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Misconceptions about routine colposcopy

P. Bösze

Department of Gynaecology, Saint Stephen Hospital, Budapest (Hungary)

Summary

Colposcopy is practised in two ways: 1) to assess women with abnormal screening findings and/or clinically suspicious cervix (called referral colposcopy), and 2) as part of a routine gynaecological examination (referred to as routine colposcopy). There are several misconceptions about routine colposcopy probably reflecting the lack of experience in using routine colposcopy. Misconceptions include: routine colposcopy is screening colposcopy, it is time-consuming, expensive, a waste of time, and the training and maintaining of colposcopic expertise is probably not sufficient in this setting. Routine colposcopy, however, is not a screening tool, it is not screening colposcopy, but capable of identifying cervical precursors and cancer, and thereby reducing the false rates of cervical cancer screening (mainly cytology). Unlike referral colposcopy, routine colposcopy is an inexpensive and rapid procedure conducted as a part of a pelvic examination and has no, or minimal, discomfort that certainly does not exceed that of smear taking, neither is it associated with any psychological burden. Routine colposcopy allows gynaecologists to be convincingly sure in their findings; ensure women having normal epithelium; evaluate abnormalities in details (without biopsy) and counsel patients immediately to alleviate the psychological effects and prepare them for a possible abnormal smear; as well as help make a diagnosis of obscure lesions.

Key words: Colposcopy; Abnormal cytology; Screening; Cervical cancer; CIN; AIS; Gynaecologic examination; Biopsy.

Definition of referral and routine colposcopy

Colposcopy is practised in two ways:

– worldwide, colposcopy is mostly used to evaluate abnormal screening findings mainly, abnormal cytology and/or clinically suspicious cervix (called referral colposcopy);
– in several countries, colposcopy is a part of the routine gynaecological examination (called routine colposcopy).

Referral colposcopy

Assessment of women with abnormal screening findings is done in a triage setting mostly in a colposcopy clinic. This way of practising colposcopy is called referral colposcopy because the patients are referred for a colposcopic evaluation, where, in most cases, a colposcopically directed biopsy is taken. The term referral colposcopy applies, irrespective of whether or not a biopsy is taken.

The definition of colposcopy per se does not include biopsy or endocervical sampling, but lightening and visualisation of the cervix (vagina, probably vulva) under magnification (colposcope) with application of acetic acid. Application of saline or Lugol’s solution is optional, as is the use of a green filter to enhance the vascular appearance.

The objectives of referral colposcopy include:

– assessment of abnormal screening findings (abnormal cytology, HPV test, clinically suspicious lesions);
– localisation of the cervical lesion;
– identification of the site of the most severe part of the abnormality for appropriate biopsy;
– exclusion of invasive cancer, if possible;
– to tailor the amount of tissue to be excised.

Referral colposcopy may also be used:

– for surveillance of non-treated patients with low-grade or equivocal findings;
– to follow-up women who underwent treatment of cervical or vaginal lesions.

Routine colposcopy

In a routine colposcopy setting, colposcopic examination of the cervix is done whenever a pelvic examination is performed irrespective of the reason women seek gynaecologists, e.g., women with amenorrhoea, infertility, abdominal pain, hormone substitution, etc., undergo colposcopy as a constituent part of their gynaecologic examination, albeit these have nothing to do with screening or cervical abnormality. i.e. routine colposcopy is practised not only in colpo-
scopic but in gynaecologic clinics as well, thus, this is the colposcopy for routine gynaecologic practice. This way of practising colposcopy might be difficult to understand for gynaecologic oncologists dealing with female cancer and their precursors only, but might not be so for general gynaecologists.

Routine colposcopy is aiming to recognise tissue structures for what they are without biopsy. It implies fundamental knowledge and training in colposcopy with the capability of understanding the tissue basis and recognition of squamous, columnar and metaplastic epithelium as well as of colposcopic patterns and signs, changes due to atrophy and radiation effects, inflammation, etc. [1]. It is also important to be familiar with the concept of the transformation zone (TZ), including type 1-3 TZ and the clinical implications, and knowledge regarding miscellaneous colposcopic findings is also essential [1]. What routine colposcopy does not imply is biopsy, ablative or excisional treatment modalities. Management of lower genital premalignancy is a further step independent from the principle of routine colposcopy. Following the guidelines of the European Federation of Colposcopy (EFC) and the International Federation of Cervical Pathology and Colposcopy (IFCPC) colposcopy training/practise is broken down into two categories, basic and advanced colposcopy [2]. With this in mind routine colposcopy can be considered as basic colposcopy.

It should be emphasised that there is only one technique of performing colposcopy. It is not different whether it is used in a routine or referral setting. The two approaches differ only in the indication and schedule of colposcopic examination: referral colposcopy to evaluate abnormal screening results, i.e., after primary screening; routine colposcopy in all instances of gynaecologic examination irrespectively from screening.

There are several misconceptions about routine colposcopy probably attributed to the lack of experience in it. Most colposcopists around the world are experts in triaging women with abnormal smears (referral colposcopy) but may not have any experience with routine colposcopy, which might explain the misconceptions.

The major misconceptions of routine colposcopy are:

1) Routine colposcopy is screening colposcopy (a screening tool)

Cervical cancer screening has been based on cytology for decades and recently the utility of primary HPV testing is under evaluation. In the past, the value of screening colposcopy has been tested in several studies and was found impractical [2-5].

Kyrgiou et al. [5] in arguing against screening colposcopy, provided a theoretical model as follows: “given the known prevalence of CIN in the general population (about 1-2%) and a general acceptable false negative rate of cytology of around 20%, in a total population of 10,000 women screened, a hundred will have a pre-invasive lesion. Of those, 80 will be detected with cytology and 20 will be missed. In other words, 9,920 women will need to be referred and undergo a colposcopic examination in order to detect the 20 missed ones, assuming that colposcopy is 100% sensitive, an assumption that clearly overestimates colposcopy’s diagnostic performance. In addition, it would be expected that the majority of false negatives would probably be detected by repetition of cytology...”. Furthermore the authors deemed that “any policy that would include colposcopy in primary screening has obvious disadvantages. Screening colposcopy is expensive, time-consuming, requires extensive training and can lead to unnecessary psychological morbidity in women. Potential long-term pregnancy-related morbidity is also an important consideration.”

Of note regarding the concept of Kyrgiou et al.: many nurses and general practitioners who take smears have been trained in colposcopy in the UK and they are doing well [6]. One may wonder what the goal of their training is; certainly it is not for triaging women.

Routine colposcopy, however, is not screening colposcopy, it is not a screening test; therefore, parameters used for assessing the utility and quality of any screening test are not relevant to routine colposcopy. Women are not referred to colposcopy for screening; they undergo colposcopy during smear taking, without biopsy, because colposcopy is done anyway, not for screening sake, but as a constituent of their pelvic examination.

As colposcopy is highly sensitive to identifying low- and high-grade precursors of the uterine cervix and vagina (sensitivity 87 to 99%) [7-9], with accuracy superior to cervicography [10], routine colposcopy is able to pick up CIN or glandular abnormalities missed by cytology and thereby reducing the false negative rates of cytology. This concept is supported by Chase et al. [11], who concluded that “colposcopy is the only means available to evaluate the cervix for more potentially advanced premalignant disease that is either missed or detected as low grade on a Papanicolaou smear alone.”

2) Routine colposcopy is a waste of time

Many gynaecologists (colposcopists) globally deem that colposcopy of women without a positive screening test has disadvantages; it is probably unnecessary and useless. One may wonder if this view results from experience or otherwise.

With the aims of basic colposcopy, routine colposcopy allows gynaecologists at the first instance, i.e., right at the time of the pelvic examination:

– to be convincingly sure in their findings;
– to detect lesions (HPV infection, precancer, etc.) not visible to the naked eye, missed by cytology.
High-grade CIN and glandular changes are subclinical and cannot be detected macroscopically. It is a devastating experience for a woman being told that her gynaecologic examination is negative and having high-grade cytology.
– to ensure women having normal epithelium;
– the negative predictive value of colposcopy approaches 100%, therefore women with negative colposcopic findings in the presence of a fully visible transformation zone (type I and II TZ) can be ensured at the time of their pelvic examination that they do not have any abnormality. This is most relaxing while waiting for the result of the screening test.
– to evaluate abnormalities in detail (without biopsy), including determination of the grade of atypia, if any; identifying microinvasion, if possible, and localisation of the lesion, and counsel patients immediately to alleviate the psychological effects and prepare them for the possibility of getting a positive screening result.

In the referral colposcopy setting patients are prompted to make an appointment at the colposcopy clinic. The waiting list may be quite substantial and the process is invariably associated with anxiety and psychological stress, etc. [12];
– to help make a diagnosis of obscure lesions (healing or granulation tissue, etc.); including ruling out high-grade abnormality.

3) Low level of training and maintaining expertise in a routine colposcopy setting

Training and education in colposcopy is based on health policy in each country and therefore there are differences even within Europe. In most parts of the European Union and in the United States, expertise in colposcopy requires special training and education in colposcopy centres and, indeed, the learning curve is quite long. Estimations include a training period of four months to accurately recognize SIL, and an additional year to identify the optimal site for directed punch biopsy. The European Federation of Colposcopy (EFC) has provided a training programme with minimum standards (51 core competencies) deemed essential for competent colposcopy practise, each of which is a learning objective [13]. This is a comprehensive and heterogeneous training programme, consisting of routine elements of gynaecological examinations (e.g., how to insert vaginal speculum, history taking, positioning of patients, etc.), which all gynaecologists should know from the outset, as well as communication skills, basic surgical techniques (e.g. biopsy) and mostly skills related to colposcopy itself. The main objective of colposcopy training includes learning the tissue basis of colposcopic findings and having the ability to accurately recognise and interpret the colposcopic features. The aiding and technique of biopsy and other treatment modalities as well as novel approaches to enhance diagnostic accuracy are secondary in the colposcopic curriculum and – as noted previously – are not included in colposcopy per se.

Like colposcopy practice, the training and education in colposcopy can also be accomplished in two ways: a) as part of residency training in obstetrics and gynaecology in the same way as cystoscopy is included in the urological residency programme or b) in colposcopic centres. Whichever applies, the core curriculum of colposcopy can and should be learned. However, the approach is basically different in these two settings:
– In the residency setting, the trainees perform colposcopy whenever they do a gynaecological examination (huge number of cases), seeing a normal cervix and vagina in the vast majority. Thus, the trainees mostly become familiar with the physiological appearance of the lower genital tract and recognise abnormalities as different from normal. A concern is that the stipulated number of abnormal colposcopic features seen by the residents cannot be achieved in this way. However, this is not the case, because during the six years of apprenticeship, even the relatively rare cases are available for studying and the caseload of abnormal colposcopic findings is usually appropriate for proper education in colposcopy; residents receive training in colposcopy and managing abnormal cytology in the same way that they are trained in ultrasound scanning, laparoscopy, etc.
– The education and training in colposcopy centres is achieved the other way round. In this setting, more abnormal colposcopic findings are evaluated and, in fact, there may be a shortage of normal colposcopic findings, since women with normal cervixes are rarely referred to a colposcopic centre. This is a real concern.

Although colposcopists in these centres invariably manage more patients with abnormal colposcopic findings, the standard number of cases required for maintaining expertise in colposcopy is available for the general gynaecologist in the routine colposcopy setting, provided he or she has at least an average workload.
Whatever the approach, training, skills and evidence-based practice with outcome-based audit is a prerequisite for professional colposcopy.

4) Routine colposcopy is time-consuming

One of the arguments against using colposcopy in a routine setting is that colposcopy takes 10-15 minutes to be performed; consequently not more than four to five patients can be examined in an hour, which makes colposcopy useless in this context. Having used colposcopy routinely, i.e., as part of every pelvic examination, on a daily basis for almost half a century, I can assure that to identify the normal cervix, TZ and whether or not there is any abnormality, does not
require more than a minute or two. Only acetic acid application is needed, and the use of Lugol’s solution does not add much, if any to it. Fine versus course punctation, mosaic, etc., and even the presence of atypical vessels can easily be diagnosed during this time-frame, as can the localisation of the lesion be determined.

Detailed analysis of abnormal lesions is important when biopsy is directed and it takes time (not more than 4-5 minutes according to my experience); this is, however, an extended diagnostic procedure and is beyond routine colposcopy per definition.

In summary, routine colposcopy is not and obviously cannot be time-consuming, because any procedure taking 5-10 minutes to perform by no means is feasible to be included in the routine gynaecologic examination.

5) Routine colposcopy is expensive

When calculating the price of referral colposcopy, one should make a clear distinction between the cost of the colposcopic examination itself and the cost of the additional biopsy and histology with or without treatment. However, depending on the national health policies, the cost may be calculated as a package price. Nevertheless, referral colposcopy is expensive: the average cost of colposcopy and biopsy was $436 per patient in 2002 in the United States [14].

The price of routine colposcopy is included in the cost of the gynaecological examination and it is not additional and it does not make gynaecologic examination more expensive. The only extra cost is the price of the colposcope. You may buy a terribly expensive colposcope and say that routine colposcopy is costly; however, in practise a normal colposcope with low and higher magnification and a green filter will be perfectly satisfactory. Extras such as a camera, video, computer imaging technology, database, etc., can be helpful in several ways, but do not improve your expertness in colposcopy. Thus, routine colposcopy is cost-effective and far less expensive than referral colposcopy. The cost of excisional or ablative treatment is irrelevant to that of routine colposcopy.

Several measures have been taken in referral settings to reduce the number of referrals for colposcopy, not only because of the price, but also due to the associated psychological burden. For instance, Pretorius et al. [15] advocated a 2-year referral interval for colposcopy, instead of yearly colposcopy, for women with CIS1 or less on biopsy whose high-risk HPV test remains positive but cytology is normal; annual colposcopy is indicated only if the cytology is also abnormal. They admit, however, that with this approach there might be a small chance of missing CIS3. With routine colposcopy these kinds of dilemmas just do not exist.

6) Routine colposcopy is associated with psychological burden

There is compelling evidence that referral to a colposcopic clinic is almost always associated with a significant negative psychological effect with the STAI (state-trait anxiety inventory) [16] score of 51 (scoring range 20-80, the average value for normal adult women is 35) [12, 17]. Some may argue that this anxiety could be due to having an abnormal smear or due to fear of the underlining HPV infection. Freeman-Wang and Walker, however, highlighted the importance of fear and anxiety from the colposcopic examination itself, as patients poorly understand colposcopy [12]. Women realise that they are facing an investigation for which they are prompted, which usually includes cervical biopsy, is uncomfortable, painful and embarrassing. Indeed, studies suggest that the level of distress and discomfort attached to referral colposcopy is more strongly related to anticipation of the procedure than its actual outcome [12]. Long waiting lists can increase the psychological burden.

Experience with routine colposcopy does not show significant, if any, anxiety or distress associated with it. Women are not referred for further assessment, and they are informed immediately. In addition, they hardly notice the colposcopic examination itself, because insertion of the vaginal speculum with exposure and cleaning of the cervix is done anyway during gynaecological examination. Thus, any “extras” such as application of acetic acid do not cause much discomfort, which certainly does not exceed that of smear taking. You may even lessen anxiety by saying “I will just examine your cervix with magnification using the colposcope. This will not cause any harm, it is not painful at all, and it only takes a minute or two, etc.”.

Discussion

This paper is not a review of the literature rather an expert opinion with the only aim to inform colposcopists worldwide about some misconceptions regarding routine colposcopy. The purpose is by no means to argue in favor of routine colposcopy, and it has no intention either to compare routine colposcopy with referral colposcopy in terms of advantages and disadvantages. The sole objective is information as to what routine colposcopy is all about. In fact, not much has been published on that.

In some countries, routine colposcopy is practised traditionally. This is fact and is not a matter of argument whether it is advantageous or a waste of time and money, etc. It is also a fact that routine colposcopy has never been tested in randomised, controlled studies whether there is statistically significant evidence for supporting its use. Tradition and experience, however, are strong arguments to substantiate routine colposcopy.
Misconceptions about routine colposcopy

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Referral and routine colposcopy are performed exactly the same way; technically there is no difference between the two. The basic difference between the two approaches is that in routine colposcopy there is no waiting list with all drawbacks attached to it, and the majority of women undergoing colposcopy has negative findings.

In conclusion: routine colposcopy is performed at the time of gynecological examination as part of it with minimal or no discomfort at the expense of 1-2 extra minutes but no extra cost. Thus, it is cheap and does not require another appointment should cytology be abnormal or during follow-up, consequently it is devoid of psychological distress of referral for colposcopy.

Routine colposcopy may identify low- or high-grade abnormalities missed or falsely diagnosed by cytology, thereby improving screening efficacy also it may help make a diagnosis of obscure lesions in the lower genital tract.

Recognising abnormal lesions at the time of smear taking, women can be prepared for possibly having abnormal cytology, minimising thereby their psychological distress. Most importantly, in the presence of normal colposcopic findings, it allows colposcopists to reassure women immediately that they do not have a significant lesion, which substantially alleviates anxiety while getting the results of a screening test.

Obtaining skills in colposcopy and maintaining a high level of expertise is deemed feasible during residency and in a routine colposcopy setting provided gynaecologists have at least an average workload and evidence-based practice with outcome-based audit is maintained.

In the author’s experience of over 40 years, routine colposcopy is an invaluable tool. One of its major advantages includes lots of information far beyond the scope of the naked eye gained during pelvic examination in terms of making confirming diagnosis or ruling out abnormality, and the opportunity to discuss those with the patient immediately; a practice most rewarding and relaxing.

References


Address reprint requests to:
P. BÖSZE, M.D.
Department of Gynaecology
Saint Stephen Hospital
Budapest (Hungary)
e-mail: bosze@axelero.hu
Repeat conisation or HPV test?
What should be done if histology of the primary conisation requires a second conisation?

R. Koiss¹, E. Babarczi², C. Jenei³, P. Gócze⁴, D. Horányi¹, P. Siklós¹

¹Department of Obstetrics and Gynaecology, St. Stephen’s Hospital, Budapest
²Department of Pathology, St. Stephen’S Hospital, Budapest
³Genoid Molecular Biology Laboratory, Budapest
⁴Clinic of Obstetrics and Gynaecology, Scientific University of Pécs, Pécs (Hungary)

Introduction

Cervical cancer

Cervical cancer is a significant cause of death and is – with precancerous lesions – a major cause of emotional and physical distress in women [1, 2]. Each year an estimated 500,000 new cervical cancer cases occur worldwide and 270,000 women die from the disease. The majority in the developing world but in the European Union a woman dies of cervical cancer every 18 minutes despite the well organised screening system.

Cervical screening programmes where they exist allow early detection of abnormal and precancerous cells and this might eventually lead to appropriate treatment. Most current mass screening programmes are based on Pap smear cytology assessment to detect precancerous cell changes [4, 5]. The limitations of cervical cytology, particularly in terms of sensitivity, are well known [1].

HR-HPV infection and CIN

High-risk human papilloma virus (HR-HPV) is a necessary prerequisite for most high-grade cervical intraepithelial neoplasias (CIN) and all cervical cancers (CC) [5-7].

CIN is a very common disease especially in women of reproductive age and a balance is needed to maximise the prevention of CC and at the same time avoid over-treatment. Management strategies of CIN include decision-making regarding the appropriateness of a conservative approach versus treatment. Conservative strategies are appropriate for women with low-grade CIN, particularly in the younger age range. High-grade CIN (CIN 2 or CIN 3) should be treated. Conservative methods reduce over-treatment as low-grade CIN lesions may regress spontaneously.

When high-grade (HG) CIN is detected the treatment is mandatory. CIN 3, the true precursor of cervical cancer, will progress to cancer if left untreated at a rate of around 30% over two years [1].

CIN 1 has been reported to progress to CIN 2/3 at a rate of 15% over two years but some of these cases may harbour undetected CIN2/3 [1, 2].

Every procedure shortening the cervical canal may lead to miscarriage and preterm delivery.

Since the loop electrosurgical excision procedure (LEEP) procedure puts less burden on the patient than cold-knife conisation, the risk of preterm delivery rises due to the increasing number of LEEP interventions [3]. Recent studies have demonstrated that women who
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Previously suffered from CIN remain at high risk of recurrent CIN [1]. CIN will evolve again in nearly 50% of these patients. The following factors increase the risk of residual dysplasia: positive surgical margins, age, and post-treatment HPV test positivity. Opinions differ on the significance of the last element [2, 4, 8-10].

The aim of the study was to assess the second HPV test as an appropriate method to reduce the number of interventions in histologically positive cases.

Method

Study design

Four hundred and thirty-eight cervical conisation procedures were performed between March 2008 and August 2010. The age range was between 22 and 65 years. In most of the cases the indication was cytological alterations. One hundred and nineteen (27.2%) out of the 438 cases were repeat (re-) conisions. The patients who were referred for re-conisation had CIN 2 or CIN 3 histopathological results with positive surgical margins. In cases where CIN 1 was confirmed we chose conservative management independently of the status of the surgical margins. The mean age of women referred for a second conisation was 34.7 years (range 22-65 years). In every case the LEEP was used.

LEEP conisation was performed under local anaesthesia using wire loop electrodes, with a diathermy apparatus set to 50 W for cutting and 50 W for coagulation. Generally only one specimen was removed by a single excision. All specimens were fixed with 10% buffered formalin and submitted to histopathology examination. Prior to the biopsy a HPV test was taken from the cervical canal and from the surface of the cervix. HPV samples were analysed by the Genoid ELISA-PCR method. The spectrum HPV Detection Kit (GenoID) was used according to the instruction manual. The cervical specimen was collected in PreservCyt medium, transferred to the laboratory and after isolation of the nucleic acids by a silica-based method, multiple HPV specific PCR was carried out. The amplicons were genotyped using a hybridisation based method; the biotinylated oligonucleotides were used as probes. The assay is capable of detecting virtually all mucosal HPV types and also type specific oligonucleotides were used as probes. The assay is capable of detecting virtually all mucosal HPV types and also high-risk genotypes (16, 31, 33, 45, 51, 52, 56, 58, 66, 68).

Pathological examination verified the histological grade. The formalin fixed preparations were sliced and embedded in paraffin for histological examination. The sections were stained with haematoxylin and eosin.

Statistical analysis of the data was performed according to the chi-square test; a p value of < 0.05 was considered significant.

We observed the relationship between the second HPV tests and residual dysplasia.

Characteristics of participants

The mean age of the 119 patients who underwent re-conisation was 34.7 years. According to this we divided the patients to two subgroups: under and over 35 years.

Of the patients 56.3% were in the younger age group while 43.7% fell into the older age group. Every patient had HG CIN with positive surgical margins at the first conisation. The re-conisation was done within eight weeks.

Results

Out of 438 cases 119 (27.2%) were re-conisations. In cases of histologically proven residual dysplasia (29 of 119) HR-HPV infection was also detected by HPV testing. In 29 patients (25.4%) of the total number of re-conisation patients, where residual dysplasia was confirmed HR-HPV infection was detected in 100%.

In 90 cases of the 119 re-conisation patients (75.6%) of the total number of re-conisation patients, residual dysplasia was not detected at re-conisation in spite of surgical margin positivity at the first biopsy.

In 77 out of this 90 patient cohort repeated HPV testing did not confirm any HPV infection.

In 13 out of these 90 patients HPV infection was detected repeatedly but only in three cases could we confirm the same HPV type. In these three cases the first histology proved to be severe cervical dysplasia.

In most of the 13 patients a new HPV type was detected at the second HPV test, showing a break of continuity of persistent infection relating to a previously detected HPV type (Table 2). Where the histology revealed persistent HG-CIN the repeated HPV test detected the same HPV type as occurred the first time (64%) (Table 3).

Futhermore in those cases where re-conisation detected a lower grade of dysplasia as seen previously, a new HR-HPV type was observed (36%). Analysing the HPV distribution we realized that HPV 16, 31 and 33 types were very common (92%) in precancerous lesions.

We analysed the HPV results of the second tests according to age distribution. We hypothesised that patient age might be a prognostic factor for residual dysplasia.

All patients who were referred for re-conisation had positive surgical margins at the first biopsy. The group was divided into two subgroups according to their age, younger or older than 35 years. Residual dysplasia was confirmed in 29 cases. There was no significant difference in occurrence of residual dysplasia between the two subgroups (below and above 35 years) by chi-square test.

Discussion

In recent years numerous publications have attempted to assess the predictive relevance of HPV status in the risk of persistent CIN [11-13]. All investigators showed a relationship between positive post-treatment HPV testing and persistent disease where surgical margins were positive.

In our study the second HPV test confirmed the same HPV type, detected before the first conisation and together with positive surgical margins were the indicators of re-conisation. According to our results there were two coherent indications for re-conisation: 1) positive surgical margin; 2) identical HPV test before and after the conisation. In these cases the existence of CIN2/3 residual dysplasia was confirmed.
Table 1. — Residual dysplasia and HPV status correlated to age.

<table>
<thead>
<tr>
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<tr>
<td>≤ 35 y</td>
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<td>6</td>
<td>47</td>
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<tr>
<td>&gt; 35 y</td>
<td>15</td>
<td>7</td>
<td>30</td>
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(Res dysp = residual dysplasia).

Table 2. — Second positive HPV test without residual dysplasia.

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<tr>
<th>Cases</th>
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<th>History of LLETZ</th>
<th>Second HPV test</th>
<th>History of re-LLETZ</th>
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<tr>
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<td>2</td>
<td>HPV16</td>
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<td>HPV66</td>
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<td>3</td>
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<td>CIS (adenoc.)</td>
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<tr>
<td>4</td>
<td>HPV33</td>
<td>CIN3</td>
<td>HPV33</td>
<td>Neg.</td>
</tr>
<tr>
<td>5</td>
<td>HPV 58, 33</td>
<td>CIN3</td>
<td>HPV59</td>
<td>Neg.</td>
</tr>
<tr>
<td>6</td>
<td>HPV16</td>
<td>CIN3</td>
<td>HPV31</td>
<td>Neg.</td>
</tr>
<tr>
<td>7</td>
<td>HPV33</td>
<td>CIN2</td>
<td>HPV59</td>
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</tr>
<tr>
<td>8</td>
<td>HPV16</td>
<td>CIN3</td>
<td>HPV33</td>
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<td>9</td>
<td>HPV 58</td>
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<td>HPV52</td>
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<td>10</td>
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<td>CIN2</td>
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</tr>
<tr>
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<td>HPV16,66</td>
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<td>HPV31</td>
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</tr>
<tr>
<td>12</td>
<td>HPV31</td>
<td>CIN2</td>
<td>HPV39</td>
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<tr>
<td>13</td>
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<td>CIN2</td>
<td>HPV52</td>
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</tbody>
</table>

LLETZ: Large loop conisation of the transformation zone; HPV: Human papilloma virus.

Table 3. — Persistent HPV infection in relationship with residual dysplasia.

<table>
<thead>
<tr>
<th>Cases</th>
<th>First HPV test</th>
<th>History of LLETZ</th>
<th>Second HPV test</th>
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<td>HPV31</td>
<td>CIN3</td>
</tr>
<tr>
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<td>HPV33,31</td>
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<td>HPV33</td>
<td>CIN2</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>HPV33</td>
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</tr>
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<td>HPV16,31</td>
<td>CIN2</td>
<td>HPV16</td>
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<tr>
<td>8</td>
<td>HPV16</td>
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<tr>
<td>9</td>
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<td>CIN2</td>
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<td>CIN2</td>
<td>HPV31</td>
<td>CIN2</td>
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<td>HPV33,16</td>
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<td>HPV16</td>
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<tr>
<td>15</td>
<td>HPV16</td>
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<tr>
<td>16</td>
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<td>CIN2</td>
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</tr>
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<td>HPV16</td>
<td>CIN2</td>
<td>HPV16</td>
<td>CIN3</td>
</tr>
<tr>
<td>18</td>
<td>HPV16,52</td>
<td>CIN2</td>
<td>HPV16</td>
<td>CIN2</td>
</tr>
</tbody>
</table>

LLETZ: large loop conisation of the transformation zone.

In our present series the sensitivity of the second HPV test was 94%, and the second HPV test had a negative predictive value of 100% for detecting residual dysplasia. Second HPV testing might be useful in reducing the number of re-conisations in those cases where HR-HPV testing is either negative or does not confirm the same HPV type as before.

These data enable us to define patient subgroups at different risks of persistent dysplasia on the basis of second HPV testing and surgical margin status in order to minimise the number of re-conisation procedures. Vice versa patients with negative second HPV tests might be followed-up only, without any subsequent treatment.

However, all patients treated for HG CIN must be carefully followed-up for at least ten years because a British study revealed that the risk of developing invasive cervical cancer among these women during the following eight years is about five times higher than that of the general population [14].

In conclusion, second HPV testing might be useful in reducing the number of re-conisations in those cases where the HR-HPV test is either negative or does not confirm the same HPV type as previously.

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References

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Address reprint requests to:
R. KOISS, M.D.
Department of Obstetrics and Gynaecology
St. Stephen’s Hospital
Budapest (Hungary)
e-mail: koiss5@freestart.hu
Clinical significance of serum growth-regulated oncogene α (GROα) in patients with gynecological cancer

R. Nishikawa¹, N. Suzumori¹, T. Nishiyama², H. Nishikawa¹, A. Arakawa¹, M. Sugiuara-Ogasawara¹

¹Department of Obstetrics and Gynecology, Nagoya City University, Graduate School of Medicine
²Clinical Research Management Center, Nagoya City University, Nagoya (Japan)

Summary

Purpose of investigation: To assess the clinical relevance of serum growth-regulated oncogene α (GROα) levels in gynecological cancer, we investigated its concentration in distinguishing patients with cervical cancer, endometrial cancer, ovarian cancer, benign ovarian tumor and control. Methods: Preoperative serum GROα levels were measured in women with cervical cancer (n = 46), endometrial cancer (n = 124), benign ovarian tumors (n = 52), and normal controls (n = 38) using an enzyme-linked immunosorbent assay. Results: Statistical analyses showed that the serum GROα concentration was significantly elevated in the cervical cancer, endometrial cancer and ovarian cancer patients compared with controls. Using GROα levels, the receiver operating characteristic (ROC) of cervical cancer (AUC = 0.775), endometrial cancer (AUC = 0.799), ovarian cancer (AUC = 0.749) and benign ovarian tumors (AUC = 0.568) vs controls were identified. Conclusion: Our findings suggest that serum GROα measurement as a molecular marker might contribute to detection and diagnosis of gynecological cancer.

Key words: Cancer; GRO; ELISA; Ovarian tumor; Serum; ROC.

Introduction

Growth-regulated oncogene α(GROα) is a neutrophil-activating chemokine and serves as a potent angiogenic factor [1, 2]. GROα was originally identified by its constitutive overexpression in transformed Chinese hamster fibroblasts [3]. Exogenously applied GROα exhibits growth-promoting activity toward melanoma cells [4]. The chemokine is also called a melanoma growth-stimulating factor [1, 2]. GROα expression is frequently detected in melanoma [4, 5], squamous cell carcinoma [6, 7], colon cancer [8], gastric carcinoma [9], oral cancer [10], and ovarian cancer [11].

Serum chemokine levels have been investigated as diagnostic and prognostic markers in gynecological cancer [12]. Chemokine α or “CXC” family members are IL-8, GRO, platelet factor 4, and IP-10 [13]. Examples of chemokine family members include upregulated on activation, normal T-cell expression and secretion (RANTES), macrophage inflammatory protein-1 (MIP-1) and monocyte chemoattractant protein-1 (MCP-1), and they are the major determinants of macrophage and lymphocyte infiltration in carcinomas of the breast, ovary, and cervix [14, 15].

Yang et al. reported that GRO-1 was expressed at significantly higher amounts in ovarian cancer than in normal tissues and was higher in serum samples from women with ovarian cancer than in serum from women without ovarian cancer [16]. Also, a recent report suggests that increased GRO levels are detected in the plasma and ascites of ovarian cancer patients [11]. To our knowledge, there are no precise clinical reports of serum GROα levels in ovarian cancer and other gynecological cancer patients.

Here we measured serum concentrations of GROα in gynecological cancer patients and evaluated the utility of preoperative GROα levels in distinguishing malignant from benign or controls.

