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Cervical screening in Hungary: why does the “English model” work but the “Hungarian model” does not?

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Summary

A comparison has been made between the English practice and the “Hungarian model” of cervical screening. In England, until 1986, extensive opportunistic screening was the practice, but – as it had no effect on cervical cancer mortality – afterwards, the screening policy was changed to be strictly in line with international recommendations. On the other hand, in Hungary, the “old practice” has been petrified: gynaecologists are the “gatekeepers”, a “gynaecological examination completed with smear-taking for cytology” makes up the screening strategy. Although in the frame of a National Public Health Programme all the prerequisites for nationwide organised screening have been provided, and an up-to-date screening strategy declared, 20-times as many smears are taken and analysed outside as inside the programme, and the efforts have had no impact on cervical cancer mortality. This is because “old habits die hard”. There is an urgent need to reconsider the screening strategy, and to reorganise the cervical screening practice in Hungary.

Key words: Opportunistic and organised screening for cervical cancer; Role of gynaecologists; Impact on mortality from cervical cancer.

Introduction

Cervical screening, as a public health policy, has proved its effectiveness in terms of reduction of mortality from the target disease in the target population [1] and today its wide application is strongly recommended by international bodies, such as the International Agency for Research on Cancer of the World Health Organization (IARC/WHO), the International Union against Cancer (UICC), and the European Council [2, 3]. Hungary (a Central-Eastern European country, one of the former “Eastern block” countries which joined the European Union in 2004) has made tremendous efforts to develop a nationwide cervical screening programme; however, the difficulties faced in this endeavour are also tremendous. This is because the country has to carry the burden of the past. It may therefore be worthwhile to develop an understanding of the history of cervical screening in Hungary, so that others can avoid the same problems.

History of screening in Hungary

Non-organised or opportunistic cervical screening has a long history in Hungary: it goes back to the late 1950s, and has gone through three phases.

First phase: colposcopy vs cytology

The first phase was characterised by choosing the proper screening tool. Originally, the screening tool was colposcopy alone, applied by oncologists engaged in practicing oncolgical-gynaecology. In 1954, a ministerial decree (MOH 8834/31/1954) was issued which described the mode of operation of the National Oncological Institute and that of the country-wide network of the some 70 oncological care units (consisting of one physician “specialist in oncology”, and one nurse in each, geographically covering the whole country, a unique but ineffective formation in the countries of the “socialistic system”. It stated that “mass screening must be conducted in such a way that each woman over 30 years of age must be screened by colposcopy”. There are no data on effect.

In the mid 1960s cytology as a screening tool had emerged in the developed countries, and in Hungary “colposcopy completed by cytology” had become the screening test. More and more cytology laboratories – based on pathology departments – had been established, and by early 1970, the entire country was fully covered. In 1972, a “School of Cytotechnology” to provide regular training for pre-screeners (5 days a week for 10 months) was established, and the
system of “pre-screening” introduced. The training has continued, and today some 300 fulltime “pre-screeners” are at work. By the end of the 1970s, there was sufficient capacity to carry out three yearly screening for all eligible women: the existing system is in place to meet the demands of mass screening [4, 5].

Second phase: oncologists vs gynaecologists

Screening to begin with was carried out almost exclusively by a limited number of oncologists who were unable to meet the workload demands. In 1978, a joint deliberation/statement was issued by the Board of Gynaecology, saying, firstly that “every gynaecological examination should be preformed as a screening”, in other words, screening had to be considered as an integral part of the gynaecological examination, and that there should be “no cervical screening without cytology” [6]. In practice, smears were taken by gynaecologists as part of a “complex gynaecological examination, including colposcopy” and then, they were analysed in cytology laboratories by pre-screener cytotechnologists, supervised by cytopathologists and finally, reported back to gynaecologists, who – depending on the results – managed the patient. As a result, the gynaecological community had become highly involved. During the 1980s, a country-wide “cervical screening programme” was declared, and extensive opportunistic screening, including a smear-taking tool had taken place [7]. The annual number of smears analysed exceeded one million. The clinical stages of the detected cervical abnormalities had shifted favourably, but the mortality from cervical cancer had not decreased, but did level off at a rather high level (10 per 100,000 population). It was admitted officially that the program had failed [8]. The reason for the failure was perceived to be the lack of individual identification and registration. Only the number of smears examined, and not the women screened had been registered. No one knew who had been screened. Certain women – a self-selected group within the target population (approximately 30% of the eligible population) – had been screened unnecessarily frequently, several times a year, while other women had never been screened.

Third phase: clinical practice vs public health measure

In the mid 1990s, as part of the World Bank-sponsored “close the gap” programme, a secondary prevention sub-component was carried out which covered three primary cancer sites: i.e., screening for cervix, breast, and colorectal cancer, the three sites where scientifically sound evidence of effectiveness is available. The establishment of a National Cancer Registry was part of the exercise [9]. The implementation of a “secondary prevention” sub-component has created a favourable policy-environment for integration of organised population screening as a core function of the health care system, thus a golden opportunity was presented to reorganise and update cervical screening in Hungary. However, at this point in time, the question presented itself as to whether what was called “screening” was a widespread clinical exercise, or a routine public health policy and programme. In the view of the gynaecological community, it seemed to be the former one. In opposition to their standpoint, the public health authority argued for a “state-of-the-art” screening strategy, and held the “English model” up as an example, where to a large extent, and from a historical perspective at the beginning, the situation was comparable to the Hungarian one.

The “English model”

Transition from opportunistic to organised screening

It is a matter of common knowledge today that, in England, the National Health Services (NHS) operates a very effective mass screening programme for cervical cancer [10]. Cervical screening began in the 1960s undertaken by enthusiastic cytologists. By the 1980s many programmes had developed at a local level, but on an ad hoc basis. Over four million smears were analysed each year. However, it was clear that cervical screening was not as effective or well managed as it could be with clear objectives, particularly due to the lack of a national call/recall system to identify and invite eligible women. Some women at greater risk of developing cervical cancer were not screened and some who were screened were not followed up appropriately [11]. The current national system was introduced in 1987. The Department of Health required each health authority in England to establish a computerised call and recall system. It also emphasised the need to introduce good local management and to nominate an individual to be responsible for the organisational effectiveness of cervical screening in each district. The NHS Cervical Screening Programme (NHSCSP) was established to bring together the health departments, professional bodies and designated members to facilitate the adoption of common standards and working practices throughout the United Kingdom.

Organisation of screening

The NHS is the provider of screening; it is funded by general taxation, and free at the point of delivery. Ninety-eight percent of the population is registered with the NHS. The NHS owns the hospitals and employs the doctors who work in the hospitals. The general practitioners are self-employed contractors to the NHS. Primary Care Trusts (PCTs) are groups of general practitioners who cover a certain geographical area. A woman needs to be registered with her general
practitioner to become a beneficiary of the system. The Department of Health gives the money to PCTs in proportion to their population. The PCTs “buy” health services from hospitals, including screening services, for their population.

In each region, screening departments have been set up from where the overall programme managing these departments are responsible for holding and updating the database of the women who are eligible for cervical screening. The source of the notification list for screening is the “Exeter System”, made up from general practitioners’ lists, which contain the personal details of everyone who is registered with the NHS. The name and address of all women eligible for screening is taken from this database which also identifies their general practitioner. The invitation letters are issued by the screening services; however, the primary care personnel play a key role, as they check the completeness and accuracy of the notification lists. Women aged 25-64 years of age are eligible for cervical screening. The screening interval is three to five years according to age. The “non responder cards” are sent to the woman’s general practitioner in the confident expectation that the general practitioner will take an interest in these non-responders and try to encourage them to have a smear test. If no result is received, the woman will remain in the programme and further invitations will be sent at the appropriate interval.

Smear taking and follow-up

Most importantly, the smears are taken in the primary care setting, sometimes by general practitioners but more usually by practice nurses, and sent to the local cytology laboratory, where the smears are registered, processed and analysed. The primary care team is fully advised on the guidelines to be followed and how to take cervical smears. As for the follow-up, an appropriate “failsafe” procedure has been established: the smear takers must assume full responsibility for seeing the result and ensuring that appropriate action is taken, including follow-up for non-attendance. When a positive result is received from the laboratory, they are to initiate follow-up action, i.e., referral to colposcopy for an appropriate gynaecological opinion. Colposcopy clinics are integral to the operation of the programme. Quality standards for colposcopy clinics are set by the national programme although gynaecologists are expected to use their own judgement. Results and management plans are communicated to the referring general practitioner.

Quality Assurance System

Quality assurance is a fundamental part of the NHS Screening Programme. To this effect, a country-wide system has been developed and implemented. The aim of quality assurance is to maintain minimum standards, and encourage continuous improvement in the performance of all aspects of screening to ensure that women have access to high quality screening services wherever they live.

Results

On 10 August, 2006, 79.5% of eligible women aged 25-64, residents of England, had been screened at least once in the previous five years, and resulted in an adequate test result. In 194 out of 303 Primary Care Organisations the coverage was 80% or higher. In 2005-2006, about 4.06 million women were invited for screening, and 3.6 million women were screened by the NHS Cancer Screening Programme. The laboratories examined just under 4 million smears, including early recalls for surveillance (14%), following findings of abnormalities (4%), and inadequate smears (6%). Ninety-two percent of the total were submitted by general practitioners. For women referred to a colposcopy clinic following persistent non-negative smears, 0.1% were found to have invasive, and 0.5% showed in situ cervical cancer (CIN3).

The NHS Cervical Screening programme is remarkably successful. The age-standardised incidence was reduced proportionally to the coverage of the program [12], and, the mortality from cervical cancer decreases by 7% a year; it has already saved a large number of lives and will continue to prevent about 4,500 deaths every year in England [13].

The “Hungarian model”

Organisation of the screening

As a result of the “model programmes” which were carried out on a limited scale, and following a thorough evaluation, a proposal was submitted to the government to introduce evidence-based organised screening as a routine service into the health care system in Hungary. The proposal was well received, and since 2000 three screening modalities – cervical screening among them - have been included in a National Public Health Programme [14]. The responsibility for implementation, coordination, monitoring quality control and evaluation of the screening programmes has been delegated by law to the Chief Medical Officer’s (CMO) office. This office – as a public health authority - is in charge of a countrywide network of medical officers, having an institution in each of 19 counties and the capital, charged with the responsibility for public health issues, population screening being included. In the CMO’s office, a Screening Coordination Department has been established, which supervises the Screening Coordinating Units in the 20 administrative areas. Most importantly, a National Screening Registry has been set up, and receives a population list from the database of the Health Insurance Fund Administration (OEP), comprising personal identification data of virtually the entire
Hungarian population. The list is broken down by county, and sent out to the primary care physicians who are able to validate it for use as a notification list.

The screening strategy

There has been a heated debate with the gynaecological community, which has revolved around three issues: (a) what constitutes a screening test, (b) the age-range during which screening should take place, and (c) the interval between screenings. The Board of Gynaecologists argued in favour of the traditional “gynaecological screening”, meaning a complete gynaecological examination with full colposcopy, and smear-taking for cytology. The physical examination of breasts is also included in the gynaecological protocol [15]. In the Board’s view, screening should start at 18 years of age, or whenever regular sexual activity commences, it should never be discontinued, and should take place once a year. This obviously contradicts the international recommendations of population screening, and there have been calls for reorganisation and updating of existing screening strategies (2). A compromise agreement has been reached on the target age-range and the interval between two consecutive screenings, but not on the method of screening. Accordingly, the screening strategy states that: “after one negative smear, once in every three years, full gynaecological examination comprising both colposcopy and cytology, of women between 25 and 64 years of age”. This means that the gynaecologists remain the “gatekeepers” of population screening.

The screening process

The personal invitation to women between 25 and 65 is centrally issued by a letter, informing the invitee about screening, and what the expected benefits and risks are (“informed decision”). It encourages women to see a gynaecologist, either a private one or one of those listed in an annex to the invitation letter. Conventional smears are taken by a gynaecologist as part of a “complex gynaecological examination, including full colposcopy”. The smears are sent to a cytology laboratory where they are processed and analysed. Cytology is not centralised: some 80 laboratories applied for financing, and some 50 have been accepted. At the end, the test-result is sent back to the gynaecologist who informs the patient. Screening is free for women. The National Screening Registry receives the screening result from the screening units in an aggregate form, because – by the data protection laws – dealing with any information on an individual’s health-related status is strictly forbidden.

The current status of cervical screening in Hungary

The experiences of the first few years of operation are disappointing. Between 2004 and 2006, about 2 million invitation letters were sent out, and fewer than 96,000 women, 5% of those invited, attended for screening. This compliance rate is totally unacceptable! In the meantime, in 2005 alone, the National Health Insurance Fund Administration paid for 960,000 smears taken from 850,000 women as “gynaecological diagnostic cytology” (OENO code 29 601), and only for 40,520 smears originating from screening (OENO code 42 700). The difference is shocking, and requires an explanation, whether the former number really covers “diagnostic gynaecological examinations”, or screening cytology is being reported under a diagnostic financial code. Most likely, the latter is true. Presumably, more women “get moving” under the influence of an the invitation letter, than reflected in the data of the Screening Registry.

Reasons for low compliance

The difficulties in the transition from an extensive opportunistic screening to an organised screening are being reflected in the current problems of population screening.

Traditionally, the “gatekeepers” of opportunistic screening were the gynaecologists, and this has become fixed in the mind of the general public. As a result, without waiting for an invitation letter, or receiving but ignoring one, means smear-taking is done on the occasion of any gynaecologist-patient encounter, disregarding the existence of the organised screening system – it is reported to the financing agency as a “diagnostic exam”, and not as a screening exam. These cases are not registered by the Screening Registry because the cytology laboratories are obliged to report only those smears that turn up with the perforated slip of the invitation letter. Furthermore, only those cytology laboratories contracted by the National Health Insurance Fund Administration for funding are obliged to report. As a consequence, the gynaecologists working in private clinics do not report the activity even though they are estimated to screen about 30% of eligible women. Similarly the cytology labs do not report the work either.

A further reason for the low compliance is that access to “screening facilities” – meaning gynaecologists – is limited. According to the list given by the Board of Gynaecologists, there are “879 workplaces suitable for screening”, however their geographical distribution is rather heterogeneous: gynaecological services are easily accessible in big cities, their number is less in small towns and in the countryside they are virtually non-existent. It is “uncomfortable” for country women to travel to towns for screening. Mobile services would be likely to alleviate this problem.

The outstanding role the primary care physicians could play in mobilising women to accept the screening offered is totally under-utilized. A “target payment” system – similar to that in England – to encourage them to take a greater part is under consideration; scarcity of resources is a major obstacle in this respect.
Discussion
There are similarities and differences in the development and operation of cervical screening programmes in England and Hungary. In both countries, screening started as an extensive non-organised, opportunistic smear-taking activity, which had reached high numbers of cervical smears analysed, but the population targeted was not adequately covered. Therefore, the programme missed those at high risk. As a result, the intensity of screening did not result in the decrease of incidence and mortality. In England, it was realised much sooner that they were on the wrong track, the old practice was discontinued, and an organised cervical screening programme was developed according to the up-to-date international recommendations, adapted to local needs. Good results followed and years later the mortality from cervical cancer started to significantly decrease.

The case of Hungary is different. The fact that we are on the wrong track was realised much later, and was not addressed appropriately. Instead of discontinuing ineffective practice, they have reached a compromise solution. The result is shameful: unacceptably low compliance, and no epidemiological impact on cervical cancer rates.

What could make cervical screening work in Hungary? By now, major progress to develop a country-wide organised cervical screening system capable of regular screening of all eligible women has been made. The management and information system, and the cytological capacity are in place. However, our hands are totally bound by the traditional mode of screening: the insistence of the gynaecological community on their “historical role” seems to be the major impediment to carry out an effective screening programme. There have been attempts to break through. In some selected areas of the country, properly trained midwives and primary care nurses – temporarily, under the supervision of local gynaecologists – are taking the smears. In case of non-negative cytology, full-scale gynaecological examination follows, colposcopy included. The results of the “model” are encouraging; however, there is a long way to go until a “state-of-the-art” cervical screening programme – such as in England – could be delivered in Hungary, because “old habits die hard”.

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The interaction of HPV with the immune system has been studied, but the results are still inconclusive for several reasons. Until now, we have not been able to understand the mechanisms of immune system regulation in the uterine cervix. HPV infection does not unleash an inflammatory response, and consequently an efficient and specific immune response against the virus. Moreover, an understanding of HPV infection and local immune response is indispensable for the development of new bioactive drugs and therapies for patients with both non invasive and invasive tumors, mainly for patients that do not present regression with radiotherapy or chemotherapy or in whom the tumors are surgically unresectable. The aim of this review is to provide support in understanding potential mechanisms used by the immune system to destroy neoplastic cells, comparing the immunotherapy used in cancer and discussing the possibility of developing new drugs based on these mechanisms of action.

Key words: Neoplasia; Cancer; Uterine cervical cancer; Immunotherapy; Human papillomavirus; Interferon; Cellular therapy; Immune system.

Introduction

Uterine cervical cancer is the second most common malignant neoplasm among women and, according to the World Health Organization, it is responsible for 300,000 deaths per year around the world. Its occurrence is only exceeded by skin cancer [1]. However, these situations could be lower, given that uterine cervical cancer could be avoided through detection and treatment of preneoplastic lesions [2].

Epidemiological and laboratory evidence has indicated that human papillomavirus (HPV) can be detected in 85 to 100% of the patients diagnosed with this type of neoplasm [3, 4].

The infection occurs more frequently in young women, particularly in those who start to be sexually active before the age of 18 years, and in those who smoke, are pregnant and have multiple sexual partners [5, 6]. Murta et al. demonstrated in cytologically normal patients that the prevalence of infection declines with increasing age [3, 7] and that, among women who present cytological alterations due to HPV, the percentage regression reaches around 85% of the cases [7, 8]. This same group, studying women with cytological alterations due to HPV, concluded that these lesions could continue to be present for a mean period of four years, in around 13% of the cases. Nonetheless, the risk factors that are thought to promote persistence of this infection need to be better studied [7].

HPV belongs to the family Papovaviridae. It is an epitheliotropic DNA virus that is sexually transmitted and causes approximately 30 million new infections every year. It is present in the lower genital tract of around 10% of women.

This family of viruses possesses tropism for keratinocytes, thereby inducing papillomas as part of its normal life cycle [9]. It is composed of around 100 described subtypes and infects a large variety of organisms in a species-specific manner. Although the majority of the types induce benign papillomas, there are some types that are classified as presenting high risk and which contribute towards the development of anogenital cancer. Some examples of these are HPV 16, 18, 45 and 56, which may give rise to malignant transformation by infecting epithelial cells [4].

Many cases of cervical cancer begin in the transformation zone, a region between the endocervix and ectocervix [10]. The first stage in malignant development is cervical intraepithelial neoplasia (CIN), in which the neoplastic cells have not yet invaded the basal membrane and are premalignant. These precursor lesions can be classified histologically into different grades (CIN I, II and III), according to the extent of the lesion [11]. CIN can regress, persist or progress to invasive cancer, and the latter occurs in varying proportions, ranging from 3% to 30% of the cases, depending on the conditions.
**Immunological characteristics of the female reproductive tract**

The immune system of the female reproductive tract behaves in a site-specific manner, i.e., the response may be different depending on the location and the degree of sterility [12-14]. This hypothesis has also been supported by functional studies using the model of *Chlamydia trachomatis*, which have indicated different T-lymphocyte patterns in the upper and lower tracts during the course of infections [13].

The upper genital tract has both an innate immune response [15] and an acquired immune response [16]. These responses keep the tract in a predominantly aseptic state and rapidly protect the reproductive organs from pathogens, thereby maintaining the function and integrity of the tissues. For this, there needs to be fine-tuning for the immune system, between the response to pathogens and either tolerance to semen or a semi-allogeneic concept. However, a large number of scientific studies will still be needed for the characteristics of the immune response in the uterine cervix to be elucidated.

**Immune response to HPV**

Several components of the innate and adaptive responses are mobilized for recognizing infections caused by HPV and eliminating the cells infected by the virus. The first line of defense consists of the innate immune response, which takes place in the epidermis and in the epithelium of the mucosa [17]. This can be considered to be the non-specific resistance to infection that occurs when the pathogens first come up against the immune system.

The innate immunity to HPV is mediated by several mechanisms, including induction of interferon (IFN) and activation of macrophages and natural killer cells. However, some infections caused by HPV are not rapidly eliminated by the immunity of the mucosa, and chronic expression of this virus at low levels may induce tolerance to the infected epithelium [18]. Several studies have demonstrated that HPV interacts with the immune system [19-21] but, despite this, it is capable of evading or inactivating the acquired immune response [22]. There are various hypotheses for such evasion mechanisms: first, the virus does not have a blood dissemination phase; second, it does not cause lysis of keratinocytes and therefore does not induce an inflammatory response; and third, production and release of the virus takes place in the differentiated squamous cells that are distant from the cytokines and immunocompetent cells of the submucosa.

**Inflammation**

Inflammatory response has a central role in innate immunity, and it is stimulated by cytokines such as IL-1 and TNF-α, which may be synthesized by keratinocytes after some type of injury has been suffered [23]. These two cytokines stimulate changes in the adhesion molecules and capillary permeability, release of other cytokines (chemokines) and also negative regulation of E6 and E7 expression in keratinocytes [24]. Acute inflammatory response leads to elimination of the infection and repair of the damaged tissue, and is responsible for triggering acquired immunity. On the other hand, the chronic inflammation that occurs when the infection persists for a long time has been considered to be one of the risk factors that could trigger various human cancers [25].

One characteristic of HPV infection is the absence of inflammatory response. Recent studies have suggested that some gene products from HPV may directly block the activity of the inflammatory mediators. The E6 protein of HPV 16 inhibits the expression of IL-18, which is proinflammatory [26], and competitively bonds with the receptor for this cytokine [27]. This same protein in the virus also bonds with the receptor for TNF-α and protects the cells from the induction of apoptosis [28].

Even though HPV infection does not readily induce an acute immune response, the expression and release of specific proinflammatory cytokines such as IL-1, TNF-α, IL-12, IL-10 and TGF-β becomes increased during the CIN III stage and in cases of invasive cervical cancer [29-31]. On the other hand, contrary to these findings, cervical carcinoma cells and cells immortalized by HPV *in vitro* present reduced quantities of proinflammatory cytokines [32, 33]. What this clearly demonstrates is that the microenvironment is fundamentally important for the release of these factors.

**Macrophages**

One important component of the innate immune response is the phagocytic cells. The recruitment of polymorphonuclear leukocytes (PMNs) and monocytes to the infection site is mediated by the release of cytokines and chemokines from the infected tissue. Several studies have reported that macrophages are present in increased quantities in infections caused by HPV or CIN [34, 35] and in cervical carcinoma cases [36]. Moreover, these cells are present in both the epithelium and stroma, and are capable of killing cells that have been transformed by HPV-16 [37]. Corroborating this idea, papillomas that present regression have significant infiltrates of macrophages that stain positively for TNF-α, thus correlating with the apoptosis of the infected epithelial cells [38].

**Natural killer (NK) cells**

NK cells are a subpopulation of lymphocytes that recognize and destroy damaged and infected cells in a non-specific manner. They may be activated by means of treatment with cytokines, to produce lymphokine-activated NK cells (LAK). The mechanism for the action of these cells basically takes place through the release of cytotoxic granules on
the surface of the target cells, thereby inducing apoptosis of these cells. In addition, through synthesizing TNF-α and IFN-γ, the inflammation is increased and other components of the immune response are activated.

Even though the lysis caused by NK cells is deficient in patients who have pre-cancerous lesions or cancer induced by HPV [39], this cell lineage is found in the CIN stroma [40]. However, epithelial cells immortalized by HPV-16 and lineages of cervical carcinoma cells are relatively resistant to NK cells, but sensitive to lysis by LAK cells [41, 42]. Data from the literature have also demonstrated that the E6 and E7 proteins of HPV-16 inhibit the ability of NK cells to synthesize IFN-γ using in vitro tests [27]. These data indicate that one of the possible mechanisms for controlling HPV infection could be by means of a reduced IL-12/IL-10 ratio [43] and increased quantities of the cytokines that are considered to be immunosuppressors, such as TGF-β and IL-10 [34, 29, 44]. Topical immunomodulators such as imiquimod, which is utilized in clinically treating HPV infection, act by inducing the secretion of cytokines of the Th1 pattern, such example IL-4, IL-10 and TGF-β (responsible for activating the humoral immune response). Recently, a further classification emerged: the Th3 type, characterized by the presence of IFN-γ and IL-10. This pattern appears to be involved in the induction of tolerance.

Regression of HPV infection has been associated with an immune response mediated by cytokines of the Th1 pattern [19, 38]. In contrast, the development of CIN is mediated by a cytokine secretion pattern of the Th2 type, with a reduced IL-12/IL-10 ratio [43] and increased quantities of the cytokines that are considered to be immunosuppressors, such as TGF-β and IL-10 [34, 29, 44]. Topical immunomodulators such as imiquimod, which is utilized in clinically treating HPV infection, act by inducing the secretion of cytokines of the Th1 pattern, such as TNF-α, IFN-γ and IL-12 in the monocytes and macrophages. These cytokines are responsible for increasing the cell-mediated immune response [45].

**Adaptive immunity**

In a simple way, the adaptive immune response could be defined as an immune response that involves the participation of cellular types with highly specific antigen receptors. However, this specific response starts only with an unspecific recognition of these antigens by antigen presenting cells (APCs), that thereafter catching and processing present peptides by MHC class II, activating auxiliary T lymphocytes (CD4+); or in the case of intracellular antigens, e.g., virus or tumor antigens, the processing results in a presentation by MHC class I molecules, thus activating cytotoxic T lymphocytes (CD8+).

The antigen presenting process is extremely complex and involves membrane molecules, soluble mediators and intracellular ways of activation such as APC as much as T lymphocytes.

**Dendritic cells**

The dendritic cells (DCs) originate from hematopoietic stem cells within the bone marrow and under physiological conditions differentiate into immature dendritic cells that circulate via blood to peripheral tissues. DCs are recognized as the most powerful antigen presenting cells (APCs) for priming both cytotoxic (CD8+) and helper (CD4+) T cells. Following an encounter with antigens the immature DCs initiate their maturation process and during this phase the cells increase their migratory capacity to the regional lymph nodes to activate T lymphocytes.

The coordination that the DCs exert between the innate and adaptive immunity is indispensable to an induction of an effective response against tumors. However, several steps are necessary to develop an effective immune response able to eliminate the tumor cells. DCs have to recognize tumor molecules, to internalize and process these antigens, to migrate to lymph nodes, and then to present the tumor antigen to T-cells to induce a cellular response. However, the immune system often fails, presumably due to alterations in the aforementioned mechanisms [46].

In patients with head and neck squamous cell carcinoma, as well as in patients with metastatic disease in breast, colorectal, gastric, lung, cervix, endometrial and renal cell carcinoma the number of blood DCs were altered [47-49]. Similarly, in primary tumors including breast, colorectal, gastric, esophageal, thyroid and bladder transitional cell carcinoma it appeared that DCs were not recruited [50-52]. These and additional data [51, 53] are clinically relevant as they are associated with a significantly poorer prognosis in patients with several types of cancer.

Several studies have suggested that DC dysfunction was indeed a systemic process and supported the notion that soluble factors derived from tumors affect DC. Several reports have now confirmed that by releasing IL-10, IL-6, M-
Potential therapeutic vaccine strategies and relevance of the immune system in uterine cervical cancer

CSF, vascular endothelial growth factor (VEGF), gangliosides and prostanoids, tumors can prevent DC differentiation and function in vitro and in vivo [54-59].

Due to the capacity of the development of cellular vaccine based in dendritic cells, they are actually used in several situations, including tumors induced by HPV. In mice with a C3 sarcoma (tumor expressing HPV16 E7) vaccination with immature DC pulsed with an MHC restricted HPV16 E7 class I peptide and eradication of tumors occurred in 80% of mice [60]. Both immature and mature autologous DC primed with antigens derived from synthethic tumor antigen peptides or tumor lysate from a variety of tumors have been shown to mediate major anti-tumor responses in humans, including cervical cancer [61].

Unfortunately, no consensus exists with respect to the key issues such as the loading method for optimum immune responses and of activating/maturation of the DC phenotype, as well as optimum route of DC administration, DC dosage schedule and DC dose.

T lymphocytes

Clues to the nature of the cellular immune response to HPV infection have come from immunohistologic studies comparing spontaneously regressing and non regressing genital warts and from recent advances in HPV vaccines.

Regressing genital warts present a large infiltrate of T-cells (both CD4+ and CD8+) and macrophages in wart stroma and epithelium, infiltrating lymphocytes express activation markers, and the cytokine milieu is dominated by proinflammatory cytokines such as IL-12, TNF-alpha and IFN-gamma. Moreover non-regressing genital warts are characterized by a lack of immune cells at the site of infection: the few intraepithelial lymphocytes are CD8+ cells, and mononuclear cells are present mainly in the stroma [62].

Immunity against HPV16 E6 and E7 oncoproteins has been tested by stimulation of peripheral blood mononuclear cells (PBMC) obtained from patients infected by HPV. To assess HPV-specific cytotoxic T-lymphocyte (CTL) activity, PBMC from HPV-16 patients were stimulated with recombinant protein, defined minimal peptide-epitopes or with recombinant adenovirus expressing HPV 16 E6 and E7 infected PHA blasts for 7-21 days. In some studies, CTL reactivity against both HPV 16 E6 and E7 was predominantly found in patients that cleared infection [63, 64]. Other studies, especially in patients with persistent infections or progressive disease, displayed CTL reactivity [65, 66].

Recently, new insights have emerged with the new vaccines with HPV peptides. Vaccination of cervical cancer patients resulted in the detection of an occasional vaccine-induced T-cell response against HPV [67, 68]. The advances of the new vaccines will clarify some important aspects of immune response to HPV necessary to improve the vaccines.

Interferons

These are a family of cytokines that have important functions in the immune system [69]. Type I IFNs, which include IFN-α and β, are produced by epithelial cells and contribute towards the first line of antiviral defense by inhibiting proliferation and inducing apoptosis of the cells that are infected by the virus [70]. On the other hand, type II IFN (IFN-γ) is produced by activated T cells and NK cells, and is an important modulator of immune function. Both types of IFN inhibit the expression of mRNA from the E6 and E7 proteins in immortalized HPV cells [71, 72]. Even though both types reduce the expression of the genes for HPV, the most effective is type II (IFN-γ). This family of cytokines has been used for treating HPV infections. However, the efficacy of the therapy has been inconsistent, given that some patients respond effectively [73], while others respond only partially.

Recent studies have demonstrated that the E6 and E7 proteins of HPV-16 and 18 have a close relationship with the synthesis of these cytokines and with tumor progression. E6 and E7 are capable of specifically inhibiting the expression and signaling of IFNs [74, 75], thereby allowing the virus to escape from the normal antiviral response [76]. These proteins are positively regulated during the progression of CIN [77], while there is a reduction in the levels of IFN-β and γ in these patients [43, 78, 79]. Although some of these studies have been carried out in vitro, this observation is compatible with the clinical studies [80], since patients who express high levels of E7 in the tissue are more resistant to treatment with IFN, while patients with low levels of E7 are sensitive. Together, these results indicate that the expression of high levels of the E6 and E7 proteins negatively regulates the expression and signaling of the IFN, thereby directly influencing the efficacy of immunotherapy using IFN.

Immunotherapy

Tumor treatment using the immune system as a tool is a very old dream within science. It may have begun in the 19th century, when surgeons and other scientists in Europe and the United States started to observe tumor regression that was associated with parallel resolution of erysipelas.

Since then, studies on the immune system have advanced significantly, and today the first steps towards manipulating this system in our favor have been taken. Several types of immunotherapy have been developed for the possible treatment of such neoplasia. Some of them involve immunostimulant therapy, antibodies, cytokines or cell therapies (Table 1). From the point of view of application, some methods have not gone beyond in vitro tests, while others have been and are being tested on animals. Just a few have gone into the phase of clinical trials on humans.
Table 1. — Principal clinical applications of immunotherapy in different types of tumors.

<table>
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<th>Clinical applications</th>
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<td>Metastatic breast cancer, leukemia, lymphoma</td>
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<td>Cytokines</td>
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<tr>
<td>IL-2</td>
<td>Renal carcinomas, melanoma, tumors of the central nervous system, hematological tumors, tumors of the head and neck</td>
<td>88-94</td>
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<tr>
<td>IL-12</td>
<td>Experimental phase in animals; use in humans has been prohibited by the FDA, because of deaths related to its use</td>
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<td>IL-6</td>
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Cellular therapies

Adoptive transfer of OF lymphocytes

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Peptide vaccination for act in OF lymphocytes

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Therapy using dendritic cells

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As mentioned above, the oldest attempts to treat tumors using immunotherapy were by means of immunostimulant therapy. Treatments for cancer patients using bacteria and their products go back to the end of the 19th century when William B. Coley started to treat cancer patients using the supernatant from cultures of Micrococcus pyogenes and Serratia marcescens. This vaccine became known as Coley’s mixed bacterial vaccine (MBV).

A variety of immunostimulants and immunomodulators have now been utilized in clinical and preclinical studies. Particular attention has been given to contact allergens, bacillus Calmette-Guérin (BCG), muramyl dipeptide, Corynebacterium parvum, and levamisole. The use of 6-mercaptopurine, doxorubicin, cisplatin and even cyclophosphamide can be cited. In large doses, the last is utilized for inhibiting the responses of T and B lymphocytes, but at low doses it eliminates suppressor cells and acts as an immunostimulant.

The utilization of antibodies in therapy against neoplasia has also been envisaged for around two decades. More specifically, monoclonal antibodies (mAbs) have been developed for different types of tumors. The problem of administering antibodies that were developed in mice has been overcome through molecular biology, with their transformation into chimeric humanized antibodies. Today, the main problem is how to characterize an appropriate target antigen for each type of neoplasm. However, for the vast majority of neoplasms, science has not yet been able to characterize these tumor proteins.

Much has been done and learned through clinical investigations utilizing cytokines as the therapy. The application that has been most published, although it is not the most effective one, is the use of IL-2 in renal tumors and melanomas. For other cytokines, experimental and basic investigation studies have been conducted with a view to their utilization. However, one of the difficulties is that the experimental models utilized, in the same way as for other diseases, are in most cases models using homozygotic animals, which definitely does not occur with patients. Thus, the treatment schemes, doses and immunological responses vary from patient to patient, and sometimes the results are completely different from those found in animals. Another limiting factor in developing these protocols for humans is that the ethical precepts only permit treatment for patients who are already in a terminal phase, with tumors that are unlikely to regress. This limits studies on other types of tumors and at phases in which the response could be better characterized and with better results. Despite these obstacles, therapy using cytokines has emerged as a promising method, particularly when allied with other types of immunotherapy.

One of the great expectations in science regarding tumor treatment relates to the utilization of IFN, since this is the first human cytokine found to be effective as a treatment for tumors. Immunotherapy using IFN-α has been employed for treating multiple myeloma, chronic myeloid leukemia, non-Hodgkin’s lymphomas, renal carcinoma, epidermoid cervical cancer, head and neck tumors and melanomas, and also for treating CIN.

Although it is recognized in the literature that IFN-α modulates the growth and differentiation of tumor cells and affects cell communication and intracellular signaling, the mechanisms through which tumor regression takes place have not been completely elucidated. Our research group has clinical studies under development along these lines, investigating which immune response cells are involved in tumor regression and what their mechanism of action is. Other points to be elucidated are the treatment schemes and the optimum doses for each type of tumor.

Nonetheless, it is known that the immune system does not act just as a mechanism for inducing tumor regression. It is a fully coordinated response that involves a range of actions from the activation of antigen-presenting cells (dendritic cells) to the activation of T-helper and cytotoxic lymphocytes and NK cells. Therapy using IFN-alpha only involves one line of action, which is possibly the activation of natural killer cells and cytotoxic lymphocytes, but we
believe that the activation of T-helper lymphocytes has fundamental importance in obtaining a much more effective antitumoral response.

As seen, immunological studies gained impetus from the middle of last century onwards. Nevertheless, many mechanisms still remain to be elucidated in order to be able to effectively manipulate this system, so that the objective of eliminating and/or preventing the emergence of tumors can be achieved by using the immune system as the tool.

Despite all these attempts to manipulate the immune system in our favor, much remains to be done. As discussed above, the desired drug must activate several cellular types of the innate and adaptive immune response; a potential drug for these are the cytokines. In a special review the most promising are alpha, beta and gamma interferons, and in spite of the results with the three types in a separate way [74], they are unique cytokines that could activate the immune system in several ways. With regard to developing antitumor drugs, a great leap forward for the pharmaceutical industry would be to develop a drug similar to interferon that would conserve in its structure the molecular and biological characteristics that are common to the three main types of IFN (alpha, beta and gamma). One way would be to exclude from the molecule the portion that could be responsible for the side-effects of these cytokines, which are extremely similar. This idealized drug could activate the principal receptors for these cytokines all at once and, at the same time, induce apoptosis and modify the high rate of mitosis in the tumor cells, but also, activate cells of the immune system, like macrophages, dendritic cells, T-helper and cytotoxic lymphocytes which could specifically destroy metastatic cells. Another extremely interesting point could be to develop this ideal drug – abolishing the structure of the sequence of the three interferons which could be responsible for the adverse effects that are actually very similar in patients treated with these drugs, as thrombocytopenia, leukopenia, fatigue and psychiatric disorders, and that are actually the main restriction in clinical practice [114-116]. Until such time, we will continue to study and investigate the possible antitumoral mechanisms in the immune system to improve the existing protocols and develop new therapies.

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Immunohistochemical bcl-2 expression, p53 overexpression, PR and ER status in endometrial carcinoma and survival outcomes

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Summary

Immunohistochemical expression of bcl-2, p53, PR and ER in cases with endometrial carcinomas arrayed on a tissue microarray (TMA) was tested and correlated with clinicopathologic features, overall survival (OS), cancer-related survival (CRS) and disease-free survival (DFS).

Seventy-seven patients with endometrial cancer were reviewed. Slides were evaluated by two pathologists blinded to patient clinical characteristics and survival data. Mean age of patients was 62.5 years (range 35-80), median follow up 60 months (range 9-120).

Seventy-nine percent of patients were FIGO Stage I; 39% of the cases showed bcl-2 cytoplasmic staining and its expression was significantly correlated with low-grade tumor differentiation and age ≤ 60 years. Nuclear p53 overexpression was detected in 23.4% of the cases and was significantly correlated with advanced stages (IIB-IV), non-endometrioid histology, nodal metastasis and advanced age (> 60 years). PR and ER were positive in 63.6% and 30% of the cases, respectively. Analysis of p53 overexpression and bcl-2 expression in relationship with PR and ER status showed a direct correlation between bcl-2 expression and PR positivity (p = 0.001). In a multivariate analysis FIGO staging was the only clinicopathologic parameter independently correlated with DFS.

