²Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Baskent University School of Medicine, Maltepe, Ankara
³Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Hacettepe University School of Medicine, Sihhiye, Ankara (Turkey)

Summary

Primary cytoreductive surgery followed by combination chemotherapy of paclitaxel and cisplatinum is the standard treatment for advanced staged epithelial ovarian cancers. Despite the maximal efforts to increase optimal cytoreductive success rates and related ultra-radical surgeries, five-year survival rates are still poor. Primary cytoreductive surgeries and their radicalities have been criticized since the early nineties. Interval debulking surgery (IDS) and neo-adjuvant chemotherapy (NAC) are the two suggested alternatives to the primary debulking approaches. In this article, the authors summarize and discuss the IDS approach with an associated literature review.

Key words: Interval debulking surgery; Interval laparotomy; Epithelial ovarian carcinoma.

Introduction

Epithelial ovarian carcinomas are the most lethal genital malignancies. About two-thirds of the patients present with advanced staged disease and five-year survival rates are between 15-30% in Stage III-IV disease [1]. Today, optimal cytoreductive surgery followed by a combination of platinum and taxane-based chemotherapy is the standard treatment of ovarian cancers. With the recent developments in the surgical treatment of ovarian cancers, survival rates have risen by 50% in some modern oncology clinics [2]. However, it is still far from being a satisfactory rise, especially when one thinks about the excess morbidity related to these treatments. Thus physicians have tried different treatments such as neoadjuvant chemotherapy and interval debulking surgery (IDS) as an alternative to the standard treatment with primary cytoreductive surgery and chemotherapy.

Criticisms of optimal cytoreduction

Optimal cytoreduction is suggested to be the unique prognostic factor for advanced staged ovarian cancer patients [3-5]. Each 10% increase in optimal cytoreduction was suggested to equal a 5.5% increase in the overall survival of these patients [6]. However, maximal cytoreductive surgeries have been criticized by some authors since the early nineties (Table 1). These objections were particularly more important in advanced stages: 1) Despite the maximal efforts, optimal cytoreduction rates are less in these patients compared to early staged patients; 2) Patients with suboptimal cytoreductive outcomes had even worse prognosis in advanced stages; 3) Median survival gain achieved by ultra-radical surgeries is less in advanced stages (5.8% gain if < 1 cm residual and 6.9% gain if microscopic residual is left); 4) To achieve optimal cytoreduction, much more radicalism and extensive surgeries should be performed which may be associated with higher morbidities (serious morbidity 8-68%) and higher mortalities (1-7%); 5) There is still not enough data about the economic burdens of these debulking surgeries; 6) None of the authors analyzed the patients’ quality of life with these radical surgeries performed to achieve optimal cytoreduction [7, 8]. These objections have directed physicians toward newer therapeutic modalities.

Terminology

As a definition, interval debulking surgery (IDS) and neoadjuvant chemotherapy (NAC) are frequently misused intervariably [9-11]. Neither are the standard treatment in epithelial ovarian carcinomas. Both strategies need a pathological diagnosis before treatment can be started and neither can be used in patients with progressive diseases. Both methods use chemotherapy but optimal regimens and numbers of chemotherapy cycles are still unknown [9-14].

NAC and IDS are basically two different approaches used in ovarian cancers. NAC is frequently used in patients with poor performance status (massive ascites, comorbid diseases or excess tumor load) that renders optimal cytoreduction or extensive debulking surgery. Following the tissue diagnosis with biopsy or cytology, patients will receive three to six cycles of chemotherapy. Patients who have a partial or complete response will undergo primary debulking surgery. Therefore, in neoad-
juvant chemotherapy we use a primary preoperative chemotherapy [9-14].

Different from NAC, interval debulking surgery is performed on patients who were not optimally cytoreduced during primary debulking surgery. It is not a procedure performed for poor performance status that renders a debulking surgery. Patients undergo debulking surgery but can not achieve optimal cytoreductive success and receive three to six cycles of postoperative chemotherapy (not primary neoadjuvant chemotherapy). Following the chemotherapy, patients with a partial or complete response are reevaluated for a second surgery (IDS) to finally achieve optimal cytoreduction [9-14].

**Interval debulking: the past**

Unlike the numerous NAC trials, interval debulking surgery (IDS) was not frequently analyzed previously. There are three randomized prospective reports up to date.

Redman et al. were the first authors who evaluated IDS [14]. Between 1986 and 1994, 79 patients with an initial suboptimal cytoreductive surgery (> 2 cm residual disease) were prospectively evaluated. All the patients received three cycles of platinum-based chemotherapy postoperatively. Following the initial chemotherapy, patients were randomized to an interval laparotomy (n = 37) vs additional chemotherapy (n = 42). While 37 underwent interval debulking surgery followed by three additional cycles of chemotherapy, the remaining 42 patients directly received an additional three cycles of platinum-based chemotherapy without any interval surgery. IDS significantly increased the optimal cytoreduction rate (73% of patients who had IDS received optimal cytoreduction). It also increased the median survival (15 vs 12 months) and decreased the mortality (OR = 0.70; 95% CI = 0.44-1.33), however these differences were not statistically significant [15].

During 1987-1993, the European Organization for Research into Therapy for Cancer (EORTC), Gynecologic Cancer Cooperative Group (GCCG) conducted a prospective randomized study: EORTC/GGCCG 55865 [15]. Two hundred and seventy-eight ovarian cancer patients with Stage IIB-IV disease with an initial suboptimal (> 1 cm residual disease) cytoreductive success received three cycles of cisplatin (75 mg/m²) plus cyclophosphamide (750 mg/m²). Following the initial debulking and three cycles of chemotherapy, patients were prospectively randomized. One hundred and forty patients underwent an interval laparotomy followed by three additional cycles of the same chemotherapy. Two-year overall survival (OS) and progression-free survival (PFS) were 56% and 38%, respectively. Median OS was 26 months and median PFS was 18 months. The remaining 138 patients received three additional cycles of the same chemotherapy without any interval debulking surgery. Two-year OS and PFS were 46% and 28%, and median OS and PFS were 20 and 13 months, respectively. These differences were statistically significant, favoring the IDS. Interval laparotomy approach increased PFS by five months, OS by six months and decreased the death risk due to disease by 33%. The authors also pointed out an important issue. Some patients had optimal debulking with the effect of three cycles of chemotherapy before the IDS (chemo-debulked). Patients who had optimal debulking before (chemo-debulked) or after the IDS had significantly higher survival rates compared to patients who remained suboptimally debulked after the IDS. Interval debulking surgery did not cause any additional morbidity or mortality for the patients and also did not cause any delay in the final treatment of patients. IDS was a significant factor on multivariate survival analysis (p = 0.0012).

However, further subgroup analyses of EORTC/GGCCG 55865 produced some confusion in the minds of some [8]: 1) Survival of patients with > 1 cm residual disease after IDS was similar to the patients in the chemotherapy arm who did not undergo the IDS procedure (19.4 months vs 20 months, respectively); 2) Another debate was the better survival of chemo-debulked patients (< 1 cm before IDS) compared to the survival of patients who were optimally debulked after IDS (41 vs 26.6 months). Perhaps intrinsic tumor chemo-sensitivity may be more important for overall survival, but not for IDS which results in optimal debulking; 3) Second-look laparotomy results were similar in both arms. Complete pathological response was achieved in 37% of the IDS and in 33% of the chemotherapy arms; 4) Only 29% of the patients were optimally debulked at IDS. About 35% were preoperatively chemo-debulked and the remaining 36% of patients in the IDS arm could not achieve optimal debulking (failed IDS); 5) There were also questions related to the quality of life and the long term follow-up [17, 18].

In 1998, three years after the initial report, the authors reported their sixth year follow-up results [12]. Decrease in risk of death due to disease was still continuing with an absolute increase up to 60% (compared to 33% in the initial report). Interval debulking was still significant on multivariate survival analysis and the authors started to ask who could benefit from IDS surgery? However, they could not find any subgroup of epithelial ovarian cancer patients who had benefitted from IDS.

Six years later, at the 10th biennial International Gynecologic Cancer Society (IGCS) Meeting held in Edin-
In 2004, the authors announced their results after a ten-year follow-up (oral presentation). They still found IDS to be an important prognostic factor for both overall and progression-free survival (10-year OS was 13.8% vs 7.4%, p < 0.0001 and PFS was 9.6% vs 3.8%, p < 0.0001). Ten-year survival was 28.9% in chemo-debulked patients while it was 20.2% in successfully debulked IDS patients and 1.55% in unsuccessfully debulked IDS patients. Decrease in risk of death due to disease (40%) was still continuing despite an absolute decrease compared to the previous report (60%).

In the year 2002, Rose et al. reported the third randomized multi-center study held in the USA: GOG-152 [11]. They prospectively randomized 425 patients with FIGO Stage III disease and who had undergone suboptimal primary debulking surgery (> 1 cm residual disease) from 1994-2001. All the patients received three cycles of paclitaxel and cisplatinum combination chemotherapy after the initial suboptimal debulking surgery. Two hundred and sixteen patients were randomized to the surgery arm (interval debulking followed by a further 3 cycles of paclitaxel and cisplatinum). The median overall and progression free survivals were 32 and 10.5 months, respectively. The remaining 209 patients were randomized to the chemotherapy arm (only an additional 3 cycles of paclitaxel and cisplatinum without any interval debulking surgery). The median overall and progression-free survivals were 33 and 10.8 months, respectively. Comparison of both the overall and progression-free survivals could not differentiate any significant difference in either arm.

GOG-152 produced new questions and confusion in the minds. In contrast to the EORTC study with a 10-year follow-up, GOG-152 could not find any differences in the arms. These different results from the two large multicenter studies may be due to some subtle differences among the designs of the trials. All the participants in the GOG study were specially trained and experienced gynecologic oncologists. They probably used maximal effort for optimal debulking in the initial cytoreductive surgery before IDS. However, most of the EORTC trial participants were gynecologists or general surgeons. Therefore, patients may have had insufficient primary surgery before IDS in the EORTC trial. Another difference between the two trials was the different regimens used (paclitaxel vs cyclophosphamide). This may also explain the different results of these trials. Therefore, one may suggest an indefinite conclusion that is ‘these two trials are totally incomparable’.

### Interval debulking: the future

The two prospective randomized trials could not resolve the ongoing debate on IDS. Also, although NAC was more frequently analyzed in the previous literature compared to the interval debulking strategy, there were still many questions to be answered regarding NAC. Furthermore, there was no report comparing these two strategies in any randomized prospective manner. These unresolved issues forced the EORTC to design a new prospective randomized trial, EORTC 55971, which started to enroll patients in 1998. The trial has four arms (Table 2) and also includes a quality of life assessment.

#### Table 2. — Clinical trial design of EORTC 55971.

<table>
<thead>
<tr>
<th>Arm</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage IIIc epithelial ovarian cancer, peritoneal cancer or fallopian tube cancer</td>
<td></td>
</tr>
<tr>
<td>Randomized (IDS vs NAC)</td>
<td></td>
</tr>
<tr>
<td>Upfront maximal cytoreductive surgery followed by taxoid (paclitaxel or docetaxel) and platin-based (cisplatin or carboplatin) chemotherapy every three weeks for three courses</td>
<td></td>
</tr>
<tr>
<td>Primary chemotherapy with taxoid (paclitaxel or docetaxel) and platin-based (cisplatin or carboplatin) chemotherapy every three weeks for three courses</td>
<td></td>
</tr>
<tr>
<td>Non-optimal primary debulking</td>
<td></td>
</tr>
<tr>
<td>IDS followed by 3 additional courses of same chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Optimal primary debulking</td>
<td></td>
</tr>
<tr>
<td>Three additional courses of same chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Disease is stable or responding</td>
<td></td>
</tr>
<tr>
<td>IDS plus three additional courses of same chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Progressive disease</td>
<td></td>
</tr>
<tr>
<td>Out of protocol</td>
<td></td>
</tr>
</tbody>
</table>

The trial not only evaluates the NAC and IDS similar to the previous reports but also directly compares the two strategies.
Conclusion (interval debulking: the present)

Interval debulking surgery is not a standardized treatment option yet. However it can be performed in a selected patient population. For a final conclusion we need to await the long-term results of EORTC 55971.

References


Address reprint requests to:
M. GULTEKIN, M.D.
Cankırı Devlet Hastanesi,
Kadın Hastalıkları ve Doğum Bolumu
Cankırı (Turkey)
e-mail: mrtgultekin@yahoo.com
Whole-body positron emission tomography with 18F-fluorodeoxyglucose is an effective method to detect extra-pelvic recurrence in uterine sarcomas

P.L. Sung¹,², Y.J. Chen¹,², R.S. Liu¹,², H.J. Shieh¹,², P.H. Wang¹,², M.S. Yen¹,², K.C. Wen¹,²
S.H. Shen¹,², C.R. Lai¹,², C.C. Yuan¹,²
¹Department of Obstetrics and Gynecology, ²Department of Nuclear Medicine, ³Department of Radiology, ⁴Department of Pathology, Taipei Veterans General Hospital, ⁵National Yang-Ming University (Taiwan)

Summary

Purpose of investigation: To assess the clinical use of F-18-fluorodeoxyglucose positron emission tomography (FDG-PET) in the post-therapy surveillance of uterine sarcoma.

Methods: Eight whole-body FDG-PET studies were performed in seven women with previously treated uterine sarcoma. Conventional image studies (computed tomography) and physical examinations were performed for follow-up. All FDG-PET studies were indicated to localize suspected recurrences noted by conventional methods.

Results: The per case sensitivity of the FDG-PET studies and CT scans was 85.7% (6/7) and 100% (7/7), respectively (p = 0.174). FDG-PET was able to detect seven extrapelvic metastatic sites below the diaphragm (7/7, sensitivity: 100%), including the liver, spleen, paraaortic lymph node, spine and paracolic gutter, as well as pulmonary lesions in five patients, while the CT scan detected only three lesions (3/7, sensitivity: 42.9%; p = 0.070). FDG-PET detected only four recurrent pelvic lesions (4/6) and CT scan detected six (6/6) recurrent pelvic lesions (66.7% vs 100%, p = 0.455).

Conclusions: The FDG-PET showed a better detection rate than the abdominal CT scan for extrapelvic metastatic lesions and a similar detection rate as well as abdominal CT scan. FDG-PET can serve as a useful detection tool for patients with uterine sarcomas because nearly 80% of recurrence involve an extrapelvic site.

Key words: FDG-PET; Recurrent uterine sarcoma; Post-treatment surveillance.

Introduction

Uterine corpus sarcomas are generally classified as leiomyosarcoma (LMS), endometrial stromal sarcoma (ESS), and carcinosarcoma (MMMT) [1]. These sarcomas are rare gynecological cancers, and represent only about 3-5% of all uterine tumors. The initial choice of treatment for these tumors is surgery. Adjuvant treatment, such as radiotherapy or chemotherapy, has shown little improvement in the survival rate or in decreasing the rate of recurrence [3-9]. The recurrence rate of uterine sarcoma is 30% to 60%, depending on the kind of sarcoma [10]. After staging and treatment, there is no standard program for post-therapy surveillance of these patients with uterine sarcoma. All follow-up modalities are performed mainly for the detection of recurrences, so that early treatment can be started. Uterine sarcoma may recur locally and/or at distant sites. Nearly 80% of all recurrences will involve an extrapelvic site [4, 5], and these cancers usually recur with a median time to recurrence of eight to 16 months [3-5]. Although the options for patients who experience a recurrence are still limited, the site of recurrence (local or distant) as well as the time of detection (early or late) may determine the options for follow-up treatment. The accuracy of conventional morphological imaging, such as computed tomography (CT) or magnetic resonance imaging (MRI) and ultrasound (US) in detecting recurrence may be decreased by postsurgical and post-radiation change [11-16]. Tumor markers could serve as a reflection of active tumor, but they have been unable to localize the site of recurrence, so the value of tumor markers for uterine sarcoma is still questionable [17].

Fluorine-18 fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) (FDG-PET) is a metabolic image, and has been useful in the detection, staging, and treatment monitoring of many kinds of cancers. Its application in uterine sarcoma has seldom been reported [18-21], and few reports have mentioned its utility in the post-treatment detection of recurrence [19-21]. In the present study, we retrospectively assessed the contribution of FDG-PET in the post-therapy surveillance of seven Asian women with uterine sarcoma.

Materials and Methods

Between July 1998 and September 2003, seven patients with uterine sarcoma were eligible for enrollment. All seven (mean age: 53.7 ± 10.5 years) had undergone staging surgery and adjuvant treatment, and received eight FDG-PET studies. One patient had undergone two FDG-PET studies. After the initial standard treatment, all patients received routine follow-up, which comprised regular outpatient visits every month, pelvic examinations, including vaginal Papanicolaou smears and image studies (X-ray, ultrasound, CT for pelvic, abdominal or...
Whole-body positron emission tomography with 18F-fluorodeoxyglucose is an effective method to detect extra-pelvic recurrence etc.

247

... and some patients had serum tests for CA-125. Uterine sarcomas were initially classified from Stage I to III, based on the international Federation of Gynecology and Obstetrics (FIGO) staging systems.

In this study, all FDG-PET studies were indicated for post-treatment patients with uterine sarcoma. All of the patients were prepared with overnight fasting to reduce serum glucose and insulin levels to near basal concentration before 18F-FDG injections. FDG-PET was performed with a Scanditronix 15WB whole body PET Scanner (Scanditronix, Sweden) for all patients. The FDG-PET scan was started 50 minutes after an intravenous injection of 10-15 mCi of 18F-FDG. To avoid bladder and urethral artifacts, an intravenous injection of diuretics (Lasix) was prescribed and a Foley catheter was implanted to empty the bladder. Images were obtained and reconstructed on the transaxial, sagittal, and coronal planes, and also in rotating fashion. The PET images were interpreted by two nuclear physicians, who were blinded to the CT imaging. Any focal uptake of 18F-FDG, which is not considered to be physiologic on FDG-PET images, was recorded.

The CT scans were obtained from Siemens Somatom Plus 4 Power; the CT images were interpreted by two radiologists, who were blinded to the PET findings. CT and FDG-PET imaging results were reported according to the sites and sizes of all detected lesions. The final diagnosis of recurrence in this study was established by local biopsy, surgery, or clinical follow-up.

Results

Eight whole body FDG-PET imaging studies were performed in seven patients with uterine sarcomas. All (8/8) of the FDG-PET studies were indicated due to a suspected recurrence in a conventional image study and/or a symptomatic presentation.

Overall sensitivity and specificity in the imaging study

The characteristics of all patients are listed in Table 1. (Figure 1-3). Six out of seven patients were confirmed to have recurrence or metastasis. The per case sensitivity of the FDG-PET studies and CT scans was 85.7% (6/7) and 100% (7/7), respectively (p = 0.174) (Tables 1 and 2), and

<table>
<thead>
<tr>
<th>Table 1. — Patient profile.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4-1</td>
</tr>
<tr>
<td>4-2</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>

MMMT: Malignant Mixed mullerian tumor; ESS: Endometrial stromal sarcoma; LMS:leiomysarcoma; LN: lymph node; Meta: metastases; TP: true positive; TN: true negative; FP: false positive; FN: False negative; CT: Chemotherapy; RT: Radiotherapy; F/U: Follow-up; US: ultrasound; LAP: lymphadenopathy; Distant: Out of pelvic region; Local: in pelvic region; Rec: recurrence with pathologic proof; Rec*: recurrence without pathologic proof but clinically proven.

Table 2. — Sensitivity and specificity of PET and CT scan.

<table>
<thead>
<tr>
<th>PET</th>
<th>CT scan</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>85% (6/7)</td>
<td>100% (7/7)</td>
</tr>
<tr>
<td>Specificity</td>
<td>100% (1/1)</td>
<td>0% (0/1)</td>
</tr>
</tbody>
</table>

Table 3. — Location of recurrent or metastatic tumor detected by image studies.

<table>
<thead>
<tr>
<th>Location below diaphragm</th>
<th>PET</th>
<th>CT scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrapelvic lesions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal tumor</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bone (spine)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Paraaortic lymph node</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Peritoneal carcinomatosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pelvic tumor</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Pelvic lymph node</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Above diaphragm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Paratracheal region</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 1. — Patient 1 (a case of MMMT): (a) whole body FDG-PET detected peritoneal carcinomatosis and spleen metastasis (black arrows, coronal view) (b, c) abdominal CT showed carcinomatosis and spleen tumor (white arrows, transaxial view).

Figure 2. — Patient 3 (a case of MMMT with disseminated metastasis): (a) whole body FDG-PET showed paraaortic lymph node metastasis (black arrows, coronal view) (b) not seen in 11C-acetate PET (coronal view) (c) abdominal CT did not show this metastasis (white arrows, transaxial view).

Figure 3. — Patient 4 (a case of low grade ESS): (a) whole body FDG-PET detected recurrence of pelvic cystic lesions which had increased intensity of 18FDG uptake in peripheral area (black arrow, coronal view) (b) abdominal CT revealed clearly recurrent cystic tumors (white arrow, transaxial view).
the per case specificity of the same two studies was 100% (1/1) and 0% (0/1), respectively (p = 1.000). The per lesion sensitivity of the FDG-PET studies and CT scans was 87.5% (14/16) and 75% (12/16), respectively (p = 0.654) (Tables 1 and 2), and the per lesion specificity of the same two studies was 100% (1/1) and 0% (0/1), respectively (p = 1.000).

Table 4. — Sensitivity according to lesion locations.

<table>
<thead>
<tr>
<th>Sensitivity according to lesion location</th>
<th>PET</th>
<th>CT scan</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Below diaphragm</td>
<td>84.6% (11/13)</td>
<td>69.2% (9/13)</td>
<td>0.645</td>
</tr>
<tr>
<td>Extrapelvic lesions</td>
<td>100% (7/7)</td>
<td>42.9% (3/7)</td>
<td>0.070</td>
</tr>
<tr>
<td>Intrapelvic lesions</td>
<td>66.7% (4/6)</td>
<td>100% (6/6)</td>
<td>0.455</td>
</tr>
<tr>
<td>Above diaphragm</td>
<td>100% (3/3)</td>
<td>100% (3/3)</td>
<td></td>
</tr>
</tbody>
</table>

Extrapelvic lesions below the diaphragm

In the present study, FDG-PET was able to detect seven extrapelvic metastatic sites below the diaphragm (7/7, sensitivity: 100%), including the liver, spleen, paraaortic lymph node, spine, and paracolic gutter, as well as pulmonary lesions in five patients (Tables 3 and 4), while the CT scan detected only three lesions (3/7, sensitivity: 42.9%; p = 0.070). The FDG-PET study detected extrapelvic metastatic lesions in the spine in two patients, an abdominal lesion in one patient, and paraaortic lymphadenopathy in one patient, all of which CT or US missed. The FDG-PET also added more information for tumor extension in three of six patients, especially paraaortic lymph node and spinal metastases.

Intrapelvic lesions below the diaphragm

FDG-PET detected only four recurrent pelvic lesions (4/6, sensitivity: 66.7%), including pelvic tumors and pelvic lymph nodes, which were also noted in the conventional imaging studies of these patients, and CT scan detected six (6/6, 100%; p = 0.455) recurrent pelvic lesions (Tables 3 and 4). Pathology confirmed the recurrence in the six patients.

Lesion above the diaphragm

The capability of the FDG-PET study to detect lesions (3/3, 100%) above the diaphragm, including lung and paratracheal lesions, was equal to that of the chest CT scan (3/3, 100%) (Tables 3 and 4).

The follow-up FDG-PET imaging study played a role in deciding the follow-up treatment. Confirmation of the recurrent pelvic lesions or operative extrapelvic lesions by the conventional imaging study, and then by the FDG-PET study, compelled the oncologist to perform surgery (Table 1). Other metastases or recurrent lesions had been treated by adjuvant chemotherapy (Table 1). Due to the disseminated diseases noted in both the FDG-PET study and CT scan in patient 3, palliative support treatment was given. The only case with a suspected lesion seen in the conventional study and a false-negative result in the FDG-PET study was conservatively followed-up. The patient expired within one year, even though she received adjuvant chemotherapy after the symptoms appeared. Patient 7, with a true negative FDG-PET image study, underwent a local biopsy for lesions seen in the CT scan, and no recurrence of the suspected lesion was confirmed. The patient had no recurrence during a one-year follow-up.

Discussion

The prognosis of uterine sarcoma has been considered to be poor, and the recurrence rate has been high, even in early stages [1,10,17, 22, 23]. In this study of seven patients with uterine sarcoma, the clinical staging was I in five, II in one, and III in one. However, six of seven (85%) patients with uterine sarcoma had recurrence (4 in Stage I, 1 in Stage II and 1 in Stage III). This was comparable with previous reports.

PET is now widely used in the field of gynecological oncology, including breast, cervical, and ovarian cancer [24-29]. It is a particularly useful tool because it evaluates the whole body in a single examination. Conventional image studies cannot survey the whole body at one time in one study. The more imaging studies that are performed, the more anxiety and medical costs the patients must endure.