Materials and Methods

Subjects. Women with cervical cancer (n = 46), endometrial cancer (n = 124), ovarian cancer (n = 52), and healthy women as controls (n = 38) were enrolled between 1998 and 2010. The preoperative serum samples were reviewed in this study. The study protocol was approved by the Institutional Review Board of Nagoya City University and informed consent was obtained from all of the study subjects. Patients with inflammatory states, e.g., allergy and infections, were excluded from this study, as well as those who had bronchial asthma, rhinitis, and atrophic dermatitis [17, 18]. Also excluded were endometriosis patients and patients with positive blood cultures, and those who showed both CRP above 5 mg/dl and peripheral leukocyte counts above 10³/mm³ were also excluded because of the possibility of bacterial infection. The benign ovarian tumor group consisted of 52 women with ovarian cysts, with no evidence of malignancies, endometriosis or pelvic adhesions. In all of the groups, the diagnosis of benign ovarian tumors was confirmed histologically. The control group consisted of generally healthy hospital personnel.

Samples. Patient charts were reviewed to obtain data regarding age, diagnosis, histology, grade, International Federation of Gynecologists and Obstetricians (FIGO) stage, presence or absence of ascites, and operative findings. All of the patients were surgically staged according to the FIGO staging system. The pathology for all patients with cancer was reviewed by a gynecological pathologist. After clarification of the samples by centrifugation at 2000 g for 10 min, the supernatants were stored at –40°C until assayed.
Clinical significance of serum growth-regulated oncogene α (GROα) in patients with gynecological cancer

**GROα-Immunoassay.** Amounts of GROα in the serum were determined with a GROα-Immunoassay kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer’s protocol. Samples from all patients were measured in parallel and in duplicate to control for interassay variance. The optical density of each well was measured at dual wavelengths of 450/570 nm. Concentrations of GROα were calculated by interpolation from a standard curve. The sensitivity of the GROα ELISA was 16 pg/ml.

**Statistical Analyses.** Calculated values were expressed as medians and interquartile ranges. All analyses were performed in the R statistical computing environment for Windows (version 2.6) [19]. All data were analyzed with the Mann-Whitney and Kruskal-Wallis non-parametric tests, a p value < 0.05 being regarded as statistically significant. Logistic regression models were used to analyze the influence of serum GROα levels on the probability of malignancy. Using the logistic regression models, sensitivity and specificity were calculated for each possible threshold value of the estimated probability of malignancy. Based on the values, receiver operator characteristic (ROC) curves were constructed to visualize the relationship among gynecological cancers [20].

**Results**

The cervical cancer group (n = 46, 51 ± 2.0; mean age ± SEM), endometrial cancer group (n = 39, 60 ± 2.4), ovarian cancer group (n = 124, 56 ± 1.3), benign ovarian tumor group (n = 52, 51 ± 2.5) and normal control group (n = 38, 45 ± 1.5) were matched for age. Serum samples from the women with ovarian cancers (n = 124) contained significantly higher concentrations of GROα (median 142 pg/ml, interquartile range 43 - 756 pg/ml) than those of the group with benign ovarian tumors (99 pg/ml, 44 - 345 pg/ml) by the Mann-Whitney U-test (p = 0.0004, Table 1 and Figure 1).

Table 1 shows descriptive statistics for preoperative GROα values by patient characteristics among those with gynecological cancers. The GROα levels were significantly higher in all gynecological cancer patients as compared to the controls. The ovarian cancer patients were staged from I to IV depending on the severity of disease based on the FIGO classification. The stage distribution among gynecological cancer patients and associated serum GROα levels (pg/ml) are shown in Table 2. In ovarian cancer, studying the differences of GROα levels based on histology (serous n = 62; non-serous n = 62; mucinous n = 22, endometrioid n = 7, clear cell n = 17), higher concentrations of GROα were observed in the patients with endometrioid carcinoma (n = 7) than the other non-serous carcinoma (n = 55, p = 0.048) or the others (n = 117, p = 0.049). At a cutoff GROα level of 170 pg/ml, the GROα levels in endometrioid carcinoma patients were detected with a sensitivity of 86% as compared to the other carcinoma and benign ovarian tumor patients.

To evaluate the utility of preoperative GROα levels in predicting malignancy, sensitivity and specificity calculations were performed for various cutoff values of GROα in predicting malignancy using all of the patients. In the ROC curves of cervical cancer, endometrial cancer, ovarian cancer, or benign ovarian tumor versus controls, the area under the curve (AUC) was examined [20]. Using GROα levels, the ROC curves of ovarian cancer (AUC = 0.749), cervical cancer (AUC = 0.775), endometrial cancer (AUC = 0.799), and benign ovarian tumors (AUC = 0.568) are shown in Figure 2. In ROC curves of ovarian cancer of endometrioid type versus non-endometrioid ovarian cancer and benign ovarian tumor, the AUC showed high values using GROα levels (AUC = 0.833). In ovarian tumors, at a cutoff GROα level of 110

In endometrial cancer, the serum levels of GROα were not associated with age, histological types and stage. Also there were no significant differences associated with age, histological types and stage in serum GROα levels of cervical cancer patients.

In ovarian cancer, studying the differences of GROα levels based on histology (clear cell n = 17), higher concentrations of GROα were observed in the patients with endometrioid carcinoma (n = 7) than the other non-serous carcinoma (n = 55, p = 0.048) or the others (n = 117, p = 0.049). At a cutoff GROα level of 170 pg/ml, the GROα levels in endometrioid carcinoma patients were detected with a sensitivity of 86% as compared to the other carcinoma and benign ovarian tumor patients.

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**Table 1.** Characteristics of gynecological cancer patients and associated serum GROα levels (pg/ml).

<table>
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<tr>
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<th>n</th>
<th>Median</th>
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**Table 2.** Characteristics of ovarian cancer patients and associated serum GROα levels (pg/ml).

<table>
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<td>118</td>
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</tr>
<tr>
<td>Clear cell</td>
<td>16</td>
<td>186</td>
<td>59-571</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>144</td>
<td>43-756</td>
</tr>
</tbody>
</table>
levels without a combination of CA125 could also be useful in distinguishing ovarian tumor cases from malignancy. CA125 has been shown to contribute to the early diagnosis of epithelial cancer [18, 19, 22]. However, only about 50% of early-stage ovarian cancers will be associated with elevated serum CA125 [23]. Elevation in serum GRO \( \alpha \) is likely to be an early event during the development of ovarian cancer of endometrioid type, although the number of serum from the patients was too small. The findings indicate that GRO \( \alpha \) might be a potentially useful biomarker for endometrioid type in distinguishing women with ovarian cancer from benign ovarian tumor patients.

Recent findings suggest that a panel of four serum biomarkers (apolipoprotein A-1, transthyretin, transferring and CA125) effectively detected early-stage ovarian cancers with the highest reported overall sensitivity of 96% [24]. They also reported that endometrioid tumors were detected at early-stages with a sensitivity of 98%. Moore et al. reported utility of a serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus [25]. Our present data suggest that preoperative serum GRO \( \alpha \) levels might have a role in the diagnostic clinical setting for discerning benign from gynecological cancers, especially ovarian cancer of endometrioid type.

GRO \( \alpha \) promotes chemoattraction, wound healing and angiogenesis through the seven-transmembrane G-protein-coupled receptor CXCR2 [26]. Recent data suggest that CXCR2 regulates the cell cycle, apoptosis and angiogenesis through multiple signaling pathways, including mitogen-activated protein kinase and NF-κB, in ovarian cancer [27]. CXCR2 thus has potential as a therapeutic target and for use in ovarian cancer.

In conclusion, GRO \( \alpha \) might play a role in oncogenesis development and metastasis of gynecological cancer, and
can be readily detected in the serum of these patients. This study has shown that GROα detection by ELISA as a molecular marker can contribute to detection and diagnosis of gynecological cancer, especially for ovarian cancer of endometrioid type. Further studies will be performed to assess how GROα production is basically regulated and to evaluate GROα and its receptor CXCR2 as a diagnostic and prognostic marker for gynecological cancer. Proof of the effectiveness will require a large prospective study in the future.

Acknowledgement

This work was supported in part by a program for developing the supporting system to upgrade education and research by the Ministry of Education, Culture, Sports, and Technology of Japan (to N.S.).

References


Address reprint requests to:

N. SUZUMORI, M.D.
Department of Obstetrics and Gynecology
Nagoya City University Graduate School of Medicine, 1 Kawaumi
Mizuho-cho, Mizuho-ku
Nagoya 467-8601 (Japan)
e-mail: og.n.suz@med.nagoya-cu.ac.jp
Correlation between preoperative endometrial sampling and final endometrial cancer histology

O. Sany, K. Singh, S. Jha
Pan-Birmingham Gynaecology Cancer Centre, Sandwell and West Birmingham NHS Trust (UK)

Summary
Objective: We conducted a study to evaluate the correlation between pre-operative endometrial sampling to the final endometrial cancer histology, in particular the non-endometrioid subtypes. Methods: This involved 191 hysterectomy specimens of patients undergoing treatment at the Pan-Birmingham Gynaecological Regional Cancer Centre (BGCC) over a two-year period (2006-2007). Results: The majority of the patients in this study were found to have endometrioid histology subtype (140/191, 73%). However, the non-endometrioid histologic subtypes were well presented in our population (51/191, 27%). We found good correlation for endometrioid histology subtype (78%) and certain types of the non-endometrioid cell types (carcinosarcoma 90%, uterine papillary serous carcinoma 67%, clear cell carcinoma 67%) but poor in sarcomas (40%). Our results also demonstrated that both pre-operative endometrial sampling methods (curettage and pipelle biopsy) were reliable in identifying endometrioid and non-endometrioid cancer cell types, with sensitivities of 96.5% and 86.5%, respectively. Conclusion: We concluded that preoperative endometrial sampling had good overall histological correlation to hysterectomised corpus specimen. This is especially so for the endometrioid and certain subtypes of the non-endometrioid endometrial cancer cells.

Key words: Complex atypical hyperplasia; Endometrial carcinoma; Endometrial sampling; Post menopausal bleeding.

Introduction
Endometrial assessments by means of pipelle biopsy or curettage are useful procedures in the assessment of patients with suspected endometrial cancer. Very few studies in the literature have evaluated the accuracy of histological tissue obtained by these techniques to the final endometrial cancer histology. These studies were limited by small numbers of patients and most of the studies do not make reference to patients with non-endometrioid histology, which generally carries a much poorer prognosis.

Material and Methods
One hundred and ninety-one hysterectomy specimens of patients undergoing treatment at the Pan-Birmingham Gynaecological Cancer Centre (BGCC) were analysed and reported for the final histology subtypes. Such patients were included in the study.

Preoperative endometrial sampling histology was retrospectively obtained from each respective referring hospitals where the patients had initially presented. These were classified into complex atypical hyperplasia (CAH) endometrioid adenocarcinoma (EA), clear cell carcinoma (CCC), uterine papillary serous carcinoma (UPSC), carcinosarcoma (CS), sarcoma and unclassified. Post-operative hysterectomy specimens were categorized as EA, CCC, UPSC, CS and sarcoma. The non-endometrioid group included CCC, UPSC, CS and sarcoma.

The concordance rate for each histological subtype was calculated. The sensitivity and positive predictive value (PPV) for preoperative sampling for both endometrioid and non-endometrioid histology were also determined.

Results
The majority of the patients in this study were found to have endometrioid histology subtype (140/191, 73%). However, the non-endometrioid histologic subtypes were well presented in this population (51/191, 27%). This group can be further divided into the following respectively: CS in 31 patients (16%), UPSC in nine patients (5%), CCC in six patients (3%) and five patients had sarcoma (2%) (Table 1).

The overall concordance rate between the initial pre-operative endometrial sampling and the final hysterectomy histology was 78% (149/191). Of these, EA accounted for 109 patients (57%), CS in 28 patients (15%), UPSC in six patients (3%), CCC in four patients (2%), and sarcoma in two patients (1%) (Table 2).

When the concordance rate for each respective histology subtype was analysed, the results were as follows: 78% in EA (109/140), 90% in CS (28/31), 67% in UPSC (6/9), 67% in CCC (4/6) and 40% in sarcomas (2/5) (Table 1).

The overall non-concordance rate between the initial pre-operative endometrial sampling and the final hysterectomy histology was 22% (42/191). Of these, CAH accounted for the majority with 22 patients (11.5%), followed by non-endometrioid type in 12 patients (6.3%), endometrioid type in four patients (2.1%) and the remaining were unclassified (2.1%) (Table 3).

The sensitivity for pre-operative endometrial sampling to accurately detect endometrioid or non-endometrioid subtypes were 96.5% and 86.5%, respectively (Table 4). The corresponding PPVs were 93.9 and 91.8, respectively.
Correlation between preoperative endometrial sampling and final endometrial cancer histology

<table>
<thead>
<tr>
<th>Final Sampling</th>
<th>EA</th>
<th>CS</th>
<th>UPSC</th>
<th>CCC</th>
<th>Sa</th>
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<tr>
<td>EA</td>
<td>109</td>
<td>2</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>UPSC</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>CCC</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CAH</td>
<td>22</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unclassified</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total (n)</td>
<td>140</td>
<td>31</td>
<td>9</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Correlation (%)</td>
<td>77.8</td>
<td>90.0</td>
<td>66.7</td>
<td>66.7</td>
<td>40.0</td>
</tr>
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</table>

Table 2. — Concordance rate.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Concordance overall:</td>
<td>149</td>
<td>78.0</td>
</tr>
<tr>
<td>Concordance by preoperative histology:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>109</td>
<td>57.0</td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>28</td>
<td>14.7</td>
</tr>
<tr>
<td>Uterine papillary serous carcinoma</td>
<td>6</td>
<td>3.1</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 3. — Non concordance rate.

<table>
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<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-concordance overall:</td>
<td>42</td>
<td>22.0</td>
</tr>
<tr>
<td>Non-concordance by preoperative histology:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complex atypical hyperplasia</td>
<td>22</td>
<td>11.5</td>
</tr>
<tr>
<td>Non-endometrioid</td>
<td>12</td>
<td>6.3</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>4</td>
<td>2.1</td>
</tr>
<tr>
<td>Unclassified</td>
<td>4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 4. — Preoperative sampling method.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity of:</td>
<td></td>
</tr>
<tr>
<td>detecting endometrioid subtype</td>
<td>96.5</td>
</tr>
<tr>
<td>detecting non-endometrioid subtype</td>
<td>86.5</td>
</tr>
<tr>
<td>Positive predictive value of:</td>
<td></td>
</tr>
<tr>
<td>detecting endometrioid subtype</td>
<td>93.9</td>
</tr>
<tr>
<td>detecting non endometrioid subtype</td>
<td>91.8</td>
</tr>
</tbody>
</table>

Discussion

The BGCC is the largest of four regional centres that cover the whole of the West Midlands area. These include the Birmingham Women’s Healthcare NHS Trust (Central and South Birmingham), Good Hope Hospital (North Birmingham) Heartlands Hospital (East Birmingham) and Walsall Manor Hospital (Walsall). The approximate catchment population of the BGCC is two million although it has been difficult to accurately establish this because of extensive cross-border flow. In addition the centre also receives tertiary referral from outwith the network.

To our knowledge, there has not been a study that evaluated the correlation between histological tissues obtained pre-operatively to the final endometrial cancer specimen histology. Most of the studies in the literature do not make reference to patients with non-endometrioid either, which generally carries a much poorer prognosis [1].

For many years, endometrial curettage and pipelle biopsy have been the methods of choice in assessing abnormal uterine bleeding, the latter being a minimally invasive method which is believed to reduce the costs of a diagnostic work up.

Our results demonstrated that both preoperative endometrial sampling methods (curettage and pipelle biopsy) were sensitive in detecting endometrial cancers. This was particularly so in identifying the endometrioid (96.5%) and to a lesser extent, the non-endometrioid histology subtypes (86.5%). This is in line with a meta-analysis which reports the accuracy of the pipelle biopsy in detecting cancer to be between 91% and 99% [2].

When we analysed each histology subtypes in turn, our study showed that the overall correlation between the initial preoperative endometrial sampling and the final hysterectomy histology to be 78%. The majority of this correlation was of the EA cell type, which showed a correlation rate of 78%. These are cells composed of malignant glandular epithelial elements [3].

Our results also showed that certain types of the non-endometrioid cell types showed good correlation (CS 90%, UPSC 67%, CCC 67%, respectively) but poor in sarcomas (40%). We do acknowledge that in our study, the numbers of patients with these non-endometrioid cell types were smaller than the endometrioid subtypes. Therefore care should be taken when interpreting these results. In addition, non-endometrioid cells are more difficult to ascertain histologically as they do often overlap in their cell characteristics.

Non-endometrioid type endometrial cancers also collectively carry poorer prognosis and have greater propensity for extrapelvic metastases. For example, UPSC is an aggressive variant of endometrial cancer, and is found in 5% of cases. Even with surgical Stage I cancer, the 5-year survival rate is 60%. UPSC resembles papillary serous carcinoma of the ovary and the fallopian tube histologically. Although adjuvant chemotherapy is helpful, UPSC does not have the same duration of response to cytotoxic agents (e.g., paclitaxel, carboplatin) as its ovarian counterpart [4].

Clear cell carcinoma is another variant of endometrial carcinoma characterised by its aggressive behaviour. It makes up about 3-6% of all endometrial carcinomas. The 5-year survival rate associated with these tumours is 45-60% [5]. Often, elements of clear cell carcinoma are associated with UPSC [4].

Carcinosarcomas or homologous mixed mullerian tumors typically have an endometrioid carcinoma, usually a higher grade, and an undifferentiated spindle cell sarcoma. These tumours can have a recurrence rate of up to 50% but demonstrate indolent growth and late recurrences. Likewise, sarcomas have a high metastatic poten-
tial and the histopathologic diagnosis can be unclear until time of definitive surgery [6].

With respect to the non-concordance results, our study showed that 52% (22/42) of those with an endometrioid type as a final histology had complex atypical hyperplasia at pre-operative sampling. This is in line with the original study by Kurman et al. that found when left untreated, complex atypical hyperplasia had a 29% rate of progression to cancer [7]. Numerous studies had also found concurrent carcinoma with these biopsies, at rates ranging from 17-52% [8, 9]. For these reasons, it is best practice to clinically treat patients with complex atypical hyperplasia similarly as per those with endometrioid adenocarcinoma histology.

Conclusions

Our findings have shown that both endometrial curette and pipelle biopsy are sensitive methods in identifying both endometrioid and non-endometrioid cancer cell types.

The data suggests that our preoperative endometrial sampling had a good overall correlation to the histological subtype of endometrial cancer in the hysterectomised corpus specimen. This is especially so for the endometrioid and certain subtypes of the non-endometrioid endometrial cancer cells.

As more than a quarter of patients were found to have non-endometrioid cancer subtypes, which generally have a propensity for extrapelvic metastases, such findings would substantiate the concept of extended surgical staging, and involvement of a gynaecology oncologist in these patients’ management.

References


Address reprint requests to:
O. SANY, M.D.
Pan-Birmingham Gynaecology Cancer Centre
Sandwell and West
Birmingham NHS Trust (UK)
e-mail: omarsany@hotmail.com
The association between polymorphisms of the RAD51-G135C, XRCC2-Arg188His and XRCC3-Thr241Met genes and clinico-pathologic features in breast cancer in Poland

H. Romanowicz-Makowska¹, B. Smolarz¹, M. Zadrożny², B. Westfal², J. Baszczyński³, G. Kokolaszwili³, M. Burzyński³, I. Połać⁴, S. Sporny⁵

¹Laboratory of Molecular Genetics, Department of Pathology, Institute of Polish Mother’s Memorial Hospital, Lodz
²Department of Oncology, Institute of Polish Mother’s Memorial Hospital, Lodz;
³Department of Obstetrics and Gynecology, Regional Hospital in Lowicz
⁴Department of Menopausal Diseases, Institute of Polish Mother’s Memorial Hospital, Lodz
⁵Department of Pathology, Medical University of Lodz (Poland)

Summary

Background: XRCC2 and XRCC3 genes are structurally and functionally related to RAD51 which plays an important role in homologous recombination, the process frequently involved in cancer transformation. Material and Methods: In the present work the distribution of genotypes and frequency of alleles of the RAD51 G135C polymorphism, XRCC2 Arg188His and XRCC3 Thr241Met polymorphism in 790 cases of breast cancer were investigated. The control group consisted of 798 cancer-free blood donors (age ± 5 years) who were sex and ethnicity-matched. The polymorphisms were determined by PCR-RFLP methods. We also correlated genotypes with the clinical characteristics of breast cancer patients. Results: Our results obtained for the 135G>C polymorphism of the RAD51 gene indicated that both the C/C genotype and the C allele are strongly associated with breast cancer. The Arg/His genotype of XRCC2 (OR = 2.16, 95% CI = 1.48-3.16) and Thr/Met of XRCC3 increased the risk of type I breast cancer occurrence (OR = 2.33, 95% CI = 1.60-3.41). We did not find any association with the RAD51, XRCC2/3 gene polymorphism and estrogen and progesterone receptor status. Conclusion: The results support the hypothesis that the polymorphism of RAD51 and XRCC2/3 gene may be associated with the incidence of sporadic breast cancer in Polish women.

Keywords: RAD51; XRCC2; XRCC3; Breast cancer; Gene polymorphism.

Introduction

Breast cancer is one of the major killers worldwide. In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women). The risk of breast cancer is increased by several factors such as sex, age, lack of childbearing or breastfeeding, higher hormone levels, race, economic status and gene mutations [1, 2]. There is growing evidence that human cancer can be induced by DNA double strand breaks (DSBs) [3]. A double strand break (DSB) is the most lethal of all DNA lesions. If unrepaired, a DSB leads to loss of chromosome segments and threatens the survival of the cell [4, 5].

DSB in DNA are repaired by two major mechanisms: homologous recombination (HR) and nonhomologous end joining (NHEJ) [6, 7]. The RAD51, XRCC2 and XRCC3 proteins are core components of DNA double strand breaks (DSBs) repair by HRR. XRCC2 and XRCC3 genes are structurally and functionally related to the RAD51 gene [8, 9].

A large number of molecular epidemiologic studies have been performed on various neoplasms, such as cancer of the breast, lung, ovarian, bladder, head and neck and skin to evaluate the role of XRCC2, XRCC3 and RAD51 polymorphisms [10-16].

The G135C, polymorphism in the 5 UTR of the RAD51 gene has been reported to be associated with altered gene transcription [17]. Two researchers showed previously that this polymorphism was not an independent marker in breast cancer, but it could be associated with an increased gastric cancer risk and an increased breast cancer risk in BRCA2 mutation carriers [18, 19]. Similar results came from other laboratories [20, 21]. A relatively rare polymorphism in the XRCC2 gene, a G>A transition resulting on Arg to His substitution at codon 188 was found to be related with breast cancer, but not with bladder cancer, colorectal adenoma, skin cancer and endometrial carcinoma [10, 22-26]. Recently, many studies have shown that the XRCC3 Thr241Met genotype has been linked to an increased risk of breast, colorectal, bladder, and head and neck cancer [27-30]. The literature data suggest that the identification of new risk factors for breast cancer in the population of women is urgently needed, and an analysis of some gene polymorphisms could be an interesting option.

Therefore in the present work the association between polymorphisms in three genes involved in the homologous recombination of double-strand breaks: RAD51 5’ untranslated region G135C, X-ray repair cross-complementing group 2 (XRCC2) Arg188His, and XRCC3 Thr241Met and breast cancer risk in polish women was investigated.
Materials and Methods

Clinicopathologic data and genotyping

Blood was obtained from 790 women of Caucasian ethnicity treated at the Department of Oncology, Institute of Polish Mother’s Memorial Hospital, Lodz, Poland (from 2000 to 2010). The mean age of the 790 patients at the time of diagnosis was 59 years (range 31-76). The controls were somewhat younger: mean age 54 years (range 44-86). No significant difference was observed in age distribution between the breast cancer patients and healthy controls. The gender distribution in breast cancer patients was also similar to that in the healthy controls.

The diagnosis of cancer was made after histopathological examination of patients’ biopsies. Patients with breast cancer involved in the study were analyzed according to TNM Classification of Malignant Tumors which describes the extent of cancer in a patient’s body: T describes the size of the tumor and whether it has invaded nearby tissue, N describes regional lymph nodes that are involved and M describes distant metastasis (spread of cancer from one body part to another). There were 320 women with node-negative and 380 with node-positive ductal breast carcinoma. No distant metastases were found in patients at the time of treatment. Median follow-up of patients still at the time of analysis was 39 months (range: 2-71 months).

The average tumor size was 20 mm (range 17-32 mm). All tumors were graded by a method based on the criteria of Scarf-Bloom-Richardson. The demographic and pathologic features of patients are summarized in Table 1. All subjects involved in the study were unrelated Caucasians and resided in the Lodz district, Poland. The study was approved by the Local Ethics Committee and written consent from each patient or healthy blood donor was obtained before participating in the study.

Determination of RAD51 genotype

RAD51 genotyping was analyzed by PCR amplification of a 175-bp region around nucleotide 135. This region contained a single Mvai site that was abolished in the 135C allele. Wild type alleles were digested by Mvai resulting in 86- and 71-bp product. The 135C allele was not digested by the enzyme, resulting in a single 157-bp product. The RAD51 genotype was analyzed using the specific primers forward 5’ TGG GAA CTG CAA CTC ATC TGG 3’ and reverse 5’ GCG CTC CTC TCT CCA GCAG 3’.

PCR was carried out in a GeneAmp PCR system 9700 (Applied Biosystems) thermal cycler. PCR amplification was performed in a final volume of 25 l. The reaction mixture contained 5 ng genomic DNA, 0.2 mol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstadt, Germany), 2.5 mM MgCl2, 1 mM dNTPs and 1 unit of Taq polymerase (Qiagen GmbH, Hilden, Germany). The PCR cycle conditions were 94°C for 60 sec, 54°C for 30 sec then 72°C for 40 sec, repeated for 35 cycles. After digestion with Mvai for 4h at 37°C (Qiagen GmbH, Hilden, Germany). The PCR cycle conditions were 94°C for 60 sec, 54°C for 30 sec then 72°C for 40 sec, repeated for 35 cycles. After digestion with Mvai for 4h at 37°C.

The wild-type allele Arg was identified by the presence of two 290 bp bands, while the mutant allele His was represented by 148, and 142 bp bands.

Determination of XRCC2 genotype

Polymorphism of the XRCC2 gene was determined by PCR-RFLP, using primers: forward 5’TGTAGTCACC-CATCTCTCTGGC3’ and reverse: 5’AGTTGCTGCCTATGCCT-TACA3’. The 25 µl PCR mixture contained about 100 ng of DNA, 12.5 pmol of each primer, 0.2 mmol/l of dNTPs, 2 mmol/l of MgCl2 and 1 U of Taq DNA polymerase. The 290 bp amplified product was digested overnight with 1 U of HpaII at 37°C. The wild-type allele Arg was identified by the presence of two 290 bp bands, while the mutant allele His was represented by 148, and 142 bp bands.

Table 1. — Pathologic features of patients with breast cancer.

<table>
<thead>
<tr>
<th>Breast cancer</th>
<th>Patients (n = 790)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal carcinoma</td>
<td>790</td>
<td>100</td>
</tr>
<tr>
<td>Scarf-Bloom-Richardson stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>230</td>
<td>29</td>
</tr>
<tr>
<td>II</td>
<td>480</td>
<td>64</td>
</tr>
<tr>
<td>III</td>
<td>80</td>
<td>7</td>
</tr>
<tr>
<td>Tumor size grade</td>
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<td></td>
</tr>
<tr>
<td>T1</td>
<td>110</td>
<td>11</td>
</tr>
<tr>
<td>T2</td>
<td>430</td>
<td>57</td>
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<td>T3</td>
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<tr>
<td>Negative</td>
<td>320</td>
<td>59</td>
</tr>
</tbody>
</table>

Data in boldface are statistically significant. <Crude odds ratio (OR), 95% CI = confidence interval at 95%; Chi square.

Table 2. — Distribution of G/G, G/C and C/C genotypes and frequencies of the G and C alleles of RAD51 G135C polymorphism in patients with breast cancer (n = 790) and controls (n = 798).

<table>
<thead>
<tr>
<th>Breed cancer patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>160</td>
<td>20</td>
</tr>
<tr>
<td>G/C</td>
<td>104</td>
<td>13</td>
</tr>
<tr>
<td>C/C</td>
<td>526</td>
<td>67</td>
</tr>
<tr>
<td>G</td>
<td>424</td>
<td>27</td>
</tr>
<tr>
<td>C</td>
<td>1156</td>
<td>73</td>
</tr>
</tbody>
</table>

Determination of XRCC3 genotype

Polymorphism of the XRCC3 gene was determined by PCR-RFLP, using codon 241 primers (5’-GCCTGGTGGTCATC-3’).
The association between polymorphisms of the RAD51-G135C, XRCC2-Arg188His and XRCC3-Thr241Met genes and breast cancer.

### Immunohistochemistry

Tumor tissue biopsies were fixed in formalin and embedded in paraffin according to standard procedures. Immunohistochemistry staining was performed as described by Sannino and Shousha [31]. The primary antibodies against ER (clone 1D5), PR (clone 1A6) and HER-2 (polyclonal anti-human HER-2) were purchased from DAKO Corporation (CA, USA).

### Statistical analysis

For each polymorphism, deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium was assessed using the standard \( \chi^2 \)-test. Genotype frequencies in cases and controls were compared by \( \chi^2 \)-tests. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% confidence intervals (CIs) by unconditional logistic regression. \( P \)-values \( < 0.05 \) were considered to be significant.

### Results

All the recruited samples were successfully genotyped for the RAD51 polymorphism. Genotype and allele frequencies for cases and controls are presented in Table 2. There were significant differences \( (p < 0.05) \) between the two investigated groups. The breast cancer showed an incidence of 20, 13, and 67%, respectively, for the G/G, G/C, and C/C genotypes of the RAD51 gene, whereas the control group showed 26, 53, and 21% for the same genotypes. In patients the observed frequencies of the G/G, G/C and C/C genotypes differed significantly \( (p < 0.05) \) from the distribution expected from the Hardy-Weinberg equilibrium. The C/C genotype frequency was statistically significant with an OR of 4.16 and 95% CI of (3.18-5.46) (Table 2). Variant 135C allele of RAD51 increased cancer risk \( [OR = 3.04 (2.62-3.53)] \). Table 5.

### No statistically significant differences were observed in the alleles or in the genotype frequencies of the XRCC2 Arg188His gene polymorphisms between the control group and breast cancer patients (Table 3).

However, a weak association between breast cancer occurrence and Met/Met genotype \( [OR = 0.72 (0.55-0.95)] \) and the Met allele \( [OR = 0.84 (0.57-0.97)] \) of XRCC3 Thr241Met was observed. The increase was statistically significant \( (p < 0.05 \) for both) (Table 4).

### Clinical pathological parameters

The histological stage was evaluated in all cases \( (n = 790) \). Stage II and III were grouped together for the purposes of statistical analysis (Table 5). There was a correlation between genotypes of the two polymorphisms XRCC2-Arg188His and XRCC3-Thr241Met and breast cancer invasiveness. We found statistically significant increase of His/His \( [OR = 0.55 (0.33-0.92)] \) and Met/Met homozygotes frequency \( [OR = 0.53 (0.31-0.89)] \) in the Stage I group by the Scarff-Bloom-Richardson classification. The higher risk of breast carcinoma of Stage 1 occurrence was associated with the Arg/His \( [OR = 2.16 (1.48-3.16)] \) and Thr/Met \( [OR = 2.33 (1.60-3.41)] \), Table 6.

### Conclusion

The distribution of genotypes and frequency of alleles in patients with lymph node metastasis (N+) and without (N-) is displayed in Table 6. We did not observe any differences in the distribution of genotypes of investigated polymorphisms between these groups. Additionally, there was no difference in distribution of genotypes and frequency of alleles in group of patients with different tumor sizes.
The association between polymorphism can modify the effect of polymorphisms of DNA. The formation of RAD51 foci represents an important step in the repair of DNA double-strand breaks. In the repair of DNA double-strand breaks (DSBs), the most dangerous damage to DNA, the concentration of the final product-the RAD51 protein. RAD51 takes part in the repair of DNA double strand breaks (DSBs), the most dangerous damage to DNA. The formation of RAD51 foci represents an important step in the repair of DNA double-strand breaks.