In conclusion p53 overexpression was directly associated with unfavorable clinicopathologic factors such as advanced stage, histologic subtype, advanced patient age and nodal metastasis. Bcl-2 expression was related with younger age, favorable grade and PR expression by tumor cells. Patient survival was not related to the tested biomarkers.

Key words: bcl-2; Endometrial adenocarcinoma; ER; p53; PR, TMA.

Introduction

Endometrial carcinoma (EC) is the most common malignancy of the female genital tract in developed countries, affecting about 2-3% of the female population [1]. The overall survival in patients with EC of all stages is 75% [2]. Traditional clinicopathologic features such as FIGO staging, tumor differentiation (grade), histological subtype, depth of myometrial invasion and extrapersitoneal spread are considered as the strongest prognostic factors of patients with EC [3]. Recently various molecular markers have been investigated providing a more accurate profile of tumor behavior in patients with EC.

The bcl-2 (B-cell leukemia/lymphoma-2) gene is a well known proto-oncogene involved in the regulation of apoptosis. The gene is located on chromosome 18q21 and encodes an oncoprotein of 24 kDa which is involved in the regulation of programmed cell death and cell survival. Bcl-2 dependent mechanisms allow the accumulation of genetic mutations contributing to neoplastic development [4-6]. In normal endometrium bcl-2 protein is expressed in the proliferative phase of the menstrual cycle and down-regulated in the secretory phase [7-9]. The prominence of bcl-2 expression in the proliferative phase is apparently under the control of steroid sex hormones [8]. Bcl-2 expression is also present in endometrial hyperplasia and it is hypothesized that this expression is related to early-stage EC. A relationship between bcl-2 expression with grade, stage and progesterone receptors status was demonstrated by previous authors, however the role of bcl-2 protein in endometrial carcinogenesis is largely unknown [4, 10].

The tumor suppressor gene p53 is located in the short arm of chromosome 17 and its mutation is the most common alteration in human malignancies. Wild type p53 protein contributes to programmed cell death by arresting cell proliferation. Thus, an inverse interaction exists between p53 and bcl-2 proteins concerning apoptosis and programmed cell death [11, 12]. P53 overexpression has been found more commonly in advanced endometrial cancer as well as in non endometrioid histologic subtypes and high-grade tumors [13-15]. Recent research showed that p53 overexpression was not related to progesterone (PR) or estrogen receptors (ER) status [16] and, the prognostic impact of p53 protein overexpression in patients with EC is still controversial [14, 16, 17].

In the present study we investigated the immunohistochemical expression of bcl-2, p53, PR and ER on a tissue microarray (TMA) of 77 endometrial carcinomas. The immunohistochemical results were correlated with clinicopathologic features and survival data.

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Material and Methods

Seventy-seven patients with endometrial carcinoma were included in the study. This is part of a cohort of 126 patients with endometrial cancer all surgically treated at our department from 1996 to 2003. Patients with a prior or concomitant diagnosis of malignancy as well as patients not fulfilling the inclusion criteria (complete immunohistochemical data, paraffin-embedded tumor material and clinical follow-up) were not enrolled.

Patients were staged according to the 1998 FIGO staging system and the histologic grading evaluation was done according to the revised FIGO grading system for endometrial adenocarcinoma [18, 19]. The surgical and postoperative approach were reported elsewhere [20]. Briefly, patients at low-risk for lymph node metastasis (endometrioid endometrial adenocarcinoma (EEA), grade 1 with myometrial infiltration (MI) ≤ 1/2 or grade 2 with MI ≤ 1/3) were treated with total abdominal hysterectomy (TAH), bilateral salpingo-oophorectomy (BSO), peritoneal washing and palpation of the peritoneal cavity while biopsy was performed when considered necessary. High-risk patients (all non-endometrioid adenocarcinomas; grade 1 with MI > 1/2 or grade 2 with MI > 1/3, and all grade 3 tumors), were further treated either with staging lymphadenectomy or with postoperative irradiation (external-beam pelvic radiation (EBR), 45-50Gy at 1.8 Gy per fraction). Paraortic node sampling was performed in case of palpable or suspicious pelvic nodes.

Tissue microarray (TMA) and immunohistochemistry (IHC)

Representative formalin-fixed and paraffin-embedded tumor blocks of EC cases were selected based on hematoxylin and eosin stained slides for TMA construction and IHC. Two TMA paraffin blocks were constructed with a manual arrayer (Beecher Instruments, Sun Prairie, WI, USA), including all 77 EC cases, five cores per case, 0.6 mm in diameter. Before the sampling of selected cases, five cores from a breast carcinoma case were arrayed to each recipient block for identification and sampling of selected cases, five cores from a breast carcinoma (n = 28)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n = 77)</th>
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<tr>
<td></td>
<td>no. (%)</td>
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<tr>
<td><strong>Age</strong></td>
<td></td>
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<tr>
<td>≤ 60 years</td>
<td>28 (36.5)</td>
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<tr>
<td>&gt; 60 years</td>
<td>49 (63.5)</td>
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<td><strong>FIGO staging</strong></td>
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<tr>
<td>I</td>
<td>61 (79)</td>
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<td>II</td>
<td>11 (15)</td>
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<td>III</td>
<td>4 (5)</td>
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<td>IV</td>
<td>1 (1)</td>
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<tr>
<td><strong>FIGO Stage I (n = 58)</strong></td>
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<tr>
<td>(endometrioid subtype)</td>
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<td>IA</td>
<td>14 (25)</td>
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<tr>
<td>IC</td>
<td>14 (25)</td>
</tr>
<tr>
<td><strong>Histology subtype</strong></td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>74 (96)</td>
</tr>
<tr>
<td>Non endometrioid</td>
<td>3 (4)</td>
</tr>
<tr>
<td><strong>Tumor diameter</strong></td>
<td></td>
</tr>
<tr>
<td>≤ 2 cm</td>
<td>39 (50.5)</td>
</tr>
<tr>
<td>&gt; 2 cm</td>
<td>38 (49.5)</td>
</tr>
<tr>
<td><strong>Uterine diameter</strong></td>
<td></td>
</tr>
<tr>
<td>≤ 8 cm</td>
<td>53 (69)</td>
</tr>
<tr>
<td>&gt; 8 cm</td>
<td>24 (31)</td>
</tr>
<tr>
<td><strong>Tumor differentiation (grade)</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32 (41.5)</td>
</tr>
<tr>
<td>2</td>
<td>37 (48)</td>
</tr>
<tr>
<td>3</td>
<td>8 (10.5)</td>
</tr>
<tr>
<td><strong>Myometrial infiltration</strong></td>
<td></td>
</tr>
<tr>
<td>≤ 50%</td>
<td>53 (69)</td>
</tr>
<tr>
<td>&gt; 50%</td>
<td>24 (31)</td>
</tr>
<tr>
<td><strong>Adnexal involvement</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>73 (95)</td>
</tr>
<tr>
<td>Positive</td>
<td>4 (5)</td>
</tr>
<tr>
<td><strong>Cervical involvement</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>65 (84.5)</td>
</tr>
<tr>
<td>Positive</td>
<td>12 (15.5)</td>
</tr>
<tr>
<td><strong>Nodal status (n = 28)</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>26 (93)</td>
</tr>
<tr>
<td>Positive</td>
<td>2 (7)</td>
</tr>
<tr>
<td><strong>Risk group</strong></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>27 (35)</td>
</tr>
<tr>
<td>High</td>
<td>50 (65)</td>
</tr>
</tbody>
</table>

* Risk group for nodal metastasis.

Interpretation of staining

The stained slides were evaluated simultaneously by two pathologists. The observers were blinded to the patient’s clinical characteristics and survival data. Bcl-2 cytoplasmic staining and p53 overexpression were scored as previously described [17, 21]. The evaluation of ER and PR staining pattern was performed according to the method described in Carcangiu’s study [22].

Statistics

The SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Mean values of the different variables with continuous outcomes were analyzed using chi-square and Fisher’s exact test, while Student’s t-test was used for the categorical variables. Survival and disease-free survival rates were estimated according to the Kaplan Meier method and the log-rank test was used to assess the prognostic significance [23]. Overall survival (OS) and cancer-related survival (CRS) were calculated from the date of the cancer diagnosis to the date
of death from any cause or death related to endometrial carcinoma, respectively, while disease-free survival (DFS) was estimated from the date of the diagnosis to the first appearance of the recurrence or death related to endometrial carcinoma (whichever occurred first). Any p value < 0.05 was considered statistically significant (all p values were two-sided). p53 nuclear overexpression, bcl-2 cytoplasmatic expression, PR and ER status were analyzed according to the different clinicopathologic characteristics of the patients. OS, CRS and DFS were estimated using univariate analysis of the conventional clinicopathologic and immunohistochemical parameters (p53, bcl-2, PR, ER) of the study. Cox-regression analysis of DFS according to the different clinicopathologic and immunohistochemical features of the study was also performed.

### Results

The mean age of the patients was 62.5 years with a range from 35 to 80 years. Distribution of patient characteristics according to the different clinicopathologic features is listed in Table 1. Seventy-nine percent of the population was classified as Stage I. Histologic subtype of the tumors was mainly endometrioid (96%), while only three cases of serous papillary carcinoma were identified (4%).

#### Immunohistochemical analysis

**Bcl-2 expression.** Thirty-nine percent of the cases showed bcl-2 cytoplasmatic staining (Figure 1A). Bcl-2 expression was significantly correlated with low-grade
In tumor differentiation (grade 1 and 2 combined) (p = 0.017) and younger age (≤ 60 years) (p = 0.045) (Table 2). Bcl-2 differed marginally (p = 0.05) when tumor differentiation (grade) was analyzed separately (grade 1 vs 2 vs 3). The same observation was made in a subgroup analysis of Stage I patients with only endometrioid histology subtype (Stage IA vs IB vs IC) (p = 0.05). Bcl-2 expression was mainly detected in cases with myometrial invasion less than half of the myometrial thickness and in low-risk patients but these differences were not statistically significant (p = 0.08 and p = 0.09, respectively).

**P53 overexpression.** Nuclear p53 overexpression was detected in 18 of 77 (23.4%) cases (Figure 1B). This overexpression was significantly correlated with advanced stage disease (IB-IIA-IV), non endometrioid histology subtype, nodal metastasis and advanced patient age (> 60 years) (Table 2). Although there was a trend of p53 overexpression in high-grade tumor differentiation (grade 1 and 2 combined vs grade 3), it did not reach statistical significance (p = 0.06).

**PR, ER status.** 63.6% of patients were PR positive and 30% were ER positive (Figures 1C and 1D). PR expression differed marginally (p = 0.05) between the low- and high-risk groups of patients for nodal metastasis (78% vs 56%, respectively). A subgroup analysis of patients with Stage I of only endometrioid histology subtype, showed a significant relationship between PR expression and stage (Table 2). Furthermore, PR positivity was higher in endometrioid histology subtype compared with ER positivity (65% vs 33%, respectively) but did not reach a statistical significance (p = 0.20). ER positivity was higher in cases with uterine diameter ≤ 8 cm (p = 0.08). The same observation was made in cases with metastatic (nodal) disease.

Analysis of p53 overexpression and bcl-2 expression in relation to PR and ER status showed a direct correlation between bcl-2 expression and PR positivity (p = 0.001) (Table 3).

| Table 3. — Bcl-2 and p53 distribution according to ER and PR status. |
|---|---|---|---|---|
| No. of patients | Bcl-2 expression | p value | p53 overexpression | p value |
| PR | (n = 77) | (n = 30) | | (n = 18) | |
| Negative | 28 | 4 (13.3) | 0.001 | 6 (33.3) | 0.80 |
| Positive | 49 | 26 (86.7) | | 12 (66.7) | |

| Table 4. — Patient prognosis according to the different clinico-pathologic and immunohistochemical characteristics. |
|---|---|---|---|---|
| Characteristics | OS (%) | P | CRS (%) | P |
| Age | | | | |
| ≥ 60 years | 93 | 0.03 | 88 | 0.25 |
| > 60 years | 75 | | 85 | |
| FIGO staging | | | | |
| I | 87 | 93 | 92 | |
| II | 91 | 91 | 9 | |
| III | 25 | 50 | 50 | |
| IV | 0 | < 0.0001 | 0 | < 0.0001 |
| FIGO stage 1 (n = 58) | | | | |
| Early | 87.5 | 93 | 92 | |
| Advanced | 25 | < 0.001 | 40 | < 0.0001 |
| FIGO stage (Grade) | | | | |
| IA | 86.5 | 93 | 93 | |
| IB | 90 | 93 | 90 | |
| IC | 81 | 90 | 94 | 90 |
| Histology subtype | | | | |
| Endometrioid | 84 | 90 | 89 | |
| Non endometrioid | 66.5 | 30 | 66 | 0.08 |
| Tumor diameter | | | | |
| ≤ 2 cm | 82 | 85 | 82 | |
| > 2 cm | 84 | 0.70 | 94 | 0.15 |
| Uterine diameter | | | | |
| ≤ 8 cm | 83 | 87 | 87 | |
| > 8 cm | 83.5 | 0.60 | 92 | 0.40 |
| Adnexal involvement | | | | |
| Negative | 86 | 93 | 92 | |
| Positive | 25 | 0.0001 | 25 | < 0.0001 |
| Tumor differentiation (Grade) | | | | |
| Low | 82 | 91 | 90 | |
| High | 75 | 0.25 | 75 | 0.10 |
| Myometrial infiltration | | | | |
| ≤ 50% | 89 | 94 | 92 | |
| > 50% | 75 | 0.03 | 79 | 0.01 |
| Nodal status (n = 28) | | | | |
| Negative | 92 | 96 | 92 | |
| Positive | 0 | < 0.0001 | 50 | 0.0004 |
| Risk group* | | | | |
| Low | 85 | 93 | 93 | |
| High | 82 | 0.90 | 88 | 0.60 |
| bcl-2 expression | | | | |
| Negative | 81 | 89 | 87 | |
| Positive | 87 | 0.20 | 90 | 0.60 |
| p53 overexpression | | | | |
| Negative | 85 | 90 | 88 | |
| Positive | 75 | 0.35 | 88 | 0.70 |
| PR | | | | |
| Negative | 86 | 89 | 86 | |
| Positive | 82 | 0.90 | 90 | 0.70 |
| ER | | | | |
| Negative | 81 | 87 | 87 | |
| Positive | 87 | 0.70 | 95 | 0.30 |

* Risk group for nodal metastasis; γ = stage I-IIA; f = stage IIB-IV; ∂ = grade 1 and 2 combined; ∀ = grade 3; PR, ER = progesterone, estrogen receptor; OS = overall survival; CRS = cancer-related survival; DFS = disease-free survival.
Survival analysis

The median follow-up was 60 months (range 9-120 months). Univariate analysis of OS, CRS and DFS are presented in Table 4. OS of patients was significantly higher in the patients age ≤ 60 years, and there was a direct correlation between the OS and FIGO staging. There was a positive relationship between poor OS and ovarian metastasis, depth of myometrial invasion (>50%) and lymph node metastasis. The same was also true concerning CRS and DFS. Furthermore, unfavorable tumor differentiation (grade 3) was significantly correlated with CRS. There were no differences between the studied immunohistochemical parameters and survival distribution. FIGO staging was the only clinicopathologic parameter independently correlated with DFS, when FIGO staging, grade, tumor diameter, myometrial infiltration, patient age and immunohistochemical features were included in a multivariate Cox regression analysis (Table 5).

Table 5. — Multivariate Cox regression analysis of DFS for the different clinico-pathologic and immunohistochemical features of patients with uterine adenocarcinoma.

<table>
<thead>
<tr>
<th>Feature</th>
<th>RR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO staging</td>
<td>0.48</td>
<td>2.7-84.4</td>
<td>.008</td>
</tr>
<tr>
<td>Tumor diameter (≤ 2 cm)</td>
<td>0.36</td>
<td>0.06-2.0</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td>0.86</td>
<td>0.06-9.7</td>
<td>NS</td>
</tr>
<tr>
<td>Myometrial infiltration (≥ 50%)</td>
<td>1.45</td>
<td>0.25-8.3</td>
<td>NS</td>
</tr>
<tr>
<td>Age (≤ 60 yrs)</td>
<td>3.10</td>
<td>0.40-24.0</td>
<td>NS</td>
</tr>
<tr>
<td>bcl-2 expression</td>
<td>1.47</td>
<td>0.22-9.7</td>
<td>NS</td>
</tr>
<tr>
<td>p53 overexpression</td>
<td>0.56</td>
<td>0.05-6.3</td>
<td>NS</td>
</tr>
<tr>
<td>PR</td>
<td>0.54</td>
<td>0.09-3.1</td>
<td>NS</td>
</tr>
<tr>
<td>ER</td>
<td>0.18</td>
<td>0.01-2.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Similar results have been shown by others [14, 25, 29]. A recent study on p53 protein overexpression also demonstrated a positive relationship between protein overexpression and recurrent disease [24]. From the previous and the present study it seems that p53 overexpression is correlated with advanced stage, unfavorable grade and non endometrioid histology subtype, suggesting the essential role of p53 overexpression in carcinogenesis of patients with endometrial adenocarcinoma.

The correlation between p53 protein overexpression and gene mutations is not absolute in human malignancy with a reported concordance of 75% for endometrial cancer [30]. Although p53 overexpression has been widely used as an indication for p53 mutation, skepticism is needed for interpretation of the results. P53 immunoreactivity does not necessarily predict gene mutation, nor does negative p53 protein staining exclude the possibility of p53 exon mutations [13, 31]. A recent study on patients with recurrent endometrial cancer using PCR for the mutation analysis of p53 exons 5-8 and 11 did not demonstrate a correlation between p53 protein overexpression and gene mutation [24]. However, in another study protein overexpression and missense mutation of the p53 gene was correlated to patients with early-stage disease [32]. The concordance between gene mutation and p53 overexpression was not the object of the present trial.

Data concerning the relationship between bcl-2 expression and the clinicopathologic characteristics of patients with endometrial carcinoma is less clear. One study showed a positive correlation between bcl-2 expression and negative lymphovascular space involvement, negative lymph nodes and superficial myometrial invasion (≤ 50%) [17], while another showed a significant correlation between bcl-2 cytoplasmatic expression and myometrial depth [33]. We showed higher bcl-2 expression of patients with superficial myometrial infiltration (≤ 50%) but with no statistically significant value (p = 0.09). Furthermore previous reports have shown a significant association of bcl-2 with all favorable clinicopathologic features of patients with endometrial cancer including early stage, favorable tumor grade, superficial myometrial infiltration, negative lymphovascular space involvement and endometrioid histology subtype [34, 35], while Taskin et al., described a direct relationship of bcl-2 only with early stage and favorable grade tumors [4].

Discussion

In the current study bcl-2 cytoplasmatic expression, p53 nuclear overexpression and PR, ER status were estimated using immunohistochemistry on paraffin-embedded endometrial adenocarcinoma tissue specimens. P53 and bcl-2 proteins have been investigated in various human cancers, including those of the female genital tract. They have been studied immunohistochemically on frozen tissue or on paraffin-embedded blocks [14, 16, 17, 24-27]. Previous studies showed a positive correlation of p53 overexpression with advanced FIGO staging, non endometrioid histology subtype and lymph node metastasis [17]. These results are similar to the present study. Ohkouchi et al. demonstrated a significant correlation of p53 overexpression with unfavorable tumor differentiation and myometrial depth (>50%). However, in our study the difference of p53 overexpression between low and high tumor grade was of borderline significance, in favor of high-grade (p = 0.06), while no significant difference was noted in relation to myometrial invasion. Koumelis et al., using a cutoff value of 10% for nuclear staining of p53 protein, showed a direct association of p53 with non endometrioid histology, high-grade tumor (grade 3) and advanced stages (Stage IIA-IV) [28].
lates carcinogenesis in EC, while p53 overexpression is probably unrelated to the former mechanism.

Survival analysis of the present study (OS, CRS and DFS) showed favorable prognosis of EC patients with early stage, no adenexal metastasis, superficial myometrial invasion and negative nodal status. Previous reports are in accordance with our findings [17, 21, 27, 37]. We detected better CRS and DFS in patients with endometrioid histology subtype but these differences were not statistically significant. Immunohistochemical marker expression in our study was not correlated significantly (univariate analysis) with patient prognosis, in accordance with a recent report [16]. In a multivariate analysis FIGO staging was the only parameter independently correlated with DFS.

Data related to the prognostic impact of p53, bcl-2 and hormonal receptor status reported in the literature are conflicting. Lukes and collaborators showed in a multivariate model that p53 overexpression and sex steroid status were not independently correlated with FIGO stage, grade, myometrial invasion and DNA ploidy [27]. A univariate analysis by Oreskovic et al. showed that p53 expression (< 15% positive cells), ER and PR status of patients with EC, correlated significantly with patient survival, while in a multivariate analysis only p53 expression remained independently correlated with patient survival [21]. Erdem et al., in a multivariate model, demonstrated independent correlation of FIGO stage and bcl-2 expression with prognosis [37]. Others, using a similar model, showed p53 overexpression and lymph node metastasis were independently correlated with prognosis [14, 17, 38, 39]. However, another report failed to show an independent correlation between recurrence and p53 overexpression [40]. Geisler et al., including bcl-2 cytoplasmatic persistence in a multivariate analysis, showed an independent association with patient prognosis [34], while Sakuragi et al. failed to demonstrate these results [33].

Deficiencies of the current study were the retrospective nature and the small number of patients, as well as the short median follow-up of 60 months. However, as previously shown, events on recurrence are mostly noted in the first 36 months after the diagnosis of malignancy in two-thirds of patients with endometrial cancer [41, 42].

Conclusion
Our findings show that FIGO staging was the only clinicopathologic parameter independently associated with patient prognosis, while the immunohistochemical markers used in the present study (p53, bcl-2, ER and PR) were not. We found that p53 overexpression was directly associated with unfavorable clinicopathologic factors of patients with endometrial adenocarcinoma such as advanced stage, non endometrioid histology subtypes, advanced patient age and lymph node positive status, while bcl-2 expression was related with younger age and favorable tumor differentiation. Furthermore, a significant relationship was noted between PR tumor positivity and bcl-2 expression.

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References
Immunohistochemical bcl-2 expression, p53 overexpression, PR and ER status in endometrial carcinoma and survival outcomes


L1 (CAM) (CD171) in ovarian serous neoplasms

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Summary

Purpose of the investigation: The evaluation of L1 (CAM) as a tumor progression marker and as a prognostic factor in serous ovarian tumors. Methods: L1 (CAM) protein expression was assessed by immunohistochemistry and Western blot in serous ovarian tumors [cystadenomas (n = 20), borderline tumors (n = 14) and carcinomas (n = 47)], and was correlated with stage, grade, progression-free survival time (PFS) and overall survival. Results: L1 (CAM) immunoreactivity correlated significantly with stage and grade. It increased from benign tumors to early carcinomas and to advanced stage carcinomas progressively and significantly. In Stage III G3 carcinoma patients, low L1 (CAM) expressing tumors exhibited better response to chemotherapy and were associated with statistically significantly longer PFS (p = 0.002). Conclusion: L1 (CAM) expression represents a novel diagnostic marker in serous ovarian neoplasms that shows characteristics of tumor progression. L1 expression was associated with chemotherapy response.

Key words: L1 (CAM); CD171; Ovarian cancer; Biomarker; Prognostic factor; Tumor progression.

Introduction

The L1 (CAM) molecule was first cloned and identified to be a 200-kDa transmembrane glycoprotein from mice [1]. L1 (CAM) belongs to the immunoglobulin (Ig) superfamily of ‘‘recognition’’ molecules and mediates cell-cell adhesion by using a Ca²⁺ independent homophilic binding mechanism [2, 3]. These homophilic interactions are considered to promote neurite outgrowth and other morphogenetic events [4, 5]. In addition, L1 (CAM) participates in heterophilic interactions with other adhesion molecules such as NCAM, TAG-1/axonin-1, integrins, CD24, laminin and proteoglycans [6-13]. Initially L1 (CAM), (subsequently designated CD 171), was extensively studied in the nervous system [14-16]. However, L1 (CAM) is expressed also by hematopoietic cells and certain epithelial cells as well as by a variety of human tumor cell lines [17-19].

Recently L1 (CAM) emerged as a promising new biomarker for the diagnosis and prognosis of human ovarian and endometrial carcinomas [20-22]. Not only was L1 (CAM) expression correlated with disease progression, even in early stage carcinomas, but serum/ tissue levels were associated with recurrent disease and short survival as well [20]. L1 (CAM) has been advocated by the same team of investigators as a marker that ‘‘provides an alternative classification of gynecologic tumors according to their aggressiveness rather than their histology’’ [21].

The reliability of L1 (CAM) as a new marker and its possible use as a prognostic marker would be benefited by additional and independent studies designed to confirm its relation with stage, grade and other histological morphologic findings (invasive fronts, tumor aggregates, etc.) that have been used as traditional indicators of ‘‘tumor aggression’’ and survival.

Materials and Methods

Out of 95 ovarian cancers that were operated on in the hospital between 2001 and 2005, all Stage I and poorly differentiated Stage III serous neoplasms that received six cycles of postoperative TC (175-180 mg/m² paclitaxel and carboplatin) after calculating the area under the concentration curve (= 5) with available specimens and follow-up were selected for further analysis.

The samples included were further selected from patients that had serous neoplasms and were matched to other risk factors (age, family history). Genetic marker analysis was not available. Control ovaries were obtained from oophorectomy specimens of women operated on for benign disease (fibroid uterus) and were matched for age with the study group. All above-mentioned samples were obtained from each patient.

One hundred and one pathological samples from paraffin-embedded tissue were included. The histology of the surgical specimens were 47 serous invasive ovarian tumors, 14 serous borderline tumors, 20 serous benign cystadenomas and 20 normal ovaries were used as the control. Tumor stage, histology and grade of these cases are summarized in Table 1.

Institutional Review Board (IRB) approval was given. Clinicopathologic information was obtained from medical records.

Cancer patients were classified after staging laparotomy (the most common initial surgical procedure consisted of abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and lymph node sampling) was performed. Nine were classified as Stage I disease (FIGO I) (4 with good differentiation - G1 and 5 with moderate differentiation - G2) and 38 as Stage III (poorly differentiated - G3) ovarian cancers.
All slides were jointly reviewed by two pathologists with special interest in gynecological pathology. The direct effects of chemotherapy were assessed using the World Health Organization criteria.

At the completion of primary chemotherapy, response was assessed on the abdominopelvic CT plus serum CA 125, physical examination and a statement in the medical record about being clinically disease-free. For survival analysis the Stage III patients were dichotomized by a L1 immunohistochemistry score of 3 (the calculated mean for Stage III L1 expression was 2.88). The two groups (high vs low expression) that were compared did not differ in age, family history or level of cytoreduction. The mean follow up was three years. At the time of the analysis 11/38 had died of their disease. Different regimens were given as second- and third-line therapy and the different groups (after further classifying them according to second-line chemotherapy) had small numbers to be analyzed separately. Therefore although both the progression-free interval and the overall survival were calculated, we report the progression-free interval as the main outcome of the study.

Immunohistochemistry Western blotting and evaluation of data

At least one tissue sample per case was studied. L1 was immunolocalized on 4-μm tissue sections using one monoclonal antibody, clone UJ127 to LICAM (Abcam laboratories, Cambridge Science Park, England) that recognizes a heavily glycosylated protein (between 200-220 kD) produced by a gene located in Xq28. The immunostaining technique included blockage of endogenous peroxidase activity with 3% H2O2 and antigen retrieval with 1 μM EDTA pH 8 (Labvision UK) in a microwave oven at 700 W for 10 min. The sections were incubated at 4°C with the primary antibody at a dilution of 1:200. Detection was performed using a commercially available kit (Envision, Dako) according to the manufacturer’s instruction. The specificity of immunostaining was checked by substituting the primary antibody with non-related isotypic (IgG1) mouse immunoglobulin at a comparable dilution. Immunoreactivity of the small nerves was used as an internal positive control for L1 (CAM).

Two pathologists evaluated L1 immunoreactivity in tumor cells independently and blindly, without knowledge of patient identity or clinical outcome. Immunoreactivity was assessed based on the intensity of immunostaining (immunointensity II) and the percentage of immunopositive tumor cells (immunopositivity IP). A two-score system for II and IP was used. Extent of immunoreactivity was estimated by multiplying the values for extent and intensity IP. The Mann-Whitney U and the the Kruskal-Wallis non-parametric tests were used for L1 (CAM) expression. Progression-free survival and overall survival were estimated by the method of Kaplan and Meier. The log-rank test was used to compare differences between survival curves. Univariate and multivariate analyses were performed using Cox proportional hazards regression. The covariates (age, family history or level of cytoreduction of the univariate analyses) were used for the multivariate analysis (all patients were Stage 3/G3).

Results

L1 immunoreactivity was not found in the normal ovarian stroma or in the normal ovarian surface epithelium. Focally, few endothelial cells showed immunoreactivity. Moreover, occasional tubal epithelial cells showed weak staining while hilar nerves showed intense immunoreactivity as expected (not shown).

Both the extent and intensity of the immunoreactivity showed variation that could not be considered patternless. The lowest immunoreactivity scores were noted in normal ovaries and benign tumors and the highest in advanced stage tumors (Figure 1a). The results of the semiquantitative evaluation of immunoreactivity are summarized in Table 1, according to the stage and grade. When the Kruskal-Wallis test was applied in benign, bor-
Figure 1a. — Extensive and intense L1 immunostaining of infiltrating ovarian serous carcinoma (original magnification 40 x).
Figure 1b. — Intense L1 immunoreactivity with predominant localization at the tumor cell membrane (original magnification 500 x).

Figure 2a. — L1 immunostaining is enhanced at the invasive fronts of solid tumor aggregates (original magnification 100 x).
Figure 2b. — L1 immunostaining is increased at the polar regions of carcinomatous glands (original magnification 200 x).
Figure 2c. — Increased L1 immunostaining was most prominent in cells invading singly or in tiny aggregates (original magnification 500 x).
Figure 2d. — Microscopic focus of early stromal invasion in a serous tumor that otherwise showed the morphology of a borderline serous tumor. Notable is the markedly enhanced L1 immunoreactivity of the few invading tumor cells (arrow) (original magnification 250 x).
Microscopic focus of early stromal invasion in a serous tumor that otherwise showed the morphology of a borderline serous tumor. Notable is the enhanced L1 immunoreactivity of invading tumor cells (original magnification 250 x).
L1 (CAM) (CD171) in ovarian serous neoplasms

derline, Stage 1 and Stage 3 the result was highly significant (p < 0.001).

Carcinomatous cells showed predominantly membranous localization of the immunoreactivity (Figure 1b). Indeed increased immunostaining was noted at the invasive fronts of solid tumor aggregates (Figure 2a), at the invading branches of carcinomatous glands (Figure 2b) and it was most prominent in cells invading singly or in tiny aggregates (Figure 2c).

In addition, microinvasive foci in two borderline tumors showed preferential L1 (CAM) immunostaining (Figure 2d), despite the absence of statistically significant increase of immunoreactivity in borderline compared to benign tumors (p = 0.35).

Advanced (Stage 3) tumors demonstrated significantly higher L1 (CAM) immunostaining scores than Stage I (p = 0.04). Tumors of higher grade showed significantly higher L1 (CAM) immunoreactivity scores (p = 0.03) (Table 1).

In the retrospective analysis of L1 (CAM) expression by Western blotting analysis on 33 serous carcinomas, 14 serous borderline tumors and ten benign serous tumors we identified L1 (CAM) in most examined ovarian carcinomas of advanced stage (in 20 of 30 specimens of women with Stage III serous-type carcinoma) (Figure 3). We did not detect L1 (CAM) in 9/10 benign serous specimens as well as in the healthy control ovaries. Moreover, L1 (CAM) protein levels varied among the different groups with higher levels detected in G3 undifferentiated ovarian carcinomas (ratio L1 (CAM)/actin: 100%) followed by G2 serous-type carcinoma (ratio L1(CAM)/actin: 80%) and borderline serous (ratio L1(CAM)/actin: 30%).

All (n = 10) low L1 (CAM) expressing tumors exhibited complete response rate to TC while in high L1 (CAM) expressing tumors (n = 28), 18 had a complete clinical response, eight had a partial clinical response, and two had progressive disease (p < 0.01).

Patients with FIGO Stage III, G3 with high (n = 28) versus low (n = 10) L1 (CAM) expression had a statistically significant (p = 0.001) shorter median progression-free survival (PFS) of 17.50 (median) (mean 18.73, standard deviation 10.27) versus 28.50 (median) months (mean 29.37, standard deviation 5.06 ), respectively. The PFS survival curve which was statistically significant (log-rank = 0.006) is shown in Figure 4. The Cox regression model for progress-free survival when L1 expression was increased showed a hazard ratio = 3.8279 (95% CI 0.91-15.92, p = 0.045). The multivariate analysis verified L1 (CAM) expression as an independent prognostic factor for PFS. Despite the statistically significant PFS in Stage III patients, the overall survival of the patients was not statistically different (p = 0.213) with a L1 (CAM) increased expression median of 28.50 months and a L1 (CAM) decreased expression median of 29 months for the two groups, respectively. Due to the short follow-up (3 years) all low stage and borderline patients were alive and disease free.

Discussion

L1 (CAM) has been advocated as a novel prognostic marker in ovarian neoplasms. Our study offers additional support to this notion, directly and indirectly. In patients with serous ovarian carcinomas, Stage III and grade 3, L1 (CAM) low expression was associated with a better response to the chemotherapy (TC = carboplatin paclitaxel) and prolonged progress-free survival (PFS). All the other risk factors (age, stage, grade, level of cytoreduction, and family history) were not statistically different between the two L1 (CAM) expression groups (low vs high) and multiple regression analysis showed it is an independent prognostic factor. This is in accordance with a recent report that suggested a link between L1 (CAM) expression and chemoresistance of ovarian carcinomas [23].

In addition, we noted that L1 (CAM) expression (as determined by IHC and Western blotting) correlated significantly with established prognostic parameters such as the stage and grade of serous ovarian carcinomas. L1 (CAM) expression increased significantly and progressively from benign to aggressive ovarian neoplasms, in a manner analogous to that of a genuine tumor progression marker.
Furthermore, certain immunohistological findings suggested that L1 (CAM) could be pivotal in tumor cell invasion. We noted increased L1 (CAM) immunoreactivity at the invasive fronts of aggressive carcinomas as well as at the invasive component of microinvasive serous tumors. L1 (CAM), being an adhesion molecule, could promote cancer invasion through interactions with other critical adhesion molecules and extracellular matrix proteins.

L1 (CAM) could find some usage in the preoperative evaluation of a given ovarian carcinoma. High L1 (CAM) expression in a small biopsy sample could predict an aggressive behavior or an advanced stage and could assist in designing a more suitable management protocol.

Recent work has shown that antibodies to L1 (CAM) have therapeutical potential and can reduce cell proliferation in vitro [24, 25], and in vivo growth in a xenograft mouse model for human ovarian carcinoma [26]. Thus, L1 (CAM) might be a novel target for antibody-based therapy as second-line therapy against aggressive human ovarian tumors.

References


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Recurrent cervical carcinoma after radical hysterectomy and pelvic lymph node dissection: a study of 32 cases

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Summary

Purpose of investigation: To investigate the characteristics of patients with recurrent cervical carcinoma after radical hysterectomy and pelvic lymph node dissection (RHND), and to evaluate the effect of clinical and surgical pathologic factors on the outcome of these patients. Methods: Data from the files of 32 patients with recurrent cervical carcinoma after RHND managed at the Soroka Medical Center from 1962 through 2005 were analyzed. Results: These 32 patients represent a recurrence rate of 25.4%. The median recurrence-free interval was 19.3 (range, 1-106) months. The prevalent signs and symptoms were obstructive nephropathy, sacral pain and bowel obstruction. Sixteen (50%) patients had loco-regional recurrence alone, 12 (27.5%) loco-regional plus distant recurrence, and four (12.5%) distant recurrence alone. Treatment modalities included radiotherapy, chemotherapy and various surgical procedures. The 5-year survival rate was 35%, with 22 (68.7%) of the patients dead of disease at the end of follow-up. Univariate analysis demonstrated a significant worsening in survival with each of the following factors: loco-regional plus distant recurrence (p = 0.010), positive pelvic lymph nodes (p = 0.010), tumor size ≥ 3 cm (p = 0.013), positive lymph vascular space involvement (p = 0.017) and RHND without bilateral salpingo-oophorectomy (p = 0.042). In a multivariate analysis, extent of recurrent disease (loco-regional alone versus loco-regional plus distant recurrence) and pelvic lymph node status (negative vs positive) at RHND were the only significant predictors of survival. Uremia was the most common cause of death. Conclusions: Recurrent cervical carcinoma after RHND is a grave disease with unfavorable prognosis. In both univariate and multivariate analyses, extent of recurrent disease and pelvic lymph node status at RHND were significant predictors of survival.

Key words: Cervical carcinoma; Radical hysterectomy; Recurrence; Prognostic factors; Survival.

Introduction

Recurrent cervical carcinoma after primary treatment with radical hysterectomy and pelvic lymph node dissection (RHND) and/or pelvic radiotherapy poses a grave therapeutic problem. Most patients with recurrent cervical carcinoma after RHND have already exhausted pelvic radiotherapy in a postoperative adjuvant setting and cannot tolerate further pelvic radiotherapy. Although these patients are usually candidates for systemic chemotherapy, and/or irradiation to extra-pelvic sites, and/or various surgical procedures in a palliative setting, the prognosis is most often dismal [1-9]. The Soroka Medical Center (SMC) in Beer-Sheva is the only tertiary care medical facility in the south of Israel that provides hospital care for a population of approximately 500,000 inhabitants. From January 1962 through October 2005, 126 patients with early-stage cervical carcinoma (FIGO clinical Stages IA1-IIA), were treated by RHND and followed-up at the SMC. Notably, 122 (96.8%) of the 126 patients were treated and followed-up between January 1981 and October 2005. In a study of these 126 patients published previously in this journal, we showed that pelvic lymph node status is the strongest factor influencing the decision of whether or not to administer adjuvant pelvic radiotherapy after RHND and the most significant predictor of survival [11]. Of these 126 patients, 32 (25.4%) had developed recurrent disease. The aims of this study were (1) to investigate the characteristics, pattern of recurrence and treatment of patients with recurrent cervical carcinoma after RHND and (2) to evaluate the effect of clinical and surgical pathologic factors on the outcome of these patients.