Studies of FDG-PET applied in the post-surveillance of uterine sarcoma are limited. Umesaki et al. reported three studies on the utility of a preoperative diagnosis of uterine sarcoma. They reported PET had 100% positive findings for five sarcomas (including one recurrent case of leiomyosarcoma) for preoperation diagnosis, compared with 80% by MR imaging study, and 40% with US [18, 19]. Jadvar et al. reported a patient with metastatic leiomyosarcoma, who had received a total abdominal hysterectomy, bilateral oophorectomy, and omentectomy six months earlier, and presented with a lower abdominal wall soft tissue mass [20]. Murakami et al. recently reported on eight patients with sarcoma after primary treatment of uterine sarcoma, who underwent FDG-PET for the detection of recurrence. Final diagnoses of recurrence were established in five cases. The recurrence sites revealed by PET were in the intraperitoneum, liver, lung, bone and retroperitoneal lymph nodes. The overall sensitivity of FDG-PET, CT and US was 100%, 60% and 60%, respectively [21]. In the present study, the rate of detection of recurrence by FDG-PET and conventional imaging studies was 87.5% (14/16) and 75% (12/16), respectively.

In extrapelvic metastatic sites below the diaphragm, FDG-PET showed a better detection rate than abdominal CT scans (7/7, 100% vs 3/7, 42.9%, respectively). The FDG-PET studies detected extrapelvic lesions of the spine in two patients and paraaortic lymphadenopathy in one patient, which were metastatic locations that the abdominal studies had missed. The diagnosis of lymph node abnormalities with CT largely depends on size. However, normally-sized lymph nodes may be diseased, and in contrast, enlarged lymph nodes may show an
inflammatory response and be free of disease. In the cases of ovarian cancer, Murakami et al. reported PET findings could detect normally-sized metastases of lymph nodes in 50% of cases of retroperitoneal metastases, which could not be detected by CT [21]. PET has a better detection rate in bone metastases patients than the CT scan. These are the reasons why PET had a better detection rate in extrapelvic lesions. The capability of the FDG-PET studies to detect these lesions (3/3, 100%) above the diaphragm, including lung and paratracheal lesions, was almost equal to that of the chest CT scan (3/3, 100%). Thus, PET is a good method to detect extrapelvic lesions, including in the lymph nodes, bone, upper abdomen, and chest. However, FDG-PET did not show a good detection rate in pelvic recurrent lesions (4/6, 66.7%) in this study, compared with abdominal CT scans (6/6, 100%). Many papers in oncology have reported that PET is of limited use in the detection of malignant tumors less then 1 cm in size. The minimum size of tumors detected by PET depended on the sites of recurrence [21]. It is hard to detect a small pelvic tumor near the bladder due to the accumulation of FDG in the urinary tract. These regions usually cannot be precisely identified in FDG-PET imaging due to bowel or urethral tracer accumulation. Combined PET/CT could efficiently contribute to distinguishing a physiological tracer uptake from tumor lesions in the abdomino-pelvic region. PET/CT allows distinguishing a physiological tracer uptake from tumor lesions in the abdomino-pelvic region. PET/CT allows distinguishing false-positive findings [32]. Further study for the quantitative assessment of post-treatment recurrence should be done.

In extrapelvic metastatic sites, PET/CT showed a better detection rate in this study than abdominal CT scanning, although FDG-PET did not have a good detection rate for pelvic recurrent lesions, compared to the abdominal CT scan. Nonetheless nearly 80% of all recurrences in uterine sarcoma will involve an extrapelvic site, so whole-body FDG-PET imaging studies can be a useful tool in the detection of recurrence and metastases in extrapelvic sites in patients with uterine sarcoma. Therefore, larger studies are needed to evaluate the complementary role of FDG-PET and conventional imaging studies in the detection of distant metastasis.

Acknowledgements
This research was supported in part by a grant from VGH95B1-010 and in part by a grant from the Bureau of Health Promotion, Taiwan.

References
Whole-body positron emission tomography with 18F-fluorodeoxyglucose is an effective method to detect extra-pelvic recurrence etc.


Address reprint requests to:
C.C. YUAN, M.D.
Department of Obstetrics and Gynecology
Taipei Veteran General Hospital, 201
Section 2, Shin-Pai Road,
Taipei 112 (Taiwan)
e-mail: chenyj@vghtpe.gov.tw
Clinical implication of medroxyprogesterone acetate against advanced ovarian carcinoma: a pilot study

K. Niwa¹, M.D.; K. Onogi¹, M.D.; Y. Wu¹, M.D.; H. Mori¹, M.D.; R.C. Harrigan³, Ph.D.; T. Tamaya¹, M.D.

Departments of ¹Obstetrics & Gynecology and ²Tumor Pathology, Gifu University Graduate School of Medicine, Gifu-City (Japan)
³Division of Complementary and Alternative Healthcare, John A. Burns School of Medicine, University of Hawaii (USA)

Summary

Purpose of investigation: The present study was performed to identify the effects of medroxyprogesterone acetate (MPA) plus adjuvant chemotherapy on advanced epithelial ovarian carcinoma (FIGO Stage III/IV). Methods: A total of 50 patients were enrolled in this study. A relatively low dose of MPA (200 mg/day) after surgery was administered in combination with platinum-based chemotherapy and the treatment was continued for two years. Patients' backgrounds were also analyzed. Results: Relapse-free survival (p < 0.05) and overall survival (p < 0.001) rates in FIGO Stage III/IV ovarian cancer patients with MPA combined chemotherapy were significantly longer than the control group. The effect was more prominent in the higher progesterone receptor expression group. The chemotherapy regimens (cyclophosphamide, doxorubicin and cisplatin vs paraplatin plus cyclophosphamide or paclitaxel) did not affect prognosis. Conclusion: MPA with platinum-based chemotherapy as an adjuvant therapy might improve the prognosis in FIGO Stage III/IV epithelial ovarian cancer cases. A randomized controlled study is still needed for further analyses.

Key words: Medroxyprogesterone acetate; Progesterone receptor; Ovarian cancer; Survival.

Introduction

Ovarian cancer is a gynecological malignancy with the highest mortality rate in Japan as well as in Western countries [1]. Most women with this advanced disease are offered some form of chemotherapy after surgery, but the 5-year survival rate after diagnosis is assumed to be approximately 30% [2]. Although paclitaxel has been introduced into the treatment for ovarian cancer, the superiority of the chemotherapy including paclitaxel to the conventional chemotherapy [cyclophosphamide, doxorubicin and cisplatin (CAP) or AP] has not yet been demonstrated [3]. As a result, combination treatment, including surgery, chemotherapy, hormonal and anti-angiogenetic therapy is needed to obtain a better prognosis, since ovarian cancer is frequently known to be hormone-dependent and angiogenetic.

Epidemiological studies have shown a decreased incidence with increased parity [4], and the use of oral contraceptives has also exerted a protective effect on the incidence of ovarian tumors [5]. Thus, progesterone is thought to have a suppressive effect on ovarian neoplasms.

The ovary is the principal source of estradiol and progesterone, and it is also recognized as a target organ for gonadal sex steroid hormones, thus acting by means of an auto-, intra- or paracrine mode [6]. Elevated sex-steroid hormones in epithelial ovarian cancer are reported to be related with the tumor volume and prognosis [7]. Progesterone has been reported to possess a preventive and growth-inhibiting effect on ovarian tumors [8, 9]. This point correlates with our present observation, especially regarding tumors with a progesterone receptor (PR) expression. The presence of PR itself has been shown to be a prognostic factor [10].

A variety of progestational agents have been shown to be effective in the treatment of recurrent and metastatic endometrial carcinoma [11]. Although the clinical response rate of progesterone to ovarian cancer patients has been reported to be 7% or lower [12], the value is suggested to be based on the use of progesterone alone to advanced or refractory ovarian cancer patients. In the present study, we used progesterone combined with platinum-based chemotherapy as a first-line treatment to treat epithelial ovarian cancer patients who had had cytoreductive surgery.

Regarding endometrial cancer patients, 400-600 mg/day of MPA orally has usually been administered. In a GOG study for recurrent or advanced endometrial cancer patients [13], the response rate of oral MPA at the dose of 200 mg/day was equally effective as that of 1000 mg/day. MPA was effective after oral administration at the dose of 200 mg/day, when the serum level was determined [14]. To avoid the most adverse effect of hypercoagulation by MPA, the dose of oral MPA in this study was determined at 200 mg/day.

The main aim of this study was to clarify the clinical implication of MPA, particularly in response to treatment and clinical outcome in a single institutional series of primary untreated advanced ovarian cancer patients.

Patients and Methods

Patients. The clinical records of 50 ovarian cancer patients who were admitted, treated, and followed-up at the Department of Obstetrics & Gynecology of Gifu University Hospital between January 1993 and December 2001 were evaluated for

Revised manuscript accepted for publication October 1, 2007
Clinical implication of medroxyprogesterone acetate against advanced ovarian carcinoma: a pilot study

Clinical prognostic factors (patient’s age, tumor stage), the survival status and causes of death (cancer association, cancer independent, unclear). Staging was performed according to the FIGO classification.

The standard surgical intervention consisted of bilateral salpingo-oophorectomy, total abdominal hysterectomy and partial omentectomy. Paraortic and pelvic lymphadenectomy and cytology of ascites or peritoneal washing cytology were also routinely performed. In addition, all patients had undergone tumor reductive surgery and were suboptimally debulked. Any patients with benign lesions, metastatic ovarian tumors, borderline tumors, stromal and germ cell tumors were excluded from the study.

Pathological study. Histological examination revealed the following subtypes according to the WHO classification [15]: serous 27 (54.0%); mucinous 13 (26.0%); clear cell seven (14.0%); endometrioid two (4.0%); others (transitional cell cancer) one (2.0%). Staging was performed according to FIGO. Thirty-seven patients (74.0%) were in Stage III and ten (26.0%) in Stage IV.

Immunohistochemistry. Four micrometer sections, fixed in 10% formalin and paraffin-embedded, were mounted on poly-L-lysine-coated slides (Sigma, St. Louis, MO). The sections were then deparaffinized, rehydrated and, to quench endogenous peroxidase activity, incubated for 30 min with 3% H2O2 in methanol. After a short rinse in Tris-buffer, the sections were boiled in a microwave oven for 3 x 5 min in citrate buffer. Following rolling and rinsing in Tris-buffered saline, normal horse serum (for ER-α, β) and goat serum (for PR antibody) were applied on the sections for 20 min to block non-specific binding. The sections were then incubated overnight at 4°C with monoclonal antibodies directed against ER-α, β and PR. The localization of antigen-antibody complex was performed with the avidin-biotin-peroxidase complex (ABC) technique using a L-lysine-coated slides (Sigma, St. Louis, MO). The sections were then deparaffinized, rehydrated and, to quench endogenous peroxidase activity, incubated for 30 min with 3% H2O2 in methanol. After a short rinse in Tris-buffer, the sections were boiled in a microwave oven for 3 x 5 min in citrate buffer. Following rolling and rinsing in Tris-buffered saline, normal horse serum (for ER-α, β) and goat serum (for PR antibody) were applied on the sections for 20 min to block non-specific binding. The sections were then incubated overnight at 4°C with monoclonal antibodies directed against ER-α, β and PR. The localization of antigen-antibody complex was performed with the avidin-biotin-peroxidase complex (ABC) technique using a Vectastain ABC kit. Peroxidase activity was demonstrated by 5-min incubation in 3,3′-diaminobenzidine tetrahydrochloride and H2O2 dissolved in citrate buffer.

The following monoclonal antibodies were used: ER-α, β (Dako, Denmark); and PR (PgR-ICA monoclonal, Abot, IL). The evaluation of all tissue sections was performed without any prior knowledge of clinical parameters by different cytopathologists by means of microscopy. Immunoreactive score for ER-α, β and PR was calculated basically based on Krajewska et al. [16]. Briefly, the intensity of immunostaining was as follows: none = 0, weak = 1, moderate = 2, strong = 3, and the percentage of positive tumor cells was as follows: none = 0, < 10% = 1, 10-50% = 2, 51-80% = 3, > 80% = 4.

Survival. Relapse-free survival (RFS) was also defined as the period from the initial surgery to the time of recurrence or death, whichever occurred first. Overall survival (OS) was calculated from the date of the first surgery to the date of death or the last contact. Medians and life tables were computed using the product-limit estimate by the Kaplan-Meier method.

Chemotherapy. All patients underwent six cycles of platinum-based chemotherapy three to four weeks after primary surgery. Chemotherapy was performed in two regimens; 12 (24.0%) with CAP [cyclophosphamide 320 mg/m², doxycarbucin 30 mg/m², cisplatin 50 mg/m²], and 38 (76.0%) with paclitaxel (AUC = 5) and cyclophosphamide (500 mg/m²) or paclitaxel (150 mg/m²).

MPA therapy. MPA was offered to all ovarian cancer patients in this study. The investigators told them the following: “The drug might be not effective for your disease, but it may be useful for your disease”. After approval from the Gifu University Hospital Ethical Committee and informed patient consent, MPA was commenced. MPA and chemotherapy were used synchronously for the patients in the MPA group. The regimen was as follows: MPA (200 mg/day) was administered from one month after the surgery for patients without hypercoagulation. If the patient’s data of coagulation tests showed beyond the normal limitations, MPA therapy was stopped and aspirin (100 mg/day) was added. The administration of such drugs continued up to two years after the start.

Clinical response and follow-up. Clinical data were obtained from the patients’ records and follow-up data were obtained from the clinical registers. The patients were followed-up every three to four months during the first three years, and then every six months at the Department of Gifu University Hospital.

Statistical analysis. Fisher’s exact probability or the chi-square test was used to analyze the cases according to several clinicopathological features.

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>MPA group</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>50</td>
<td>22</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Mean age</td>
<td>57.7 ± 12.4</td>
<td>61.1 ± 12.4</td>
<td>55.0 ± 12.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>12</td>
<td>12</td>
<td>0.22</td>
</tr>
<tr>
<td>2 and 3</td>
<td>26</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Histologic type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>27</td>
<td>11</td>
<td>16</td>
<td>0.084</td>
</tr>
<tr>
<td>Mucinous</td>
<td>13</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Clear cell</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Others (TCC*)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III vs IV</td>
<td>37 vs 13</td>
<td>14 vs 8</td>
<td>23 vs 5</td>
<td>0.14</td>
</tr>
<tr>
<td>Residual tumor</td>
<td>(+)</td>
<td>30</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>(−)</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Chemotherapy **</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAP</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>0.98</td>
</tr>
<tr>
<td>CBDCA plus CPA or PTX</td>
<td>38</td>
<td>16</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

* TCC, transitional cell carcinoma; ** CAP, cyclophosphamide, doxycarbucin and cisplatin; CBDCA, carboplatin; CPA, cyclophosphamide; PTX, paclitaxel.

![Figure 1. — Immunohistochemical staining for PR in a representative section of high-grade serous carcinoma is shown. High PR expression was restricted mainly to the tumor cells (original magnification x 200).](image-url)
Results

Patient characteristics and background. Follow-up data were available from 50 patients. As of June 2007, the median follow-up period was 4.5 years, and the mean follow-up period was 4.4 ± 4.3 years. The background ratios including histological grades, residual tumors, and chemotherapy between the control and MPA group showed no differences (Table 1).

Immunohistochemical expression. Immunohistochemical staining for PR is shown in Figure 1. Immunohistochemical expression scores in the MPA and control groups are summarized in Table 2. No differences were found in the ER-α, β and PR scores between the two groups.

Survival analysis. Figure 2 shows the OS for the MPA and control groups. The MPA group did significantly better than the control group (Figure 2, p < 0.001). The RFS group treated with MPA also did significantly better than that without (p < 0.01, data not shown). Figure 3 reveals the OS by different chemotherapy regimens. No significant difference was found in the OS (CAP vs para-platin plus cyclophosphamide or paclitaxel). Figure 4 shows the OS by MPA on/off related with PR expression. The OS for PR high expression with MPA was significantly better than without (p < 0.05) in the high PR expression group.

Discussion

In the present study, a relatively low-dose MPA combined with platinum-based chemotherapy significantly improved the survival as well as recurrence rates of advanced stage (FIGO Stage III/IV) epithelial ovarian cancer patients. These results imply that MPA combined with platinum-based chemotherapy could improve the prognosis of advanced epithelial ovarian cancer. In the present study, the chemotherapy regimens did not affect the prognosis. The effect of MPA was more prominent in the PR high expression group.

The possible mechanisms of progesterone on ovarian cancer are proposed to be 1) the induction of apoptosis of cancer cells [8], 2) the inversion of multi-drug resistance of cancer cells [17], 3) inhibition of angiogenesis [18], 4) causing cells to starve to death by inhibition of the synthesis and esterification of cholesterol [19], 5) inhibition of ovarian cancer growth by release of FSH and LH through negative feedback [19].

There is a clinical report that progesterone was used to treat ovarian cancer and the response rate was 7% [12]. However, such evidence is basically based on the use of progesterone alone to treat advanced ovarian cancer or for those patients who had no longer responded to other drugs. In the present study, we used MPA combined with

Table 2. — Immunohistochemical expression scores in the MPA and control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ER-α score</th>
<th>ER-β score</th>
<th>PR score</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPA group</td>
<td>6.5 ± 1.6*</td>
<td>3.5 ± 1.6</td>
<td>5.4 ± 1.4</td>
</tr>
<tr>
<td>(n = 22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.2 ± 1.6</td>
<td>3.3 ± 1.4</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>(n = 28)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD
systemic chemotherapy as a first-line treatment to treat epithelial ovarian cancer patients who had undergone cytoreductive surgery. Since MPA inhibits angiogenesis and the growth of cancer cells, the administration of MPA after cytoreductive surgery may inhibit the angiogenesis and growth of cancer cells that remain in the abdominal cavity in advanced ovarian cancer patients. Related with anti-angiogenic effects of MPA, it has also been reported to improve the long-term survival for chemo-resistant breast cancer [20] and the quality of life for non-hormone-sensitive cancer patients [21].

The presence of PR itself is thought to be a prognostic factor [10]. As the expression of ER-α, β and PR showed the same tendencies (Table 2), the differences for PR expressions between the MPA and control groups seemed small. In the MPA-treated group, however, the efficacy of MPA on the higher PR expression subgroup was more prominent than the lower PR expression subgroup (Figure 4).

Although the number of cases in this study was relatively small, adding MPA to systemic chemotherapy in advanced ovarian cancer patients statistically induced better survival. A randomized controlled study is still needed for further analyses.

References


Address reprint requests to:
K. NIWA, M.D. 
Department of Obstetrics and Gynecology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu-city, Gifu 501-1194 (Japan) 
e-mail: kniwa@gifu-u.ac.jp.
Synchronous ovarian and endometrial carcinoma: a strong link to endometriosis?

A. Kondi-Pafiti¹, D. Grapsa¹, A. Liapis¹, K. Papadias², E. Kairi-Vassilatou¹, D. Hasiakos²

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

Summary

Purpose: To further study the clinicopathological features of synchronous ovarian and endometrial carcinomas. Methods: We retrospectively studied all cases of synchronous ovarian and endometrial carcinomas diagnosed in our laboratory over the last 15-year period. The pathological findings were correlated with the clinical records of the patients. Results: Seven cases of synchronous primary ovarian and endometrial carcinomas were retrieved. The most common presenting symptom was abnormal vaginal bleeding (5 cases, 71.4%). Five patients (71.4%) were postmenopausal and two (28.6%) were nulliparous. All seven patients had Stage I ovarian and endometrial carcinomas of endometrioid histology. Moreover, in all seven ovarian carcinomas endometriosis foci were observed, while atypical endometriosis was found in four of them. With the exception of one patient, who received adjuvant post-operative radiation, all remaining patients were treated with surgery alone. All patients were alive and free of disease at completion of the study. Conclusion: The correct classification of synchronous primary ovarian and endometrial carcinomas is often problematic because of the frequent confusion with their metastatic counterparts. Although the exact etiology remains unclear, endometriosis seems to be a major risk factor for their development.

Key words: Ovarian; Endometrial; Synchronous carcinomas; Endometriosis.

Introduction

Multifocal presentation of primary carcinomas in various sites of the female genital tract is a relatively rare but well recognized entity of unknown etiology. Most of these neoplasms seem to involve the ovary and the endometrium, with the most reported incidences approximating 10% of all women with ovarian cancer and 5% of those with endometrial cancer [1]. However, many authors believe that these percentages are overestimated, including metastatic rather than true primary neoplasms [1, 2]. On the other hand, the pathological diagnostic criteria of synchronous ovarian and endometrial tumors, as originally described by Ulbright and Roth in 1985 and completed by Scully et al. 13 years later, although extremely useful in resolving these diagnostically challenging cases, seem mostly empirical and remain to be validated [1, 3, 4]. As a result of all this controversy, the staging and therapeutic strategies employed in these cases varies significantly among different authors and institutions, and the optimal management of – at least – some of these patients is likely to be compromised.

Endometriosis is an enigmatic disease of unclear pathogenesis, which is defined as the implantation of endometrium-like glandular and stromal cells outside their normal location in the uterus [5]. Although benign in nature and clinical behavior, endometriosis shares many of the features of neoplasia, while it is associated with an increased risk of malignant transformation [5]. Thus, it is estimated that the risk of ovarian cancer is considerably higher (about 4-fold) in the presence of endometriosis, as compared to that in the general population [5, 6]. Furthermore, ovarian endometriosis is identified in about 30% of synchronous endometrial and ovarian cancers, especially of endometrioid type [5, 7, 8].

The aim of the present study was to review the clinicopathological features of all synchronous ovarian and endometrial carcinomas diagnosed in our laboratory over the last 15-year period and shed more light in the pathogenesis of this puzzling clinical entity.

Materials and Methods

After reviewing the archival files of our laboratory over the last 15-year period (years from 1991 to 2005), we retrieved seven cases of synchronous ovarian and endometrial carcinomas, among 1,680 cases of ovarian carcinomas and 300 cases of endometrial carcinomas. Only those cases that fulfilled the criteria proposed by Ulbright and Roth were included [4]. For the exclusion of cases of endometrial carcinoma metastatic to the ovaries, similar criteria proposed by Ulbright and Roth were also followed (Table 1) [3]. The relative clinical and pathology reports as well as representative slides for each case were also retrieved.

The clinical data, including patient age, presenting symptoms, parity, menopausal status and outcome were correlated with the pathologic data (both gross and histological). The latter comprised the following features: histological type and grade of tumor, presence and type of endometrial hyperplasia, presence and extent of myometrial, lymphatic and blood vessel invasion, fallopian tube involvement, coexistence of endometriosis, ovarian size and pattern of ovarian involvement (unilateral or bilateral and multinodular or solitary development) and pelvic extension of disease. Staging was also reviewed and updated in order to conform to the current criteria put forth by the International Federation of Gynecology and Obstetrics (FIGO).
Tumors were histologically classified according to the World Health Organization (WHO) guidelines. Follow-up data were available for all patients, for a period ranging from six years to 84 months (mean 35 months).

**Results**

**Clinical data**

A summary of the clinical findings of all cases included in our study is provided in Table 2.

The patients' age at diagnosis ranged from 48 to 62 years (mean 55.3 years). The most common presenting symptom was abnormal vaginal bleeding (5 cases, 71.4%), followed by lower abdominal pain (2 cases, 28.6%). Five patients (71.4%) were postmenopausal, and two (28.6%) were nulliparous. All patients were submitted to total abdominal hysterectomy and bilateral salpingo-oophorectomy. For the purpose of staging, peritoneal washings, omentectomy and pelvic lymphadenectomy had also been performed, with negative results. With the exception of one patient, who received post-operative radiation, all remaining patients were treated with surgery alone, without any adjuvant therapy. No disease recurrence or death of a patient was noted during the time of the follow-up.

**Pathological data**

Table 3 shows the clinicopathological features of all cases included in our study.

All seven patients had Stage IA ovarian carcinomas of endometrioid histology four ovarian carcinomas (57.1%) were grade 1 and three (42.9%) were grade 2. Similar results were found with regard to endometrial carcinomas: all cases were of endometrioid histology, stage tumors: five (71.4%) were Stage IA and two (28.6%) Stage IB. There were three cases (42.9%) with grade 1 tumor, three (42.9%) with grade 2 and one case (14.3%) with grade 3. In all patients, lymphatic and blood vessel invasion were absent and the invasion of the uterine myometrium was either absent (2 cases, 28.6%) or in the upper third (5 cases, 71.4%). The size of the involved ovary ranged from 7 to 25 cm (mean 12.3 cm) and the tumor was solitary in all cases. Moreover, in all seven ovarian carcinomas endometriosis foci were observed (Figure 1). In four of them (57.1%), atypical endometriosis was also found.

**Discussion**

To the best of our knowledge, the two largest series of patients with synchronous primary cancers of the ovary and the endometrium are those reported by Zaino et al. in 2001 and Soliman et al. three years later, comprising 84 and 74 cases, respectively [1, 9]. On the basis of their results, it seems that in the majority of cases these dual primary tumors are of endometrioid histological type, of low stage and grade, present in younger age (about 50 years) than the median age of onset of either ovarian or endometrial carcinomas (63 and 60 years, respectively) and are associated with a surprisingly favorable prognosis. What is also of interest is that most of the remaining smaller series, including our own, present similar results, thus consistently reaffirming the same findings [2, 3, 10-15].