Recently, several studies have shown that the 135G>C polymorphism can modify the effect of polymorphisms of the BRCA2 and XRCC3 genes on breast cancer occurrence [32-35].

In the literature the 135G/C polymorphism of RAD51 gene may be identified as a susceptibility locus for breast cancer [36, 37].

Hormone receptor status

Distributions of genotypes of the three polymorphisms of DNA repair gene for cancer patients with different hormone receptor status is shown in Table 7. We did not find any association with the RAD51, XRCC2/3 gene polymorphism and estrogen and progesterone receptor status for breast cancer patients.

Discussion

The present study examined whether polymorphism Arg188His of XRCC2, Thr241Met of XRCC3 and 135G/C of RAD51 gene is related to the development of breast cancer. We found an association between breast cancer and three investigated polymorphisms in this study population. The single nucleotide polymorphism (SNP) has been studied as a risk factor for various cancers [10-16].

The polymorphisms chosen for this study have been shown to have functional significance and may be responsible for a low DNA repair capacity phenotype characteristic of cancer patients including breast carcinoma. A single nucleotide polymorphism, 135G/C, is located in the 5'-untranslated region of the RAD51 gene, so it could affect the gene expression and, as a consequence, alter the concentration of the final product-the RAD51 protein. RAD51 takes part in the repair of DNA double strand breaks (DSBs), the most dangerous damage to DNA. The formation of RAD51 foci represents an important step in the repair of DNA double-strand breaks.

Recently, several studies have shown that the 135G>C polymorphism can modify the effect of polymorphisms of the BRCA2 and XRCC3 genes on breast cancer occurrence [32-35].

In the literature the 135G/C polymorphism of RAD51 gene may be identified as a susceptibility locus for breast cancer [36, 37].

Table 6. — RAD51, XRCC2 and XRCC3 gene polymorphism and breast cancer progression.

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>Node status</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 T2 N = 250</td>
<td>T1 T2 N = 540</td>
</tr>
<tr>
<td>RAD51 G135C</td>
<td>Number (%)</td>
</tr>
<tr>
<td>G/G 57 (22%)</td>
<td>103 (20%)</td>
</tr>
<tr>
<td>G/C 33 (11%)</td>
<td>71 (12%) 0.83 (0.49-1.41)</td>
</tr>
<tr>
<td>C/C 160 (67%)</td>
<td>366 (68%) 0.79 (0.54-1.44)</td>
</tr>
<tr>
<td>G 147 (29%)</td>
<td>277 (26%) 1.00 Ref.</td>
</tr>
<tr>
<td>C 353 (71%)</td>
<td>803 (74%) 0.82 (0.65-1.04)</td>
</tr>
</tbody>
</table>

*H. Romanowicz-Makowska, B. Smolarz, M. Zadrożny, B. Westfal, J. Baszczyński, G. Kokołaszwiili, M. Burzyński, I. Pocha, S. Sporny

CC genotype may be associated with an elevated tumor risk in European populations in sporadic breast cancer [36, 38].

Our results is in line with data of other reports introducing the important role of RAD51 G135C polymorphism for breast carcinoma occurrence.

In our work CC genotype was associated with an increased risk for the development of breast cancer compared with the GG and GC genotype. The C allele also increased the risk of breast cancer compared with the G allele.

In our earlier study RAD51 G135C polymorphism was not related to breast cancer occurrence. The reason for this could be the relatively small group of patients enrolled in our study [39].

We also analyzed the distribution of genotypes and frequency of alleles in groups of patients suffering from breast cancer according to different cancer staging by the Scarf-Bloom-Richardson classification. The heterozygote Thr241Met genotype was associated with type I breast cancer. The similar relationships for Arg188His allele.

For Arg188His allele, the combined Thr241Met-XRCC3/135G/C-RAD51 genotype was slightly increased the risk of local metastasis in breast cancer patients [41, 42]. Moreover, the combined Thr241Met-XRCC3/135G/C-RAD51 genotype decreased the risk of breast cancer occurrence [41].

Similarly to our observation, according to recent reports, the XRCC3 Thr241Met allele seems to be associated with elevated breast cancer risk in non-Chinese subjects [43].

The role of position 188 in the functioning of the XRCC2 protein is unknown. Indeed, very few sites of functional significance within the protein have been iden-
The association between polymorphisms of the RAD51-G135C, XRCC2-Arg188His and XRCC3-Thr241Met genes and et al.

<table>
<thead>
<tr>
<th>Stage*</th>
<th>ER− (n = 340)</th>
<th>ER+ (n = 450)</th>
<th>OR (95% CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD51 G135C</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>70 (13%)</td>
<td>90 (20%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>50 (5%)</td>
<td>54 (12%)</td>
<td>1.19 (0.72-1.95)</td>
<td>0.571</td>
</tr>
<tr>
<td>C/C</td>
<td>220 (82%)</td>
<td>306 (68%)</td>
<td>0.92 (0.64-1.32)</td>
<td>0.729</td>
</tr>
<tr>
<td>G</td>
<td>190 (28%)</td>
<td>234 (26%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>490 (72%)</td>
<td>666 (74%)</td>
<td>0.90 (0.72-1.13)</td>
<td>0.420</td>
</tr>
<tr>
<td>XRCC2 Arg188His</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>94 (28%)</td>
<td>116 (25%)</td>
<td>1.09 (0.73-1.60)</td>
<td>0.740</td>
</tr>
<tr>
<td>Arg/His</td>
<td>152 (46%)</td>
<td>222 (50%)</td>
<td>0.85 (0.61-1.16)</td>
<td>0.324</td>
</tr>
<tr>
<td>His/His</td>
<td>310 (50%)</td>
<td>442 (49%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>XRCC3 Thr241Met</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>99 (29%)</td>
<td>121 (27%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>Thr/Met</td>
<td>151 (44%)</td>
<td>227 (50%)</td>
<td>0.83 (0.59-1.16)</td>
<td>0.324</td>
</tr>
<tr>
<td>Met/Met</td>
<td>331 (49%)</td>
<td>431 (48%)</td>
<td>1.02 (0.89-1.33)</td>
<td>0.324</td>
</tr>
</tbody>
</table>

* p = 0.05, according to Scarf-Bloom-Richardson criteria; Crude odds ratio (OR), 95% CI.

Table 7. — Genotype distribution and odds ratios (OR) of the RAD51, XRCC2 and XRCC3 polymorphism gene for breast cancer patients with different hormone receptor status.

<table>
<thead>
<tr>
<th>Stage*</th>
<th>ER− (n = 340)</th>
<th>ER+ (n = 450)</th>
<th>OR (95% CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD51 G135C</td>
<td>Number (%)</td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>62 (20%)</td>
<td>98 (20%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>39 (13%)</td>
<td>65 (14%)</td>
<td>0.94 (0.79-1.19)</td>
<td>0.841</td>
</tr>
<tr>
<td>C/C</td>
<td>201 (67%)</td>
<td>317 (66%)</td>
<td>0.96 (0.72-1.45)</td>
<td>0.887</td>
</tr>
<tr>
<td>G</td>
<td>250 (50%)</td>
<td>458 (51%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>472 (50%)</td>
<td>699 (73%)</td>
<td>1.04 (0.83-1.27)</td>
<td>0.307</td>
</tr>
<tr>
<td>XRCC2 Arg188His</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>84 (27%)</td>
<td>110 (24%)</td>
<td>1.13 (0.76-1.68)</td>
<td>0.283</td>
</tr>
<tr>
<td>Arg/His</td>
<td>142 (46%)</td>
<td>222 (50%)</td>
<td>0.95 (0.67-1.34)</td>
<td>0.841</td>
</tr>
<tr>
<td>His/His</td>
<td>310 (50%)</td>
<td>472 (52%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>XRCC3 Thr241Met</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr/Thr</td>
<td>99 (29%)</td>
<td>121 (27%)</td>
<td>1.00 Ref.</td>
<td></td>
</tr>
<tr>
<td>Thr/Met</td>
<td>151 (44%)</td>
<td>227 (50%)</td>
<td>0.83 (0.59-1.16)</td>
<td>0.324</td>
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<td>Met/Met</td>
<td>331 (49%)</td>
<td>431 (48%)</td>
<td>1.09 (0.89-1.33)</td>
<td>0.324</td>
</tr>
</tbody>
</table>

* p = 0.05, according to Scarf-Bloom-Richardson criteria; Crude odds ratio (OR), 95% CI.

Finally we suggested that Arg188His, Thr241Met and the G135C polymorphisms may be associated with the occurrence of breast cancer in Poland. Further studies, conducted on a larger group, are required to clarify this point.

References

The association between...risk of breast or ovarian cancer".


Zienolddiny S., Campa D., Lind H., Ryberg D., Skaug V., Stange

et al. (2010) observed a meta-analysis of 21 studies, which showed a significant association between the XRCC2 Arg188His polymorphism and breast cancer risk.


Jacobsen N.R., Nexo B.A., Olsen A., Overvad K., Wallin H., Tjon-


Address reprint requests to: H. ROMANOWICZ-MAKOWSKA, M.D. Laboratory of Molecular Genetics Department of Pathology Institute of Polish Mother’s Memorial Hospital Rzegowska 281/289, 93-338 Lodz (Poland) e-mail: smolbea@wp.pl.
Sentinel node dissection in the treatment of early stages of vulvar cancer

A. García-Iglesias¹, M.O. Rodríguez-Martín¹, R. Ruano¹, D. Beltrán¹, L. Peñalosa¹, B. Hernández-Barreiro¹, A. Martín de Arriba², J.L. Lanchares¹

¹Obstetrics and Gynaecology Department, ²Nuclear Medicine Department, University Hospital of Salamanca, Salamanca
³Madrileño Institute of Public Health, Madrid (Spain)

Introduction

Vulvar cancer is not one of the most common tumours in women [1]. However, if we take into account the characteristics of the patients, in which the age of appearance is usually between the seventh and eighth decades of life, together with the associated medical procedures and the aggressiveness of the surgical treatments that are performed, as well as the complications derived from them, we can observe that there is a high morbidity associated to these types of patients [2]. Prognostic factors for vulvar cancer have been established, not only in order to know the possible evolution of the oncologic process, but also in order to individualise treatment [3]. One of the most important factors in the prognosis of vulvar cancer is lymph node involvement, which leads to large lymphadenectomies being performed in surgery, in order to remove any affected inguinal lymph nodes, because the survival of the patient is determined by the presence or absence of affected unilateral or bilateral lymph nodes [4]. This factor accounts for the fact that morbidity after treatment for conventional vulvar cancer reaches rates of 85%; 69% of the patients can present chronic lymphedema. Identification of the sentinel lymph node (SLN) may reduce the aggressiveness of the surgical treatment, because it is defined as the first node that receives lymphatic drainage in a specific area (the vulva, in this case) [5]. Several techniques (US, CT, NMR), together with puncture cytology [6] have been used to identify the inguinal lymph nodes that have been infiltrated by the tumour.

Material and Methods

We present a retrospective, observational, multicenter study in which 76 patients with initial stages of vulvar cancer were assessed. Histologically, they presented squamous cell carcinomas and melanomas with a maximum diameter of less than 4 cm. The data collection period started in the year 2000, and it ended in 2010 with the control stage. All data were sent to the Department of Gynaecology of the University Hospital of Salamanca, which is considered as a reference centre for the treatment of these processes.

Vulvar cancer was diagnosed with a vulvoscopy and a punch biopsy together with the corresponding imaging tests to assess the extension of the disease, according to current protocols. The study was completed with a preoperative assessment. All patients were informed about the procedure and treatment they were being subjected to, and the corresponding informed consents were signed. Then, the protocol for the location of the SLN was programmed together with the Department of Nuclear Medicine.

Procedure: Early in the morning on the day of the operation,
in the Department of Nuclear Medicine, the radiotracer, 2-3 mCi (71-111 Mbk) Tc99m-colloid rhenium sulphide (nanocis), was injected intradermally in the area of the tumour. Afterwards, anterior and lateral planar images were taken with an Elscint monohead gamma camera (model Apex SP-4HR), and the projection of the SLNs that had been observed were marked on the skin. Then, isosulfan blue or methylene blue vital dye was injected intradermally around the tumour.

When the surgical operation started, the SLN was explored with a small incision on the area that had been previously marked. Detection of the sentinel node was carried out with a gamma detection device model Europrobe, so that the SLN could be identified and subsequently extracted. The location of the SLN was found due to the increase in radiation count with regard to basal levels, and to the blue dye. The SLN was removed separately and it was labelled for the histopathological study. In cases in which the unilateral or bilateral SLN showed a positive result, a lymphadenectomy with separate incisions was performed. The incisions were parallel to the inguinal ligament, and the anatomical margins of the incision were limited by the inguinal ligament, the abductor muscle and the inferolateral sartorial muscle. After the incision, the fascia cribrosa was opened, releasing the femoral vein. The treatment of the vulvar lesion consisted of a radical vulvectomy with margins of 2 cm. Adjuvant radiation therapy was applied in cases of lymph node metastases, according to the protocol of the Department of Radiation Therapy. Inguinal recurrence was monitored, and the operation site was verified in later protocol-guided controls up to 36 months after the operation.

Histopathology: Lymph nodes that were identified as SLNs were sent unfixed to the laboratory. There they were macroscopically assessed, and some of the sections were studied with hematoxylin and eosin (HE). The samples were also subjected to immunohistochemical study.

The statistical study was performed with PASW for PC version 18.

Results

An observational study in T1 and T2 stages was performed. It included 76 patients who presented vulvar cancer, with ages between 65 and 88 years. Of the patients 11.84% were 70 years old or younger, and 88.15% of the patients were over 70 years old. The average age was 76 years (Table 1). The primary tumour was 2cm in diameter or smaller in 36 patients (47.36%), and it was bigger in 40 patients (52.53%). Tumour invasion was less than 3 mm in 33 patients (17.10%), between 3 and 4 mm in 53.94% of the patients, and more than 4 mm in 22 patients (28.93%). The most common location of the tumour was the labia majora, with lateral location in 50 patients (65.58%). The most common central location was the periclitoral region in 26 patients (34.21%). In five patients (6.57%), the tumour was multifocal, and in 71 patients (93.42%), it was unifocal. The histologic study identified 65 tumours as squamous cell carcinoma (85.52%), and 11 lesions (14.47%) were identified as melanomas.

Identification of the SLN was positive and unilateral in 52 patients (68.42%), and was bilateral in 24 patients (31.57%). The treatment was hemivulvectomy in 11 patients (14.47%), vulvectomy in 62 patients (81.57%) and radical vulvectomy in three patients (3.94%). The SLN was identified with Tc99m and blue dye in 61 patients (80.26%), and with Tc99m alone in 15 patients (19.73%). None of the patients had their SLNs identified with dye alone. The total number of identified SLNs was 90 in the 76 patients of the study, including unilateral and bilateral nodes (Table 2).

In the group of lymph nodes that were identified as SLNs, 20 (22.22%) showed a metastasis of the primitive carcinoma, and the remaining 70 SLNs were negative for metastasis (77.77%). There were no false negatives identified, which means that sensitivity was 100% and the negative predictive value (NPV) was also 100 (Table 3).

The distribution of patients for T1 and T2 groups was 24 (31.57%) and 52 (68.42%), respectively. Controlled recurrences after 36 months were observed in three patients. One of them (1.31%) was in the T2 group. She showed a negative SLN and she did not undergo lymphadenectomy. The remaining two patients (2.63%) were also in the T2 group, and they presented metastatic positive SLNs with histology results of melanoma in both cases. The rest of the patients did not show any local or inguinal recurrence (Table 4).
Table 3. — Sentinel lymph node biopsy.

<table>
<thead>
<tr>
<th>Sentinel lymph node biopsy</th>
<th>No. of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metastatic sentinel node</td>
<td>20</td>
<td>22.22</td>
</tr>
<tr>
<td>No metastatic sentinel node</td>
<td>70</td>
<td>77.77</td>
</tr>
<tr>
<td>False negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>–</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4. — Recurrence data. Follow-up: 36 months.

<table>
<thead>
<tr>
<th>Sentinel node detection</th>
<th>No. of patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN (-) + Recurrence</td>
<td>24 (31.57%)</td>
<td>52 (68.42%)</td>
</tr>
<tr>
<td>SN (+) + Recurrence</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>SN = Sentinel node.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The use of non-invasive or microinvasive techniques in the treatment of vulvar cancer has led to an evident improvement in the morbidity rate of patients, compared with the conventional block exeresis of the vulva and the lymph nodes. In these last cases, complications after surgery were common, and they included surgical wound dehiscence, the presence of cellulitis, lymphedema of the lower extremities and prolonged hospital stays as a consequence of radical treatments, plus the appearance of possible complications of medical conditions that were already affecting the patient prior to surgery [9]. For these reasons, biopsy of the SLN is currently a rational method in the treatment of vulvar cancer and vulvar melanoma, because it reduces the aggressiveness of lymphadenectomy. Biopsy of the SLN show lower rates of the previously mentioned complications. Thus, surgical wound dehiscence and lymphedema become rare, and they significantly reduce hospital stays [10].

The characteristics of inguinal lymph nodes in vulvar cancer are considered as the best prognostic factor and as a crucial element for adjuvant therapy [11], due to their histological assessment and identification during the clinical and surgical management [12].

The location of the tumour is particularly valuable when identifying the SLN because bilateral lymph drainage of the vulva needs be taken into account, as it involves the existence of a SLN in both inguinal regions. The use of Tc99m-colloid is considered as the most accurate technique for the detection of the SLN in breast cancer, vulvar cancer and vulvar melanoma [13]. Tc99m has been combined with a dye (isosulfan blue or methylene blue) with good results that reached global percentages between 95% and 100%, according to Brunner [14], Hampi [15] and Moore et al. [13], among others. The radioactive technique seems to be more effective than the vital dye, with an identification rate of the SLN of 100% vs 82.5%. de Hullu [16] detected that 56% of all SLNs that had been identified with Tc99m had been dyed with the vital stain. Nevertheless, when the tumour is next to the inguinal region, the combination of Tc99m and blue dye may be useful because the isolated use of the radiotracer can be compromised by the proximity between the tumour and the bladder, even though the stain is not considered as a fundamental element in the detection of the SLN and it is only recommended when preoperative lymphoscintigraphy shows any difficulty. Isolated use of the vital dye would significantly reduce the identification rate of the SLN [17]. In any case, the sensitivity and NPV are next to 100% [15, 18]. In spite of the good results described, false negatives have been observed in women with vulvar carcinoma [19]. Blocked lymph canals due to a metastasis in the SLN are a factor that might hinder the identification of said SLN [20]. The use of a histopathological technique, together with immunohistochemical techniques, makes it possible to identify micrometastases that might go unnoticed [21], and that should be clinically assessed due to the risk of metastasis.

The significant increase in the number of melanomas of the female genital tract, and particularly of the vulva, has made it possible to achieve significant advances in the diagnosis, staging, surgical treatment, and adjuvant therapies. The initial treatment of choice in vulvar melanoma is surgery. Recent results show that resections are less radical, as well as lymphadenectomies. The study of the SLN for the assessment of lymph nodes, together with immunohistochemical studies are especially important factors in the treatment of vulvar melanoma [22].

The results that have been established for the control of recurrences have been determined by the rate of false negatives. Brunner et al. [14] and Hampi et al. [15] described three false negatives in each of the studied series, with a sensitivity of 90% and 92%, respectively. The NPV was 97% in both cases. These parameters establish the reliability of this technique. However, both the sensitivity and the predictive values show a very small variability among studies, and they reach 100% in some cases [4, 23].

The prevalence rate of inguinal recurrences is highly variable, and depends on the retrospective studies that have been performed, reaching values that range between 0% and 5.8% in patients with negative nodes [24]. The months that pass until the monitoring control is verified are an important variable because recurrences usually take place between 24 and 36 months after treatment. Some control studies have been done with patients with a negative lymph node that did not go through lymphadenectomy, and no recurrences were found [25], whereas other references registered a recurrence rate of 14.3% [7]. Some authors also said that the size of the vulvar lesion can influence the appearance of recurrences, and that a smaller lesion means a lower risk of lymph node recurrence. Like in any other line of research, more observations and controls are needed to assess the recurrence rates in longer periods than what have been studied so far. Currently, and according to the results published by the International Sentinel Node Society, the biopsy of the SLN is a reasonable alternative to total inguinal lymphadenectomy in patients with vulvar cancer Stage I and II [26].
Acknowledgments

The authors want to thank Mrs. Maria-Humi (secretary) and the nurses of the Department of Gynecology of the Clinical Hospital of Salamanca for their collaboration in the registration and control of patients who were included in this study.

References

Minimally invasive mastectomy: minimal incisions for better aesthetic quality of breast reconstruction

M.P. Costa¹, M.C. Ferreira², J.M. Soares Jr.¹, A.G.Z. Rossi¹, E.C. Baracat⁶

¹Division of Plastic Surgery, Hospital das Clínicas, University of São Paulo Medical School; ²Brazilian Society of Plastic Surgery; ³Division of Plastic Surgery, Hospital das Clínicas, University of São Paulo Medical School; ⁴American Association of Plastic Surgeons; ⁵Gynecology Department of UNIFESP and Researcher of Divisão de Ginecologia do Departamento de Obstetrícia e Ginecologia do Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo; ⁶Gynecology Department of UNIFESP; ⁷Professor and head of Divisão de Ginecologia do Departamento de Obstetrícia e Ginecologia do Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo (Brazil)

Summary

Background: Women with a family history of breast cancer who develop this disease are confronted with important situations regarding the increased risk for development of a second cancer in the contralateral breast. Prophylactic contralateral mastectomy (PCM) reduces by approximately 95% the risk for contralateral breast cancer. In spite of an increase in indications for PCM, the technical difficulties are many regarding the accomplishment of these procedures. The aim of this study is to describe the technique of mastectomy with preservation of the nipple-areola complex and a small incision, reducing surgical difficulties and complications attributed to this technique, thus allowing better aesthetic results in breast reconstruction. Methods: Forty-six patients with indications for PCM (28 bilateral) were submitted to minimally invasive mastectomy from March 2005 to November 2007. A small incision in the superior pole of the areola, sufficient to pass a liposuction 4 mm cannula is made. With the help of this cannula, detachment of the skin from the gland tissue is performed. Then a 3.5 to 4.5-cm long incision in the inframammary fold is made. Glandular detachment is completed using cautery in the subglandular portion and scissors in the upper breast portion cutting the restraints left by the cannula. The mammary gland tissue is removed through this incision. Results: Seventy-four breasts were operated on. The resected breast mass ranged from 285 g to 475 g. All 43 patients were reconstructed with prostheses. There was no necrosis of the nipple-areola complex or of the skin. Conclusions: This technique is an option for cases of patients with indications for PCM.

Key words: Mastectomy; Minimally invasive; Breast cancer; Prophylactic contralateral mastectomy.

Introduction

Women with a family history of breast cancer who develop this disease are confronted with important situations regarding the initial cancer treatment and increased risk for development of a second cancer in the contralateral breast [1]. The risk for cancer in the contralateral breast in the general population of women with a history of cancer is approximately 0.7% to 1% per year, with a lifetime cumulative risk of approximately 15%. This risk significantly increases in women with BRCA1/2 mutation, with an incidence of 12% to 20% in the following five years [2, 3] and cumulative risk of 52% at the age of 70 [4]. Young women with BRCA1 alterations and less than 50 years of age at onset of breast cancer have a 40% chance of developing a second primary cancer in a 10-year follow-up [5]. These high-risk women have several options regarding management of the contralateral breast. Follow-up through screening, chemoprevention, prophylactic oophorectomy and prophylactic contralateral mastectomy (PCM) [1]. PCM reduces by approximately 95% the risk for contralateral breast cancer in women with a family and personal history of breast cancer [1].

In spite of the increased indications for prophylactic mastectomy and the emergence of mastectomy with preservation of the nipple-areola complex, the technical difficulties are many regarding the accomplishment of such procedures. Several studies show cases of patients with indications for mastectomy and with tumors far from the nipple (more than 2 cm from the tumor border); mastectomy with skin preservation associated with negative intraoperative frozen sections of the lower portion of the nipple-areola complex offers the opportunity of conservation of the nipple-areola complex without increasing the risk of local relapse [6-9].

Another possibility in order to attempt the maintenance of the nipple-areola complex is the use of preoperative subareolar mammotomy, which has been found to be efficient when evaluating the impairment of the complex, becoming an alternative to freezing [10].

Difficulties regarding glandular detachment such as intraoperative bleeding, irregularities in the cutaneous flap which make good reconstruction quality difficult, large scars, surgical time, skin necrosis and nipple-areola complex necrosis overshadow this type of procedure.

It is important to emphasize in the cases of prophylactic mastectomy where the patient does not present cancer, as the mentioned problems related to mammary reconstruction may lead to concern on part of the medical team responsible for the surgery. A poor mammary reconstruction result may raise doubts in the patient regarding the need for such surgery.

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The aim of this study is to describe the technique of mastectomy with preservation of the nipple-areola complex and a minimal incision, reducing surgical difficulties and complications attributed to this technique, thus allowing better aesthetic results in breast reconstruction.

Patients and Methods

Forty-six patients with indications for prophylactic adeno-mastectomy (28 bilateral) were submitted to minimally invasive mastectomy from March 2005 to November 2007.

Forty patients presented BRCA1+ and six BRCA2+. All cases had a positive family history (mother or sister) and cancerophobia. Ten patients were diabetic and hypertensive, four had heart disease, 14 were smokers (one pack per day) and one patient had systemic lupus erythematosus. Mean age of the patients was 34 ± 1.2 years (30-44).

Eighteen patients (39.13%) presented previous breast cancer with diagnosis by biopsy or mammotomy and stage T1N0M0. Of these tumors, 12 (66.66%) were of the ductal invasive type, four (22.22%) lobular invasive and two (11.11%) medullar. From a histochemical viewpoint, 16 patients (88.88%) were triple negative (estrogen, progesterone and HER2 receptors) and two patients (11.11%) HER2 + E- P-.

Regarding physical examination, 24 patients (52.17%) presented small breasts and 22 (48.83%) with medium hypertrophies. Six patients (13.04%) presented breasts with grade 1 Rees ptosis, 31 (73.91%) with grade 2 and six (13.04%) with grade 3.

Surgical technique

With the patient in the horizontal dorsal decubitus position, and the breast to be resected is demarcated with a marker on the skin. During this stage, it is important to observe the positioning of the intercostal arteries, noting that they are important for the maintenance of viability of the cutaneous flap (Figure 1A).

After marking, infiltration of a vasoconstrictor anesthetic solution (2% xylocaine, 1: 200,000) in the flap is performed between the skin and the gland and between the gland, and the pectoral muscle.

A small incision in the superior pole of the areola, sufficient to pass a 4 mm liposuction cannula is made. With the help of this cannula, and back and forth movements, detachment of the skin from the gland tissue is achieved (Figure 1A).

On finishing the detachment of the upper portion of the gland, a 3.5 to 4.5-cm long incision in the inframammary fold is performed, reaching the subglandular plane. Glandular detachment is completed with the help of a breast retractor using cautery in the subglandular portion and scissors in the upper breast portion cutting the restraints left by the liposuction cannula (Figure 1B). The mammary gland tissue is removed through this incision (Figures 1C-F). Drainage of all patients was done using vacuum assisted drains.

Results

Seventy-four breasts were operated on. The incision in the breast sulcus ranged from 3.8 cm to 4.9 cm (mean 4.2 cm ± 0.2 cm). The resected breast mass ranged from 285 g to 475 g (mean 350 g ± 15 g).

All 46 patients were initially reconstructed with an expander and later replaced with mammary silicone gel implants. Three and a half months was the average between the first and second time of reconstruction.

Mean time of mastectomies with preservation of the
The frequency of accomplishing prophylactic mastectomies is still undefined, however in the study by Perlata et al. [11] approximately 2.2% of all patients were submitted to this procedure between 1973 and 1998. Studies of the Cancer Research Network demonstrated that prophylactic mastectomy not only protected against contralateral cancer development but also led to a reduction in the total mortality due to breast cancer [2].

The Society of Oncologic Surgery considers such indication for some selected patients [12]. As such, this procedure should be considered more frequently than before, thus increasing interest of bilateral prophylactic mastectomy as reported in the literature [12]. The number of these surgeries has become larger. Therefore the establishment of its role in the treatment of cancer becomes important as well as how it should be done with the least amount of sequelae.

The study by Frost et al. showed that, in spite of the satisfaction regarding the accomplishment of a PCM, the reduction in the level of satisfaction was associated with worsening of body appearance, complications of reconstruction after PCM when the great majority of patients did not observe favorable effects with respect to their self-esteem (83%). Problems related to femininity and sexual relationships were also observed [1].

Minimally invasive mastectomy attempts to fulfill safe surgical criteria allowing the best possible esthetic result. As any new technique, this one requires a learning curve. However, minimally invasive mastectomy with preservation of the nipple-areola complex showed to be of easy execution and easy learning. Mean time of surgery also seems to be less than the approaches by former techniques.

The resulting scar has a minimal extension and varies from 3.4 to 4.5 cm according to the size of the resected mammary gland. The determining factor for the size of the incision is not the need for a larger field for dissection and detachment of the gland but the space necessary for removal of the mammary tissue (285 g to 475 g). It is the same incision through which mammary reconstruc-
tion can be made, be it a prosthesis or an expander. In the case of a myocutaneous abdominal rectus, great dorsal or any other type of flap for reconstruction, there is maximum skin preservation, also facilitating the procedure.

Mammary reconstruction was facilitated not only by the same incision which may also be used for implant placement, but also by the fact of having more homogeneous flaps, due to detachment performed initially with the liposuction cannula and not by detachment performed with scissors and cautery.

Infiltration with vasoconstrictor solution together with liposuction cannulas (which do not impair the blood vessel) and detachment of the lower portion of the mammary gland being performed with cautery has greatly decreased bleeding in this type of procedure.

Absence of skin and nipple-areola complex necrosis is also an important factor in the attempt to use this type of approach.

In conclusion, the technique presented in this study is an option for cases of patients with indications for mastectomy with preservation of the nipple-areola complex.

References

Prognostic importance of selected molecular immunohistochemical markers and DNA ploidy in endometrial cancer

M. Kudela¹, R. Pilka¹, M. Lubusky¹, P. Hejtmanek ¹, P. Dzubak², S. Brychtova³

¹Department of Gynaecology and Obstetrics, Faculty and University Hospital, Olomouc;
²Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc;
³Institute of Pathology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc (Czech Republic)

Summary

The aim of the study was the analysis of the new molecular genetic immunomarkers (p53, c-erbB-2, Ki 67, bcl-2) hormonal receptors (ER, PR) and ploidy disturbances and their relation to the most important prognostic factors for endometrial cancer. The study group consisted of 135 endometrial cancer patients. Biopsies of the tumours obtained at operations were routinely histopathologically examined. Subsequently, the immunohistochemical tumour markers were determined. The same biopsies were examined by microdissection and flow cytometric ploidy analysis and karyotyping. The findings were compared with the most important prognostic factors for endometrial cancer, mainly with clinical stage of the disease and grade. Results: High expression of p53, Ki 67, c-erbB-2 and low rate of progesterone receptors was found in the prognostically unfavourable group (G 3). Aneuploidy was found in 72% in the group of poorly differentiated endometrial cancers (G 3) in contrast to 27% in the group of G1 and G2 tumours, but this difference was not statistically significant. Conclusions: Identification of p53, Ki 67, c-erbB-2, PR and determination of DNA ploidy is a useful tool to specify a group of prognostically unfavourable patients.

Key words: Endometrial cancer; DNA ploidy; Molecular immunomarkers; Prognostic factors.

Introduction

Endometrial carcinoma is the second most frequent gynaecological malignancy (after breast carcinoma) affecting female reproductive organs. Continuing increase of incidence, mainly in economically developed countries, is evidently related to the complex influence of civilization factors such as prolonged survival, reproductive behaviour and living standards of contemporary populations. In spite of the relatively low mortality, almost as many women in the Czech Republic die from endometrial carcinoma as those from cervical carcinoma due to the lower incidence of the latter.