Patients and Methods

The clinical and pathological records of 32 patients with recurrent cervical carcinoma after RHND treated and followed-up at the SMC between January 1962 and October 2005 were reviewed. The surgical technique performed with RHND was consistent with a Class III extended hysterectomy as described by Piver et al. [12]. The pelvic lymph node dissection consisted of removal of all lymphatic tissue around the common, external, and internal iliac vessels and anterior to the obturator nerve. For patients who received postoperative adjuvant pelvic radiotherapy, it consisted of external megavoltage photon irradiation employing a 10 MeV linear accelerator usually delivering 4,500-5,040 cGy to the whole pelvis in daily fractions of 180 cGy via an AP-PA opposed fields or the four-field box technique. This was usually followed by two vaginal intracavitary...
applications of brachytherapy using cesium-137 (each application, 2,000 cGy) via a vaginal cylinder (Delcos) or ovoids (colpostats). Since January 2000, external pelvic radiotherapy was given as a rule concomitantly with intravenous chemotherapy composed of weekly cisplatin 40 mg/m². After a thorough record review, all patients were retrospectively staged according to the revised FIGO staging system for gynecologic cancer [13]. Recurrences were documented with histopathologic examination when possible. Thorough investigations were conducted to delineate the site and extent of recurrent disease including physical examination, chest X-ray, abdominal and pelvic computerized tomography, cystoscopy, rectoscopy, and bone isotopic scan. Sites of tumor recurrence were categorized as vaginal cuff, pelvic, vaginal cuff plus distant recurrence, pelvic plus distant recurrence, and distant alone. Vaginal cuff recurrence was defined as recurrence involving the vaginal cuff only without involvement of other pelvic organs. Pelvic recurrence was defined as recurrence involving at least one of the following pelvic organs: the bladder, rectum, pelvic lymph nodes, and pelvic sidewall. If recurrent disease was found to involve both the vaginal cuff and at least one of the aforementioned pelvic organs, the recurrence was categorized as pelvic recurrence. Vaginal cuff recurrences and pelvic recurrences were grouped under the heading of loco-regional recurrences. Distant recurrence was defined as tumor involving organs outside the pelvis. The following data were retrieved from the files of the patients: age at initial diagnosis, menopausal status, clinical stage at initial diagnosis, handling of ovaries during RHND, pathologic findings, administration of adjuvant radiotherapy, length of recurrence-free interval, signs and symptoms of recurrence, sites of recurrence, method of therapy for recurrent disease, results of follow-up and direct cause of death (if applicable).

Differences between patient groups were tested by the chi-square test with Yates’ correction for small numbers [14]. Survival was calculated using the Kaplan-Meier method [15] and compared statistically with use of the log-rank test [16]. Multivariate analysis using the Cox proportional hazards regression analysis [17] was employed to evaluate the joint effects of clinical and surgical pathologic variables on survival. Only p values < 0.05 were considered statistically significant.

### Results

Patient characteristics are displayed in Table 1. The mean number of pelvic lymph nodes removed per patient during RHND was 25.9 (median, 21.5; range, 1-62). Positive pelvic lymph nodes were found in 14 (43.7%) of the 32 patients. The mean number of positive pelvic lymph nodes per patient in patients with positive pelvic lymph nodes was 4.4 (median, 3; range, 1-15). Lower paraaortic lymph node sampling was performed during RHND in 15 (46.8%) of the 32 patients with a mean of 0.72 (median, 1; range, 0-2) lymph node removed per patient. All paraaortic lymph nodes removed were negative for metastases. Eighteen (56.2%) of the 32 patients had adjuvant pelvic radiotherapy after RHND; 11 (34.3%) received external pelvic irradiation (XRT) followed by brachytherapy (BT), six (18.7%) received XRT alone, and one (3.1%) received BT alone. In two of the 11 patients who received XRT followed by BT and in one of the six patients who received XRT alone, the XRT was given concomitantly with intravenous systemic chemotherapy (5 courses of weekly cisplatin 40 mg/m²). The total dose of XRT per patient ranged from 3,960 cGy to 9,960 cGy (mean, 5,482 cGy; median, 5,040 cGy). The total dose of BT per patient ranged from 1,500 cGy to 6,080 cGy (mean, 3,223 cGy; median, 3,500 cGy).

The median time to recurrence was 19.3 (mean, 24.9; range, 1-106) months; 20 (62.5%) patients recurred within two years after RHND (12 during the first year and 8 during the second year), whereas 12 (37.5%) recurred from two to 8.83 years after RHND. Signs and symptoms of recurrent disease are listed in Table 2. The prevailing signs and symptoms were obstructive nephropathy (14 patients, 43.7%), sacral pain (9 patients, 28.1%) and bowel obstruction (5 patients, 15.6%). Specific sites of recurrence were the vaginal cuff alone in five (15.6%) patients, pelvic – 11 (34.4%), vaginal cuff + distant recurrence – two (6.2%), pelvic + distant – ten (31.2%), and distant recurrence alone – four (12.5%). Overall, 16 (50%) patients had loco-regional recurrence alone (vaginal cuff and/or pelvis), 12 (27.5%) loco-regional plus distant recurrence, and four (12.5%) distant recurrence alone. Thus, loco-regional recurrence occurred in 28 (87.5%) patients and distant recurrence in 16 (50%). Distant sites of recurrence are listed in Table 3. The most common sites of distant recurrence were the lungs (8 patients, 25%) and bones (7 patients, 21.8%). Treatment modalities for recurrent disease are listed in Table 4. Twenty-four (75%) of the 32 patients had radiotherapy and/or chemotherapy for recurrent disease; nine had radiotherapy, six radiotherapy followed by

| Table 1. — Patient characteristics (32 patients). |
|---|---|---|
| Age at initial diagnosis (years) | No. | % |
| Mean | 46.8 | |
| Range | 24-71 | |
| Menopausal status | | |
| Premenopausal | 17 | 53.1 |
| Postmenopausal | 15 | 46.9 |
| Stage at initial diagnosis | | |
| IA<sub>1</sub> | 1 | 3.1 |
| IB<sub>1</sub> | 17 | 53.1 |
| IB<sub>2</sub> | 7 | 21.9 |
| IIA | 7 | 21.9 |
| Histologic type | | |
| Squamous cell carcinoma | 22 | 68.8 |
| Adenosquamous carcinoma | 5 | 15.6 |
| Adenocarcinoma | 3 | 9.4 |
| Verrucous carcinoma | 1 | 3.1 |
| Clear cell carcinoma | 1 | 3.1 |
| Grade | | |
| 1 | 8 | 25 |
| 2 | 8 | 25 |
| 3 | 16 | 50 |
| Handling of ovaries during RHND | | |
| Both ovaries removed | 20 | 62.5 |
| At least one ovary preserved | 12 | 37.5 |
| Adjuvant radiotherapy | | |
| No | 14 | 43.7 |
| Yes | 18 | 56.2 |

RHND, radical hysterectomy and pelvic lymph node dissection.
chemotherapy, seven chemotherapy followed by radiotherapy, and two chemotherapy. Overall, 22 (68.7%) of the 32 patients had radiotherapy and 15 (46.9%) chemotherapy. Of the 22 patients who had radiotherapy for recurrent disease, 13 had not received adjuvant pelvic radiotherapy after RHND and nine had received adjuvant pelvic radiotherapy after RHND. All 13 radiotherapy-naïve patients had pelvic radiotherapy for loco-regional recurrent disease, whereas the nine patients who had already exhausted pelvic radiotherapy for their primary disease received radiotherapy for recurrent disease to sites other than the pelvis. Of the 15 patients who had chemotherapy for recurrent disease, six patients had cisplatin + 5-fluorouracil (5-FU), three - single-agent cisplatin, one - cisplatin + 5-FU followed by carboplatin + ifosfamide, one - cisplatin + 5-FU followed by doxorubicin, one - BIP (bleomycin + ifosfamide + cisplatin), one - cisplatin + 5-FU followed by BIP, one - BIP followed by paclitaxel (taxol), and one - BIP followed by BIC (bleomycin + ifosfamide + carboplatin) followed by vinorelbine. Five (15.6%) of the 32 patients had various surgical procedures for recurrent disease; these included inguinal lymphadenectomy for inguinal lymph node metastases in two patients, anterior exenteration for central pelvic recurrence involving the bladder in one, amputation of the leg below the knee for tibial metastases in one, resection of the ischial bone in one, and resection of an abdominal mass in one. Obstructive nephropathy was managed by unilateral percutaneous nephrostomy (PCN) in ten (31.2%) patients, and insertion of ureteric stent in one patient. Bowel obstruction was handled by colostomy in two patients and ileostomy in one patient. Pleural effusion was managed by pleurodesis in one patient.

Follow-up after detection of recurrent disease ranged from 1-241 months, with eight (25%) of the 32 patients followed for at least five years or until the time of death. No patients were lost to follow-up; 22 (68.7%) patients had died of disease, one (3.1%) had died of intercurrent disease, eight (25%) were alive free of disease, and one (3.1%) was alive with disease. The cumulative 1-, 2-, 3-, 4-, and 5-year survival rates after detection of recurrence were 66%, 46%, 39%, 35% and 35%, respectively. Notably, none of the five patients with vaginal cuff recurrence alone were dead of recurrent disease at the end of follow-up. Two (50%) of the four patients with distant recurrence alone were dead of disease at the end of follow-up. Outcome, pelvic lymph node status at RHND and length of recurrence-free interval (≤ 2 years vs > 2 years) in relation to recurrence site in the 28 patients with loco-regional ± distant recurrence are displayed in Table 5. Eight (50%) of 16 patients with loco-regional recurrence alone were dead of disease at the end of follow-up, whereas all 12 patients (100%) with loco-regional + distant recurrence were dead of disease at the end of follow-up. The difference in the proportion of patients dead of disease between loco-regional recurrence alone and loco-regional + distant recurrence is of borderline significance (X² = 3.3; 0.1 > p > 0.05). The difference in the proportion of patients with recurrences free interval ≤ 2 years between loco-regional recurrence alone and loco-regional + distant recurrence was not significant (X² = 1.6; p > 0.1).

### Table 2. Signs and symptoms of recurrent cervical carcinoma (32 patients).

<table>
<thead>
<tr>
<th>Sign and symptoms</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstructive nephropathy</td>
<td>14</td>
<td>43.7</td>
</tr>
<tr>
<td>Sacral pain</td>
<td>9</td>
<td>28.1</td>
</tr>
<tr>
<td>Bowel obstruction</td>
<td>5</td>
<td>15.6</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Leg deep vein thrombosis</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>Inguinal lymphadenopathy</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>Leg lymphedema</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Back pain,</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Leg pain</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Perineal lesion</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Hematuria</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Abdominal swelling</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Mediastinal lymphadenopathy</td>
<td>1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Note: Some patients had more than one sign or symptom; therefore, the number of patients adds up to more than 32.

### Table 3. Distant sites of recurrent disease (16 patients).

<table>
<thead>
<tr>
<th>Site</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Bone</td>
<td>7</td>
<td>21.8</td>
</tr>
<tr>
<td>Liver</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Inguinal lymph node</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>Paraaortic lymph node</td>
<td>2</td>
<td>6.2</td>
</tr>
<tr>
<td>Perineum</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Abdominal wall</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Omentum</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Mediastinum</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td>Brain</td>
<td>1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Note: Some patients had more than one distant recurrent site; therefore, the number of patients adds up to more than 16.

### Table 4. Treatment modalities for recurrent disease (32 patients).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Nephrostomy</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Ureteric stent</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Colostomy</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ileostomy</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pleurodesis</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Note: Some patients had more than one treatment modality; therefore, the number of patients adds up to more than 32.

### Table 5. Recurrence site (locoregional vs loco-regional + distant) in relation to pelvic lymph node status at RHND, recurrence-free interval, and number of patients dead of disease at the end of follow-up (28 patients).

<table>
<thead>
<tr>
<th>Site of recurrence</th>
<th>No. of patients</th>
<th>pelvic lymph node status at RHND</th>
<th>recurrence-free interval</th>
<th>Dead of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loco-regional</td>
<td>16</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Loco-regional + Distant</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>16</td>
<td>12</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: The difference in the proportion of patients dead of disease between loco-regional recurrence alone and loco-regional + distant recurrence is significant (X² = 11.02; p < 0.001). The difference in the proportion of patients with positive pelvic lymph node at RHND between loco-regional recurrence alone and loco-regional + distant recurrence is of borderline significance (X² = 3.3; 0.1 > p > 0.05). The difference in the proportion of patients with recurrence-free interval ≤ 2 years between loco-regional recurrence alone and loco-regional + distant recurrence was not significant (X² = 1.6; p > 0.1).
follow-up; this difference was statistically significant ($X^2 Y = 11.02; p < 0.001$) (Table 5). The difference with respect to pelvic lymph node status at RHND between patients with loco-regional recurrence alone and patients with loco-regional + distant recurrence was of borderline significance ($X^2 Y = 3.3; 0.1 > p > 0.05$) (Table 5). The difference with respect to length of recurrence-free interval ($\leq 2$ years vs $> 2$ years) between patients with loco-regional recurrence alone and patients with loco-regional + distant recurrence was not significant ($X^2 Y = 1.6; p > 0.1$) (Table 5). Univariate analysis with use of the log-rank test demonstrated a significant worsening in survival from recurrent cervical carcinoma with each of the following factors: loco-regional recurrence in conjunction with distant recurrence ($p = 0.010$), positive pelvic lymph nodes at RHND ($p = 0.010$), tumor size $\geq 3$ cm at RHND ($p = 0.013$), positive lymph vascular space involvement at RHND ($p = 0.017$) and RHND without bilateral salpingo-oophorectomy ($p = 0.042$), whereas positive parametrial and/or paracervical involvement at RHND ($p = 0.069$) and positive or close vaginal margin involvement at RHND ($p = 0.089$) were of borderline significance (Table 6). Univariate analysis failed to demonstrate a significant worsening in survival with each of the following clinical and surgical pathologic factors: penetration $\geq 50\%$ of the thickness of the cervical wall at RHND ($p = 0.219$), administration of adjuvant radiotherapy after RHND ($p = 0.310$), Stage IB2 + IIA at RHND ($p = 0.398$), recurrence-free interval $\leq 2$ years ($p = 0.448$) and positive lower uterine segment involvement at RHND ($p = 0.998$) (Table 6). Multivariate analysis with use of the Cox proportional hazards regression analysis of clinical and surgical pathologic variables with endpoint death demonstrated after sequential elimination by backward stepwise logistic regression of non-significant factors (“better fit” model) that recurrence site (loco-regional alone vs loco-regional + distant recurrence) and pelvic lymph node status at RHND were the only significant predictors of survival from recurrent cervical carcinoma (Table 7). Uremia was recorded as the direct cause of death in 12 (54.5%) of the 22 patients who were dead of disease at the end of follow-up; other direct causes of death included sepsis, pulmonary embolism and brain metastases.

### Table 6. — Univariate analysis with use of the log-rank test of clinical and surgical pathologic factors in relation to 5-year survival in patients with recurrent cervical carcinoma after radical hysterectomy and pelvic lymph node dissection.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of patients</th>
<th>5-year survival</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loco-regional</td>
<td>16</td>
<td>48.1%</td>
<td></td>
</tr>
<tr>
<td>Loco-regional + distant</td>
<td>12</td>
<td>0.0%</td>
<td>0.010</td>
</tr>
<tr>
<td>Positive pelvic lymph nodes at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>51.8%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>14.3%</td>
<td>0.010</td>
</tr>
<tr>
<td>Tumor size at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;3$ cm</td>
<td>13</td>
<td>56.4%</td>
<td></td>
</tr>
<tr>
<td>$\geq 3$ cm</td>
<td>12</td>
<td>16.7%</td>
<td>0.013</td>
</tr>
<tr>
<td>Lymph vascular space invasion at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>54.1%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>7.7%</td>
<td>0.017</td>
</tr>
<tr>
<td>RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without BSO</td>
<td>12</td>
<td>22.2%</td>
<td></td>
</tr>
<tr>
<td>With BSO</td>
<td>18</td>
<td>50.0%</td>
<td>0.042</td>
</tr>
<tr>
<td>Parametrial/paracervical involvement at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>21</td>
<td>40.4%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>0.0%</td>
<td>0.069</td>
</tr>
<tr>
<td>Vaginal margin involvement at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>25.3%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>45.7%</td>
<td>0.089</td>
</tr>
<tr>
<td>Penetration of cervical wall at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;50%$</td>
<td>9</td>
<td>53.3%</td>
<td></td>
</tr>
<tr>
<td>$\geq 50%$</td>
<td>18</td>
<td>26.7%</td>
<td>0.219</td>
</tr>
<tr>
<td>Adjuvant radiotherapy after RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>48.2%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>25.9%</td>
<td>0.310</td>
</tr>
<tr>
<td>Stage at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA2+IB1</td>
<td>18</td>
<td>41.9%</td>
<td></td>
</tr>
<tr>
<td>IB2+IA1</td>
<td>14</td>
<td>25.7%</td>
<td>0.398</td>
</tr>
<tr>
<td>Recurrence-free interval</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq 2$ years</td>
<td>20</td>
<td>30.0%</td>
<td></td>
</tr>
<tr>
<td>$&gt;2$ years</td>
<td>12</td>
<td>45.7%</td>
<td>0.448</td>
</tr>
<tr>
<td>Lower uterine segment involvement at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>34.1%</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10</td>
<td>25.0%</td>
<td>0.998</td>
</tr>
</tbody>
</table>

**Note:** Information regarding survival was available for 32 patients. Some factors were not available for all patients; therefore, the number of patients in some of the patient groups adds up to less than 32.

RHND, radical hysterectomy and pelvic lymph node dissection; BSO, bilateral salpingo-oophorectomy.

### Table 7. — Multivariate analysis (Cox proportional hazards regression analysis) of clinical and surgical pathologic variables with endpoint death in patients with recurrent cervical carcinoma after RHND. This "better fit" model was achieved after sequential removal of non-significant factors by backward stepwise logistic regression.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds Ratio</th>
<th>95% confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loco-regional</td>
<td>1.000</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Loco-regional + distant</td>
<td>2.785</td>
<td>1.055-7.352</td>
<td>0.039</td>
</tr>
<tr>
<td>Positive pelvic lymph nodes at RHND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.000</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.738</td>
<td>1.040-7.208</td>
<td>0.041</td>
</tr>
</tbody>
</table>

RHND, radical hysterectomy and pelvic lymph node dissection.

**Discussion**

The presently reported 32 patients represent a recurrence rate of 25.4%. Other authors have reported a recurrence rate after RHND ranging from 7.5% to 19.8% [5,6,8,18]. In this study, the median time from RHND to recurrence was 19.3 (mean, 24.9; range, 1-106) months; approximately one-third (37.5%) of the patients developed recurrent disease within one year after RHND and nearly two-thirds (62.5%) within two years. Samlal et al. [6] observed a median recurrence-free interval of 14 (range, 3-64) months, with 77% of the recurrences occurring within three years after RHND. Grisaru et al. [18] noted that the median time from initial surgery to recurrence was 14 months, with 67% of the patients having...
Recurrent cervical carcinoma after primary treatment carries a poor prognosis, which is reflected in this series by a 5-year survival of only 35%. This survival, however, is better than the 5-year survival rate of 10.1% reported by Wang et al. [5] in their series of 177 patients with recurrent cervical carcinoma after RHND. In this series, 22 (68.7%) of the 32 patients were dead of disease at the end of follow-up. Notably, all five patients with vaginal cuff recurrence alone were successfully treated with pelvic radiotherapy for recurrent disease and were alive with no evidence of disease at the end of follow-up. These patients could undergo pelvic radiotherapy for recurrent disease because they had not already previously exhausted pelvic radiotherapy in an adjuvant setting after RHND. Nevertheless, eight (72.7%) of the remaining 11 patients with recurrence in the pelvis beyond the vaginal cuff were dead of disease at the end of follow-up. Overall, we have observed that eight (50%) of 16 patients with loco-regional plus distant recurrence were dead of disease at the end of follow-up, whereas all 12 (100%) patients with distant recurrence died of disease. Of the 11 patients who had pelvic recurrence alone, only one of the four patients with an isolated pelvic sidewall recurrence died of disease at the end of follow-up. Thus, we have shown that recurrence site is a significant predictor of survival. Samlal et al. [6] observed that 22 (81.5%) of 27 patients with recurrent disease after RHND had died of disease. Of the 11 patients who had pelvic recurrence alone, one of the four patients with an isolated pelvic central recurrence died of disease compared with all seven patients with an isolated pelvic sidewall recurrence (p = 0.02) [6]. All three patients with pelvic plus distant recurrence died of disease, whereas 11 (84.6%) of 13 patients with distant disease alone died of disease [6]. Wang et al. [5] demonstrated a significantly better 5-year survival rate for patients with vaginal vault recurrence alone as compared to patients with extra-vaginal recurrence (23.9% vs 7.8%, respectively, p = 0.0007). They demonstrated by means of univariate analysis that each of the following factors: positive pelvic lymph nodes at RHND, recurrence beyond the vaginal cuff, and avoidance of any therapy for recurrent disease was significantly associated with a worse survival. In a multivariate analysis, they confirmed that positive pelvic lymph nodes
at RHND and avoidance of any therapy for recurrent disease were significantly associated with a worse survival [5]. Wang et al. [5] also demonstrated that the 5-year survival rate of patients who had not exhausted pelvic radiotherapy in an adjuvant setting after RHND was significantly better than that of patients who had already received pelvic radiotherapy in an adjuvant setting after RHND (19.6% vs 3.6%, respectively, \( p = 0.0054 \)). This can be explained by the fact that patients who do not need adjuvant treatment after RHND represent a low-risk group (i.e., absence or minimal presence of high-risk factors) with an a priori better prognosis. Moreover, since these patients had not already exhausted pelvic radiotherapy in an adjuvant setting after RHND, pelvic radiotherapy can be given for loco-regional recurrent disease. We have demonstrated by means of univariate analysis a significant worsening in survival from recurrent cervical carcinoma after RHND with each of the following factors: loco-regional recurrence in conjunction with distant recurrence, positive pelvic lymph nodes at RHND, tumor size \( \geq 3 \) cm at RHND, positive lymph vascular space involvement at RHND, and RHND without bilateral salpingo-oophorectomy. In a multivariate analysis, however, extent of recurrent disease (locoregional alone vs loco-regional plus distant recurrence) and pelvic lymph node status (negative vs positive) at RHND were the only significant predictors of survival from recurrent disease.

Conclusion

One-quarter of patients with cervical carcinoma treated primarily by RHND at the SMC developed recurrent disease. Obstructive nephropathy and sacral pain were the most common signs and symptoms. Overall, loco-regional recurrence occurred in nearly 90% of the patients with recurrent disease and distant recurrence in 50%. Treatment modalities employed for recurrent cervical carcinoma included radiotherapy, chemotherapy and various surgical procedures. The prognosis was unfavorable with a 5-year survival rate of 35%. Uremia was the most common direct cause of death. Extent of recurrent disease (loco-regional alone vs loco-regional plus distant) and pelvic lymph node status (negative versus positive) at RHND were significant predictors of survival from recurrent cervical carcinoma on both univariate and multivariate analysis.

References


Concentrations of follicle stimulating hormone are increased in ovarian tumor fluid: implications for the management of ovarian cancer

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Summary

Purpose of investigation. Significant progress has been made in recent years in the understanding of the mechanisms postulated by the gonadotropin theory of ovarian carcinogenesis. In the present study we compare FSH concentrations between serum and fluid from cysts or the rectouterine pouch of patients with epithelial tumors and non-neoplastic lesions. Methods. We enrolled 277 patients. They were divided into five groups: I (n = 44) - ovarian cancer patients, II (n = 16) - borderline tumors, III (n = 40) - benign epithelial cystadenomas, IV (n = 137) - non-neoplastic lesions and V (n = 22) - admitted for “second-look” laparoscopy. Results. There were any significant differences between FSH concentrations in serum and tumor fluid in patients with ovarian cancer (36.46 vs 28.11 mIU/ml) and borderline epithelial tumors (31.5 vs 22.7 mIU/ml). For benign cystadenomas the respective concentrations were 28.96 mIU/ml in serum and 6.93 mIU/ml in tumor fluid in these groups p < 0,0000001. The same highly significant differences were found in non-neoplastic lesions (24.97 vs 4.77 mIU/ml), p < 0,0000001. Patients who underwent “second-look” laparoscopy demonstrated significant differences (p < 0.05) as FSH concentration in serum and peritoneal fluid when neoplastic cells were not disclosed, but the difference was not significant (p = 0.752) when fluid from the rectouterine pouch was positive for carcinoma cells. Conclusions. The results of our study can reflect an ineffective tumor: blood barrier and easy diffusion of gonadotropins into the tumor tissue. Local reduction of FSH levels through administration of GnRH analogs may in some clinical situations produce clear therapeutic benefits for the management of ovarian malignancies.

Key words: Ovarian cancer; Etiopathogenesis; Gonadotropins; GnRH analogs; Treatment.

Introduction

Ovarian cancer is notable for the highest mortality among gynecological tumors [1]. A mean survival time of three years in women with advanced ovarian cancer is currently all that can be achieved with the traditional approach consisting of surgical resection of the tumor followed by chemotherapy (platinum-paclitaxel) [2]. Clearly, investigations must continue for more effective and less toxic methods of treatment based on what is known about the etiopathogenesis of this neoplasm. Significant progress has been made in recent years in the understanding of mechanisms postulated by the gonadotropin theory of ovarian carcinogenesis [3-7]. Evidence is accumulating that gonadotropins (FSH, LH) participate in the neoplastic transformation of the normal ovarian epithelium [8-10]. Gonadotropin receptors have been revealed in several ovarian cancer cell lines [11-13] and it has conclusively been demonstrated that overexpression of the FSH receptor (FSHR) markedly increases the risk of cancer by enhancing the proliferation of pre-neoplastic ovarian surface epithelial cells [14]. Receptor and non-receptor mechanisms by which gonadotropins act as hormonal promoters of carcinogenesis in the ovary are much better understood today [3-5, 7].

Schiffenbauer et al. [15] found an association between neoangiogenesis which is profoundly important in carcinogenesis and gonadotropin stimulation. According to these researchers, gonadotropin-related tumor growth can be attributed to the activation of vascular endothelial growth factor (VEGF) by gonadotropins. We have previously reported that gonadotropin concentrations are significantly higher in the fluid from malignant tumors than from benign or non-neoplastic tumors [16, 17].

Indirect proof for the involvement of gonadotropins in the pathogenesis of ovarian cancer arises from the fact that ovarian cancer is virtually absent in the prepubertal period as long as the gonadostat is operational and only germ-cell tumors are observed in this period. By suppressing the pulse generator in the hypothalamic arcuate nucleus, the gonadostat inhibits pulsatory release of the gonadotropin-releasing hormone (GnRH) and gonadotropins and in consequence reduces their concentrations. Our previous studies showed a tendency to premature menopause in patients with BRCA 1 gene mutation. We believe that hypergonadotropic activity in these patients may predispose them to ovarian cancer at a younger age [19].

Understandably, the gonadotropin theory of ovarian cancer has paved the way to the use of GnRH analogs in ovarian cancer patients aimed at reducing gonadotropin levels [20-23].

The present work was undertaken to compare FSH concentrations in serum and fluid from the cyst or rectouter-
ine pouch of patients with epithelial tumors and non-neoplastic lesions of the ovary. We also hoped to gather information on the effectiveness of vascular endothelium as a barrier preventing diffusion of FSH from the blood to tumor tissue.

**Material and Methods**

We enrolled 277 patients treated because of a cyst, tumor or fluid in the rectouterine pouch at the Chair and Department of Gynecological Surgery and Oncology of Adults and Adolescents, Pomeranian Medical University. Some of the patients were previously operated on for ovarian cancer and appeared for “second-look” laparoscopy. Blood was sampled prior to the procedure. Fluid was obtained from the tumor, cyst, or rectouterine pouch during laparoscopy, laparotomy, or culdocentesis. Definitive diagnosis was based on the results of histopathology or cytology and the patients were allocated to one of the following groups: I (n = 54) ovarian cancer (44 cases when tumor fluid was obtained and 10 cases of solid tumors when fluid could only be obtained from the rectouterine pouch); II (n = 16) borderline tumors; III (n = 40) benign epithelial cystadenomas; IV (n = 137) non-neoplastic lesions including 121 patients with cysts and 16 patients with fluid in the rectouterine pouch; V (n = 30) “second-look” laparoscopy (22 cases without neoplastic lesions and 8 cases with carcinomatous cells in the aspirate).

FSH concentrations were measured in serum and fluid using commercial MEIA test kits from Abbott and the AxSYM robotic analyzer. FSH binds to the anti-bFSH coated microparticles forming an antibody-antigen complex. An aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles is transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix. The matrix cell is washed to remove unbound material. Then anti-αFSH subunit specific alkaline phosphatase conjugate is dispensed into the matrix cell and binds with the antibody-antigen complex. The matrix cell is once again washed to remove unbound material. The substrate, 4-methylumbelliferyl phosphate, is added to the matrix cell and the fluorescent product is measured by the MEIA optical assembly. Sensitivity of the FSH AxSYM assay was determined as 0.01 mIU/ml and represents the lowest measurable concentration of FSH that can be distinguished from zero.

Histopathologic and cytologic diagnosis was established at the Department of Genetics and Pathomorphology, Pomeranian Medical University.

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

**Results**

We failed to find any significant differences between FSH concentrations in serum (36.46 mIU/ml) and tumor fluid (28.11 mIU/ml), as well as between serum (24.41 mIU/ml) and peritoneal fluid (19.99 mIU/ml) in patients with ovarian cancer (group I). In borderline epithelial tumors (group II), concentrations of FSH in serum and tumor fluid were 31.5 and 22.47 mIU/ml (n.s.), respectively. For benign cystadenomas (group III), the respective concentrations were 28.96 mIU/ml and 6.93 mIU/ml.
Concentrations of follicle stimulating hormone are increased in ovarian tumor fluid: implications for the management of ovarian cancer

The same highly significant difference was found for FSH concentrations in serum and fluid of patients with non-neoplastic lesions (24.97 vs 4.72 mIU/ml; p < 0.0000001; Table 2). In the subgroup of patients with ovarian cysts, FSH concentrations in serum and cyst fluid were 24.53 and 3.75 mIU/ml, respectively (p < 0.0000001) mIU/ml). When the pathology was limited to the presence of fluid in the rectouterine pouch, FSH concentrations in serum and fluid were 28.31 and 12.04 mIU/ml, respectively (p < 0.05). Finally, patients who underwent second-look laparoscopy demonstrated significant differences (p < 0.005) as to FSH concentrations in serum and peritoneal fluid when neoplastic cells were not disclosed. The difference was not significant (p = 0.752) in cases testing positive for carcinomatous cells (Table 3).

### Table 1. — FSH concentrations in serum and fluid of patients with epithelial ovarian tumors (groups I, II, and III).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>FSH in serum [mIU/ml]</th>
<th>FSH in fluid [mIU/ml]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>W</td>
<td>34.2</td>
<td>30.44</td>
<td>26.6</td>
</tr>
<tr>
<td>n = 54</td>
<td>[1.32-117.86]</td>
<td>[26.91-41.5]</td>
<td>[11.12-78.39]</td>
</tr>
<tr>
<td>I tumor fluid</td>
<td>36.43</td>
<td>36.025</td>
<td>28.11</td>
</tr>
<tr>
<td>n = 44</td>
<td>[1.32-117.86]</td>
<td>[27.78-45.09]</td>
<td>[11.12-78.39]</td>
</tr>
<tr>
<td>rectouterine pouch fluid</td>
<td>24.41</td>
<td>24.05</td>
<td>19.99</td>
</tr>
<tr>
<td>n = 10</td>
<td>[7.87-54.21]</td>
<td>[14.16-34.65]</td>
<td>[3.86-55.3]</td>
</tr>
<tr>
<td>II</td>
<td>31.5</td>
<td>11.38</td>
<td>22.47</td>
</tr>
<tr>
<td>n = 16</td>
<td>[2.07-96.66]</td>
<td>[14.01-49.01]</td>
<td>[0.05-99.86]</td>
</tr>
<tr>
<td>III</td>
<td>28.96</td>
<td>14.18</td>
<td>6.93</td>
</tr>
<tr>
<td>n = 40</td>
<td>[2-106.31]</td>
<td>[19.79-38.14]</td>
<td>[0.01-64]</td>
</tr>
</tbody>
</table>

W - whole group, analysis of tumor fluid and rectouterine pouch fluid together; group I - cancers, group II - borderline tumors, group III - cystadenomas.

### Table 2. — FSH concentrations in serum and fluid of patients with non-neoplastic ovarian pathologies (group IV).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>FSH in serum [mIU/ml]</th>
<th>FSH in fluid [mIU/ml]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>W</td>
<td>24.97</td>
<td>7.25</td>
<td>4.72</td>
</tr>
<tr>
<td>n = 137</td>
<td>[0.1-126.99]</td>
<td>[19.45-30.48]</td>
<td>[0.01-95.93]</td>
</tr>
<tr>
<td>IV cyst fluid</td>
<td>24.53</td>
<td>7.92</td>
<td>3.75</td>
</tr>
<tr>
<td>n = 121</td>
<td>[0.1-124.28]</td>
<td>[18.81-30.24]</td>
<td>[0.01-78]</td>
</tr>
<tr>
<td>rectouterine pouch fluid</td>
<td>28.31</td>
<td>6.21</td>
<td>12.04</td>
</tr>
<tr>
<td>n = 16</td>
<td>[0.61-126.99]</td>
<td>[7.13-49.51]</td>
<td>[0.95-95.93]</td>
</tr>
</tbody>
</table>

W - whole group, analysis of cyst fluid and rectouterine pouch fluid together.

### Table 3. — FSH concentrations in serum and rectouterine pouch fluid of ovarian cancer patients undergoing second-look laparoscopy (group V).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>FSH in serum [mIU/ml]</th>
<th>FSH in fluid [mIU/ml]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>W</td>
<td>50.75</td>
<td>51.99</td>
<td>27.69</td>
</tr>
<tr>
<td>n = 30</td>
<td>[0.98-105.94]</td>
<td>[38.54-62.96]</td>
<td>[0.14-79.2]</td>
</tr>
<tr>
<td>V S-L(-)</td>
<td>52.79</td>
<td>65.17</td>
<td>23.73</td>
</tr>
<tr>
<td>n = 22</td>
<td>[0.98-105.94]</td>
<td>[37.82-67.77]</td>
<td>[0.14-79.2]</td>
</tr>
<tr>
<td>S-L(+)</td>
<td>45.14</td>
<td>41.62</td>
<td>38.59</td>
</tr>
<tr>
<td>n = 8</td>
<td>[2.68-91.92]</td>
<td>[19.26-71.01]</td>
<td>[1.71-70.49]</td>
</tr>
</tbody>
</table>

W - whole group V with presence and absence of neoplastic cells in rectouterine pouch fluid; S-L(-) - neoplastic cells absent in rectouterine pouch fluid; S-L(+) - neoplastic cells present in rectouterine pouch fluid.

Discussion

The hormonal model of tumorigenesis dating back to the 1940s [24] has lost nothing of its validity and remains the object of much discussion in the literature [3-7]. In support of this statement is the long list of direct and indirect proof for the involvement of gonadotropins in proliferative processes of ovarian surface epithelial cells [4, 5, 9, 15-17]. Mandai et al. [25] demonstrated the presence of gonadotropin receptors (LH/hCG) on ovarian cancer cells and Choi et al. [14] showed in addition that overexpression of FSHR in ovarian epithelium is accompanied by enhanced proliferation and induction of oncogenesis. Parrot et al. [11] reported that the gonadotropin receptor (OCC1) is present in some cell lines only which are also notable for a proliferative reaction in response to gonadotropins in vitro.

The discovery by Kakar et al. [26] and Irmer et al. [27]
that 80% of epithelial ovarian tumors carry receptors specific for gonadoliberin with properties identical to pituitary receptors has paved the way for therapeutic applications of gonadoliberin analogs in ovarian cancer and enabled clinical practice to benefit from what is known about the role of gonadotropins in proliferative processes of ovarian cancer cells. Clinical trials with gonadoliberin analogs began in the end of 1980s and continuously provide new insights [28-30]. Hasan et al. [30] used tamoxifen with goserelin in ovarian cancer patients not responding to chemotherapy and observed a therapeutic effect in 50% of cases (CR 3.8%, PR 7.7%, SD 38.5%). A similar observation was made by Zidan et al. [22] in patients with relapse of advanced ovarian cancer. Gonadoliberin analogs produced full remission lasting eight months in one out of 15 patients (6.8%), partial remission of 14 months in one patient (6.8%), and stabilization of symptoms for 7.5 months in three patients (20%). Similar findings were reported by Pasceviciute et al. [29]. Keeping in mind that the response to second and third chemotherapy cycles is observed in just 15% and less than 10% of cases, respectively, hormonal therapy with its low toxicity is certainly an important alternative in selected cases [29]. We have previously reported that the best therapeutic effect can be expected when therapy is supplemented with GnRH analogs [31].

Two aspects have been distinguished in the mechanism of action of GnRH analogs: firstly, they reduce the levels of gonadotropins, and secondly they act directly on receptors and suppress in various ways the proliferation of neoplastic cells [32-36]. In spite of structural similarities between GnRH receptors in the pituitary gonadotrope and tumor cells, the mechanism of signaling differs markedly [36]. Binding of ligand to the receptor on tumor cells (G1 protein ai) activates phosphotyrosine phosphatase (PTP) leading to dephosphorylation of the EGF receptor and suppression of neoplastic growth induced by EGF [37]. In some cell lines, antiproliferative activity is synonymous with induction of apoptosis [38]. It has further been shown that triptorelin, an analog of GnRH I, activates the JNK/c-jun pathway thereby suppressing neoplastic growth [39].

We have found that concentrations of FSH in serum and epithelial tumor (malignant or borderline) fluid do not differ significantly. No significant differences were noted when FSH concentrations in serum were compared with concentrations in peritoneal fluid obtained during the first cytoreductive surgery or during the second-look laparoscopy which disclosed the presence of carcinomatous cells. We take this finding as indirect evidence for an ineffective barrier allowing FSH to diffuse from the blood and accumulate in the tumor or peritoneal cavity. On the other hand, the barrier appears to be more tight in cystadenomas and non-neoplastic cysts, resulting in significantly (p < 0.0000001) lower concentrations of FSH in cyst fluid. The difference was also significant (p < 0.005) when second-look laparoscopy proved negative for carcinomatous cells.

Our present results consistently show that FSH concentrations in the fluid surrounding proliferating cells (ovarian cancer, borderline tumors, relapse) do not differ significantly from serum concentrations. As neoplastic cells are not capable of synthesizing gonadotropins, FSH seems to pass to fluid through an ineffective barrier separating neoplastic cells from the blood. In view of over-expression of gonadotropin receptors on tumor cells [11, 12], induction of proliferation processes and promotion of tumor growth is facilitated in this situation [10, 13]. Of particular interest is the observation that FSH passes freely into the peritoneal fluid. This diffusion opens the way to further tumor growth if cytoreductive surgery is unsuccessful in removing neoplastic cells from the peritoneum, and gonadotropin levels in serum remain unreduced. We believe that the use of GnRH analogs is theoretically well founded if aimed at reducing accumulation of gonadotropins in tumor tissue, an objective no less important than direct inhibition of receptor-mediated antiproliferative activity. Elimination of gonadotropins from the tumor means that all adverse effects of their action would disappear concomitantly.