Therefore, there is uniform agreement that synchronous primary ovarian and endometrial carcinomas represent a distinct clinicopathologic entity, whose most prominent feature is their significantly improved overall prognosis in comparison to their metastatic counterparts (Stage II ovarian carcinoma and Stage III endometrial carcinoma) or even to their single primary counterparts (early-stage, low-grade ovarian and endometrial carcinomas) [12, 13]. Most previous studies of synchronous ovarian and endometrial primaries report high 5-year survival rates ranging from 73.3 to 100%, while the corresponding rates for Stage II ovarian carcinoma and Stage III endometrial carcinoma are as low as 60 and 43-58%, respectively [10, 13-17]. In accordance with these data, the overall survival in our series was 100%, with a median follow-up of 35 months. The mean age of our patients at presentation was 53.3 years, which is close to the reported range, and the commonest presenting symptom was abnormal uterine bleeding. Given the insidious nature of ovarian cancer, it is conceivable that its early diagnosis and the subsequent improved survival of patients could at least be partly attributed to the coexistence of a symptomatic endometrial tumor.
Discriminating between two independent primaries and metastatic disease is therefore of crucial importance with regard to the clinical implications in each case. Nevertheless, many of the cases included in some previous reports do not meet all the existing pathological criteria. Furthermore, several researchers support the view that a definite distinction of patients with multiple primary tumors from those with metastatic disease requires the evaluation of molecular data in addition to the standard clinicopathological parameters [1, 2, 18-20]. Molecular profiling of these cases might ideally lead to an improved stratification of patients, and the administration of individualized modes of treatment, thus further improving their outcome [20]. Although most recent molecular studies of synchronous tumors of the ovary and endometrium have thus far failed to prove the diagnostic efficacy of molecular pathology techniques in this field, some of them succeeded in providing significant information regarding the pathogenesis of these cancers [18-22]. However, the differential diagnosis still relies on the evaluation of conventional clinicopathological findings, while the importance of a careful and extensive clinicopathologic evaluation as a prerequisite for accurate classification is undisputable [2, 3, 13, 18]. In our study only those cases that strictly fulfilled the criteria described by Ulbright and Roth [3] and Scully et al. [4] were included for analysis, thus producing a group of patients with the highest possibility of representing true independent primaries. Previously reported incidences of synchronous ovarian and endometrial primaries range from 2-8.5% of endometrial carcinomas and 4.5-30% of ovarian carcinoma cases [10, 11, 23, 24]. The incidence reported in our series is relatively low (2.33%) of endometrial carcinoma and 0.42% of ovarian carcinoma patients in comparison to the aforementioned percentages, especially with regard to ovarian carcinoma. This could be attributed to the limited number of patients included in our study and/or the strict application of the proposed diagnostic criteria.

Despite the fact that the etiology of the synchronous development of carcinoma in the ovary and the endometrium remains unclear, several theories have been proposed for the explanation of this enigmatic entity. The theory of an extended or secondary Mullerian system, comprising the ovarian epithelium, fallopian tube, uterine corpus and cervix and behaving as a single morphologic unit, explains this phenomenon as a response of this entire system towards the development of primary carcinomas in multiple sites [25, 26]. From a molecular point of view, as recently described by Furlan et al., this “field effect” in the upper genital tract and the ovaries “could be the result of either independent molecular events affecting multiple cells separately under the action of a common carcinogenic agent, or one molecular event in a single clonal progenitor that gives rise to multiple foci of tumorigenesis via mechanisms of widespread clonal expansion” [20]. Pathologically, this multifocal oncogenic transformation is reflected in the synchronous detection of early-stage and low-grade primary cancers both in the ovary and the endometrium [2, 14]. In our study all of the patients had both ovarian and endometrial Stage I tumors, mostly low grade, a fact further supporting the separate and independent rather than metastatic nature of these cases.

Despite an abundance of epidemiologic, histopathologic and molecular data, linking endometriosis to ovarian cancer, it is still unclear whether these two diseases are directly or indirectly associated [27]. Two current theories support a) that endometriotic implants may undergo direct malignant transformation, often through an atypical endometriosis transition phase, and b) that cancer and endometriosis have in common many environmental, immunological, hormonal or genetic pre-
Synchronous ovarian and endometrial carcinoma: a strong link to endometriosis?

Disposing factors [5]. Ovarian endometriosis is a common finding in many cases of synchronous primary ovarian and endometrial carcinomas of endometrioid type, providing an explanation for the synchronous pathogenesis of the dual tumors. Around 60–80% of cases of endometriosis-associated ovarian cancer occur in the presence of atypical ovarian endometriosis [5, 28, 29]. Atypical endometriosis is characterized histologically by endometrial glands with cytological or architectural atypia and has been observed in 12–35% of ovarian endometriosis [5, 30]. In our study, atypical endometriosis was found in the majority of cases, thus further supporting the hypothesis of a potential transition phase of non-atypical to atypical endometriosis and malignancy [5]. As already suggested by other investigators, the association of an endometrioid ovarian tumor with endometriosis represents reasonable evidence of its independent development [3, 31, 32]. Thus, the coexistence of ovarian endometriosis in all of our cases further supports the independent development of ovarian and endometrial carcinomas in our studied material.

In conclusion, the results of our study provide further evidence in support of the involvement of endometriosis in the pathogenesis of synchronous primary ovarian and endometrial carcinoma. To safely discriminate these independent primaries from their metastatic counterparts we should refine our currently applied diagnostic criteria. For this purpose, additional data, both pathological and molecular, are needed which should be derived from large, prospective series including carefully selected and eligible patients.

References


Address reprint requests to:
A. KONDI-PAFITI, M.D.
Pathology Laboratory
Aretaieion Hospital
Vas Sofias 76
11528 Athens (Greece)
e-mail: akondi@med.uoa.gr
Prognostic factors in patients with carcinoma of the vulva – our own experience and literature review

P. Blecharz¹, K. Karolewski¹, T. Bieda¹, M. Klimek¹, J. Pudelek¹, E. Kojs², K. Zur³, P. Dzialak⁴, K. Urbanski⁴

¹Center of Oncology, Gynecologic Oncology Department, M. Skłodowska-Curie Memorial Institute, Krakow Branch
²Center of Oncology, Medical Oncology Department, M. Skłodowska-Curie Memorial Institute, Krakow Branch
³Students’ Scientific Society of the Medical College of Jagiellonian University
⁴Krakow Medical Center, Krakow (Poland)

Summary

Aim of the study: The objective was the analysis of prognostic factors and treatment outcomes of 104 patients with vulvar cancer, treated between 1990 and 2003 in the Center of Oncology, Maria Skłodowska-Curie Memorial Institute, Cracow, Poland. Material and Methods: The median age of patients was 67. Advanced disease (TNM III and IVA) was found in 54 (51.9%) patients and grade 2 and 2 in 50 (48.1%). Inguinal lymph nodes were clinically uni- or bilaterally involved in 40.4% of patients. Fifty-seven (54.8%) patients underwent radical vulvectomy with bilateral inguinal lymphadenectomy and 47 (45.2%) radical vulvectomy only. Cancer differentiation was well in 38 (36.2%) of patients, moderate in 38 (36.2%) and poor in 28 (36.6%). Adjuvant radiotherapy was applied in 30 (28.8%) cases. Results: Five-year overall survival rate was observed in 44.4% of patients. Depending on TNM grade, 5-year OS rates were 61.4% for grade 1, 54.9% for grade 2, 40.1% for grade 3 and 13.3% for IVA. In patients aged < 70, 5-year OS rate was 54.7% compared to 30.5% for those ≥ 70. Among patients with G1 cancer differentiation 64.4% survived five years, with G2 39.1% and with G3 24.9%, respectively. Conclusion: Univariate analysis revealed a statistically significant, unfavorable impact of age ≥ 70, with G3 cancer differentiation, clinically confirmed inguinal lymph node involvement and TNM classification stage on 5-year overall survival. Cox multivariate analysis demonstrated that independent prognostic factors for 5-year survival were the age of the patient, clinical status of inguinal lymph nodes and TNM classification grade.

Key words: Carcinoma of the vulva; Prognostic factors.

Introduction

Carcinoma of the vulva is an uncommon disease, representing approximately 2.5-5% of female reproductive tract malignancies [1]. Vulvar cancer typically develops in women in their 7th and 8th decade of life, but recently the incidence of vulvar tumors has increased in women younger than 40 years of age [1]. Studies suggest two different etiologic types of vulvar cancer. The first, related to HPV infection and smoking, is commonly anticipated by vulvar intraepithelial neoplasia (VIN) and is seen in younger patients. The other, more common type, unrelated to HPV and seen usually in elderly women, is connected with a high incidence of dystrophic lesions adjacent to the tumor [1, 2]. The most common histological type of vulvar cancer is squamous cell carcinoma (85%), whereas adenocarcinomas, melanomas and basal cell carcinomas are much less common [1].

Surgery is the first choice for the treatment of patients with carcinoma of the vulva [2]. Radiotherapy is often applied, especially as adjuvant therapy. There are many population, pathologic and clinical factors which influence treatment methods and prognosis. The problem of prognostic indicators in patients with vulvar cancer is controversial. The range of prognostic factor values (i.e. age, tumor growth, depth of infiltration, width of surgical margin) remains unclear. The aim of this study was to analyze the value of prognostic factors, on the basis of our own clinical material and literature review.

Material and Methods

One hundred and four patients with invasive vulvar carcinoma were followed at the Center of Oncology, M. Skłodowska-Curie Memorial Institute, Krakow Branch in Poland between 1990 and 2003. Medium follow-up time from the date of initial treatment for all patients at the date of diagnosis was 58 months. The medium age of patients was 67. Classification of cancer stage was based on clinical TNM score [3]. Advanced disease (TNM III and IVA) was found in 54 (51.9%) patients and grade 1 and 2 in 50 (48.1%). Inguinal lymph nodes were clinically uni- or bilaterally positive in 40.4% patients and in 59.6% there was no clinical evidence of nodal metastasis. The cancer was well differentiated in 38 (36.2%) patients, moderate in 38 (36.2%) and poor in 28 (36.6%). Radical vulvectomy with bilateral inguinal lymphadenectomy from separate incisions was performed in 57 (54.8%) patients and radical vulvectomy alone in 47 (45.2%) patients. Among 57 (100%) patients treated with lymphadenectomy, inguinal metastases were found unilaterally in 28 (49.1%) and bilaterally in 17 (29.8%) patients. Uninvolved groin nodes were found in 12 (21.1%) patients. Adjuvant radiotherapy was applied in 30 (28.8%) patients due to positive surgical margins or inguinal node involvement. Survival was estimated for all of the indicators of interest using the Kaplan-Meier method. Differences between characteristics were tested with...
the two-sided chi-square test, and differences in continuous characteristics like age, were tested with a t-test; p values ≤ 0.05 were considered significant. Cox’s proportional hazards model was used to compute risk reduction among analyzed factors [4].

Results

The five-year overall survival rate in the study group was 44.4%. Three patients (2.9%) died due to postoperative complications and two (1.8%) due to cerebral stroke, with no evidence of cancer. Six (5.4%) patients survived five years with clinical evidence of cancer recurrence and 52 (50%) died due to treatment failure. Univariate analysis showed that BMI value, histology, clitoral involvement, tumor dimension and status of the surgical margins had no significant impact on treatment outcomes. However, age, differentiation of cancer, clinical inguinal node status and TNM stage were found to be associated with overall survival. A significantly higher 5-year overall survival rate was found in patients < 70, with well differentiated cancer, clinically negative groins and with disease in Stage I and II by TNM. Differences in 5-year overall survival are shown in Table 1. Furthermore, on multivariable analysis by forward stepwise Cox model regression, age, clinical node status and cancer stage by TNM were independent risk factors for cancer-related death (Table 2).

Table 1. — Treatment results of patients depending on population and clinical features.

<table>
<thead>
<tr>
<th>Characteristics of study group</th>
<th>No. of pts</th>
<th>5-year prognosis overall survival rate</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>under 70</td>
<td>60</td>
<td>54.7</td>
<td>0.00</td>
</tr>
<tr>
<td>≥ 70</td>
<td>44</td>
<td>30.5</td>
<td>6</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>under 31</td>
<td>86</td>
<td>47.5</td>
<td>NS</td>
</tr>
<tr>
<td>≥ 31</td>
<td>18</td>
<td>28.8</td>
<td></td>
</tr>
<tr>
<td>Cancer histological differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>well G1</td>
<td>38</td>
<td>64.4</td>
<td>0.05</td>
</tr>
<tr>
<td>moderate G2</td>
<td>38</td>
<td>39.1</td>
<td></td>
</tr>
<tr>
<td>poor G3</td>
<td>28</td>
<td>24.9</td>
<td></td>
</tr>
<tr>
<td>Clitoral involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>18</td>
<td>44.7</td>
<td>NS</td>
</tr>
<tr>
<td>no</td>
<td>86</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Primary tumor size (by TNM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>23</td>
<td>57.9</td>
<td>NS</td>
</tr>
<tr>
<td>T2</td>
<td>61</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>19</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>1</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Inguinal node status (by TNM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>62</td>
<td>55.4</td>
<td>0.00</td>
</tr>
<tr>
<td>N1</td>
<td>27</td>
<td>33.3</td>
<td>3</td>
</tr>
<tr>
<td>N2</td>
<td>15</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Vulvar cancer stage by TNM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>20</td>
<td>61.4</td>
<td>0.00</td>
</tr>
<tr>
<td>II</td>
<td>30</td>
<td>54.9</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>39</td>
<td>40.1</td>
<td></td>
</tr>
<tr>
<td>IVA</td>
<td>15</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>Surgical margins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninvolved</td>
<td>67</td>
<td>50.8</td>
<td>NS</td>
</tr>
<tr>
<td>involved</td>
<td>37</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The population clinical and microscopic features of the study group are similar to others described in the literature [2-10]. The medium age of patients at the time of diagnosis was 67 years which is similar to other studied groups (range 60-70 years). Comparable to other authors [1], squamous cell carcinoma was found in over 90% of cases. Primary tumor location was also similar to that reported in the literature, e.g. clitoral involvement was described in 27% by Hopkins et al. [8].

There are some differences between the study group and other series described in the literature. Fifty-two percent of patients had Stage III and IVA. This proportion in the literature is different and ranges from 18.3% to 51% [2, 4, 6-10]. In our study group of 104 patients the 5-year survival rate was 44.4%, which was lower than that described in the literature (50-80% rate) [6-7, 10-15]. This difference is probably due to the characteristics of our material, which consists of mostly advanced patients.

The clinical composition of the study group was unfavorable (52% of cases had Stage III and IVA) and only clinical TNM classification was applied; microscopic verification of inguinal lymph nodes was performed only in half of the cases. All these features had an important influence on survival which was lower than reported in the literature.

Treatment failure occurred in 58 (55.8%) of the patients studied. In 46.5% of the group it was only vulvar recurrence, in 37.9% recurrence was found in the vulva and groin lymph nodes and in 6.9% only in the groin lymph nodes. Distant metastases was the reason for failure in 6.9% of uncured patients. The literature review showed that loco-regional recurrence is the major reason for treatment failure in vulvar cancer patients, which was found in 60-80% of patients with treatment failure [16].

Stage of cancer

Clinical stage is considered to be a principal prognostic factor in patients with carcinoma of the vulva (according to AJCC, FIGO, TNM) [3, 4, 6-8, 15, 17]. In Stage I vulvar carcinoma the 5-year survival rate described in the literature ranges from 90% to 98%, in Stage II - from 60% to 91%; in Stage III - from 36% to 77% and in Stage IV - from 7% to 31% [6-8, 10, 12, 14, 18, 19]. Treatment results in our group were poorer, probably because of lack of full, surgical verification of inguinal node status in all patients. In the investigated group, the 5-year survival rate
was 61.4% of cases with Stage I by TNM, 54.9% of cases with Stage II, 40.1% of cases with Stage III and 13.3% with Stage IV. TNM stage was found to be an independent prognostic factor in Cox’s multivariate analysis.

**Microscopic status of the inguinal lymph nodes**

The microscopic status of inguinal lymph nodes is widely considered to be the major prognostic factor for vulvar carcinoma [6, 7, 12-15, 18, 20, 21]. In most studies regional lymph node metastases were positive in 21-42% of cases [14, 18, 22, 23]. The frequency of positive inguinal nodes depends on the stage of cancer, depth of invasion, tumor size, and location and histological differentiation [3, 5, 18, 22].

In the literature, successful treatment of patients with negative inguinal nodes varies between 70 and 100% and decreases to 20%-50% if positive lymph nodes are present [6, 13, 15, 18, 24]. Many authors highlight the prognostic meaning of the number of positive lymph nodes [6, 13, 22, 23, 25], while others suggest that survival of patients with vulvar cancer is associated with the size of positive groin nodes. Origoni et al. evaluated patients with positive lymph node size less than 5 mm and 5-year survival rate reached 90.9%. Inguinal lymph nodes larger than 15 mm were related with a 20.6% 5-year survival rate [13]. Our data do not contain information about the microscopic status of the inguinal lymph nodes of all patients, however, in our material clinical status of the groin nodes was an independent prognostic factor (Cox’s multivariate analysis). The 5-year survival rate was 55.4% in patients with clinically negative groin nodes (N0), 33.3% in patients with unilateral lymph node metastasis, and 20% in patients with bilateral lymph node metastasis.

**Tumor size**

Size of the primary lesion is one of the major criteria of vulvar carcinoma stage, however the impact of tumor size on prognosis remains unclear [3, 8, 12, 17, 21-23]. In the study of Rutledge et al. tumor size had a significant impact on survival in patients with a lesion diameter over 6 cm. Relative risk (RR) of death in this group was 2.4 times higher compared to those with a tumor size of 0-2 cm [12].

According to Rodolakis et al. 79.5% of patients with lesion size 2-3 cm survived five years, whereas this rate was only 52.6% in patients with lesion size over 3 cm [16]. Hopkins et al. suggest that size of the tumor is a prognostic factor for Stage III and IV but not in Stages I and II [8]. Other authors reported that tumor size is correlated with the hazard of local lymph node metastasis [3, 22]. In our study the statistical correlation between dimension of the primary lesion and the clinical and microscopic status of groin nodes was found but tumor size had no significant impact on survival.

**Surgical margin**

Most authors accept that size of the surgical margin is a prognostic factor for local recurrences and agree that an 8 mm margin is sufficient [4, 9, 10, 12, 15]. In a group of 135 patients, Heaps et al. discovered that none of 91 women with a negative surgical margin ≥ 8 mm had had a local vulvar recurrence and 21 (48%) of 44 women with a margin < 8 mm had had a local vulvar recurrence [6]. The influence of the surgical margin on overall survival is controversial [9, 10, 15]. In the study group positive surgical margins had no statistical influence on 5-year survival rate.

**Stage of cancer differentiation**

Some authors suggest that the stage of differentiation has a prognostic value in vulvar carcinoma patients [5, 6, 8, 14]. Moreover, the stage of differentiation influences the frequency of lymph node metastases [5]. Homesley et al. reported groin metastases in 26.8% of patients with G1 stage, 36.1% with G2, and 54.8% of patients with G3 stage [7].

According to Rosen and Malmstrom [10] cancer stage, tumor differentiation and age at time of diagnosis are prognostic factors in vulvar carcinoma; 5-year survival rate was 70% for well-differentiated tumors and 55% for moderately and poorly differentiated tumors. The prognostic value of tumor differentiation was reported by Malmstrom et al., however in the study by Hopkins et al. tumor differentiation of vulvar carcinoma was a prognostic factor in a group of patients only with Stages I and II but not for patients with Stages III and IV [8, 27]. Some authors emphasize the fact that multivariate analyses do not demonstrate the prognostic value of tumor differentiation stage [3, 10, 21], and many suggest that tumor differentiation has no influence on 5-year survival [4, 6, 7, 10]. In the study group cancer differentiation had a prognostic value for 5-year survival in univariate analysis but not in Cox’s multivariate analysis. Patients with well-differentiated cancer (G1) survived five years in 57.5%, with moderately differentiated (G2) in 34.9%, and with poorly differentiated (G3) in 9.1%.

**Age**

Most authors accept that age is an independent prognostic factor for vulvar carcinoma [5, 6, 8, 9, 10, 18]. Rosen and Malmstrom showed that there were significant differences in survival when comparing patients older than 69 years [10]. In a group of 588 patients, Homesley et al. discovered that local lymph node metastases was present in 25.2% of patients younger than 55 years, in 25.4% cases between the age of 55 and 64 years, in 36.4% in the group between 65 and 74 years, and in 46% of patients aged over 74 [7]. In the study group the age of patients at the time of diagnosis had a prognostic value for 5-year survival in Cox’s multivariate analysis. In the group of patients under 70 years, 5-year survival was achieved in 47.1% of cases and in those aged ≥ 70 in 18.8% only.

**Primary tumor location**

Most authors accept that location of the tumor on the clitoris is an unfavorable prognostic factor. In a group of patients with lesions located on the clitoris Boyce et al.
and Curry et al. reported groin nodes metastases in about 40% of patients [23, 27]. In the group of 225 patients Magrina et al. discovered that urethra invasion had a significant, unfavorable impact on survival in patients with vulvar carcinoma, and it decreased the 5-year survival rate from 84.9% to 42.9%. The local recurrence rate was 57.1% and 12.4% in patients with or without invasion of the urethra, respectively [9]. In the study group cancer location on the clitoris had no prognostic value.

Conclusions

Univariate analysis of our material showed that age, differentiation of cancer, clinical inguinal node status and TNM stage are associated with overall survival rate. Patients aged under 70, with well differentiated cancer, differentiation of cancer, clinical inguinal node status and cancer stage by TNM were independent risk factors for cancer-related death in our patients. Those results are similar to the literature data, however, treatment outcome and 5-year overall survival were less satisfactory.

References


Address reprint requests to:
P. BLECHARZ, M.D., Ph.D.
Center of Oncology,
M. Sklodowska-Curie Memorial Institute,
Cracow Branch,
Gynecologic Oncology Department,
31-115 Krakow, ul. Garsnarska 11 (Poland)
e-mail: pawel.blecharz@interia.pl
KIT protein expression in uterine sarcomas: an immunohistochemical study and review of the literature

M. Zafrakas¹, T.D. Theodoridis¹, L. Zepiridis¹, I.D. Venizelos¹, T. Agorastos¹, J. Bontis¹

¹1st Department of Obstetrics & Gynecology, Aristotle University of Thessaloniki
²Department of Pathology, “Hippokration” General Hospital, Thessaloniki (Greece)

Summary

Purpose: The aim of the present study was to investigate the possibility of treating uterine sarcomas with imatinib mesylate. Imatinib mesylate, a selective tyrosine kinase inhibitor, is very efficient against mesenchymal tumors of the gastrointestinal tract, known as GISTs. Imatinib mesylate acts against a tyrosine kinase encoded by the KIT gene in GISTs, and is more effective in tumors expressing this protein. Methods: Expression of KIT was analyzed immunohistochemically (n = 12) in formalin-fixed paraffin-embedded primary uterine sarcomas. Results: Using a semi-quantitative immunohistochemical score we found that KIT expression was very weak in the majority of tumors, while none of the uterine sarcomas tested showed strong expression. Overall, published studies addressing this issue in small series of uterine sarcomas yielded similar results. Conclusion: Current data suggest that it is unlikely that imatinib mesylate could be used effectively as a single agent in patients with uterine sarcomas.

Key words: Uterine sarcoma; KIT; Imatinib mesylate.

Introduction

Uterine sarcomas are rare malignant mesenchymal tumors of the female reproductive tract, comprising less than 1% of gynecologic malignancies [1]. Like most malignant mesenchymal tumors in other organ systems, uterine sarcomas have a very poor prognosis. According to published series, although they represent only 2-5% of the total, they account for more than 25% of deaths due to malignancies of the uterus corpus [1, 2]. This is largely due to the fact that uterine sarcomas are commonly diagnosed in advanced stages, making complete surgical resection virtually impossible in most of these cases [2, 3]. Moreover, the majority of uterine sarcomas respond poorly to chemotherapy and radiation, if at all [3-5]. Thus, development of new therapeutic strategies for the treatment of uterine sarcomas is needed.

A recent advance in cancer treatment has been the administration of novel targeted therapeutic agents in patients whose tumors have specific molecular characteristics, as determined by molecular analyses prior to initiation of therapy. One of the most promising among these novel agents is imatinib mesylate, which has shown very encouraging results when given to patients with mesenchymal tumors of the gastrointestinal tract - gastrointestinal stromal tumors (GISTs) [6-8]. Imatinib mesylate is a selective inhibitor of tyrosine kinases, acting in GISTs against a protein, which is expressed on the surface of tumor cells and has tyrosine kinase activity; this protein, also known as CD117, is encoded by the KIT gene [9]. To evaluate the possibility of applying imatinib mesylate in uterine sarcomas, we have analyzed immunohistochemically the expression of KIT in a series of these tumors.