Adequate, complex and timely initiated treatment is essential for good therapeutic results. Adequate therapy of malignant tumours in general must bring about the maximal therapeutic effect with the minimal burden for the patient. Undertreatment as well as overtreatment should be avoided.

To select the right therapeutic strategy it is necessary to take into account a whole array of factors among which prognostic factors are very important. Even though the clinical and pathological parameters such as grade and histopathologic type of the tumour are still the most important factors, there are several other factors which can influence and predict the course of the disease and are, therefore, important for the estimation of the extent of surgery and eventually the necessity of following radiotherapy or other adjuvant therapies. Molecular immunomarkers and DNA ploidy of malignant cells are also among those important prognostic factors [1, 2].

Research in the field of molecular genetic biomarkers is at present rapidly growing in oncology and in oncogynaecology as well. New biomarkers are important not only for the theory where they contribute to answering the basic questions concerning the course of cancerogenesis, but they also have an impact in clinical medicine [3]. It is already known that the malignant process is initiated by changes in genetic information in normal cells. The main event in the initial phase of cancerogenesis is the activation of protooncogenes, inactivation of tumour suppressor genes, microsatellite instability and several other genetically related changes. The chromosomal genome often undergoes important changes which occur either in an individual nucleotide or also, in the whole chromosome. This often leads to the loss of heterozygosity, aneuploidy or in some cases also polyploidy. The extent and character of these defects is thereafter directly reflected in the biological behaviour of the tumour and in this way determines the clinical course of the disease.

The aim of this study was to evaluate selected immunomarkers (p53, c-erbB-2, Ki 67, bcl-2), hormonal receptors (ER, PR) and changes in cell ploidy of malignant cells and compare them with the most important prognostic factors, mainly with the clinical stage and grade and eventually histopathological type of the tumour [4].
Material and Methods

The study included 135 patients with histopathologically proven endometrial carcinoma. The diagnosis was based on the examination of biotic material obtained by diagnostic hysteroscopy or D&C. The patients underwent surgery which in most cases included hysterecctomy with bilateral salpingo-oophorectomy. If indicated pelvic and paraaortal lymphadenectomy were performed. The surgery was done from the abdominal, vaginal or laparoscopic approach.

Indirect immunohistochemistry on formalin-fixed paraffin-embedded 5-8 μm thick tissue sections was performed in the staining automaton Vetana Benchmark, using mouse or rabbit mouse primary monoclonal antibodies against p53, c-erbB-2, Ki 67, bcl-2, PR and ER. For negative controls, the samples were taken through the procedure with omission of primary antibody. Positive staining was expressed in percent. The median value was set as a limit of positivity for p53, c-erB-2, Ki 67 and bcl-2. The cut-off limit for p53 and bcl-2 was 20%, for Ki-67 40%, and 10% for c-erbB-2. As for the PR and ER the lower limit of positivity was 5%.

DNA ploidy was also examined from the samples of microdissected paraffinized tumorous tissue using flow-cytometry. The stained nuclei were measured by the flow-cyto meter FACS Calibur and the obtained data were analysed by ModFit-LT software. DNA histograms were classified as diploid or aneuploid with respect to the content of DNA in the region of the Go/G1 peak. The findings were compared with the internal standard of nuclei isolated from normal diploid cells. If the quality of the sample enabled further measurements, the analysed nuclei were also evaluated for the cell cycle proliferation.

The study group was divided into two groups according to surgical staging. The first group with a good prognosis comprised 58 patients with FIGO classification IA and IB. The group with a poor prognosis included 77 patients with advanced disease, classified as FIGO IB, II, III and IV.

According to the grade of tumour the patients were divided into 113 with a good prognosis (FIGO G1 and G2) and 22 with a poor prognosis (G3).

The main histopathological classification of the tumour, endometroid cancer, was found in 130 patients. The remaining five patients had serous papillary adenocarcinoma or mixed types of tumours. With regard to the relatively small number of prognostically unfavourable cases, these were not evaluated separately.

According to karyotype, the tumours were divided in euploid or aneuploid groups. The aneuploid group included hypoploid and polyploid nuclei.

The results of immunomarkers, estrogen and progesterone receptor analysis as well as cell ploidy were evaluated in relation to the expected prognosis of the disease. Inclusion criteria for a good or bad prognosis were based either on FIGO staging or the grade of tumour.

Statistical evaluation was performed by arranging the data into contingency tables (cross tabulation) and calculating Fisher’s tests for homogeneity of the compared groups of patients. The value of p = 0.05 was taken as significant in Fisher’s tests. The statistical software used was SPSS Inc Chicago, USA. The results with both-sided exact significance less than 0.05 were considered statistically significant.

Results

Table 1 shows the division of the groups according to the grade of tumour.

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<th>Table 1. — Tumour grades.</th>
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<td>euploid</td>
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<td>42.5%</td>
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<tr>
<td>aneuploid</td>
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<td>29.2%</td>
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<tr>
<td>G3</td>
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<td>83.3%</td>
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<td>aneuploid</td>
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In the group of 113 patients with the prognostically favourable grade (G1 and G2) there were 48 tumours (42%) with euploid karyotype, while in 65 tumours (57%) there were hypo- or polyploid nuclei. The respective expression of the studied immunomarkers and steroid receptors in euploid and aneuploid tumours is given in Table 1.

Among 22 patients with biologically immature tumours (G3) the euploid karyotype was found in six (27%) while aneuploid karyotype was found in 16 cases (72%). Though the difference seems to be high, it was not statistically significant.

Expression of p53 in the group with the prognostically favourable grade (G1 and G2) occurred in 34 patients (29%) while in the group G3 it occurred in 13 (59%). The difference was statistically significant. The same was valid also for the expression of Ki 67 (28 vs 64%) and c-erbB-2 (11 vs 27%). Expression of bcl-2 was not significantly statistically different.

Expression of steroid receptors differed significantly only in case of progesterone receptors. The well differentiated tumours were positively stained in 100%, while the less differentiated ones only in 59% (Table 2).

Clinical stage FIGO IA + IB was found in 58 (43%) patients. Euploid cells were found in 20 patients (34%)
and aneuploid karyotype was found in 38 (66%) cases. Expression of studied immunomarkers and steroid receptor positivity in the same group of euploid and aneuploid tumours is given in Table 2.

Prognostically unfavourable stages FIGO IC, II, III, IV were found in 77 (57%) patients. There were 34 (44%) euploid tumors and 43 (56%) aneuploid tumours. The difference was not statistically significant.

Comparison of studied immunomarkers between prognostically favourable stage FIGO IA, IB and stages FIGO IC, II, III, and IV did not show statistically significant differences. On the contrary, statistically significant differences were found for progesterone receptors, which were more frequently positive in the initial, prognostically favourable stage FIGO IA, IB and stages FIGO IC, II, III, and IV.

Discussion

At present it is generally accepted that the genesis of malignant tumour is determined by a primary genetic disorder, either sporadically arising or based on hereditary disposition. Cancerogenesis is a multistep process, originating as imbalance between normal cellular proliferation and apoptosis, i.e. programmed cell death. Many genes can participate in the formation of tumours. The main events in the initial phase of cancerogenesis are changes in activation prononcogenes, inactivation of tumour suppressor genes, microsatellite instability, aneuploidy, point mutations, translocations, amplification, loss of heterozygosity and others.

Oncogenes which arise by a defect – mutation – of prononcogenes are pathologic. They encode proteins which, if they occur in an abnormal amount or form, can lead to the tumorous transformation of cells. Genes as c-erbB-2, also known as HER2/neu and bcl-2, belong to the well known oncogenes [5]. Oncogenes can also form complexes with the products of tumour suppressor genes which leads to their inactivation.

Amplification with increased expression of c-erbB-2 was reported in 10-40% of endometrial cancers [6, 7]. Oncogene c-erbB-2 encodes in a similar way as does EGRF (epidermal growth factor receptor) and is therefore frequently found in aggressive types of tumours and should correlate with a worse prognosis. Nevertheless, the conclusions of clinical studies are ambiguous. Our study shows an increased expression of this marker in prognostically unfavourable cases according to the grade, but not according to FIGO stage.

Oncoprotein bcl-2 inhibits apoptosis and prolongs the cellular lifespan. Its expression changes in the course of the menstrual cycle; it is high in the proliferative phase and low during menstruation. High levels are also found in endometrial hyperplasia. Increased expression has been documented in endometrial cancer, especially in carcinoma of the type I. It is suggested to be related to the greater depth of myometral invasion, stage, grade and a worse prognosis [8-10]. Decreased expression is reported in type II carcinoma and namely in unfavourable histopathological types of tumour [10, 11]. We found increased levels of bcl-2 in prognostically unfavourable cases according to the grade, but not according to the stage. The results, however, did not reach statistical validity.

Tumour suppressor genes play an important role in cell division. They encode proteins which inhibit/regulate cell division. They can control cell division in two ways - either they rectify the arising errors or they stop further division of cells. They function as the so called “safety catch” which switches off the cell cycle in case of abnormal proliferation or a defect of genetic information. The defect of tumour suppressor genes such as mutation can result in the escape of defective cells from their control mechanisms and in this way contribute to the formation and growth of malignant tumours. The most known suppressor gene is p53. With regard to its important role in the process of apoptosis it is also called the “genome guardian”. It encodes a nuclear phosphoprotein and as a transcription factor influences expression of other genes which regulate growth and division of cells. Gene p53 relatively often undergoes mutation and its specific regulatory role may change or is inhibited. Protein product of the mutated gene is slowly degraded in malignant cells; its regulation does not respond to the dynamic changes in cells and therefore it can be detected by immunohistochemical methods.

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<td>Figo IA+IB</td>
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very often and its increased expression correlates with the clinical stage of disease, poor prognosis and aggressive histopathological types of tumour [6, 7, 11-14]. Our results show almost three times higher values in poorly differentiated tumours compared to well differentiated ones. The difference was highly statistically significant (p = 0.01). The difference in expression of p53 according to the stage was not statistically significant.

Marker Ki-67 is connected with cell proliferation which is a characteristic feature of all malignant tumours. This protein is encoded by the MK 167 gene and can be detected in cell nuclei in all phases of the cell cycle except for the quiescent phase (G0). The majority of endometrial cancers express a low proliferative index, while increased expression usually correlates with unfavourable grade, clinical stage and means a poor prognosis. Unfavourable histopathological types of endometrial cancer also show high expression [15-17]. Our study documented increased expression in prognostically unfavourable tumours according to grade. High positivity was found mainly in aneuploid and immature tumours (69%). The results were statistically significant. We could not evaluate the importance of Ki-67 in non-endometroid cancers due to the small number of patients.

Estrogen and progesterone receptors, when activated, bind to the specific target sites in DNA where they modulate expression of respective genes. Besides the direct activation of target genes, the indirect mechanism of effect through the binding on transcription factors has also been reported [18]. Steroid receptors play an important role not only in the healthy endometrium, but participate also in the process of endometrial carcinogenesis. The absence of steroid receptors is considered a negative prognostic factor and is most often found in case of unfavourable histopathologic types and aggressive tumours of the endometrium [9, 18-20]. In agreement with published data our study showed a statistically significant lower occurrence of progesterone receptors in biologically immature tumours as well as in advanced stages of tumour (FIGO IC-IV).

A typical karyotype of healthy human cells consists of 46 chromosomes assigned in 23 pairs. In contrast, genetic material in tumour cells is characterised by a certain degree of genetic instability. As a consequence of mitotic defects this may lead to important changes of a genome. The marked elements of tumour progression are defects in genes which are connected with the maintenance of chromosome stability and integrity. The excess or loss of one or more chromosomes leads to genetic instability in tumour cells. It has been documented that this instability is an early manifestation of malignant transformation and that it is typical mainly for some types of malignant tumours [21]. Aneuploid changes are reported in a wide range (15-40%) in endometrial carcinoma [22-27]. In general, the well differentiated prognostically favourable tumours show the prevalence of diploid cells, while the aggressive tumours have typically aneuploid karyotype [29-32]. From the clinical point of view it is important that most authors consider ploidy as an independent prognostic factor [38-42]. In the prognostically borderline cases (FIGO IC, G1-2) the determination of ploidy can contribute to the decision about adjuvant therapy.

In our group of patients the aneuploid forms of karyotype were more frequent in biologically immature tumours (G3). Though, the difference between prognostically favourable stages G1 and G2 and unfavourable grade G3 was not statistically significant.

Furthermore a higher proportion of aneuploidy in tumours of stages FIGO IC-IV was not found.

Conclusion

The determination of DNA ploidy, steroid receptors and selected immunohistochemical markers, mainly p53, Ki 67 and c-erbB-2 should contribute to specify the groups of endometrial carcinoma with favourable and unfavourable prognoses. This should lead to the selection of an optimal therapeutic strategy. However the DNA ploidy did not prove to be a significant prognostic factor in our study.

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References

Prognostic importance of selected molecular immunohistochemical markers and DNA ploidy in endometrial cancer


Address reprint requests to:
M.M. KUDELA, PhD.
Department of Gynaecology and Obstetrics
University Hospital
I.P. Pavlova 6
779 20 Olomouc (Czech Republic)
e-mail: kudelam@fnol.cz
Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer

A. Frega1, L. Lorenzon1, M. Bononi1, A. De Cesare1, A. Ciardi4, D. Lombardi1, C. Assorgi1, M. Gentile1, M. Moscarini1, M.R. Torrisi5, D. French5

1Department of Woman’s Health and Territorial Medicine, Sant’Andrea Hospital, Faculty of Medicine and Psychology
2Surgical and Medical Department of Clinical Sciences, Biomedical Technologies and Translational Medicine, Sant’Andrea Hospital, Faculty of Medicine and Psychology
3Department of Surgery “Pietro Valdoni”, Universitary Hospital, Faculty of Medicine and Odontoiatry
4Department of Experimental Medicine, Universitary Hospital, Faculty of Medicine and Odontoiatry
5Department of Clinical and Molecular Medicine, Sant’Andrea Hospital, Faculty of Medicine and Psychology

University of Rome “La Sapienza”, Rome (Italy)

Summary

Several studies have suggested a possible role for HPV in the pathogenesis of the breast cancer. We investigated the presence of the HPV DNA in breast cancers and non malignant disease breast tissues by the use of a standard HPV detection method (INNO-Lipa HPV), in order to detect HPV DNA in metastatic nodes, to investigate a possible cervical HPV co-infection, and to evaluate the E6/E7 mRNA expression in HPV DNA positive breast cancer tissues. The rate of HPV infection was significantly higher in the cancer group than in controls (9/31 vs 0/12, p = 0.04). One out of eight metastatic axillary nodes was positive for HPV infection; 2/3 of the positive HPV breast cancer patients were co-infected at the cervical site. The role of the virus in breast oncogenesis is still unclear, since our analysis failed in demonstrating the expression of viral E6 and E7 in positive HPV positive breast tumor tissues.

Key words: Breast cancer; E6; E7; HPV; HPV 16.

Introduction

According with the Italian Network of Cancer Registries (AIRTUM), from 1998 to 2002 breast cancer was the most frequent cancer, representing 24.9% of all the cancer diagnoses in the AIRTUM area; mortality was 17.1% of all cancer deaths among females [1].

Although many risk factors have been established for this disease (e.g. age, family history or mutations of the BRCA1/2 genes), the etiology of the majority of breast cancers is unknown.

Over the last few years several studies have reported the detection of HPV within breast cancer tissues, suggesting a possible role for HPV in the pathogenesis of this tumor. HPVs are classified in a “low-risk group” which generate benign lesions, and an oncogenic or “high-risk group” (e.g. HPV-16, HPV-18) which is recognized as an etiologic agent of cervical and anal cancer [2, 3].

Papillomaviruses are a group of small non-enveloped DNA tumor viruses: to date, more than one hundred human and animal papillomavirus genotypes (types) have been completely sequenced. Viral gene expression leads to the expression of six nonstructural viral regulatory proteins (E1, E2, E4, E5, E6 and E7) and two structural viral capsid proteins (L1 and L2). E5, E6 and E7 are the viral oncoproteins and their expression induces cell immortalization and transformation. In particular, E6 and E7 are two viral oncoproteins that inactivate, respectively p53 and Rb, two cellular tumor suppressor proteins [2].

The correlation between high-risk HPV infections and cervical and anal carcinomas is widely accepted, but the correlation between HPV and malignancies of other tissues, including the breast, is still unclear.

Although the rate of HPV infection in breast cancer has been reported up to 86% [4], several authors failed in detecting the virus, resulting in an ample range of positivity among different studies [5-19].

Because of the contradicting results of the past studies, we conducted a search for HPV DNA in 31 breast cancers and 12 non malignant disease breast tissues through a gold standard detection method routinely employed for HPV detection and genotyping at the cervix; secondary aims of the present study were: a) to detect the HPV DNA in metastatic nodes, b) to investigate a possible cervical HPV co-infection in HPV positive breast cancer patients through DNA extraction and genotyping, and c) to evaluate E6 and E7 mRNA expression in HPVs DNA positive breast cancer tissues.

Material and Methods

Thirty-one women with an histological diagnosis of primary breast cancer were enrolled from 1994 to 2002. Mean age of the selected patients was 57 years (range 35-78); 29 were ductal carcinomas and two were lobular carcinomas.

Ten patients with diagnoses of breast fibroadenoma and two with diagnoses of breast papilloma were selected as a control group (mean age 27 years, range: 25-35).

Exclusion criteria were: past history of breast and cervical cancer and neo-adjuvant treatments. All molecular diagnostic investigations were performed on paraffin-embedded surgical
The detection of HPV was carried out through the INNO-Lipa HPV Genotyping kit, which can detect 28 different HPVs by DNA amplification followed by reverse hybridization of the SPF10 fragment (Innogenetics).

Stored frozen cancer tissues, positive for HPV 16, 18 and 31 DNA, were further investigated after RNA extraction for E6 and E7 mRNA expression by the NASBA assay Pre-Tect HPV-Proofer (Norchip), when the kit became available.

Briefly, NASBA is based on isothermal mRNA amplification, accomplished by the simultaneous enzymatic activity of avian myeloblastosis virus (AMV) reverse transcriptase, T7 RNA polymerase and RNase H. For detection primers and molecular beacon (MB) probes directed against E6/E7 mRNA for HPV types 16, 18, 31, 33, 45, 52 and 58 were employed. The final

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Table 1. — Clinical and pathological features of breast cancer patients and controls.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histology</th>
<th>TNM</th>
<th>Breast HPV genotype</th>
<th>Cervical HPV genotype</th>
<th>Nodal HPV genotype</th>
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<tr>
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<tr>
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<tr>
<td>43</td>
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</table>
concentration of MBs used in the reaction was 2.5 mM. NASBA amplification was carried out in a volume of 20 µl at 41°C for 2.5 h. A 5 µl volume of nucleic acids, diluted five times after the extraction was included in the reaction. The expression of U1A mRNA was evaluated as a performance control.

Statistical analysis was performed to compare the rate of HPV infection between cancers and controls: differences between groups were analyzed using the Fisher’s exact test (MedCalc Software 11.3).

Results

HPV DNA was detected in nine out of 31 breast cancer patients (29%), and among these HPV 16 was the most frequent type detected (44%), followed by HPV 6 (22.2%). All controls resulted negative for HPV infection (Fishier’s exact test p = 0.04). Table 1 summarizes the results of the study.

Patients who tested positive at the breast cancer site successively underwent cervical sampling to investigate a possible HPV co-infection at the genital site; six patients resulted to be (66%) co-infected. All these patients that tested positive for HPV at the genital site shared the same type (or at least one type) at both the breast and cervical sites.

Patients who tested positive for HPV at the cervix were successively referred for clinical gynecological evaluation and follow-up.

Eight patients in the cancer group had metastatic axillary nodes; among these just one patient (16%) tested positive for HPV infection, and the others resulted negative. Interestingly, this patient tested positive for HPV type 16 at the breast, node and cervical site.

After extraction of the RNA from stored frozen cancer tissues, we conducted a search for the expression of the E6 and E7 mRNAs in five patients who tested positive for HPV type 16, 18 or 31 at the breast site. Although human mRNA expression was positively validated by the housekeeping expression, the analysis failed in detecting the viral mRNA expressions in all the five samples.

Discussion

The relationship between HPV and breast cancer was first reported in 1992 [6-19]. Since then several authors investigated the presence of the virus in breast cancers through different techniques, resulting in an ample range of positivity among studies, ranging from 0 to 86.2% [4-19].

A literature review of previous studies reveals a high heterogeneity among HPV detection techniques, primer sets employed, tissues investigated (e.g. paraffin-embedded tissues, frozen tissues), population investigated and selection of controls. In our study, we investigated patients through a genotyping kit, which is routinely employed as a gold standard method for cervical HPV detection.

According to our results, the rate of HPV infection within breast cancer patients was significant if compared with controls (p = 0.04); moreover our detection rate was similar to what was previously reported by Khan and colleagues with the same detection method [20].

The possible role of HPV virus in carcinogenesis is indeed not clear: presence of the virus within the tumor could be coincidental, otherwise it might play a role causing chromosomal instability, inactivation of p53, activation of oncogenes or inactivation of tumor suppressors by insertionally mutagenesis caused by integration of the virus within the host genome.

Key points in HPV-related cancers are integration of the virus in the host genome and expression of viral oncoproteins. Integration represents a consequence of viral infection and is detected in almost 90% of cervical carcinomas. The mechanism of integration is not completely understood, although there is a clear predilection for chromosomal common fragile sites due to their accessibility for insertion of foreign DNA. Indeed integration seems to be a direct consequence of chromosomal instability and an important molecular event in the progression of pre-neoplastic cervical lesions [21].

To clarify the viral status (integrated/episomal), Khan investigated the E2/E6 ratio and detected an integrated virus in the vast majority of the HPV-positive breast cancer tissues. However a mixed/episomal form was detected in the cervical cancer tissues (positive controls) and in the surrounding normal breast epithelium of the HPV positive tissues [20].

The authors moreover investigated the HPV viral load in positive breast tissues comparing results with HPV-positive cervical cancer tissues (positive controls), and highlighted that viral load of HPV-positive breast tissues was much lower than the one observed in cervical cancer tissues (geometric mean per 10³ cells: 5.4 vs 130 480) [20].

We decided to investigate the viral oncoproteins, evaluating E6 and E7 mRNA expression in HPV DNA positive breast cancer tissues. According to our investigation, however, positive breast tumors did not express the viral E6/E7 transcripts, leaving unresolved the issues regarding the role of HPV in breast oncogenesis.

Two theories have been proposed to explain the possible mechanism through which the virus reaches the breast: one regards a mechanical transmission and the other one systemic spreading [4, 13]. De Villiers et al. detected the viruses in the nipple and the areola in patients with breast carcinoma, supporting the theory of a retrograde way in a retrograde fashion via the nipple through the breast ducts [4]. Widschwendter et al., hypothesized that the virus could reach the breast through the blood-stream after detecting the same HPV in nodes and breast cancers of cervical cancer patients [13].

In our study two-thirds of the patients who tested positive for HPV at the breast site resulted HPV positive at the cervical site, all of them shared at least one of the HPV types; however the mechanism of transmission remains unclear. To the best of our knowledge this is the first study aimed also to investigate the expression of HPV E6 and E7 mRNAs, the two major viral oncoproteins in breast cancer tissues. In conclusion, these data indeed suggest that
References


Comparison of risk of malignancy indices; RMI 1-4 in borderline ovarian tumor

M.C. Yenen¹, İ. Alanbay², E. Aktürk¹, C.M. Ercan², H. Coksuer², E. Karaşahin², H. Ozan¹, M. Dede¹

¹Obstetrics and Gynecology Department, Gulhane Military Medical Faculty, Etilik, Ankara
²Obstetrics and Gynecology Department, Uludag University Medical Faculty, Bursa (Turkey)

Summary

Purpose: The aim of this study was to evaluate prognostic values of the risk of malignancy index (RMI)/1-4 in patients with borderline ovarian tumors (BOTs). Methods: The study consisted of 50 patients with BOT diagnosed and treated between 2005-2010 and 50 patients with benign adnexal masses between 2009-2010 as a control comparison group in the retrospective study. Preoperative serum CA125, U score, tumor size (S), and menopausal status were recorded. The RMI 1-3 was calculated according to the formula; U×M×CA125 and RMI 4 formulation was; U×M×CA125×S. S equaled 1 for tumor size < 7 cm and was 2 when size ≥ 7 cm. The RMI 1-4 indices were calculated for all patients together with the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy (DA). The performances of RMI indices were evaluated by McNemar’s test and determined the best score cutoff value by the receiver operating characteristic (ROC) curve. Results: The mean age, median value of CA125, ultrasound score, menopausal status, median values of RMI 1-4 of BOTs were statistically higher than benign adnexal masses. The sensitivity of RMI 1-4 was 26, 36, 62, and 60% at cutoff 200 level, respectively. The areas under curve of RMI 1-4 were found to be 0.676, 0.665, 0.668 and 0.734, respectively. DA of RMI 1-4 was found to be 56, 59, 50, and 71, respectively. When RMI 1-4 indices were compared with each other RMI 4 was the best RMI for BOTs. Conclusion: RMI 4 was the best predictive RMI for preoperative discrimination of BOT at a cutoff level of 200.

Key words: Borderline ovarian tumor (BOT); Risk of malignancy index (RMI).

Introduction

Borderline ovarian tumors (BOTs) were first described by Taylor in 1929 [1] and were introduced in 1971 by FIGO as a category of epithelial ovarian tumors [2]. BOTs account for 10-15% of all epithelial ovarian tumors. BOTs are accepted as malignant, although they are different from both benign epithelial ovarian tumors and invasive epithelial ovarian cancers. BOTs are diagnosed at clinically earlier stages, such as Stage I, and often affect young women who wish to preserve their fertility, have low potential for malignancy, have longer patient survival, and have later recurrence as compared with invasive epithelial ovarian tumors [3-7].

The preoperative evaluation of adnexal masses is rather complicated and the main purpose is to discriminate between benign and malignant lesions. Several parameters are used to assess the risk of malignancy, such as gray scale sonographic parameters, color Doppler ultrasonography, gynecologic examination, biochemical markers as tumor markers, and demographic characteristics i.e., menopausal status [8]. Instead of using these parameters alone, combined methods have been proposed for accuracy in differentiation of adnexal masses. Jacobs et al. in 1990 developed a risk of malignancy index (RMI) depending on serum CA125, menopausal state, and ultrasound findings [9]. Then RMI 2 was described by Tingulstad et al. in 1996 [10], RMI 3 was described by Tingulstad et al. in 1999 [11], and RMI 4 was described by Yamamoto et al. in 2009 [12].

BOTs have fewer scores of RMI characteristics; menopausal status, CA125 levels, and ultrasound scores. First, since the majority of women with BOTs are at reproductive age, absolute contribution of age at RMI is less [3, 5]. They are usually diagnosed at an early stage and only 50% early-stage ovarian cancers have elevated CA125 [13], and their tumor marker secretions are less than invasive ovarian cancers. Elevated serum tumor markers such as CA125, CEA and CA19-9 have been reported in only 25% to 60% of women with BOTs [14-18]. BOTs also have a borderline sonographic appearance and do not have a pathognomonic or typical sonographic pattern [19]. These factors may affect RMI calculation in BOTs. But when we searched the literature, we did not find any study that used RMI in BOTs separately from benign and malign adnexal masses. Usually BOTs are included in malignant groups, although BOTs are actually different from malignant groups.

The aim of this study was to evaluate the diagnostic performance of RMI 1-4 for BOTs and determine the best RMI index and cutoff level for preoperative evaluation of BOTs. To the best of our knowledge, this is the first study evaluating and comparing RMI indices of BOTs.

Materials and Methods

The clinical data were obtained from 50 women with BOTs diagnosed and treated between 2005-2010, and 50 women with benign adnexal masses between 2009-2010 as a control comparison group in this retrospective study. Only serous and mucinous BOTs with complete laboratory and clinical data were
included in this study; all others were excluded. Preoperative serum CA125, ultrasound findings, tumor size (single greatest diameter; S), menopausal status, and histopathologic results were recorded. If the adnexal masses were bilateral, the more morphologically complex the tumor was considered, and if both masses were morphologically similar, the largest one was considered in the statistical analysis. An ultrasound score of 1 was assigned for each of the following ultrasound features suggesting malignancy: a multicystic cystic lesion, solid areas, bilateral lesions, ascites, and intraabdominal metastases findings [9]. A total ultrasound score was calculated for each patient. Premenopausal status was defined as more than one year of amenorrhea, or being 50 years or older among women who had undergone hysterectomy. All other women were considered premenopausal. Final diagnoses of the participants were based on the histopathologic examination of surgical specimens.

The RMI was calculated according to the formula: the ultrasound scores (U), the menopausal score (M), and the serum CA125 level: RMI: U x M x CA 125 for RMI 1-3. RMI 4 formulation was: U x M x CA125 x S. S equaled 1 for tumor size < 7 cm, was taken as 2 when tumor size was ≥ 7 cm. The scoring system of menopausal status and total ultrasound score were based on originally described RMI indices, for example menopausal status was scored as 3 at RMI 1, while it was scored as 4 at RMI 4. The scoring system of RMI 1-4 is shown in Table 1.

The RMI 1, RMI 2, RMI 3, and RMI 4 indices were calculated for all patients together with the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of the four methods.

Tumors were classified according to World Health Organization definitions and borderline ovarian tumors were staged according to the International Federation of Gynecology and Obstetrics [20]. Pathologically, BOTs were characterized by features of malignant epithelial ovarian tumors, including stratification of epithelial lining of the papillae, formation of microscopic papillary projections, epithelial pleomorphism, atypia, and mitotic activity, without invasion of stroma [21].

All statistical analyses were done using the Statistical Package for Social Sciences 12.0 (SPSS Inc., Chicago, IL). With all sonographic parameters and patient age, a univariate statistical analysis was performed. The Kolmogorov-Smirnov test was used to assess the normal distribution of continuous data. Continuous data were compared with the use of the Student’s t and Mann-Whitney U tests according to their distribution. An ultrasound score of 1 was scored as 4 at RMI 4. The scoring system of menopausal and ultrasound characteristics is shown in Table 1.

To determine the best score cutoff value to discriminate between BOTs and benign adnexal masses, a receiver operating characteristic (ROC) curve was plotted. The best cutoff value was chosen according to the highest sensitivity with the lowest false-positive rate. A probability value \( p < 0.05 \), was considered to be statistically significant.

**Results**

A total of 100 patients were included in the study. Fifty patients had benign adnexal masses, whereas 50 patients had BOTs. Histopathological diagnoses of the study are shown in Table 2. Histopathological results revealed 30 (60%) serous and 20 (40%) mucinous BOTs. The rates of benign lesions were simple serous cysts 10%, endometriomas 24%, dermoid cyst 14%, serous cystadenomas 26%, mucinous cystadenomas 22%, paratubal cyst 1% and tuboovarian complex 2%.

The characteristics of the study group according to age, menopausal status, tumor markers, tumor size, and ultrasound scores are shown in Table 3. The mean age of the patients
A pelvic mass is one of the most frequent indications for referral to a gynecologist [12] and differentiating between benign and malignant is the most important step for both appropriate preoperative assessment and optimal surgical choice [8]. Jacobs et al. in 1990, developed a RMI depending on serum CA125, menopausal state, and ultrasound findings, and recommended its use in benign-malignant determination of adnexal masses [9]. Then, RMI was modified by Tingulstad et al. as RMI 2 and RMI 3 [10, 11], and later modified by Yamamato et al. as RMI 4 [12], who added the parameter of the tumor size score (S) to the RMI formulation.

Being a simple scoring system and being applied directly into clinical practice without the introduction of

<table>
<thead>
<tr>
<th>Cutoff</th>
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<th>PPV (%)</th>
<th>NPV (%)</th>
<th>DA (%)</th>
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</tr>
<tr>
<td>200</td>
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<td>250</td>
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<td>400</td>
<td>18</td>
<td>24</td>
<td>20</td>
<td>38</td>
<td>98</td>
</tr>
</tbody>
</table>

Table 5. — Sensitivity, specificity, PPV, NPV, and DA for predicting borderline tumors at different cutoff levels of RMI 1-4.