It has been demonstrated that gonadotropins support cell proliferation by activating transcription of protein kinase Cα [40]. In OVCAR-3 cells, gonadotropins stimulate secretion of estradiol which subsequently induces cell growth [41]. By activating proteolysis and promoting invasiveness of tumor cells mediated by protein kinases (PKA, P13K), gonadotropins favor metastasis. FSH promotes the activity of several genes responsible for metabolic processes and cell proliferation [9]. It has been suggested that neoplastic spread in the peritoneum is the result of greater adhesiveness of ovarian cancer cells induced by gonadotropins [42].

We tend to relate the ineffective barrier in malignant and borderline tumors to neoangiogenesis which is necessary for uncontrolled proliferation, invasiveness, and metastases [43, 44]. Angiogenesis in tumors takes place in stages resembling the physiologic process but the structure of tumor vessels is different: the vascular wall is formed by endothelial cells only, the basal membrane is much thinner, and the composition of the extracellular matrix is altered. In consequence, and because of increased synthesis and secretion of VEGF [43, 44], permeability of tumor vessels is increased. Elevated concentrations of VEGF have been reported in serum and fluid from epithelial ovarian tumors, suggesting its role in tumor growth [45] and its potential use as a tumor marker [46]. Increased expression of VEGF-C and VEGF-2 is associated with aggressiveness of the tumor and its ability to spread rapidly [47].

In conclusion, similarly high levels of FSH in serum, tumor fluid, and peritoneal cavity fluid from patients with malignant epithelial neoplasms of the ovary reflect an ineffective tumor: blood barrier and easy diffusion of gonadotropins into the tumor tissue. Local reduction of FSH levels through administration of GnRH analogs may in some clinical situations produce clear therapeutic benefits for the management of ovarian malignancies.
Acknowledgments

This study was supported by grant N. 4P05A01719 from the State Committee for Scientific Research.

References


Summary

Objective: To identify risk factors for the appearance of vaginal intraepithelial neoplasia (VAIN). Material and methods: A total of 485 women with abnormal cytologies were followed over three years (2003-2006). They underwent cytology and colposcopy, and testing for human papillomavirus virus (HPV) infection. If the colposcopy was atypical, a biopsy was performed. Results: A total of 256 women were treated: 161 by cone biopsy, 103 by LLETZ, 12 by repeat conization, and 44 by total hysterectomy. In eight cases VAIN was diagnosed following hysterectomy. The average age at which VAIN appeared was 49.8 years (age range 39-61). Hysterectomy was indicated in two cases of cervical cancer, four cases of persistent high-grade cervical SIL, and two cases of recurrent high-grade cervical SIL. The mean time for the appearance of VAIN following hysterectomy was 3.8 years (range 1-9 years). Of these eight women, seven had HPV infections at high risk for carcinogenesis. Conclusions: Long-term follow-up cytology is necessary for women treated for high-grade SIL, even after hysterectomy, because of the increased risk of a primary vaginal VAIN lesion, especially in women with high-risk HPV infection.

Key words: Vaginal intraepithelial neoplasia; Human papillomavirus; Cervical SIL.

Introduction

Vaginal intraepithelial neoplasia (VAIN) is a rare disorder of the lower genital tract in contrast to cervical intraepithelial neoplasia (CIN). VAIN represents only 1% of all intraepithelial neoplasias of the lower genital tract [1, 2]. The etiopathology of both types of lesions, along with that of vulvar intraepithelial neoplasia (VIN), is closely linked to human papillomavirus (HPV) infection [3]. It is known that although the cervix is the site most frequently affected by HPV infection due to the histological characteristics of the epithelium, the infection is multicentric, simultaneously affecting the cervix, the vulva and the vagina, in this order of frequency [4].

As the vagina is less frequently involved, cytology screening to detect vaginal pathology in women who have undergone hysterectomy is less frequently performed than cervical cytology screening. The American College of Obstetricians and Gynecologists recommends periodic follow-up vaginal cytology in women who present risk factors, but without establishing what those risk factors are or at what intervals cytology should be repeated [5].

This prospective study attempts to determine the factors that place women at risk of developing VAIN.

Material and Methods

This was a prospective study carried out between 2003 and 2006 in which 485 women followed for abnormal cytology were followed. In all cases the women underwent colposcopy and genotyping of HPV using a microarray-based method. In cases of atypical colposcopy, a colposcopically guided punch biopsy was also performed.

In this study, the frequency of the appearance of VAIN was analyzed, focusing on those women who required hysterectomy. VAIN was diagnosed cytologically, and the diagnosis was subsequently confirmed by colposcopically directed punch biopsy.

Risk factors for VAIN presented by the women who had undergone hysterectomies were analyzed, with special attention to HPV infection and its different genotypes. Subsequently, the women diagnosed with VAIN and HPV infection were compared with the total sample.

Results

Of the 485 women included in the study, 316 were diagnosed with HPV infection (65%). The most frequent reason for medical consultation was cytological diagnosis of high-grade squamous intraepithelial lesions (SIL) in 200 women (41.1%) followed by low-grade SIL in 160 cases (32.9%). The remaining cytological diagnoses were ASCUS, AGUS, and cases suggestive of carcinoma.

Of the women with a cytological diagnosis of high-grade SIL, 86.5% presented HPV infection as compared with 65% of the women with low-grade SIL.

The most frequently observed colposcopy result was leukoplakia (91 cases), followed by punctuation (51 cases). Colposcopically guided biopsy confirmed the presence of high-grade SIL diagnosed cytologically in 84 women (42%), and cervical cancer in seven cases (3.5%).

In women with low-grade SIL diagnosed cytologically, colposcopically guided biopsy identified low-grade SIL in 53 cases (33.1%), and high-grade SIL in 12 cases (7.5%).

Of the total number of women followed in this study, 256 were treated; 161 by cone biopsy, 103 by LLETZ, 12...
by repeat conization, and 44 by hysterectomy. Some of the women were treated by more than one type of procedure. In 36 of the 44 cases requiring hysterectomy, a previous conization for high-grade SIL had been performed, and the indication for hysterectomy was persistent or recurrent disease. In the eight remaining cases, the indication for hysterectomy was cervical cancer.

Of the 44 women who underwent hysterectomy, eight subsequently presented a vaginal lesion (18%). The indications for hysterectomy were as follows: cervical cancer (2 cases); persistent high-grade SIL following conization, with the lesion being detected within the year following the procedure (4 cases); and recurrent high-grade SIL in which the lesion reappeared more than a year following treatment (2 cases).

The mean age of the women who presented VAIN was 49.8 years (age range 39-57 years).

The average time elapsed between hysterectomy and the appearance of VAIN was 3.8 years (range 1-9 years). In all cases, the diagnosis was made cytologically and subsequently confirmed by colposcopy and guided biopsy, and the colposcopic image was leukoplakia located in the vaginal cupula. The histologic diagnosis of the colposcopically guided biopsy was VAIN I in three cases, VAIN II in one case, and VAIN III in four cases.

Of the eight women with VAIN, seven (87.5%) had HPV infection with a high-risk genotype for carcinogenesis. In three cases it was possible to isolate two different genotypes. The most frequently isolated HPV genotypes were types 51 and 53 (Table 1).

The presence of HPV infection in women with VAIN was similar to that observed in women presenting high-grade cervical SIL (87.5% and 86.5%, respectively).

Treatment of the women with VAIN III was carried out by partial colpectomy of the vaginal cuff. In three cases the lesion was confirmed, and in one case the result was negative.

Table 1. — HPV genotypes isolated in patients with VAIN.

<table>
<thead>
<tr>
<th>Case</th>
<th>HPV genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51/56</td>
</tr>
<tr>
<td>2</td>
<td>53/35</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
</tr>
<tr>
<td>7</td>
<td>66/70</td>
</tr>
</tbody>
</table>

No HPV infection was detected in one case.

Table 2. — Risk factors for the development of VAIN.

VAIN risk factors

| Abnormal cytology* |
| History of CIN* |
| History of cervical cancer* |
| Condylomas |
| Vaginal radiotherapy* |
| Immunosuppression |
| Low level of education |
| Low social class |

* Risk factors found in the 8 women with VAIN in the study group.

Discussion

VAIN is the least frequent type of intraepithelial neoplasia of the lower genital tract [1]. Although it is possible to diagnose primary VAIN, or to diagnose it in conjunction with cervical intraepithelial neoplasia (CIN) or vulvar intraepithelial neoplasia (VIN), it is usually diagnosed in women who have previously undergone hysterectomy.

In a study analyzing the incidence of VAIN in a given population, 0.6% of all cytologies performed yielded a diagnosis of VAIN [1]. In our study, since the population was selected for abnormal cytology results, the VAIN diagnosis rate was just over 1%.

VAIN diagnoses among women who have undergone hysterectomy correspond to 70% of all VAIN diagnoses [1]. If we consider only women with previous hysterectomies, the incidence of VAIN depends on the indication for hysterectomy. Women with previous hysterectomies for CIN account for 87% of VAIN diagnoses, and women who underwent hysterectomy for benign pathology of the uterus account for 13% of VAIN diagnoses [1].

The percentage of VAIN diagnoses in women with previous hysterectomies for CIN varies between 1% and 6% [6, 7]. In this study, it was slightly over 16.6%. It should be emphasized that we were extremely conservative, performing 12 repeat conizations in cases of persistent CIN III instead of hysterectomy. In this study as well as others, these percentages may be influenced by the number of hysterectomies performed for CIN [6, 7]. Other indications for hysterectomy that lead to higher risk for detection of VAIN in follow-up may be cervical cancer [8], of which there were two cases in our study.

The average age at appearance of VAIN varies between 57 and 58 years in different studies [9, 10]. In this study, it was 49 years (age range 39-57 years).

Several risk factors that may influence the development of VAIN have been described in the literature [10, 11] (Table 2). Among these, a history of hysterectomy for cervical cancer and CIN, vaginal radiotherapy, and HPV infection, especially by high-risk genotypes. In this study, as shown in Table 2, several of these risk factors were present.

The HPV genotypes most frequently involved in VAIN are usually 16 and 18 [3, 11]. In this study, the most frequently isolated genotypes were 51 and 53 (Table 1).

The definitive diagnosis is attained by biopsy, since the initial diagnosis is cytological. VAIN is usually located in the upper third of the vagina [9, 12] and the lesion usually presents as a leukoplasic area [1], which was true in all the cases in this study.

The time elapsed between hysterectomy and the appearance of VAIN varies between one and nine years [1, 9, 13]. In this study, the mean was 3.8 years (range 1-9 years).

The recommended treatment for VAIN is surgical, based on excision or destruction techniques. Excision is recommended because it makes it possible both to confirm the lesion and to rule out the presence of invasive disease [10]. The most frequent complication [10] is...
excessive bleeding (10%), which was observed in one case in this study. In 22% of cases [10] histologic study of the vaginal cuff was negative, as found in one case in our study.

In conclusion, it should be stressed that it is important to continue long-term follow-up vaginal cytology for women who have undergone hysterectomy for CIN for a period of at least five years (the average time during which VAIN may appear following hysterectomy). Detection of HPV in these women may be of value in identifying those who are at greater risk of developing VAIN. In our view, women infected with a high-risk HPV genotype should continue to have follow-up cytologies and colposcopies until the infection disappears.

Acknowledgment

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

References


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Results of postoperative radiotherapy in the treatment of uterine sarcomas: a retrospective analysis of 46 patients

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¹Department of Radiation Oncology, Ege University Faculty of Medicine, İzmir
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³Department of Pathology, Ege University Faculty of Medicine, İzmir (Turkey)

Summary

Purpose: The aim of this study was to evaluate treatment outcome, survival data and prognostic factors in patients with uterine sarcoma treated by postoperative radiotherapy. Materials and Methods: The records of 46 patients treated between 1993 and 2003 were reviewed. Median age was 55 (range 31-75). There were 21 mixed mullerian tumors, 12 leiomyosarcomas, 11 endometrial stromal sarcomas and two adenosarcomas. According to FIGO classification 65.2% were Stage I, 17.4% Stage II, 13% Stage III and 4.3% Stage IV. All patients received external radiotherapy with 1.8 Gy daily fractions up to 50.4-64 Gy (median 50.4 Gy). Intracavitary brachytherapy was applied to 39 patients. Twelve patients received adjuvant chemotherapy. Results: Median follow-up time was 48 months (6-144 months). Seventeen patients (37%) developed distant metastases and one patient had local failure. Five-year overall, disease-free and local recurrence-free survival rates were 57.8%, 60.5% and 97.8%, respectively. Univariate analysis demonstrated that stage (p = 0.009), positive peritoneal cytology (p = 0.000) and the use of chemotherapy (p = 0.002) had a significant effect on overall survival. Prognostic factors influencing disease-free survival were stage (p = 0.005), positive peritoneal cytology (p = 0.000) and the use of chemotherapy (p = 0.002). The only prognostic factor affecting local control was stage (p = 0.000). Conclusion: Postoperative radiotherapy seems to be an effective adjuvant treatment providing high local control rates in uterine sarcomas. However its efficacy should be clarified by randomized trials. The important prognostic factors influencing the treatment results were stage, histologic subtype, tumor size and positive peritoneal cytology. Key words: Uterine sarcoma; Radiotherapy; Prognostic factors.

Introduction

Uterine sarcomas are rare tumors which comprise 3% to 7% of all malignancies of the uterus [1]. They carry a poor prognosis with a 2-year overall survival rate of less than 50% even at an early stage [1-3]. Due to the rarity of these tumors and the different characteristics and prognosis of the various histological subtypes optimum treatment strategy has not yet been defined. The recommended primary treatment is total abdominal hysterectomy and bilateral salpingo-oophorectomy. The role of adjuvant treatment after surgery is controversial and the overall prognosis remains poor. The impact of radiotherapy on local recurrence has been demonstrated by some authors [4-13] whereas others have demonstrated no benefit from adjuvant radiotherapy [14-16]. The role of adjuvant chemotherapy is also controversial [2, 6, 17-19]. Several prognostic factors influencing survival have been proposed. Most notably tumor stage, grade, and histologic subgroup are predictive of outcome [1, 2, 4, 5, 14, 20]. Lymph node involvement, depth of myometrial invasion, lymphovascular invasion, peritoneal cytologic findings, mitotic count, age and menopausal status are the other proposed factors [4, 7, 20-22].

In the present study 46 cases of uterine sarcoma who received adjuvant radiotherapy were evaluated retrospectively regarding treatment outcome, survival and prognostic factors.

Materials and Methods

The records of 46 patients with histologically verified uterine sarcoma who were treated by postoperative radiotherapy at the Radiation Oncology Department of Ege University Hospital between January 1993 and December 2003 were reviewed retrospectively. Total abdominal hysterectomy and bilateral salpingo-oophorectomy (TAH-BSO) were performed in 41 patients. Wertheim’s radical hysterectomy with pelvic lymphadenectomy (Type III hysterectomy) was performed in three and a subtotal hysterectomy only in one patient. The adnexa were conserved in one young patient (31 years old). Patients were staged using the FIGO staging system for endometrial cancer. Complete blood count, liver and kidney function tests, chest X-ray and abdominopelvic computed tomography (CT) were performed in all patients before radiotherapy.

According to our clinical protocol, patients with one or more adverse prognostic factors such as high histologic grade, deep myometrial invasion, mitotic count above 10 per 10 high-power fields, and suboptimal surgery were offered adjuvant radiotherapy. External RT was delivered by a 6-25 MV linear accelerator (Philips SL 25) through individually shaped pelvic portals including the tumor bed and regional lymph nodes using an AP/PA or a four-field technique (AP/PA and opposed laterals) with 1.8 Gy daily fractions up to 50.4-64 Gy (median 50.4 Gy). In one patient the paraaortic field was added because of...
enlarged paraaortic nodes detected in abdominopelvic CT. Intracavitary vaginal vault irradiation with ovoids was applied to 39 patients via the microSelectron high-dose rate remote afterloader Ir-192. One fraction of 9.25 Gy or two fractions of 6.5 Gy were given to a depth of 5-7 mm from the vaginal surface. Mainly adriamycin-based chemotherapy was administered to 12 patients in an adjuvant setting.

The patients were followed by physical and radiological examinations (chest X-ray, abdominopelvic ultrasound or CT every other 6 months) with 3-month intervals for the first two years, 6-month intervals for the second two years and annually thereafter.

Overall survival was defined as the time from diagnosis to death or to last follow-up. Local recurrence-free survival was defined as the time from diagnosis to first clinical or radiological evidence of local recurrence. Survival analysis was performed using the Kaplan-Meier method. Potential prognostic factors such as age, menopausal status, FIGO stage, histologic subtype, grade, tumor size, mitotic count, peritoneal cytology, lymphovascular invasion, total tumor dose and the use of chemotherapy were analyzed to assess their impact on local control, disease-free and overall survival. Univariate analysis using the Log-rank test and multivariate analysis using the Cox regression model were performed to assess the significance of prognostic factors.

**Results**

The median age of the patients was 55 years (range: 31-75) which differed according to histology: 58 years for mixed mullerian tumors (MMT), 56 years for leiomyosarcomas (LMS), 44 years for endometrial stromal sarcomas (ESS) and 59 years for adenosarcomas (AS). Sixty-five percent of the patients were postmenopausal. The majority of the patients were multiparous (84.8%) and the median number of parity was three (range: 0-8). The most common presenting symptom at diagnosis was bleeding (82.6%) followed by pelvic or abdominal pain (10.9%) and vaginal discharge (6.5%). One patient had previous colon cancer.

Histologically MMT accounted for 45.6%, LMS 26.1%, ESS 23.9%, and AS 4.3%. According to the FIGO classification 30 (65.2%) patients were classified as Stage I, eight (17.4%) as Stage II, six (13.0%) as Stage III, and two (4.3%) as Stage IV. The median uterine size was 6.5 cm. In most of the patients (58.7%) tumor size was greater than 5 cm. The grade was not clearly specified in 18 patients (39%); most of the identified ones were high grade (67.9%). Mitotic count was not identified in 30 patients (65.2%); among the remaining 16 patients two had less than ten mitoses, seven had 10-19 mitoses and seven had more than 20 mitoses per 10 high-power fields. Lymph-node sampling or pelvic lymphadenectomy was performed in 15 patients (32.6%); nine with MMT and six with LMS. Among these one patient with MMT had lymph node metastasis. Lymphovascular invasion was documented in ten patients (21.7%), necrosis in 11 (23.9%) and positive peritoneal cytology in three patients (6.5%). Patient and tumor characteristics are indicated in Table 1. Table 2 outlines identified tumor characteristics according to stage.

<table>
<thead>
<tr>
<th>Table 1. — Patient characteristics.</th>
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<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
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<td>Median 55 (range 31-75)</td>
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<th>%</th>
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<td>Postmenopausal</td>
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<th>%</th>
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<td>Stage IV (IVA)</td>
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<th>%</th>
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<td>45.6</td>
</tr>
<tr>
<td>LMS</td>
<td>12</td>
<td>26.1</td>
</tr>
<tr>
<td>ESS</td>
<td>11</td>
<td>23.9</td>
</tr>
<tr>
<td>AS</td>
<td>2</td>
<td>4.3</td>
</tr>
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<th>%</th>
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<td>13.0</td>
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<tr>
<td>2</td>
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<td>6.5</td>
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<td>19</td>
<td>41.3</td>
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<th><strong>Tumor size</strong></th>
<th>No. of patients</th>
<th>%</th>
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<tbody>
<tr>
<td>≤ 5 cm</td>
<td>16</td>
<td>34.8</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>27</td>
<td>58.7</td>
</tr>
<tr>
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<td>6.5</td>
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<th><strong>Necrosis</strong></th>
<th>No. of patients</th>
<th>%</th>
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<tr>
<td>(+)</td>
<td>11</td>
<td>23.9</td>
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<tr>
<td>(−)</td>
<td>35</td>
<td>76.1</td>
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<th><strong>Mitotic count</strong></th>
<th>No. of patients</th>
<th>%</th>
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<td>&lt; 10</td>
<td>2</td>
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<tr>
<td>10-19</td>
<td>7</td>
<td>15.2</td>
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<tr>
<td>≥ 20</td>
<td>7</td>
<td>15.2</td>
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<tr>
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<td>65.2</td>
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<th><strong>Lymphovascular invasion</strong></th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
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<tr>
<td>(+)</td>
<td>10</td>
<td>21.7</td>
</tr>
<tr>
<td>(−)</td>
<td>36</td>
<td>78.3</td>
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<th><strong>Peritoneal cytology</strong></th>
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<th>%</th>
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<tr>
<td>(+)</td>
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<td>6.5</td>
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<tr>
<td>(−)</td>
<td>43</td>
<td>93.5</td>
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<th>Table 2. — Tumor characteristics by stage.</th>
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<td><strong>Histologic subtype</strong></td>
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<td>-------------------------</td>
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<tr>
<td>MMT</td>
</tr>
<tr>
<td>LMS</td>
</tr>
<tr>
<td>ESS</td>
</tr>
<tr>
<td>AS</td>
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<table>
<thead>
<tr>
<th><strong>Grade</strong></th>
<th>No. of patients</th>
<th>%</th>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>4</td>
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<table>
<thead>
<tr>
<th><strong>Tumor size</strong></th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5 cm</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>18</td>
<td>3</td>
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<table>
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<tr>
<th><strong>Mitic count</strong></th>
<th>No. of patients</th>
<th>%</th>
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<tr>
<td>&lt; 10</td>
<td>1</td>
<td>−</td>
</tr>
<tr>
<td>10-19</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>≥ 20</td>
<td>7</td>
<td>−</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th><strong>Necrosis</strong></th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>(−)</td>
<td>22</td>
<td>8</td>
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<table>
<thead>
<tr>
<th><strong>Lymphovascular invasion</strong></th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>(−)</td>
<td>26</td>
<td>6</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th><strong>Distant metastasis</strong></th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>(−)</td>
<td>18</td>
<td>7</td>
</tr>
</tbody>
</table>
Median follow-up was 49 months (range 6-148 months). Seventeen patients (37%) had developed distant metastases mainly in multiple sites (41.2%). Locoregional recurrence was detected in one patient both inside and outside the radiation field six months after radiotherapy. This patient had a Stage IVa tumor with rectal involvement and died four months after the detection of recurrence.

During follow-up 16 patients (34.8%) had died of cancer, and two patients had died of intercurrent disease. Five-year overall, disease-free and local recurrence-free survival rates were 57.8%, 60.5% and 97.8%, respectively. Univariate analysis for overall survival demonstrated statistical significance for stage (p = 0.011), histologic subtype (p = 0.010), tumor size (p = 0.044), positive peritoneal cytology (p = 0.006) and the use of chemotherapy (p = 0.005). With respect to histology LMS predicted the worst prognosis followed by AS, MMT and ESS with an overall survival rate at 5 years of 20.8%, 50%, 70.2% and 80.8%, respectively (Figure 1). In multivariate analysis tumor size (p = 0.019) and positive peritoneal cytology (p = 0.026) affected overall survival. According to univariate analysis prognostic factors influencing disease-free survival were stage (p = 0.009), positive peritoneal cytology (p = 0.000) and the use of chemotherapy (p = 0.002). The only prognostic factor affecting local control was stage (p = 0.000) (Table 3).

**Discussion**

Uterine sarcomas are rare and highly malignant tumors of the female genital tract. Optimal management consists of complete surgical removal. The role of adjuvant radiotherapy is controversial as different conclusions have been reached by different series [2, 4-16], and the results with chemotherapy have been disappointing [2, 6, 12, 16-19].

In a Gynecologic Oncology Group study to determine the role of adjuvant radiotherapy in patients with Stage I and II uterine sarcoma, 60 patients out of 157 received radiotherapy. Although no difference was observed in the survival rates, local control rates increased from 23% to 54% (except for LMS) in those who received radiotherapy (p = 0.028) [6]. Preliminary results of the only randomized trial of adjuvant pelvic radiation for all histologic types of uterine sarcomas showed significantly lower pelvic relapse rate in the radiotherapy arm (12% vs 21%; p = 0.004) without a difference in overall or disease-free survival and in this EORTC-GCG study radiotherapy appeared to be more beneficial for MMT [13]. The only statistically significant improvement in

---

**Table 3.** — Prognostic factors for overall, disease-free and local progression-free survival.

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>No. of patients</th>
<th>5-year overall (%)</th>
<th>5-year disease-free (%)</th>
<th>5-year local progression-free (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 55</td>
<td>23</td>
<td>68.4</td>
<td>68.1</td>
<td>100</td>
</tr>
<tr>
<td>&gt; 55</td>
<td>23</td>
<td>48.5</td>
<td>40.7</td>
<td>95.6</td>
</tr>
<tr>
<td>(p = 0.081)</td>
<td>(p = 0.088)</td>
<td>(p = 0.317)</td>
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<td></td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Premenopausal</td>
<td>16</td>
<td>73.8</td>
<td>72.7</td>
<td>100</td>
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<tr>
<td>Postmenopausal</td>
<td>30</td>
<td>49.5</td>
<td>47</td>
<td>96.6</td>
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<tr>
<td>(p = 0.074)</td>
<td>(p = 0.071)</td>
<td>(p = 0.465)</td>
<td></td>
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</tr>
<tr>
<td>Stage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>30</td>
<td>57.5</td>
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</tr>
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<td>II</td>
<td>8</td>
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<td>III</td>
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<td>(p = 0.011)</td>
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<td>(p = 0.000)</td>
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<td>Histologic subtype</td>
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<td>MMT</td>
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<td>ESS</td>
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<td>80.8</td>
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<td>AS</td>
<td>2</td>
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<td>(p = 0.010)</td>
<td>(p = 0.061)</td>
<td>(p = 0.418)</td>
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<td>Grade</td>
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<td>(p = 0.519)</td>
<td>(p = 0.456)</td>
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<td>10-19</td>
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<td>&gt; 20</td>
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<td>(p = 0.919)</td>
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<td>Tumor size</td>
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<td>≥ 5 cm</td>
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<td>72.2</td>
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<tr>
<td>&gt; 5 cm</td>
<td>27</td>
<td>43.5</td>
<td>49.9</td>
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<td>(p = 0.145)</td>
<td>(p = 0.441)</td>
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<td>(+)</td>
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<td>60</td>
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<td>57</td>
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<tr>
<td>(p = 0.940)</td>
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<td>Peritoneal cytology</td>
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<td></td>
</tr>
<tr>
<td>(+)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>(–)</td>
<td>43</td>
<td>62.1</td>
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<td>(p = 0.006)</td>
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<td>(p = 0.791)</td>
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<td>Chemotherapy</td>
<td></td>
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<td></td>
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<tr>
<td>(+)</td>
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<td>(–)</td>
<td>34</td>
<td>73.6</td>
<td>80.5</td>
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<tr>
<td>(p = 0.005)</td>
<td>(p = 0.002)</td>
<td>(p = 0.113)</td>
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</table>

NA: Not available.
Results of postoperative radiotherapy in the treatment of uterine sarcomas: a retrospective analysis of 46 patients

Survival from adjuvant radiotherapy was reported in surveillance, epidemiology, and end results data which included 2,677 cases treated between 1989 and 1999. The survival rate of women with Stage II disease who received adjuvant radiotherapy was 55% compared with 31% for those women who did not (p < 0.01) and the survival rate of women with Stage III-IV disease was 33% with adjuvant radiotherapy compared with 25% without radiotherapy [23]. Given the importance of preventing local recurrence adjuvant pelvic radiotherapy is recommended by many clinicians [4, 5, 7, 10, 11, 21, 24]. Higher doses delivered to tumor volume are radiobiologically expected to give better local disease control. Livi noted that the best results were obtained after postoperative external radiotherapy plus brachytherapy with a total dose higher than 50 Gy (p = 0.001) and the reduction in local recurrence seemed to be influenced by brachytherapy dose to the vaginal vault [4]. Larson et al. in a study of 147 patients with MMT reported a better local control rate for patients treated with adjuvant combined brachytherapy and external beam therapy compared with patients treated with each modality alone [9]. In another series improvement both in local control and overall survival was reported in 84 patients with Stage I disease treated with a combination of pelvic external beam radiotherapy followed by intracavitary brachytherapy [24]. In Knocke et al.’s study 72 patients were given postoperative external radiotherapy and brachytherapy. The five-year local control rate was 77.9% and overall survival was 52.3% [10]. In our study 39 patients were treated with a combination of external radiotherapy and intracavitary brachytherapy. Our local control rate of 97.8% was excellent when compared with data from the literature regarding local control by surgery alone [21, 25-27].

Several prognostic factors have been identified that have a profound influence on outcome [1, 4, 5, 10, 14, 16, 21].

### Table 4. — Comparison of the results of various retrospective trials on uterine sarcoma and prognostic factors.

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of patients</th>
<th>Treatment</th>
<th>Median follow-up (months)</th>
<th>5-year OS (%)</th>
<th>5-year LC (%)</th>
<th>5-year DFS (%)</th>
<th>Prognostic factors</th>
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</thead>
<tbody>
<tr>
<td>Livi et al. [4]</td>
<td>141</td>
<td>Only surgery 36 pts</td>
<td>36</td>
<td>27.7</td>
<td>–</td>
<td>–</td>
<td>Stage, Histologic subtype, Grade, Age, RT is favored</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT 74 pts, (37 with BRT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>CT 20 pts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kelly et al. [5]</td>
<td>87</td>
<td>Surgery 82 pts</td>
<td>–</td>
<td>48</td>
<td>–</td>
<td>–</td>
<td>Stage, Histologic subtype, CT (adverse factor)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT 16 pts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT 25 pts</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chauveinc et al. [7]</td>
<td>73</td>
<td>Surgery 73 pts</td>
<td>96</td>
<td>45</td>
<td>25.7%</td>
<td>–</td>
<td>Stage, Histologic subtype, Grade, Menopausal status</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT 37 pts</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>CT 24 pts</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Knocke et al. [10]</td>
<td>72</td>
<td>Surgery 72 pts</td>
<td>92</td>
<td>52.3</td>
<td>77.9</td>
<td>58.5</td>
<td>Stage, Age RT is favored</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT 72 pts</td>
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<tr>
<td></td>
<td></td>
<td>(55 with BRT)</td>
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<tr>
<td>Ferrer et al. [11]</td>
<td>103</td>
<td>Surgery 103 pts</td>
<td>49</td>
<td>56</td>
<td>57.4</td>
<td>48.7</td>
<td>Stage, Histologic subtype, Grade, Age RT is favored</td>
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<tr>
<td></td>
<td></td>
<td>(24 with BRT)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT 33 pts</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Major et al. [21]</td>
<td>360</td>
<td>Surgery 360 pts</td>
<td>–</td>
<td>–</td>
<td>LF 56%</td>
<td>–</td>
<td>Histologic subtype, Grade, Myometrial invasion, LVSI, Peritoneal cytology, Adnexal involvement, Tumor size, Mitotic index</td>
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<tr>
<td></td>
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<td>RT 132 pts</td>
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<td></td>
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<td>Yamada et al. [33]</td>
<td>62</td>
<td>Surgery 62 pts</td>
<td>22</td>
<td>74</td>
<td>LF 55%</td>
<td>–</td>
<td>Myometrial invasion, LVSI, Peritoneal cytology, Adnexal involvement, Serosal involvement, Lymph node involvement, RT is favored</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT 20 pts</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(early stage)</td>
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<td></td>
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</tr>
<tr>
<td>Ege University</td>
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<td>Surgery 46 pts</td>
<td>49</td>
<td>57.8</td>
<td>97.8</td>
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<td>Stage, Histologic subtype, Tumor size, Peritoneal cytology, CT (adverse factor)</td>
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<tr>
<td></td>
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<td>(39 with BRT)</td>
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<td></td>
<td>CT 12 pts</td>
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</table>

OS: Overall survival; LC: Local control; DFS: Disease-free survival; RT: Radiotherapy; BRT: Brachytherapy; CT: Chemotherapy; LVSI: Lymphovascular space involvement; pts: Patients.
20, 24, 28]. Prognosis has been reported to vary inversely with the initial stage at presentation and according to the literature approximately 50-75% of all patients present with clinical Stage I disease [1, 4, 5, 10, 11, 29]. The corresponding rate was 65.2% in the present series. As in all published data, stage was the main prognostic factor in this series for overall, disease-free and local-recurrence free survival. However our overall and disease-free survival of Stage II patients were better than that of Stage I patients (Table 3). Twelve patients out of 30 with Stage I disease (40%) had developed distant metastases and ten patients had died due to distant metastases, whereas the distant metastases rate of Stage II patients was 12.5% (1/8). Also there were more high-grade tumors, tumors > 5 cm, and mitotic count ≥ 20 in Stage I patients than in Stage II patients (Table 2).

Histologic grade is also a powerful parameter for predicting outcome [2, 4, 7, 11, 21, 28]; however it was not a prognostic factor in the current study which could be attributed to patients with unspecified grade. These patients were referred to our university hospital from other hospitals and their histologic material was unable to be retrieved for review.

With respect to histology LMS tends to have the worst prognosis among the other subtypes [4, 7, 21, 30]. Although adjuvant radiotherapy may reduce the risk of local recurrence in early-stage disease, overall survival tends to be low because of the high rate of pulmonary metastases [21]. Livi et al. stated an 18.8% survival rate for LMS at five years [4]. Dinh and associates reported a 65% 2-year survival for 27 patients with uterine LMS although only 19% survived with no evidence of disease and the crude 5-year survival was 42% [20]. In the current study LMS also predicted the worst prognosis with a survival rate of 20.8% at five years. Fifty-eight percent of these patients developed single or multiple organ metastases mainly to the lungs. The patients with ESS had better overall survival (80.8%) like those of some other studies [4, 5, 7, 28, 30-32].

Tumor size greater than 5 cm was an adverse prognostic factor for overall survival. In Major et al.’s study univariate analysis showed that tumor size was an important prognostic factor for the progression-free interval for MMT [21].

Positive peritoneal cytology was an adverse prognostic factor for overall and disease-free survival in this study. Husseiny et al. and Yamada et al. also reported similar results [28, 33] and Major et al. found that positive peritoneal cytology was significantly related to progression-free survival in early-stage MMT [21]. Other factors including age, menopausal status, mitotic count, lymph node status, depth of myometrial invasion, lymphovascular invasion that have been found as prognostic variables in several studies [2, 4, 5, 10, 11, 20-22, 24, 31] were not statistically significant for prognosis in this study. A comparison of the results of this study with the results of other studies are shown in Table 4.

The role of adjuvant chemotherapy in uterine sarcomas is also controversial [2, 6, 7, 12, 16-19]. It has been suggested that adjuvant chemotherapy may afford a survival benefit by controlling subclinical metastatic disease but this has not been proven yet and the ideal chemotherapeutic agent has yet to be established. Until recently the most active chemotherapy regimen in uterine sarcomas was doxorubicin and ifosfamide yielding a response rate of around 30% in LMS [18]. Lately a phase II study of gemcitabine plus docetaxel exhibited a 53% response rate [34]. Pautier et al. published the results of a multidrug regimen combining dacarbazine, cyclophosphamide, or ifosfamide, cisplatin, adriamycin and vindesine [35]. The objective response rate of 54% achieved in their series was high, but the median duration of response was low. Newer agents such as paclitaxel have been tried in combination with some encouraging results [36]. Recently the results of the Gynecologic Oncology Group randomized trial of whole abdominal irradiation versus cisplatin-ifosfamide-mesna in optimally debulked Stage I-IV carcinosarcoma of the uterus were presented at the ASCO 2006 meeting. Adjuvant chemotherapy reduced the recurrence rate and prolonged overall survival; however the authors concluded that due to a high relapse rate and poor overall survival the imperative for new adjuvant therapies remains [37]. In our study the use of chemotherapy was a significant adverse factor for overall and disease-free survival. This finding is likely confounded by the fact that the patients who were given chemotherapy tended to have higher stage and poor prognostic factors.

Researches on uterine sarcomas are being carried out to understand the biology of this malignancy at the molecular level. Recent trials have been investigating COX-2, c-KIT and HER-2/neu expressions in uterine sarcomas hoping to find an association between the expression of these oncogenes and clinicopathologic factors and also to find potential markers for targeted therapies [37-39].

Conclusion

The data presented here are comparable with other published studies [4, 5, 7, 10, 11, 24, 28-31]. Although the prognostic factors proposed in various series differ widely, tumor stage seems to be the most important factor mentioned in each of these studies as well as histologic subtype. These tumors have a poor prognosis and the majority of patients die because of distant metastases. Many authors have suggested that a multimodality approach would be a logical treatment of these aggressive tumors [1, 5, 12, 17, 19, 27, 35]. Therefore radiotherapy should be employed to control local disease and chemotherapy to prevent potential metastases. This is a retrospective study and a control group of patients who were treated with surgery alone is lacking. Although our local control rate was very high favoring radiotherapy the exact value of adjuvant radiotherapy can not be proven and given the small number of patients receiving adjuvant chemotherapy it was not possible to address the value of adjuvant chemotherapy. The low number of patients in a single institution prevents the development of clinical trials. The optimal adju-
vant therapy hopefully will be elucidated through multi-centric randomized trials of radiotherapy, active chemotherapy agents, investigational treatments and molecular studies.

References


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Migrant women and cervical cancer: background of a prevention study

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Summary

The study was scheduled in order to organize a program of prevention against cervical cancer in female migrants in Rome, and therefore to facilitate access to appropriate preventive oncological facilities for discriminated women. Moreover, the study will also investigate the risk factors and social conditions (HPV-subtypes, sexual behavior, smoking habits) of such women since their migration to Italy. This is scientific and cultural background of a longitudinal, observational study on the cervical cancer risk in Roman migrant population. By means of a mother language questionnaire (with the presence of a cultural mediator) it will be possible to achieve data on social conditions and the new life-style. An HPV-testing (HC2) combined with Pap-test (with further genotype distribution) will be performed in all women enrolled in the study. Further diagnostic/therapeutic decisions will depend on the results of both tests. Scientific results are expected in the next two years, but an increasing of cancer prevention awareness among female migrant populations is expected from the beginning of the program. The present study was aimed at culturally appropriate intervention strategies to limit the disparities that migrants usually suffer in most of the developed Western nations in respect to the native counterparts.

Key words: Cervical cancer; Prevention; Migrants; Health discrimination.

Introduction

Key indicators suggest that health in Western countries continues to improve, while in less developed countries it is still much poorer than in the past. This socio-economic imbalance represents the main engine for human migration. Moreover, the reproductive health of migrants, and their political and economic condition, is lagging behind that of host populations. Indeed in some situations it may be worsening. Migrants different nationalities constitute a significant and growing proportion of the national populations of the “old” Western Europe. For public health reasons, as well as for ethical and human rights reasons, the reproductive health of migrants calls for urgent attention.