Materials and Methods

 Archived tumor specimens from 12 patients with uterine sarcomas were obtained from the Pathology Department of “Hippokration” General Hospital in Thessaloniki Greece. All tumor specimens were fixed in formalin and embedded in paraffin. All patients were surgically treated at the 1st Department of Obstetrics and Gynecology. Patient age ranged between 40 and 69 years. Hematoxylin and eosin stained slides were reviewed to confirm histological diagnoses. Tumors were classified according to the World Health Organization (WHO) classification (2003) for uterine sarcomas. The tumors included four leiomyosarcomas, four mixed mullerian mesenchymal tumors (MMMT), two low-grade endometrial stromal sarcomas (LGESS), and two high-grade endometrial stromal sarcomas (HGESS).

Representative tissue blocks were selected for immunohistochemistry. Immunoperoxidase staining for KIT (CD117) was performed in 4.0-μm-thick tissue sections from all tumors. The BioGenex Automatic Staining System (BioGenex, San Ramon, CA) was used. In brief, tissue sections were deparaffinized, rehydrated, and soaked in 0.6% hydrogen peroxide for 30 min in order to block endogenous peroxidase activity. Microwave antigen retrieval in citrate buffer with pH 6.0 (BioGenex, San Ramon, CA) for 25 min followed. Tissue sections were incubated with the polyclonal rabbit anti-KIT antibody A4502 (Dako, Glostrup, Denmark) at a dilution of 1:250 for 30 min. Incubation with a peroxidase-streptavidin conjugate (BioGenex, San Ramon, CA) for 20 min followed. Diaminobenzidine tetrahydrochloride was then used as a chromogen and sections were counterstained with hematoxylin, dehydrated and mounted. Tissue sections from a gastrointestinal stromal tumor (GIST) with strong membranous and cytoplasmic staining for KIT were used as a positive control.

For evaluation of immunohistochemical data a semi-quantitative scoring system was used, as described previously [10]. In brief, staining intensity was characterized using the following scale: 0 = negative, 1+ = low, 2+ = middle and 3+ = strong.

The percentage of stained cells varied between: 0 = negative, 1 = < 10%, 2 = 10-50%, 3 = 51-80% and 4 = > 80% positive cells. According to the scores, tissues were classified as having low (0 to 2 points), middle (3 to 6 points) or strong (8 to 12 points) KIT expression.

Revised manuscript accepted for publication June 28, 2007
**Results**

Immunostaining results for KIT expression are summarized in Table 1 according to sarcoma histological type. KIT expression was detected in all four leiomyosarcomas (two 2+/10-50%, one 1+/10-50%, and one 1+/<10%), and both LGESS (both 1+/10-50%) tested. Three of the four MMMTs were KIT-negative and one was positive (1+/10-50%). One of the two HGESSs tested was KIT-negative and one was positive (1+/<10%). Altogether, the majority of uterine sarcomas showed weak to moderate intensity (1+ or 2+ in 8 out of 12 tumors) and focal (< 10%) to moderate (10-50%) staining distribution (8 out of 12 tumors) of KIT. Four of the 12 tumors were entirely KIT-negative. Neither strong intensity (3+) nor extensive intensity distribution was observed in any of the tumors tested. Cytoplasmic staining was seen in all positive sarcomas, while staining of the cell membrane was also seen in most, but not all, positive tumors. Figure 1 shows a representative section of sarcoma cells positive for KIT.

Results of the semi-quantitative immunohistochemical scores are presented in Table 2. Ten out of 12 tumors had a low immunohistological staining score (0-2), and two had a moderate score, while none of the tumors showed strong KIT expression.

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>Staining intensity</th>
<th>Tissue staining distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1+</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>LGESS ‡</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HGESS §</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

MMMT: mixed mullerian mesenchymal tumor, LGESS: low-grade endometrial stromal sarcomas, HGESS: high-grade endometrial stromal sarcomas.

Table 2. — Immunohistochemical scores of KIT expression in uterine sarcomas.

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>Low (0-2 points)</th>
<th>Moderate (3-6 points)</th>
<th>Strong (8-12 points)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leiomyosarcoma</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MMMT †</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>LGESS ‡</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>HGESS §</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

MMMT: mixed mullerian mesenchymal tumor, LGESS: low-grade endometrial stromal sarcomas, HGESS: high-grade endometrial stromal sarcomas. 

**Discussion**

Imatinib mesylate (Glivec, Novartis International AG, Basel, Switzerland) is a selective inhibitor of the enzymatic activity of several tyrosine kinases. Its main advantages include oral administration and favorable safety profile, with minimal side-effects under standard doses [7-9, 11]. Imatinib mesylate was first used in chronic myeloid leukemia, in which a new gene is created by chromosomal translocation, leading to fusion of two genes (bcr and abl); the new gene encodes a protein kinase, whose spontaneous activity is responsible for leukemia [9]. In the case of GISTs, a mutation of the KIT gene leads to production of an activated protein kinase and subsequent uncontrolled cell growth and proliferation [9,12]. Imatinib mesylate is active against KIT-positive tumors, and has not shown any activity in KIT-negative GISTs [9]. Since 2001, more than 2,000 patients with GISTs have been included in therapeutic trials with imatinib mesylate, with a clinical benefit of 80-90% in patients whose chance of survival had been less than 30% at one year [8, 9].

Based on the fact that both GISTs and uterine sarcomas are mesenchyme-derived tumors, the application of imatinib mesylate in uterine sarcomas seems to be a reasonable treatment option. The expression of KIT in uterine sarcomas has been previously analyzed [13-22], but due to the rarity of this pathological entity the total number of tumors studied so far is limited, not allowing definitive conclusions to be drawn. Thus, we analyzed immunohistochemically the expression of KIT in a panel of archival formalin-fixed paraffin-embedded tissue specimens of this rare entity. For this purpose we used an anti-KIT antibody, which was found to be the most sensitive in a recent study comparing seven different antibodies with the use of tissue microarrays [23]. The majority of the tumors we tested had a low immunohistological staining score (10 out of 12, with 4 entirely negative), only two had a moderate staining score, while none showed strong KIT expression.

Our results are in line with those of previous studies, most of which found only rare expression of KIT in uterine sarcomas by immunohistochemistry [13-22]. Winter et al. [13] found KIT immunopositivity in nine of 21 MMMTs, and one of 17 leiomyosarcomas. Likewise, Klein and Kurman [14] found KIT expression in one out of 24 and Nakayama et al. in four out of 26 uterine sarcomas [15]. In three studies KIT expression was analyzed only in MMMTs. Sawada et al. [16] found KIT overexpression in the mesenchymal component in six out of 16 cases, Menczer et al. [17] did not find any KIT-stained sarcoma cells (n = 20), and Raspolliini et al. [18] found KIT expression only in four of 24 uterine MMMTs. In two other studies, KIT expression was analyzed only in leiomyosarcomas. Raspolliini et al. [19] found immunopositivity in 17 out of 32 cases, while Serrano et
al. [19] did not find any positive tumors (18 cases). In contrast to the above studies [13-20], Rushing et al. [21] and Leath et al. [22] found KIT to be positive in all uterine sarcomas tested (25 cases and 11 cases, respectively).

The differences among various studies in KIT expression, as determined by immunohistochemistry, are most likely due to different antibodies used, differences in staining methods, and different patient populations [20]. A rough overall estimate from the above studies, with the total number of uterine sarcomas tested hardly exceeding 200 cases, is that KIT is expressed was no more than 36%. However, this could well be an overestimate, since as previously shown [20] mast cells infiltrating uterine sarcomas stain strongly for KIT and possibly lead to false-positive results. Such non-specific staining was ruled out in the present study by careful histological evaluation. Thus, it seems unlikely that patients with uterine sarcomas could benefit from imatinib mesylate treatment. Furthermore, molecular analyses suggest that even KIT-positive uterine sarcomas would probably not respond to imatinib mesylate: tumors that respond frequently to imatinib mesylate have mutation(s) in exon 11, and KIT needs to be phosphorylated in order to start its signaling cascade, but neither mutations [19-21], nor KIT phosphorylation [21] were found in uterine sarcomas.

Conclusion

Our data together with those from previous studies, as presented above, suggest that it is unlikely that patients with uterine sarcomas might respond to imatinib mesylate. However, treatment of uterine sarcomas with imatinib mesylate might be feasible in a small subgroup of patients with KIT-positive tumors, possibly in combination with other therapeutic modalities.

References


Address reprint requests to: M. ZAFRAKAS, M.D. 1st Department of Obstetrics & Gynecology Aristotle University of Thessaloniki Papageorgiou General Hospital Periferiaki Odos Thessalonikis N. Efkarpia 56403 Thessaloniki (Greece) e-mail: mzafrafas@gmail.com
Cl-channel blockers inhibit cell proliferation and arrest the cell cycle of human ovarian cancer cells

M. Li, B. Wang, W. Lin
Department of Obstetrics and Gynecology, Qilu Hospital, Shandong University, Jinan (China)

Summary

Objective: To investigate the role of chloride channels in cell proliferation and cell cycles of human ovarian cancer cell line A2780. Methods: Chloride channel blockers were used to observe the effects of chloride channels on A2780 cells with MTT assay and flow cytometry. Results: NPPB (100 μM) significantly inhibited the cell proliferation and affected the cell cycle, which increased the percentage of cells in the G1 phase, and reduced it in the S phase. NFA (100 μM) and TAM (30 μM) had similar inhibitory effects. Glibenclamide (100 μM), however, had no effect on cell proliferation or cycle. Moreover, chloride channel blockers could inhibit Ca2+ influx in these cells. Conclusion: Chloride channels, voltage-gated chloride channels, and volume-sensitive chloride channels especially, play an important role in the cell proliferation and cycle of A2780 cells. It is likely that the influence of chloride channels on cell proliferation and cell cycle is mediated by a Ca2+-dependent mechanism.

Key words: Chloride channels; Cell proliferation; Cell cycle; Ovarian cancer.

Introduction

Chloride channels are ubiquitous transmembrane proteins which have been implicated in salt and fluid movements across epithelia, cell differentiation and migration, cell volume regulation and intracellular organelle acidification [1-4]. According to their gating mechanisms, there are, from the functional point of view, five classes of chloride channels, including voltage-gated chloride channels (CLCs), cystic fibrosis transmembrane conductance regulator (CFTR), volume-regulated anion channels (VRACs) (also named volume/swelling-sensitive/-activated chloride channels), calcium-activated chloride channels (CLCAs), and glycine or γ-aminobutyric acid (GABA) activated channels which mainly form synaptic channels. Cl-channels are also the main targets for many drugs that change cellular function to produce beneficial effects or to cause toxicity. Cl-channel blockers inhibit cell proliferation in many types of cells, including pulmonary artery endothelial cells and liver cells [5,6]. Diverse types of Cl-channels have also been documented in tumor cells. Accumulating evidence supports the essential role of plasma membrane chloride channels in tumor cell proliferation control [7-10]. Tumor cells of unlimited proliferation are usually accompanied by an abnormal cell cycle. Lots of evidence has indicated that chloride channel activity plays an important role in cell cycle progression. For example, volume-activated Cl-currents displayed cell cycle-dependent expression in nasopharyngeal carcinoma cells which was high in the G1 phase, downregulated in the S phase, but increased again in the M phase [8]. Pharmacological blockage of VRAC causes proliferating cervical cancer cells to arrest in the G0/G1 phase, demonstrating that activity of this channel is critical for G1/S checkpoint progression [11]. These observations suggest an important role for chloride channels in cell proliferation and cell cycles.

Ovarian cancer, one of the most gynecological malignant epithelial tumors, is characterized by its high potential for unlimited proliferation and metastasis. There is a need to explore new therapeutic targets to have a better understanding of the mechanisms involved in the unlimited proliferation of ovarian carcinoma. The factors correlated with the proliferation of ovarian cancer, however, are poorly understood. Up to date, there are no data available as to whether Cl-channels are related to the cell proliferation and cell cycle progression of ovarian cancer. In this study, we investigated the representative Cl-channel expressions and the role of Cl-channels in cell proliferation and cell cycles of human ovarian cancer cell line A2780.

Materials and Methods

Reagents. 5-nitro-2-((3-phenylpropylamino)-benzoate (NPPB), niflumic acid (NFA), glibenclamide, tamoxifen (TAM), propidium iodide (PI), and MTT were purchased from Sigma, MO, USA. Trizol reagent, dNTP and M-MLV reverse transcriptase were obtained from Takara, Japan. Blockers were prepared in stocks (100- to 1000-fold concentrated) in DMSO. The final concentration of DMSO in electrophysiological studies, cell proliferation and cell cycle experiments never exceeded 0.2%. We have tested that this concentration has no effect on current measurements, cell proliferation and cell cycle. Blockers were diluted to the desired final concentrations using corresponding solutions for different experiments. Fura2/acetoxymethylester (Fura2/AM) was obtained from Molecular Probes, Inc., OR, USA.

Cell culture. Ovarian cancer cell line A2780 was obtained from Basic Medicine Research Institute, Qilu hospital, Shandong University, China. Cells were cultured in 90% RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS),...
100U/ml penicillin, 100 μg/ml streptomycin and maintained at 37°C in a humid atmosphere of 5% CO₂ in air.

RT-PCR. Total RNA was isolated from the cultured A2780 cell line using the Trizol reagent according to the manufacturer's procedure. mRNA was transcribed into first strand cDNA using oligo-dT primers and M-MLV reverse transcriptase. The cDNA was used for subsequent PCR using primers specific for human CLC-3, CFTR and hCLCA-2. The sequences used were: CLC-3 primer, sense, 5’-GGCAGCATTACAGTTCTACAC-3’; antisense, 5’-TTTCCAGGCCACAGGCATAG-3’. CFTR primer, sense, 5’-GGCGGAGGGAAGCGGTGTA-3’; antisense, 5’-ATCTCTCAGTGTCCAGCCCATAC-3’; hCLCA-2 primer, sense, 5’-ACTATGCCAGGAAAGCCATT-3’; antisense, 5’-CAGGGAACCTCAAGCAGTGG-3’. β-actin primer, sense, 5’-AATCCATCATGAAGTGTG-3’; antisense, 5’-ACTCTGGTGCTGATCCAC-3’. Control reactions without reverse transcriptase were performed for each PCR amplification experiment.

Electrophysiology and solutions. The whole-cell mode of the patch-clamp technique was used to measure membrane potentials and membrane currents. Currents were measured with an EPC-9 patch clamp amplifier (HEKA Electronics, Lambrecht, Germany). Patch electrodes had a resistance of between 3 to 5 MΩ. The step protocol consisted of a 1s voltage step, applied every 15s from a holding potential of -40 mV to test from -100 to +100 mV with an increment of 20 mV. All experiments were performed at room temperature (22–26°C). The standard extracellular medium was a Krebs solution. VRACs were activated in A2780 cells by superfusing the cells with the same solution without mannitol, resulting in a 25% hypotonicity (240 ± 5 mOsm). To measure VRAC activity, the pipette solution contained (mM): CsCl 40, caesium aspartate 100, MgCl₂, 1, CaCl₂ 0.81, BAPTA 5, NaATP 4 and Hapes 10. In this pipette solution, the free Ca²⁺ concentration was 25 nM. The pipette solution was adjusted to pH 7.2 with CsOH.

Proliferation assay. MTT assay was used to analyze the effect of different Cl-channel inhibitors on A2780 cell proliferation. One hundred microliters of cell suspension (1×10⁴ cells/ml) was distributed into each well of flat-bottomed 96-well culture plates. After the 24 h incubations, 100 μl reagent solutions or media at the desired blocker concentrations were distributed into each well. The well containing only media served as a positive control. Two hundred microliters of the medium alone without cells and reagent were used as a negative control. The culture plate was incubated for 48 h. Thereafter, 20 μl of the MTT dye (5 mg/ml) was added into each well. Four hours later, 150 μl of DMSO was added to each well after discarding media. The absorbance (A) values of each well at 540 nm were read.

Statistics. Data are presented as the mean ± standard error. The Student’s t-test (SPSS version 12.0; Chicago, IL, USA) was used for statistical analyses and differences were considered significant at p < 0.05. All experiments were performed at least three times with representative data presented.

Results

RT-PCR assay

To determine whether Cl-channels are expressed in the A2780 cell line, RT-PCR analysis was performed using primers specific for CLC-3, CFTR, hCLCA-2 and β-actin. Figure 1 shows that PCR amplified 235-bp CLC-3, ~300-bp CFTR and 247-bp β-actin from total RNA isolated from A2780 cells. Employing the same strategy to examine the expression of hCLCA-2, no product was yielded on A2780 cells. No product was detected in the absence of reverse transcriptase yet.

Hypotonic cell volume-activated chloride channels in ovarian cancer cells

Because the VRAC has not yet been identified at the molecular level, we used whole-cell voltage-clamp recordings to detect whether volume-activated Cl-cur-
Cl-channel blockers inhibit cell proliferation and arrest the cell cycle of human ovarian cancer cells

Membrane currents recorded during the step protocol applied to A2780 cells in isotonic solution were small and time independent. A hypotonic solution induced cell swelling which was accompanied by activation of large outwardly rectifying currents. The current-voltage relationship in hypotonic solution, obtained from the step protocols, reversed close to the theoretical equilibrium potential for Cl- (E_Cl = -25mV), indicating that the volume-regulated currents were carried mainly by Cl-. Results suggested that addition of NPPB (100 μM) inhibited the hypotonic volume-activated outward current along with the inward current of A2780 cells. Tamoxifen (30 μM) also inhibited the hypotonic volume-activated outward current and inward current of A2780 cells.

The effect of a variety of chloride channel blockers on cell proliferation and colony formation

Different channel inhibitors were used to examine the effects on A2780 cell proliferation. NPPB is a conventional non-selective chloride channel blocker. TAM is thought to exert its antiproliferative action by binding competitively to estrogen receptors (ERs) and thereby blocking the mitogenic effect of estradiol. However its effectiveness in the treatment of the estrogen-independent neoplasia indicates that TAM has other mechanisms underlying the antiproliferative action. In this study, human ovarian cancer A2780 cells did not express ERs [12], which was by using immunocytochemistry (data not shown). Tamoxifen has been demonstrated to be a specific high-affinity inhibitor of VRACs in several cell types, including endothelial cells [5]. TAM was found to specifically inhibit the volume-sensitive Cl-currents used as blockers of VRAC. Niflumic acid is considered to be able to inhibit the CLC family. CFTR is an ATP-binding cassette family, blocked by glibenclamide at 100 μM/L[13]. Results indicated that A2780 cell proliferation was inhibited by 100 μM NPPB, 30 μM TAM and 100 μM NFA. In contrast, 100 μM glibenclamide had no effect on cell proliferation (Table 1). These data showed an important role of CLC and VRAC, but not CFTR, in the proliferation of A2780 cells.

Table 1. — Effect of chloride channel blockers on absorbance values in cultured A2780 ovarian cancer cell lines.

| Control | 100 ± 4 |
| Glibenclamide (100 μM) | 96 ± 6 |
| NPPB (100 μM) | 9.1 ± 0.6 |
| Tamoxifen (30 μM) | 23.4 ± 2.1 |
| NFA (100 μM) | 31.3 ± 3.4 |

* Data are presented as means ± SE in percentage (n = 8-20). Control with no inhibitor treatment is normalized to 100%.

In addition, 100 μM NPPB strongly reduced the number of colonies. A moderate decrease was also observed on treatment with NFA and TAM. The colony formation rate was 43.7 ± 1.2% in the control group (no additives), 10.6 ± 3.1%, 17.0 ± 2.3%, 21.2 ± 3.5% in the 100 μM NPPB, 30 μM TAM, and 100 μM NFA group, respectively. Compared to the control group, changes of colony formation rate at various groups were significant (n = 6, p < 0.05). Glibenclamide, however, had no effect on the number of the colonies compared to the control group. The colony formation rate was 41.5 ± 1.7% in the glibenclamide group (n = 6, p > 0.05).

The effect of chloride channel blockers on cell cycle

Results showed that NPPB at 100 μM significantly blocked the progression of the cell cycle with a 72-h treatment of A2780 cells. In NPPB-treatment cells, the proportion of G1 cells increased significantly to 84.19 ± 2.32% (n = 8, p < 0.05), while it decreased significantly to 12.14 ± 2.33% (n = 8, p < 0.05) in the S phase. NFA at 100 μM and TAM at 30 μM had a similar effect on the progression of the cell cycle to NPPB. On the other hand, no effect of 100 μM glibenclamide on cell cycles was observed (Table 2, Figure 2).
Table 2. — Distribution of cell cycle phases of A2780 cells (%).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>55.41 ± 2.08</td>
<td>34.03 ± 2.45</td>
<td>9.05 ± 0.91</td>
</tr>
<tr>
<td>Glib</td>
<td>53.09 ± 3.21</td>
<td>36.89 ± 1.57</td>
<td>9.98 ± 0.86</td>
</tr>
<tr>
<td>NPPB</td>
<td>84.19 ± 2.32*</td>
<td>12.14 ± 2.33*</td>
<td>3.20 ± 0.67*</td>
</tr>
<tr>
<td>TAM</td>
<td>79.37 ± 3.05*</td>
<td>15.26 ± 1.61*</td>
<td>5.44 ± 0.97*</td>
</tr>
<tr>
<td>NFA</td>
<td>74.55 ± 2.09*</td>
<td>18.72 ± 1.93*</td>
<td>7.93 ± 0.89</td>
</tr>
</tbody>
</table>

A2780 cells were incubated in media without (control) or with chloride channel blockers, NPPB (100 μM), NFA (100 μM), TAM (30 μM) and Glibenclamide (100 μM). Flow cytometric analysis was carried out 72 h after treatments of A2780. Data are shown as means ± SE. n = 8,*p < 0.05 vs control.

The effect of chloride channel inhibitors on [Ca²⁺]i

To further explore the mechanism of Cl-channel involvement in A2780 cell proliferation, we examined the effect of Cl-channel inhibitors on Ca²⁺ influx. Ca²⁺ influx was evoked by adding 0.5 mM CaCl₂ to the bathing medium. The addition of extracellular Ca²⁺ caused a rapid rise in cytosolic Ca²⁺ and the cytosolic Ca²⁺ level remained elevated for several minutes. Results displayed that 100 μM NPPB almost completely abolished the increase of [Ca²⁺]i evoked by the application of external Ca²⁺. NPPB reduced the peak Ca²⁺ response by 39 ± 2.1% (n = 5, p < 0.05) while 100 μM NFA decreased the Ca²⁺ response by 28 ± 2.7% (n = 6, p < 0.05), and 30 μM TAM by 35 ± 1.7% (n = 6, p < 0.05).

Discussion

In this study we determined, for the first time, the presence of Cl-channel genes in ovarian cancer A2780 cells by using RT-PCR. Our data showed that A2780 cells expressed CLC-3 and CFTR genes. Furthermore we used whole-cell voltage-clamp recordings to find out if volume-activated Cl-currents exist in A2780 cells. Quite surprisingly, we had no way to detect the expression of hCLCA-2 genes using RT-PCR, which shows that the distributions of Cl-channels possess tissue-specific expression patterns.

Chloride channels have been implicated in the proliferation of normal as well as tumor cells. Chloride channels can control mouse cell proliferation and human mast cells [6, 14]. Volume-activated Cl-currents play an important role in human nasopharyngeal carcinoma cell proliferation [10]. In our study we observed the effect of diverse Cl-channel blockers on the proliferation of the human ovarian cancer cell line A2780. Our data showed NPPB, a non-selective inhibitor for chloride channels, NFA, a voltage-gated chloride channel inhibitor, and TAM, a volume-activated chloride channel blocker, obviously inhibited cell proliferation, whereas glibenclamide, a CFTR chloride channel blocker, had no effect on A2780 cell proliferation, indicating that CLC and VRAC were critical for A2780 cell proliferation, whereas CFTR channels might not be important. In addition to the inhibition of cell proliferation, Cl-channel activity has been tightly linked to the cell cycle. CLC-2 is regulated by cdc2/cyclinB activity, suggesting a mechanism for M phase activation [15]. The Cl-channel blocker NPPB inhibits progression through the cell cycle in synchronized NIH3T3 cells, further supporting a role in the cell cycle [16]. Our data indicated that glibenclamide had no effect on the A2780 cell cycle. NPPB, NFA and TAM, in contrast, could arrest the progression of the A2780 cell cycle. Distribution of the G1 phase obviously went up, and distribution of the S phase, surprisingly declined, thus demonstrating that Cl-channels modulated the cell cycle of A2780 cells. Taken together with the present study, Cl-channel blockers inhibited the proliferation and arrested the cell cycle of A2780 cells. This also suggests that Cl-channel blockers are a useful tool for studying the physiologic role of these channels.