<table>
<thead>
<tr>
<th>Cutoff level</th>
<th>RMI 1</th>
<th>RMI 2</th>
<th>RMI 3</th>
<th>RMI 4</th>
<th>RMI 1</th>
<th>RMI 2</th>
<th>RMI 3</th>
<th>RMI 4</th>
<th>RMI 1</th>
<th>RMI 2</th>
<th>RMI 3</th>
<th>RMI 4</th>
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<td>50</td>
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<td>0.001</td>
<td>0.008</td>
<td>0.063</td>
<td>0.063</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
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<td>0.25</td>
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<td>0.5</td>
<td>0.5</td>
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<td>0.002</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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</tr>
<tr>
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<td>1</td>
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<td>0.063</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
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<td></td>
</tr>
<tr>
<td>250</td>
<td>0.031</td>
<td>1</td>
<td>0.008</td>
<td>&lt; 0.001</td>
<td>0.08</td>
<td>1</td>
<td>0.004</td>
<td>0.001</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td>300</td>
<td>0.031</td>
<td>0.016</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<td>&lt; 0.001</td>
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<tr>
<td>350</td>
<td>0.031</td>
<td>0.016</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table 6. — Comparison of RMI 1-4 values with each other by using MacNemar’s test.

<table>
<thead>
<tr>
<th>Cutoff level</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>350</th>
<th>400</th>
<th>450</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMI 1-2</td>
<td>0.125</td>
<td>0.001</td>
<td>0.008</td>
<td>0.063</td>
<td>0.063</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>RMI 1-3</td>
<td>0.125</td>
<td>0.125</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>RMI 1-4</td>
<td>0.002</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RMI 2-3</td>
<td>1</td>
<td>0.016</td>
<td>0.063</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>RMI 2-4</td>
<td>0.031</td>
<td>1</td>
<td>0.008</td>
<td>&lt; 0.001</td>
<td>0.08</td>
<td>1</td>
<td>0.004</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>RMI 3-4</td>
<td>0.031</td>
<td>0.016</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

with benign lesions was 35.2 ± 12.3, and BOTs was 42.1 ± 14.4 years, and there was a significant difference in ages of the groups (p < 0.05). The premenopausal rate of benign and borderline were 44 (88%) vs 36 (72%), respectively. There was a significant difference between the two groups according to their menopausal status (p < 0.05). The median value of CA125 serum levels of the patients with benign cases were 34.18 ± 24.98 mIU/ml, and BOTs were 152 ± 355.47 mIU/ml, and the difference was statistically significant (p < 0.05). The ultrasound scores obtained from sonographic morphologic findings were U= 0: 31 (62%) cases; U=1: ten (20%) and U=2-5: nine (18%) were found to be benign and 12 (24%), 20 (40%), and 18 (36%) were BOTs, respectively. There was a significant difference between the two groups (p < 0.05).

The median RMI 1-4 values of BOTs and benign adnexal masses are shown in Table 4. The median values of the calculated RMI 1-4 for BOTs were higher than benign adnexal masses, and there was a significant difference between the two groups (p < 0.001).

When we compared the ROC curve evaluation of RMI 1-4 according to the area under curve (AUC), RMI 4 was found to be significantly superior to other RMIs. The AUC of RMI 1-4 was found as 0.676, 0.665, 0.668, and 0.734, respectively. The ROC curve analyses of RMI 1-4 are shown in Figure 1.

The performance of RMI 1-4 with respect to different cutoff levels is shown in Table 5. When a cutoff level was set at 200, diagnostic accuracy of RMI 1-4 was found to be 56, 59, 50, and 71, respectively.

When RMI scores 1-4 were compared with each other, e.g, RMI 1 vs RMI 4, by using MacNemar’s test, RMI 4 was found to be the best RMI for BOTs (p < 0.05) (Table 6).

Discussion

A pelvic mass is one of the most frequent indications for referral to a gynecologist [12] and differentiating between benign and malignant is the most important step for both appropriate preoperative assessment and optimal surgical choice [8]. Jacobs et al. in 1990, developed a RMI depending on serum CA125, menopausal state, and ultrasound findings, and recommended its use in benign-malignant determination of adnexal masses [9]. Then, RMI was modified by Tingulstad et al. as RMI 2 and RMI 3 [10, 11], and later modified by Yamamato et al. as RMI 4 [12], who added the parameter of the tumor size score (S) to the RMI formulation.

Being a simple scoring system and being applied directly into clinical practice without the introduction of
expensive or complicated methods are the main advantages of RMI compared with other diagnostic tests [22]. In comparison with age, menopause score, the ultrasound score and serum CA125 level, the RMI was significantly superior to all of them [23]. Although the RMI index has been used to differentiate benign and malignant ovarian masses, there is not yet a study in the literature using a RMI index in BOTs as a separate group. Usually, studies of RMI consisted of malignancy and benign subjects and BOTs were included in the malignant group. Moreover, the number of BOTs were small and even their numbers were not described separately.

An appropriate triage of BOTs is important since like ovarian invasive malignancies, especially BOTs with invasive implants do require proper staging (still recommended) and need fertility sparing surgery for women desiring future fertility [3-5]. However, BOTs and malignant ovarian cases have different characteristics. Considerable numbers of BOTs are premenopausal, have low CA125 level, and exhibit simple cyst characteristics. These differences may influence RMI values in BOTs, and low values for RMI characteristics result in low RMI scores in BOT groups [3-7]. BOTs have low scores both on ultrasound scores and CA125 levels [24]. RMI performance is different in premenopausal and postmenopausal groups [21]. The RMI predicts invasive malignancy best in the postmenopausal group; also there is a higher incidence of ovarian cancer in postmenopausal women compared with premenopausal women [22]. Ovarian enlargements and ovarian masses are more frequently detected in premenopausal age [25, 26]. The other factor is the lower diagnostic accuracy of serum CA125 in premenopausal patients because CA125 levels fluctuate during the menstrual cycle, being the highest during menstruation. Also, diseases such as endometriosis and pelvic inflammatory disease are more frequent in premenopause. These diseases are known to cause elevated CA125 [23]. BOTs have low CA125 levels compared to invasive ovarian cancer. Elevation of CA125 depends mainly on ovarian cancer stage [27]. CA125 levels were increased in 50% of patients with ovarian cancer in FIGO Stage I, in 90% of those in Stage II, in 92% of those in Stage III, and in 94% of those in Stage IV disease [28].

BOTs are usually diagnosed at an early stage and also mucinous BOTs tend to have lower tumor stages compared with serous BOTs. Therefore serum tumor markers are different for both serous and mucinous tumors at related stages [6, 29]. Also, elevated CA125 levels of serous BOTs are higher than for mucinous BOTs [30].

In our opinion, BOTs need to be studied as separate groups from both benign and malignant adnexal masses. Similar to our proposed theory, a study indicated that borderline malignancies were allocated to a non-invasive group when calculating the sensitivity and specificity levels, leaving only invasive malignancies to be detected for referral to a gynecologic oncologist [22]. A study using RMI 1/2 and Tailor’s regression model, found that the sensitivity of RMI 1/2 for BOTs was only 25% and indicated that RMI performed best in diagnosing invasive epithelial ovarian malignancies and not non-epithelial and borderline tumors [31]. Therefore, we used the RMI 1-4 index for BOTs to study them as a separate group.

In our study, when the cutoff level was set at 200, the sensitivity of RMI 1-4 was 26, 36, 32 and 60% (extremely lower than the literature) and specificity was defined as 86, 82, 86 and 80%, respectively, which was similar to the literature. Also when the cutoff was set at 200, the sensitivity of RMI 4 was better than RMI 1-3; (60% vs 26, 36, 32). The DA of RMI 4 was found higher than RMI 1-3, at a cutoff of 200 (71% vs 56, 59, and 50%, respectively. The specificity of RMI at a cutoff of 200 was found similar to RMI 1-3; 80% vs 86, 82 and 86%, respectively. The PPV of RMI 4 was found to be higher than RMI 1-3 (75% vs 65, 67, and 70%, respectively (Table 5). This predominance of RMI 4 markedly continued at different cutoff levels of RMI (Table 4). For example, when the cutoff level was set at 50, the sensitivity of RMI 1-4 was 54, 62, 62, and 74, respectively (lower than the literature). The DA, PPV, and NPV of RMI 4 were higher than the other RMI 1-3 values.

There are several studies for RMI in the evaluation of adnexal masses. Sensitivity, specificity and cutoff values of RMI studies vary largely, and most studies evaluated a range of cutoff levels varying between 25 and 250, although many authors suggest that the best cutoff value for RMI is 200 [9-11, 32]. Contrary to others, one study recommends the use of a RMI cutoff level of 153 [23]. Sensitivity of RMI 1 at a cutoff level of 200 was reported to be 85, 71.7, 90, and 88.5%, respectively [9, 23, 33, 34]. In a review of 109 studies comparing risk scores to predict differentiation of benign from malignant lesions; the pooled estimate for sensitivity was 78% and specificity was 87% when 200 was used as a cutoff level [8]. Our results are lower than the above-mentioned studies.

In a study where BOTs were included in invasive malignant groups, sensitivity was especially low (25%), similar to our study [30]. In different studies, sensitivity of RMIs in BOTs had a large range of sensitivity of 25%, 40% and 75%, respectively [31, 10, 34]. According to a systematic review, the pooled estimate for sensitivity of RMI 2 at a cutoff of 200 was 79%, and specificity was 81% [8]. In our results, the sensitivity and specificity of RMI 2 were 36% (lower than the literature), and 82% (similar to the literature), respectively.

In a study evaluating RMI 3 the sensitivity and specificity were found to be 74% and 91%, respectively [8]. In our study the sensitivity of RMI 3 was lower than the literature, and the 200 cutoff level sensitivity was 32%.

When we analyzed AUC of RMI 1-4 by ROC analysis, we found that AUC of RMI 4 was the highest of all (0.734 RMI 4 vs 0.676, 0.665, 0.668 RMI 1-3, respectively). In one study, the AUC of RMI was found to be 0.893 for the whole study, where it was 0.839 for the premenopausal and 0.911 for the postmenopausal group. Our results were lower compared to the results of this study [22].
Nemar’s test, different cutoff levels were determined such as RMI 4 vs RMI 1-3; RMI 4 was the best RMI in all different cutoff levels, $p < 0.001$ (Table 6). Our results showed that RMI 4 is the best predictive RMI model for differentiating benign and BOTs.

There are few studies in the literature comparing RMI with each other. In one study, a direct comparison of the RMI 1-3 indices at a cutoff level of 200 showed that there is no statistically significant difference in performance of the three methods in identifying malignancy, according to sensitivity, specificity, NPV, PPV and DA. For example, the specificities of RMI 1, RMI 2, and RMI 3 were shown as 91, 82, and 91%, respectively [32]. In another study RMI 1-3 in patients with abnormal pelvic masses were compared and RMI 2 was indicated as better than others (sensitivity 79% vs 71 and 71) but the specificities were similar [35]. A direct comparison of the RMI 1-4 showed that RMI 4 at a cutoff level of 450 was significantly better at predicting malignancy than RMI 1, 2 and RMI 3 at a cutoff level of 200 [12].

The differences between our results and the literature may also result from the numbers of BOT studies, because generally BOTs take place in small groups in RMI studies, and RMI usually differentiates benign and malignant disease and usually BOTs are not included as separate groups. Consequently, borderline malignancies tend to have lower RMI values compared to invasive malignancies and are therefore less detectable. Thus, low score results in lower RMI values compared to invasive malignancies and separate groups.

RMI studies, and RMI usually differentiates benign and may also result from the numbers of BOT studies, comparison of the RMI 1-4 showed that RMI 4 at a cutoff level of 200 < 0.001 (Table 6). Our results

References


Address reprint requests to:
I·. ALANBAY, M.D.
Gulhane Military Medical Faculty
Obstetrics and Gynecology Department
06018, Etilik, Ankara (Turkey)
e-mail: coksuer@gmail.com

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Laparoscopic management of unexpected borderline ovarian tumors in women of reproductive age

G. Pados1,2, D. Tsolakidis1,2, H. Bili1,2, D. Athanatos1,2, Τ. Zaramboukas3, B. Tarlatzis1

1st Department of Obstetrics & Gynaecology, School of Medicine, Aristotle University of Thessaloniki, “Papageorgiou General Hospital”, Centre for Endoscopic Surgery “Diavalkaniko” Hospital, Thessaloniki (Greece)

Summary

Purpose of investigation: The aim of this study was to review the clinical features of women with unexpected borderline ovarian tumours.

Methods: Between October 1992 and December 2010, 1,332 out of 4,016 laparoscopies were performed for adnexal masses in women of reproductive age and 1,838 cysts were removed. When ultrasonographic findings did not meet the criteria for low risk malignancy, tumour markers, colour Doppler and MRI/CT were applied. At laparoscopy any solid component or papilla was sent for rapid frozen section.

Results: Borderline ovarian tumours were found in eight (0.6%) out of 1,332 patients, two of which were bilateral. The mean age was 28.75 ± 9.27 years and the mean diameter of the cysts was 5.1 ± 1.7 cm. In two cases unexpected malignancy was discovered during the diagnostic and in six cases during the operative phase of the intervention. Conclusion(s): Risk of failure to diagnose cancer could be minimised with careful patient selection preoperatively. Adequate training on laparoscopic oncology is the necessary prerequisite for a safe laparoscopic approach.

Key words: Laparoscopy; Ovary; Surgery; Ultrasound; Borderline ovarian tumors.

Introduction

Laparoscopic approach compared with laparotomy is considered the “gold standard” for the management of preoperative diagnosed benign adnexal masses in women of reproductive age [1, 2].

The advances in accurate preoperative diagnosis of suspicious adnexal masses have reduced unnecessary laparotomies without sacrificing the principles of oncologic surgery in cases of unexpected malignancy since the incidence of ovarian cancer is 15.7/100,000 at the age of 40 years [3, 4]. In the reproductive age group, malignancy is found in 7%-13% of cases [5]. In 1973, the term borderline ovarian tumours (BOT) was adopted by the World Health Organization (WHO) and they represent approximately 10-15% of all ovarian tumours. One third of these tumours tend to occur in women younger than 40 years old, who want to preserve their childbearing capacity, and appear in 50-80% of cases at an early stage with a favourable prognosis [6].

However, neither sonographic features nor CA-125 levels are considered as adequate sensitive markers for discriminating a benign from a malignant lesion in premenopausal women [7, 8]. The aim of this study was to review our experience by presenting the clinical features and reproductive outcome of eight cases with unexpected BOT in women of reproductive age, on whom laparoscopic conservative surgery was performed.

Materials and Methods

This is a retrospective review of 1,332 out of 4,016 consecutive operative laparoscopies in women of reproductive age who were treated for adnexal masses; 1,838 cysts were removed at the Centre for Endoscopic Surgery of ‘Diavalkaniko’ Hospital during a 17-year period (October 1992-December 2009). All operative laparoscopies and ultrasounds were performed by the same laparoscopist and also, all frozen sections and pathology reports were read by the same pathologist.

In our centre, adnexal masses in women of reproductive age should fit the sonographic criteria listed in Table 1 in order to be characterized preoperatively as non-suspicious and, therefore, to be managed laparoscopically. Magnetic reasonance imaging (MRI), due to its high cost, was only ordered in sonographically undetermined ovarian lesions. Finally, as a routine, all patients had a preoperative CA-125 test done, despite its limited usefulness in women of reproductive age. Informed consent was signed by each patient about the possibility of cyst spillage during the intervention, and the possibility of a required staging laparotomy in case of unexpected malignancy confirmed on frozen section, if staging could not be accomplished by laparoscopy.

The protocol for laparoscopic management of adnexal masses followed in our department is depicted in Figure 1.

Results

Eight patients (0.6%) out of 1,332 were discovered to have BOT according to the results of their rapid frozen sections; two (25%) were bilateral. Totally, intraoperative frozen sections were ordered in 69 out of 1,838 cysts (3.7% per cyst) (Table 2). The findings of the preoperative investigation are summarized in Table 3. Of eight cases of unexpected malignancy, two were macroscopically evident during the diagnostic phase of laparoscopy and six were macroscopically recognised during the operative phase of the intervention. No “late” unsuspected ovarian malignancy was encountered. In all patients laparoscopic salpingo-oophorectomy was performed except for one nulliparous woman with a borderline malignancy of both ovaries. In this case, ovarian cystectomy was initially per-
Laparoscopic management of unexpected borderline ovarian tumors in women of reproductive age

Table 1. — Ultrasonography evaluation of adnexal masses.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Benign</th>
<th>Suspicious</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>&lt; 8 cm</td>
<td>&gt; 8 cm</td>
</tr>
<tr>
<td>Septum thickness</td>
<td>≤ 3 mm</td>
<td>&gt; 3 mm</td>
</tr>
<tr>
<td>Cyst wall thickness</td>
<td>≤ 3 mm</td>
<td>≥ 3 mm</td>
</tr>
<tr>
<td>Papillary excrescences projection</td>
<td>≤ 3 mm</td>
<td>&gt; 3 mm</td>
</tr>
<tr>
<td>Solid part</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Free fluid</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Doppler RI</td>
<td>&gt; 0.42</td>
<td>&lt; 0.42</td>
</tr>
</tbody>
</table>

Table 2. — Histological types of adnexal cysts.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Cases</th>
<th>Cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometriotic cysts</td>
<td>634</td>
<td>917</td>
</tr>
<tr>
<td>Dermoid cysts</td>
<td>167</td>
<td>198</td>
</tr>
<tr>
<td>Paraovarian-paratubal cysts</td>
<td>189</td>
<td>251</td>
</tr>
<tr>
<td>Simple cysts</td>
<td>287</td>
<td>413</td>
</tr>
<tr>
<td>Fibroadenomas</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>Borderline cysts</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1,332</td>
<td>1,838</td>
</tr>
</tbody>
</table>

Table 3. — Incidence of malignancy during laparoscopic removal of ovarian neoplasms.

<table>
<thead>
<tr>
<th>Case</th>
<th>Patient initials</th>
<th>Age</th>
<th>Preoperative diagnosis</th>
<th>Phase of laparoscopy</th>
<th>Final histology of borderline cyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RV</td>
<td>21</td>
<td>R: endometrioma</td>
<td>Diagnostic</td>
<td>Serous cystadenoma</td>
</tr>
<tr>
<td>2</td>
<td>BK</td>
<td>32</td>
<td>R: endometrioma</td>
<td>Operative</td>
<td>Endometrioid</td>
</tr>
<tr>
<td>3</td>
<td>AV</td>
<td>38</td>
<td>R: simple cyst</td>
<td>Operative</td>
<td>Serous-papillary</td>
</tr>
<tr>
<td>4</td>
<td>TE</td>
<td>16</td>
<td>R: simple cyst</td>
<td>Operative</td>
<td>Brenner</td>
</tr>
<tr>
<td>5</td>
<td>ZN</td>
<td>27</td>
<td>R: endometrioma</td>
<td>Operative</td>
<td>Endometrioid</td>
</tr>
<tr>
<td>6</td>
<td>ZM</td>
<td>42</td>
<td>R: cystadenoma</td>
<td>Diagnostic</td>
<td>Epithelial</td>
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<tr>
<td>7</td>
<td>PM</td>
<td>20</td>
<td>R: dermoid</td>
<td>Operative</td>
<td>Epithelial</td>
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<tr>
<td>8</td>
<td>LZ</td>
<td>34</td>
<td>L: cystadenoma</td>
<td>Operative</td>
<td>Serous-papillary</td>
</tr>
</tbody>
</table>

Discussion

This retrospective study presents 17 years experience of our endoscopic center and according to our protocol the appearance of unsuspected BOT was limited only to eight patients. All of them are alive with favourable prognoses. The rate of unexpected borderline and invasive tumours ranges between 0.4% [9] and 4.2% [10], and our results are one of the lowest compared to the reported outcomes of other experienced endoscopic centres. The strength of this study can be attributed to the fact that all preoperative ultrasound (US) images were personally performed or reviewed in case of referral patients by the laparoscopist. In addition, other studies demonstrated that the risk of malignancy in simple cysts, defined as unilocular with smooth inner wall, rises from 0.8% in premenopausal women to 9.6% in postmenopausal [11]. However, cyst wall papillaries may sometimes be undetected during preoperative workup, especially in cysts with a diameter of > 50 mm because of the larger inner surface that has to be examined. This occurs because small papillary formations of 2-3 mm can easily be missed by US [7] due to poor US penetration and resolution. Taking into consideration the above studies, we can justify our inability to correctly characterise our eight borderline cases. Furthermore, three out of ten BOT were characterised as simple unilocular cysts without any visible papillae or solid mass. Also, four cysts were described preoperatively as endometriomata but histology revealed three of them as endometrioid BOT and the other as borderline serous cystadenoma. Thus, in cases with a previous history of endometriosis a high index of suspicion must always be maintained with strict adherence to pre- and intraoperative protocols.

Laparoscopy or laparotomy, conservative or radical treatment of Stage I BOT are fields of conflict between laparoscopists and oncologists. However, borderline and early ovarian cancers may also be missed after inadequate management by laparotomy [12]. This means that the crucial point is the accuracy of preoperative and intraoperative diagnosis rather than the preferred method of access to the abdominal cavity. In all of our unexpected eight cases, frozen sections were performed due to correct intraoperative laparoscopic diagnosis and were in 100% agreement with the permanent histological results without missing any case of malignancy.

When an unexpected malignancy is encountered in case of a presumed benign cyst based on the findings of preoperative investigation and laparoscopic inspection, three completely different subgroups with different implications for prognosis can be distinguished: a) the malignancy is macroscopically evident during the diagnostic phase of laparoscopy and no operative maneuver is considered on the cyst, b) the malignancy is macroscopically evident during the operative phase of laparoscopy, when the cyst wall is opened, and c) no macroscopic evidence has been found either in the diagnostic or operative phase, and no frozen section has been performed or in case of a false negative frozen section the operative procedure is completed by laparoscopy. In the first subgroup no adverse
laparoscopy and six were macroscopically recognized during the operative phase of the intervention. No “late” unsuspected ovarian malignancy was encountered.

After correct diagnosis, the next important step is the appropriate management of a borderline adnexal mass, which depends on the laparoscopist’s experience and patient’s expectations with regard to the maintenance of fertility capacity in case of unexpected borderline or malignant tumour. In favour of fertility sparing treatment is a recent Gynaecologic Oncology Group (GOG) study suggesting that even cystectomy may be adequate thera-

Figure 1. — Our protocol for laparoscopic management of adnexal masses.

effect on prognosis from the surgical approach can be expected, except for the theoretical negative effect from the increased intraperitoneal pressure. In the second subgroup, when an accidental rupture of the cyst occurs, the patient is upstaged from Stage IA to IC. For this reason in suspicious cysts all operative maneuvers should take place using an endoscopic bag. In the third subgroup, since there is a delay until the final pathology which has a detrimental effect on the prognosis. From the eight cases of unexpected malignancy of our study, two were macroscopically evident during the diagnostic phase of laparoscopy and six were macroscopically recognized during the operative phase of the intervention. No “late” unsuspected ovarian malignancy was encountered.

After correct diagnosis, the next important step is the appropriate management of a borderline adnexal mass, which depends on the laparoscopist’s experience and patient’s expectations with regard to the maintenance of fertility capacity in case of unexpected borderline or malignant tumour. In favour of fertility sparing treatment is a recent Gynaecologic Oncology Group (GOG) study suggesting that even cystectomy may be adequate thera-
py for women of reproductive age considering an the eventual relapse may be successfully managed by re-operation [13]. Seven out of our eight patients chose salpingo-oophorectomy in case of unexpected BOT and the other one of our nulliparous patients initially gave her consent for cystectomy, but later, after two successful IVF cycles and pregnancy, underwent bilateral oophorectomy.

In the absence of prospective clinical trials with regard to the spillage of the cyst content after intentional or no cyst perforation, the prognostic value of intraoperative rupture of cysts in Stage IA BOT or invasive cancer is a controversial topic. However, spilling should be avoided until otherwise confirmed.

In conclusion, in women of reproductive age, pre-laparoscopic evaluation and selection for the endoscopic approach are of utmost importance in the reduction of cases of unrecognised borderline or malignant ovarian cysts. Finally, in young women wishing to preserve their fertility, a conservative approach is reasonable followed by definitive surgery after successful pregnancies or by recurrence or malignant invasion at an early stage.

References

Address reprint requests to:
G. PADOS, M.D.
40, Mitropoleos Street
Thessaloniki 54623 (Greece)
e-mail: padosgyn@hol.gr
Expression of survivin and VEGF-C in breast cancer tissue and its relation to lymphatic metastasis

Xiangqi Li, Xiangguo Dang, Xibo Sun
Department of General Surgery, Affiliated Hospital of Taishan Medical College, Taian (China)

Summary
Purpose: To study the expression of survivin and VEGF-C in breast cancer tissue and its relation to lymphatic metastasis. Methods: The expression level of survivin and VEGF-C in breast cancer tissue is determined by immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR). Results: Among the 60 breast cancer tissues, the percentage of positive expression of survivin or VEGF-C was 85.0% and 78.3%, respectively. The expression rate of VEGF-C was 84.3% (43/51), 44.4% (4/9) in the survivin positive and negative expression group, respectively. Linear correlation analysis showed a correlation coefficient of survivin and VEGF-C in breast cancer was 0.80 (p = 0.035), which indicates a positive correlation between the two biomarkers. Conclusions: Survivin and VEGF-C are highly expressed in breast cancer tissues with a positive correlation. The regulation of survivin expression can cause excessive expression of VEGF-C, leading to the generation of breast cancer lymphangiogenesis, thus causing lymphatic metastasis of breast cancer.

Key words: Breast cancer; Lymphatic metastasis; Survivin; Vascular endothelial growth factor-C.

Introduction
The major cause of death in breast cancer patients is widely spread metastasis. Metastasis usually first occurs in the axillary lymph node; therefore, lymphatic metastasis is frequently used as one of the main standards to evaluate prognosis and choose treatment methods for breast cancer patients [1]. During the diagnosis and treatment of breast cancer, early detection of a lymphangiogenesis and control of lymphatic metastases are very important in the prevention of the patient dying from breast cancer. Vascular endothelial growth factor-C (VEGF-C) is a new member of the vascular endothelial growth factor family, and is highly expressed in most human tumors. In vivo studies confirm that VEGF-C can induce a generation of tumor lymphatic metastasis and participate in the metastasis of tumor lymphatics through vascular endothelial growth factor receptor-3 (VEGFR-3), which is considered a regulatory factor of specific lymph angiogenesis [2]. Survivin belongs to the inhibitor of apoptosis protein (IAP) family, and is highly expressed in human embryonic tissues. It plays a role in tissue differentiation and organ generation in the course of embryonic development. Survivin expression cannot be detected in mature differentiated adult tissues, but is widely detected in many kinds of human tumors. Studies have shown that survivin not only is expressed in cancer tissues, but also is expressed in many pre-cancer tissues [3]. Here we investigated whether survivin expression is related to the expression of VEGF-C in breast cancer and their roles in the generation of lymphangiogenesis. At present there are no related reports of whether survivin expression is related to VEGF-C expression in breast cancer. This paper focuses on studying the expression of survivin and VEGF-C in breast cancer and their effects and relationship in the course of lymphatic metastases, suggesting potential targets for comprehensive therapy of breast cancer.

Patients and Methods

Patients
Sixty cases of breast cancer postoperative specimens diagnosed by hematoxylin-eosin (HE) staining pathology [4] were collected from female patients with an average age of 43.6 (range 27-66) between January 2007 and December 2009 in a subsidiary hospital, Taishan Medical College. The collection was approved by the Medical Ethics Committee of the subsidiary hospital, Taishan Medical College. Among the 60 cases, there were 54 cases of infiltrative ductal carcinoma, one case of infiltrative lobular carcinoma, four cases of medullary carcinoma, and one case of infiltrative ductal carcinoma with squamous carcinoma. Thirty-two cases had lymph node metastasis while the remaining cases did not. All patients were not given radiotherapy or chemotherapy prior to the surgery. Based on the 7th edition staging of UICC (International Union Against Cancer and AJCC American Joint Committee on Cancer) [5], there were 11 Stage I cases, 38 Stage II cases and 11 Stage III cases of breast cancer. In addition, among the 60 cases we also collected 23 fresh specimens of breast infiltrative ductal carcinoma [4] diagnosed by pathology, with 12 cases having lymph node metastases and 11 cases free of lymph node metastasis. All of the specimens were excised to about 1.2 cm³ in size and quickly frozen in a -70°C freezer.

Main reagents
Rabbit anti-human polyclonal antibody against survivin and VEGF-C were purchased from Beijing Boaosen Biotechnology Limited Company (Beijing). Streptavidin-horseradish peroxidase, S-P kit (sp-9001), and enrichment-type 3,3N-diaminobenzidine tetrahydrochloride (DAB) chromomeric kit were obtained from Beijing Zhongshanjinqiao Biotechnology Co.
Expression of survivin and VEGF-C in breast cancer tissue and its relation to lymphatic metastasis

Experimental Methods

Detection of survivin and VEGF-C by immunohistochemistry

Paraffin-embedded specimens were deparaffinized and then embedded again to restore antigenicity. Specimens were cut into sections of 4 µm in thickness and the immunohistochemical S-P method was used following the manufacturer’s protocol. The sections of known VEGF-C and survivin served as positive controls in each experiment while phosphate buffered saline (PBS) was used as the negative control. All sections were stained with DAB and read under light microscope within 48 hours.

Results determination: The positive expression of VEGF-C was localized in the cytoplasm with a distribution of brownish yellow or sepia colored particles. Survivin was localized in the cytoplasm with yellow to brownish yellow particles. Two expert pathologists evaluated the experimental results using the double blind method. Three visual fields were randomly chosen in each section of 4 µm in thickness and the immunohistochemical S-P sections were cut into 2.0% agarose gel by electrophoresis. Gel pictures were taken under ultraviolet light after staining with ethidium bromide. The separation of density values between the target bands and internal reference primer sequence F: GGC-CTGGCAGCCCTTTC-3', R: 5'-CTGGCTCCCAGCCTTCCA-TGCTTAGCTGACACTTGT, the length of PCR primer sequence F: CACGGCTTATGCAAGCAAAGA, R: 5' the fragment of amplified product was 188 bp. cDNA was first synthesized by reverse transcription in 50°C for 5 min, then the PCR reaction began after pre-degeneration in 95°C for 15 min followed by 35 cycles of amplification at 94°C for 45 sec, 52°C –58°C for 45 sec, 72°C for 1 min and a final 10 min extension at 72°C. The amplification product was separated on 2.0% agarose gel by electrophoresis. Gel pictures were taken under ultraviolet light after staining with ethidium bromide. The graphical analysis software of BandScan Version 4.3 was used to analyze the densities of the electrophoresis bands. The ratio of density values between the target bands and internal reference bands was the relative expression quantity of survivin and VEGF-C mRNA.

Statistical analysis

Applying SPSS 13.0 statistics analysis software, experimental data was analyzed using χ² and relative linear regression analysis. Ranks table χ² inspection and the four-layer table χ² inspection, Spearman correlation analysis, independent sample t inspection and Wilcoxon two-sample comparison were performed according to the type of information and different statistical purposes. A p value less than 0.05 was considered statistically significant.

Results

Expression of survivin and VEGF-C protein in breast cancer tissue

In 60 cases of breast cancer specimens, the positive expression rate of survivin was 85.0% (51/60) and 78.3% (47/60) for VEGF-C. The expression rate of survivin in the lymph node positive group was 96.9% (31/32), which

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<th>VEGF-C</th>
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<tr>
<td>Tumor size (cm)</td>
<td>a</td>
<td>χ²</td>
</tr>
<tr>
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<td>21</td>
<td>17 (81.0)</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>39</td>
<td>34 (87.2)</td>
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<tr>
<td>Other cancer</td>
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</tr>
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<tr>
<td>No</td>
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<td>20 (71.4)</td>
</tr>
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<td>49</td>
</tr>
<tr>
<td>Stage III</td>
<td>11</td>
<td>10 (90.9)</td>
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<th>VEGF-C mRNA</th>
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</tr>
<tr>
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<td>8</td>
<td>3</td>
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<td>VEGF-C: Vascular endothelial growth factor-C.</td>
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<th>VEGF-C</th>
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<tr>
<td>+</td>
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</tr>
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</tr>
<tr>
<td>++++</td>
<td>1</td>
<td>5</td>
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<tr>
<td>VEGF-C: Vascular endothelial growth factor-C.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Expression of survivin and VEGF-C in breast cancer tissues

In 23 cases of fresh breast cancer specimens, the expression of survivin mRNA in breast invasive ductal carcinoma tissue with lymph node metastasis was higher than that without lymph node metastasis. The difference between these two groups was statistically significant ($u = -2.869$, $p = 0.0041$). The difference of VEGF-C expression in the two groups of breast invasive ductal carcinoma tissues was also statistically significant ($u = -2.2794$, $p = 0.0226$) Table 3, Figure 2.