The increased pace of migration has been mostly marked in and between developing countries, but it has also become very evident in Western Europe where rapidly changing economic and demographic conditions are attracting and necessitating new human resources, while offering political sanctuary to others.

Reproductive health of migrants

As with health and health care in general, migration places people under such conditions affecting their reproductive health and their access to, and use of, reproductive health care services.

Gender issues run through the gamut of reproductive health and health care issues. The fact that female migrants are now outnumbering men in many parts of Western Europe calls for special attention to be given to problems: such as work-place exploitation, discrimination in terms of remuneration and, sometimes, sexual abuse. The status of migrant women within migrant communities also calls for attention, especially since there is evidence that in “strong” ethnic communities many of the gender-based abuses associated with previous life often persist and place women in situations that are replete with contradictions for health.

The findings from different studies suggest that migrant women do not access gynecological care services in the same way and or to the same extent as the host-country population. A UK study reported that refugee women from former Yugoslavia were far less likely to

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access cervical screening programs than “nationals”. For example, 30.5% of refugee women (compared to 17.5% nationals) said they had never had a cervical smear. Among those that had a Pap-test, the proportion with abnormal smears was 40% compared to 21.2% in the national population [2], highlighting the fact that many women may be delaying their check-ups until it is too late.

Ethnic background and migrant status also appear to play a role in the risk of poor breast cancer detection. A study in Denmark found that 71% of Danish-born women readily accepted mammography, compared to far fewer migrant women (from Pakistan, ex-Yugoslavia, Turkey). Moreover, almost half of the migrants failed to show up for their appointments, with the result of more advanced and less easily treatable breast cancer in such population [3, 4].

If the reproductive health of migrants is not promoted and protected, the health of all Europeans will suffer. Reproductive health affects family and community health as well as individual health, and when major disparities are allowed to emerge and persist among different groups of people, the health of everyone is gradually affected.

Cervical cancer background

Country-specific behavioral, health, economic and demographic measures are strictly related to the cancer risk profile in developing areas. It is estimated that in low-resource countries all of the cancer-related deaths (by viral agents such as HBV or HPV, tobacco consumption, and Western-type diets) will exceed, in the near future, the infectious deaths. Among these populations the risk of cervical cancer is particularly high. Of the estimated yearly worldwide 470,000 cervical cancers, 81% (380,000 women) occur in the developing world [5]. Indeed, the definition of the word developing is represented by these areas where cervical cancer is still the leading cause of cancer death: the African continent (mainly the Sub-Saharanian territories), South/Western Asia, and Central/South America.

Nevertheless, some Eastern Europe areas (Poland, Estonia, Slovakia) report lower survival rates after cervical cancer treatment compared to the richest northern countries (Sweden, Netherlands, Norway) [6]. Moreover, socioeconomic transition within some parts of Europe has been shown in the loss of previous improvements in the health of women, and this has generated health disparities. In Central and Eastern European countries and the newly independent states of the former USSR poverty has caused a reduction of female life expectancy [7].

The same poor levels of human development and low economic resources, which contribute to the high risk for cervical cancer incidence and mortality, sustain the immigration flux to the industrialized world. World Health Organization reports that most of the nearly 175 million migrants (2.9% of the world’s population) generally belong to the lower social level, have limited access to health facilities and thus migrants must be considered at high-risk for any disease. Indeed, most women from undeveloped countries themselves are not aware of cancer screening or facilities available, as revealed from a Nigerian study: only 15% of the women had ever heard of cervical cancer [8].

Cervical cancer screening

Cervical cancer can be prevented by using relatively inexpensive screening (Papanicolau test) to detect abnormal cervical tissue before it progresses to invasive cancer. Although Pap smear screening remains the best available method for reducing cervical cancer [9], its application is still geographically related. In industrialized countries cytological screenings strongly decreased incidence and mortality from cervical carcinoma (about 80% in Finland [10]), while screening programs have rarely been implemented and virtually never sustained in most underdeveloped countries [11]. Indeed, as quoted by Sankaranarayanan [12], cytology screening is yet to be effectively implemented in many developing countries or has failed to reduce the cervical cancer burden to an appreciable extent in some developing countries.

Cytologic screening programs are expensive and require trained cytotechnicians, high-quality laboratories that maintain adequate quality-control programs, an infrastructure for transporting smears to the laboratory and results back to the clinical site, and the capacity to recall, diagnose, and treat women with abnormal results [13].

In these areas the lowest doctor ratio for the population worldwide is also reported [14], with scarce possibility of medical opportunities and, as a consequence, any preventive consciousness.

In other words, in such poor countries only 5% of women have been screened for cervical dysplasia, compared with some 40-50% in developed nations. The effects of these discrepancies are that, up-to-now, the incidence rate for cervical cancer is about 3.8/100,000 women in Finland, compared to more than 30/100,000 women in South Africa [15].

Visual cervical inspection after acetic acid application (VIA) [16] and HPV-DNA testing have been recently proposed [17] as reasonable screening strategies in low-resource settings.

Indeed, in such situation the HPV-prophylactic vaccination has a tremendous potential to lower the incidence and mortality from cervical carcinoma. An HPV vaccine could have the greatest utility in such developing countries, but there are many difficulties for an extended vaccination program in these areas. First of all, there may be significant differences in HPV vaccine immune responses among malnourished populations, compared to Western countries. Moreover, papillomavirus vaccine can be locally produced, easily stored (which means thermo-stable) as well as easily/cheaply distributed and protective after only a single-dose.
Cancer disparities in developed countries

Cancer disparities (in terms of incidence and mortality) by race/ethnicity are strictly related to a complex interplay of economic, social and cultural factors. The condition of “poverty” has been defined as the most critical factor affecting health and longevity [18]. Indeed, the status of migrants influences the prevalence of risk factors for cancer (see above) and affects the access to appropriate early detection, cancer treatment (particularly the conservative approach), follow-up, and pain management.

For all cancer sites residents of poorer countries have a higher cancer death rate than residents in more affluent countries, and also within each racial/ethnic group those living in poorer countries have the lowest survival rates [19].

Moreover the American Cancer Society has focused on the trend in cancer disparities (breast, colon-rectum, lung, stomach, uterine cervix) between the racial segregates of the American population: African, Asian, Pacific Islander, American-Indian, Hispanic-Latino [20]. Although biological and inherited features are less important than socioeconomic characteristics in explaining the disparities, different rates of cervical cancer in migrant women may be due to the prevalence of HPV-subtypes [21].

Furthermore, as it has already been highlighted for the United States (but is valid for all of Western countries), “…there is a critical disconnection between what we discover and what we deliver to all Americans in the form of prevention, diagnosis, and treatment of cancer. Cancer disparities exact an extraordinarily high human cost and a significant economic cost to this nation” [22].

It is also important to put in evidence the higher prevalence of comorbid conditions, the higher rate of patients’ treatment refusal or the lack of physician recommendation in the minorities groups [23]. All these factors may therefore interfere with the survival rate for cervical cancer [24], which seems to be significantly lower in Afro-Americans (57.5%, 5-yrs for all stages) versus Whites (71.5%).

The risk-status of migrant women

Variables that may enhance the risk for cervical cancer in the migrant female population depend on:

a) social integration in the host-country: communication and racial difficulties, utilization of preventive care services, discrimination, type of employment;

b) personal life-style: sexual behavior, number of partners, smoking and dietary habits, etc.

c) personal life conditions: homeless, refugees, legal status (irregular), temporary or permanent mobile status, victim of prostitution (trafficked women) with the possible major risk under such sexual conditions.

Most of them are not aware of cancer screening or facilities available, and the knowledge of all cancer-related problems is also very limited. Indeed, it seems that the discrepancy between the north and south of the world concerning cervical cancer incidence-mortality is paralleled by a similar discrepancy regarding education and knowledge of this problem.

The possibility to have access to the preventive national tools for mobile women is also limited because traditional public-health programs are only directed at sedentary communities. This appears also true for the so-called ethnic minority groups which suffer from barriers to cancer screening compared with the majority of the population [25]. Some studies on Vietnamese migrants to the USA [26] revealed that the number of women who adhere to cervical cancer screening guidelines is low and, as a consequence, the most commonly occurring cancer in such mobile females is indeed cervical cancer. Vietnamese-American women were more likely to have high-risk HPV types [27] and had the highest rates of cervical cancer (43/100,000 women) of any racial/ethnic group in USA.

The human papillomavirus infection

Up-to-now it is well stated that human papillomavirus (HPV) infection is the necessary cause of cervical cancer. The risk of acquiring such infection, which represents worldwide the first sexually transmitted disease, is directly related to sexual activity and particularly to the number of sexual partners.

Thirteen out of the 100 and more identified genotypes are considered as high-risk for cervical carcinogenesis. While low-risk HPV infection is very common and transient, only a very small proportion of high-risk infections will further develop into cervical carcinoma. On the other hand, cervical cancer contains fragments of integrated HPV-DNA in the host-genome.

As stated in a series of population-based HPV surveys coordinated by the International Agency for Research on Cancer (IARC), there are many variations in HPV prevalence worldwide. The HPV prevalence in poor-resource settings, by means of PCR techniques, is reported from 18% in Nigeria [28] up to 44% in Kenya [29].

The identification of HPV infection has many and substantial implications in terms of global public health, particularly for migrant women. By using detecting methods for HPV, such as Hybrid Capture 2 (HC2, Digene®) inte-
grated with the traditional Pap test, it would be possible to select women with high-risk HPV-subtypes to submit to further diagnostic procedures. Moreover, it may be more feasible to set up HPV-DNA testing on the low-resource site, than to provide on-site cytology services. In a South-African study a prevalence of high-risk HPV-DNA up of 22% was detected by the HC2 technique (12) in a urban female population also affected by other STD: chlamydia or gonorrhea (6%), trichomonas vaginalis (18%).

On the contrary, such integrated preventive tools could reassure women with both negative results (Pap and HC2), which are at very low-risk (near zero) for the next five years.

**Migratory waves in Rome**

During the decade 1970-80 the city of Rome was the objective of a relevant “first” migratory wave: mainly from the Philippines, South and Central America. In such period there was a relevant demand for private domestic labor, so that a “healthy migrant effect” was seen. It means that migrant women were submitted to self-selection. Only those who were in good health and physical/psychological aptitudes arrived in Rome. Such migrant populations were mostly legal, tended to live in communities, were characterized by a high social integration in the host-country population and had a sedentary profile.

From 1999 we assisted with a “second” relevant migratory wave, mainly from the former USSR, North-Africa or Albania. The lifestyle of these women was completely different from the other previously described. Most of them arrived in Rome for reconjunction, or because they were refugees, displaced, victims of human trafficking, and easy targets for illegal employment. They do not usually live in communities, tend to have a low profile of integration and continue to be migrant. Moreover, most of them suffer from other comorbidities.

Up-to-now (Table 2) most female migrants (42.5%) in the Roman district are in the second to third decade of life and therefore represent an ideal target for cervical cancer screening.

<table>
<thead>
<tr>
<th>Table 2. — Age distribution of female migrants in the District of Rome (2003).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
</tr>
<tr>
<td>0-19 yrs</td>
</tr>
<tr>
<td>20-39 yrs</td>
</tr>
<tr>
<td>40-59 yrs</td>
</tr>
<tr>
<td>50-69 yrs</td>
</tr>
<tr>
<td>&gt;70 yrs</td>
</tr>
</tbody>
</table>

**Present Study**

**A cervical cancer prevention study in a migrant Roman community**

Little data are available on cancer incidence for inter-European migrations and on all the strategies that reduce barriers to screening.

The present study, just started in Rome, is a longitudinal, observational study on HPV prevalence among the migrant Roman community. The enrollment will be done at the Department of Preventive Medicine of Migration, Tourism and Tropical Dermatology of the San Gallicano Dermatological Institute, which historically represents the main health meeting point for migrant populations in Rome.

The objective is to increase the confidence of women as well as their consciousness of cervical cancer risk. It will also be important to value the health belief model factors, socio-demographic variables and the barriers to screening related to cervical cancer prevention.

After signing an informed consent, the women will be asked to fill in a questionnaire in their mother-tongue language about the risk factors and to attend a face-to-face gynecological interview. Such questionnaire, in order to value the impact of migration, has been divided into two parts concerning the life-style before and after arriving in Italy. A cultural woman mediator (for each language) will help the patient fill in the questionnaire.

After this preliminary step, the Pap smear and HPV test (HC2) will be performed. In all women positive to HC2 a genotype evaluation (by means of PCR) will also be performed.

All women with both negative tests (cytological and viral) will be invited after 24 or 36 months for follow-up regarding the presence of other risk factors.

Women with only a HC2 positive test will be followed at 6-12 months.

A second diagnostic level (colposcopy, with further biopsy if required) will immediately be performed in the following cases:

- women with cytological abnormalities \( \geq \) LSIL (regardless of the viral test);
- women with ASCUS and HC2 positive for high-risk subtypes.

Scientific results are expected in the next two years, after enrollment of about 800 women.

**Conclusions**

Culturally sensitive educational interventions and cervical cancer preventive programs for migrant women are needed to increase the early detection of cervical cancer. Appropriate intervention strategies, employed in conjunction with clinical services, can be successful in increasing cancer prevention awareness and screenings among female migrant populations.

The present prevention program for migrant women aims to enhance the consciousness of cancer risk in such high-risk populations, and is directed to increase the participation of women and to facilitate access to health preventive services.
References


Outcome of uterine clear cell carcinomas compared to endometrioid carcinomas and poorly-differentiated endometrioid carcinomas

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Summary

Objectives: Our aim was to compare the survival between patients with clear cell carcinoma (CC) and patients with endometrioid carcinoma (EC). Methods: Through the population-based Geneva Cancer Registry, we identified 1,380 resident women diagnosed with uterine cancer between 1970 and 2000. We excluded those with papillary serous endometrial carcinoma and uterine sarcomas. We categorized patients as CC (n = 32, 2.8%) or EC (n = 1,145, 97.2%). Uterine cancer-specific survival rates were calculated by Kaplan-Meier analysis. We used Cox proportional hazards analysis to compare uterine cancer mortality risks between groups, and adjusted these risks for other prognostic factors. Results: CC patients presented with a more advanced stage at diagnosis than EC patients (p = 0.002). Compared to women with EC, women with CC had a significantly greater risk of dying from their disease (hazard ratio [HR] 2.9, 95% confidence interval 1.7-4.9). After adjustment for age, stage and adjuvant chemotherapy, the risk of dying from uterine cancer was still significantly higher for CC patients (HR 2.0, 95% CI 1.2-3.4). By univariate analysis, the risk of dying of endometrial cancer was not significantly higher in CC patients than in patients with poorly-differentiated EC (HR 1.3, 95% CI 0.7-2.3). Conclusion: This population-based investigation shows that patients with CC have a poorer outcome than those with EC. Studies to determine if CC patients should be considered as a high risk and if they should be treated in a different manner than patients with endometrioid carcinoma (EC).

Key words: Adjuvant chemotherapy; Endometrioid carcinoma; Clear cell carcinoma; Survival.

Introduction

Uterine clear cell carcinoma (CC) is recognized as a distinct histological variant of endometrial carcinoma, and accounts for 2-4% of all endometrial adenocarcinomas [1-3]. Only a few series have reported on the management and clinical outcome of CC, and most of them regrouped CC with other potentially high-risk histologies such as uterine papillary serous carcinoma [3-10]. Moreover, most studies included a small number of patients with limited follow-up.

The prognosis of CC is still controversial as it is unclear if the worse prognosis is due to a more advanced stage at diagnosis or a more aggressive histological cell type. Giri et al. have suggested a favourable outcome in patients with clear-cell tumours, similar to classical adenocarcinomas in their behaviour and response to therapy [11]. Other authors observed that CC is an aggressive carcinoma with a poor outcome similar to uterine papillary serous carcinoma [1, 3, 8, 11]. One of the reasons for the poor prognosis of CC is that it generally presents at a more advanced stage at diagnosis than other uterine cancers. However, when analysing different stages separately, it is still not clear if CC behaves more aggressively than other endometrial cancers. Therefore, it is difficult to determine if CC patients should be considered as a high risk and if they should be treated in a different manner than patients with endometrioid carcinoma (EC).

In this population-based study, we aimed to elucidate whether CC is really associated with a worse outcome, while taking other prognostic factors into account.

Materials and Methods

The current investigation was performed with information from the population-based cancer registry of the Swiss canton of Geneva (approximately 420,000 inhabitants). The registry records all incident cases of malignant neoplasms occurring in the Canton’s resident population were accessed. Information was collected from various sources (i.e., pathology reports, medical files from public hospitals and private physicians), and is considered very accurate, confirmed by the very low percentage (≤ 1%) of cases recorded from death certificates only [12].

Recorded data included socio-demographic information, diagnostic circumstances, modalities of diagnostic assessment, and tumour characteristics (coded according to the International Classification of Diseases for Oncology) [13]. The cause of death was established from clinical records according to the World Health Organisation’s classification. In addition to passive follow-up (routine examination of death certificates and hospital records), active follow-up was performed routinely each year through the files of the Cantonal Population Office, which is in charge of registration of the resident population. The Geneva Cancer Registry regularly assesses survival. The incidence index date refers to the date of confirmation of diagnosis.
or to the date of hospitalisation if it precedes the diagnosis and is related to the disease. Active follow-up was last done in December 2004.

We identified all 1,380 patients diagnosed with uterine cancer between January 1970 and December 2000, who were residents in the Swiss canton of Geneva. We excluded patients with uterine sarcoma (n = 127), uterine papillary serous carcinoma (n = 76) or endometrial cancer diagnosed at autopsy (n = 17). The study finally included 1,160 patients.

Disease stages were recorded according to the 1988 International Federation of Gynaecology and Obstetrics (FIGO) staging system: Stage I, tumour confined to the uterus; Stage II, tumour invading the cervix; Stage III-IV tumour associated with positive peritoneal cytology or with macroscopic or histological involvement of the serosa or adnexa or tumour invading the vagina, mucosa of the bladder, bowel, regional lymph node or distant metastases. Tumour grade was only coded for EC patients, because FIGO tumour grading is not applicable to CC; these tumours are generally considered as high-grade tumours [6]. We classified differentiation as good (grade 1), moderate (grade 2), poor (grade 3), or unknown. Information on grade was only available after 1985. Types of surgery included hysterectomy (with or without salpingo-oophorectomy) and no surgery. Radiotherapy and chemotherapy were categorized as yes versus no.

Statistical methods: We compared women with CC or EC in terms of age, stage and treatment by the chi-square test for heterogeneity. Five- and 10-year disease-specific survival rates were calculated according the actuarial method, taking only deaths from uterine cancer as terminal events. Cox’s proportional hazard analysis was used to compare the risk of dying from endometrial cancer between CC and EC patients adjusted for all other prognostic factors. Subgroup analysis was then performed in which we compared survival and endometrial cancer mortality risks between CC patients and patients with poorly-differentiated EC. Statistical analysis was carried out with SPSS (version 11.5) and differences were considered statistically significant if the 2-sided p value was < 0.05.

Results

Of the 1,160 patients included in the study, 32 (2.8%) had CC. The characteristics of the 32 CC and 1,128 EC patients are summarised in Table 1. Patients with CC were somewhat older than EC patients (70 versus 66 years, respectively, p = 0.052). Patients with CC had a more advanced stage at diagnosis (p < 0.001). Only 52% had Stage I disease at diagnosis, whereas 77% of EC had Stage I and II disease only, the survival differences persisted (5-year survival of 64% for CC patients was 50% vs 80% for EC patients. For Stages III and IV, the survival difference between CC and EC persisted, but the number of CC patients in this category was rather small.

In subgroup analysis, we compared the characteristics, survival and mortality risks of CC patients and patients with poorly-differentiated EC. There were no significant differences in age between CC and poorly-differentiated EC patients (70.3 vs 67.4 years, respectively). Both groups had comparable stage distribution (25% of CC

Table 1. — Characteristics of uterine cancer patients according to histology (clear cell vs endometrioid carcinoma).

<table>
<thead>
<tr>
<th></th>
<th>Clear cell carcinoma</th>
<th>Endometrioid carcinoma</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(years)</td>
<td>70.3</td>
<td>66.5</td>
<td>0.052**</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>445 (59%)</td>
<td>445 (59%)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>193 (26%)</td>
<td>116 (15%)</td>
<td></td>
</tr>
<tr>
<td>Poor/undifferentiated</td>
<td>374 (-)</td>
<td>374 (-)</td>
<td></td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>15 (52%)</td>
<td>742 (77%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>II</td>
<td>6 (21%)</td>
<td>75 (8%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>3 (1%)</td>
<td>73 (7%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>7 (24%)</td>
<td>78 (8%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (3%)</td>
<td>160 (-)</td>
<td></td>
</tr>
<tr>
<td>Surgical procedure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hysterectomy/BSO ± LN</td>
<td>26 (81%)</td>
<td>981 (88%)</td>
<td>0.608</td>
</tr>
<tr>
<td>No surgery</td>
<td>15 (47%)</td>
<td>425 (41%)</td>
<td>0.224</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (–)</td>
<td>80 (–)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (9%)</td>
<td>36 (13%)</td>
<td>0.056</td>
</tr>
<tr>
<td>No</td>
<td>29 (91%)</td>
<td>1,092 (97%)</td>
<td></td>
</tr>
</tbody>
</table>

*p-value of the chi-square test for heterogeneity; **t-test; ***tumour grading was not applicable for clear cell carcinomas; BSO = bilateral salpingo-oophorectomy; LN = lymph node dissection.

Radiotherapy was equally frequently administered to CC patients as to EC patients (53 vs 59%, respectively). Adjuvant chemotherapy was, as expected, uncommon for both CC and EC patients (9% vs 13% respectively).

Figure 1 shows the disease-specific survival curves for CC and EC patients. Table 2 summarises the 5-year disease-specific survival rates for CC versus EC patients, for all patients together and for Stages I and II and Stages III and IV, separately. Important survival differences were observed. Overall, the 5-year disease-specific survival for CC patients was 50% vs 80% for EC patients. For patients with Stage I and II disease only, the survival differences persisted (5-year survival of 64% for CC patients vs 88% for EC patients). Also, for Stages III and IV, the survival difference between CC and EC persisted, but the number of CC patients in this category was rather small.

Patients with CC had a 3-fold increased risk of dying from endometrial cancer compared to EC patients (unadjusted hazard ratio [HR] 2.9; 95% confidence interval [CI]: 1.7-4.9) (Table 2). After adjustment for age and stage, the risk of dying from endometrial cancer was still significantly increased for CC patients (HR 2.0, 95% CI: 1.2-3.4). Further adjustment for treatment did not modify the results.

In subgroup analysis, we compared the characteristics, survival and mortality risks of CC patients and patients with poorly-differentiated EC. There was no significant difference in age between CC and poorly-differentiated EC patients (70.3 vs 67.4 years, respectively). Both groups had comparable stage distribution (25% of CC
and 27% of poorly-differentiated EC patients had Stage III or IV disease). Nineteen percent of CC versus 10% of poorly-differentiated EC patients did not undergo surgery (p = 0.25). CC patients less often received radiotherapy (53% vs 72%, p = 0.057). No differences in the use of adjuvant chemotherapy were observed.

The 5-year disease-specific survival of patients with poorly-differentiated EC was 58% (95% CI: 49%-67%), not significantly different from that of CC patients, 50% (95% CI: 32%-68%). The corresponding mortality risks are presented in Table 3. By univariate analysis, the risk of dying from endometrial cancer was not significantly higher in CC patients than in patients with poorly-differentiated EC (HR 1.3, 95% CI: 0.7-2.3). Adjustment for age and stage resulted in a HR of 1.1 (95% CI: 0.6-2.0). Further adjustment for therapy did not modify the results.

### Table 2. — Five-year endometrial cancer survival according to histology and FIGO stage.

<table>
<thead>
<tr>
<th>FIGO stage</th>
<th>Clear cell carcinoma</th>
<th>Endometrioid carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>All stages</td>
<td>32 15 50% (32-68%)</td>
<td>1,128 208 81% (79-83%)</td>
</tr>
<tr>
<td>Stages I-II</td>
<td>21 7 64% (42-86%)</td>
<td>817 91 88% (86-90%)</td>
</tr>
<tr>
<td>Stages III-IV</td>
<td>8 6 25% (0-55%)</td>
<td>151 84 40% (32-48%)</td>
</tr>
</tbody>
</table>

*Number of deaths at 5 years; DSS = disease-specific survival.

### Table 3. — Effect of adjustment for age and stage on the risk of dying of endometrial cancer for patients with uterine clear cell carcinoma (CC) compared to all patients with endometrioid carcinoma (EC) and to patients with poorly differentiated EC.

<table>
<thead>
<tr>
<th></th>
<th>Clear cell carcinoma</th>
<th>Endometrioid carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>Unadjusted HR (95% CI)</td>
<td>HR (95% CI) adjusted for age and stage</td>
</tr>
<tr>
<td>EC</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>CC</td>
<td>2.9 (1.7-4.9)</td>
<td>2.0 (1.2-3.4)</td>
</tr>
</tbody>
</table>

*Number of deaths at 5 years; DSS = disease-specific survival.

### Discussion

CC is a well-established histopathological entity that comprises about 2-4% of endometrial carcinomas [1-3]. In our study, it represented 2.8% of all endometrial cancers, which is consistent with the three largest reports published in the literature, where the observed incidence rate was 3.3-1% [1, 2, 6].

A literature review revealed that the prognosis of this type of uterine cancer is still regarded as somewhat controversial, though many investigations suggest that it is an aggressive disease [1, 2, 8]. As a result, the optimal management of these patients remains undefined. The aim of our work was to compare the outcome of CC with EC in a population-based study after exclusion of uterine serous papillary carcinoma and uterine sarcomas which are well-known high-risk uterine cancers.

In our series, the uterine cancers were diagnosed at a later stage of disease compared to EC. High rates of recurrence have been observed in patients with CC, even in those with early-stage disease. The disease-specific survival for Stages I-II was poorer for CC compared to EC (64% vs 88%). This is in agreement with most previous reports where the outcome of CC patients is generally inferior to that of endometrial cancer patients. However, ranges reported in the literature for 5-year survival rates in CC patients are large, between 59% and 72% for Stages I-II and 38-64% for Stages I-IV [2, 3, 5, 6, 14, 15]. The large difference in survival may be attributed to the fact that most published works have included small series recruited over a long period with possible variation in treatment modalities.

In our study, when only poorly-differentiated EC was compared to CC, a similar percentage of patients had extrauterine disease and the survival rate appeared to be similar between the two groups. This result points to a similar aggressive behaviour of CC and poorly-differentiated EC, in agreement with two previous studies, but the small sample size of these series limits the statistical power of the analysis and may have failed to show a difference [1, 2]. A recent large, population-based investigation has shown that CC and uterine papillary serous carcinoma have a worse outcome than grade 3 EC [2].

A shortcoming of our study was that surgical staging was performed at the discretion of the physicians (non-standardised surgical approach). The current trend is that CC needs comprehensive surgical staging similar to ovarian cancer, including peritoneal washing, omentectomy and peritoneal-blind biopsies, and pelvic and paraaortic lymphadenectomy. Therefore, the frequency of extraperitoneal metastasis in CC (and in EC) could be underestimated. On the other hand, our study is a population-based selection with long-term follow-up (median follow-up 8 years).

In conclusion, CC comprises a small percentage of endometrial carcinomas, presents with older age, advanced stage at diagnosis, and is associated with poor outcome. In the future, adjuvant chemotherapy and radio-
therapy need to be explored in patients with early-stage disease in an attempt to improve disease outcome. However, due to disease rareness, it will not be easy to recruit patients in an appropriately powered and randomised study, and only multicentre trials will be able to give a final response.

References

Sociodemographic and clinicopathologic characterization of cervical cancers in northern Nigeria

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Summary

Objective: To evaluate the sociodemographic and clinicopathological characteristics of patients with cervical cancer seen in a tertiary referral center in northern Nigeria. Materials and Methods: Between January 2002 and December 2004, 70 consecutive patients with histologically confirmed cervical cancer, with a median age of 48 years (range, 30-75 years), were interviewed on the basis of a structured pro forma. Results: Of these patients, 39 (56%) had had no formal education, and 36 (51%) were unemployed housewives. Sixty (86%) had become sexually active before 17 years of age; 44 (63%) were in polygamous families, and 25 (36%) patients were in at least a second marriage. There was an average of 6.8 live births per patient. Vaginal bleeding was seen in all patients, and 55 (79%) had vaginal discharges; 50 (71%) had a bulky cervical mass, and 46 (66%) presented with at least Stage IIIA disease. Squamous cell carcinoma was the commonest histology. The three HIV-seropositive patients were young and had advanced disease. Conclusion: Sociodemographic factors, such as low socioeconomic level, early age at first sexual intercourse and multiple sexual partners, place women at high risk of developing cervical cancer in northern Nigeria. Late presentation with advanced disease predominates.

Key words: Cervical cancer; Sociodemographic factors; Epidemiology; Clinical features; Africa.

Introduction

The incidence of cervical cancer in developed countries has been dropping drastically over the past two decades owing to successful screening programs with “Pap smears”. On the contrary, the incidence and prevalence of cervical cancer are rising in most African countries [1, 2]. This reflects lack of screening programs and the persistence of sociocultural factors that place the population at risk by increasing the prevalence of human papillomavirus (HPV) infection, the central and necessary cause of cervical cancer [3]. The burden of this cancer in developing countries is alarming, as they have about 80% of all cases worldwide [4].

The true incidence of cervical cancer in Nigeria remains unknown because of lack of a cancer registry, but it remains a major challenge in this environment because of the very low uptake of Pap smear screening and the associated late presentation. The sociocultural practices of the population include marriage at a very young age, polygamy and many divorces and remarriages. It has been observed that cervical cancers are commoner in the northern part of the country, which is predominantly Muslim, than in the southern part, which is predominantly Christian and where breast is the commonest cancer. The aim of this study was to analyse the sociodemographic and clinicopathologic characteristics of cervical cancers in a major tertiary institution in northern Nigeria.

Materials and Methods

The study was conducted between January 2002 and December 2004, accruing a total of 70 patients with histologically confirmed cervical cancer. Patients presenting for the first time and those referred to the institution with histologically confirmed carcinoma of the cervix were included. Histologic confirmation was done either by the referring hospital or at presentation if the patient had no histology report. For each patient, data including sociodemographic characteristics, symptoms and signs at presentation, and histology, were collected prospectively on a structured pro forma by a single investigator, by one-to-one interviews with the patients. The data were entered into Epi-Info software and analysed.

The socio-demographic information obtained from the patients included age at time of presentation, age at first marriage, number of marriages, age at first coitus, marital status, religion, parity, menopausal status, history of sexually transmitted diseases, knowledge and practice of Pap smears for screening, number of miscarriages warranting dilatation and curettage, and history of cigarette smoking. Postmenopause was defined as complete cessation of menstruation for at least six months. Bulky cervical mass was defined as a cervical mass greater than 5 cm in at least one dimension by ultrasound measurement.

Written informed consent was obtained from all participants. The first 37 patients in the study were not screened for HIV because this aspect was not included in the initial study protocol cleared by the institutional ethical committee, but subsequent patients were ethically cleared for HIV screening.

Results

Table 1 shows the characteristics of the patients, with the age distribution at the time of presentation. Two-thirds of the patients were in polygamous families, and 25 (36%) were in at least a second monogamous or polyga-
eight (83%) women had had at least five live births, with an average of 6.8 live births per patient and a range of 1-14. Similarly, 37 patients had had at least one miscarriage warranting dilatation and curettage. Thirty-nine (56%) patients had no formal education, 16 had completed primary education, 11 had completed secondary education and four patients had a tertiary education. Thirty-six patients were unemployed housewives, seven had white-collar jobs, and the others were farmers, petty traders and artisans. Of the 15 patients with knowledge about Pap smears, only three had had a smear, and only after the onset of symptoms. Fifty-five (79%) of the patients had never smoked cigarettes.

Table 2 shows that vaginal bleeding was a common symptom in all the patients, and 55 (79%) had vaginal discharges, which were malodorous in 30 (43%). Forty-seven patients (67%) presented with symptoms of more than six months’ duration (Table 3). The gross findings on digital and speculum examination revealed 49 patients with exophytic, 20 with ulcerative and only one patient with endophytic growth. Fifty (71%) patients had a bulky cervical mass. After adequate staging investigations (without CT scan and MRI), only five (7%) patients were found to have Stage IB, 19 (27%) had Stage II, 30 (43%) had Stage III, 14 (20%) had Stage IVA and two (3%) had Stage IVB disease. The histologic types seen were squamous cell carcinoma, accounting for 90% (63), adenocarcinoma (9%; 6) and adenosquamous carcinoma (1%; 1).

Table 1. — Characteristics of patients by age.

<table>
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<tr>
<th>Characteristics</th>
<th>30-39</th>
<th>40-49</th>
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<td>14</td>
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<td>3</td>
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<td>3</td>
<td>9</td>
<td>8</td>
<td>6</td>
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<td>28</td>
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<td>Muslim</td>
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<td>18</td>
<td>6</td>
<td>9</td>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>Residence</td>
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<tr>
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<td>9</td>
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<td>8</td>
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<td>18</td>
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<td>Type of marriage</td>
<td></td>
<td></td>
<td></td>
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<td>6</td>
<td>4</td>
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<td>4</td>
<td>1</td>
<td>2</td>
<td>–</td>
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</tr>
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<td>5-9</td>
<td>6</td>
<td>19</td>
<td>10</td>
<td>11</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>≥ 10</td>
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<td>4</td>
<td>3</td>
<td>2</td>
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<td>No. of abortions warranting dilatation and curettage</td>
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<td></td>
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<td>2</td>
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<tr>
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<td>–</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>5</td>
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<td></td>
<td></td>
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<td>11</td>
<td>24</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>38</td>
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<tr>
<td>Post</td>
<td>–</td>
<td>3</td>
<td>11</td>
<td>15</td>
<td>3</td>
<td>32</td>
</tr>
</tbody>
</table>

Figure 1. — Age distribution at time of first marriage.
Three of the 33 patients screened for HIV infection were found to be seropositive. Their ages were 30, 32 and 35 years, while the age range of the HIV-negative patients was 32-75 years with a median of 48 years. All three patients with HIV presented with advanced disease: one each with Stage IIIB, IVA and IVB.

Discussion

The results show that most of these patients were married before the age of 17 years and had therefore become sexually active at that time. A large proportion of the patients had high parity and multiple sexual partners, not as a result of sexual promiscuity but because the culture and religion permit divorce, remarriage and polygamy. This directly and indirectly increases the risk of exposure to HPV, the central and necessary cause of cervical cancer [3, 5, 6] and HIV. The educational level of the patients was low, and they presented late to hospital, with symptoms and signs of advanced disease. A significant proportion of the patients were postmenopausal. Cigarette smoking or tobacco consumption was not common. There was poor knowledge and attitudes about screening.

The patients in this study did not represent all the cervical cancers in the region because many patients go to private hospitals, Government peripheral hospitals and traditional or herbal practitioners for treatment, and may never be referred to the teaching hospital for diagnosis and treatment. Other factors that limit patient attendance are the prevailing poverty, the cost of medical services, especially for diagnosis and treatment of cancers, and the bias against women and children, whose health issues are not considered a priority by their immediate families. Not all the relevant investigations necessary for staging and treatment, especially CT scan and MRI, were done for most patients.

None of the patients was tested for HPV infection or checked for the presence of inclusion bodies in histologic specimens, as there are no facilities for these tests. Thus, the role of these infections could not be established. However, in a separate study carried out by the International Agency for Research on Cancer (IARC), the prevalence of HPV types was determined in a random sample of 932 asymptomatic women aged 15 to > 65 years in Ibadan, Nigeria. The overall HPV prevalence was very high (26%), and the most frequent types were HPV 16, 35, 31 and 58. In contrast to populations studied in similar surveys, the HPV prevalence was high not only among young women but also among those in middle and old age [7].

Epidemiological studies conducted over the past three decades have consistently indicated that the risk for cervical cancer is strongly influenced by measures of sexual activity, such as the number of sexual partners, age at first coitus and sexual behavior of the male partner [4], which are correlates of exposure to HPV [3]. We confirmed these observations in our study environment.

The peak age and cluster age for this cancer in our study occurred in the parturient age group of 40-49 years. In terms of age distribution, parity and menstrual status, cervical cancers in the northern savannah of Nigeria were similar to those reported elsewhere in Africa [8, 9] but significantly different from those in developed countries [10]. The patients not only had had first coitus before the age of 17 years but were sexually active by virtue of their marriage. This also contributes to the high parity, as the religion discourages use of family planning of any kind. A decline in fertility rates in North America over the past four decades has reduced the incidence of and mortality from cervical cancers [11, 12].

Lack of cervical cancer screening, coupled with delay in presentation to hospital, might account for the predominantly advanced stage of disease in developing countries. Cigarette smoking has been incriminated in cervical cancer, as the tobacco components lower local immunity in female genitalia [13]. In our study, however, most of the patients had never smoked cigarettes, and those that did did so very occasionally. In this study, all the male partners were circumcised and therefore did not play a role in spreading HPV [14].

The symptoms at presentation in this environment were that of advanced disease, with unprovoked bleeding, offensive vaginal discharges, lower abdominal pain and waist pain and, in some cases, the presence of fistula. This is the norm in most developing countries but differs significantly from the presentation in developed countries, where patients are seen at an early stage, with post-coital bleeding and occasionally, as a result of screening, with no symptoms [15]. The pattern of histology seen in our study was similar to that in the developed world, adenocarcinoma and adenosquamous carcinoma accounting for about 10% of all cervical cancers [11, 16, 17].

Although only about half of the patients had been screened for HIV, those with HIV antibodies were younger, with more advanced disease and, in terms of histology, had poorly differentiated squamous cell carcinoma. This result, although observed in only a few patients, supports reports in the literature of the pattern of carcinoma of the cervix in HIV-positive patients, suggesting that HIV-infected women with cervical cancer are more likely to have advanced disease at presentation and to have a higher recurrence rate than non-HIV-infected women [18]. Furthermore, cervical intraepithelial neoplasia occurs more frequently in women with HIV infection [18]. Our findings support previous observations on cofactors of HPV [19].

This study lays the foundation for a more comprehensive prospective study of cervical cancer and, on the basis of these findings, in promoting cervical cancer screening and discouraging the population from practicing lifestyles that place the individual at risk. We consider the results to be revealing despite the limitations of the study. Much needs to be done to change these sociocultural factors, and it may take time.

There is also a need for broader, all-encompassing research to address the remaining questions about cervical cancers in African countries, especially in Nigeria, and also to study the impact of the high prevalence of
HIV/AIDS on the natural history and therapeutic outcome of cervical cancers. A multidisciplinary protocol for prospective research on cervical cancers is currently being developed to evaluate the outcome of treatment and prognostic factors. HPV vaccines, which have been shown recently to be safe and to be highly effective in preventing precancerous lesions of the cervix and which are being commercialized, offer great hope for the prevention of cervical cancer in Africa and other developing countries where screening programs have been difficult or impossible to establish [20].