How Cl-channels are involved in control or regulation of cell proliferation and cell cycle progression is, at present, not clearly understood. Cell proliferation can also be affected by intracellular pH [17]. Chloride channels can influence the intracellular pH or the pH in various organelles. CLC-3 is a representative member of the CLC family, and because it might be associated with VRAC, it attracts further interest in terms of testing whether it is involved in cell proliferation. A previous study revealed that CLC-3 chloride channels contribute to regulate intracellular pH [18]. Thus it has been postulated that CLC may affect cell proliferation by changing cellular pH. Furthermore, efficient concentrations of cyclin/cyclin dependent kinase (CDK) and other key factors used to control cell cycle progression need to be maintained by the volume-activated chloride current [7]. The volume-activated chloride current may facilitate cells to pass through the restriction point in the G1 phase. Having passed through the restriction point, cells can enter into the next cell cycle by virtue of helping cyclin/cyclin dependent kinase (CDK) even if the stimulation of growth factors is deficient. In addition, Ca²⁺ is an intracellular second messenger and correlated with a restriction point which controls progression from the G1 to the S phase [19]. The intracellular Ca²⁺ concentration which is involved in cell proliferation and cycle progression is controlled by Ca²⁺ entry pathways in the plasma membrane. Ca²⁺ affects cell proliferation by pathways of Ca²⁺-dependent signal transduction. Blocking the Ca²⁺ signal transduction pathway can arrest the G1 phase resulting in cessation of cell progression [20, 21]. We found that NPPB, TAM and NFA abrogate the increase of [Ca²⁺]i evoked by external Ca²⁺, indicating that chloride channel activity may regulate Ca²⁺ influx into ovarian cancer cells, and subsequently modulate the proliferation of these cells. It, however, is unknown how the chloride channels interact with the Ca²⁺ channels.

In summary, the present findings show that ovarian cancer cell line A2780 expresses CLC, VRAC and CFTR chloride channels. Chloride channels, voltage-gated chloride channels and volume-sensitive chloride channels especially, play an important role in cell proliferation and the cycle of ovarian cancer cells. It is likely that the Cl-channel activity may modulate cell volume and regulate Ca²⁺ influx into A2780 cells, therefore affecting the pro-
Cl-channel blockers inhibit cell proliferation and arrest the cell cycle of human ovarian cancer cells

References


Address reprint requests to:
B. WANG, M.D.
Department of Obstetrics and Gynecology, Qilu Hospital, Shandong University, WenHua West Road 107#, Jinan, 250012 Shandong (China)
e-mail: wangboshandong@yahoo.com.cn
Cervical cancer associated with genital prolapse - 
a brief review of the literature and long-term results 
of successful treatment with radiochemotherapy and surgery 
in a very frail patient

D. Reimer¹, A. Sztankay², I. Steppan¹, E. Abfalterm¹, H. Lunzer¹, C. Marth¹, A.G. Zeimet¹

¹Department of Obstetrics and Gynecology, ²Department of Radiotherapy, 
Innsbruck Medical University (Austria)

Introduction

About one-third of the adult female population suffers 
from prolapse of the pelvic organs associated with 
urinary and fecal incontinence [1]. At 14/100,000 cases 
the incidence of carcinoma of the uterine cervix has 
remained unchanged in Europe over recent years. 

The combination of both disorders is very rare and is 
most frequently observed in multiparous and elderly 
women. Since 1950, 55 cases have been published world-
wide. The few cases reported during the last two decades 
were observed mainly in underdeveloped countries [2-9]. 
Although the prolapsed uterine cervix is exposed to con-
stant irritation and ulceration, malignant changes remain 
a rare complication. This remarkably low risk may be 
explained by the displacement of the cervix from its 
natural environment, made harmful by exudates or viral 
infection [2]. Nonetheless, the continual regeneration of 
repetitive ulcerous injuries of the epithelium is regarded 
as the main causative background of malignant transfor-
mation. In general, these cancers are characterized by 
slow growth due to low-grade malignancy and often 
advanced age of patients. The majority of reported 
cancers were staged FIGO IIA disease or less and virtu-
ally all tumors were histologically defined as keratinizing 
squamous cell carcinoma. Considering all published 
cases, development of cervical cancer seems to be a late 
complication of procidentia and strongly related to its 
untreated duration. In 75% of cases, procidentia had been 
present for ten years or more. One case was published in 
which a huge carcinoma of the cervix led to sudden 
icarcerated procidentia [9]. Schraub et al. pointed out a 
correlation between the development of cervical cancer 
and the treatment of uterovaginal prolapse by pessary 
insertion. In their series, pessary-associated cancers were 
again found to be a late adverse event as the mean inter-
val between first pessary use and cancer diagnosis was 18 
years. As almost all tumors occurred at the site of pessary 
insertion, the authors concluded that foreign body-related 
chronic inflammations associated with viral infection 
might be the main etiologic factors [9]. 

The curative treatment of cervical cancer is based on 
radical surgery and radiotherapy alone or in combination 
with chemotherapy. Both therapeutic principles are 
equivalent in early tumor Stages (Ib-IIa) [10], whereas in 
locally advanced Stages (IIb-Iva) radiochemotherapy is 
the preferred treatment [11]. By contrast, management of 
uterovaginal prolapse associated with a carcinoma of the 
cervix is not standardized and therapy strategies vary 
considerably between published cases. When operable, 
most cases were treated either by radical vaginal hyster-
tomy (Schauta-Amreich) with extraperitoneal lymphadenectomy as proposed by Mitra [12] or without lymphadenectomy but complemented with adjuvant radiotherapy [13]. To date, the addition of laparoscopic lymphadenectomy to the radical vaginal approach seems to be the treatment of choice, but to our knowledge this strategy remains unreported in this patient population.

Summary

Background: A case of cervical cancer associated with irreducible procidentia successfully treated with external beam radiation and extracorporeal HDR-AL with concomitant chemotherapy followed by obliterate vaginal surgery is reported for the first time. 

Case: A 73-year-old woman presented in frail condition suffering from a huge, irreducible uterovaginal procidentia combined with a squamous cell carcinoma of the cervix in FIGO Stage IIA. Successful treatment consisted of sequential application of combined radiotherapy with concurrent cisplatin chemotherapy followed by total vaginal hysterectomy and partial colpectomy with colpocleisis according to the Labhardt method. The five-year follow-up documents the excellent long-term results with regard to cervical cancer and pelvic floor stability. Conclusion: Especially in patients ineligible for extended surgery, radiochemotherapy followed by an obliterate surgical approach is feasible without aberrant wound healing and constitutes a suitable and efficient option for treating carcinomas of the cervix associated with irreducible genital prolapse.

Key words: Cervical cancer; Procidentia; Squamous cell carcinoma.
We report the long-term results of a case involving a 73-year-old very frail patient suffering from irreducible uterogenital prolapse combined with FIGO Stage IIa squamous carcinoma of the cervix, who was successfully treated with combined radiotherapy and concomitant chemotherapy followed by oblitterative vaginal surgery.

**Case Report**

A 73-year-old cachectic patient with ten births in her anamnesis presented at our oncological outpatient unit with severe vaginal bleeding from a uterovaginal prolapse persisting for more than ten years. The patient complained of pain, lack of appetite, increasing malodor and anuresis. Gynecological examination revealed that the prolapse was irreducible and that the entire surface of the cervix was a ragged exophytic tumor showing verrucous hyperkeratotic features with punctual bleeding (Figure 1A). The tumor extended to the proximal vagina and had a horizontal and vertical diameter of 14.3 and 12.5 cm, respectively. Rectovaginal examination gave no evidence of parametrial or rectal tumor involvement. According to FIGO classification, the disease was clinically Stage IIa. Performed biopsies resulted in the histological diagnosis of a well-differentiated, keratinizing squamous cell carcinoma. No pathologically enlarged mediastinal or retroperitoneal lymph nodes or signs of distant metastatic disease were detected by computed tomography (CT) scan. However, bilateral hydrenephrosis was diagnosed. Pelvic magnetic resonance (MR) imaging showed a large prolapse comprising the entire uterus, the caudal parts of the urinary bladder, the parametria as well as extra- and parieto-peritoneal parts of the peritoneal cavity (Figure 2A). Serum creatinine was elevated to 1.7 mg/dl, and SCC was 3.1 ng/ml.

Because of inoperability due to the patient’s frail general condition on the one hand and the tumor extension on the other, the decision was made to administer neoadjuvant radiochemotherapy: pelvic external irradiation at a total dose of 52.2 Gy, subdivided into 29 fractions of 1.8 Gy each, was administered with concomitant weekly cisplatin therapy at a dose of 40 mg/m². On completion of external irradiation the tumor mass showed partial regression (Figure 1B). External radiotherapy was complemented with three sessions of extrarectal high-dose-rate intracavitary brachytherapy (HDR-AL) of 7.5 Gy per session at point A. To minimize irradiation-induced injury of adjoining pelvic organs because of the altered anatomical situation caused by the still irreducible procidentia, the patient’s radiation portal was adjusted by MRT-based planning. Despite meticulous computerized planning, hemorrhagic cystitis could not be avoided after an administered dose of 36 Gy. After radiotherapy had been discontinued for three weeks, all symptoms of radio-cystitis improved and finally resolved. Recurrent grade 1 anemia was treated with recombinant erythropoietin. One episode of grade 2 neutropenia that occurred towards the end of radiochemotherapy was promptly treated with filgrastim to avoid further protraction of treatment.

On completion of radiochemotherapy no macroscopic tumor residues were visible and the prolapse was reducible (Figure 1C). As the patient was widowed and no longer sexually active,
a simple vaginal hysterectomy was performed with a two-third colpectomy and a colpoceleisis according to the Labhardt method (Figure 1D). Histologic examination revealed minor residues of the squamous cell carcinoma with no lymphatic space involvement. All margins of the specimen were found to be clear.

The patient has been followed-up in accordance with our aftercare program over the past five years. On her last visit, the patient was in very good physical condition with excellent quality of life; her considerable weight gain of 14 kg, reached 18 months after treatment was completed, remained unchanged. Neither CT scan nor gynecologic examination revealed evidence of recurring cervical cancer or any signs of weakness of the pelvic floor. Serum SCC was 0.1 ng/ml.

Discussion

Although treatment concepts are well-established for the various stages of cervical cancer [10, 11] as well as for assessment of uterovaginal prolapse and related pelvic floor disorders, the best standard of care remains unclear when both conditions coincide. The completely changed anatomical situation in the case of a massive, irreducible uterovaginal prolapse and the frequently associated severe comorbidity, especially in frail geriatric patients, often make radical surgery impossible.

We have reported the case of an elderly and very frail patient suffering from a huge, irreducible uterovaginal prolapse associated with cervical cancer. Despite the existing bilateral hydronephrosis at diagnosis, we classified the tumor as FIGO Stage IIA and not Stage IIB, because we were convinced that the hydronephrosis was not related to tumor spread but rather due to third-degree procidentia as recently reported [13, 14]. Even though the tumor was operable, the patient’s severely compromised general condition, the recurring hemorrhage and the onset of renal failure prompted us to adapt the available therapy modalities to meet the requirements of the special situation. We therefore decided to concomitantly administer radiotherapy of the lower pelvis and chemotherapy as a first measure with the intention to significantly down-stage the tumor and make the procidentia reducible.

In the literature, successful treatment with external beam therapy was reported only by Kriplani et al. in an incarcerated procidentia secondary to a huge cervical cancer. However, in that case, prior to therapy, the prolapse was successfully reduced manually under deep sedation and remained in situ during radiotherapy [9]. In contrast, the uterovaginal prolapse in our patient was irreducible due to the bulky tumor. On completion of radiochemotherapy successful down-staging was achieved, the prolapse became reducible and no residual tumor was visible.

In all reported cases so far, reduction of genital prolapse prior to radiation therapy was clearly recommended and the authors pointed out an increased risk of visceral injury and vesico- or rectovaginal fistula formation from ionizing radiation in the case of persisting uterine prolapse [6, 7]. Accordingly, in our case, cautious MR-based planning of radiotherapy with patient-specific adjustment of the radiation fields for external beam treatment and extracorporeal HDR-AL was not able to prevent an episode of severe hemorrhagic cystitis and a subsequent treatment delay of three weeks. We wonder if hyperfractionized scheduling with smaller single doses per session would have been able to prevent this complication without compromising oncologic outcome. On the other hand, no late adverse events have been recorded during the five-year follow-up. Taken together, the radiochemotherapy-induced early side-effects were manageable and ultimately acceptable in this special case.

In our frail and sexually inactive patient we opted for an uncomplicated surgical procedure consisting of a simple vaginal hysterectomy and a colpoceleisis according to the Labhardt method to enable safe reconstruction of the pelvic floor. It is worth noting that the patient had an unremarkable postoperative recovery without aberrant wound healing as a potential consequence of previous radiotherapy.

Although a radical vaginal surgical approach with either laparoscopic or extraperitoneal lymphadenectomy should be the treatment of choice for operable cervical cancer associated with procidentia, we conclude from our own experience that in elderly and frail patients considered ineligible for radical surgery, an up-front radiochemotherapeutic approach is a valuable treatment option, even in the case of unreduced genital prolapse. This may also be true for the neoadjuvant approach in primary inoperable locally advanced cervical cancers associated with procidentia.

Initial surgery of huge tumor masses in combination with an altered anatomical situation is often associated with excessive hemorrhage and involves great effort. Preceding radiochemotherapy could ease subsequent surgery and prevent unforeseen complications by reducing tumor mass. Moreover, prior administered external irradiation including the pelvic lymph nodes reduces the extent of required surgery and the risk of postoperative complications.

Although literature is available on the best quality therapy for cervical cancer, the question of a standard therapy concept for uterovaginal prolapse associated with cervical cancer remains unsolved. Reports on the treatment of several cases involving this problem and critical appraisal thereof will be helpful in choosing the best therapeutic strategy.

References

Cervical cancer associated with genital prolapse - a brief review of the literature and long-term results of successful treatment etc


Address reprint requests to:
A.G. ZEIMET, M.D., Ph.D.
Department of Gynecology and Obstetrics
Innsbruck Medical University
Anichstrasse 35
A-6020 Innsbruck (Austria)
e-mail: alain.zeimet@i-med.ac.at
Stage 1B cervical cancer in a pregnant woman at 25 weeks of gestation

E. González Bosquet¹, A. Castillo¹, M. Medina², M. Suñol², A. Capdevila¹, J.M. Lailla¹

¹Department of Obstetrics and Gynecology, ²Department of Pathology, ³Department of Radiological Diagnostics
Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Barcelona (Spain)

Summary
Cervical cancer associated with pregnancy is rare (0.05%), although it is the most frequently diagnosed malignancy in pregnant women. We present the case of a 28-year-old woman at 25 weeks of gestation diagnosed with Stage 1B cervical cancer. Treatment was delayed until fetal maturity, and an elective cesarean section was performed at 33 weeks’ gestation, followed by a total hysterectomy preserving the ovaries, and a pelvic lymphadenectomy. A review of the literature on the treatment of cervical cancer during pregnancy relevant to the case described is also presented.

Key words: Cervical cancer; Pregnancy; Hysterectomy; Delay in treatment.

Introduction
Cervical cancer is the malignancy most frequently diagnosed in pregnant women [1-4]. Nevertheless, cervical cancer associated with pregnancy is rare, diagnosed in only 0.05% of pregnant women [5]. Because of this low frequency, it is difficult to establish a clear set of guidelines for treatment, a circumstance further complicated by the pregnancy and the woman’s preference for continuing it or terminating it. Until recently, the usual recommendation was to abort the pregnancy if diagnosis occurred before 20 weeks of gestation, and to delay treatment until fetal maturity if the diagnosis occurred during the second half of the third trimester. The main problem lies in the management of cervical cancer diagnosed between the end of the second trimester and the beginning of the third. There are published studies showing no negative effect of pregnancy on the progression of the disease during this period [6-9]. Consequently, delaying treatment in these women is acceptable as long as the woman is willing to accept the risk, and it can be demonstrated that the prognosis and risk of relapse for cervical cancer is not significantly high. Another element to take into consideration is that the stage at which the disease is diagnosed may alter its management, especially in advanced cases in which delaying treatment may have a significant impact on the prognosis.

We present the case of a pregnant woman in whom Stage 1B cervical cancer was diagnosed at 25 weeks gestation, and a discussion of the management of this case in light of the recent literature.

Case Report
A 28-year-old woman at 25 weeks of gestation was referred to our hospital presenting with cervicovaginal cytology results indicating a diagnosis of squamous cell carcinoma. She had no family history of cancer. Her gynecological history included a conization performed in her country of origin (Ecuador) in 1999, normal cytology results until 2003, and irregular menstrual cycles prior to pregnancy. She had had three prior pregnancies, two of which ended in a normal delivery and one in a spontaneous abortion that did not require curettage. The pregnancy in question was followed starting at 20 weeks, when the above-mentioned cytology was performed because no cytology results were available for the previous three years.

On examination the external genitalia were normal. Through a vaginal speculum a white lesion 1.5 cm in diameter was observed on the anterior cervical labium, while the vagina was normal. In a colposcopy performed with prior application of acetic acid, the above-mentioned lesion was observed, and the colposcopic image showed a variegated exophytic leukoplasia. The cytology was repeated, a cervical smear taken for HPV testing, and a colposcopically directed biopsy was performed. On vaginal examination the cervix was found to be closed, of normal length, and mobile. Rectal examination ruled out the presence of parametrial involvement.

The cytology results showed high-grade SIL with cells suggestive of squamous cell carcinoma. The result of the microarray-based HPV test was negative, and the biopsy revealed a moderately differentiated squamous cell carcinoma (Figure 1). Since it was difficult to determine how invasive the lesion was, large loop excision of the cervical transformation zone (LEEPZ) was indicated. This confirmed the presence of squamous cell carcinoma in the entire specimen, measuring 1.2 x 1 x 0.5 cm, clinically Stage 1B. The results of blood work were normal, and ultrasound imaging showed a single normally developed fetus.

The treatment possibilities were discussed with the patient, and with her approval it was decided to delay treatment until fetal maturity, since the malignancy was detected at an early stage. The patient was scheduled for clinical and obstetric follow-up visits, which included magnetic resonance imaging at 32 weeks of gestation to rule out the possibility of disease progression and parametrial involvement.
Stage 1B cervical cancer in a pregnant woman at 25 weeks of gestation

Discussion

The incidence of cervical cancer in pregnant women is approximately one case per 1200-10,000 pregnancies [10], although in one study a decline in the number of cases of cervical cancer associated with pregnancy was observed recently [7]. Cervical cancer is diagnosed in the same way during pregnancy as it is in non-pregnant women, on the basis of cytology, colposcopy and biopsy results. It should, however, be remembered that cytology is less reliable during pregnancy because of the changes that take place in the cervix, but it is imperative in cases in which recent cytology results are unavailable or the woman is at risk, as in our case, in which the patient had a history of prior conization but had had no cytologies during the previous three years.

It is noteworthy that although in nearly 100% of women with cervical cancer it is possible to detect human papillomavirus infection [11], in our case the patient tested negative for the virus.

The most frequent form of clinical presentation of cervical cancer is generally vaginal bleeding [12]. In our case, the initial diagnosis was cytological. Occasionally it is necessary to confirm a diagnosis by conization even if the woman is pregnant [13]. In our case, LLETZ was performed at 26 weeks of gestation. Once a diagnosis was reached and discussed with the patient, it was decided to postpone treatment until fetal maturation, but only after reviewing several published studies supporting this approach to management of Stage 1B cervical cancer [7, 14-16]. In these studies, delay of treatment does not worsen the prognosis, nor is it usually associated with progression of the disease [7, 14-16].

Although the standard approach until recently was to treat immediately in cases under 20 weeks’ gestation, the literature reports cases of this type in which treatment was delayed until fetal maturation. In some of these cases neoadjuvant chemotherapy was administered [1, 17] and in others laparoscopy and lymphadenectomy were performed [18] during pregnancy and prior to delay of surgical treatment.

At 32 weeks a dose of intramuscular betametasone was administered with a repeat dose 24 hours later to achieve fetal pulmonary maturation. Third-trimester ultrasound showed a normal fetus weighing approximately 2,050 g. At 33 weeks an elective cesarean section was performed by median laparotomy and longitudinal hysterotomy without surgical manipulation of the bladder. The premature female neonate, weighing 2420 g and with Apgar scores of 7, 9 and 10, was admitted to the neonatal unit for mild respiratory distress, and discharged at 13 days. During surgery the parametrical area was evaluated. There was no appearance of involvement, and no apparent pelvic or paraaortic adenopathies were observed. The postpartum period was uneventful except for a urinary tract infection, and the patient was discharged six days following the cesarean section.

At 19 days following the cesarean (11 weeks after the cytological diagnosis) the patient underwent a radical hysterectomy preserving both ovaries, and a bilateral pelvic lymphadenectomy with complete examination of the abdominal cavity. Lysis of minor adherences was performed without difficulty, and during the procedure the patient received one unit of red blood cells for moderate bleeding. The results of the postoperative hemogram were 10.8 mg/dl hemoglobin and 32.2 hematocrit.

The postoperative period was complicated by several fever spikes and abdominal pain. A hematoma measuring 11.1 x 9.4 cm was diagnosed by CAT scan, and severe anemia required transfusion of two units of two red blood cells prior to another surgical intervention to drain the hematoma, which was located in the vaginal fornix. In addition, a small abscess in the abdominal wall was observed, and cultured positive for Pseudomonas aeruginosa. Intravenous antibiotic treatment was administered without incident during the postoperative period following the second surgery, and the patient was discharged 19 days following the first surgery.

Study of the surgical specimen showed squamous cell carcinoma of the cervix to a depth of 4 mm, moderately differentiated and focally keratinized (Figure 2), with marked lymphocytic infiltration in the transformation zone. The 15 pelvic lymph nodes removed were negative. The tumor board of our hospital concluded that the diagnosis of Stage 1B moderately differentiated cervical cancer did not warrant further treatment. In subsequent follow-up visits, both the patient’s cytology results and physical examinations were normal. Her last follow-up visit was a year following the intervention.

Figure 1. — Moderately differentiated squamous cell carcinoma with a microinvasive focus.

Figure 2. — Squamous cell carcinoma of the cervix moderately differentiated and focally keratinized to a depth of 4 mm.

Discussion

The incidence of cervical cancer in pregnant women is approximately one case per 1200-10,000 pregnancies [10], although in one study a decline in the number of cases of cervical cancer associated with pregnancy was observed recently [7]. Cervical cancer is diagnosed in the same way during pregnancy as it is in non-pregnant women, on the basis of cytology, colposcopy and biopsy results. It should, however, be remembered that cytology is less reliable during pregnancy because of the changes that take place in the cervix, but it is imperative in cases in which recent cytology results are unavailable or the woman is at risk, as in our case, in which the patient had a history of prior conization but had had no cytologies during the previous three years.

It is noteworthy that although in nearly 100% of women with cervical cancer it is possible to detect human papillomavirus infection [11], in our case the patient tested negative for the virus.

The most frequent form of clinical presentation of cervical cancer is generally vaginal bleeding [12]. In our case, the initial diagnosis was cytological.

Occasionally it is necessary to confirm a diagnosis by conization even if the woman is pregnant [13]. In our case, LLETZ was performed at 26 weeks of gestation. Once a diagnosis was reached and discussed with the patient, it was decided to postpone treatment until fetal maturation, but only after reviewing several published studies supporting this approach to management of Stage 1B cervical cancer [7, 14-16]. In these studies, delay of treatment does not worsen the prognosis, nor is it usually associated with progression of the disease [7, 14-16].

Although the standard approach until recently was to treat immediately in cases under 20 weeks’ gestation, the literature reports cases of this type in which treatment was delayed until fetal maturation. In some of these cases neoadjuvant chemotherapy was administered [1, 17] and in others laparoscopy and lymphadenectomy were performed [18] during pregnancy and prior to delay of surgical treatment.
In the majority of cases (74.2%) of cervical cancer in pregnant women, the diagnosis is made in the initial stages of the disease, as in our case. In 93% of cases, cervical cancer during pregnancy is diagnosed during the first and second trimesters [19]. Another factor affecting treatment is the stage of the disease, since at advanced stages delaying treatment is more likely to worsen the prognosis. In the literature, we found reports of cases of advanced cervical cancer diagnosed during the first trimester and treated with chemotherapy, delaying surgery and/or radiotherapy [20], although the possible long-term consequences of this for both mother and child should be evaluated with care.

Magnetic resonance imaging (MRI) is used in follow-up for pregnant women with cervical cancer in cases of planned delay in treatment to detect progression of the disease [21]. In our case, an MRI was performed prior to planned delay in treatment to detect progression of the disease, since at advanced stages delaying treatment is more likely to worsen the prognosis. In the literature, we found reports of cases of advanced cervical cancer diagnosed during pregnancy: report of a case and review of the literature. [22] report delaying treatment until 31-41 weeks in pregnant women with Stage 1B cervical cancer, the mean delay was between 3.5 and 20.5 weeks.

The week chosen for definitive treatment depends on the stage of the disease and fetal maturity. Takushi et al. [22] report delaying treatment until 31-41 weeks in pregnant women with disease ranging between Stage 1A1 and 1B2, while in only those women with Stage 1B disease, treatment was delayed until 31-32 weeks. In our case, a cesarean section was performed at 33 weeks, and definitive treatment delayed until 19 days later.

The data from three studies [10, 12, 22] in which treatment was postponed until fetal maturity show that of a total of 26 pregnant women, in 11 cases vaginal delivery was chosen and in 15 cesarean delivery was performed, as in our case. In all cases in which a cesarean section was performed, a hysterectomy was also performed immediately afterward, in the course of the same surgical intervention. In our case, a complete hysterectomy was performed 19 days after the cesarean section, which had the effect of decreasing intraoperative bleeding and facilitating the surgery, since involution of the uterus was more advanced and we found no significant adhesions.