Discussion

In recent years, with the discovery of lymphatic growth factor and some specific lymphatic endothelial markers, the study of lymphatic formation and lymphatic metastasis has gradually become a new hotspot [8]. Among lymphatic formation factors, VEGF-C, first discovered as a member of the vascular endothelial growth factor (VEGF) family, is a specific lymphatic formation growth factor. The affinity of VEGF-C to VEGFR-3 is three times stronger than that of VEGFR-2. VEGF-C activates VEGFR-3 on lymphatic endothelial cells, induces phosphatidylinositol 3-kinase signaling pathways and subsequently activates p42/p44 mitogen activated protein kinase and kinase B signal pathways. These cascade reactions protect the lymphatic endothelial cells from serum induced apoptosis, promote proliferation and migration of lymphatic endothelial cells, and promote the generation of lymphangiogenesis [9]. At the same time, the adhesions between lymphatic endothelial cells are reduced, improving permeability of lymphatic endothelial cells. Improved permeability of the lymphatic endothelial cells allows cancer cells to infiltrate more...
Expression of survivin and VEGF-C in breast cancer tissue and its relation to lymphatic metastasis

Our research results also coincide with those of Nakamura et al. [11] indicating VEGF-C plays an important role during the generation and metastasis of lymphatics in breast cancer. In matched breast cancer specimens, VEGF-C mRNA expression was significantly higher in lymph node-positive cases (90.6%, 29/32) than in lymph node-negative cases (64.3%, 18/28) (χ² = 6.10, p = 0.01), which suggests that the positive expression of VEGF-C is related to breast cancer lymphatic metastasis, indicating VEGF-C plays an important role during the generation and metastasis of lymphatics in breast cancer. These results indicate survivin may play an important role during the generation and development of breast cancer, and thus promote lymphatic metastasis. Our research also showed that the positive expression rate of VEGF-C is 78.3% (47/60), the expression rate in the lymph node-negative group [90.6%, (29/32)] was significantly higher than that of lymph node-negative group [64.3% (18/28)] (χ² = 5.72, p = 0.02), suggesting that survivin may play an important role during the generation and development of breast cancer by inhibiting apoptosis of cancer cells, directly or indirectly promoting the expression of VEGF-C protein, and thus promoting the generation of breast cancer lymphangiogenesis, increase the metastasis of local lymphangiogenesis and distant metastasis (lung). Their results also suggest that newly formed lymphangiogenesis exists in malignant tumors. By activating the receptor VEGFR-3 on lymphatic endothelial cells, VEGF-C can promote the generation of lymphangiogenesis in tumors, and thus promote lymphatic metastasis. Our research showed that the positive expression rate of VEGF-C is 78.3% (47/60), the expression rate in the lymph node-negative group [90.6%, (29/32)] was significantly higher than that of lymph node-negative group [64.3% (18/28)] (χ² = 6.10, p = 0.01), which suggests that the positive expression of VEGF-C is related to breast cancer lymphatic metastasis, indicating VEGF-C plays an important role during the generation and metastasis of lymphatics in breast cancer. Our research results also coincide with those of Nakamura et al. [12]. There was no difference on VEGF-C expression between invasive ductal carcinoma and other pathological breast cancer types and between each TNM staging, which suggests that VEGF-C expression is not related to breast cancer size and tumor type.

Survivin is a member of inhibitors of the apoptosis protein family, and participates in controlling apoptosis and cell division with dual functions of inhibiting apoptosis and regulating cell proliferation [13]. The expression of survivin has high specificity; there is no expression or low expression in normal tissue, but highly specific expression in tumor tissue, related to the loss of apoptosis of tumor cells and the generation of tumors. In addition, the expression of survivin is always positively related to the grade of malignancy of breast tumors and poor prognosis [14]. A previous study by Tan et al. [15] showed that survivin can effectively maintain micro blood vessel structure, promote VEGF protection effects on endothelial cells, and resist apoptosis of endothelial cells induced by chemotherapy drugs.

In all studied 60 cases of breast cancer specimens, the positive expression rate of survivin was 85.0% (51/60), and the expression rate of the lymph node-positive group [96.9% (31/32)] was significantly higher than that of the lymph node-negative group [71.4% (20/28)] (χ² = 5.72, p = 0.02), suggesting that survivin may play an important role during the generation and development of breast cancer by inhibiting apoptosis of cancer cells. In addition, we found that the expression rate of survivin in breast cancer tissues with lymphatic metastasis was significantly higher than that of breast cancer tissue without lymphatic metastasis. There was no obvious difference in survivin expression between invasive ductal carcinoma and other pathological breast cancer types or between each TNM staging, which suggests that VEGF-C expression is not related to breast cancer size and tumor type. These results indicate survivin may play a promoting role in the activity of breast cancer cell metastasis and invasion. The presence of lymphatic metastasis was an independent index related to breast cancer therapy and prognosis. The expression of survivin mRNA and VEGF-C mRNA in breast invasive ductal carcinoma with lymphatic metastasis was higher than that without lymphatic metastasis. Survivin mRNA and VEGF-C mRNA were positively related to lymphatic metastasis, indicating survivin and VEGF-C jointly promote lymphatic metastasis. Further research results showed a positive relation between the expression of survivin and VEGF-C in breast cancer tissues and that they were closely related to lymphatic metastasis of breast cancer. Therefore, we conclude that elevation of the expression of survivin can upregulate the expression of VEGF-C in breast cancer, induce lymphangiogenesis via VEGFR-3 action on endothelial cells, consequently promoting lymphatic metastasis.

We also conclude that survivin and VEGF-C play a common role during lymphatic metastasis of breast invasive ductal carcinoma. It is likely that survivin inhibits the apoptosis of breast cancer cells, directly or indirectly promotes the expression of VEGF-C protein, and thus promotes lymphatic endothelial cell growth and lymphatic metastasis of breast cancer. However, due to limited number of specimens in this study, it is still not clear how survivin promotes the expression of VEGF-C, which needs to be confirmed by relevant in vivo studies.

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References


Address reprint requests to:
XIANGQI LI, M.D.
Department of General Surgery
Affiliated Hospital of Taishan Medical College
#706 Taishan Street
Taian 271000 (China)
e-mail: drlixqi@hotmail.com
Accuracy of physician and nurse practitioner colposcopy to effect improved surveillance of cervical cancer

G. Kilic1, J. England1, M. Borahay1, D. Pedraza1, D. Freeman2, R. Snyder1, A.K. Ertan3

1Department of Obstetrics & Gynecology, The University of Texas Medical Branch, Galveston, TX
2Department of Preventive Medicine and Community Health, Office of Biostatistics, The University of Texas Medical Branch, Galveston, TX (USA)
3Department of Obstetrics and Gynecology, Klinikum Leverkusen gGmbH, Leverkusen (Germany)

Summary

Purpose: To compare physician and nurse practitioner accuracy in recognizing cervical dysplasia during colposcopy. Materials and Methods: A retrospective review was performed of cervical excisional biopsies from 2007 to 2009 performed by gynecologists and nurse practitioners in the same patient population. Cervical cone biopsy and loop electrosurgical excision procedure (LEEP) pathology were used as a gold standard compared to the previous colposcopy biopsies. Results: Four hundred fifty-five patients qualified for the study. Patients were stratified according to age: under 30 years, 30-39, and 40 and above. For physicians, 77% of high-grade colposcopy biopsy results agreed with high-grade pathology on cone biopsy or LEEP. This was statistically similar to nurse practitioner results (p = 0.12). Likewise, there was no significant difference between physician and nurse practitioner accuracy within the various patient age strata. Conclusion: Colposcopy biopsy results compared to cone biopsy or LEEP results were statistically similar between gynecologists and nurse practitioners.

Key words: Colposcopy; Colposcopic training; Cervical dysplasia; LEEP; Cold knife conization.

Introduction

Cervical cancer mortality rates have steadily declined in the past 30 years by 50%, and this is attributed to early detection of cervical dysplasia due to prevention and as a result of screening [1, 2]. Their early detection has led to the treatment of preinvasive lesions [3]. Success in cervical cancer prevention has occurred due to the ability to detect and treat grade 3 cervical intraepithelial neoplasia (CIN3) prior to development of invasive disease [4]. Therefore, a special interest for clinicians is to recognize high-grade lesions at the time of colposcopy.

Screening for cervical dysplasia involves various modalities, including the Papanicolaou (Pap) smear and colposcopy. In the US, approximately 3.5 million of 5 million women undergoing Pap smear screening annually will require additional intervention for cytologic abnormalities [5]. In some European countries, colposcopy is performed routinely during the gynecologic visit, often in conjunction with the annual Pap smear [6]. However, in the United Kingdom and the United States, colposcopic exam usually follows an abnormal cytology result [6]. Colposcopic biopsies are directed at the most apical site of the cervix identified by the colposcopist [7].

It is imperative to have comprehensive, evidence-based teaching of physicians and nurse practitioners in colposcopy and management of cytologic abnormalities in order to continue to decrease the morbidity and mortality of cervical cancer. The increased numbers of colposcopists has improved access to care for women with abnormal clinical cytology and has likely decreased the waiting time for their evaluation [8]. The retrospective review of colposcopic examinations by Baum et al. revealed favorable colposcopy results of gynecology residents; their study compared colposcopic impressions to cervical biopsy results. They concluded, however, that a more structured colposcopy training program would be preferred [5].

A standardized teaching of colposcopy does not currently exist in the United States for resident physicians and nurse practitioners, and there is discrepancy regarding the training requirements and experience required to obtain proficiency with colposcopy. Caruthers and Sheets recommend 30 supervised colposcopies [6]. Brotzman and Appar advise 25-50 colposcopic examinations, including ten colposcopies for high-grade lesions [7].

The Council on Resident Education in Obstetrics and Gynecology (CREOG) suggests that residents should “perform and interpret the results of diagnostic procedures for cervical dysplasia” [9]. Additionally, the Accreditation Council for Graduate Medical Education does not define a required training program for colposcopy [5]. The American Society for Colposcopy and Cervical Pathology (ASCCP) currently recommends a 3-tiered system for colposcopy training. The first tier consists of a didactic program during residency training or through an accredited colposcopy course. The second tier of the system consists of mentored colposcopic instruction. The final tier of the program consists of completion of an exit examination [10]. The ASCCP offers a colposcopic mentorship program and reviews colposcopic cases with a written examination of colposcopic principles and practice [11].

Reduced cervical cancer rates are due to a number of factors, including technical expertise, high coverage rate, and rigorous quality assurance in the laboratory, in the colposcopy clinic, and in administrative offices [12].
Development of accurate and reproducible methods of colposcopic assessment are needed to manage abnormal cervical screening results being referred for diagnosis and possible treatment [13].

Colposcopic examination has several restrictions to take into account. First, colposcopy is not a sufficient diagnostic method when the squamocolumnar junction is not entirely visible. A diagnostic cone biopsy is indicated then, although some colposcopists consider the large loop biopsy as a good diagnostic method in those patients. Second, colposcopy includes the subjective assessment of the impression by colposcopists, which consequently results in observer variability. Levels of agreement among experienced colposcopists increase as the cervical lesion becomes more severe. In the ASCUS-LSIL Triage Study (ALTS), the sensitivity of initial colposcopy for CIN3 identified during two years of observation was only 54% [14].

In our study, the gynecologists’ accuracy in recognizing high-grade cervical dysplasia during the colposcopy exam was compared to that of nurse practitioners in the same department serving the same patient population. Having these two groups coming from two different backgrounds and colposcopy training methods gave us the opportunity to compare the two training techniques.

Materials and Methods

All cold-knife cone and LEEP pathology results were retrospectively collected between 2007 and 2009. A total of 455 patients qualified for this study. After institutional IRB approval was obtained, all the cases were divided into two groups: colposcopy performed by physicians and those performed by nurse practitioners. Patients were also stratified by age: less than 30 years of age, 31-39 and 40 years and above.

Women who had colposcopy performed followed by successive cone biopsy or LEEP were included. Colposcopy was performed on patients with high-grade squamous intraepithelial lesions (HSIL) on pap smear cytology, persistent low-grade squamous intraepithelial lesions (LSIL), atypical squamous cells (ASC), or atypical glandular cells (AGC). Patients with cervical cancer were excluded due to the small number of cases to avoid statistical inconvenience, and patients with inconclusive biopsy results unable to be classified (n = 17) were also excluded.

All colposcopic exams were performed and documented the same way: the key concepts of the system included the dimensions of color, vessel, border, and surface pattern. Descriptions of cervical findings in each category were classified as normal, preinvasive disease, and invasive disease. The procedure utilizes low power (3.5x) on colposcopy to obtain a general impression of the surface architecture of the cervix. Medium and high (7x, 15x) powers are utilized to evaluate the vagina and cervix, and various light filters are available to highlight different aspects of the surface of the cervix. Three percent acetic acid solution is applied to the surface to achieve hemostasis. Monsel’s solution is applied with large cotton swabs to the surface of the cervix to achieve hemostasis.

Interpretation of results was calculated with Cohen’s κ Statistic [15] in order to determine the strength of agreement between colposcopy versus cone biopsy or LEEP pathology.

Results

The gynecologist group performed a total of 147 of colposcopy exams which required cervical excisional biopsy, and the nurse practitioner group performed a total of 308 exams that ended up with excisional biopsy in the same time frame. For agreement between colposcopy biopsy and cone biopsy, gynecologists had a kappa of 0.24 and RNs had a kappa of 0.1542 (Table 1). These values were not significantly different. The highest agreement was for patients 40 and older. Among the patient group 40 years of age and older, the number of exams for gynecologists and nurse practitioners were 30 and 57, respectively. The provider-by-age stratified kappas still were not significantly different.

Excisional biopsy showed high-grade cervical dysplasia in 315 patients (Table 2). Considering excisional biopsy as the gold standard, the sensitivity of colposcopy examination for the MD group was 77.14% while the nurse practitioner group was 82.38% (p = 0.12). None of the age bracket differences between nurse practitioners and MDs was statistically significant, but the difference for under age 30

<table>
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<th>Kappa value n</th>
<th>95% Confidence Limits</th>
<th>Test of Equality</th>
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</tr>
<tr>
<td>MD</td>
<td>0.1923</td>
<td>0.0983</td>
<td>-0.0004</td>
<td>0.385</td>
</tr>
<tr>
<td>NP</td>
<td>0.1022</td>
<td>0.0814</td>
<td>-0.0574</td>
<td>0.2617</td>
</tr>
<tr>
<td>40 and older</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>0.3902</td>
<td>0.137</td>
<td>0.1218</td>
<td>0.6587</td>
</tr>
<tr>
<td>NP</td>
<td>0.2332</td>
<td>0.0987</td>
<td>0.0397</td>
<td>0.4267</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Provider</th>
<th>Sensitivity</th>
<th>Exact p value n</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>77.14</td>
<td>0.1231 315</td>
</tr>
<tr>
<td>NP</td>
<td>82.38</td>
<td></td>
</tr>
<tr>
<td>Under 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>71.05</td>
<td>0.0564 151</td>
</tr>
<tr>
<td>NP</td>
<td>84.96</td>
<td></td>
</tr>
<tr>
<td>30 to 39</td>
<td></td>
<td>0.8268 122</td>
</tr>
<tr>
<td>MD</td>
<td>76.59</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td>82.19</td>
<td></td>
</tr>
<tr>
<td>40 and older</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>83.33</td>
<td>0.2348 42</td>
</tr>
<tr>
<td>NP</td>
<td>70.83</td>
<td></td>
</tr>
</tbody>
</table>
was “borderline” ($p = 0.06$). For patients from ages 30 to 39, the MD group sensitivity was 76.59%, and nurse practitioner group sensitivity was 82.19% ($p = 0.82$). The only age bracket for which the MD group sensitivity was higher than nurse practitioners was patients 40 and older. The MD group compared to nurse practitioners was 83.33% vs 70.83%, respectively ($p = 0.23$).

Both sets of provider patients had similar age distributions. The mean age for the MD group was 32.74 ± 7.53 versus 31.72 ± 9.65 for the nurse practitioner group (Table 3). On average, the time lapse from the colposcopy exam to the excisional biopsy was 66 ± 54.26 days. Being the only tertiary care hospital partially explains the lag time from colposcopy to excisional biopsy. Additionally, our teaching hospital provides indigent care, and the scheduling, transportation, and follow-up obstacles for these patients also prolong the lag time. We do, however, have a strong patient follow-up system, which helps us to eventually reach patients initially lost to follow-up.

### Discussion

In this study, we focused on the patients who received excisional biopsy after colposcopy exam following ASCCP guidelines. All consecutive excisional biopsies were used as a gold standard compared to the last colposcopy biopsy results. Furthermore, all the colposcopies were performed by trained nurse practitioners or gynecologists. Colposcopic impressions for nurse practitioners and gynecologists were not statistically different. Nurse practitioners are able to perform the colposcopic exam within their first year of training to the same ability as medical doctors.

Table 3: — Age distribution and timeframe between colposcopy and excisional biopsy.

<table>
<thead>
<tr>
<th>Provider</th>
<th>N</th>
<th>Mean Age</th>
<th>Standard Deviation</th>
<th>Minimum Quartile</th>
<th>Median Quartile</th>
<th>Upper Quartile</th>
<th>Maximum Quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>147</td>
<td>32.74</td>
<td>7.53</td>
<td>20</td>
<td>27</td>
<td>31</td>
<td>38</td>
</tr>
<tr>
<td>NP</td>
<td>308</td>
<td>31.72</td>
<td>9.65</td>
<td>17</td>
<td>25</td>
<td>29</td>
<td>36</td>
</tr>
</tbody>
</table>

Days to excisional biopsy: 308 | 66.16 | 54.26 | 5 | 34 | 53 | 78.5 | 565 |

MD = medical doctor; NP = nurse practitioners.

It is imperative for colposcopists to perform accurate colposcopies in order to appropriately care for these patients. The training involves multiple steps, which include understanding the indication for colposcopy, patient counseling, formation of a colposcopic impression, and taking cervical biopsies. During training, the goal of the colposcopist is to master these steps. Our nurse practitioners went through intense colposcopy training: a 3- to 5-day didactic course to evaluate and manage patients with abnormal Pap smears, including classroom instruction and clinical skills practice with live models. The course consists of description of preinvasive disease of the cervix, information regarding normal and abnormal features of the transition zone of the cervical os, performing colposcopy, and patient education. Nurse practitioners are required to perform a minimum of 50 supervised colposcopies before certification. In this study, only post-certification cases were included. Gynecologists certified by the American Board of Obstetrician and Gynecologists train for colposcopy during their residency programs and also complete one of ASCCP’s 2-day colposcopy courses. At the time of study, the experience of the gynecologists ranged from 3-25 years post residency.

Cervical excisional biopsy may not be the best gold standard in certain situations. For example, in some cases, the whole lesion might have been removed at the time of biopsy done during colposcopy. However, both groups in our study were subject to the same shortcomings of the gold standard.

The ASCUS LSIL Triage Study (ALTS) Group looked at the effects the type of medical training and number of biopsies performed have on sensitivity of colposcopically guided biopsies [16]. In their study, the sensitivity of the procedure did not vary significantly by type of colposcopist. However, the sensitivity was significantly greater when the colposcopists took two or more biopsies instead of one ($p < 0.01$), a pattern observed across all types of colposcopists, including nurse practitioners, general gynecologists, gynecologic oncology fellows, and gynecologic oncologists. Their results in terms of similarities between nurse practitioners and gynecologists in their colposcopy impressions also agree with our results.

The role of the nurse colposcopist has been established in some countries for a number of years. McPherson et al. published their experience in a New Zealand nurse colposcopist training program. They reported on a clinical audit undertaken to assess the diagnostic skills of the nurse colposcopist measuring colposcopy: histology: cytology correlation. An 82% (82/100) colposcopy: histology: cytology correlation was achieved by the nurse in the third phase of her training program. The results were almost identical with our study; our nurse practitioner group was 82.38%.

Effective screening to detect and treat cervical dysplasia is essential. Residency programs must strive to properly educate residents on the performance of colposcopy to decrease the morbidity and mortality of cervical cancer. The nurse practitioners can be an effective resource for cervical cancer screening using colposcopy as a preliminary screening method. Initial didactic training and continued training are needed to maintain colposcopic skills in both medical doctors and nurse practitioners.

### References


Address reprint requests to:
G.S. KILIC, M.D.
301 University Blvd.
Galveston, TX 77555-0587 (USA)
e-mail: gokilic@utmb.edu
Impact of sampling origin on molecular detection of high-risk human papillomavirus and oncogene expression

S. Kahla1, M. Achour1, S. Oueslati1, L. Kochbati2, M.B. Chanoufi3, M. Maalej2, R. Oueslati1

1Unit of Immunology Microbiology Environmental and Carcinogenesis (IMEC), Science Faculty of Bizerte, University of Carthage
2Radio-Oncology Department, Salah Azaiez Institute, Tunis; 3Service of Gynaecology Obstetrics A, Center of Maternity and Neonatology, La Rabta Hospital, Tunis (Tunisia)

Summary

Purpose of investigation: The recognition of high-risk human papillomavirus (HR-HPV) as an etiological agent of cervical cancer has increased the importance of testing for HPV, and this might contribute to better risk stratification. Methods: Eighty-eight randomly selected cervical cancer specimens including biopsies and their respective smears were used in this study. Control scappings were obtained from ten healthy women. The presence of HPV16 and HPV18 was investigated using the technique of polymerase chain reaction (PCR) with the specific primers for the L1 region, while mRNA expression of HPV16 E6-E7 was evaluated by a reverse transcription PCR method (RT-PCR). Results: The positivity for the viral genotype was influenced by the quantity of amplified DNA used. In tumor biopsies the higher positivity for HPV16 (54.5%) and HPV18 (15.9%) was obtained using 687.4 ng of DNA. At smears level sole- tion PCR method (RT-PCR). Conclusion: Our data provide prospective evidence that HPV16/18L1 revelation at biopsy toward pathological types is efficient and correlates well with oncogenic transcript findings. Subtle changes in viral oncogene dynamics highlight the presence of other regulating proteins serving as additional biomarkers.

Key words: Biopsies; Cervical cancer; DNA load; E6-E7 oncogenes; Human papillomavirus; L1 gene; Smears.

Introduction

Cervical cancer is a major problem in women’s health. It is the second most common cancer in the world and the leading cause of cancer death among women in developing countries. Worldwide, an estimated 500,000 new cases occur and 250,000 women die annually from this tumor [1]. In Tunisia, the standardized cervical cancer incidence is 5.91 per 100,000 women yearly [2]. Clinical, molecular and epidemiological investigations have identified HPV as the major cause of cervical cancer and cervical dysplasia [3, 4]. This virus is sexually transmitted and the male is the carrier. More than 100 HPV genotypes have been described and 20 of them have been associated with cervical cancer [5]. Among the high-risk types, HPV16 and HPV18 are the most closely associated with cervical carcinoma [6, 7]. HPV18 is particularly interesting, since it is reported to be mainly associated with adenocarcinoma (AC), while HPV16 is more frequent in squamous carcinoma (SC) [8, 9]. Cytological examination of cervical smears is the most widely applied screening method for cervical cancer and its precursors. However, success of the smear test is limited with respect to sensitivity. Histological testing will be required in order to evaluate HPV detection. Because of the strong association between HPV infection and cervical cancer, detection of HPV DNA in cervical samples may be an available option to identify women at risk of developing cancer [10]. Numerous molecular techniques have been used, essentially the polymerase chain reaction (PCR) which is widely used for routine clinical practice. For comparison and additional evaluation, the detection of E6 and E7 transcripts of HR-HPV could serve as a better risk evaluation factor than DNA detection for the development of a high-grade squamous intraepithelial lesion and the progression to cervical carcinoma [11]. Specific HR-HPV transcripts E6 and E7 have been shown to act as oncogenes [12]; E6 and E7 proteins inactivate the tumor suppressor p53 and retinoblastoma (Rb) respectively and induce the breakdown of cell cycle regulation. Hence, HR-HPV infected cells develop genomic instability which can lead to the progression of cancer [13, 14]. Due to methodological reasons in large studies, we investigated in this work whether the origin of sampling affects viral load and molecular presence of the main high-risk viruses. Furthermore, we studied the screening impact on AC and SC of the cervix in different tumor stages. Ultimately the HPV16 E6-E7 transcripts were evaluated to correlate with HPV16 DNA findings.

Materials and Methods

Clinical samples

The study was retrospectively performed on 88 cervical cancer specimens including biopsies and respective smears. From each patient, cervical scraping was taken using an Ayre spatula harvested at once with biopsy then collected in 1 ml PBS (phosphate-buffered saline, pH 7.4). These samples were...
KCl, 10 mM Tris HCl, 200 μM of each dNTP (deoxynucleoside triphosphate) and 2.5 U of taq polymerase (Fermentas). DNA sample, 1.5 μl of each primer, 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris HCl, 200 μM of each dNTP (deoxynucleoside triphosphate) and 2.5 U of taq polymerase (Fermentas). DNA was amplified using a PCR thermocycler (Applied Biosystem). Primers and thermal cycler programs for identification of each virus are listed in Table 1 [15]. Each PCR experiment was performed with positive (HPV plasmids) and negative (water) controls. The quality of DNA obtained was controlled by amplification with primers detecting the housekeeping gene β-Globin. Finally, amplified DNA obtained by PCR from each sample was examined by electrophoresis on 1.5% agarose gels (Sigma) stained with ethidium bromide, visualized under ultraviolet light and photographed. DNA bands with the appropriate size were identified by comparison with a DNA ladder of known molecular weight (promega).

Reverse transcription PCR for detection of HPV16 E6 and E7 transcripts

HPV16 positive samples were further subjected to amplification of E6 and E7 transcripts. To control RNA integrity, PCR reactions using β-actin specific primers were performed as described previously [16] (Table 1). We processed 1 μg total RNA that was reverse-transcribed using the one-step RT-PCR Kit (Qiagen) in a 50 μl reaction containing 1X Qiagen one step RT-PCR buffer, 400 μlM of each dNTP, 0.6 μM random primers and 2 μl Qiagen one-step RT-PCR enzyme. The reaction was allowed to proceed for 30 min at 50°C for reverse transcription and 15 min at 95°C for initial PCR activation step. Cycling programs performed are illustrated in Table 1. The amplified products were electrophoresed in 1.5% agarose gel, marked by a 100-bp DNA ladder (Gene Ruler, Fermentas), stained with ethidium bromide, and visualized under UV light.

Statistics

Statistical analyses were performed using Statistica, version 6.0, for windows. The relationships between the different variables were assessed using Fisher’s exact test. Differences in detection of HPV16 DNA L1 and mRNA E6-E7 in biopsies were evaluated using the two-tailed McNemar’s test. When the p value less than 0.05 the difference was considered statistically significant.

Results

Prevalence of HPV genotypes in cervical cancer cells

The amount of DNA quantified by ultraviolet spectrophotometer varied widely, and the mean value was 56.2 μg. Variations in DNA quantities reflects the difference on number cells in each sample. The quantity of PCR assay affects viral detection. Thus, in tumor biopsies...
Table 2. — Quantitative evaluation of DNA according to cellular rates and prevalence of the HPV types on the level of tumor biopsies and smears.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Whole DNA recovered (μg)</th>
<th>Cells number X 10^6</th>
<th>DNA used for PCR (μg) in positive</th>
<th>HPV prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV16</td>
<td>91.6 ± 24.4</td>
<td>15.2 ± 4</td>
<td>687.4 ± 182.9</td>
<td>54.5%</td>
</tr>
<tr>
<td>HPV18</td>
<td>20.9 ± 22.9</td>
<td>3.2 ± 4</td>
<td>157.2 ± 178.4</td>
<td>31.8%</td>
</tr>
<tr>
<td>HPV16+HPV18</td>
<td></td>
<td></td>
<td></td>
<td>6.8%</td>
</tr>
<tr>
<td>Smears</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV16</td>
<td>91.6 ± 24.4</td>
<td>15.2 ± 4</td>
<td>687.4 ± 182.9</td>
<td>54.5%</td>
</tr>
<tr>
<td>HPV18</td>
<td>20.9 ± 22.9</td>
<td>3.2 ± 4</td>
<td>157.2 ± 178.4</td>
<td>31.8%</td>
</tr>
</tbody>
</table>

Data is presented as mean ± S.D.

(P: Fisher’s exact test).

Pathologic type

| SC         | 39 | 23 (58.9%) | 3 (7.6%) | 0.005* |
| AC         | 5  | 1 (20%)    | 4 (80%)  |        |

Stage

| Early (I, II) | 39 | 20 (51.2%) | 4 (80%)  |        |
| Late (III)    | 5  | 4 (10.2%)  | 3 (60%)  | 0.17   |

SC = squamous carcinoma; AC = adenocarcinoma.

Table 3. — Prevalence of HPV DNA types according to the clinicopathologic data.

<table>
<thead>
<tr>
<th>Items</th>
<th>Total no. of samples</th>
<th>HPV 16 positive</th>
<th>HPV 18 positive</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathologic type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>39</td>
<td>23</td>
<td>3</td>
<td>0.005*</td>
</tr>
<tr>
<td>AC</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Early (I, II)</td>
<td>39</td>
<td>20</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Late (III)</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>0.17</td>
</tr>
</tbody>
</table>

(p value for the differences between HPV DNA types distribution in smears and biopsies. (P: Fisher’s exact test).

Table 4. — Comparison between L1 DNA and E6-E7 RNA for HPV16 detection.

<table>
<thead>
<tr>
<th>Items</th>
<th>No. of specimens</th>
<th>HPV 16 Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR L1 gene</td>
<td>44</td>
<td>24 (54.5%)</td>
</tr>
<tr>
<td>RT-PCR E6-E7 transcripts</td>
<td>44</td>
<td>22 (50%)</td>
</tr>
</tbody>
</table>

(P: two-tailed McNemar’s test).

the higher positivity for HPV16 (54.5%) and HPV18 (15.9%) was obtained using 687.4 ng of DNA. Among smear specimens only HPV16 (31.8%) was found with a lower DNA amount (157.2 ng). The relationship between the detectability of HPV types in smears and biopsies is approximated to a statistically significant level (p = 0.05) (Table 2). In the cancer specimens, three cases were positive for HPV16 and HPV18 (6.8%). In all scraping samples of the control group, HPV DNA was not detected.

HR-HPV infection was determined by the PCR method. Figure 1 demonstrates the results of agarose gel electrophoresis of PCR products. The β-globin gene was used as an internal control to ensure the quality of DNA in all samples. The desired strips were clear and conspicuous (Figure 1A).

Using L1 primer, HPV DNA was detected according to sampling origin. In Figure 1 B and C, HPV16 and HPV18 positive cases are shown as examples.

Correlation between HPV status and clinicopathologic data

The prevalence of HPV types according to the histological data and stages of cervical carcinoma is listed in Table 3. There was a significant correlation between the frequency detection of HPV genotypes according to histological types (p = 0.005, Table 3). Differences in frequency between HPV16 and HPV18 were not statistically significant with regard to the various stages of cervical carcinoma (Table 3). Meanwhile these data revealed that there was a tendency of increased HR-HPV positivity when the lesion was more severe. HPV16 L1 amplification indicated that 20% (1/5), 62% (18/29) and 80% (4/5) of cervical cancer tissues were Stage I, II and III of the disease, respectively in SC. Exceptionally one case of HPV16 was found at Stage II of AC (20%). However, samples found positive for HPV18 or dually positive for HPV16 and HPV18, were classified at advanced stages of SC (60%, 40% Stage III respectively) and AC (80%, 20% Stage II, respectively) (Figure 2).