Conclusion

In northern Nigeria, the risk factors for cervical cancer include active sex at a young age, multiple sexual partners (due to polygamy, divorce and remarriage) and multiparity. In this environment, because of sociocultural factors (due to polygamy, divorce and remarriage) and multiparity, many women are exposed to these risk factors that increase the chances of acquiring genital HPV infection, the central and necessary cause of cervical cancer. The situation is worsened by poor uptake of screening programs.

References


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FIGO Stage I endometrial carcinoma: evaluation of lung metastases and follow-up

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Summary

**Purpose:** The aim of our study was to evaluate the incidence of lung metastases in the follow-up of women submitted to surgery for endometrial carcinoma, in particular for FIGO Stage I which is the lowest risk stage for this metastatic site. **Methods:** The study was conducted on 210 patients affected by FIGO Stage I endometrial cancer in the years 1990 to 2005 distributed as follows: 35 patients with Stage IA (limited to the endometrium), 150 patients with Stage IB (invasion up to and including half the myometrial thickness), 25 patients with Stage IC (invasion greater than half the myometrial thickness). They underwent follow-up. **Results:** Only one patient out of the group studied has developed lung metastasis six years after surgery. She was staged as FIGO IB (T1b Mx G1). **Conclusion:** We are still following the cases and evaluating the biological behavior of this specific endometrial carcinoma and its reaction to further therapies. We are also looking for possible clinical characteristics in disagreement with those reported in the literature, which would thus make it necessary to reconsider the prognosis and therapy of this stage of disease.

**Key words:** Endometrial cancer; Lung metastasis; Follow-up; Lung metastasis therapy.

Introduction

The aim of the study was to evaluate the incidence of lung metastasis in the follow-up of women submitted to surgery for endometrial carcinoma, in particular for FIGO Stage I [1, 2], which is the lowest risk stage for this metastatic site [3-5].

According to the studies of the American Cancer Society of the Centers for Disease Control and Prevention and of the Gynecologic Oncology Group we can affirm that endometrial carcinoma is the most common pelvic malignant neoplasia in women [6-8].

It mainly spreads to less than half of the myometrium (Stage IB), over half of the myometrium (Stage IC), endocervical glands (Stage IIA), cervical stroma (Stage IIB), serosa and/or adnexa and/or peritoneum (Stage IIB), vagina (Stage IIIB), pelvic and pre-aortic nodes (Stage IIIC), bowel and bladder mucosa (Stage IVA), distant organs including inguinal and intraabdominal nodes (Stage IVB).

On the basis of the architecture and cytologic atypia, endometrial carcinoma is classified as: well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) [9].

The lung is the most frequent site of distant metastasis. The incidence of metastasis varies according to stage and pathology as shown by many studies [3-5] and in particular by a study conducted by a Japanese group which demonstrated that lung metastases due to endometrial cancer FIGO Stage I/G1 have a very low incidence compared to those at a higher stage and with a more aggressive pathology [10].

Materials and Results

Our study was conducted on 210 patients affected by endometrial cancer FIGO Stage I in the years 1990 to 2005 distributed as follows:

- 35 patients with Stage IA (limited to the endometrium);
- 150 patients with Stage IB (invasion up to and including half the myometrial thickness);
- 25 patients with Stage IC (invasion greater than half the myometrial thickness).

All the patients underwent laparotomic surgery in our Institute and more specifically they were submitted to total hysterectomy, bilateral salpingo-oophorectomy, peritoneal washing (with negative results), and lymph node sampling [11-13].

On the basis of substaging and grading, some patients underwent adjuvant therapy [14-18].

Surveillance after treatment was every three months the first and second years, every six months the third year, every six months the fourth and fifth years, and once a year for the following period.

The investigation included a general and gynecologic check-up with vaginal smear, markers, pelvic, kidney and hepatic ultrasounds, lung X-ray (yearly), bilateral mammography (yearly), bone scintigraphy (yearly), abdominal CAT scan before surgery, 2 years after surgery, and afterwards once a year [19-21].

Results

Only one patient out of the study group developed lung metastasis six years after surgery. She was staged as FIGO IB (T1b Mx G1).

The patient, 55 years old with negative anamnesis for risk factors, underwent a total hysterectomy with bilateral salpingo-oophorectomy, removal of the vaginal cuff and pelvic lymphadenectomy. Peritoneal washing was negative. The pathologic diagnosis was highly differentiated G1 endometrial adenocarcinoma. Follow-up was totally
negative up to the sixth year, when a routine lung X-ray followed by a CAT scan showed bilateral lung metastasis.

The patient was submitted to various hormonal therapies without results [22-24]. A second-line treatment included chemotherapy with cisplatinum [25-28] up to volume reduction of the metastasis followed by bilateral lung metastasectomy [29]. She had been free of disease for six months when she developed a new bilateral lung metastasis. The patient is now in treatment with aromatase inhibitor.

Discussion

According to the clinical history of the patient who developed metastasis, we can assert that this case, based on the biological behavior, was an endometrial sarcoma rather than an endometrial carcinoma:

– family and personal history were negative for risk of developing endometrial adenocarcinoma;

– the first symptom of the patient was an episode of metrorrhagia lasting 12 hours two years after menopause, typical of uterine sarcoma rather than of endometrial carcinoma which causes abnormal bleeding;

– atypical incidence of recurrence (after 6 years), response to hormonal treatment (negative) and surgery of recurrence (recurrence of metastasis six months afterwards).

Conclusion

Two different pathologists reexamined the histological samples to exclude a wrong evaluation between endometrial adenocarcinoma which causes abnormal bleeding; typical of uterine sarcoma rather than of endometrial carcinoma which was an endometrial sarcoma rather than an endometrial carcinoma: an endometrial sarcoma. We can assert that this case, based on the biological behavior, was an endometrial sarcoma rather than an endometrial carcinoma.

According to the clinical history of the patient who developed metastasis, we can assert that this case, based on the biological behavior, was an endometrial sarcoma rather than an endometrial carcinoma: an endometrial sarcoma rather than an endometrial carcinoma.

Further therapies

We are still following the case and evaluating the biological behavior of this specific endometrial carcinoma as well as its reaction to further therapies.

We are also investigating possible eventual clinical characteristics not in agreement with those reported in the literature, which would make it necessary to reconsider the prognosis and therapy of this stage of disease.

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[16] Chen S.S.: “Operative treatment in Stage I endometrial carcinoma with deep myometrial invasion and or grade 3 tumor surgically limited to the corpus uteri”. Cancer, 2000, 63, 1834.


TV sonographic assessment in postmenopausal women with bleeding

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Summary

The aim of this study was to evaluate retrospectively the usefulness of transvaginal sonography for the detection of endometrial disease in postmenopausal women with bleeding. This study involved 275 postmenopausal women aged 47-81 years (median 62). None of them were on hormone replacement therapy and all had had amenorrhea for more than one year. Concerning the age of the study patients, we confirm that endometrial cancer occurs at any age, but more commonly in ages above 58 years. Transvaginal sonography was performed in all women. About 89.2% of malignant diseases were discovered in the study women whose endometrial thickness was above 4 mm, but we also found endometrial cancer in 10.2% of the cases in women whose endometrial thickness was below 4 mm. In postmenopausal symptomatic women premalignant or malignant causes of bleeding can not be excluded with just transvaginal ultrasound.

Key words: Transvaginal ultrasound; Age; Endometrium thickness; Postmenopausal women with bleeding.

Introduction

Thirty-three percent of women referred to gynecologic clinics have abnormal bleeding and this figure rises to 69% in peri- and postmenopausal women [1-3]. Postmenopausal bleeding occurs in 80-95% of patients with endometrial cancer [4]. The differential diagnosis includes a broad range of conditions, but the vast majority of postmenopausal women who present with irregular or excessive vaginal bleeding have benign disease [4]. Endometrial cancer accounts for approximately 7-14% of cases of postmenopausal bleeding [5-8]. Endometrial adenocarcinoma is the most common gynecological malignancy in females in North America and Europe [5]. Incidence is rising due to increased life expectancy and rise in incidence of obesity in women. Due to early detection of women with Stage I disease (75-80%), which mainly depends on the use of transvaginal ultrasound, it does not constitute a leading cause of cancer deaths [5, 9].

All women with the clinical appearance of postmenopausal bleeding should immediately undergo a clinical examination, cervical smear test and transvaginal ultrasound examination [10]. Ultrasound signs of endometrial carcinoma include heterogeneity and irregular endometrial thickening. However, these signs are non-specific and ultrasound cannot reliably distinguish between benign proliferation (hyperplasia, submucosal myoma, polyps) and cancer [11]. Endometrium contains estrogen receptors and responds to circulating estrogens. Endometrial thickness constitutes a potential biological marker of estrogen status even in postmenopausal women [12]. The aim of this retrospective study was to estimate the efficacy of transvaginal sonography (TVS) as a non-invasive method in the detection of endometrial pathology in symptomatic postmenopausal women, and to determine whether endometrial thickness can predict the likelihood of endometrial cancer and thus reduce the need for endometrial biopsy in patients with postmenopausal bleeding.

Method

This retrospective study was conducted to investigate the diagnostic prognostic value of only endometrial thickness in postmenopausal patients with postmenopausal bleeding. The women were examined at the Department of Gynecology of Democritus University Hospital in Alexandroupolis during the time period from January 2000 to December 2006. All subjects had not been menstruating for more than a year. None of the women included in the study had ever received hormone replacement therapy or had been treated with anti-estrogens such as tamoxifen. Women who had any gynecological malignancy or severe medical conditions such as heart, pulmonary or renal disease or other pathology in the past were excluded. All study patients underwent TVS. We used a scanner transducer 6.5 MHz in all patients on the same day of surgery and one to two measurements of endometrial thickness were taken. TVS evaluation of the uterus was done both in the transverse and longitudinal axis. Measurements of endometrial thickness on sonograms were calculated by placing calipers in outer borders of the junction zone along the long axis of the endometrium at the level of the greatest anterior to posterior diameter. The surrounding echolucent layer was considered inner myometrium and it was not included in the measurement of the thickness. The midline echo was considered to be normal when a straight endometrial lining with well defined margins and without echodense foci was found. In the cases where the endometrial layers were separated by intracavity fluid, both layers were measured independently and the total endometrial thickness included both endometrial layers. Except for endometrial thickness, the endometrial cavity was also examined for the presence of polyps or submucosal myoma or other pathological conditions.

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Reliability of the recordings was usually confirmed by a single trained clinician and in difficult cases a second opinion was sought from one of the consultants involved in the study. A questionnaire completed by the patients themselves before ultrasound examination provided information on sociodemographic characteristics, body weight, height, reproductive and obstetric history, family history of cancer, personal medical history and cigarette smoking. At the end of the TVS procedure, fraction curettage was performed and the samples from the cervical canal and uterine cavity were sent for histopathologic examination to the Institute of Pathology of Democritus University Hospital in Alexandroupolis.

Results

The study group included 275 postmenopausal women. The patients ranged in age from 47 to 81 years, with a median age of 62 years (mean age ± SD = 62.37 ± 7.72 years). The time since menopause ranged from one to 33 years (mean = 7.82 median = 7.00, SD = 4.98; min = 1.00, max = 33.00). Parity ranged from 0-7 (mean 3.86, median 4.00, SD =1.11, min = 1.00, max = 7.00). The presence or absence of hypertension had no impact on the accuracy of TVS. Seventy-six women with diabetes or obesity but without malignancy or premalignancy, were found to have thicker endometrium (> 10 mm) than women without these risk factors. Satisfactory visualization of the endometrium was obtained in all 275 examined cases. The thinnest endometrium was 0 mm while the thickest was 25 mm.

Histological findings are shown in Table 1. Of the 275 study patients, 6.54% (18) had endometrial cancer, 6.9% (19) had atypical hyperplasia, 4% (11) had complex hyperplasia, 18.9% (52) had simple hyperplasia, 27.63% (76) had atrophic endometrium and 20% (55) had polyps. Endometritis was found in 12.38% (34) of the study population and 2.19% (6 cases) were identified with submucosal fibroids. Histology was not available for three patients as no adequate material was obtained at biopsy without explanation and the remaining 0.37% (1 case) had intrauterine adhesions. The area of sonographic endometrial thickness-value measurements by histological findings in relation to patient age.

### Table 1. — Distribution of histological findings.

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<th>Patients</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range (min-max)</th>
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<tr>
<td>Atypical hyperplasia</td>
<td>19</td>
<td>9.58 ± 3.37</td>
<td>8.00</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>22</td>
<td>12.91 ± 5.85</td>
<td>10.50</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>53</td>
<td>10.64 ± 4.93</td>
<td>9.00</td>
</tr>
<tr>
<td>Polyps</td>
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<td>16.43 ± 4.33</td>
<td>16.00</td>
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<tr>
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<td>Endometritis</td>
<td>34</td>
<td>6.32 ± 3.14</td>
<td>6.00</td>
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<tr>
<td>Myoma</td>
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<td>17.67 ± 5.24</td>
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<td>Unsuitable</td>
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<td>1.67 ± 0.58</td>
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<tr>
<td>Adhesions</td>
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<tr>
<td>Total</td>
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<td>100%</td>
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</table>

### Table 2. — Thickness of endometrium versus histological diagnosis.

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Patients</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial cancer</td>
<td>18</td>
<td>12.33 ± 8.22</td>
<td>12.00</td>
<td>1.00-25.00</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>19</td>
<td>9.58 ± 3.37</td>
<td>8.00</td>
<td>4.00-19.00</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>22</td>
<td>12.91 ± 5.85</td>
<td>10.50</td>
<td>5.00-24.00</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>53</td>
<td>10.64 ± 4.93</td>
<td>9.00</td>
<td>1.00-22.00</td>
</tr>
<tr>
<td>Polyps</td>
<td>65</td>
<td>16.43 ± 4.33</td>
<td>16.00</td>
<td>0.00-25.00</td>
</tr>
<tr>
<td>Atrophy</td>
<td>52</td>
<td>4.73 ± 2.23</td>
<td>4.50</td>
<td>0.00-10.00</td>
</tr>
<tr>
<td>Endometritis</td>
<td>34</td>
<td>6.32 ± 3.14</td>
<td>6.00</td>
<td>0.00-12.00</td>
</tr>
<tr>
<td>Myoma</td>
<td>6</td>
<td>17.67 ± 5.24</td>
<td>17.00</td>
<td>12.00-26.00</td>
</tr>
<tr>
<td>Unsuitable</td>
<td>3</td>
<td>1.67 ± 0.58</td>
<td>2.00</td>
<td>1.00-2.00</td>
</tr>
<tr>
<td>Adhesions</td>
<td>1</td>
<td>2.4 ± 0</td>
<td>–</td>
<td>2.4-0.0</td>
</tr>
<tr>
<td>Total</td>
<td>275</td>
<td></td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. — Incidence of malignancy and benign diseases in relation to age and endometrial thickness.

<table>
<thead>
<tr>
<th>Age</th>
<th>Malignancy cancer + atypical hyperplasia</th>
<th>Benign all others</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>47-57 years</td>
<td>5 (13.5)</td>
<td>159 (66.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>58-70 years</td>
<td>14 (37.8)</td>
<td>51 (21.4)</td>
<td></td>
</tr>
<tr>
<td>&gt; 70 years</td>
<td>18 (48.6)</td>
<td>28 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>0.473</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 4 mm</td>
<td>4 (10.8)</td>
<td>45 (18.9)</td>
<td></td>
</tr>
<tr>
<td>4-8 mm</td>
<td>12 (32.4)</td>
<td>66 (27.7)</td>
<td></td>
</tr>
<tr>
<td>&gt; 8 mm</td>
<td>21 (56.8)</td>
<td>127 (53.4)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>37 (100.0)</td>
<td>238 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. — Histological findings in relation to patient age.

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Patient age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>40 (72.7)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>33 (63.5)</td>
</tr>
<tr>
<td>Polyps</td>
<td>53 (81.5)</td>
</tr>
<tr>
<td>Submucosal myoma</td>
<td>4 (66.6)</td>
</tr>
<tr>
<td>Endometritis</td>
<td>25 (73.5)</td>
</tr>
<tr>
<td>Adhesions</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>Nothing</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are presented as number of patients and percentages.
Concerning the measurements of endometrial thickness, we divided the patients into three groups. The distribution frequency of histological diseases in each of the three groups is described in Table 7. When comparing the histological findings in the first group (endometrial thickness < 4 mm) malignancy and premalignancy occurred in 8.16% of all cases with endometrial thickness < 4 mm. In the other two groups with greater endometrium (4-8 mm, > 8 mm) the malignancy and premalignancy rate increased.

Table 7.— Histological findings in relation to endometrial thickness in relation to the total number of patients with the same thickness.

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>&lt; 4 mm</th>
<th>4-8 mm</th>
<th>&gt; 8 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>4 (8.16%)</td>
<td>3 (3.84%)</td>
<td>11 (7.43%)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>0</td>
<td>9 (11.53%)</td>
<td>10 (6.75%)</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>2 (4.08%)</td>
<td>7 (8.97%)</td>
<td>13 (8.78%)</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>2 (4.08%)</td>
<td>16 (20.51%)</td>
<td>37 (25%)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>32 (65.3%)</td>
<td>20 (25.64%)</td>
<td>0</td>
</tr>
<tr>
<td>Polyps</td>
<td>0</td>
<td>3 (3.84%)</td>
<td>62 (41.89%)</td>
</tr>
<tr>
<td>Submucosal myoma</td>
<td>0</td>
<td>0</td>
<td>6 (4.05%)</td>
</tr>
<tr>
<td>Endometritis</td>
<td>5 (10.2%)</td>
<td>20 (25.64%)</td>
<td>9 (6.08%)</td>
</tr>
<tr>
<td>Adhesions</td>
<td>1 (2.04%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nothing</td>
<td>3 (6.12%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>78</td>
<td>148</td>
</tr>
</tbody>
</table>

Data are presented as number of patients and percentages.

Table 8.— Correlation between histological findings and thickness of endometrium by transvaginal scan in postmenopausal women with bleeding.

<table>
<thead>
<tr>
<th>Authors</th>
<th>E.T.* frequency</th>
<th>E.C* frequency</th>
<th>E.T.* frequency</th>
<th>E.C* frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randelzhofer et al. 2002</td>
<td>&lt; 5 mm 3%</td>
<td>6-10 mm 12%</td>
<td>&gt; 10 mm 43%</td>
<td></td>
</tr>
<tr>
<td>Taskin et al. 2006</td>
<td>&lt; 4 mm 2%</td>
<td>6-10 mm 12%</td>
<td>&gt; 10 mm 17%</td>
<td></td>
</tr>
<tr>
<td>Buchim et al. 2004</td>
<td>&lt; 5 mm 0%</td>
<td>5-9 mm 10.5%</td>
<td>&gt; 9 mm 18.5%</td>
<td></td>
</tr>
<tr>
<td>Gull et al. 2003</td>
<td>&lt; 4 mm 0%</td>
<td>5-7 mm 33.3%</td>
<td>&gt; 8 mm 14.3%</td>
<td></td>
</tr>
<tr>
<td>Phillip et al. 2004</td>
<td>&lt; 4 mm 50%</td>
<td>5-10 mm 37.5%</td>
<td>&gt; 10 mm 12.5%</td>
<td></td>
</tr>
<tr>
<td>Tsikouras et al. 2007</td>
<td>&lt; 4 mm 8.16%</td>
<td>4-8 mm 3.84%</td>
<td>&gt; 8 mm 7.43%</td>
<td></td>
</tr>
</tbody>
</table>

E.T.* Endometrial thickness; E.C* Endometrial cancer.

Discussion

Today no standardized methodology is used in the evaluation of women with abnormal vaginal bleeding other than endometrial biopsy or curettage [13]. There is great interest in the role of transvaginal sonography in the evaluation of patients with postmenopausal bleeding [14]. Tissue sampling is required to confirm the presence of carcinoma. The endometrium in postmenopausal women normally appears as a uniform, thin echogenic line. The postmenopausal double-layer endometrial thickness should measure < 8 mm and typically measures < 5 mm. A small amount of anechoic fluid in the uterine cavity may be seen and does not necessarily indicate significant pathologic conditions. The most common cause of post-

### Table 5. — Histological findings in relation to endometrial thickness.

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>&lt; 4 mm</th>
<th>4-8 mm</th>
<th>&gt; 8 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>4 (22.2%)</td>
<td>3 (16.7%)</td>
<td>11 (61.1%)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>0 (15.8%)</td>
<td>9 (47.4%)</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>2 (9.1%)</td>
<td>7 (31.8%)</td>
<td>13 (59.1%)</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>2 (3.6%)</td>
<td>16 (29.1%)</td>
<td>37 (67.3%)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>32 (61.5%)</td>
<td>20 (38.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Polyps</td>
<td>0</td>
<td>3 (4.6%)</td>
<td>62 (95.4%)</td>
</tr>
<tr>
<td>Submucosal myoma</td>
<td>0</td>
<td>6 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Endometritis</td>
<td>5 (14.7%)</td>
<td>20 (58.8%)</td>
<td>9 (26.5%)</td>
</tr>
<tr>
<td>Adhesions</td>
<td>1 (100.0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nothing</td>
<td>3 (100.0)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are presented as number of patients and percentages.

### Table 6. — Histological findings in relation to the total number of patients of the same age.

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>47-57 years</th>
<th>58-70 years</th>
<th>&gt; 70 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>2 (1.21%)</td>
<td>5 (7.81%)</td>
<td>11 (23.91%)</td>
</tr>
<tr>
<td>Atypical hyperplasia</td>
<td>3 (1.82%)</td>
<td>9 (14.06%)</td>
<td>7 (15.21%)</td>
</tr>
<tr>
<td>Complex hyperplasia</td>
<td>3 (1.82%)</td>
<td>12 (18.75%)</td>
<td>7 (15.21%)</td>
</tr>
<tr>
<td>Simple hyperplasia</td>
<td>40 (24.39%)</td>
<td>10 (15.62%)</td>
<td>5 (10.86%)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>33 (20.12%)</td>
<td>13 (20.31%)</td>
<td>6 (13.04%)</td>
</tr>
<tr>
<td>Polyps</td>
<td>53 (32.31%)</td>
<td>7 (10.93%)</td>
<td>5 (10.86%)</td>
</tr>
<tr>
<td>Submucosal myoma</td>
<td>4 (2.43%)</td>
<td>1 (1.56%)</td>
<td>1 (2.17%)</td>
</tr>
<tr>
<td>Endometritis</td>
<td>25 (15.24%)</td>
<td>7 (10.93%)</td>
<td>2 (4.34%)</td>
</tr>
<tr>
<td>Adhesions</td>
<td>1 (0.6%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nothing</td>
<td>0 (0%)</td>
<td>1 (1.56%)</td>
<td>2 (4.34%)</td>
</tr>
<tr>
<td>Total</td>
<td>164</td>
<td>64</td>
<td>46</td>
</tr>
</tbody>
</table>

Data are presented as number of patients and percentages.
menopausal bleeding is endometrial atrophy [15]. Atrophic endometrium typically appears on TVS as a uniformly thin echogenic line. Bleeding can also be caused by other benign conditions including endometrial polyps, hyperplasia, and submucosal fibroids. Endometrial polyps may be sessile or pedunculated and can be multiple in ≥ 20% of cases. An endometrial polyp is typically a focal homogeneous, hyperechoic endometrial mass [15]. Hyperplasia often manifests itself as echogenic endometrium with detectable small cysts. Atypical hyperplasia may appear as an inhomogeneous, irregular endometrial stripe [15]. Endometrial carcinoma tends to appear in greater endometrial thickness than do benign endometrial diseases. The sonographic appearance of endometrial carcinoma is typically a thick, solid, heterogeneous, ill-defined endometrial tissue [15].

The gold standard for diagnosing abnormalities in the endometrial tissue of patients with postmenopausal bleeding is endometrial sampling [16, 17]. Since the sensitivity of endometrial sampling has been estimated to range from 85% to 95%, there has been a growing trend towards using a noninvasive procedure, such as high-resolution transvaginal sonography, to measure the endometrial thickness so as to classify cases as being at low or high risk for malignancy and thus avoid unnecessary sampling [18-25]. Vaginal sonography would be preferred over uniform biopsy of postmenopausal women with vaginal bleeding because it a) is a less invasive procedure, b) is generally painless, c) has no complications, and d) may be more sensitive for detecting carcinoma than blind biopsy. Unfortunately according to our study ultrasonography does not provide a completely safe differentiation between benign and malignant endometrial disease. Ultrasound cannot distinguish between hyperplasia and malignancy and normal tissue, so an endometrial biopsy should be performed to eliminate the possibility of cancer and proceed rapidly to further treatment, which may include hysterectomy. Occasionally (in 5% to 10% of cases), a woman’s endometrium cannot be identified on ultrasound, and these women also need further evaluation [11]. In various studies limitations of TVS have been reported regarding evaluation of the endometrium, and recently saline hysterosonography has been used by investigators to show correlated morphology and thickness of the endometrium. They concluded that combining the measurement and morphologic aspects of the endometrium improved the predictive ability of pathologic findings [28, 29]. Various studies using endometrial thickness as criterion for the detection of endometrial intracavity pathology are listed in Table 8.

Thickened endometrium during menopause is the most significant ultrasonographic criterion implicating its pathology [30]. Most authors agree that there is a positive correlation between the thickness of endometrium and its pathological conditions. The most often used limit values are 3 and 4 mm. Higher limit values of endometrial thickness increase the sensitivity of the method even to 100%, but affect its specificity negatively [28]. An ultrasound measurement of endometrial thickness ≥ 4 mm in postmenopausal women with bleeding warrants further examination [31].

Concerning our findings, we could confirm that endometrial cancer occurs at any age, but more commonly in ages above 58 years. It was found that the frequency of malignancies tend to increase with increasing age, unlike other diseases which are more frequent in younger ages. Despite that, 13.5% of malignancies were found in younger women. In the study women most causes of postmenopausal bleeding were benign.

Endometrial polyps usually occur in women between 40 and 50 years old and more frequently in postmenopause [32]. Our results confirm this with age peak between 47 and 57 years (Table 4).

The presentation of complex or simple hyperplasia, polyps and submucosal myoma was also associated with increased endometrial thickness, unlike endometritis, atrophy and adhesions which were more prevalent in thinner endometrial thickness. Regarding endometrial thickness, the incidence of malignancies tend to increase as endometrial thickness increases. About 89.2% of malignant diseases were discovered in the study women, whose endometrial thickness was above 4 mm. However, despite this finding, we also found endometrial cancer in 10.2% of women whose endometrial thickness was below 4 mm.

We conclude that a postmenopausal double-layer endometrial thickness even less than 4 mm as measured by transvaginal ultrasound does not appear to safely exclude endometrial cancer as a cause of postmenopausal bleeding.

References


TV sonographic assessment in postmenopausal women with bleeding


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Risk factors for cervical cancer in China: a case-control study

H.B. Cai, X.H. Ding, Y.F. Zhou, D.M. Lie

Department of Gynecological Oncology, Zhongnan Hospital, *Institute of Virology, Wuhan University, P.R. (China)

Summary

Objectives: To determine the prevalence of human papillomavirus types and investigate the risk factors for cervical cancer in Hubei, China. Methods: We conducted a case-control study to investigate risk factors. Results: HPV DNA was detected in 94.55% of patients with cervical carcinoma, and 23.64% of control subjects. The most common HPV type in cervical cancer was HPV type 16 (81.82%), followed by HPV 58 (6.36%). HPV infected patients have a higher risk of developing cervical carcinoma, which is 75.79 times more than non-infected people. The other risks were age at first intercourse (p = 0.017) and number of live births (p = 0.032). A history of previous cytologic screening was associated with a substantial reduction in risk (p = 0.001). Conclusions: The three principal reasons that Hubei has a high rate of women developing cervical carcinoma are HPV infection, age at first sexual intercourse and number of live births. Cervical cytology screening provides efficacious protection.

Key words: Cervical cancer; Risk factors; Case-control study; HPV.

Introduction

Cervical cancer is the second commonest type of cancer in females worldwide. Incidence rates of this disease varies from about 10 cases per 100,000 women per year in many developed countries to more than 40 per 100,000 in some developing countries [1]. Of the half million cases of cervical cancer estimated annually in the world, nearly 80% occur in developing countries [2]. China has one of the highest incidence rates of cervical cancer with 135,000 new cases detected every year, approximately one-fourth of those in the world [3]. However, there are quite different frequencies in various zones of China. Wufeng (Hubei province) in the central region of China, is one of the highest prevalence areas of cervical carcinoma (1,073.34 per 100,000). Beijing (2.54 per 100,000) and Shanghai (3.80 per 100,000) have the lowest incidence of cervical cancer. The rate in Hubei is 422 times that in Beijing [4]. The high incidence of cervical carcinoma may reflect a poor screening program [5] and differences in the human papillomavirus (HPV) infecting the Chinese population. In addition, HPV infection appears to be a necessary but not a sufficient cause of cervical cancer. The role of specific viral host or environmental factors for progression from infection to invasive disease has not been clarified.

The incidence of cervical carcinoma is relative to some risk factors, including three aspects. The first is biological factors, such as HPV infection [6]. The second is behavioral risk factors, such as early sexual intercourse, multiple sex partners, more pregnancies, more births, early age at first birth [7], low social economic status [8], smoking [9], malnutrition [10], and long-term use of oral prophylactics [11]. The third factor is genetic susceptibility, and familial aggregation has been observed in recent years [12].

To better assess the role of HPV infection and other risk factors in the development of cervical carcinoma, we carried out an analysis of data from case-control studies of cervical carcinoma in the Hubei province, China. Our aim was to determine the prevalence of human papillomavirus types in cervical carcinoma and investigate risk factors for this disease in Hubei. It would be helpful to know the specific types of HPV operating as cervical carcinogens in Hubei regions and to know whether it is necessary to tail interventions to the specific needs of particular areas. The definition of risk factors can help identify groups at particularly higher risk and can lead to new preventive strategies.

Materials and Methods

Study population

Recruitment of study subjects was conducted from 2003 through 2004. Case subjects were women with cervical cancer that was newly diagnosed and histologically confirmed at Zhongnan Hospital, Wuhan University in Hubei. The subjects had not received previous treatment for cervical cancer, and all were in sufficiently good physical and mental condition to provide reliable answers. Histologic slides were reviewed by two expert pathologists. Control subjects were women without cervical cancer who were selected from the same hospital. These women had no history of treatment with conization or hysterectomy. In addition, as was required for the case subjects, control subjects had to be in sufficiently good physical and mental condition to provide reliable answers. Other reasons for ineligibility of control subjects included diagnoses of cancer and tobacco-related disease.

The 110 patients were compared with 110 healthy women (ratio of 1:1). The condition of comparison was that both patients and healthy women lived in the same area and had similar ages (with differential no more than 2 years). The two groups were residents of Hubei province.
Data and specimen collection

Study subjects were interviewed at the hospital by use of a standardized questionnaire to elicit information on sexual behavior, reproductive history, contraceptive practice, genital hygiene, history of sexually transmitted diseases, screening history, and various measures of socioeconomic status. Two specially trained female technicians administered the interview to all case and control subjects. An effort was made to keep them blinded to the case or control status of the study subjects.

A pelvic examination was performed on both case and control subjects to obtain biopsy specimens. Samples were transferred into sterile tubes and transported to the laboratory in medium at 4°C. On receipt of tissues in the laboratory, they were cut into small pieces, weighed, and stored as multiple aliquots at -80°C.

The study protocol was cleared by the local ethical committee. Written informed consent was obtained from all subjects - total of 110 eligible case subjects and 110 eligible control subjects. Refusal to participate was the main reason for nonparticipation of control subjects.

Detection and typing of HPV DNA

Cervical specimen DNA was extracted from the tissues using following standard techniques [13]. DNA amplification for HPV detection was performed using primers MY11/MY09 (Primer1: 3-GCA CAG GGT CAG AAC AAT GG-5 and Primer 2: 3-CGT CCA AGG GGA TAT TGA TC-5).

PCR reactions were performed using sterile 0.5-ml RNAse-DNase-free tubes and each PCR reaction was made up to a final volume of 50 ul. A typical 50 ul of PCR reaction contained 100 mM Kcl, 20 mM Tris-HCL pH 8.0, 2.0 mM Mgcl2, 2.5 mM of dNTPs, 1.5 units of Taq polymerase (Promega Corp. Madison, WI) 25 pmol of each primer, and 10 ul of sample. After thermal cycling (initially for 90 sec at 94°C for 1 cycle; then 40 cycle at 55°C for 1 min, 72°C for 1 min, and at 94°C for 1 min; and finally 1 cycle at 72°C for 10 min), 10 ul of the PCR reactions were analyzed by agarose gel electrophoresis. All HPV-negative carcinomas were retested for HPV-using the methods described previously to ensure that a positive result had not been “missed” initially.

HPV DNA sequencing

HPV DNA detection by PCR was further verified by sequencing of the DNA in all samples. Products for sequencing were generated using the sample primers as described for the amplification of HPV DNA. Amplified products were subjected to cycle sequencing PCR with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequencing reactions were then run on the ABI PRISM 310 Genetic Analyzer. Viral sequencing was analyzed by sequencing analysis software and sequencing navigator.

Statistical analysis

To estimate the risk of cervical cancer associated with various HPV types and other risk factors, we calculated the odds ratio (OR) and 95% confidence interval (CI) as approximation of relative risk by using unconditional logistic regression. The software used for statistics was SPSS 11.0 of which the level of significance was α = 0.05.

Results

For patients with cervical carcinoma, the mean age was 48.06 ± 24.85 years (range 22-72 years) and for control subjects, it was 48.23 ± 25.23 years (range 23-72 years).

Table 1 presents the mono-factor analysis of risk factors relative to cervical carcinoma in Hubei province, China. The following factors were associated with risk: HPV infection (p = 0.0001), age at first intercourse (p = 0.001), number of live births (p = 0.042), number of lifetime sexual partners (p = 0.003), and education level (p = 0.001). In addition, a history of previous cytologic screening was associated with a substantial reduction in risk (p = 0.0001). However, a history of venereal disease (p = 0.291), number of pregnancies (p = 0.633) and use of hormonal contraceptives (p = 0.381) were not associated with risk.

Table 1. — Mono-factor analysis risk factors related to cervical carcinoma.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Case group (n = 110)</th>
<th>Control group (n = 110)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>104</td>
<td>26</td>
<td>0.0001</td>
</tr>
<tr>
<td>negative</td>
<td>6</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>Age at first intercourse (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 23</td>
<td>16</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>21-23</td>
<td>32</td>
<td>24</td>
<td>0.633</td>
</tr>
<tr>
<td>18-20</td>
<td>42</td>
<td>37</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt; 18</td>
<td>20</td>
<td>41</td>
<td>0.001</td>
</tr>
<tr>
<td>Lifetime no. of sexual partners</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>67</td>
<td>87</td>
<td>0.003</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>43</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>No. of live births</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>13</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>66</td>
<td>60</td>
<td>0.042</td>
</tr>
<tr>
<td>2-3</td>
<td>26</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>&gt; 4</td>
<td>18</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Use of hormonal contraceptives (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>74</td>
<td>83</td>
<td>0.381</td>
</tr>
<tr>
<td>1-3</td>
<td>17</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>&gt; 4</td>
<td>19</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Interval since last Pap smear (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>92</td>
<td>49</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>5</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>≥ 5</td>
<td>13</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Education</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Higher</td>
<td>10</td>
<td>9.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Primary</td>
<td>77</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>23</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Any veneral disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>64</td>
<td>72</td>
<td>0.001</td>
</tr>
<tr>
<td>Once</td>
<td>21</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>More than once</td>
<td>25</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 presents the multivariate regression analysis of risk factors associated with cervical carcinoma. The variables that remained associated with risk were HPV infection (p for trend = 0.0001), age at first intercourse (p for
Table 2. — Multivariate regression analysis risk factors related to cervical carcinoma.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Case subjects</th>
<th>Control subjects</th>
<th>B</th>
<th>Wald</th>
<th>OR</th>
<th>95% CI for OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV negative</td>
<td>6</td>
<td>84</td>
<td>2.32</td>
<td>51.41</td>
<td>75.79</td>
<td>23.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>HPV positive</td>
<td>104</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first intercourse (years)</td>
<td>&gt; 23</td>
<td>18</td>
<td>6</td>
<td>0.09</td>
<td>1.09</td>
<td>0.41</td>
<td>0.08</td>
</tr>
<tr>
<td>21-23</td>
<td>32</td>
<td>24</td>
<td>0.37</td>
<td>0.23</td>
<td>1.46</td>
<td>0.31</td>
<td>6.79</td>
</tr>
<tr>
<td>18-20</td>
<td>42</td>
<td>37</td>
<td>1.31</td>
<td>2.90</td>
<td>3.71</td>
<td>1.82</td>
<td>16.81</td>
</tr>
<tr>
<td>&lt; 18</td>
<td>20</td>
<td>41</td>
<td>&gt; 23</td>
<td>1.25</td>
<td>1.96</td>
<td>3.49</td>
<td>0.61</td>
</tr>
<tr>
<td>No. of sexual partners</td>
<td>0-1</td>
<td>67</td>
<td>87</td>
<td>0.01</td>
<td>0.00</td>
<td>1.01</td>
<td>0.32</td>
</tr>
<tr>
<td>2-3</td>
<td>26</td>
<td>27</td>
<td>0.51</td>
<td>0.63</td>
<td>1.67</td>
<td>0.47</td>
<td>5.92</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>18</td>
<td>23</td>
<td>1.25</td>
<td>3.49</td>
<td>8.66</td>
<td>9.52</td>
<td>2.23</td>
</tr>
<tr>
<td>Interval since last Pap smear (years)</td>
<td>&lt; 5</td>
<td>13</td>
<td>30</td>
<td>1.56</td>
<td>7.29</td>
<td>4.75</td>
<td>1.53</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>&gt; 2</td>
<td>2.35</td>
<td>8.86</td>
<td>9.52</td>
<td>2.23</td>
<td>49.54</td>
</tr>
</tbody>
</table>

B: partial regression coefficient; Wald: B/standard error^2; OR: odds ratio.

Discussion

The results of this case-control study conducted in Hubei, China confirm the finding of the investigators indicating that HPV DNA is present in the vast majority of cervical cancers (94.55%). The outcome of this research showed that human papillomavirus is not only a high-risk factor of cervical carcinoma, but a principal risk factor, which increased the risk by 75.79 times.