The histological diagnosis confirmed the presence of Stage 1B disease and squamous cell carcinoma, the histologic type most frequently found [12]. In the studies we reviewed [10, 12, 22] on postponing treatment for pregnant women with Stage 1B cervical cancer, the mean delay was between 3.5 and 20.5 weeks (range 2-30 weeks). In our case the delay was 11 weeks. In none of these cases, including ours, was disease progression observed, and all the women are now disease free.

From this literature review, we conclude that it is possible to delay treatment safely in pregnant women with Stage 1B cervical cancer if they are past 20 weeks’ gestation. Judging by the results reported in the studies cited above, it even appears to be safe to postpone treatment prior to 20 weeks’ gestation.

Management of more advanced cases must be tailored to the circumstances; the week of gestation in which cervical cancer is diagnosed, and the woman’s preference.

Acknowledgment
The authors are grateful to Susan M. DiGiacomo, Ph.D., Universitat Rovira i Virgili, Tarragona (Spain), and University of Massachusetts at Amherst (USA), for preparation of the English-language version of the manuscript.

References
Stage IB cervical cancer in a pregnant woman at 25 weeks of gestation


Address reprint requests to:
E. GONZÁLEZ BOSQUET, M.D.
Hospital Sant Joan de Déu, Barcelona
Passeig Sant Joan de Déu, 2
08950 Esplugues, Barcelona (Spain)
es-mail: egonzalezb@hsjdbcn.org
The role of ovarian transposition in patients with early stage cervical cancer - two case reports

N. Salakos, K. Bakalianou, C. Iavazzo, G. Paltoglou, K. Papadias, A. Liapis, A. Kondi-Pafiti

2nd Department of Obstetrics and Gynecology, Medical School, University of Athens, Aretaieion Hospital, Athens (Greece)

Summary
The aim of the study is to present two cases of premenopausal women with early stage cervical carcinoma who underwent ovarian transposition. The role and the advantages of this technique are discussed.

Key words: Early stage cervical carcinoma; Ovarian transposition; Preservation; Menopause; Fertility.

Introduction
Early cervical carcinoma (Stage IA2 to IIA) can be cured in 95% of patients [1]. Although, the average age at diagnosis is 51 years [1], the disease may also occur in younger non-gravid women in the second decade of their life (10-15%) [2]. Patients with early-stage disease can be treated with either radical hysterectomy and pelvic lymphadenectomy or with primary radiation with concomitant chemotherapy with approximately equal 5-year survival [1]. The major advantage of surgical treatment is the fact that the ovaries might be transposed out of the radiation field in case of postoperative irradiation. The most common locations for ovarian transposition are laterally within the pelvis, in the lower paracolic gutters, anterior to the psoas muscles and more usually in the paracolic gutters [3].

Case 1
A 22-year-old nulliparous woman with Stage IB2 cervical carcinoma underwent radical hysterectomy with preservation of the right ovary plus pelvic lymphadenectomy. The remaining ovary was transposed in the right paracolic fossa outside the pelvis. Histological examination revealed a moderately differentiated squamous cell carcinoma. The patient received adjuvant radiotherapy, and two years after the operation is free of disease with excellent ovarian function.

Case 2
A 27-year-old nulliparous woman with Stage IB1 cervical cancer underwent radical hysterectomy without salpingo-oophorectomy plus pelvic lymphadenectomy with transposition of the right ovary in the right paracolic gutter out of the pelvis. The left ovary was left in situ in the pelvis. Histological examination revealed a moderately differentiated squamous cell carcinoma. The patient also received adjuvant radiotherapy and two and a half years after the operation she is free of disease with excellent ovarian function.

Discussion
The major advantage of ovarian translocation in patients with early cervical cancer is the prophylaxis of premenopausal women from menopausal symptoms such as vaginal atrophy, hot flushes and osteoporosis. In a prospective study, it has been shown that preservation of ovarian function in patients with cervical carcinoma is effective in 100%, 90% and 60% of those treated exclusively by surgery, vaginal brachytherapy or external radiation, respectively [4].

According to Ishii et al. ovarian preservation is safe in patients under 40 years old who could preserve ovarian function for one up to nine years [5]. For the best follow-up of ovarian function, Olejek et al. suggested that patients’ hormone levels and bone density should be closely monitored [6]. The ovarian function of our patients was investigated by measurements of FSH, LH and estradiol levels in combination with bone mineral density measurements. The levels of FSH, LH and estradiol were within normal ranges in both our patients and moreover no menopausal symptoms were mentioned in the follow-up period (2 and 2.5 years, respectively). The bone mineral density was stable in both our patients.

However, Wu et al. reported that ovarian failure occurred in 35% of women after 12 months of such a preservative operation [7]. Buekers et al. showed that 41% of their patients retained ovarian function for a mean of 43 months and a mean age of menopause of 36.6 years [8], whereas ovarian function was preserved in 50% of patients in a retrospective study of Feeney et al. [9].

Bidzinski et al. showed that ovarian function even after transposition could be better preserved when (in case of external irradiation) the distance of the upper margin of the field and the ovary was more than 3 cm [10], whereas van Eikeren et al. proposed that the limit to preserve ovarian function is 300 cGy [11].

It is known that ovarian hormonal production or stimulation does not adversely affect the course of the disease [11]. In non-gravid women when preservation of fertility is desired, ovarian translocation could offer the option of fertility with a donor uterus. Duska et al. reported two
cases of young nulliparous women with Stage IA2 cervical cancer who underwent ovarian stimulation and oocyte retrieval followed by radical hysterectomy. Of course even these authors suggest that there are many ethical and practical aspects that should be resolved regarding the risk of metastasis in young women. Among them there are very young nulliparous patients such as the two reported cases. Translocation of ovaries could be safely performed in young patients with early stage squamous cell carcinoma, with macroscopically normal ovaries and with preserved peripheral unaffected cervical stroma [15].

Conclusion

Over half of the women with cervical cancer are premenopausal. Among them there are very young nulliparous women who underwent ovarian stimulation and oocyte retrieval followed by radical hysterectomy. Of course even these authors suggest that there are many ethical and practical aspects that should be resolved regarding the risk of metastasis in young women. Among them there are very young nulliparous patients such as the two reported cases. Translocation of ovaries could be safely performed in young patients with early stage squamous cell carcinoma, with macroscopically normal ovaries and with preserved peripheral unaffected cervical stroma [15].

References

Squamous cell carcinoma arising in a mature cystic teratoma of the ovary in young patient with elevated carbohydrate antigen 19-9


1Department of Obstetrics and Gynecology, 2Department of Pathology, 3Department of Surgery, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar; 4Department of Obstetrics and Gynecology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir (Turkey)

Summary

Squamous cell carcinoma is the most common type of malignant transformation in mature cystic teratomas. It mainly affects postmenopausal women but is rarely seen in young patients. Carbohydrate antigen 19-9 (CA19-9) tumor marker is a high-molecular-weight glycoprotein, frequently elevated in gastrointestinal adenocarcinomas. CA19-9 levels can increase in both dermoid cysts and in malignant transformation of dermoid cysts. Herein we report a case of squamous cell carcinoma originating from a dermoid cyst in a 31-year-old, gravida 0, para 0, single woman with high levels of CA19-9 and normal levels of CEA. Preoperative CA19-9 was 1000 U/ml (normal range below 27 U/ml). The patient underwent unilateral salpingo-oophorectomy, omentectomy, appendectomy, pelvic and paraaortic lymphadenectomy. After the pathologic analysis of the material, the patient was categorized as FIGO Stage IIa due to metastasis to the left tube. She received six cycles of cisplatin and paclitaxel at 21-day intervals. The postoperative first day, second month, and sixth month CA19-9 values were 602 U/ml, 33.5 U/ml and 22.3 U/ml, respectively. She is now doing well without recurrence of disease six months after the surgery. Squamous cell carcinomas originating from dermoid cysts are rare tumors especially seen in elderly patients with high levels of tumor markers (like CEA, SCCA). Every case may not have the same characteristics and management should be individualized.

Key words: Squamous cell carcinoma; Mature cystic teratoma; Carbohydrate antigen 19-9 (CA19-9).

Introduction

Mature cystic teratomas, also known as dermoid cysts, are the most common ovarian tumors in women during reproductive years. Rarely does this type of tumor show malignant transformation (approximately 2% of cases) [1]. Any component may become malignant and squamous cell carcinoma is the most frequent type (80% of cases) of malignant transformation in dermoid cysts [1, 2]. Most of the patients are postmenopausal women and the mean age ranges from 51 to 55 years (minimum 21 and maximum 87 years old) [2-4].

Carbohydrate antigen 19-9 (CA19-9) tumor marker is a high-molecular-weight glycoprotein, frequently elevated in gastrointestinal adenocarcinomas. CA19-9 levels may also increase in dermoid cysts and in malignant transformation of dermoid cysts [5-7].

We report a case of squamous cell carcinoma originating from a dermoid cyst in a young patient with high levels of CA19-9.

Case report

A 31-year-old, gravida 0, para 0, single woman was referred to our clinic due to new onset of lower left quadrant abdominal pain. She had an approximately 10-cm left ovarian complicated cyst detected by abdominal ultrasonography (US). On physical examination she had a palpable mass in the lower quadrant. Pelvic/abdominal US and computerized tomography (CT) determined a 13 x 10 x 11-cm heterogeneous left ovarian cyst including calcific and oily structures without any papillary projections or ascites. Initial complete blood count, serum electrolytes, and liver and kidney function tests were normal. The preoperative CEA (carcinoembriogenic antigen), AFP (alphafetoprotein) and hCG (human chorionic gonadotropin) levels were in the normal range but CA19-9 was 1000 U/ml (normal range below 27 U/ml) and CA 125 was 126 U/ml (normal range below 35 U/ml). We could not look for squamous cell carcinoma antigen (SCCA) because of techniqual difficulties in our laboratory. In light of the findings the patient was thought to have a mature cystic teratoma and underwent exploratory laparotomy.

During the operation, an irregularly shaped left ovarian mass 13 cm in diameter was seen. The mass was not adherent to the other pelvic structures. The uterus and the left ovary and the other intraabdominal organs were macroscopically normal. Left cystectomy and peritoneal washing were performed. The cyst contained sebaceous material, teeth, and hair. The serial cut section of the cyst revealed a solid area 9 cm in diameter. The intraoperative diagnosis was consistent with carcinoma, thus unilateral salpingo-oophorectomy, omentectomy, appendec-
Squamous cell carcinoma arising in a mature cystic teratoma of the ovary in young patient with elevated carbohydrate antigen 19-9

Surgery, pelvic and paraaortic lymphadenectomy were performed. Biopsies were taken from suspicious peritoneal nodules. As the CA19-9 levels were increased and the frozen section diagnosis was malignant, an intraoperative gastroenterology consultation was done. Gastroscopy and rectosigmoidoscopy were performed and accepted as normal. Pathologic analysis of material revealed a dermoid cyst containing foci of moderately differentiated squamous cell carcinoma (Figure 1). The carcinoma appeared to have spread to the left tube (Figure 2). The cyst capsule was intact. All lymph nodes, peritoneal washings, the omentum and appendix were negative for cancer. Consequently, the patient was categorized as FIGO Stage IIa.

After the operation there were no complications. The postoperative first day, second month, and sixth month CA19-9 levels were 602 U/ml, 33.5 U/ml and 22.3 U/ml, respectively and CA 125 was 79 U/ml, 19.8 U/ml and 19.2 U/ml, respectively. The patient was treated with six cycles of combination chemotherapy consisting of 75 mg/m2 cisplatin and 175 mg/m2 paclitaxel at 21-day intervals. She is now doing well without any recurrence of disease six months after the surgery.

Discussion

Malignant transformations are extremely rare complications of mature cystic teratomas. Squamous cell carcinoma accounts for 80% of malignant transformations in dermoid cysts [1, 2]. Although germ cell tumors are seen in younger age groups this type of tumor is especially observed in older age [2-4]. Older age seems to be related with malignant transformation. Squamous cell carcinoma secondary to a dermoid cyst has very seldomly been seen in younger patients (10% in younger than 35 years) [2, 4].

Preoperative diagnosis of squamous cell carcinoma arising from a dermoid cyst is very difficult as it does not have specific symptoms. Only intraoperative diagnosis is possible for such cases. Researchers have worked on various tumor markers and clinical parameters. Kikkawa et. al. stated that tumor size and patient age are important clinical factors in the differential diagnosis of squamous cell carcinoma from a dermoid cyst. They also studied the significance of SCCA and CEA and finally concluded that a good clinical strategy would be to examine SCCA and CEA levels if the patient is over age 45 or if the tumor volume is greater than 99 mm in the greatest diameter [8].

The most useful tumor markers have been SCCA and CEA for squamous cell carcinoma arising in a dermoid cyst. Some authors explain that these markers are very important in detecting malignancy earlier and to follow postoperative recurrence [4, 8]. Unfortunately other tumor markers like CA19-9 and CA125 do not seem to have any place in clinical application and also CA19-9 has not been a useful screening marker due to a high positive rate in dermoid cysts [5, 8]. CA19-9 is frequently elevated in gastrointestinal adenocarcinomas such as the pancreas and could be increased in dermoid cysts and also squamous cell carcinoma of dermoid cysts [5-7].

In the literature, squamous cell carcinomas arising in a mature cystic teratoma are seen mainly in postmenopausal ages and generally together with tumor markers like CEA and SCCA. However our patient differs from cases in the literature as she was young, had a normal CEA level and a very high CA19-9 level. The CA19-9 level normalized rapidly following surgical treatment in our patient. CA19-9 levels may be an important tumor marker indicating the effectivity of treatment in patients with high preoperative or pretreatment CA19-9 levels.

As our patient was a virgin and fertility was expected in the future, we performed full staging and left salpingo-oophorectomy although conservative surgical interventions have been defined for FIGO Stage Ia nulliparous women in the literature [4].

In conclusion, although having a standard approach for squamous cell carcinoma cases is an advantage for diagnosis and follow-up, it should be kept in mind that every case may not have the same standards and the management should be individualized.

References


Address reprint requests to:
D.T. ARIÖZ, M.D.
Dumlupınar Mah. Menderes Cad. Özgürler Apt. No: 8/7
03200/Afyonkarahisar (Turkey)
e-mail: drarioz@yahoo.com
Primary vaginal melanoma: a case report and literature review

M. Schmidt¹, M.D.; A. Honig¹, M.D.; M. Schwab¹, M.D.; P. Adam², M.D.; J. Dietl¹, M.D.

¹Department of Obstetrics and Gynecology, ²Department of Pathology, University of Wuerzburg, Wuerzburg (Germany)

Summary

Background: Malignant melanoma of the vagina is a rare malignancy associated with high risk of recurrence, distant metastasis and short survival time. Due to the rarity of the disease, no prospective studies or validated treatment recommendations exist. Case: We describe the case of a 54-year-old patient with a locally advanced melanoma located on the anterior vaginal wall. At the time of diagnosis there was no evidence of nodal or distant metastasis. Conclusion: In view of retrospective data in the literature, we treated the patient with colpectomy in terms of a wide local excision only. A more radical approach, adjuvant radio- or chemotherapy did not seem to be justifiable since there are no data demonstrating prolonged survival.

Key words: Vaginal melanoma; Colpectomy.

Introduction

Malignant melanoma of the vagina accounts for only 0.3% of all melanomas in women [1, 2]. Less than 3% of primary vaginal tumors are malignant melanomas and they have the worst prognosis of all vaginal malignancies [3] with five-year-survival rates of 5-25% [1, 3-5]. The most common presenting sign is vaginal bleeding and locally advanced disease is frequently found at the time of first diagnosis [5, 6]. In the majority of cases pigmented melanomas are found; only 10-23% of the cases are amelanotic melanomas [5]. For diagnosis of malignant melanoma in unusual locations, immunohistochemical evaluation is useful. Expression of S100 protein is found in virtually all melanomas with low specificity. MART1 (melanoma antigen recognized by T cells) and MITF (microphthalmia transcription factor) are antibodies to melanocyte/melanogenesis related proteins and are very specific for cells of the melanocytic lineage. The combination of these antibodies leads to greater diagnostic accuracy as reviewed by Carson et al. [7]. Different surgical treatment options have been discussed in the past including wide local excision with or without sentinel node biopsy, pelvic lymphadenectomy, and more extensive surgical procedures such as exenteration of all organs involved. Radiotherapy has been reported as the exclusive treatment for locally advanced disease that was considered to be surgically unresectable. In addition, radiotherapy was applied as an adjuvant approach especially if the pathologic analysis revealed positive microscopic margins [3, 5, 8-11]. In the adjuvant setting, different chemotherapy regimens and immunotherapy have been administered [5, 12]. Surgical treatment was the only therapeutic procedure that was shown to prolong survival in retrospective analyses [5, 9, 13-14].

Case Report

A 54-year-old Caucasian women, gravid 2, para 2, presented with four weeks of vaginal discharge. Her medical history included hysterectomy which had been done four years before for menopausal bleeding disorders. Examination revealed a healthy female in good general condition. The gynaecological examination showed a 3 x 3 cm pigmented and ulcerated lesion at the anterior vaginal wall (Figure 1a). Clinically, the lesion was about 1 cm thick and relocatable against the posterior wall of the urinary bladder. The lateral and posterior fornices appeared to be free of cancer and no superficial nodal involvement was observed. Representative biopsies of the lesion confirmed the presence of a malignant melanoma. The chest X-ray, abdomen and pelvic computed tomography (CT) gave no evidence of metastases or lymph node involvement. We performed a vaginal colpectomy (Figure 1b). Lymph node sampling was not performed. Histopathology of the entire specimen showed a tumor thickness of 1.6 cm and a maximum diameter of 2.5 cm with superficial ulcerations (Figure 2a-c). The lateral and deep tumor margins were clear. Tumor staging analogous to staging of skin tumors was pT4b. Immunohistochemical staining for S100 (Figure 2d) as well as for MART1 (Figure 2e) was strong and specific. We also found nuclear staining for MITF (Figure 2f). Proliferation rate, measured as Ki67-index, was about 50%.

Following the operation, the patient recovered uneventfully with excellent healing of the wound site. We recommended no further treatment and the patient is followed-up regularly. At the three-month follow-up visit, the patient was well with no evidence of recurrence.

Discussion

Malignant melanoma of the vagina is a rare tumor entity with less than 300 cases described in the literature. Most of the articles are case reports and a few small series with 35 cases at most. To our knowledge, there are no prospective data regarding therapeutic options. Most vaginal melanomas are diagnosed in postmenopausal women in their fifth and sixth decade [15]. For the majority of patients, vaginal melanoma is associated with poor...
clinical outcome. The median survival reported in a study of 35 cases was 20 months [5]. Histopathological staging of vaginal melanoma can follow the International Federation of Gynecology and Obstetrics (FIGO) system for vaginal carcinoma, the Breslow system or the TNM-staging system for skin tumors. Prognostic factors established for other tumor entities such as age, stage, depth of invasion, pigmentation, ulceration, and adjuvant therapy did not correlate with patient outcome in the study of Miner et al. [5]. Actually, pathologically negative or positive margins of resection did not correlate with recurrence-free survival in this study.

Surgical resection is considered the treatment of choice. Different methods such as wide local excision, colpectomy, radical resection with total abdominal hysterectomy and bilateral salpingo-oophorectomy, and even extenteration have been described. Van Nostrand et al. [14] found improved survival with radical surgery whereas most authors have stated that the type of surgery did not influence survival [5, 9, 13, 16]. Since the outcome of malignant vaginal melanoma is poor, most authors recommend avoiding radical surgery and favour local excision with the aim of complete resection. Regarding lymph node dissection, most authors do not recommend performing routine staging of the pelvic lymph nodes since nodal metastases are rare and the morbidity associated with lymphadenectomy is high [5, 16]. As an alternative to identify lymphatic metastasis without the morbidity of complete lymphadenectomy, sentinel node biopsy has been described [5, 17-19].

Radiation therapy has also been described to be effective in the treatment of vaginal melanoma. In the majority of reports, radiation therapy was applied for surgically unresectable disease or as adjuvant treatment in case of pathologically positive margins [5, 8, 20-22]. Radiotherapy as an alternative to surgery was recommended by Petru et al. [3] for patients with lesions less than 3 cm in diameter. Radiation therapy appeared to provide good local control in patients with surgically unresectable disease. The value of adjuvant radiation therapy cannot be evaluated considering the limited and only retrospective data available.

Most of the recurrences are distant, therefore different chemotherapy regimens and immunotherapy with interferon-γ or interferon-α-2b have been discussed as treatment options for vaginal melanoma in the adjuvant setting [23, 24]. As yet, no prospective randomised trials have been completed. Therefore, we have no rationale for adjuvant chemo- or immunotherapy.

In conclusion, primary vaginal melanoma is a rare disease with limited prognosis. Complete surgical resection is the treatment of first choice and seems associated with prolonged overall survival. Primary radiation therapy should be reserved for patients with surgically unresectable disease. There is no evidence for elective lymph node dissection. The value of sentinel lymph node biopsy, adjuvant radiation therapy, chemo- or immunotherapy has not been evaluated yet. Due to the rarity of the disease, multicenter trials will be required to answer these questions.

References

Primary vaginal melanoma: a case report and literature review


Figure 2. — Gross finding of the formalin-fixed specimen (a, b). Microscopic findings by H&E staining (c), immunohistochemical staining for S100 (d), MART1(e) and MITF (f).


Address reprint requests to:
M. SCHMIDT, M.D.
Department of Obstetrics and Gynecology
University of Wuerzburg
Josef-Schneider-Strasse 4
97080 Wuerzburg (Germany)
e-mail: melanie_weigand@hotmail.de
Malignant mixed müllerian tumor of primary mesenteric origin associated with a synchronous ovarian cancer: case report and literature review


Departments of 1Surgery and Anesthesia, Kaohsiung Municipal Hsiao-Kang Hospital; Departments of 2Pathology, 3Obstetrics and Gynecology, and 4Surgery, Kaohsiung Medical University Hospital 5Department of Pathology, Kaohsiung Chang Gung Memorial Hospital 6Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung (Taiwan)

Summary

Malignant mixed müllerian tumor (MMMT) is a rare tumor in females and extragenital MMMT is even more so. We report a patient with MMMT primarily in the mesentery with synchronous ovarian cancer. In the English literature, 42 cases of extragenital MMMT have been reported other than the presented case, and this is only the second MMMT arising from the mesentery. Furthermore, among the cases reviewed, MMMTs tend to be associated with synchronous or metachronous colonic cancer or gynecologic tumors originating from the müllerian duct, including ovarian tumors, fallopian tube cancer, endometrial cancer, cervical cancer, and serous carcinoma of the peritoneum (14 out of 43 patients; 32.6%). The risk factors for MMMT include obesity, nulliparity, exogenous estrogen, and long-term tamoxifen use. The prognosis of MMMT is catastrophic and the treatment is based on the experience of those of uterine sarcomas, which is composed of operation, radiotherapy and chemotherapy.

Key words: Malignant mixed müllerian tumor; Mesentery; Ovarian cancer.

Introduction

Malignant mixed müllerian tumor (MMMT) is a rare tumor with both epithelial (carcinoma) and mesenchymal (sarcoma) components. MMMT is further classified into homologous or heterologous type according to the sarcomatous component. MMMTs generally originate in the organs of the müllerian duct: uterus, ovaries, fallopian tubes, cervix, and vagina in descending order of frequency and rarely occur in the extragenital area. To the best of our knowledge, 42 cases have been reported in the English literature with extragenital MMMTs and only one case is of primary mesenteric origin. Here, we reported a patient with MMMT arising from the mesentery along with synchronous right ovarian cancer.

Case Report

A 62-year-old, gravida 4, para 2, abortion 4, postmenopausal female presented with abdominal fullness and lower abdominal pain of more than two weeks duration. She was not on hormonal replacement therapy. Physical examination revealed a large, firm, nontender mass in the right lower abdomen and pelvis. Ultrasonography revealed a solid tumor with mixed internal components in the right side of the pelvis, measuring 11 cm in the largest diameter. Computed tomography confirmed a large tumor in the right side of the pelvis with compression and displacement of the uterus and the bladder to the left with ascites in the peritoneum (Figure 1). The serum CA125 level was 24.7 U/ml while lactate dehydrogenase (LDH) and CA 19-9 were high at 1041 IU/l and 48 U/ml, respectively. She was admitted to the Department of Obstetrics and Gynecology with the suspicion of right ovarian cancer.

At laparotomy, the uterus and the left adnexa were intact. There was a tumor measuring 11.5 × 10 × 7.5 cm arising from the mesentery involving the terminal ileum, the greater omentum, the right fallopian tube and the right ovary. Therefore, a right salpingo-oophorectomy, excision of the mesenteric tumor, and segmental resection of the ileum with end-to-end anastomosis were performed after consulting the Department of Surgery. Gross findings showed a well-capsulated tumor in the mesentery and the cut surfaces were yellow to white in color and soft in consistency on macroscopic observation. Areas of hemorrhage and necrosis were observed (Figure 2).