Prevalence of oncogene-derived transcripts for HPV16 compared with HPV16 DNA L1 finding

All samples were positive for the RNA control, β-actin, used to avoid false-negative results that could be due to degradation of RNA. Using the RT-PCR method, the major E6 and E7 HPV16 transcripts were detected in 22 of the 44 (50%) cancer cases (Table 4). The transcriptional activity of these oncogenes shows different levels of expression. The discrepancy in finding HPV16L1 DNA and HPV16 RNA E6-E7 (PCR and RT-PCR data) was not statistically significant.

Discussion

Epidemiological and molecular studies have shown that cervical infection by certain types of human papillomavirus is the precursor event in the genesis of cervical
neoplasia [17], and identification of HPV genotypes in clinical specimens is an important prognostic indicator for clinical screening and disease management [18]. Walboomers et al. [19] reported that the association between HPV and cervical cancer is high and up to 99.7%. Since papillomaviruses are difficult to culture and poorly detected by serological assays, molecular techniques remain the gold standard to detect the presence [12]. HPV PCR, a frequently used diagnostic tool for epidemiological investigations, involves amplification of HPV DNA by primers that bind to highly conserved regions within the L1 open reading frame of all genital HPV genotypes [20]. We wanted to rule out the possibility that the HPV late 1 region did not integrate into the cellular genomic DNA, and therefore could not be detected by PCR. Our findings demonstrated that HPV16 was the most prevalent type among HPV positive specimens (38/88), and HPV 18 (7/88) was the second. These results agree with the reported association of these genotypes with malignancy [21-24]. Moreover, our data showed that HPV16 was distributed differently in biopsies and smears with a rate of 54.5% versus 31.8%, respectively. False-negative rates for cervical premalignant lesions and cervical cancer in smears can be explained by the fact that the initial infection requires access of infectious particles to the cell in the basal layer [25] and therefore could not be detected. The prevalence of multiple HPV infections is a common phenomenon that can have clinical significance. In our study group 6.8% of cervical cancer specimens were found dually positive for HPV16 and HPV18. This rate is lower than what has been reported in recent studies showing rates between 9% and 32%, depending on ethnicity [26, 27]. Patients with multiple HPV types may have a higher risk of persistent infection compared to those with a single HPV type [28]. Persistent HPV infection, in turn, is necessary for the development of cervical cancer [29, 30]. In our study, we found that DNA yield recuperated varied widely. In fact, the quantity of DNA affects molecular detection; therefore, it represents a limitation for PCR assay. The higher positivity for HPV was found in biopsies having the highest amounts of DNA. This affectation could also be related to the viral type since this limiting factor was seen especially for HPV18 detected in biopsies with a high DNA amount, however with a lesser quantity of DNA extracted from smears HPV18 was lacking. This result is also consistent with Prêtet et al.’s report. Their study confirmed that the presence of HPV is significantly associated with viral load [31]. In relation to HPV16 and 18 distributions according to the histological type we found statistically significant differences ($p = 0.005$). These findings are in accordance with several previous studies that have consistently found HPV16 to be preferentially associated with SC rather than AC of the cervix [8]. HPV 18 has been associated with more aggressive forms of cervical intraepithelial neoplasia, invasive cervical cancer (adeno- and adenosquamous carcinomas) higher genome integration rate, and a greater likelihood of cancer recurrence and lymph node metastasis [32, 33]. We agree with other reports on concluding that HPV18 is strongly associated with AC of the cervix, which rapidly progresses through the preinvasive stages of cervical neoplasia [34, 35]. HPV18 plays a relatively minor role (15.9%) among HPV infection in tumor biopsies, whereas it was absent in
smears. Hence, cytological screening entails substantially lower protection against AC than SC of the cervix. A possible explanation could be that HPV18 infections preferentially increase in cervical AC and more often are localized in the endocervical canal [36, 37]. We agree with others who have concluded [38] that the use of smears might lower the sensitivity of HPV analyses and thus underestimate the true HPV prevalence in our cohort. The proportion of both HPV types clearly augments in accordance with the stage of cervical carcinoma. This is supported by previous data of Schelcht et al., where the presence of HR-HPV types was higher as the lesion evolved [39]. In the same way, such multiple HPV infections are frequently in advanced stages and have recently been detected in invasive cervical cancer [26, 27]. After screening cervical carcinoma specimens, the rate of HR-HPV DNA types detection was found to be 31.8% and 63.6%, respectively in smears and biopsies. On this basis, our results prompted us to focus on mRNA HPV16 E6/E7 expression in biopsies, of which expression is required for maintenance of malignancy. There are only minor differences between the mRNA E6-E7 and DNAL1 HPV16 detection rates. It has been shown that E7 promotes the formation of benign lesions whereas E6 works to complete the malignant transformation [40]. Cuschieri et al. recently reported that the detection of E6 or E7 transcripts in baseline samples helped predict those patients who were likely to carry a persistent infection [41]. Further elucidation of these findings utilizing quantity of mRNA could be more revealing. Therefore HPV1.1 PCR is especially useful in screening, while the detection of oncogene transcripts could serve as a marker for risk of the development of cervical cancer.

Conclusions

In summary, this study provides further insights into Tunisian cervical cancer specimens with implications of HPV-based prevention strategies. Further investigations will be required to define more precisely the impact of practical conditions on the quality of viral genome, and on viral revelation. Nevertheless, type-specific HPV testing is valuable to address the burden of HPV infections epidemiologically and to gain more insights into the natural history and dynamics of HPV infections. To further establish the potential of HPV E6 and E7 mRNA other promising biomarker molecules that regulate these viral oncogene expressions will be predictive for prevention, early diagnosis, and treatment for cervical carcinoma.

References


The impact of presurgical magnetic resonance in early breast cancer: an observational study

C. De Felice, V. Cipolla, A. Stagnitti, A. Marini, E. Pasqualitto, M.L. Meggiorini

Department of Radiological Sciences, University of Rome “Sapienza”, Rome (Italy)

Summary

The aim of this study was to evaluate the impact of presurgical breast magnetic resonance imaging (MRI) on the surgical management of selected patients with early-stage breast cancer who were candidates for BCT. The sample was built up according to the EUSOMA (European Society of Breast Cancer Specialists) recommendations enrolling women with unifocal unilateral early-stage breast carcinoma diagnosed by mammography, ultrasound (US) examination and in some cases also by histological analysis; all were scheduled for wider local excision. All eligible patients underwent presurgical breast MRI and findings were classified according to the BI-RADS system. In the presence of additional foci classified as BI-RADS 3-4, a targeted second-look US study was performed. If second-look US confirmed the presence of foci, needle biopsy was performed. Possible changes in the therapeutic approach resulting from preoperative MRI findings were decided upon by a multidisciplinary team. Outcome of histological examination of the surgical specimen and particularly analysis of tumor infiltration of the resection margins was the standard for determining the appropriateness of surgical strategy. A total of 123 patients underwent presurgical breast MRI. Additional foci were detected in 41.6% of patients, a greater local extension of the index lesion in 6.4%, whereas MRI confirmed local staging established by conventional imaging in 52%. However, 13.8% of additional foci were not confirmed by second-look and needle biopsy. More extensive surgery as a result of MRI findings was performed in 34.2%. This decision proved to be appropriate in 29.3% thus resulting in an over-treatment rate of 4.9%. Presurgical breast MRI resulted in confirmation of planned surgical strategy in 65.8% with an appropriateness rate of 54.5%. Surgical resection margins were positive for malignancy in 11.3% and repeated surgery was therefore required. Therapeutic strategy established on the basis of MRI was appropriate in 83.8% of cases. This study confirms the utility of MRI in presurgical workup of selected breast cancer patients. The results obtained suggest the importance of a sensitive tool such as MRI in the local staging of breast cancer before treatment planning.

Key words: Early-stage breast cancer; Breast magnetic resonance imaging; Presurgical staging.

Introduction

Breast conserving treatment (BCT), including wider local excision or quadrantectomy plus radiation therapy, is generally accepted as a preferable alternative to mastectomy for tumors up to 3 cm in diameter, since there is no significant difference between mastectomy and BCT in terms of mortality rate [1].

Surgical treatment within the framework of BCT has always aimed at complete excision of the tumor tissue and at obtaining clear margins. In order to obtain the best results in BCT and to reduce the risk of recurrence, accurate local staging of breast cancer is essential (extent of index lesion, multifocality, multicentricity, contralateral cancer) [2, 3]. Various studies have demonstrated that breast magnetic resonance imaging (MRI) has a higher sensitivity in local staging than conventional imaging, such as X-ray mammography (X-RM) and breast ultrasound (US) [4-13], particularly in conditions where the sensitivity of these techniques is reduced, e.g., in women with elevated mammographic density. In these patients, US examination can reduce the number of false-negatives produced by mammography [14-16]. However, a significant number of multifocal and multicentric breast carcinomas are still missed at routine diagnostic imaging [17]. Mammographic density has consistently been one of the strongest risk factors for breast cancer, with risk estimates that are three- to five-fold greater for women with high breast density [18].

According to international oncology guidelines [19] MRI as a staging procedure in women with breast cancer is optional, but according to EUSOMA (European Society of Breast Cancer Specialists) [20] breast MRI staging before treatment planning presents potential advantages and is indicated in the following cases:

1) patients newly diagnosed with invasive lobular cancer; 2) patients at a high risk for breast cancer; 3) patients under 60 years of age with discrepancy in size > 10 mm between X-ray mammography and US with expected impact on treatment decision; 4) patients eligible for partial breast irradiation (PBI) on the basis of clinical breast examination (CBE) and conventional imaging. EUSOMA furthermore recommends preoperative MRI as a scientific research issue in: 1) patients with dense breasts: 1a) dense breast in young women (< 40 years of age); 1b) dense breast associated with intermediate lifetime risk (15-20%) for other factors, 2) patients with unilateral unifocal pure ductal carcinoma in situ (DCIS) at conventional imaging (to exclude synchronous ipsilateral or contralateral invasive cancers).

The aim of this study was to evaluate the impact of presurgical breast MRI in the operative management of selected patients with unilateral unifocal early breast cancer, candidates for BCT.
Materials and Methods

Approval for this single-center, observational study was granted by the Medical Research Ethics Committee of our institution, and written informed consent was obtained from all patients.

The sample was built up from January 2009 to September 2011 at the Department of Radiological Sciences, University of Rome “Sapienza” among women with unilateral unifocal early breast cancer. Diagnosis was based on clinical examination, X-RM and US and in some cases also on needle biopsy; all patients were candidates for BCT. The initial palpable lesion and/or suspicious mammographic or US findings are in the following analysis called the “index lesion”.

In all cases conventional X-RM was performed using digital image formation and computed radiography.

At least two views per breast were obtained. In addition to this, further views or spot magnification were performed at the discretion of the interpreting radiologist. US and Doppler US studies were performed by the same radiologist according to previously reported standards [21]. Mammograms and US were interpreted in accordance with the guidelines of the American College of Radiology (ACR) Breast Imaging and Data system (BI-RADS) by a radiologist with 20 years of experience in the field of breast imaging, blinded to the clinical data. Based on the BI-RADS lexicon, patients were then assigned to one of the four categories of breast parenchymal density distribution [22]: type A, the breast is almost entirely fat (glandular parenchyma < 25% of the total area of both breasts); type B, scattered fibroglandular densities (25%-50%); type C, heterogeneously dense breast tissue (51%-75%); type D extremely dense (> 75% glandular).

Before MRI, US guided needle biopsy of the index lesion was performed in some cases by an expert to clarify diagnostic doubt.

After recruitment, the women were interviewed by a physician to collect information including: age at diagnosis of breast cancer, family history of breast cancer (positive: at least two first-degree relatives age ≤ 50), positive for BRCA1/2 gene mutations (subjects with a positive test for deleterious mutation in breast cancer susceptibility genes BRCA1, BRCA2), age at menarche, menopausal status (absence of menstrual cycles for at least 12 months), parity (nulliparous or with at least one full-term pregnancy), lactation for at least three months (yes/no).

Patient population was selected according to the following inclusion criteria:

– mammography: elevated mammographic density (BI-RADS C or D), suspicious microcalcifications (pleomorphic or heterogenous calcifications (granular) or fine linear, fine linear branching (casting) calcifications);
– discordant mammographic and US outcome in the identification of the index lesion and/or its dimensions (significant if ≥ 10 mm)
– histology of the index lesion (histological diagnosis of invasive lobular carcinoma, ILC);
– hereditary factors (positive for BRCA1/2 gene mutations, with at least two first-degree relatives age ≤ 50 years with a clinical history positive for breast carcinoma);
– characteristics of the lesion and treatment plan: the study included only women with unilateral unifocal lesions smaller than 3 cm in diameter for whom the interdisciplinary medical team had indicated wider local excision on the basis of conventional imaging findings.

Patients were excluded if they presented with contraindications to MRI (pace-maker, ferromagnetic clips, claustrophobia, gadolinium allergy, acoustic hearing implants and intraocular lens implants incompatible with 1.5 T magnetic field), if they were eligible for PBI on the basis of CBE and conventional imaging and/or eligible for radiotherapy or neoadjuvant chemotherapy.

Patients who were eligible for this study underwent MRI a maximum of 30 days from diagnosis of unifocal breast cancer. In premenopausal women, presurgical breast MRI was performed on day 6-13 of the menstrual cycle, including those who were receiving oral contraception [4]. Patients receiving hormone replacement therapy underwent MRI a minimum of four weeks after discontinuation of treatment [5].

The examination was carried out using a 1.5 T magnet (Sonata, Siemens Medical Solutions, Germany) equipped with bilateral multichannel dedicated coil with an integrated compression mechanism. The patient was positioned face down on a moveable examination table, the breasts were placed inside the dedicated coil in order to avoid an incorrect position which might have prevented the study of the entire mammary gland. The built-in compression mechanism guaranteed the stability of the breasts in the coil so as to minimize any motion artifacts.

Morphological study was performed using T2-weighted short tau inversion recovery (STIR) unenhanced axial-plane sequences, whereas dynamic study was carried out in six consecutive T1-weighted Flash 3D Dynamic (FL 3D DYN) sequences in the axial plane after intravenous injection of paramagnetic contrast medium followed by a T1-weighted fat saturation (FS) sequence in the coronal plane.

T1-weighted sequences presented the following characteristics: repetition time (TR) = 4.23 msec; echo time (TE) = 1.24 msec; flip angle = 10°; matrix = 384 x 384; pixels = 0.9 x 0.9 x 4; FOV = 340 x 340; slice thickness = 1 mm; interslice gap = 0.2 mm.

T2-weighted sequences presented the following characteristics: TR = 5280 msec; TE = 51 msec; flip angle = 160°; matrix = 384 x 384; pixels = 0.9 x 0.9 x 4; FOV = 340 x 340; slice thickness = 4 mm; interslice gap = 0.8 mm.

Contrast medium was gadoterate meglumine (Dotarem, Guerbet, France) administered in a concentration of 0.1 mmol/kg; it was injected through a 20 G intravenous cannula at the rate of 2 ml/sec using an automatic injector and followed by infusion of 20 ml saline solution at the same speed.

Image post-processing included temporal subtraction (contrast-enhanced minus unenhanced image) for dynamic studies without fat saturation and maximal intensity projection (MIP). Dynamic analysis with generation of percent enhancement versus time curves was performed through positioning of regions of interest (ROI) for all identified enhancing lesions with a diameter ≥ 5 mm and mass-like morphology according to the MRI BI-RADS classification [6].

Analysis of the obtained MRI results took the following into account:

1) Shape (round, oval, lobular, irregular), margin (circumscribed, microlobulated, obscured, indistinct, spiculated) and the characteristics of the baseline signal in T1- and T2-weighted sequences of the main index lesion and possible additional foci (iso-hypo-hyperintense compared to the glandular parenchyma).
2) Kinetics of enhancement assessed by the intensity/time curve.
3) Local extension. Criteria applied to establish local extent of disease were: a) size of the index lesion defined as the largest diameter of the lesion; b) infiltration of the skin; c) infiltration of the pectoralis major muscle; d) infiltration of the nipple. With regard to size, a difference of > 10 mm between the size measured at conventional imaging techniques and the size measured at MRI was considered significant.
4) Presence of additional foci were considered only if > 5 mm. Multifocality was diagnosed in the presence of multiple foci of malignancy in the same breast quadrant. Multicentricity was diagnosed when two or more foci of disease occupied more than one quadrant. Bilaterality was diagnosed if neoplastic lesions were found in both breasts (bilateral synchronous breast cancer) [7, 23]. All lesions were classified in one of the six BI-RADS categories according to their probability of being malignant [6].

Targeted second-look US was performed to identify MRI findings classified as BI-RADS 3-4, and US guided needle-biopsy procedure was performed on additional foci confirmed at second-look US. In cases where additional foci were classified as BI-RADS 5 and/or the index lesion was larger than established by conventional imaging techniques, no further diagnostic investigation was performed. The multidisciplinary team consisting of a radiologist, a pathologist, a surgeon/gynecologist and an oncologist reviewed all cases establishing therapeutic strategy in view of the evidence provided by MRI. Total treatment delay due to preoperative MRI and possible workup did not exceed one month. Histological examination of the surgical specimen and particularly analysis of tumor infiltration of the resection margins was the standard for determining the appropriateness of therapy. The surgical procedure was considered appropriate in the presence of disease-free resection margins.

Results

The sample was selected from 374 patients with clinical, mammographic, US and in some cases histological diagnosis of unilateral unifocal breast cancer; all were candidates for conservative surgery (wider local excision or quadrantectomy).

A total of 206 patients with unifocal breast cancer < 3 cm in diameter for whom the multidisciplinary team had planned wider local excision based on conventional imaging findings were selected. Of these patients 123 were found eligible for this study and underwent presurgical breast MRI. The main characteristics of the eligible patients are presented in Table 1. All MRI examinations were performed according to EUSOMA guidelines and considered technically adequate and of good diagnostic quality.

With regard to MRI-guided local staging, there was concordance with the results obtained by conventional imaging techniques in 52%, whereas MRI provided better local staging in 48%:
- in 6.4% MRI showed greater local extent of the index lesion (in 0.8% for infiltration of the nipple, in 1.6% for infiltration of the skin, in 1.6% for infiltration of the pectoralis major muscle and in 2.4% because the lesion was > 10 mm larger than measured at conventional imaging);
- in 41.6% MRI detected further post-contrast enhancements of > 5 mm in diameter (multifocal carcinoma in 16.5% and bilateral carcinoma in 3.2%).

In 10.7%, morphology and dynamics of the additional foci were highly suggestive of malignancy (BI-RADS 5), whereas the remaining 30.9% were classified as BIRADS 3-4 and underwent second-look US. In 9.7% second-look US was negative, whereas the additional lesions detected by MRI were confirmed in 21.2% cases, and US-guided needle biopsy was therefore performed. Histological examination was positive for carcinoma in 17.1% and for typical ductal hyperplasia in 4.1% cases. Overall, 13.8% of additional foci were not confirmed by second-look and needle biopsy.

Re-evaluation of each case by the multidisciplinary team led to confirmation of therapeutic strategy in 65.8% (9.7% as additional lesions were not confirmed after targeted second-look US; 4.1% as US-guided needle biopsy of additional focal lesions was negative (typical ductal hyperplasia); 52% as MRI confirmed local staging established by conventional imaging techniques).

Histological examination of the surgical specimen showed that resection margins were free of disease in 54.5% thus confirming that therapeutic strategy was appropriate; in 11.3% resection margins showed neoplastic infiltration and repeat surgery was required.

More extensive surgery was performed in 34.2% including: 6.4% due to greater local extent of the unifocal lesion and 27.8% due to the presence of additional foci, classified as BI-RADS 5 in 10.7% or confirmed by needle biopsy in 17.1%.

Planned therapeutic strategy was substituted with quadrantectomy plus radiation therapy in 20.3% due to greater local extent of the index lesion (6.4%) or multifocality (13.9%) (Figure 1), with unilateral mastectomy in 10.7% due to multicentricity (Figure 2) and with bilateral mastectomy in 3.2% due to bilaterality.

Modified therapeutic strategy was assessed by histological examination of the surgical specimens showing appropriateness in 29.3%:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sample (N = 123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at cancer diagnosis (years; mean)</td>
<td>50.2 ± 10.4</td>
</tr>
<tr>
<td>Menopausal status (%)</td>
<td></td>
</tr>
<tr>
<td>Menopause</td>
<td>55.2%</td>
</tr>
<tr>
<td>Non menopause</td>
<td>44.8%</td>
</tr>
<tr>
<td>Parity (%)</td>
<td></td>
</tr>
<tr>
<td>Nulliparity</td>
<td>43.9%</td>
</tr>
<tr>
<td>At least one full-term pregnancy</td>
<td>56.1%</td>
</tr>
<tr>
<td>Age at menarche (years, mean)</td>
<td>13.6 ± 3.8</td>
</tr>
<tr>
<td>Lactation for at least 3 months (yes, %)</td>
<td>42.2%</td>
</tr>
<tr>
<td>Mammographic breast density (%)</td>
<td></td>
</tr>
<tr>
<td>BI-RADS AB (non dense breast)</td>
<td>49.5%</td>
</tr>
<tr>
<td>BI-RADS CD (dense breast)</td>
<td>50.5%</td>
</tr>
<tr>
<td>Suspicious microcalcification</td>
<td>15.4%</td>
</tr>
<tr>
<td>Discordance (&gt; 10 mm) between mammographic and US detection of the main index lesion and/or its dimensions (%)</td>
<td>13%</td>
</tr>
<tr>
<td>Positive for BRCA1/2 (%)</td>
<td>2.4%</td>
</tr>
<tr>
<td>First-degree family history of breast carcinoma* (%)</td>
<td>22.7%</td>
</tr>
<tr>
<td>ILC**</td>
<td>4%</td>
</tr>
</tbody>
</table>

* At least 2 first-degree relatives diagnosed with breast carcinoma at age ≤50.
** Assessed by needle biopsy of the index lesion before MRI.
Surgical planning is commonly based on clinical examination and conventional breast imaging techniques, such as mammography and US, although the impact of breast MRI on presurgical staging of patients with primary breast cancer is evolving [1, 24-26].

The value of breast MRI is based on the capability of this modality to depict: (a) multicentric and multifocal disease [27-30], (b) an invasive component in ductal carcinoma in situ lesions [31], (c) the tumor in a three-dimensional way [27, 32], and (d) cancer in dense breast tissue [33-35]. Thus, MRI has facilitated improved local staging (extent of index lesion, multifocality, multicentricity, contralateral cancer) [4-8] and safer breast-conserving surgery in patients with breast lesions, thereby reducing the risk of local recurrence [36, 37]. Furthermore, contrary to initial assumptions, MRI has also proved to be able to detect invasive lobular carcinoma and ductal carcinoma in situ (DCIS) as well as the extensive intraductal component that can appear as “non mass like” enhancement [31, 38-42].

Numerous studies have been performed to assess the diagnostic performance of MRI in the evaluation of breast lesions [43, 44]. Sensitivity and specificity varied widely among the included studies: sensitivity ranged from 0.63 to 1.00, and specificity ranged from 0.21 to 1.00. At a sensitivity of 0.95, the corresponding specificity was 0.67 [45].

On the other hand, suboptimal specificity of breast MRI often leads to the need for further diagnostic workup (second-look US and US-guided needle biopsy) and changes in therapeutic management have a frequency of about one fifth compared with a well-known lower rate of local recurrence after breast-conserving treatment combined with radiotherapy [7]. Furthermore, a more complete local staging of the disease may be associated with a risk of surgical overtreatment. To date there is no evidence from randomized controlled studies in favor or against a positive impact of presurgical breast MRI on disease-free or overall survival.

Our results confirm the high sensitivity of MRI in presurgical local staging of breast cancer reported in the literature [46]. In this study, MRI detected additional foci in 41.6% and more extensive surgery was performed in 34.2%. This decision proved appropriate in 29.3% with an overtreatment rate of 4.9%. Surgical resection margins were positive for malignancy in 11.3% and repeat surgery was therefore required. Overall appropriateness of therapeutic strategy as a result of MRI was 83.8%.

Our results confirm the importance of an accurate selection of patients for MRI on the basis of risk factors such as mammographic features, family history of breast cancer and/or histological analysis as indicated in the EUSOMA recommendations [20]. In accordance with these recommendations, patients eligible for PBI on the basis of CBE and conventional imaging were excluded from this study as PBI is not performed in our institution.

The low overtreatment rate due to false-positive find-
The impact of presurgical magnetic resonance in early breast cancer: an observational study

In our opinion, the combination of patient selection and identification of additional foci using second-look US and needle biopsy is essential for an accurate interdisciplinary assessment and for a correct therapeutic approach, despite the increase in time and costs. However, in the present patient population the total treatment delay due to preoperative MRI and possible workup did not exceed one month.

The main strength of this study was that our center performs more than 150 MRI examinations per year and has extensive experience in conventional breast imaging, i.e., X-RM, breast US and US guided needle-biopsy procedures as well as in targeted second-look US to analyze MRI findings missed at conventional imaging prior to MRI. It was furthermore an advantage that histological examination was carried out exclusively by a pathologist specialized in breast diseases.

Technical procedures (MRI protocol and post-processing images) and methodology (MRI was always performed according to the phase of the menstrual cycle and at least four weeks after discontinuation of hormone replacement therapy) were performed according to the EUSOMA recommendations, and a standardized method such as BI-RADS lexicon was employed for the interpretation. Furthermore, changes in therapeutic planning were decided on by a multidisciplinary team.

On the other hand, the lack of a control group, the randomization in the selection of patients for presurgical MRI and follow-up makes it impossible to evaluate some parameters, such as the impact of MRI on the risk of repeat surgery and the real benefit of more extensive surgery in case of detection of additional malignant lesions followed by radiotherapy and/or adjuvant systemic chemotherapy or hormone therapy. Mammographic breast density was established by a single radiologist using a qualitative visual system.

In conclusion, preoperative MRI remains a hot topic and a complex problem which will probably remain unresolved for several years. We have in our hands a technique which is surely the best option for evaluating ipsilateral disease extent and possible contralateral cancers, but we are not sure that, using this technique, we can provide our patients with a better treatment. We might in fact provide a worse treatment, i.e., an avoidable more aggressive treatment.

The present experience confirms the utility of a highly sensitive diagnostic tool such as MRI in the presurgical workup of breast lesions. However, in our opinion an improved advantage/disadvantage relationship includes a careful selection of patients and US as well as histological confirmation of additional foci detected by MRI.

Changes in therapeutic management resulting from preoperative MRI findings should be decided on by a multidisciplinary team.

Figure 2. — A 54-year-old patient with high mammographic density (BI-RADS C) and suspicious micro calcifications detected at mammography (a, b) in the lower inner quadrant of the right breast. MIP reconstructions of FL 3D DYN T1-weighted sequences (c): in addition to the main lesion located in the lower inner quadrant of the right breast, more foci are evidenced in the same quadrant (BI-RADS 4) involving also the upper outer quadrant. Diagnosis: multicentric carcinoma confirmed by second-look and needle biopsy. The patient underwent unilateral mastectomy. Postoperative histological analysis confirmed the appropriateness of this modified therapeutic strategy.
Careful prospective randomized trials are required to determine whether MRI in the preoperative assessment of women with diagnosis of breast cancer leads to a decrease in tumor recurrence and to determine the cost-effectiveness of this approach.

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Address reprint requests to:
ML. MEGGIORINI, M.D.
Department of Gynecology and Obstetrics
Sapienza University of Rome
Viale Del Policlinico, 155
00162 Roma (Italy)
e-mail: marialetizia.meggiorini@uniroma1.it
Radical abdominal trachelectomy is a safe and fertility preserving option for women with early stage cervical cancer

A. Karateke, C. Kabaca

Department of Gynecologic Oncology, Zeynep Kamil Women and Children Diseases Education and Research Hospital, Istanbul (Turkey)

Summary

Purpose of investigation: To present the surgical, oncological and obstetrical outcomes gained from patients who underwent radical abdominal trachelectomy (RAT) in Zeynep Kamil Women and Children Diseases Education and Research Hospital and radical Yeditepe University Hospital. Methods: A total of eight RATs were performed between 2003-2010. Data were obtained from medical and pathological records of the patients. Results: The mean age of the patients was 27.37 ± 6.39 years. The mean follow-up time of the patients was 33.62 ± 27.47 months. Three (37.5%) patients had a tumor size smaller than 2 cm, and five (62.5%) patients had a tumor size larger than 2 cm. Seven (87.5%) patients had Stage IB1 and one (12.5%) patient had Stage IIA tumor. Three (37.5%) patients had late post-operative complications: uterotubal abscess, severe lymphedema and lymphocyst. There were no recurrences. Three patients became pregnant which resulted in two live births and one abortus. The spontaneous pregnancy rate was 50%. Conclusion: We think that RAT is a reliable surgical option for a patient with early stage cervical cancer who wants to preserve fertility.

Key words: Radical abdominal trachelectomy; Early stage cervical cancer; Pregnancy; Fertility.

Introduction

Cervical cancer is the third most common cancer in women, with an estimated 530,000 new cases in 2008. Cervical cancer is the ninth most common cancer among women in Turkey and ranks 12th among cancer-related deaths [1]. Historically, the recommended surgical treatment for women with Stage IA2 -IB1 cervical cancer is radical hysterectomy and bilateral pelvic lymphadenectomy. More than 40% of the diagnosed early invasive cervical cancer is found in women younger than 45 years of age and many women within this age group have not completed their childbearing [2, 3]. According to the literature, radical trachelectomy, which aims at the preservation of the body of the uterus, is claimed to be an acceptable approach for young women with cervical cancer who wish to preserve their fertility [4-6]. Dargent et al. [4] popularized the procedure known as radical vaginal trachelectomy. In 1997, Smith et al. [7] introduced radical abdominal trachelectomy (RAT). In the literature, many authors have reported that RAT is safe and applicable with results similar to those seen with radical vaginal trachelectomy [8-11].

The aim of the present study was to present the surgical, oncological and obstetrical outcomes gained from the patients who had undergone RAT in the Gynecologic Oncology Clinics of Zeynep Kamil Women and Children Diseases Education and Research Hospital and Yeditepe University Hospital.

Materials and Methods

The medical records of all patients who had undergone RAT were reviewed. All surgical interventions were carried out by the same operator in the two institutes between July 2003 and November 2010. Institutional review board approval was obtained. Data were obtained from medical and pathological records of the patients and included age, stage, histopathologic subtype, tumor size, evidence of lymphovascular space invasion, number and malignant invasion of the lymph nodes removed, disease status of the surgical specimen, duration of hospitalization, intraoperative and postoperative complications, number of perioperative blood transfusions, oncologic follow-up and fertility outcomes.

Patients with a confirmed diagnosis of cervical cancer were considered eligible for RAT if they met the criteria for radical abdominal hysterectomy in addition to a strong desire for future fertility preservation. The parametrium should be intact by bimanual rectovaginal examination under general anesthesia. There should be no evidence of the disease in the parametrium according to preoperative pelvic magnetic resonance imaging (MRI) examination. MRI was employed preoperatively to decide whether there was any tumor near the isthmus to accomplish the surgical intervention with a safe tumorless margin of approximately 0.5-1 cm at the upper cervical border. Positron emission tomography-computed tomography (PET-CT) was used to determine whether there were any lymph nodes and distant metastasis. All patients were advised that the standard treatment for women with early cervical cancer still remained as radical hysterectomy rather than trachelectomy and patient approvals were obtained.

Surgical technique

Entry into the abdominopelvic cavity is performed through either a Maylard or median incision. The round ligaments are divided. The paravesical and pararectal spaces are exposed and the bladder is dissected caudally to the mid-vagina. The infundibulopelvic ligaments with the ovarian vessels are kept intact and maximum attention must be established during the operation. The uterine vessels are then ligated and divided at their origins from the hypogastric vessels. The ureters are then dissected bilaterally to their insertion into the bladder with lateral mobilization. The uterosacral ligaments are identified and transected. Then, the parametrium and paracolpos are divided. The

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Radical abdominal trachelectomy is a safe and fertility preserving option for women with early stage cervical cancer. Cervix is amputated from the vagina. Cervical tissue is divided from the uterine corpus close to the isthmus according to size of the tumor. The specimen is sent for frozen section evaluation to assure that at least a 5 mm margin is free of tumor. Bilateral complete pelvic and paraaortic lymphadenectomies are performed from the level of the inferior mesenteric vessel to the circumflex iliac vein. If the frozen section evaluation is benign, it means at least a 5 mm clear margin obtained at the endocervical edge is provided; the uterine body is reconstructed to the vagina with 4-6 interrupted # 0 Vicryl sutures. In the present series a cerclage suture was not placed except for the first case of the series. The abdominal wall is then closed. A standard antibiotic prophylaxis with routine postoperative care is performed.