The HPV types identified in this study were, in order of decreasing prevalence, HPV 16, 58, 31, 18, 52, 33, 59, 35, 11, and 6 were detected. The other HPV types were not identified in any specimens. Out of the 110 case subjects, HPV DNA was identified in 104 cases (94.55%), with HPV16 being detected in 90 (81.82%) cases, HPV 58 in seven (6.36%) cases, HPV31 in five (4.55%) cases, HPV 18 in four (3.64%) cases, and the remaining six (5.45%) were HPV negative. Among control subject, the HPV prevalence was 23.64% (26/110). HPV type 11 (9.09%) and 6 (6.36%) were the most common types, followed by types 16 (1.82%), 18 (1.82%) and 35 (1.82%). When the prevalence rates of HPV types detected in case subjects were compared to control subjects only women with HPV 16 and 11 infection showed a statistically significant difference between the two groups (p = 0.001, p = 0.01, respectively). Two or more different HPV types were detected in 12 (10.91%) case subjects and 0 (0.00%) control subjects. The majority of instances of double infections included infections with HPV 16 or HPV 31.
Risk factors for cervical cancer in China: a case-control study

...genic factors. Thus incidence ratio of cervical carcinoma is significantly higher.

The number of live births is also a high risk factor for cervical carcinoma. When compared with women reporting only one live birth, those reporting two had a 6.05-fold increased risk and those reporting three had a 9.06-fold increased risk (p for trend = 0.032). Multiple pregnancies and fertilities may result in birth injury to the cervix, thus increasing the risk of cervical carcinoma.

In addition, there was a strong protective effect with the interval since last Pap smear. Women who were screened in the previous five years had a 9.52-fold reduction in risk (p for trend = 0.001). Similar associations have been observed in Spain and Colombia, Brazil and the Philippines [19-24]. Thus using Pap smears cervical lesions can be found, patients can be treated in time, and further development of disease may be prevented.

In conclusion, the three principle reasons that Hubei has a high rate of cervical carcinoma are HPV infection, young age at first sexual intercourse and number of live births. Cervical cytology screening provides efficacious protection. The high prevalence of HPV16 and HPV58 in Hubei deserves special attention in future vaccination programs to effectively lessen the burden of cervical cancer in China.

References


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Knowledge and interest of Turkish women about cervical cancer and HPV vaccine

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3Department of Obstetrics and Gynecology, Ministry of Health Pazarcik County Hospital, Kahramanmaraş (Turkey)

Summary

Objective: We aimed to evaluate the knowledge and interest level of Turkish women about HPV, HPV vaccines and cervical cancer using a questionnaire. Method: A 25-item questionnaire was distributed to women in three different cities located in separate sociocultural locations. Results: At the closure of the study 143 women responded and returned the survey. Of the participants 62.2% (89) had a university degree, 36.4% (52) a high school education, and 1.4% (2) had lower school degrees; 98.5% of the women would consent to have their daughter vaccinated for HPV and 94.7% would consent to have their son vaccinated if vaccine provided prevention against cancer and related diseases. However in both cases women gave importance to the “cost” - unless vaccine could be free. On logistic regression analyses none of the variables (i.e., questions) in the survey predicted women's willingness to accept the vaccine for themselves or their children. Conclusions: Women in Turkey would be willing to have themselves and their children receive HPV vaccine against cervical cancer and related diseases.

Key words: Cervical cancer; Human Papillomavirus; HPV; Vaccine.

Introduction

Cervical cancer is the second most common cause of cancer-related death in women worldwide. Five hundred thousand new cases of cervical cancer are diagnosed and more than 280,000 deaths occur worldwide [1]. Any prevention method that leads to a decreased incidence of cervical cancer together with preinvasive cervical diseases is very important for public health and health economy. Cervical mass screening using the Pap smear was the only way of effective prevention and early diagnosis until 2006 when HPV prophylactic vaccines became available.

Human Papillomavirus (HPV) is the most common sexually transmitted infection (STI) and a known risk factor for cervical cancer [2, 3]. Approximately 5.5 million people develop genital infections each year in the USA [4]. Although most infections are transient, persistent HPV infections can lead to anogenital warts, cervical intraepithelial neoplasia (CIN), and cervical cancer [2, 5, 6]. The highest rates for HPV occur in women between the ages of 18 and 28 [4]. It is estimated that 24% of 15-year-old girls, 38% of 16-year-old girls and 62% of 18-year-old women have had sexual intercourse in the USA [7]. These figures are nearly the same in Turkey according to recent public statistics giving the result that the age at first intercourse is 17.5 for men and 18.5 for women [8]. Persistent HPV infection and preinvasive cervical diseases are more frequent in girls who have their first sexual intercourse at early ages because of cervical im-maturity [9]. Currently we know that up to 80% of patients with intraepithelial neoplasia and almost all cases of invasive squamous cell cervical cancer are associated with HPV infections [10-13].

HPV prophylactic vaccine became available in 2006 with the main aim of vaccination of preadolescent girls before the first sexual intercourse which will result ideally with an immunized female population against HPV high-risk types. Efficacy and prevention of vaccines have been very good and acceptable in clinical trials which report nearly 100% [14]. The vaccine type in public usage now is providing immunity against four HPV types. The two most prevalent anogenital condyoma causing types (HPV Type 6 and 11) and the two most prevalent CIN and cervical cancer causing types (HPV Type 16 and 18) [1].

There is no doubt that universal immunization of HPV-negative women and men would reduce the incidence of cervical cancer worldwide [15]. In Turkey, Gardasil (Merck Sharp Dohme) has just gone on the market. We still have no mass cervical cancer screening program used nationwide but we have a very effective national vaccination program for childhood diseases. Currently in Turkey, about 1,400 cases of cervical cancer are diagnosed each year, and the prevalence of HPV infection is estimated to be lower than Europe or the USA [16]. Whether or not the vaccine should be compulsory has caused a polarizing debate on cost-effectiveness, potential social barriers and religious conservatism. Previously, many authors reported different aspects of vaccine acceptance and different knowledge levels about HPV, HPV-related diseases, vaccine and cancer politics in different countries [17-22].
The primary objective of this study was to evaluate the knowledge status and potential acceptance of HPV vaccination by women in different geographical areas of Turkey.

Methods

A survey was distributed to women admitted to outpatient gynecology clinics in three different cities before HPV vaccine became available in Turkey commercially. The cities were selected to represent different socio-economic and geographic locations in Turkey (Istanbul, Erzurum and Kahramanmaras). The main characteristic of the end-responders was aimed at women who had social security and did not have any HPV-related disease. Those who were unable to read or write were excluded.

The survey comprised 25 questions (Table 1). It was adapted from Solomovitz et al. with modifications [17]. Personal information of subjects was not collected, and the survey did not include any personal question that would make a woman's identity accessible. All participants received an educational component consisting of current knowledge about cervical cancer and HPV vaccine (Table 2) and then they responded to the questions (Table 1). Replies to the questionnaire were collected in sealed envelopes.

Descriptive statistics were used to evaluate patient responses. A logistic regression analysis was performed to predict women's willingness to accept the vaccine for themselves and their children. Statistical analyses were performed with the SPSS 10.0 statistical package (SPSS Inc, Chicago, IL).

Results

At the closure of the study 143 women had responded and returned the survey. Table 3 gives the demographic information of the participants.

Table 3. — Demographic information (n: 143 participants).

<table>
<thead>
<tr>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
</tr>
<tr>
<td>17-27</td>
</tr>
<tr>
<td>28-35</td>
</tr>
<tr>
<td>&gt; 35</td>
</tr>
<tr>
<td>Place of residence</td>
</tr>
<tr>
<td>Istanbul</td>
</tr>
<tr>
<td>Erzurum</td>
</tr>
<tr>
<td>Kahramanmaras</td>
</tr>
<tr>
<td>Household income</td>
</tr>
<tr>
<td>&lt; 1000 USD</td>
</tr>
<tr>
<td>1000-1500 USD</td>
</tr>
<tr>
<td>&gt; 1500 USD</td>
</tr>
<tr>
<td>Education</td>
</tr>
<tr>
<td>University</td>
</tr>
<tr>
<td>High School</td>
</tr>
<tr>
<td>Lower education</td>
</tr>
<tr>
<td>Marital status</td>
</tr>
<tr>
<td>Single</td>
</tr>
<tr>
<td>Married</td>
</tr>
<tr>
<td>No. of children</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>≥ 1</td>
</tr>
</tbody>
</table>

Descriptive statistics were used to evaluate patient responses. A logistic regression analysis was performed to predict women's willingness to accept the vaccine for themselves and their children. Statistical analyses were performed with the SPSS 10.0 statistical package (SPSS Inc, Chicago, IL).

Table 2. — Educational paragraph for the participants.

Human papillomavirus is a type of virus that causes cervical cancer. Recent progress against this virus brought about development of a vaccine. Anyone receiving this vaccine will be immune against the virus and will be protected from related diseases. This vaccination is advised before the beginning of sexual activity. Because of this fact, the vaccine should be given beginning from the age of 9. There are many women experiencing cervical cancer and precancer diseases in our country. Turkish women may be protected against these diseases and cancer by the usage of HPV vaccine. Vaccine may have minor side-effects like allergic reactions, pain and fever at the site of infection. All of these side-effects go away without a need for treatment.
would have themselves vaccinated if it were possible for prevention from cancer. Only 6% of the women (9) did not need such protection. Ninety-six percent of women (n = 38) would consent to have their daughter vaccinated for HPV and 92% (n = 131) would consent to have their son vaccinated if vaccine would provide prevention against cancer and related diseases.

Ninety-eight percent of mothers (n = 54) have had their children fully vaccinated through the national vaccine program of Turkey. Ten percent of their children (n = 6) experienced some side-effects from these vaccinations.

Sixty-four percent of the women (n = 91) believed that sexual education should be given by mothers, 5% (n = 7) by fathers, 8% (n = 11) by grand-parents and 24% (n = 34) thought this sexual education should be given by the school.

Fifty-six percent of the women (n = 80) believed that prohibition of premarital sexual intercourse is not a way to prevent sexually transmitted disease. Fifty-one percent of the women (n = 73) believed that their children should not have sexual relations before marriage, 13% (n = 19) said that it might happen but the child should not talk about it with the parents, and 36% (n = 51) thought that it might happen, and that the child should speak about it with the mother.

Forty-five percent of women (n = 65) believed that men in Turkey have their first sexual intercourse with paid sex, 26% (37) believed it happens when married, and 29% (41) believed that it is with girlfriends. Cumulatively 74% of men are believed to have their first sexual intercourse before marriage.

On logistic regression analyses none of the variables (i.e., questions) in the survey predicted women’s willingness to accept the vaccine for themselves and their children.

Discussion

In the current study, an overwhelming ratio of women would accept a vaccine against cancer. Similarly, more than 90% of the women would accept HPV vaccination for themselves and for their sons and daughters. Nearly all women in the survey reported that their children had received all of the childhood vaccinations. Generally, study participants believed that their children should not have sexual intercourse before marriage or did not want to be aware of this issue themselves. However, they pointed out that prohibition of sexual intercourse is not an effective way to impede engagement in sexual relations nor to prevent sexually transmitted disease. Similarly, most of the women believed that men in Turkey begin sexual activity before marriage. Many of the responders did not accept the idea of sexual education in schools. In light of these ideas most of the women in the survey seemed to have a conservative attitude about the sexual practices of their children but this did not affect their decision about HPV vaccination of their children. The final decision of the women about HPV vaccination seemed to be unaffected by their social or cultural attitudes.

HPV vaccine may be categorized as having been developed against a STD which might cause conservative groups and some non-governmental organizations to reject the vaccine with the claim of promoting safer sexual practices for adolescents. Parents also may feel that consenting to a vaccine for an STD may inadvertently encourage their adolescent children to engage in sexual intercourse. Current knowledge and surveys show that this can be surpassed by adequate and effective education [18, 23-26].

Successful and effective application and usage of a vaccine depends on public knowledge and acceptance in every country or culture. Public acceptance and usage of a prophylactic vaccine is related to the degree of knowledge about the disease or illness from which vaccine will protect from. This knowledge should normally be provided by healthcare professionals. High-risk HPV types (oncogenic types) have been shown to cause preinvasive cervical diseases (cervical intraepithelial neoplasia) and cervical cancer. A vaccine that protects women against these viral infections and cervical cancer is a totally new development that many medical professionals are still not aware of.

Education about HPV prophylactic vaccines, cervical cancer and related topics is needed in every country where vaccine is available commercially. Prophylactic vaccination against any disease – infectious or malignant – may only be possible if the target population has enough knowledge about causes, findings, diagnosis and treatment of disease.

Incidence of cervical preinvasive diseases and cervical cancer is strictly known in developed countries like European and North American societies, where they can make future projections about disease and treatment. This affords a good opportunity to evaluate the cost-effectiveness and public health usefulness.

Many similar studies about HPV prophylactic vaccines in different countries and cultures resulted with higher rates of unacceptance, although sexual relationships before marriage are considered socially normal [22]. There are studies reporting 55% refusal rates at the beginning but this number decreases to 35% after education of the parents [27]; refusal rate was only 1.5% in our survey.
One of the main rejection causes of vaccination in other countries is the belief that children will feel more secure about having sexual relationships since they would be protected by vaccine [27]. Whether Turkish women who replied to this questionnaire believe that the vaccine causes this secure feeling or not, they do believe their daughters should be protected from such a virus-causing cancer.

In contrast to other studies [17, 27-29], the parents did not point out possible “side effects” as a cause for rejecting vaccination. They trust the vaccine in light of previous and current childhood vaccination program experiences.

As a result we want to state that Turkish women accept the idea of HPV vaccination to protect their children from cervical cancer. This acceptance rate will increase with optimal and detailed public education. The cost of the vaccine is still a problem for families and it seems nearly impossible to add HPV vaccine to the national childhood vaccination program, at least in the near future.

References


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Relationship between risk factors and tumor stage in breast cancer patients in a university hospital - Brazil

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¹Department of Biological Sciences, ²Research Institute of Oncology (IPON)/Discipline of Gynecology and Obstetrics, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais (Brazil)

Summary

Purpose: To verify the relationship between clinical variables and tumor stage in breast cancer. Methods: This retrospective study (1998 to 2001) analyzed data of 176 women with breast cancer attending a university hospital. Patients were divided into groups according to the clinicopathological variables studied. Results: The disease had a similar frequency at age under 50 years (44.3%) or above (55.7%) 50 years. Stage II was more frequent. Most patients were white (69.9%), non-smokers (69.3%) and were not using oral contraceptives (71%). Stages 0-II were mainly detected in the white (74.8%) vs non-white (60.4%) group. Monthly breast self-exams were performed by 62.5% of women, in which earlier stages (0, I) were more frequently detected than in those who did not perform self-exams (27.3% vs 12.1%, p = 0.01). Conclusion: Breast cancer occurred mainly in white women in Stage II, and with similar frequency at age under or over 50 years. Breast self-exam was associated with early detection of the disease.

Key words: Breast cancer; Tumor stage; Risk factors; Breast self-exam.

Introduction

Breast cancer represents the first cause of death by cancer among women and is considered an important public health problem worldwide [1]. The interaction of genetic factors with lifestyle, reproductive habits, and environment are involved in the development of the disease [2]. Epidemiological studies indicate that environmental factors account for 80% cases of breast cancer, while genetic factors correspond to 5%, increasing to 25% when disease is detected in women under 35 years of age [3, 4]. Age exerts a strong influence on predisposition to breast cancer. It is more frequent in women from 45 to 65 years old, while at ages under 35 years epidemiological data are controversial [5, 6].

One important problem in breast cancer diagnosis is the failure to detect tumor at initial stages [7]. In Brazil, it was reported that 60% cases are detected at advanced stages, having as consequence increased relapses, risk of metastasis and reduced survival [8]. Breast self-examination is considered an important strategy for detection of breast tumors at earlier stages [9], although this procedure was not found to alter the mortality rate [10].

The study was carried out in a university hospital with the aim of examining the relationship between tumor stage and diverse variables considered as risk factors, such as age, race, oral contraceptive use, smoking, and performing breast self-exams in women with breast cancer.

Patients and Methods

A retrospective study was conducted with data collected from the medical records of 176 breast cancer patients attending the mastology outpatient service of a public tertiary reference university hospital in Brazil for patients with a low socioeconomic level. All patients with anatomopathologic diagnoses of breast cancer from 1998 to 2001 were enrolled.

Patients were further analyzed in groups according to the following characteristics upon diagnosis: age (< 40, 40-50, > 50), race (white, non-white), smoking status (yes, no), oral contraceptive use (yes, no), monthly breast self-examination (yes, no) and tumor stage (0, I, II, III, IV, according to Beahrs [11]).

Statistical analysis was performed by GraphPad InStat software. Data were compared by the chi-square and Fisher exact test and significance level was established at p < 0.05.

Results

Patients enrolled (n = 176) had a mean age (± SD) of 53.7 ± 11.9 years (range 23-80 years). The diagnosis of breast cancer was more frequent in white women, non-smokers and in those who were not using oral contraceptive at diagnosis (Table 1). Stage II was the most frequently detected, comprising around half the patients (Table 1).

General and clinical features of the patients were determined on the basis of tumor stage and classified in three groups: 0/I, II and III/IV to ascertain any possible relationship between these variables (Table 2). Considering the age intervals, Stage II was the most frequently found in all groups but without any statistical differences (Table 2). Also, no differences were detected when considering age (± SD) at diagnosis for white (53.2 ± 12.7) and non-white (54.9 ± 9.9) women. Stage II was also the most frequent in both white and non-white women (Table 2). Nevertheless, if considering Stages 0, I and II altogether a higher incidence was detected in white women (74.8%) compared to non-white (60.4%) (p < 0.05, Fisher’s exact test). Considering smoking and oral contraceptive use, again Stage II was mainly detected, without statistical differences among groups.

Among the enrolled women, the majority (62.5%) performed monthly breast self-exams (Table 1). Breast
cancer was mainly detected at Stage II in both groups but advanced stages (III, IV) were also found with fairly high frequency (Table 2). However, patients performing the breast self-exam had earlier detected stages (0, I); 27.3% more frequently detected than women who did not (12.1%, p = 0.01), (Table 2). Nevertheless, the complaint of a “tumor finding” was reported with similar frequency by patients who did or did not perform (60.9% vs 69.7%, respectively) breast self-exams.

Discussion

In our population study most cases of breast cancer occurred in white women, as reported in the United States, Europe and Korea [12]. However, the mortality rate was found to be higher among black women in the United States, independent of age and mainly due to diagnosis at advanced stages [13]. It is possible that differences in response to treatment or access to newer medical interventions might largely account for these trends [14]. Although not reaching statistical significance, our study showed that Stages III/IV were more frequent in non-white compared to white women (Table 2).

Epidemiological studies have demonstrated that breast cancer is increasingly affecting younger women. In 1990, up to 50% cases of breast cancer in the United States comprised women over 65 years [14], while in 1997 the disease had an increased incidence and mortality in women under 55 years [15]. Our results showed that 44% of breast cancer cases comprised women < 50 years and, from these, 11% of cases were < 40 years (Table 1). These findings suggest that mammography should be indicated as a routine exam for women under 40 years of age. In Lebanon, 49% of breast cancer cases were detected in patients < 50 years, with 4.7% being cases < 30 years of age [16]. In Nigeria, patients affected had a mean age of 42.7 years and in 40% of cases of infiltrating disease women were < 40 years old [17]. Age under 40 at diagnosis could be considered an independent poor prognostic factor since the 10-year survival for breast cancer was found to be significantly lower compared to middle-aged women (40-69 years) [18]. Although the influence of cultural environmental and socio-economic factors in each population could not be ruled out, the hypothesis that breast cancer occurs more frequently in industrialized countries or urbanized regions in response to lifestyle should be reevaluated.

Oral contraceptive use was reported to be associated with a small increase in breast cancer risk [19]. Norwegian-Swedish women under oral contraceptive use for a long time were at greater risk for the development of breast cancer than those that had never used such drugs [20]. Most cases from our study were not using oral contraceptives when diagnosed, in line with studies that did not find oral contraceptive use as a risk factor for breast cancer [21]. A large prospective cohort study enrolling women with a familial history of breast cancer found that long duration of oral contraceptives was inversely associated with breast cancer risk [22]. In addition, the use of low-dose oral contraceptives did not increase the risk of early-onset breast cancer for mutation carriers [23].

An overview of studies evaluating the relationship between smoking and breast cancer has shown a neutral effect on the incidence of the disease [24]. However considering prognosis, a cohort study showed that smokers had a higher mortality from breast cancer [25]. In our study, most patients were non-smokers at time of diagnoses, but some could have been ex-smokers since our data did not differentiate between these features. It was already reported that women who start smoking as teenagers and continue to smoke for at least 20 years may increase their breast cancer risk [26].

Mortality rates for breast cancer are high throughout the world, probably because of diagnoses at advanced stages [27]. Our results demonstrated that breast cancer

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**Table 1. — Clinicopathological characteristics of 176 women with a first diagnosis of breast cancer in a university outpatient service.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td></td>
</tr>
<tr>
<td>&lt; 40 (n = 20)</td>
<td>20 (11.3)</td>
</tr>
<tr>
<td>40-50 (n = 58)</td>
<td>58 (33.0)</td>
</tr>
<tr>
<td>&gt; 50 (n = 98)</td>
<td>98 (55.7)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>123 (69.9)</td>
</tr>
<tr>
<td>Non-white</td>
<td>53 (30.1)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>54 (30.7)</td>
</tr>
<tr>
<td>no</td>
<td>122 (69.3)</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>51 (29.0)</td>
</tr>
<tr>
<td>no</td>
<td>125 (71.0)</td>
</tr>
<tr>
<td>Breast self-exam</td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>110 (62.5)</td>
</tr>
<tr>
<td>no</td>
<td>66 (37.5)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
</tr>
<tr>
<td>0/1</td>
<td>38 (21.6)</td>
</tr>
<tr>
<td>II</td>
<td>86 (48.9)</td>
</tr>
<tr>
<td>III/IV</td>
<td>52 (29.5)</td>
</tr>
</tbody>
</table>

**Table 2. — Distribution of general and clinical features of 176 breast cancer patients according to tumor stage.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>0/I (n = 38)</th>
<th>II (n = 86)</th>
<th>III/IV (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 40 (n = 20)</td>
<td>4 (10.5)</td>
<td>12 (14.3)</td>
<td>4 (7.7)</td>
</tr>
<tr>
<td>40-50 (n = 58)</td>
<td>12 (31.6)</td>
<td>31 (36.5)</td>
<td>15 (28.8)</td>
</tr>
<tr>
<td>&gt; 50 (n = 98)</td>
<td>22 (57.9)</td>
<td>43 (50.0)</td>
<td>33 (63.5)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (n = 123)</td>
<td>30 (78.9)</td>
<td>62 (72.1)</td>
<td>31 (59.6)</td>
</tr>
<tr>
<td>Non-white (n = 53)</td>
<td>21 (11.1)</td>
<td>24 (27.9)</td>
<td>21 (40.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 54)</td>
<td>11 (28.9)</td>
<td>31 (36.5)</td>
<td>12 (23.1)</td>
</tr>
<tr>
<td>No (n = 122)</td>
<td>27 (71.1)</td>
<td>55 (64.5)</td>
<td>40 (76.9)</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 51)</td>
<td>9 (23.7)</td>
<td>24 (27.9)</td>
<td>18 (34.6)</td>
</tr>
<tr>
<td>No (n = 125)</td>
<td>27 (76.3)</td>
<td>62 (72.1)</td>
<td>34 (65.4)</td>
</tr>
<tr>
<td>Breast self-exam</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n = 110)</td>
<td>30* (78.9)</td>
<td>48 (55.8)</td>
<td>32 (61.5)</td>
</tr>
<tr>
<td>No (n = 66)</td>
<td>8 (21.1)</td>
<td>38 (44.2)</td>
<td>20 (38.5)</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with patients at Stage 0/I who did not perform breast self-exams (Fisher exact test). Percentages in parenthesis refer to tumor stage.
affected women under and over 50 years old with similar frequency, without any significant differences in tumor stage between these groups. Although Stage II accounted for around half the cases in all variables studied, at least one-fifth of cases were diagnosed at Stages III/IV. If we consider that women over 40 years of age should be routinely submitted to mammography, a relatively high number of cases detected at advanced stages would suggest that public health programs have failed to recruit women for detection of early alterations suggestive of breast cancer and annual exams should be considered.

In Brazil, the breast self-exam represents an important national strategy in the detection of breast cancer but it is uncertain as to whether breast self-exam has any effectiveness at all in reducing breast cancer morbidity and mortality. It was reported that even a high level of compliance with breast self-exams may not lead to any appreciable degree of early detection of cancer and, therefore, no appreciable degree in the reduction of mortality. In our study, earlier stages were significantly more frequent in patients who performed breast self-exams. Nevertheless, independent of performing breast self-exams, a “tumor finding” was the main complaint at the consultation reported by the majority of patients. It is possible that the observed association between breast self-exam compliance and breast cancer diagnosis at earlier stages better reflects that women from this group are more worried about disease and, as consequence, have undergone regular clinical breast exams and mammography.

Conclusions

Breast cancer was mainly diagnosed in white women, at Stage II, and with similar frequency at ages under or over 50 years old. A relationship between age and tumor stage was not observed but an association was found over 50 years old. A relationship between age and tumor stage between these groups. Although Stage II accounted for around half the cases in all variables studied, at least one-fifth of cases were diagnosed at Stages III/IV. If we consider that women over 40 years of age should be routinely submitted to mammography, a relatively high number of cases detected at advanced stages would suggest that public health programs have failed to recruit women for detection of early alterations suggestive of breast cancer and annual exams should be considered. In Brazil, the breast self-exam represents an important national strategy in the detection of breast cancer but it is uncertain as to whether breast self-exam has any effectiveness at all in reducing breast cancer morbidity and mortality. It was reported that even a high level of compliance with breast self-exams may not lead to any appreciable degree of early detection of cancer and, therefore, no appreciable degree in the reduction of mortality. In our study, earlier stages were significantly more frequent in patients who performed breast self-exams. Nevertheless, independent of performing breast self-exams, a “tumor finding” was the main complaint at the consultation reported by the majority of patients. It is possible that the observed association between breast self-exam compliance and breast cancer diagnosis at earlier stages better reflects that women from this group are more worried about disease and, as consequence, have undergone regular clinical breast exams and mammography.

Acknowledgements

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Cold-knife conization versus the loop electrosurgical excision procedure for treatment of cervical dysplasia

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Institute of Gynecology and Obstetrics, Clinical Centre of Serbia (Serbia)

Summary

Introduction: Classical conization is a standard procedure for treatment of cervical lesions. Conization with loop diathermy is well established and lesions can be excised in more than 90% of cases. Objective: To compare two methods of conization for the treatment of cervical dysplasia. Method: The study included 172 patients who had conization for diagnosed cervical dysplasia. A retrospective analysis was conducted on incidence of complications and presence of dysplasia on the specimen edges after classical conization compared to conization with loop diathermy. The possibilities for analyzing specimen edges were reviewed. Results: A significantly higher incidence of complications was found among patients who underwent classical conization compared to those who had the loop diathermy procedure. The loop procedure is sufficient for treatment of cervical dysplasias. Conclusion: The authors suggest loop diathermy conization as the method of choice for treatment of cervical dysplasia.

Key words: Conization; Complications; Resection edges; Leep; Cold-knife.

Introduction

Cold-knife conization has been the standard procedure for treatment of cervical dysplasia (CIN). Significant changes have been made with the introduction of the loop electrosurgical excision procedure (LEEP) for treatment of cervical dysplasia since it is easily performed and the efficacy rate for complete excision of a lesion is 90% [1, 2]. Randomized studies have suggested that conization with LEEP is followed by fewer complications than cold-knife conization [3, 4].

There have been several reports regarding detection of residual disease on resection edges after a loop electrosurgical procedure and cold-knife conization as well. Results differed from different histopathology departments worldwide. The success in respect to identifying residual disease on resection edges depended mostly on whether an experienced pathologist performed the analysis. Detection of residual disease on resection edges is often difficult after a LEEP and an experienced and well-trained pathologist is needed. Girardi et al. conducted a study on the possibilities of detection of residual disease on resection edges and concluded that there was no difference in interpretation of resection edges after conization with the LEEP compared to cold-knife conization [6]. Methivet and colleagues performed a study on the evaluation of resection edges and concluded that it was not sufficient in 31% of tissue specimens after a loop electrosurgical procedure [5]. If there is residual disease present on resection edges, repeat conization should be performed only if there is atypia on the cytology reports on follow-up examinations [5].

In this study we compared the incidence of complications after cold-knife conizations versus conizations with the LEEP. In addition we evaluated the histopathology reports to see if there was any difference in analyzing resection edges after the procedures.

Material and Method

A retrospective analysis of the histopathology and clinical reports of patients who underwent conization at the Institute of Gynecology and Obstetrics in Belgrade was carried out over two years. Patients included in the study were operated on for cervical dysplasia (CIN). Patients who had repeat conization for cervical dysplasia were excluded from the study. Data were obtained from medical reports and histopathology reports with detailed examination of cone specimen and resection edges. We compared the incidence of complications after cold-knife conizations (Stumdorf stitch) versus conizations with the LEEP. Incidence of intraoperative and postoperative complications was analyzed as well as presence of residual disease on resection edges after both the standard cold-knife conization and loop electrosurgical procedures.

Cold-knife conizations were performed as excision of the cone to a depth of 10 mm and application of Stumdorf stitches. Conizations with the LEEP were also performed to a depth of 10 mm with different diameters of loops (30 to 40 W), using ball electrodes for coagulation.

Classical conization was performed on 100 patients; 72 patients underwent the loop electrosurgical procedure. Histopathology reports were evaluated and data analyzed with the chi-square.

Results

All patients who were diagnosed with cervical dysplasia were surgically treated by conization of the cervix. Indications (degree of dysplasia) for each technique of conization are shown in Tables 1 and 2.
All degrees of dysplasia were included in the indications for both types of conization. Cold-knife conization was more often the method of choice for high-degree dysplasia while the LEEP was used more often for low-degree dysplasia ($\chi^2 = 16.60; p = 0.001$).

Several complications were noted after cold-knife conizations including bleeding, infections and stenosis of the cervical canal (Table 3).

After conization with the loop electrosurgical procedure 2.8% of patients experienced light bleeding. More complications were noted after cold-knife conizations compared to the LEEP but no statistical significance was found ($\chi^2 = 1.768; p = 0.184$).

Evaluation of resection edges from histology reports was completed for all patients and revealed differences in cold-knife conizations compared to loop electrosurgical procedures. In 8% of patients residual disease was found ($\chi^2 = 16.60; p = 0.001$). Several authors have reported that in more than 30% of cones samples that could have been analyzed after both types of conization (Table 4).

Discussion

Our experience has shown that both classical conization and the LEEP have been good choices for successful treatment of cervical dysplasia. At the Institute for Gynecology and Obstetrics in Belgrade standard cold-knife conization is still the preferred method of choice for treatment of severe cervical dysplasia, although the loop electrosurgical procedure is likely to be used for low-grade cervical dysplasia. This might be an explanation as to why resection edges after the LEEP have often been clear of residual disease. The complication rate in our experience is consistent with the data from the literature and it is significantly higher after cold-knife conization compared to the LEEP. Bleeding in patients after standard cold-knife conization was managed with application of surgical knots. There was no heavy bleeding after LEEP. Infections and cervical canal stenosis occurred after classical conizations, though no such problems were observed after loop procedures. Several studies have shown that incidence of cervical canal stenosis was dependent on depth of excised cervical tissue, and it was more often observed when cervical tissue was cut more than 20 mm in depth [2]. In our experience the excision depth of more than 20 mm only occurred in cones cut with cold knife, and that might be an explanation as to why stenosis was found after standard conizations and not after loop procedures. Randomized studies have confirmed that there are fewer complications after loop procedures compared to cold-knife conizations [4]. Several papers have reported that in more than 30% of cones obtained after loop electrosurgical procedures, resection edges could not be analyzed due to the presence of residual disease. Our experience has shown that the resection edges could not be interpreted in 4.1% of cones. Similar results have been found in different studies. Giacalone et al. stated that the resection edges could not be evaluated in 7% of cones after loop procedures [1]. In our experience 2.3% of cones could not be analyzed with respect to the resection edges after the LEEP. Several authors have suggested that the experience of the surgeon plays an important role in decreasing the damage of the cone specimen with the loop procedure. An experienced pathologist is important as well so that resection edges can be properly evaluated [2, 5, 8].
Cold-knife conization versus the loop electrosurgical excision procedure for treatment of cervical dysplasia

In our study total excision of the cervical dysplasia was obtained in 92% of patients treated with standard cold-knife conization compared to 82% who had conization with the loop procedure. There is no statistical difference in the efficacy of cold-knife conization versus loop procedures for treatment of cervical dysplasias.

Several authors have come out with similar results: total excision of cervical dysplasia in 90% of patients treated with standard cold-knife conization versus 80% of lesions totally excised with the loop electrosurgical excision procedure [5, 6]. They also noted that recurrences were not any more frequent after the loop procedure versus standard conization.

Conclusion

Conization with the loop electrosurgical excision procedure is efficient method for therapy of cervical dysplasia. Standard cold-knife conization is also the method of choice for successful treatment of cervical dysplasia, however more complications occur after cold-knife conization versus the loop procedure.

Conization with the loop electrosurgical procedure is easier to perform than standard cold-knife conization, the complication rate is lower, and it is as successful as standard conization in treating cervical dysplasias. Conization with the loop diathermy is well established and widely accepted. It also costs less and lesions can be totally excised in more than 90% of patients.

Conization with the loop procedure should be recommended for patients with cervical dysplasias.

References


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Recurrence of granulosa cell tumor 25 years after initial diagnosis. Report of a case and review of the literature

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12nd Department of Obstetrics and Gynecology, 2Department of Pathology, University of Athens, School of Medicine, Aretaieion Hospital, Athens (Greece)

Summary

Granulosa cell tumors (GCTs) are rare functional sex cord-stromal ovarian tumors constituting approximately 2-3% of all ovarian malignancies. They are characterized by low malignant potential, local spread, late recurrence and high survival rates. We report a case of recurrent ovarian GCT in a 60-year-old woman 25 years after the initial diagnosis. The patient underwent surgical resection of the pelvic masses and refused to receive any adjuvant treatment, considering the late recurrence and high survival rates of this tumor. This case illustrates an example of a very late recurrence and emphasizes the importance of the extended follow-up required for these patients.

Key words: Adult granulosa cell tumor; Recurrence.

Introduction

Granulosa cell tumors (GCTs) are rare functional sex cord-stromal ovarian tumors constituting approximately 2-3% of all ovarian malignancies. Two distinct types exist, known respectively as adult and juvenile. The adult granulosa cell tumors (AGCTs) account for 95% of all granulosa cell tumors [1]. They are characterized by low malignant potential, local spread, late recurrence and high survival rates [2]. Recurrences as late as 20 or more years after the initial diagnosis have been reported [3, 4]. We report a case of recurrent ovarian GCT 25 years after the initial diagnosis together with a review the literature.

Case Report

A 60-year-old, para 2 woman presented with abdominal and pelvic discomfort. Physical and pelvic examination revealed a right lower quadrant mass. Her past medical history was significant for a total abdominal hysterectomy and bilateral salpingo-oophorectomy in 1982 for a solid left ovarian mass. Pathologic evaluation revealed a GCT of the ovary and no significant abnormality of the uterus, tubes or contralateral ovary. No additional therapy was prescribed.

Transvaginal pelvic sonography (TVS) confirmed a tumor in the anatomical location of the right ovary measuring 52 x 40 mm in diameter, with both solid and cystic components (Figure 1). Doppler examination showed a normal vascular pattern. Magnetic resonance imaging (MRI) of the abdomen revealed a right-sided solid and cystic pelvic mass, 45 mm in diameter, adherent to the right rectus abdominal muscle. Another complex cystic lesion 25 mm in diameter was also detected below the previous one in the lower pelvic wall. Laboratory work-up revealed normal levels of CA-125 (15.35 U/ml), CEA (1.7 ng/ml), inhibin-B (8.9 pg/ml) and 17β-estradiol (30.6 pg/ml).

At laparotomy, a 4 x 5 cm mass, partially solid and cystic, adherent to the right rectus abdominal muscle and above the pubic symphysis was found. Another solid mass 3 cm in diameter was found in the lower pelvic wall, adhering to the rectum. The liver, hemidiaphragms, intestines, abdomen and pelvis were all normal, with the exception of dense pelvic adhesions. Peritoneal lavage for cytology, extensive adhesiolysis and resection of the pelvic masses were performed.

The specimen that was attached to the rectus abdominal muscle consisted of fibrofatty tissue with an attached fragment of skeletal muscle and measured 10 x 6 x 3.5 cm. On sectioning, a cyst measuring 4 cm in diameter was identified. Its lining consisted of hemorrhagic friable tissue, which microscopically was fibrin and focally a recurrent granulosa cell tumor (Figure 2). Focal involvement of the striated muscle by granulosa cells was also identified (Figure 3). The specimen that was excised from the lower abdominal wall was well circumscribed and measured 3.5 x 2.5 x 2 cm. The cut surface was solid and focally cystic. Microscopically it was a recurrent granulosa cell tumor growing in a diffuse and trabecular pattern with rare Call-Exner bodies. The neoplastic cells had low mitotic activity (< 1 mitotic figure/10 HPF) and were positive for inhibin and vimentin by immunohistochemistry. Cytologic examination of the peritoneal washings was negative.

Postoperative recovery was uncomplicated. Adjuvant chemotherapy and radiotherapy were recommended. However, despite the consultation from her doctors, the patient refused to follow any adjuvant treatment, keeping in mind the late recurrence and high survival rates of this tumor. The patient has been followed-up regularly for one year without any signs of recurrence.

Discussion

GCTs of the ovary are hormonally active tumors which account for a small percentage of ovarian malignancies. They occur more often in postmenopausal women, with a
Recurrence of granulosa cell tumor 25 years after initial diagnosis. Report of a case and review of the literature

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manifestations due to hormone secretion by the tumor, leading to early diagnosis. The majority of these neoplasms are estrogenic [6]. Rarely, are they androgenic and cause virilization [7]. The consequences of estrogen production depend on the age of the patient. The most common symptom in postmenopausal women is vaginal bleeding. Due to prolonged exposure to tumor-derived estrogen, about 50-60% of patients develop endometrial hyperplasia and 5-10% have concomitant uterine cancer [2]. Occasionally, swelling and tenderness of the breast are prominent symptoms. In contrast, premenopausal women may develop irregular menses or less often, amenorrhea. A juvenile form of GCT in prepubertal girls is associated with isosexual precocious puberty. Elevated levels of estrogens and inhibin have been reported and vaginal cytologic smears typically show increased maturation of squamous epithelial cells [1].

Stage of disease at presentation is the most important prognostic factor. About 85-90% of patients present with Stage I disease, which is associated with a greater than 90% 5-year survival rate. Bilateral tumors are uncommon, accounting for 2-8% of cases. Poor prognostic factors include tumor size greater than 10-15 cm, high mitotic index, tumor rupture and lymphatic invasion [2].