Histopathologic examination revealed sheets of spindle cells, which demonstrated positive reaction to vimentin (BioGenex, San Ramon, CA) and scattered islets of epithelial cells forming solid nests, which showed positive reaction to cytokeratin AE1/AE3 (Dako, Glostrup, Denmark) (Figure 3). Marked nuclear pleomorphism with frequent bizarre tumor giant cells and geographic tumor necrosis were also noted. A diagnosis of MMMT with homologous type was confirmed. Furthermore, there was a small nodular lesion composed of malignant glandular structures in bland-looking fibrous stroma in the right ovary and adenocarcinoma was ultimately diagnosed (Figure 4). The right fallopian tube was also invaded by the ovarian adenocarcinoma.

Unfortunately, tumor recurrence developed three months after the first operation. The patient received adjuvant chemotherapy with regimens composed of ifosfamide, carboplatin and etoposide. She is currently able to carry on normal activity six months after chemotherapy, though the best response condition is only stabilization of the disease.
### Table 1. — Extragenital MMMTs in the English literature.

<table>
<thead>
<tr>
<th>Case</th>
<th>Year</th>
<th>Author</th>
<th>Age</th>
<th>Primary site</th>
<th>Tissue type</th>
<th>Associated tumor</th>
<th>Treatment</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1955</td>
<td>Ober and Black</td>
<td>74</td>
<td>Pelvic peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation RT</td>
<td>Death at 5 months</td>
</tr>
<tr>
<td>2</td>
<td>1967</td>
<td>Fernie and Ross</td>
<td>47</td>
<td>Abdominal retroperitoneum</td>
<td>Homologous</td>
<td>Hydatidiform mole</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>3</td>
<td>1977</td>
<td>Weiz-Carrington et al.</td>
<td>77</td>
<td>Cecal peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Death at 1 week, from pulmonary embolism</td>
</tr>
<tr>
<td>4</td>
<td>1982</td>
<td>Marchevsky et al.</td>
<td>40</td>
<td>Pelvic retroperitoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (adriamycin, cisplatin)</td>
<td>Death at 12 months</td>
</tr>
<tr>
<td>5</td>
<td>1983</td>
<td>Hermann and Tessler</td>
<td>72</td>
<td>Abdominal retroperitoneum</td>
<td>Heterologous</td>
<td>Ovarian serous papillary carcinoma, metachronous</td>
<td>Operation CT (adriamycin, cytoxan, DTIC, vincristine)</td>
<td>Deag at 6 months</td>
</tr>
<tr>
<td>6</td>
<td>1984</td>
<td>Hausk et al.</td>
<td>77</td>
<td>Abdominal retroperitoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Biopsy</td>
<td>Death at 20 days</td>
</tr>
<tr>
<td>7</td>
<td>1986</td>
<td>Campins et al.</td>
<td>58</td>
<td>Pelvic peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>1986</td>
<td>Chimas et al.</td>
<td>67</td>
<td>Rectosigmoid peritoneum</td>
<td>Heterologous</td>
<td>Mucinous cystadenoma, metachronous</td>
<td>Operation CT</td>
<td>Death at 24 months</td>
</tr>
<tr>
<td>9</td>
<td>1986</td>
<td>Nguyen and Berendt</td>
<td>58</td>
<td>Greater omentum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation RT</td>
<td>Death at 6 months</td>
</tr>
<tr>
<td>10</td>
<td>1988</td>
<td>Chen and Wolb</td>
<td>58</td>
<td>Pelvic peritoneum</td>
<td>Homologous</td>
<td>Ovarian serous papillary adenocarcinoma, metachronous</td>
<td>Operation RT</td>
<td>Death at 11 months</td>
</tr>
<tr>
<td>11</td>
<td>1989</td>
<td>El-Jabbour et al.</td>
<td>82</td>
<td>Sigmoid colon peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (cisplatin, adriamycin, cytoxan)</td>
<td>Death at 5 months</td>
</tr>
<tr>
<td>12</td>
<td>1989</td>
<td>Ohno et al.</td>
<td>66</td>
<td>Descending, sigmoid colon peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation CT (cyclophosphamide)</td>
<td>Death at 21 months, from MI</td>
</tr>
<tr>
<td>13</td>
<td>1991</td>
<td>Garde and Jonesaz</td>
<td>65</td>
<td>Diaphragmatic peritoneum</td>
<td>Heterologous</td>
<td>Ovarian endometroid adenocarcinoma, metachronous</td>
<td>Operation CT (cisplatin, adriamycin, ifosfamide)</td>
<td>Death at 6 months</td>
</tr>
<tr>
<td>14</td>
<td>1991</td>
<td>Solis et al.</td>
<td>54</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>Serous carcinoma of the peritoneum, synchronous</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>15</td>
<td>1993</td>
<td>Nimaroff et al.</td>
<td>82</td>
<td>Sigmoid colon peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (cisplatin, adriamycin, cytoxan)</td>
<td>Death at 5 months</td>
</tr>
<tr>
<td>16</td>
<td>1994</td>
<td>Choong et al.</td>
<td>63</td>
<td>Sigmoid colon peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>17</td>
<td>1994</td>
<td>Garamvoglgi et al.</td>
<td>59</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>Endometrial adenocarcinoma, synchronous</td>
<td>Operation CT (cisplatin, doxorubicin, ifosfamide)</td>
<td>Death at 24 months</td>
</tr>
<tr>
<td>18</td>
<td>1994</td>
<td>Garamvoglgi et al.</td>
<td>64</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>Fullopian tube carcinoma in situ, synchronous</td>
<td>Operation CT (ifosfamide)</td>
<td>Death at 8 months</td>
</tr>
<tr>
<td>19</td>
<td>1994</td>
<td>Garamvoglgi et al.</td>
<td>84</td>
<td>Retroperitone peritoneum</td>
<td>Heterologous</td>
<td>Colonic adenocarcinoma, synchronous</td>
<td>Operation</td>
<td>Death at 2 months, from heart disease</td>
</tr>
<tr>
<td>20</td>
<td>1994</td>
<td>Westra et al.</td>
<td>55</td>
<td>Spleen</td>
<td>Homologous</td>
<td>None</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>21</td>
<td>1995</td>
<td>Mira et al.</td>
<td>62</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>Ovarian endometroid adenocarcinoma, synchronous</td>
<td>Operation</td>
<td>Survival for 28 months</td>
</tr>
<tr>
<td>22</td>
<td>1995</td>
<td>Mira et al.</td>
<td>83</td>
<td>Cecal peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Death at 6 months</td>
</tr>
<tr>
<td>23</td>
<td>1997</td>
<td>Rose et al.</td>
<td>57</td>
<td>Peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (cisplatin, ifosfamide)</td>
<td>Survival for 42 months</td>
</tr>
<tr>
<td>24</td>
<td>1997</td>
<td>Rose et al.</td>
<td>71</td>
<td>Peritoneum</td>
<td>Homologous</td>
<td>Uterine cervical adenocarcinoma, synchronous</td>
<td>Operation CT (cisplatin, ifosfamide)</td>
<td>Death at 6 months</td>
</tr>
<tr>
<td>25</td>
<td>1997</td>
<td>Rose et al.</td>
<td>67</td>
<td>Peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (cisplatin, ifosfamide)</td>
<td>Death at 3 months</td>
</tr>
<tr>
<td>26</td>
<td>1997</td>
<td>Ibanez-Manlapaz et al.</td>
<td>58</td>
<td>Abdominal peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (adriamycin, cisplatin)</td>
<td>Death at 20 months</td>
</tr>
<tr>
<td>27</td>
<td>1997</td>
<td>Ibanez-Manlapaz et al.</td>
<td>75</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation CT (ifosfamide)</td>
<td>Death at 6 months</td>
</tr>
<tr>
<td>28</td>
<td>1999</td>
<td>Ari et al.</td>
<td>56</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>29</td>
<td>2001</td>
<td>Shen et al.</td>
<td>67</td>
<td>Greater omentum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Survival for 8 months</td>
</tr>
<tr>
<td>30</td>
<td>2001</td>
<td>Shen et al.</td>
<td>33</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>Endometrial adenocarcinoma, synchronous</td>
<td>Operation</td>
<td>Death at 12 months</td>
</tr>
<tr>
<td>31</td>
<td>2001</td>
<td>Shen et al.</td>
<td>66</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Death at 12 months</td>
</tr>
<tr>
<td>32</td>
<td>2001</td>
<td>Shen et al.</td>
<td>53</td>
<td>Retroperitoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Death at 1 month</td>
</tr>
<tr>
<td>33</td>
<td>2001</td>
<td>Shen et al.</td>
<td>40</td>
<td>Pelvis</td>
<td>Heterologous</td>
<td>Fullopian tube carcinoma, metachronous</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>34</td>
<td>2001</td>
<td>Shintaku and Matsumoto</td>
<td>51</td>
<td>Pelvic peritoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation CT (epirubicin, carboplatin)</td>
<td>Survival for 42 months</td>
</tr>
<tr>
<td>35</td>
<td>2002</td>
<td>Wei et al.</td>
<td>67</td>
<td>Omentum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation CT (cisplatin, etoposide)</td>
<td>Liver metastasis after 8 months</td>
</tr>
<tr>
<td>36</td>
<td>2002</td>
<td>Sumathi et al.</td>
<td>77</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>Benign endometrial polyp, synchronous</td>
<td>Operation</td>
<td>Death at 2 hours</td>
</tr>
<tr>
<td>37</td>
<td>2002</td>
<td>Sumathi et al.</td>
<td>87</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation</td>
<td>Unknown</td>
</tr>
<tr>
<td>38</td>
<td>2002</td>
<td>Dincer et al.</td>
<td>50</td>
<td>Pelvic peritoneum</td>
<td>Heterologous</td>
<td>None</td>
<td>Operation CT (cisplatin, ifosfamide)</td>
<td>Unknown</td>
</tr>
<tr>
<td>39</td>
<td>2004</td>
<td>Booth et al.</td>
<td>71</td>
<td>Retroperitoneum</td>
<td>Homologous</td>
<td>None</td>
<td>Operation RT</td>
<td>Survival for 8 months</td>
</tr>
<tr>
<td>40</td>
<td>2005</td>
<td>Ko et al.</td>
<td>45</td>
<td>Pelvic peritoneum</td>
<td>Homologous</td>
<td>none</td>
<td>Operation RT CT (cisplatin, ifosfamide)</td>
<td>Disease free for 60 months</td>
</tr>
<tr>
<td>41</td>
<td>2005</td>
<td>Mikami et al.</td>
<td>53</td>
<td>Mesentery</td>
<td>Heterologous</td>
<td>Fullopian tube carcinoma, synchronous</td>
<td>Operation CT</td>
<td>Survival for 6 months</td>
</tr>
<tr>
<td>42</td>
<td>2005</td>
<td>Shaco-Levy</td>
<td>85</td>
<td>Omentum</td>
<td>Heterologous</td>
<td>Colonic adenocarcinoma, synchronous</td>
<td>Operation</td>
<td>Survival for 3 months</td>
</tr>
<tr>
<td>43</td>
<td>2006</td>
<td>Current case</td>
<td>62</td>
<td>Mesentery</td>
<td>Homologous</td>
<td>Ovarian adenocarcinofibroma, synchronous</td>
<td>Operation CT (cisplatin, etoposide)</td>
<td>Survival for 6 months (still alive)</td>
</tr>
</tbody>
</table>

CT: Chemotherapy; RT: Radiotherapy; MI: Myocardial infarction.
Malignant mixed müllerian tumor of primary mesenteric origin associated with a synchronous ovarian cancer etc.

Discussion

MMMT generally arises in the female reproductive organs of the müllerian system, including the uterine, ovaries, fallopian tubes, cervix and vagina consecutively in frequency. The incidence of MMMT is extremely low, accounting for about 2-5% of all tumors arising from the uterine area and about 1% of those arising from other female reproductive organs. Extragenital origin is even rarer, which was first described by Ober and Black in 1955 [1]. In the English literature, there have been only 42 other cases reported until now [1-31]. It was previously described as occurring on peritoneal surfaces, including visceral peritoneum of the cecum, the rectosigmoid colon, the parietal peritoneum of the abdomen, pelvis and diaphragm, and the retroperitoneum. This is the second patient with MMMT originating primarily in the mesentery, which was first reported by Mikami et al. in 2005 [30].

Among all the cases reported, the majority were postmenopausal with a median age of 64 years (range 33-87 years). There were 14 out of 43 patients (32.6%) with synchronous or metachronous colon cancer (3 cases) or tumors of müllerian duct origin, including ovarian tumors (4 cases of cancer, including the present case, and 1 case of benign tumor), fallopian tube cancer (3 cases), endometrial tumors (2 cases of cancer and 1 case of benign polyp) and cervical cancer (1 case). This may indicate that either MMMTs could be found incidentally when treating gynecological tumors or the female genital organ should be checked carefully when managing MMMTs, especially at the time of surgery.

The risk factors for MMMT include obesity, nulliparity, and exogenous estrogen, similar to those for endometrial carcinoma [32]. Long-term use of tamoxifen, a synthetic nonsteroidal triphenyl antiestrogen with partial estrogenic effects serving as hormone therapy for breast cancer, is another risk factor for MMMT [33-36]. Curtis et al. [33] determined that the relative risk was 4.62 for MMMT and increased 8-fold for breast cancer patients surviving five years or longer. McCluggage et al. [34] reported 19 patients who had used tamoxifen for one to 15 years (median: 7.1 years) developed MMMT, and Kloos et al. [35] reported five patients who had used tamoxifen for five to 20 years (median: 9 years). Seven out of 43 patients (16.3%) altogether had endometriosis,
and Dincer et al. [27] also suggested that MMMT seems to be associated with endometriosis. It was reported that MMMT could occur following irradiation [5, 13, 17, 23, 37, 38]. Callister et al. [38] analyzed 300 patients with MMMT of the uterus and 32 patients (11%) had a history of previous pelvic irradiation. The median interval from radiotherapy to development of MMMT was 14 years (range 1–43); however, the role that radiotherapy plays in MMMT is still unclear.

The treatment of MMMT is generally based on the experience of treating sarcomas of the uterus. The prognosis is poor and survival is usually several months to less than a year, however, some patients could survive till 21 to 42 months with aggressive treatment comprising surgery, chemotherapy, and radiotherapy. Ohno et al. [12] reported a complete response to cyclophosphamide with surgery, chemotherapy, and radiotherapy. Ohno experience of treating sarcomas of the uterus. The prognosis of MMMT is still unclear. The role that radiotherapy from radiotherapy to development of MMMT was 14 years (range 1-43); however, the role that radiotherapy plays in MMMT is still unclear.

Conclusion

Extragenital MMMTs are rare and usually associated with female reproductive tumors. The female reproductive area should be well investigated during surgery of MMMTs or of gynecologic tumors. Although the prognosis is poor, long-term survival can be achieved with treatment by surgery, chemotherapy and radiotherapy in some cases.

References


Address reprint requests to:
J.Y. WANG, M.D.
Department of Surgery, Faculty of Medicine,
College of Medicine and Kaohsiung Medical University Hospital,
Kaohsiung Medical University,
100 Tzyou 1st Road,
Kaohsiung 8007, Taiwan
e-mail: cy614112@ms14.hinet.net
Pelvic actinomycosis mimicking ovarian malignancy: three cases

S.E. Akhan1, M.D.; Y. Dogan1, M.D.; S. Akhan2, M.D.; A.C. Iyibozkurt1, M.D.; S. Topuz1, M.D.; O. Yalcin1, M.D.

1Department of Obstetrics and Gynecology, Istanbul Faculty of Medicine, Istanbul University
2Department of Infectious Disease and Clinical Microbiology, Medical School, Kocaeli University, Istanbul (Turkey)

Summary

Objective: Three cases of pelvic actinomycosis initially diagnosed as pelvic malignancy and treated surgically are reported. Cases: The first case was a 38-year-old multiparous woman who was referred to our clinic because of bilateral ovarian solid masses. With the impression of ovarian carcinoma, a laparotomy was performed. During surgery adhesiolysis, total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, appendectomy, peritoneal washings, and peritoneal abscess drainage were performed. The second patient was a 37-year-old woman who presented with a left-sided fixed solid mass highly suggestive of pelvic malignancy. Both ureters were found to be dilated with hydropnephrosis in the right kidney supporting the diagnosis of retroperitoneal fibrosis. Excision of the mass, colectomy and temporary diverting colostomy and stent insertion to the left ureter were performed. Colostomy repair was performed five months later. On the fifth day postoperatively, fascial necrosis developed so a Bogota-bag was placed on the anterior abdominal wall and left for secondary healing. The third patient was a 51-year-old postmenopausal woman incidentally diagnosed as having a pelvic mass while having been investigated for constipation and nausea. She had had a colostomy one year before and a reanastomosis two months after. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. In all cases, histopathologic staining of the specimens revealed chronic inflammation containing actinomycosis abscesses confirmed with microbiologic identification. Conclusion: Pelvic actinomycosis is an uncommon cause of a pelvic mass. However, it should be kept in mind in the differential diagnosis of pelvic masses, especially in the patients with a history of IUD use to avoid an unnecessary extensive surgical procedure.

Key words: Pelvic actinomycosis; Ovarian carcinoma; Retroperitoneal fibrosis; Colon carcinoma; IUD.

Introduction

Actinomycosis is a slowly progressive bacterial infection caused by a variety of gram-positive filamentous anaerobic or microaerophilic rods, genus Actinomyces, most commonly by Actinomyces israeli. It has the ability to cause suppurrative lesions and diseases that have the classical actinomycotic clinicopathologic stigmata: granulation tissue, severely dense fibrosis, multiple small abscesses and draining sinuses. Once established, actinomycosis spreads contagiously in a low, progressive manner infiltrating the tissue planes. The disease usually presents as an abscess or a mass lesion that is often fixed to underlying tissue and mistaken for a tumor [1].

Actinomycotic infection of the pelvis occurs most commonly in association with an IUD [2]. Symptoms are typically indolent such as fatigue, anorexia, weight loss and abdominal pain. The earliest stage of disease progresses to a limited pelvic mass or tuboovarian abscess. Since the diagnosis is often delayed, a frozen pelvis mimicking malignancy may develop by the time of recognition.

Definitive diagnosis is usually established on the basis of the histology of infected tissue obtained by biopsy or culture of Actinomyces spp. or both. A correct diagnosis is almost impossible based on clinical findings alone, and actinomycosis is often not considered in the differential diagnosis because of the rarity of the condition.

We describe three cases of pelvic actinomycosis treated surgically, diagnosed as pelvic masses suggesting malignancy preoperatively.

Case Reports

Case 1

A 38-year-old gravida 3, para 2 woman was referred to our clinic with weight loss and fatigue of three months duration. With the diagnosis of typhoid fever, she had been treated with ciprofloxacin for 14 days. Aside from typhoid fever, her past history was unremarkable and her periods were regular.

On admission, her temperature was 37.2°C. Her blood pressure was 110/70 mmHg and her pulse was regular at 80 beats/min. Gynecologic examination and ultrasound showing bilateral fixed adnexal masses were confirmed with abdominopelvic magnetic resonance imaging (MRI) which showed 6-cm heterogeneous solid masses with focal areas of diminished attenuation in the left adnexal area as well as right-side involvement. A 3-cm solid mass in the right adnexal area infiltrating the right ureter was also noted. Laboratory data included hematocrit of 26%, white blood cell count of 10,200 mm3/ml with all the other parameters including tumor markers within normal limits.

The patient was referred for surgery with the clinical diagnosis of a pelvic mass suspicious for malignancy despite a true-

Revised manuscript accepted for publication August 23, 2007
A highly vascularized mass densely adhering to the rectum obliterating the Douglas pouch. The left tube and ovary seemed to be stuck to the peritoneum at generally with a normal uterus. The right ovary contained an 8-cm solid mass conglomerated with the bowels on the left side and peritoneal washing, and peritoneal abscess drainage were performed.

Peritoneal abscess culture demonstrated actinomycosis. The patient was discharged on the 8th day postoperatively in a stable condition with intravenous high-dose penicillin treatment for six months. Her follow-up has been uneventful.

**Case 2**

The second patient was a 37-year-old, gravida 3, para 3 woman who presented with vertigo and fatigue for the previous two months. Her menstrual cycles were regular, and her past history and familial history were unremarkable.

The vital signs were stable and the patient was afebrile. A left-sided nontender fixed firm mass arising from the left side of the pelvis highly suggestive of malignancy was found at gynecologic examination under anesthesia. An abdominopelvic MRI showed the presence of a cystic lesion of 8 cm lateral to the right-sided hydronephrosis expanding to the pelvic rim, both ureters dilated, and irregular soft tissue densities lying retroperitoneally, suggesting retroperitoneal fibrosis in the differential diagnosis (Figure 1). The patient’s hematocrit was 28%, and leucocytosis of 12,600 mm$^3$/ml was present. Tumor markers and all other parameters were within normal limits.

Exploratory laparotomy revealed an approximately 20-cm solid mass conglomerated with the bowels on the left side and the left ovary and tube densely adhering to the pelvic wall laterally with a normal uterus. The right ovary contained an 8-cm multiloculated serous cystic lesion with smooth contours, and the left tube and ovary seemed to be stuck to the peritoneum at the posterior side of the uterus obliterating the Douglas pouch. A highly vascularized mass densely adhering to the rectum was excised, and colectomy and temporary diverting sigmoid colostomy were performed. A stent was inserted into the left ureter via cystoscopy. Frozen section of the mass did not contain any malignant cells but did show fibrosis. Histopathologic staining of the specimen confirmed chronic inflammation containing actinomycosis abscesses. The patient was discharged on the 13th day postoperatively with a treatment plan of penicillin for six months.

Colonic reanastomosis was performed five months later. A foul smelling purulent material draining from the subcutaneous hemovac drain and a temperature rise in the 5th day postoperatively were the warning signs of fascial necrosis. The patient’s temperature was 39°C. Leucocytes were 18,100 mm$^3$/ml and CRP was 179 mg/l. Immediate exploratory laparotomy was performed: anastomosis was intact, and anterior abdominal fascia seemed to be necrotic. The necrotic tissue was debrided and a Bogota-bag was placed on the anterior abdominal wall, and left for secondary healing with daily dressings. Cultures collected from necrotic tissues revealed ampicillin resistant *Escherichia coli*. After 14 days of ceftriaxone 1 x 2 g IV, metronidazole 4 x 500 mg IV treatment, her WBC was 11,400 mm$^3$/ml and CRP was 5 mg/dl. She was discharged on the 35th day postoperatively with the abdominal wall almost closed and her general condition improved.

**Case 3**

The third patient was a 51-year-old postmenopausal woman who was referred from the general surgery clinic due to detection of pelvic masses while being evaluated for constipation and nausea. She had been on a triple antibiotic regimen of metronidazole, ceftriaxone, and penicillin for 14 days for suspected pelvic inflammatory disease. In her obstetric history there were two vaginal deliveries and six curettages.

An important note in the medical history was the colostomy operation one year before because of a bowel injury at explorative laparotomy for suspected colon carcinoma and reanastomosis two months later. One year before, she had been admitted to a clinic with the complaint of constipation; the proctosigmoidscopy revealed a hard fragile mass compressing the mucosa 6 cm above the anal sphincter as well as severe narrowing of the bowel lumen 15 cm above the anal sphincter resulting from extrinsic compression. Biopsies revealed edema and focal mucosal erosion but no malignancy. Besides the 5-cm cystic mass containing multiple septations and soft tissue densities in the right adnexal area, her abdominopelvic computed tomography (CT) showed an 8-cm mass with poorly defined margins constricting the rectal lumen and infiltrating the perirectal space. To rule out malignancy and to relieve her symptoms she was submitted to surgery. At laparotomy, the mass extended to the pelvic sidewall involving the uterus and bladder. Colectomy and sigmoid loop colostomy were performed. Histopathological specimens revealed chronic inflammatory granulation tissue, chronic colitis and fibrosis. Two months later, colonic reanastomosis was performed. In her follow-up, symptoms of constipation and nausea recurred so proctosigmoidoscopic balloon dilatation was performed twice.

The patient was referred to us because of the adnexal mass detected at CT. On admission, her body temperature was 36.6°C and blood pressure was 130/80 mmHg. Gynecologic examination revealed a frozen pelvis. CT imaging showed a 4-cm cystic mass with thick walls in the right adnexal area adhering to the ovary, uterus and bowels. Proximal rectal wall thickening, which caused narrowing of the lumen and posterior bladder wall thickening, was also seen. Leukocyte count was 7300, CRP was 1 mg/l with all the other parameters including tumor markers within normal ranges.

Ileal and colonic segments were adhering to the anterior...
abdominal and pelvic sidewall and posterior wall of the uterine corpus at laparotomy. The bladder was adhering to the entire anterior wall of the uterus ascending to the fundus. Both ovaries and fallopian tubes were stuck to the bowels superiorly and the pelvic sidewalls laterally. Adhesiolysis, total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. Histopathological samples were correlated with the cultural identification of chronic actinomycotic infection. She was discharged on the 13th day postoperatively with ampicillin 4 x 1 g treatment in good general condition. Six months of oral penicillin treatment was planned. Her follow-up has been uneventful.