Treatment for GCTs is primarily surgical. The extent of initial surgery is somewhat controversial. During the reproductive years, conservative surgery with unilateral salpingo-oophorectomy with careful staging and endometrial biopsy (to exclude a concomitant uterine cancer) appears to be adequate for Stage I disease. Radical surgery with total abdominal hysterectomy, bilateral salpingo-oophorectomy and complete tumor debulking is preferable for patients with greater than Stage IA disease or if infertility is not an issue. Adjuvant chemotherapy or radiation therapy in the setting of completely resected Stage I tumors does not appear to reduce the risk of recurrence. Advanced metastatic GCTs are typically treated with aggressive surgical resection, followed by postoperative radiation therapy or systemic chemotherapy. Radiation therapy may have some effect in minimal residual disease [6].

It is well established that GCTs of the ovary have a tendency to recur late. The median time to relapse is four to six years after initial diagnosis [2], although disease has recurred as late as 37 years [3]. Due to the long interval to relapse, assessing the impact of any adjuvant treatment on overall survival is difficult. The total recurrence risk for these tumors is difficult to assess, since it may be obscured by deaths from other diseases. Given this, any abdominal, retroperitoneal or pelvic mass in these patients should be considered recurrent GCT until proven otherwise, regardless of the time of initial diagnosis. There is no standard approach to the management of relapsed disease. Repeat surgical resection for optimal cytoreduction is a reasonable option given the tumor’s indolent growth and late recurrence. Radiation treatment in the metastatic or recurrent setting may lead to prolonged disease-free survival [8]. Chemotherapy may be an option for patients with large residual disease, metastases and

peak incidence between 50 and 55 years of age. Patients with GCTs often present with nonspecific symptoms of abdominal pain or distension due to large tumor size [5]. Also, about 10% of patients present with acute abdominal pain and hemoperitoneum due to torsion or rupture of the tumor [2]. About two-thirds of patients have endocrine

Figure 1. — Transvaginal ultrasonogram demonstrating a tumor measuring 52 x 40 mm in diameter with both solid and cystic components.

Figure 2. — Low power view of the cystic granulosa cell tumor. Fibrofatty tissue, cyst wall and granulosa cell tumor (arrow) (H&E x 25).

Figure 3. — Granulosa cells involving skeletal muscle (H&E x 160).
inoperable tumors. Cisplatin is the most active agent in GCT of the ovary [9]. Hormonal therapies, such as progestins (medroxyprogesterone acetate), antiestrogens (tamoxifen) and GnRH agonists (leuprolide) have been used for recurrent GCTs and have the advantage of low side-effect profiles [10].

The long natural history of this disease highlights the importance of extended follow-up for patients with GCT. The use of tumor markers for surveillance is controversial. Serum estradiol levels are too insensitive to be a reliable marker of disease activity, particularly in premenopausal women. Serum inhibin, which is a hormone produced by granulosa cells, may be a useful tumor marker. In one prospective study, serum inhibin levels were found to be elevated preoperatively in patients with GCTs and became elevated up to two years before secondary surgery was performed for recurrent disease [11]. Serial inhibin levels may be useful in monitoring patients posttherapy, but should be interpreted with caution since they may be elevated in other conditions as well (during the menstrual cycle, pregnancy) [12].

Our case represents a recurrence of a GCT 25 years after initial diagnosis, which is one of the latest recurrences reported in the literature [3,4]. These recurrences emphasize the necessity for lifetime follow-up. The recurrent tumor was treated only with surgical resection, which remains an acceptable approach given the natural history of the disease.

References


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Ovarian cancer after kidney transplantation

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Summary

Malignancies are one of the major causes of morbidity and mortality in transplant patients. The incidence is progressively increasing either because of the increased age transplant patients and the increase of immunosuppressive therapy or the increased follow-up range post-transplantation. The main causes of increased tumor incidence in transplant patients with respect to the general population are the reduced immunosurveillance and the high incidence of infections due to oncogenic viruses. This problem might become more and more serious in the near future due to the introduction of new immunosuppressive strategies that significantly extend allograft survival. A case of ovarian cancer in a kidney transplant patient is described. Attention is focused on the potential dual role of immunosuppressive therapy in the development of malignancies in transplant patients.

Key words: Ovarian cancer; Kidney transplantation; Immunosuppressive drugs.

Introduction

Over the last ten years marrow and solid organ transplants have grown at an exponential rate in Europe and the United States. Furthermore, the prognosis for patients who have a solid organ transplant has been improving over time, with a four-year survival rate for 85-92% of those with a transplanted kidney and 72% of those with a heart transplant [1]. This increase in transplantation activity has been accompanied by an increased frequency of problems related to immunosuppressive activity, which is necessarily applied to reduce rejection risk. Post-transplant problems basically consist of infections and malignancies.

The frequency of neoplastic disease in patients who had a transplant is on the rise (6%) [2, 3], being four to five times higher than among the broader population [4]. This can be linked both to the longer survival of transplanted patients, which results in greater exposure to immunosuppressive therapy, and to the increasingly older age of receivers.

The link between immunosuppression and malignancies became particularly clear at the onset of the transplantation era, in relation to the use of very aggressive and few selective protocols [5-7]. In addition to immunosuppressive therapy, other risk factors have been identified for carcinoma development such as receiver’s age, smoke, viral infections and male sex [8].

The most frequent tumors are skin cancer (melanoma, spino- and basocellular carcinoma), Kaposi’s sarcoma, lymphoproliferative disorders, anal and genital carcinomas (anus, vulva, penis scrotum), hepatobiliary carcinomas and kidney carcinoma [9].

Case Report

A 43-year-old woman came under our observation at the Department of Obstetrics and Gynecology of the University of L’Aquila, where she had been referred from the Transplant Unit of San Salvatore Hospital after being diagnosed with bilateral ovarian neoformation and ascites.

The patient had a kidney transplant in July 1996 following chronic kidney failure and has since been on immunosuppressive therapy: cyclosporin A (150 mg/day) and prednisone (2.5 mg/day).

Family history revealed a predisposition to hypertension and diabetes mellitus type II. The patient had menarche at age 12 and two natural childbirths. She reported that she has been suffering from hypertension for about 12 years. At the time of the examination she was being treated with valsartan (80 mg/day) and felodipine (10 mg/day).

Objective examination showed a good general condition, but there was abdominal extension due to the presence of ascites. Outcome of the gynecological examination showed outer genitalia typical of a woman who had given birth, a regular vagina, and a well epithelialized uterine neck. Outcome of latest Pap test carried out four months before was normal. The uterine corpus was hard and mobile with increased volume. Adnexa were considerably enlarged with hard and elastic consistency.

The patient underwent hematochemical examinations, tumoral markers, kidney and hepatic functionality assessment, basal and peak cyclosporine treatment, RX thorax, ECG and abdominal CAT scan with and without contrast medium.

Hematochemical examinations showed normal results as well as normal kidney and hepatic functionality.

The patient had high CA125 levels (353.23 IU/ml), CA19-9 was 42.80 IU/ml and CEA within normal range.

CAT scans carried out with and without contrast medium revealed presence in the adnexa of a large bilateral cystic formation measuring about 10 cm in diameter, with significant vascularized endocysts which were thicker on the left side,

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infiltration of fatty tissue adhering to the ovary was noted. The
left tube was free of infiltration. There was well differentiated
serous papillary cystoadenocarcinoma in the right ovary, not
appearing on the ovarian surface. The right tube was free of
neoplastic infiltration. The lymph nodes examined were found
to be free of infiltration. Cytologic analysis of the peritoneal
washing revealed some clusters of adenocarcinomatous cells.
The patient was discharged on the tenth day in good general
condition while still on immunosuppressive therapy.

She has undergone outpatient checks at the Transplant Unit
of San Salvatore Hospital in L’Aquila, which as yet have not
revealed any changes in kidney functionality, nor any second
offense with regard to neoplastic disease.

Discussion

For many years transplants have been a concrete and
effective therapeutic approach for terminal diseases of
many organs such as the kidney, heart, liver and lungs. In
the United States 300,000 new transplants are performed
every year [10]. As for Italy, the latest estimates show that
250,000 organ transplants were carried out between 1992
Survival has also considerably increased for transplanted patients due to improvements in surgical methods as well as, most importantly, to the advancement of immunosuppressive therapy.

It is a fact that immunosuppressive therapy increases the risk for infections and malignancies in patients with transplanted organs compared with the general population due to its very operational mechanism.

Most reports on post-transplantation malignancies have been limited by a small number of relevant cases. Kasikse et al. [12] studied the frequency of tumors in kidney transplants carried out in various centers in the period 1995-2001, examining a total of 35,756 patients. For the most common tumors, such as those affecting the colon, lung, stomach, esophagus, pancreas, ovary and breast, the incidence after kidney transplant was found to be twice as high as among the general population. Melanoma, leukemias, hepatobiliary tumors, tumors of the uterine neck and vulvovaginal tumors all turned out to be five times as common, with kidney cancer being 15 times as common. Testicular and vesical neoplasia were about three times as frequent, with kidney cancer 15 times as frequent. Kaposi’s sarcoma, non-Hogkin lymphomas and skin tumors other than melanoma were 20 times as frequent as among the general population. Hence the incidence of most forms of neoplasia after kidney transplant is found to be higher than among the general population.

Transplant patients as well as other groups of patients with different immunodeficiency conditions (congenital or HIV) are not at high risk of developing the most common forms of malignancies such as breast, prostate or colon carcinoma. On the contrary, the tumors most frequently developing in transplant patients are seldom found in the broader population and are often etiologically associated with viral infections such as lymphomas that are often associated with infections caused by the Epstein-Barr virus (EBV) and Kaposi’s sarcoma, which is invariably associated with infections caused by human herpes virus 8 (HHV-8) [13].

Reduction of immunosuppressive treatment is the first therapeutic option which, while effective in causing a remission of tumor in some cases, almost invariably leads to the organ being rejected. Some types of treatment, including those based on adoptive cell immunotherapy, are either still in the trial stage or have come into use in medical practice only recently.

The role of immunosuppression in tumor development has been documented by the comparison between patients with a transplanted kidney and patients on kidney transplant waiting lists. This analysis has highlighted that transplantation, while reducing the overall death risk for the patient, significantly increases the chance of death from malignancies, especially among older patients [14].

Obviously, intense immunosuppression, needed to curb immune response to the transplanted organ, inevitably limits the body’s ability to eliminate the cells transformed as a result of tumor.

However, many believe that the significant immune response reduction in transplanted patients alone cannot fully explain the increased post-transplant incidence of neoplastic disease. Among the responsible factors a role has been suggested for chronic antigenic stimulation due to external antigens found in transplanted organs and to frequent infections. Indeed, this condition may cause excessive stimulation of the already depressed immune system, thereby further “diverting” it from its surveillance of neoplastic cells [4, 15].

The direct oncogenous potential of immunosuppressive drugs should also be considered. Indeed, it is a well known fact that these drugs can cause direct damage to DNA by strengthening the effects of other carcinogenic factors [4, 15]. Nevertheless, recent studies suggest that not all immunosuppressive drugs lead to the development of tumors in transplanted patients, with some of them having potentially antineoplastic properties [16].

In our particular case we ought to consider the role played by cyclosporine in the onset of ovarian tumors. Its introduction in immunosuppressive transplant therapy has significantly improved the short- and long-term outcome of organ transplants; this result can be ascribed to the drug’s powerful immunosuppressive effect [17]. In contrast, the development of malignant neoplasia with an unusual aggressive phenotype has been linked to immunosuppression by cyclosporin, through the drug’s direct action on the phenotype of neoplastic cells. The latter, under exposure to the effect of cyclosporin, show an increase in proliferation and migration speed [18, 19].

An additional mechanism that may be behind the carcinogenic effect of cyclosporin is thought to result from inhibition by this drug of the molecular mechanisms responsible for DNA repair processes [20].

However, cyclosporin’s pro-neoplastic effect is complemented by the inhibiting action of neoplastic cells on the production of glycoprotein p, a membrane pump which enables cells to eliminate anti-neoplastic drugs [21].

Since tumors are one of the main morbidities and causes of death among transplant patients, it seems advisable to adopt a set of preventive treatment measures in an attempt to reduce tumor incidence within this category of patients: careful anamnestic history and accurate clinical and laboratory analyses, avoidance of heavy immunosuppressive therapies, careful and regular clinical surveillance of transplanted patients, limiting exposure to carcinogenic agents, and prophylaxis of viral infections.

Conclusion

The introduction of immunosuppressive therapies has considerably improved survival after kidney transplantation but, at the same time, it causes profound changes in the immunological surveillance mechanism that plays a vital role in restraining tumor growth [22].

Tumors are indeed a major cause of death and morbidity among patients receiving a kidney transplant, not least because the natural history of neoplastic diseases shows greater aggressiveness in transplanted patients compared with the general population [23].
It would be advisable to use a careful monitoring protocol for patients on the transplant list as well as for transplanted patients – one that allows diagnosis of tumors at an early stage.

Tumor management in transplanted patients requires a multidisciplinary approach, aggressive treatment, and involves modifying immunosuppressive treatment according to the histotype and natural history of the tumor.

The emergence of new types of immunosuppressive drugs offers an opportunity to take advantage of the neoplastic action of some of them, thus ensuring good immunological protection in combination with the oncologic protocol.

References


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Solitary ovarian mass: a case of metastatic malignant melanoma

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Summary
Malignant melanoma involving the ovaries is rare and in most cases metastatic in origin. We present a case of ovarian malignant melanoma presenting as a large adnexal mass in a patient with no previous history of malignant melanoma.

Key words: Malignant melanoma; Ovary; Metastases.

Introduction
Malignant melanoma involving ovaries is rare and in most cases metastatic in origin. In the majority of patients with multi organ spread ovarian involvement is not clinically significant, therefore the diagnosis of ovarian metastases is often made on autopsy [1]. Diagnosis of primary ovarian malignant melanoma is difficult since it is based on exclusion of the extraovarian source which could be an occult or regressed primary site in the skin, choroid plexus, or elsewhere.

We describe a case of ovarian malignant melanoma presenting as a large adnexal mass in a patient with no previous history of malignant melanoma.

Case Report
A 23-year-old woman was referred to our institution because of lower abdominal pain, abdominal swelling and a palpable pelvic mass. She had no significant gynecologic history. Pelvic ultrasound scan revealed a solid mass with anechoic foci measuring 9 cm situated in front of the uterus. The origin of the tumor could not be identified and a scan of the ovaries was inconclusive. Secondary deposits in an enlarged liver and presence of ascites were also visualized. Blood results on admission showed mild iron-deficiency anemia, increased erythrocyte sedimentation rate, normal renal function and abnormal liver function tests. CA125 was mildly elevated. The patient underwent laparotomy nine days following admission. At laparotomy, two liters of ascites were evacuated. A left ovarian tumor measuring 10 x 11 x 5 cm with an intact, smooth capsule and without adhesions to its surroundings was identified. The right ovary and uterus appeared normal. Metastatic spread to the peritoneal surface, omentum and liver was noted during exploration of the abdominal cavity. The pelvic lymph nodes were not enlarged. We performed total abdominal hysterectomy, bilateral salpingo-oophorectomy and infracolic omentectomy.

On sectioning, the left ovarian tumor appeared yellowish brown in the central parts and darker in the periphery. The tumor was predominantly solid with two large cystic formations filled with pink proteinaceous fluid, measuring 3.5 x 5 cm and 3 x 7 cm. On microscopic examination there was no evidence of teratoma. The tumor was almost entirely composed of large epithelioid cells with abundant eosinophilic cytoplasm. Nuclei were large, polymorphic with prominent eosinophilic nucleoli. Numerous atypical mitoses were observed. The following characteristics were also noted: areas of focal necrosis, mild lymphocyte response and absence of lymphovascular invasion. Immunohistochemistry was positive for S-100, HMB-45 and anti-Melan-A staining confirming the diagnosis of malignant melanoma. Similar lesions, forming nevoid nests, were identified in the uterine isthmus, mesosalpinx and omentum. Similar, but rare, changes were also noted in the right ovary.

The postoperative recovery was uneventful. A complete ophthalmologic exam was initiated due to swelling of the right upper eyelid and was normal. The patient was directed to a referral oncology center.

Discussion
Melanoma involving the ovary is a rare and usually lethal disease, predominantly seen in young women of reproductive age. Since melanomas of the female genital tract account for only 3% of all melanomas [2], metastases to the female genital tract often pose diagnostic and therapeutic dilemmas, both for the clinician and the pathologist.

Clinically, although occasionally reported [3], it is uncommon for melanoma to present as an ovarian mass. Since a previous history of melanoma may not be available or is remote, this entity is often overlooked. Metastatic melanoma is unilateral in the majority of cases and this finding could be misleading since most metastatic tumors involve both ovaries. Ultrasound and computed tomography findings are nonspecific but usually with a growth pattern which excludes benign ovarian pathology [4]. If a sufficient amount of melanin pigment is present, magnetic resonance imaging can demonstrate high signal changes on T1-weighted images consistent with melanin deposition [5].
Pathologically, a gross feature that is helpful in making the diagnosis of melanoma is the presence of pigment. In contrast, the various appearance of malignant melanoma on microscopic examination can mimic primary ovarian neoplasms, mostly sex cord stromal tumors [6,7]. In the absence of primary lesions immunohistochemical staining can be conclusive. The most sensitive markers are S-100 and HMB-45 with a sensitivity of 95% and 85%, respectively [6]. Once a diagnosis is made, it is important to make a distinction between primary and secondary lesion. The diagnosis is hardened by the fact that the extraovarian source could be an occult or regressed primary site in the skin, choroid plexus, or elsewhere. Paradoxically, the pathologist evaluating the sample is rarely properly informed about the patient’s previous history.

Finally, the optimal management of ovarian melanoma has been a subject of debate and has not yet been established [8]. Procedures currently used include cytoreductive surgery with or without adjuvant chemotherapy or radiotherapy. Also, various immunotherapy modalities have been proposed. So far, the value of such adjuvant treatments is not clear.

Conclusion

The case presented had the characteristic features of metastatic malignant melanoma, yet the clinician and the pathologist were in a diagnostic cul-de-sac. Emphasizing, once again, the rarity, clinical variability and unpredictable biologic behavior of the entity, we can only call to mind that, once in a while, looking at particles we can see in a wider perspective.

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A case of synchronous relapse of breast cancer and uterine müllerian adenosarcoma post tamoxifen in a premenopausal woman

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Summary

Purpose & Methods: We report a case of a 42-year-old multigravida, premenopausal woman with breast carcinoma, who presented after four years of use of adjuvant tamoxifen with synchronous liver, bone, and lung metastasis of breast cancer with müllerian adenosarcoma. Results: Immunohistochemical stains on the uterine tumor for estrogen and progesterone receptors showed positivity for both epithelial and stromal cells, actin, and desmin while the proliferative index (MIB-1) showed positivity for stromal cells only. The patient underwent a hysterectomy followed by palliative chemotherapy. She died 14 months after her relapse because of progressive disease (cerebral, bone, liver and lung metastases). Conclusion: Our case is the only one reported in the literature with synchronous relapse of breast adenocarcinoma and a Müllerian adenosarcoma. Moreover, it is one of the rare cases occurring in a premenopausal woman since all except two cases were postmenopausal.

Key words: Premenopausal, Müllerian adenosarcoma, Relapse, Tamoxifen, Uterine.

Introduction

Tamoxifen, a non-steroidal antiestrogen, is used at all stages of breast cancer in pre- and postmenopausal women as an adjuvant or palliative therapy, as well as a prophylactic drug for women identified at high risk of hormone-dependent breast cancer [1]. Several studies have shown its association with an increased incidence of endometrial cancer [2] which was explained by the fact that tamoxifen shows various responses from different estrogen sensitive tissues. Effectively, it is anti-carcinogenic in the breast, while it is carcinogenic in the endometrium [3].

Uterine müllerian adenosarcoma is increasingly being reported as a side-effect of tamoxifen adjuvant therapy [4-12]. This tumor has been documented to be associated with long-term estrogen exposure.

Case Report

A 42-year-old multigravida premenopausal woman was diagnosed as having a breast cancer, ductal type, Stage IIA T2 N(2+)/27) M0, with positive estrogen and progesterone receptors, a high proliferative index (MIB1), and a score of 3 for cerb-B2 using the immunohistochemical method. At that time she underwent a modified radical mastectomy followed by six cycles of chemotherapy (5FU, doxorubicin, and cyclophosphamide) and loco-regional irradiation. Tamoxifen was then initiated in an adjuvant setting at a dose of 20 mg. No other medications were used.

Forty-five months after the initial diagnosis, the patient presented with bone pain. The work-up revealed the presence of generalized relapse with liver, bone, and lung metastases. The appearance of vaginal bleeding led to a gynecological exam which revealed a protruding polypoid lesion and enlarged uterus. The patient underwent an ultrasonography that confirmed the enlargement of the uterus with an increase in the thickness of the endometrium; therefore, a dilatation and curettage was performed. The uterus weighed 760 g with a polypoid mass arising from the endometrium measuring 10.5 cm in the maximum dimension; no myometrial invasion was noted. The cut surface was soft with cystic areas containing mucinous material (Figure 1).

The diagnosis of tamoxifen-associated adenosarcoma in this case was readily apparent and the patient underwent hysterectomy.

Immunohistochemistry showed estrogen and progesterone receptor positivity in both epithelial and stromal cells (30%), c-erbB2 (Her 2 neu) negativity, smooth muscle actin and desmin positivity for stromal cells, and a high positive proliferative index (MIB-1).

Discussion

This report documents another case of the rare uterine tumor Müllerian adenosarcoma associated with tamoxifen therapy. In 1974, Clement and Scully first introduced the term müllerian adenosarcoma to designate a very rare uterine malignancy composed of benign or mildly atypical glands admixed with a sarcomatous, usually low-grade, stromal component [4]. The literature reviewed revealed around 15 cases [4-12] and 12 cases from seven papers were compared clinically and histologically with our case (summarized in Table 1). Our case is one of the rare cases occurring in a premenopausal woman since this tumor occurs mainly in postmenopausal women and all except two cases were postmenopausal [4]. Additionally, our case is the only case reported in the literature.
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with synchronous relapse of the breast adenocarcinoma and a müllerian adenosarcoma.

All but three of the reported cases presented with vaginal bleeding: one with a polyp protruding from the vaginal vault [3], another after a regular gynecological check-up, where endometrial thickening was noted [11] and one asymptomatic case [3]. The lymph node status varied from no metastasis to 12 out of 29 (2 out of 27 axillary lymph nodes in our case). As in our case, all patients received adjuvant chemotherapy and radiotherapy, except one case where only radiotherapy was employed [9]. The duration of 20 mg adjuvant tamoxifen administration varied from one case to another ranging from four months to five years. The shorter period of appearance of Müllerian sarcoma was observed in the reported case presented by Martin-Loeches [11] where the patient was found to have increased endometrial thickness two months after tamoxifen initiation and the biopsy revealed the diagnosis of uterine Müllerian sarcoma. In our case, the patient was recommended to have a repeated gynecological follow-up after tamoxifen intake but she did not show up and was lost to follow-up.

In all reported cases smooth muscle actin was positive, indicating the muscular origin of these tumor cells. Desmin was variable but vimentin was positive. Estrogen and progesterone receptors were also positive. In the cases reported in the literature all except one were negative [9] suggesting that the loss of these receptors is due to their proliferative activity [5].

The cases of uterine müllerian adenosarcoma associated with tamoxifen, as reported from the papers reviewed, revealed similar histological features to those occurring without tamoxifen intake history. The prognosis of women with uterine sarcomas or adenocarcinomas who had taken tamoxifen was not any poorer than that of women not exposed to tamoxifen; in fact for both histologic subgroups, the probabilities of survival were greater among women treated with tamoxifen (median follow-up, 85 months) [12].

In conclusion, the benefit to risk ratio of tamoxifen in the treatment of breast cancer patients is definitely high. Even though adenosarcoma represents a rare endometrial malignancy and its association with tamoxifen therapy makes its occurrence even rarer, pathologists should keep in mind this differential diagnosis whenever examining endometrial material.

References

A case of synchronous relapse of breast cancer and uterine müllerian adenosarcoma post tamoxifen in a premenopausal woman


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Leiomyosarcoma of the uterine corpus with ovarian metastases in a 28-year-old woman: case report

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Summary
A case of invasive leiomyosarcoma of the uterine corpus with right ovarian metastases in a 28-year-old woman is presented. The patient was submitted to surgery including total abdominal hysterectomy with left salpingo-oophorectomy, dissection of the pelvic and paraaortal lymph nodes and fixation of the right ovary to the psoas muscle. Postoperative radiation therapy was applied. A year after treatment, the patient was well.

Key words: Leiomyosarcoma uteri; Ovarian metastases; Hysterectomy.

Introduction
Sarcoma of the uterine corpus is a rare malignant tumor of the female genital tract. It accounts for less than 1% of all gynecological malignancies and 2-5% of all uterine malignancies [1]. The most common histological types of uterine sarcoma are: carcinosarcoma in 50%, leiomyosarcoma in 30% and endometrial stromal sarcoma in 15% of cases [2]. The incidence of this sarcoma in America is about 0.67 per 100,000 women [3]. The incidence of sarcomatous alterations in benign uterine leiomyoma is between 0.13 and 0.81% [4]. The average age of women with leiomyosarcoma ranges from 40 to 50 years. Treatment is surgical and involves total abdominal hysterectomy with bilateral salpingo-oophorectomy and pelvic and paraaortal lymphadenectomy and peritoneal lavage [5]. There is a high risk of recurrence so it is not uncommon to also apply postoperative adjuvant chemotherapy and radiation therapy [6]. The prognosis is poor [7]. Patient survival with uterine leiomyosarcoma is between 20 and 63%. It is correlated with the degree of mitotic tumor activity, as well as the disease stage [8].

Case Report
A 28-year-old patient, gravida 0, para 0, presented for gynecological examination due to slight pelvic pain. She had menarche at the age of 12, her menstrual cycles were normal every 28 days and lasting about five days. History data showed that she had had uterine myoma and a right ovarian cyst for three years. Vaginal gynecological examination was performed which revealed an enlarged uterus with posterior wall myoma and right ovarian cystic formation. Ultrasound examination showed the uterus was 10 cm x 8 cm x 6 cm in size, with a posterior uterine intramural myoma 6 cm x 5 cm x 5 cm in size. A cystic formation of 5 cm x 4 cm with hematic contents was found in the right ovary. The left ovary was 3 cm x 2 cm x 1 cm.

Laboratory data showed no abnormality of the complete blood count, serum urea, creatinine, sodium, potassium, calcium and phosphate concentrations. A liver function test was also within normal limits. Lung X-ray, abdominal ultrasound and internal findings were within normal limits. The patient was submitted to laparotomy. Myomectomy and cystectomy were performed on the right ovary.

Histologic examination of the excised tumor mass showed that it was leiomyosarcoma of the uterine corpus with over 10 MF/10 HPF, presumed to be transformed from pre-existing uterine leiomyoma, and the right ovarian endometriotic cyst. A decision was made to reoperate on the patient with radicalization including conservation and transposition of the right ovary. Relaparotomy was performed. Classic total abdomen hysterectomy with left salpingo-oophorectomy was performed together with a right ovarian biopsy. The right ovary was fixed to the psoas muscle. Pelvic and paraaortal lymphadenectomy was performed as well as omental resection. Pelvic and abdominal discharge was taken for cytological analysis. Swabs from the diaphragm and the right and left paracolic area were taken for cytological analysis. The liver, intestines, stomach, spleen and diaphragm were examined and no signs of disease signs were found.

The final histopathological finding was: infiltrating leiomyosarcoma (Figure 1). It primarily infiltrated vascular areas. Metastases of leiomyosarcoma were found in the capsule and cortex of the right ovary (Figure 2). The omentum, pelvic and paraaortal lymph nodes were without malignancy signs. Cytological analysis of the abdominal and pelvic discharge was negative. The final diagnosis was leiomyosarcoma of uterine corpus in Stage III a. The patient underwent postoperative transcutaneous radiation therapy (50 Gy) applied in 25 courses. After therapy the patient was well.

Discussion
Leiomyosarcoma of corpus uteri is a rare malignant tumor of the female genital tract. It is characterized by a high malignancy potential. Out of all histological types of uterine sarcomas, leiomyosarcoma accounts for 42% [9]. It may arise from the uterine myometrium de novo or may be transformed from a pre-existing benign leiomyoma [10]. The risk factors for development of sarcoma of the uterine corpus have not been completely elucidated. Application of radiation therapy in the area of the small pelvis has been considered to have an impact on the
Leiomyosarcoma of the uterine corpus with ovarian metastases in a 28-year-old woman: case report

Metastatic changes in the pelvic and paraaortal lymph nodes. The incidence of ovarian metastases and lymph node metastases in leiomyosarcoma is very low. In patients with Stages I and II of the disease, ovarian metastases were found in 2.8% of cases, while there were no metastases in the lymph nodes. In advanced stages of the disease with extragenital disease, the percentage of ovarian metastases was about 5.4%, and in the lymph nodes about 8.1% [13]. A multidisciplinary approach to treatment of patients with uterine sarcoma is crucial for optimal treatment [14].

Treatment is surgical and includes total abdominal hysterectomy with bilateral salpingo-oophorectomy, as well as pelvic and paraaortal lymphadenectomy and peritoneal lavage [5]. The risk of recurrence is high so upon surgical therapy adjuvant chemotherapy is frequently applied as well as radiation therapy [6,9]. Pelvic radiation therapy has not significantly improved patient survival [15]. Disease prognosis is poor [7]. It primarily depends on disease extent at time of diagnosis and the degree of mitotic tumor activity [8]. High mitotic indices and cellular atypia are significant indicators of tumor malignancy. A histological indicator of poor tumor prognosis includes the presence of anaplasia and tumor necrosis [15]. Some authors think that for leiomyosarcoma the tumor size is the most important prognostic factor and that tumors with a maximal diameter of over 5 cm have poor prognoses [16].
Survival of patients with uterine leiomyosarcoma is 20-63%. Five-year survival for patients with Stage I disease is approximately 50% and for other stages 0-20% [17]. For leiomyosarcomas, overall survival has been 67% at one year and 33% at five years, but relapse-free survival has been 33% at one and five years [18].

References
Endometrial metastasis of a primitive neuroendocrine ovarian carcinoma: management and treatment of a case

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Summary

Background: Neuroendocrine tumours are a heterogeneous group of separate clinico-pathological entities which have a common characteristic, i.e., expression of potential endocrine differentiation. In the ovary, the term "neuroendocrine" relates mainly to widely known carcinoids, but it may also be applied to rare neuroendocrine carcinomas as non-small cell type and small cell carcinomas of pulmonary type. In the literature only 11 cases of primary ovarian non-small cell neuroendocrine carcinomas have been described and ten of these were associated with a surface epithelial ovarian tumour. Small cell neuroendocrine carcinoma of the ovary is a rare malignant tumour of the ovary. Advanced small cell carcinoma of the ovary is a very aggressive tumour with an overall poor prognosis and unfavourable outcome. Case report: The case reported is unique in the literature because the authors describe a rare case of endometrial metastasis of a primary ovarian non-small cell neuroendocrine carcinoma without any surface epithelial ovarian tumour association. The tumour invaded up to less than half of the myometrium. The first symptoms were related to endometrial metastasis as metrorrhagia and pelvic pain while the asymptomatic presence of primary ovarian carcinoma was not acknowledged with physical examination, routine biochemistry, tumour markers, blood count and traditional transvaginal greyscale ultrasound. Conclusion: Magnetic resonance and three-dimensional (3D) ultrasonography with power Doppler are a great help in the diagnosis of ovarian localisation but only immunohistochemistry on histological material can provide a correct diagnosis. Immunohistochemistry expression of Ki67 is a useful marker of malignancy. Due to the rarity of this neoplasm, a general consensus for optimal treatment has yet to emerge. The reported biological aggressiveness of these tumours prompts combined treatment with radical surgery and adjuvant polychemotherapy.

Key words: Neuroendocrine ovarian tumours; Endometrial metastasis.

Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of separate clinico-pathological entities which have a common characteristic, i.e., expression of potential endocrine differentiation. These tumours can be classified as biologically active (BANETs) and inactive (BINETs) neuroendocrine tumors. The diagnosis is based on the use of cytoplasmatic markers (N-CAM, NSE, PGP 9.5, chromogranin A, synaptophysin, cytokeratin (l mw, Ki67). In the ovary, the term "neuroendocrine" relates mainly to widely known carcinoids, but it may also be applied to rare neuroendocrine carcinomas as non-small cell type and small cell carcinomas of pulmonary type [1]. Moreover neuroendocrine differentiation may be expressed in a variety of ovarian tumours including epithelial tumours, Setoli Leydig cell tumours, teratomas, carcinoid tumour, small cell carcinoma of pulmonary type and non-small cell undifferentiated carcinoma of neuroendocrine type [2]. The last has previously been described in association with surface epithelial tumours of the ovary [3, 7]. In the literature only 11 cases of primary ovarian non-small cell neuroendocrine carcinomas have been described and ten of these were associated with a surface epithelial ovarian tumour. Small cell neuroendocrine carcinoma of the ovary is a rare malignant tumour [2, 8]. It is the most common undifferentiated ovarian carcinoma in young women. Approximately two-thirds of patients with ovarian small cell carcinoma have hypercalcemia [9, 10]. The few cases reported in the literature account for less than 1% of all cases of carcinoids in the body [11]. Advanced small cell carcinoma of the ovary is a very aggressive tumour with an overall poor prognosis and unfavourable outcome [12].

A rare case of endometrial metastasis of a primary ovarian non-small cell neuroendocrine carcinoma without any surface epithelial ovarian tumour association is reported.

Case Report

A 56-year-old postmenopausal white female with no significant past medical history came under our care in March 2006 for abnormal uterine bleeding and pelvic pain. She complained of alterations in defecation and abnormal spotting of three months duration. She did not report any paraneoplastic or virilizing symptoms and her laboratory values, including calcium levels, were normal.

Vaginal examination showed the womb had increased volume and was deformed on the left by a mass of 80 x 80 mm. Rectal exploration confirmed the vaginal examination. The mass was fixed to the wall of the sigma rectum pouch deforming the caliber and profile.
Infracolic omentectomy, appendectomy, paraaortic lymph node dissection and pelvic side wall biopsies were performed. Subsequent clinical work up did not disclose any evidence of an extrapelvic tumour. Pathologic examination revealed a diagnosis of undifferentiated small cell carcinoma with neuroendocrine immunophenotype positivity for CK35betaH11 (low mw), NSE and with high mitotic activity (> 10/HPF), and clone Ki67 K-2 equaled 60%. The primary origin was determined to be ovarian carcinoma involving the left endosalpinx and uterus. Tumour invaded up to less than half the myometrium.

Transvaginal ultrasonography (TVS) was performed (Figure 1a) and an urgent fractional curettage was carried out due to intense bleeding. TVS confirmed the suspicion of a mass on the left side fixed to the uterus, ovary and sigma rectum pouch with increased thickness of the endometrium.

Fractional curettage revealed a diagnosis of undifferentiated small cell carcinoma of the endometrium with neuroendocrine immuno-phenotype positive for CK35betaH11 (low mw), NSE and with high mitotic activity (> 10/HPF), and clone Ki67 K-2 equaled 60%. The staging was performed in accordance with FIGO guidelines.

Clinical staging was performed: biochemistry and blood count, tumour markers, chest X-ray, magnetic resonance imaging (MRI), three-dimensional (3D) ultrasonography with power Doppler (Figure 1b), esophagastroduodenoscopy (EGDS) and colonscopy.

MRI showed the tumour involved the left ovary with implants in the uterus, ascites and bilateral pleural effusion. There was hydrometra and presence of a subserous leiomyoma (80 x 80 mm) on the left side. No metastasis nor lymph node (LN) involvement was found, confirmed by preoperative lymphoscintigraphy with technetium-99m (Tc-99m).

3D ultrasonography with power Doppler confirmed the diagnosis of ovarian cancer associated with endometrial involvement.

Explorative laparotomy (Figure 2) was performed revealing a left ovarian tumour fixed to the leiomyoma and the wall of the sigma rectum pouch deforming the caliber and profile but without involving the wall of the rectum.

Laparo hysterectomy and a bilateral salpingo-oophorectomy,
There were no malignant cells in the ascites. FIGO Stage II A/T2aN0M0/G3 (WHO) was determined. Following surgery the patient received six cycles of taxol and carboplatinum. Today, after a follow-up of ten months including total body CT and tumour markers, the patient shows no evidence of disease.

Discussion and Conclusions

NETs are a heterogeneous group of separate clinico-pathological entities which have a common characteristic, i.e., expression of potential endocrine differentiation. In the ovary, the term "neuroendocrine" relates mainly to widely known carcinoids, but it may also be applied to rare neuroendocrine carcinomas of non-small-cell type and small cell carcinomas of pulmonary type. Ovarian carcinoids develop in pure form or in association with other tumours, mainly teratomas. They originate from endocrine cells, either of teratomatous origin or possibly also indigenous [1]. Ovarian neuroendocrine carcinomas belong most probably to surface epithelial neoplasms, which express endocrine pathway differentiation [1]. NETs of the ovary are rare neoplasms with only 11 cases reported in the literature [2, 8] and they account for less than 1% of all cases of carcinoids in the body [11]. Advanced small cell carcinoma of the ovary is a very aggressive tumour with an overall poor prognosis and unfavourable outcome [12]. An extremely rare case of endometrial metastasis of a primary ovarian non-small cell neuroendocrine carcinoma without a surface epithelial ovarian tumour association has been presented. The tumour invaded up to less than half the myometrium. The initial symptom was abnormal bleeding in a postmenopausal woman. The metrorrhagia and pelvic pain were suggestive of endometrial metastasis while the asymptomatic presence of primary ovarian carcinoma was not acknowledged at first physical examination with routine biochemistry, tumour markers, blood count and traditional TVS. Moreover, the 3D ultrasonography with power Doppler and the MRI were very useful in the diagnosis of ovarian localisation however, in these cases, only immunohistochemistry on histological material can provide an accurate diagnosis. Also immunohistochemistry expression of Ki67 is a useful marker of malignancy.

In comparison to transvaginal 2D grayscale or 3D...
sonography, 3D power Doppler – and especially the combined use of 3D sonography and power Doppler imaging – significantly improve diagnostic accuracy in preoperative sonographic assessment of suspected ovarian lesions. Moreover they can enhance and facilitate the morphologic and functional evaluation of both benign and malignant ovarian lesions [13, 18].

The management and treatment of ovarian NETs are very difficult. In fact, due to the rarity of this neoplasm, a general consensus for optimal treatment has yet to emerge [8]. The reported biological aggressiveness of these tumours prompts a combined treatment with radical surgery and adjuvant polychemotherapy.

Numerous experiences using monochemotherapies show an unsatisfactory benefit in comparison to the use of polychemotherapies [Level C].

It is not always possible to perform radical surgery due to the often advanced stage of disease, nevertheless cytoreductive surgery together with a polychemotherapy often improves the quality of the life and offers a sure advantage in long-term survival. In fact, this case showed no progression of disease over ten months and the overall disease outcome has been excellent.

References