**Discussion**

An anaerobic, gram-positive, branching filamentous bacterium *A. israeli*, a common inhabitant of the mouth, often cultured from the gastrointestinal tract, bronchi, and female genital tract, is the microorganism responsible for most cases of actinomycosis. It acts as an opportunistic infection, usually with other bacterial invasion. It tends to follow a break in the normal mucosal barrier. More than 50% of actinomycosis infections occur in the cervicofacial region. Other sites are the thoracic region (22%), ileocecal region (15%), and less commonly other locations (6%) [2].

Actinomyces colonization and pelvic abscesses occur most commonly in women using an intrauterine device (IUD) which was the common history of our three cases. In a systematic review, Fiorino identified 92 actinomycotic IUD-associated abscesses reported from 1926 to 1995 [3]. Gupta and associates identified Actinomyces in a Pap smear of a woman with an IUD in 1976 [4]. Later investigations of pap smears among IUD users reported a prevalence of Actinomyces-positive smears ranging from 0% to 31%, with an average of 7% [3, 5]. In a review, Lippes emphasized that removal of the IUD of a patient with a positive culture is not necessary and in the absence of evidence for pelvic infection, antibiotics are not required [6]. Actinomycotic abscesses are extremely rare so the pap test has a high false-positive rate and an extremely low positive-predictive value. Furthermore, only half of the women with abscesses described in published reports had had a previous pap test that was positive for Actinomyces-like organisms. In predicting Actinomycotic abscess formation, the prognostic significance of a pap test is minimal [7].

The atypical, noninfectious nature of the clinical presentation is a challenge to clinicians in distinguishing pelvic actinomycosis from intraabdominal or pelvic malignancies. The presenting symptoms are often non-specific, such as weight loss, lower abdominal pain, and fatigue, as in our patients. Generally, neither fever nor leukocytosis is present. The diagnosis of abdominopelvic actinomycosis is seldom made preoperatively (10%) [8]. Because of its chronicity and ability to cross conventional tissue planes, it will often simulate malignancy in a range of sites, including the genitourinary tract. Only a few case reports have been published in which an advanced actinomycotic pelvic infection mimicked a pelvic malignancy [8-13]. Hoffman et al. reported two cases of actinomycotic pelvic inflammatory disease simulating advanced ovarian carcinoma and advanced cervical carcinoma [9]. Perlow et al. reported a case of disseminated pelvic actinomycosis presenting as metastatic carcinoma associated with the Progestasert IUD [10]. Like our first case, Powell et al. reported the case of a patient with a large pelvic mass simulating ovarian carcinoma both by clinical presentation and by imaging modalities [11]. As a result of misdiagnosis, both patients were treated surgically.

The infection can be infiltrative, causing marked induration and extensive fibrosis. In the literature, there are cases with retroperitoneal fibrosis like the second case we presented [14, 15]. Milam et al. described a case with right hydronephrosis and abdominal CT images showing a right retroperitoneum consistent with retroperitoneal fibrosis as in our second case. In that case, right ureteral stent placement and true-cut biopsies were performed confirming the diagnosis of idiopathic retroperitoneal fibrosis. Worsening of the patient’s condition on methylprednisolone treatment was the cause for proceeding to exploratory laparotomy which revealed a tuboovarian abscess [15]. Ureteral stricture [16, 17, 19] and rectal stricture [20] due to pelvic actinomycosis have been reported. Like our second patient, Haj et al. presented a case with right hydro-ureteronephrosis and a large board-like pelvic mass infiltrating the retroperitoneum, involving the distal part of the right ureter. In their case, a diverting sigmoidostomy was created and the ureter was drained by a pig-tail catheter introduced through the urinary bladder, as in our patient. Invasion of infection and fibrosis beyond the abdominal viscera may necessitate extensive surgery including ureteral stent insertion and colostomy which was the procedure performed in two of our cases.

Cases mimicking colon cancer have also been reported [20, 21]. Like the third patient we have presented, Rose et al. reported a case with an abdominal mass that after colonoscopy the preoperative presumptive diagnosis was carcinoma of the colon [20]. The same surgical intervention – colectomy and colostomy – was the operation performed in both their case and ours. Also Scribner et al. described a patient with a pelvic mass suggesting colorectal carcinoma revealing actinomycosis after colectomy [8].

The infection is characterized by abscesses or indurated masses with hard, fibrous encasement and soft central loculations that contain purulent debris. Microscopically, a typical actinomycosis abscess consists of an outer zone of granulation around central purulent loculations that contain variable numbers of granules. The granulation zone consists of thick cellular tissue containing collagen fibers, fibroblasts, lymphocytes, plasma cells, and sometimes, giant cells. Necrosis is rarely seen. The central zone is characterized by typical ‘sulfur’ granules, which is of great significance for diagnosis [22]. With routine culture techniques it is difficult to identify this fastidious, obligate anaerobic and slowly growing bacterium.
Imaging modalities have a minimal contribution in the differential diagnosis. However Ha et al. published a retrospective study of ten patients whose CT scans showed predominately solid masses with focal areas of diminished attenuation as in our first patient, or cystic masses with thickened walls as in our third patient. The aggressive nature of invasion and infiltration of contagious tissues is confirmed with CT as seen in our patients [23]. In the literature there is only a small number of published data about abdominopelvic MRI of actinomycosis. In a case report, Hawnaur et al. reported that relatively low signal intensity in T2-weighted sequences in abdominopelvic MRI of a tuboovarian mass may suggest a fibrotic process rather than malignancy [24].

The disease has an excellent prognosis with appropriate antibiotic management. Since antimicrobial therapy alone can cure extensive disease, it is unclear how often surgical intervention is actually necessary. However, surgical intervention can still play a role in facilitating recovery in selected patients and is useful to rule out malignancy in some instances. Penicillin is the drug of choice. A prolonged treatment regimen is required because of the poor penetration of antibiotics into the fibrotic tissues. Dose recommendations include 18 to 24 million units of penicillin intravenously for two to six weeks, followed by oral therapy with penicillin or amoxicillin for six to 12 months. If therapy is extended beyond the point of resolution of measurable disease, the risk of relapse will be minimized [1]. All our patients responded well to penicillin treatment without recurrence. Tetracycline, minocycline, erythromycin and clindamycin are alternatives for penicillin-allergic patients.

Conclusion

Actinomycosis remains a rare cause of pelvic masses, but should be kept in mind in the differential diagnosis. A previous history of IUD use may be the only clue directing the clinician to such a rare condition which has a favorable prognosis with prior antibiotic therapy instead of extensive surgery.

References


Address reprint requests to:
S.E. AKHAN, M.D.
Department of Obstetrics and Gynecology
Istanbul Faculty of Medicine
Istanbul University
34290 Capa-Topkapi
Istanbul (Turkey)
e-mail: akhan93@hotmail.com
A case of endometrial carcinoma arising in a 36-year-old woman with uterine atypical polypoid adenomyoma (APA)

K. Bakalianou, N. Salakos, C. Iavazzo, G. Paltoglou, K. Papadias, A. Kondi-Pafiti

2nd Department of Obstetrics and Gynecology, University of Athens, Aretaieion Hospital, Athens (Greece)

Summary
Atypical polypoid adenomyomas are tumors of low malignant potential. We present a case of endometrial carcinoma arising in a 36-year-old woman with atypical polypoid adenomyoma. The diagnosis and treatment of such a tumor is discussed through an English literature review.

Key words: Atypical polypoid adenomyoma (APA); Endometrial cancer; Differential diagnosis; Treatment; Prognosis.

Introduction
Endometrial polyps are lesions of the endometrial cavity which usually present with abnormal uterine bleeding. A less usual category of such lesions are atypical polypoid adenomyomas (APA). Longacre et al proposed that these lesions could be designated as low malignant potential tumors [1]. The differential diagnosis should be made from atypical endometrial hyperplasia, infiltrating carcinoma or malignant mixed mesodermal tumors.

Case Report
We present a case of a 36-year-old nulliparous obese woman with a history of menometrorrhagia for the previous three years. Pelvic examination was normal. The Papanicolaou smear was negative and transvaginal ultrasound showed a hyperechogenic lesion measuring 30 x 20 mm with poorly defined margins. The patient underwent dilatation and curettage which revealed an endometrioid endometrial cancer with grade 1 squamous differentiation. The patient underwent further investigation. Tumor markers (CA 19-9, CA15-3, CA125) were within normal ranges. MRI showed a hypointense endometrial lesion with hyperintense foci measuring 3.6 cm on T2-weighted magnetic resonance (MR) images and computed tomography (CT) revealed no enlarged lymph nodes. The patient underwent a total abdominal hysterectomy with bilateral salpingo-oophorectomy plus bilateral lymphadenectomy up to the level of the common iliac arteries. Cytologic analysis of free peritoneal fluid was negative. The histologic diagnosis was APA (Figure 1) with focal areas of invasive endometrioid endometrial carcinoma grade 1 (Figure 2) which invaded less than half of the endometrium (Stage Ia). No lymph node metastasis was found. The decision of the oncologists was follow-up with no need of further treatment. The patient was free of disease six months postoperatively.

Discussion
APAs are usually found in premenopausal women. However, in the literature we were able to find some cases of APA in postmenopausal women [2-4]. Longacre et al. in 1996 reported that 96% of 55 patients with APA were premenopausal with a median age of 39 years. In the same study, 28/55 patients were nulliparous, 15/55 had a history of infertility and 13/55 were obese [1]. Moreover, it should be mentioned that APA has also been described in patients with Turner’s syndrome [5]. For these reasons, hyperestrogenism could be proposed to be the pathogenetic mechanism. The question which arises is whether the lesion is related with endometrial cancer. Our case is one of the rare cases of the coexistence of endometrial cancer with APA [6-8]. It was found that some APAs exhibit MLH-1 promoter hypermethylation with focal lack of MLH-1 immunostaining, a molecular abnormality involved in the transition from complex atypical hyperplasia to endometrioid adenocarcinoma [9].

It should be noted that ultrasound characteristics of APAs (heterogeneous or homogeneous isoechogenic polypoid tumors with solid areas ± cystic areas and poorly defined margins) can not usually help in making the diagnosis preoperatively as the clinical suspicion should be increased to do so [10]. Furthermore, Kimura et al. suggested that even endometrial smears or biopsies might be inaccurate methods for the diagnosis of APA [11].

Mazur et al. reported the histologic characteristics of APAs in 1981. Microscopically APA is characterized by atypical and hyperplastic glands within stroma which are surrounded by smooth muscle [12, 13]. Cytologic atypia is characterized by enlarged nuclei with prominent nucleoli. The smooth muscle might show some mitotic activity (< 2 mitoses/10 hpf). Sometimes extensive squamous metaplasia with central necrosis may coexist, a fact which raises suspicions of carcinoma. However, carcinomas usually show greater cytologic atypia and more glandular crowding and architectural complexity. It should be noted that the stroma in APA and adenocarcinoma may show similar immunohistochemical markers such as smooth muscle actin, desmin and CD 34 [14].
APA is a low malignant potential tumor which could be cured by curettage in order to permit the preservation of fertility. Furthermore, Vilos et al proposed hysteroscopic resection of such tumors as a fertility sparing option [15]. In a recent case report from Hong Kong, a live pregnancy is described in a woman with APA [16]. However, it should be mentioned that Longacre et al. in their retrospective study found a 45% recurrence rate in APAs treated conservatively. For this reason, close follow-up of these patients is proposed so that such recurrences can be found in early stages.

References

Immature teratoma in pregnancy: a case report and literature review

A. Daponte1, M.D., F.C.O.G., E. Kostopoulou2, M.D.; A. Zavos1, M.D.; H. Skentou1, M.D.
A. Kallitsaris1, M.D.; G. Koukoulis2, M.D.; I.E. Messinis1, M.D.

1Department of Obstetrics and Gynecology, 2Department of Pathology, University Hospital of Larissa (Greece)

Summary

Background: The management of a Stage I immature teratoma during pregnancy with a review of the literature is reported. Case Report: A growing adnexal mass was removed at 12 weeks of gestation. Although the frozen section was negative, because of intraoperative clinical suspicion, a right salpingo-oophorectomy and surgical staging were performed. Histological examination revealed a Stage Ia, grade 1 immature ovarian teratoma. Appropriate surgical staging enabled avoidance of chemotherapy despite the unexpected histological diagnosis. The pregnancy was terminated because of fetal distress, with cesarean section at 34 weeks of gestation. At that time the peritoneal cavity was inspected and biopsies were taken as in second-look laparotomy. Two years after the first operation the patient remains disease free. Conclusion: For adnexal masses removed during pregnancy frozen section is useful but when there is clinical suspicion surgical staging must be performed.

Key words: Immature teratoma; Pregnancy; Adnexal mass in pregnancy; Explorative laparotomy; Surgical staging.

Introduction

Germ-cell malignancies are just as common as epithelial ovarian malignancies in pregnancy [1].

Immature ovarian teratomas are germ cell tumors, characterized by a variety of tissues derived from all three germ cell layers. Most commonly, the immature elements are of neural origin [2, 3]. The occurrence of immature teratoma with a coexisting pregnancy is exceedingly rare. Only 15 papers could be detected in the world literature [3-17] (Table 1). The management of a Stage I, grade 1, (only three reported as yet) immature ovarian teratoma in a 33-year-old pregnant woman is reported and the literature reviewed.

Case Report

A 5-week pregnant 33-year-old primigravida was referred to our hospital because of a 7 x 7 cm mass in the right adnexa. The tumor was increasing in size at the examinations and at the 12th week it measured 20 x 20 cm. The tumor was by then multilocular with both solid and cystic components. Two tumor markers were slightly elevated; serum alpha-fetoprotein was 15.94 IU/ml (normal range 0-7 IU/ml outside pregnancy), and serum CA-125 was 89.6 U/ml (normal range 0-35 IU/ml). Laparotomy with frozen section was performed. Ascites was absent, the contralateral ovary and tube had normal morphology and there were no macroscopic implants or palpable lymph nodes in the peritoneal cavity. Although the frozen section was negative for malignancy a right salpingo-oophorectomy and surgical staging (peritoneal washings, peritoneal and omentum biopsies) were performed because of clinical suspicion of a non benign tumor. Histological examination revealed a grade 1 immature ovarian teratoma with rare nests of neuroepithelial tissue (limited to low magnification field in every slide (x 40) according to the criteria of Norris et al. [19] (Figure 1). Peritoneal washings and all biopsies were negative for malignancy. Thus surgical stage was designated as FIGO Stage Ia (AJCC TNM and FIGO staging classification 2002) [20] and after discussion in the Multidisciplinary Oncology Meeting no adjuvant treatment was given. The pregnancy continued without complications until 34 weeks of gestation, when the patient gave birth to a healthy infant by cesarean section which was indicated for fetal distress. Cesarean section was performed through a midline incision, the peritoneal cavity was inspected, and biopsies were taken as in second-look laparotomy. Laboratory tumor markers were at normal levels during the pregnancy and at the time of the cesarean section. Two years after the first operation the computed tomography and tumor markers were negative for any recurrence of the teratoma.

Discussion

Already in 1963 Munnell [20] suggested that removal of an adnexal mass during pregnancy was indicated for: 1) elimination of a possible cause of dystocia, 2) danger of torsion, rupture, or hemorrhage, and 3) danger of malignancy.

Our patient had a growing complex adnexal mass (> 30% increase) [2, 21] with suspicious ultrasound characteristics which persisted after the 12th week [2, 22-24]. Fetal survival is markedly improved if surgery is delayed until after the 12th week of gestation as in our case, since up to one-third of all surgeries performed in the first trimester may result in spontaneous abortion [25, 26].

AFP and CA-125 were found slightly elevated but it would be difficult to indicate laparotomy on this finding alone. CA-125 can be elevated in the first trimester of pregnancy [22]. In cases of germ cell tumors of either gonadal or extragonadal origin only AFP levels > 7.0
Table 1. — Literature review of immature teratoma in pregnancy.

<table>
<thead>
<tr>
<th>Name</th>
<th>No. of Age</th>
<th>Patient findings, outcome, surgery, time type</th>
<th>Stage</th>
<th>Grade</th>
<th>Chemotherapy/ Radiation</th>
<th>Fetal outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Klein 1953</td>
<td>1 26</td>
<td>Growing tumor of the ovary</td>
<td>31st excision of the tumor of the (L) ovary</td>
<td>Frozen and permanent sections.</td>
<td>Radiation.</td>
<td>At SC fetal good</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33rd week preeclampsia metastasis found; 8 months after initial surgery carcinoma lung, abdominal cavity, ascites; Death 10 months after initial surgery</td>
<td>SC and excision of remaining ovary</td>
<td>Benign adult teratoma.*</td>
<td>Radiation, no chemotherapy</td>
<td></td>
</tr>
<tr>
<td>2 Robboy Scully 1970</td>
<td>1 18</td>
<td>Recently postpartum left ovarian teratoma; Recurrence 4 months postoperatively; Death 9 months postoperatively; Autopsy revealed metastatic carcinoma in the lungs and lymph nodes. Glial tissue lungs, peritoneum, pericardium, pleura.</td>
<td>Hysterectomy BSO</td>
<td>a IIc G3</td>
<td>Postoperatively RT therapy; After the recurrence thio-TERA and later methotrexate</td>
<td>Postoperatively RT therapy; After the recurrence thio-TERA and later methotrexate</td>
</tr>
<tr>
<td>3 Montz et al. 1989</td>
<td>1 27</td>
<td>19th week AFP &gt; 2.5 MoM. Amniotic AFP level normal. 21st week complex mass in the cul-de-sac</td>
<td>22nd week exploratory laparotomy, (R) SO pelvic washings, intraperitoneal evaluation and retroperitoneal nodes palpation. No further staging</td>
<td>23-37 weeks, 3 courses vincristine, dactinomycin, cyclophosphamide</td>
<td>3.285 g, female apgar 8.9 (1st, 5th min) alopecia, haemoglobin level normal</td>
<td>Disease-free 1.5 years after initial surgery.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37th week spontaneous vaginal delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No gross or micro malignant disease at second-look laparotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disease-free 1.5 years after initial surgery.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Charles et al. 1989</td>
<td>2 24</td>
<td>13th week gross cystic mass of the ovary; Recurrent at 30th week;</td>
<td>13th week (R) SO</td>
<td>Ia G3</td>
<td>After CS actinomycin, oncovin, cisplatin adriamycin, bleomysine Male 2.430 g cried immediately.</td>
<td>Male 2.430 g cried immediately.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24th week CS + TAH (L) SO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>patient refused second-look laparotomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No recurrence 3.5 years after CS.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Christman et al. 1990</td>
<td>1 29</td>
<td>6th week mixed echogenic mass (R) adnexal</td>
<td>15th week (R) SO and surgical staging</td>
<td>Ic G3</td>
<td>After surgery adriamycin, cyclophosphamide, cisplatin</td>
<td>Good 3.5 years after CS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>At term spontaneous delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>After the 4th cycle of chemotherapy, second-look only mature glial elements.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>61 months post discovery of the teratoma doing well.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>No. of Age</td>
<td>Patient findings, outcome, surgery</td>
<td>Surgery stage</td>
<td>Grade</td>
<td>Chemotherapy/ radiation</td>
<td>Foetal outcome</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------</td>
<td>------------------------------------</td>
<td>---------------</td>
<td>-------</td>
<td>-------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Poremba et al. 1993</td>
<td>1 27</td>
<td>Late in gestation hydrocephalus of fetus was diagnosed; at the 38th week CS was decided during which tumor of the ovary was revealed. Follow-up N/R</td>
<td>a lc</td>
<td>a G1</td>
<td>N/R</td>
<td>Died 9 weeks after delivery. Autopsy revealed intracranial immature teratoma of deferent origin of the mothers.</td>
</tr>
<tr>
<td>O'Connor** 1994</td>
<td>3 N/R</td>
<td>N/R</td>
<td>I N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Whitecar*** et al. 1999</td>
<td>1 N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Quesada et al. 2002</td>
<td>30</td>
<td>At 28th week great mixed echogenic mass (L) adnexal diagnosed with US during examination for bleeding; Conservative management; 34th week elective CS</td>
<td>34th week CS (L) SO staging</td>
<td>High grade (G2 or G3)</td>
<td>Postoperatively (postpartum) 6 cycles of carboplatin, bleomycin, etoposid</td>
<td>Normal 2,430 g male infant was delivered.</td>
</tr>
<tr>
<td>Kishimoto et al. 2002</td>
<td>28</td>
<td>At 35th week palpable mass in Douglas’ pouch. At 38 weeks elective CS.</td>
<td>At 38th week CS, simple TAH-BSO and staging</td>
<td>IIIc</td>
<td>G2</td>
<td>2,308 g foetus, good at CS.</td>
</tr>
<tr>
<td>Agarwal 2003</td>
<td>1 N/R</td>
<td>Mass detected during pregnancy Enormous recurrence during pregnancy</td>
<td>During pregnancy excision of growth</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Barki 2004</td>
<td>2 33</td>
<td>Further-follow-up N/R 10 weeks post abortion abdominal pain and palpable mass 8th week abdominal pain, Died 2nd trimester</td>
<td>(L) SO staging</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Han 2004</td>
<td>1 27</td>
<td>16th week AFP &gt; 7.25 MoM; 18th week AFP &gt; 12.55 MoM; Amniotic AFP level normal; Normal karyotype; 24th week (R) mass. Died of follow-up</td>
<td>26th weeks (R) SO staging</td>
<td>G3</td>
<td>30th weeks, 2 cycles bleomycin, etoposide, cisplatin</td>
<td>Apgar 9, 10 (1st, 5th min), no evidence of gross malformations; 3 cycles after pregnancy 7.5 months of age infant suffered from intussusception; 26 months after birth 13 k normal physiological and neurological development.</td>
</tr>
<tr>
<td>Leiserowitz 2005****</td>
<td>12 N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Zhao 2006</td>
<td>2 24</td>
<td>17th week of gestation adnexal mass; 30 months after operation disease-free survival; 8th week of gestation adnexal mass; 18 months after operation disease-free survival.</td>
<td>17th (L) SO</td>
<td>G1</td>
<td>No chemotherapy</td>
<td>Term infant</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>13th (L) SO</td>
<td>I G1</td>
<td>No chemotherapy</td>
<td>Term infant</td>
<td></td>
</tr>
</tbody>
</table>

CS: cesarean section; B: Bilateral; SO salpingo-oophorectomy; N/R not recorded. *Regions suggesting neuroepithelium and resembling somewhat immature brain substances were found, as also glial structures, but were not so unorganized or irregular as to warrant the diagnosis of malignancy. **Large series for 244 Stage I immature teratomas where the reproducibility of grading was investigated; and it was reported that 3 of the patients were pregnant women. ***Large series for adnexal masses during pregnancy where in one table of 118 cases of adnexal masses in pregnancy; one with a histological diagnosis of immature teratoma was reported. ****Large series of 9,375 adnexal masses during pregnancy where 12 cases of immature teratoma in pregnancy were reported.
MoM in the absence of any fetal malformation or maternal disease, should be considered diagnostic [6].

When trying to preserve the pregnancy a first estimation of the histological type of the tumor with frozen section is useful for planning the extention of the surgery although many times the results from the frozen section are incorrect [4]. Clinical assessment of possible malignancy as in our case can justify more extensive surgery to achieve optimal staging.

Bilateral malignant teratoma was not observed by Norris et al. [18] or in the Gynecologic Oncology Group study; therefore, conservative surgery consisting of unilateral salpingo-oophorectomy and staging is acceptable [27, 28].

All reported cases are summarized in Table 1. Most authors agree that survival is related to the stage and grade of the tumor. Our case is the fourth reported case Stage I/grade 1 in the literature. In order to decide the management we assumed that as in non pregnant women the prognosis seems dependent on the histologic grading and stage of the tumor at the time of discovery [2].

Tumors are graded according to the degree of immaturity of the tissue, and the presence and quantity of neuroepithelium. There are two grading systems: Thulderberg and Scully, and Norris (the most commonly used grading system) [18, 29]. Later on, Norris proposed only two grades to select the treatment in Stage I tumors: low grade for grade 1, and high grade for grade 2 and 3 [10].

Immature teratomas as well as others germ cell tumors can be treated and are very sensitive to chemotherapy [2, 17] with a 75% cure rate for advanced stage disease [6, 8, 15, 30, 31]. In the three reported cases (Table 1), investigators treated patients with Stage Ia/grade 1 immature teratomas as our case with unilateral oophorectomy alone [17] and only patients with high-grade Stage Ia as well as more advanced lesions with chemotherapy post surgically [2, 3]. Chemotherapy is also proposed in incompletely staged patients and can be avoided if no relapse has occurred at second-look laparotomy [14]. Our patient was Stage Ia/grade 1 and because she was staged appropriately we managed to avoid chemotherapy during pregnancy. Also although cesarean section was indicated for obstetric reasons it was performed as a second-look laparotomy and no sign of a recurrence was observed.

In neoplasmatic masses which are removed during pregnancy frozen section is useful but when there is clinical suspicion surgical staging must be performed. It enables, in selected cases, avoidance of chemotherapy during pregnancy.
References


Address reprint requests to:
A. DAPONTE, M.D., FCOG
M. Skylakou, 10
41334 Larissa (Greece)
e-mail: daponte@otenet.gr