Routine oncological follow-up includes a pelvic examination, pelvic ultrasonography, and Pap smear every two months for the first year, then every four months for the second year, and every six months for the subsequent years. Dilatation with hysteroscope to prevent granulation and synechia is performed two times a month for the first three months after the surgical intervention. Dilatation with a hysteroscope is discontinued if the patient has regular menstrual bleeding and no dysmenorrhea.

**Results**

A total of eight RATs were performed. Six patients were operated on in Zeynep Kamil Women and Children Diseases Education and Research Hospital and the remaining two patients were operated on in Yeditepe Medical Faculty Hospital. The data of the patients and the pathological results are summarized in Tables 1 and 2, respectively.

The mean age of the patients was 27.37 ± 6.39 years (range: 18-35). Four (50%) patients had squamous cell carcinoma, three (37.5%) patients had adenocarcinoma, and one (12.5%) patient had clear cell carcinoma. The case with a diagnosis of a clear cell carcinoma is the first case in the literature with this histological subtype and a follow-up time of 92 months has still revealed no recurrence. The mean follow-up time of the patients was 33.62 ± 27.47 months. Three (37.5%) patients had a tumor size smaller than 2 cm, and five (62.5%) patients had a tumor size larger than 2 cm. Seven (87.5%) patients had Stage IB1 and one (12.5%) patient had Stage IIA tumor. Three patients (37.5%) had lymphovascular space invasion. The median surgical intervention time was 163.75 ± 33.77 minutes (range: 120-210 min). The median length of hospitalization was 8.25 ± 3.10 days (range: 5-12 days). No intraoperative complications occurred. Menses resumed within the first six weeks after the operation. There were no recurrences during the follow-up period.

Three (37.5%) patients had late postoperative complications. One of them was reoperated after the third year following the first surgical intervention due to suspicion of metastasis. An abdominal MRI revealed a right adnexal mass of 10 × 15 cm. Re-laparotomy revealed an abscess formation within the uterus and tubing. The uterine wall was thinned due to the ongoing abscess. Her second operation was right-side salpingo-oophorectomy with a uterine corpus resection. Because the first patient presented with a late abscess formation within the uterine...
cavity due to obstruction, a cerclage suture was not placed for the subsequent patients. The second patient with a late complication presented with a severe lymphedema of her left leg after two months following her operation and lymphedema was reduced with regular lymph massage. The third patient with late complications had a lymphocyst in the pelvis.

Three patients became pregnant during their follow-up periods. The first of them experienced fetal loss in the 21st week of pregnancy, whereas the second patient delivered a healthy baby with a cesarean section in the 31st week of gestation and the third patient delivered a term baby in the 38th week of gestation and the third patient delivered a term baby in the 38th week of gestation. Prolonged pregnancy and the third patient delivered a term baby in the 31st week of pregnancy, whereas the second patient delivered a healthy baby with a cesarean section in the 31st week of gestation. Pro lugton depot, 250 mg, was injected every week intramuscularly to both cases with continuing pregnancies until delivery.

Discussion

The results of this study showed that RAT can be performed safely in well selected patients with early-stage cervical cancer who wish to preserve their fertility. In the literature review, patients with tumor size smaller than 2 cm were selected for radical vaginal trachelectomy whereas patient selection can potentially be extended to patients with a larger lesion size up to 4 cm for RAT [11-13]. In one of the latest reviews, approximately 116 RATs were described in the literature [13]. Five patients in our series were unusual due to a tumor size of larger than 2 cm. The mean follow-up was 33.62 ± 27.47 months and no recurrences were noted. Our results regarding the duration of the operation, hospitalization time, intra- and postoperative complications resemble other similar reports [8, 12]. The oncologic safety of trachelectomy has been demonstrated with extensive retrospective data [14]. Lower recurrence rates were reported when RAT had been performed compared to a vaginal approach since extended parametrial resection was carried out by the abdominal route [8, 15].

According to our experience and literature review, RAT operations have some potential advantages including extended parametrial resection and ability to more accurately determine the site of cervical amputation from the uterine isthmus. Disease-free upper surgical margin ranged between 6 and 40 mm in our series. We also think that the disease-free margin should be at least 5 mm as also recommended by the related literature [13]. Moreover, there is neither a necessity to be trained in laparoscopic surgery or to be trained specifically in radical vaginal surgery since RAT is identical to radical hysterectomy with the exception that the uterine corpus is not removed. The potential disadvantages of RAT include poor cosmesis, longer hospitalization, delayed return of bowel function and delayed return to daily activities [10].

Normal menstruation was resumed within six months in all our patients. In our series, when the patient with uterine corpus resection and the last and newest case with insufficient follow-up time (5 months) were not taken into account, the spontaneous pregnancy rate was 50%. While one patient had a second trimester abortus, the second one had a preterm delivery, whereas the third patient had a term pregnancy. We think that bilateral ligation of the uterine arteries of a nonpregnant uterus does not influence spontaneous pregnancy rates and obstetrical outcomes.

In the literature, the procedure of isthmic cerclage is controversial because of the complications, like abscess or cerclage expulsion. Although there are reports that recommend performing isthmic cerclage routinely [11, 12], because of the late complication that occurred in the first case of our series, we did not perform any cerclage in the subsequent cases. Some similar experiences about complications as well as complete cerclage expulsion have been reported in the literature [6, 10]. We could not decide whether cerclage should really be added to the procedure since abortus occurred in one of our patients in the 21st week of pregnancy.

We performed consecutive dilatation with a hysteroscope routinely for three months to keep the isthmic patency and to prevent hematocolpos and pyometra formation caused by synechia. Dilatation with bougie has also been reported by other authors [8].

As the published cases are increased in the literature, RAT would be a safe alternative surgery to Type III hysterectomy for women with early-stage cervical carcinoma who wish to preserve fertility or the uterus. The successful results could increase the popularity of trachelectomy. RAT has been carried out by the same technique as Type III hysterectomy except for the preservation of the utero-ovarian ligament and uterine corpus. We think that our report could be one of the encouraging studies to enhance and universalize the RAT procedure because of successful obstetrical and oncological outcomes gained from unusual cases. The oncological outcomes of the RAT and Type III hysterectomy are similar in cases with intact parametrium since cervical cancer progresses to the parametrial tissues rather than in the caudocranial direction.

Conclusion

We think that RAT is a reliable surgical option for a patient who desires fertility preservation and in whom postoperative chemoradiotherapy could be predicted as unnecessary in the preoperative period.

References

Radical abdominal trachelectomy is a safe and fertility preserving option for women with early stage cervical cancer


Address reprint requests to:
C. KABACA, M.D.
Semsettin Gunaltay Cad. No: 197
Medine apt. D. 23
34738, Kadikoy, Istanbul (Turkey)
e-mail: canankabaca@yahoo.com
Distribution of human papillomavirus types in Turkish women

Z.S. Tuncer¹, G. Boyraz¹, N. Şahin¹, A. Alp²

¹Gynecologic Oncology Unit, Department of Obstetrics and Gynecology, ²Department of Medical Microbiology, Hacettepe University Faculty of Medicine, Ankara (Turkey)

Summary

Purpose of investigation: Since oncogenic types of human papillomavirus (HPV) are associated with a higher risk of cervical cancer and certain types can be controlled by a vaccine, a study has been performed to determine the HPV genotype distribution among Turkish women. Methods: The study included patients with abnormal cytology or in the follow-up for cervical intraepithelial neoplasia between 2002 and 2009 at Hacettepe University Hospital. The results of 1,797 consecutive cervical samples were analyzed retrospectively. INNO-LiPA HPV genotyping, HPV-Typing and Seeplex HPV 18-plex genotyping tests were used to determine the types of HPV. Results: HPV was detected in 404 (22.4%) of 1,797 samples studied. HPV DNA was identified in 194 cases by using HPV-Typing test but the specific genotype was not available. The most frequent genotype was HPV 16 which was observed in 103 cases (49.0%). Conclusion: HPV 16 was the most common genotype observed among Turkish women with abnormal cytology. It suggests that HPV vaccination may be useful for prevention of cervical cancer in this population.

Key words: Human papillomavirus; Cervical cancer; Pap test; Genotyping.

Introduction

Carcinoma of the uterine cervix continues to be one of the most common female genital cancers worldwide despite availability of effective screening [1]. Infection by human papillomavirus (HPV) has long been recognized as a main causal factor for almost all cases of cervical cancer. More than 100 genotypes of HPVs are characterized to date and over 30 types that infect the anogenital tract have been described [2, 3]. Tumorigenicity of the virus differs markedly among genotypes and several high-risk types have been implicated in cervical carcinogenesis. HPV 16 is the most common type observed in women with normal cervical cytology and in those with cervical neoplasia [4]. The pattern of HPV type distribution may vary among countries and regions. With the advent and routine application of HPV vaccines, data on regional HPV genotype distribution would be more useful to predict the potential benefits. However, data regarding community based distribution of HPV genotypes are still limited in Turkey. Determination of HPV genotypes will also allow monitoring of the impact of vaccination on HPV type replacement and enhancing further research.

Since oncogenic types of HPV are associated with a higher risk of cancer and certain types can be controlled by a vaccine, a study has been performed to determine the HPV genotype distribution among Turkish women.

Methods

A study including 1,797 cases between 2002 and 2009 was carried out in Hacettepe University Hospital, Ankara, Turkey. The research protocol was approved by the institutional ethics committee. The study included patients with abnormal cytology or in follow-up of cervical intraepithelial neoplasia at the Department of Obstetrics and Gynecology, Gynecologic Oncology Unit. The mean age of the study population was 36.8 years (± 9.3). The mean values for gravidity and parity were 3.0 and 2.0, respectively. The mean ages of menarche and first sexual intercourse were 13.5 years (± 1.4) and 20.5 years (± 4.1), respectively.

The results of 1,797 consecutive cervical samples were analyzed retrospectively. INNO-LiPA HPV Genotyping Test (Innogenetics, USA) was used between 2002 and 2005 while the HPV-typing test (GenID, Germany) was used between 2005 and 2007; and the Seeplex HPV 18-plex genotyping test (Seegene, Korea) was used between 2007 and 2009 to determine the types of HPV.

The INNO-LiPA HPV Genotyping Test is based on the reverse hybridization principle. Part of the L1 region of the HPV genome was amplified and denatured biotinylated amplicons were hybridized with specific oligonucleotide probes immobilized on the strip. After hybridization and stringent washing, streptavidin-conjugated alkaline phosphatase was added and binded to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen gave a purple/brown precipitate and results were interpreted visually. Amplification and detection methods were performed according to instructions of manufacturer.

The HPV-typing test is based on the reverse hybridization assay for the differentiation of high- and low-risk genotypes. After DNA was isolated from clinical samples, PCR and subsequent reverse dot blot hybridization with sequence-specific oligonucleotide probes that represented particular HPV genotypes immobilized on nitrocellulose membranes were applied according to the manufacturer’s instructions.

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The Seeplex HPV 18-plex Genotyping Test utilizes amplification of target DNA by multiplex PCR based on dual priming oligonucleotide technology and detects 18 different HPV DNA genotypes. Following DNA extraction with the Nucleospin DNA extraction kit (Macherey-Nagel, Germany) per instructions of the manufacturer, amplification was carried out in GeneAmp PCR Systems 9700 (Applied Biosystems, USA). The PCR amplicons were analyzed by a capillary electrophoresis-ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). Typing of HPV was achieved by separation of the amplicons according to their sizes. Evaluation of this data was performed by using Applied Biosystems GeneMapper Software. Amplification and detection methods were performed according to manufacturer’s instructions.

ThinPrep system was used for liquid-based cytological analysis. Pap test results were evaluated in terms of the Bethesda III classification.

Results

HPV was detected in 404 (22.4%) of 1,797 samples studied. HPV DNA was identified in 194 cases by using the HPV-typing test but the specific genotype was not available. In the remaining 210 cases analyzed by INNO-LiPA HPV and Seeplex HPV 18-plex genotyping tests, 22 different HPV types were detected. While 68 (32.3%) of the samples had infection with multiple genotypes, 142 (67.7%) had single genotype infection. The most frequent genotype was HPV 16 which was observed in 103 (49.0%) cases (Table 1). The following common types were HPV 51 (19.5%), HPV 31 (17.1%), HPV 6 (10.0%), HPV 42 (8.0%), HPV 33 (7.6%), HPV 68 (5.2%), and HPV 18 (4.7%). Among HPV 16 positive samples, 62 (60.1%) had single type of HPV infection. HPV 66 and HPV 54 were observed as only single infections whereas HPV 35, HPV 40, HPV 44 and HPV 45 were detected only in multiple infections.

Of the 404 cases with HPV infection, Pap smear results were negative in 243 (60.1%) cases (Table 2). The most common epithelial cell abnormality observed at Pap smear was LSIL in 67 (16.6%) cases. Among 103 cases with HPV 16 infection, Pap tests were negative in 59 (57.3%) patients while the remaining 44 had various cytological abnormalities.

Discussion

The present study on 1,797 consecutive patients from Turkey undergoing HPV testing for abnormal cytology or follow-up of cervical intraepithelial lesion shows a 22.4% prevalence of HPV infection. Worldwide prevalence of HPV infection is estimated to be approximately 10% among women with normal cytology [4]. Figures from Europe and Asia (where Turkey belongs) are even lower reaching approximately 8%. A review of several previous studies including approximately 3,000 cases from Turkey reported a 5% HPV prevalence among low-risk women [5]. The study population of the current work represents a high-risk group for HPV infection since they had either an abnormal Pap test or in the follow-up cervical intraepithelial neoplasia. Thus, this figure is definitely higher than the global crude HPV prevalence as expected.

HPV prevalence rates are reported to be greater in high-risk populations. Menegazzi et al. reported 45.9% rate of HPV infection in an Italian group of patients undergoing opportunistic screening and evaluation of HPV associated lesions [6]. Tsao et al. found 32.4% of HPV infections among 343 Taiwanese women who visited clinics for screening or for follow-up of cervical intraepithelial neoplasia [7]. HPV infection rates of 22.4% in a high-risk population of Turkish women may be evaluated to be lower than the figures reported from similar study groups in the literature.

HPV 16 was found to be the most common genotype in this study. According to a meta-analysis including 48 studies from different regions of the world that provided type-specific information, the most common genotype was HPV 16 followed by HPV 18, HPV 31, HPV 58 and HPV 52 [4]. Determination of HPV 16 as the most common genotype in the current study is in agreement

<table>
<thead>
<tr>
<th><strong>Table 1. — HPV genotype distribution.</strong></th>
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<tbody>
<tr>
<td>Total (n, %)</td>
</tr>
<tr>
<td>HPV 16</td>
</tr>
<tr>
<td>HPV 51</td>
</tr>
<tr>
<td>HPV 31</td>
</tr>
<tr>
<td>HPV 6</td>
</tr>
<tr>
<td>HPV 42</td>
</tr>
<tr>
<td>HPV 33</td>
</tr>
<tr>
<td>HPV 68</td>
</tr>
<tr>
<td>HPV 18</td>
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<tr>
<td>HPV 43</td>
</tr>
<tr>
<td>HPV 56</td>
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<tr>
<td>HPV 39</td>
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<tr>
<td>HPV 52</td>
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<tr>
<td>HPV 58</td>
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<tr>
<td>HPV 11</td>
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<tr>
<td>HPV 59</td>
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<tr>
<td>HPV 70</td>
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<tr>
<td>HPV 35</td>
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<tr>
<td>HPV 66</td>
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<tr>
<td>HPV 40</td>
</tr>
<tr>
<td>HPV 54</td>
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<tr>
<td>HPV 44</td>
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<tr>
<td>HPV 45</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Table 2. — Pap smear results in HPV positive cases.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap test</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Atypical glandular cells (AGC)</td>
</tr>
<tr>
<td>Atypical squamous cells (ASC)</td>
</tr>
<tr>
<td>ASC-US</td>
</tr>
<tr>
<td>ASC-H</td>
</tr>
<tr>
<td>Low-grade squamous intraepithelial lesion (LSIL)</td>
</tr>
<tr>
<td>High-grade squamous intraepithelial lesion (HSIL)</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
with worldwide genotype distribution of HPV. However, the second most commonly reported HPV 18 genotype was found to be less common in this series. Distribution of individual HPV genotypes varies across geographic areas and ethnic groups [2, 4]. Determination of HPV 51 as one of the most common types of infection has been reported in studies from Italy, Taiwan, Germany and Denmark [6-9]. The finding of HPV 51 as the second most common type of infection in this series is in accordance with these studies. HPV 31 was found to be the third most common type of HPV infection which correlates well with the literature [4].

Current vaccines protect against HPV 16 and HPV 18 infections. Furthermore, cross reactivity for HPV 31, HPV 33 HPV 45 and HPV 52 suggest an even higher percentage of efficacy [10]. Observation of HPV 16 as the most common genotype as well as the other common HPV types profiled among Turkish women suggests that HPV vaccination may be useful for prevention of cervical cancer in this population. HPV vaccination should be a national priority in light of the findings in this study. However, besides genotypes which are targeted by current vaccines, other types should also be taken into consideration in the development of enhanced vaccines with larger coverage.

References

Address reprint requests to: S.Z. TUNCER, M.D.
Gynecologic Oncology Unit
Department of Obstetrics and Gynecology
Hacettepe University Faculty of Medicine
06100 Sihhiye, Ankara (Turkey)
e-mail: zstuncer@hacettepe.edu.tr
Do high levels of CA 19-9 in women with mature cystic teratomas of the ovary warrant further evaluation?

M.G. Ugur¹, E. Ozturk¹, O. Balat¹, E. Dikensoy¹, S. Teke¹, A. Aydin²

¹Department of Obstetrics and Gynecology, ²Department of Pathology, Gaziantep University, Faculty of Medicine, Gaziantep (Turkey)

Summary

Purpose: To evaluate the serum levels of tumor markers (particularly CA 19-9) in patients with ovarian mature cystic teratomas (MCT) with respect to age, size, bilaterality, menopause, presence of adhesions, complications and the postoperative levels. Methods: We evaluated clinical characteristics and tumor markers of 157 patients with MCT of the ovary operated at our clinic. Results: CA 19-9 was the only tumor marker with a mean serum level (46.95 ± 101.11 U/ml) above the cut-off value and the elevated rate was 33.1%. Tumor size, presence of adhesions and CA 125 levels were significantly higher in patients with elevated CA 19-9. Bilaterality rate was 10.8%. The most common complication was torsion (6.4%). Conclusion: We suggest that elevated levels of CA 19-9 may be expected in MCTs of the ovary and that they will probably be decreased postoperatively. Therefore, postponing evaluation of other possible sources of CA 19-9 elevation in asymptomatic and young patients is more common sense.

Key words: Tumor markers; CA 19-9; Mature cystic teratoma; Ovary; Adhesion.

Introduction

Mature cystic teratomas (MCT) or dermoid cysts are germ cell tumors of the ovary. They are the most common type of ovarian tumor accounting for 27-44% of all primary ovarian tumors [1]. A vast majority of these tumors are benign and in about 1% of MCTs, one tissue element shows malignant transformation, most often to squamous cell carcinoma [2]. MCTs generally occur during reproductive years with a mean age of 32 years and are commonly unilateral (about 88% of cases) [1].

Quite discrete surgical approaches in MCTs and malignant tumors emphasize the need for making a differential diagnosis of patients with ovarian tumors, and serum tumor markers have been shown to be useful in providing additional information [3-6]. There are few studies concerning the clinical value of tumor markers in the diagnosis and management of MCT [4, 5, 7-9]. Almost all studies have suggested that CA 19-9 may be the only tumor marker with clinical significance.

In this study, our aim was to evaluate the serum levels of tumor markers (particularly CA 19-9) in patients with ovarian MCTs with respect to age, size, bilaterality, menopause, presence of adhesions, complications and the postoperative levels.

Materials and Methods

Patients with MCT of the ovary, who underwent surgery in Gaziantep University, Faculty of Medicine, Department of Obstetrics and Gynecology between November, 2001 and August, 2010 were reviewed through hospital charts and pathology archives. Ultrasound examinations were performed with a 3.5-MHz transabdominal sector probe and/or a 7.5-MHz transvaginal probe (Applio, Toshiba, Japan). MCTs were typically diagnosed when there was a homogeneous ovarian hypoechoic mass with regular capsule and posterior shadowing or a heterogeneous mass with irregular hypo- and hyperechoic appearance with posterior shadowing without any septa [10].

Age of the patients, average tumor size (according to operative records and gross pathologic descriptions), bilaterality, preoperative and postoperative serum tumor marker levels [including CA 19-9, CA 125, CA 15-3, alpha-fetoprotein (AFP), and carcino-embryogenic antigen (CEA)], presence of any adhesions and complications observed during surgery were all recorded.

No further imaging and endoscopic procedure had been performed in patients in order to rule out any possible gastrointestinal tract disease.

The determination methods and cut-off values were immunoassay for AFP (11.3 ng/ml), CA 19-9 (37 U/ml), CA 125 (35 U/ml) and CA 15-3 (30 U/ml) and enzyme immunoassay for CEA (3.4 ng/ml). Analyses were performed on the Modular Analytics E170 module (Roche Laboratory Systems, Mannheim, Germany).

Surgeries performed for MCTs were cystectomy, oophorectomy or hysterectomy with unilateral or bilateral salpingo-oophorectomy according to age, desire of future fertility or presence of other pathology.

According to serum level of CA 19-9 patients were divided into an elevated level group (Group I) and a normal level group (Group II). Groups were compared in terms of clinical characteristics and preoperative or postoperative serum tumor markers. Also the correlation of diameter of tumor to CA 19-9 was analyzed.

Statistical analysis was performed with SSPS 13 software (SSPS Inc, Chicago, IL). Statistical evaluation of the data was performed by the chi-square test, Student’s t-test, Mann-Whitney U test and Pearson’s test. Differences were considered significant when p < 0.05 for the two-tails.
Results

There were 157 patients with MCT of the ovary who underwent an operation in our clinic. The mean age of all patients was 37.45 ± 14.90 years (median 35; range 15-84). Overall, 33 patients (21%) were in the post-menopausal period. Tumor size ranged from 2.5 to 28 cm in diameter, with a median and mean ± SD, 7 cm and 7.88 ± 3.62 cm, respectively. The overall bilaterality rate after pathologic examination was 10.8%. Unilateral tumors were more abundantly observed on the right side (77 patients, 49%) than the left (63 patients, 40.1%). Intraoperative adhesions were detected in seven patients (4.5%). The most commonly observed complication was torsion, followed by infection, rupture and malignant transformation, observed in ten patients (6.4%), six patients (3.8%), one patient (0.6%) and one patient (0.6%), respectively. Infection resulted in a tubo-ovarian abscess in one patient who had a CA19-9 level of 60.9 U/ml. The malignant transformation was reported as squamous cell carcinoma in a 67-year-old woman who had only preoperative serum CA125 level evaluated, which was 7.82 U/ml.

Preoperative serum CA 125 (157 patients), CA19-9 (148 patients), CA 15-3 (53 patients), AFP (25 patients) and CEA (52 patients) levels of the patients were: 19.59 ± 22.01 U/ml, 46.95 ± 101.11 U/ml, 21.45 ± 8.66 U/ml, 2.37 ± 2.54 ng/ml and 2.60 ± 3.96 ng/ml, respectively. CA19-9 was the only tumor marker with a mean serum level above the cut-off value. Elevated rates of serum tumor markers from most common to least were CA 19-9 (33.1%), CA 125 (19.1%), CA 15-3 (18.9%), CEA (15.4%) and AFP (4%).

As shown in Table 1, there was no difference in terms of patient age, menopause and bilaterality of tumor between groups I and II. The mean tumor diameter of group I was significantly greater than group II (p = 0.001). We determined a weak positive correlation of CA19-9 levels to diameter of tumor (r = 0.33, p < 0.001) in all patients. In group I there was a moderate positive correlation of CA 19-9 levels to diameter of tumor (r = 0.53, p < 0.001) in all patients. In group I there was a moderate positive correlation of CA 19-9 levels to diameter of tumor (r = 0.53, p < 0.001) in all patients. In group I there was a moderate positive correlation of CA 19-9 levels to diameter of tumor (r = 0.53, p < 0.001) in all patients. In group I there was a moderate positive correlation of CA 19-9 levels to diameter of tumor (r = 0.53, p < 0.001) in all patients. In group I there was a moderate positive correlation of CA 19-9 levels to diameter of tumor (r = 0.53, p < 0.001) in all patients.

When complications were reviewed, torsions observed in the patients of group I and II were three (6.2%) and seven (7.1%), respectively (p = 0.829). Also, no significant difference was revealed between the groups, as there was one patient with a complication of infection in group I (2.0%) and four patients in group II (4.0%) (p = 0.528). There was only one patient with rupture and again only one patient with malignant transformation in group I, with no such complications in group II (p = 0.155).

Postoperative assessment of CA 19-9 levels in 33 patients of group I revealed that elevated rate was decreased to 0%, and mean CA 19-9 level was decreased from 157.28 ± 172.78 to 15.11 ± 10.48 U/ml (p < 0.001).

Figure 1. — Correlation of serum CA 19-9 levels and diameter of tumors in group I.

Table 1. — Clinical characteristics and preoperative serum levels of tumor markers of groups.

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Group I</th>
<th>Group II</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49</td>
<td>99</td>
<td>0.422</td>
</tr>
<tr>
<td>Diameter of tumor (cm)</td>
<td>8.95 ± 3.21</td>
<td>7.10 ± 3.13</td>
<td>0.001</td>
</tr>
<tr>
<td>Elevated rate (%)</td>
<td>12 (24.5%)</td>
<td>19 (19.2%)</td>
<td>0.458</td>
</tr>
<tr>
<td>Bilaterality</td>
<td>6 (1.2%)</td>
<td>10 (10.1%)</td>
<td>0.694</td>
</tr>
<tr>
<td>Intraoperative adhesions</td>
<td>6 (1.2%)</td>
<td>1 (0.01%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA 19-9 (U/ml)</td>
<td>120.36 ± 151.06</td>
<td>10.67 ± 11.45</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Elevated rate (%)</td>
<td>100.0%</td>
<td>0%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CA 125 (U/ml)</td>
<td>25.94 ± 15.20</td>
<td>17.37 ± 24.97</td>
<td>0.029</td>
</tr>
<tr>
<td>Elevated rate (%)</td>
<td>36.7%</td>
<td>12%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CA 15-3 (U/ml)</td>
<td>21.14 ± 9.00</td>
<td>21.59 ± 8.63</td>
<td>0.865</td>
</tr>
<tr>
<td>Elevated rate (%)</td>
<td>18.7%</td>
<td>18.9%</td>
<td>0.989</td>
</tr>
<tr>
<td>AFP (ng/ml)</td>
<td>1.87 ± 0.53</td>
<td>2.60 ± 3.06</td>
<td>0.515</td>
</tr>
<tr>
<td>Elevated rate (%)</td>
<td>0.0%</td>
<td>5.9%</td>
<td>0.493</td>
</tr>
<tr>
<td>CEA (ng/ml)</td>
<td>2.69 ± 1.23</td>
<td>2.58 ± 4.54</td>
<td>0.932</td>
</tr>
<tr>
<td>Elevated rate (%)</td>
<td>15.3%</td>
<td>15.4%</td>
<td>1.00</td>
</tr>
</tbody>
</table>


Similarly, postoperative mean CA 125 level of 14 patients, who had elevated levels preoperatively, was decreased from 60.91 ± 49.37 U/ml to 21.78 ± 14.25 U/ml (p = 0.001).

Discussion

MCTs constitute about 58% of the benign ovarian tumors and are mainly encountered in women of reproductive age [2, 11, 12]. Consistently, median age of patients with MCT of the ovary was 35 in our study and only 21% were postmenopausal women.

Mean tumor diameter of MCTs range between 6.4 and 8.8 cm in the literature, and in accordance mean tumor diameter was 7.9 cm in our study [13, 14]. MCTs of the ovary can be reliably diagnosed with ultrasound [3, 10].
MCTs of the ovary consist of a combination of peculiar kinds of tissues due to their origin in parthenogenetic development of germ cells and therefore, increased levels of certain tumor markers can be detected. There are few reports concerning the usefulness of tumor markers in clinical management, especially CA 19-9, in patients with MCT of the ovary [4, 5, 7].

CA 19-9 is a tumor associated cancer antigen with a glycolipid structure [15]. Initially, CA 19-9 was described in gastrointestinal adenocarcinomas particularly of the pancreas but its specificity extends to any tissue malignancy [11].

In the literature, elevated rate of CA 19-9 varies between 59% and 31.9% and extreme levels up to 1430 U/ml have been reported [7, 14, 16]. We found that a considerable portion (33.1%) of patients in our study had an elevated rate of CA 19-9, which is of clinical significance. Larger tumor size was reported to be associated with increased serum levels of CA 19-9 [5-7], whereas no significant correlation was reported in other studies [4, 8].

Our results support that diameter of MCTs are significantly larger in patients with increased levels of CA 19-9 and there is a moderate correlation of CA 19-9 levels to tumor size. This may be mainly due to a weakened cyst wall and CA 19-9 leakage from cystic cavities into the blood circulation [17].

Similar controversy is true for the relation of CA 19-9 and bilaterality of MCTs. Dede et al. [4] reported a significant relation but other studies including our present study did not agree with this finding [5-7].

A complication rate of 20% is expected in MCTs, which may present as torsion, rupture, infection and malignant transformation [18]. Total complication rate was 11.5% in our study and torsion (6.4%) was the most common one as expected. Kyung et al. [7] reported that elevated CA 19-9 levels were correlated with higher torsion rates, however, we did not find such a relation with torsion or any other complications with elevated CA 19-9 levels.

Concerns may raise thoughts about postoperative evaluation of CA 19-9 for follow-up of increased levels in MCTs but there are no such data in the literature. We demonstrated a postoperative decrease in CA 19-9 levels in 33 patients.

To the best of our knowledge, this study is the only one that has investigated the association of elevated CA 19-9 levels and intraoperative presence of adhesions. Although there was a significant difference, only seven patients could be evaluated from this perspective, which is a limitation of our study.

CA 125 is the most widely investigated ovarian tumor marker in ovarian cancer and may be elevated in about 1% of healthy women [19]. In accordance with the literature, elevated rate of CA 125 was 19.1% in our study, as varying elevated levels between 28.0% and 12.7% have been reported [8, 14]. CA 125 alone cannot be considered as useful in the diagnosis. We also observed a postoperative decrease of these elevated serum CA 125 levels, which is noteworthy.

The value of CA 19-9 in asymptomatic patients is a more dubious issue. In a study concerning diagnostic value of CEA, CA19-9 or AFP in asymptomatic individuals with slightly elevated tumor markers, despite continuous and repeated meticulous examinations, no specific pathology was revealed to explain these increases [20]. In the literature, there are reports regarding abnormally high elevations of CA 19-9 with MCTs of the ovary, but no gastrointestinal or other pathology could be detected [5, 21]. We suggest that especially in asymptomatic young women with MCTs of the ovary that can be demonstrated by ultrasound, elevated rates of CA 19-9 should not warrant further evaluation, which may be complicating matters for clinicians, a cause of anxiety for patients and a reason for unnecessary and expensive further clinical examinations such as colonoscopy, complex imaging techniques and so forth.

Conclusion

We suggest that there may be a relation between elevated levels of CA 19-9 and diameter of tumor and presence of adhesions. Because elevated levels of CA 19-9 may be expected in some patients with MCTs of the ovary that will probably be decreased postoperatively, postponing evaluation of other possible sources of this elevation to the postoperative period is more common sense.

References


Address reprint requests to:
M.G. UGUR, M.D.
Batikent Mahallesi, 74 Nolu Cadde
No: 4 A Blok Daire: 3
27560, Gaziantep (Turkey)
e-mail: metegurolugur@hotmail.com
The role of surgery in patients with advanced gynaecological cancers participating in phase I clinical trials


Drug Development Unit, The Royal Marsden NHS Foundation Trust, Downs Road, Sutton, Surrey (UK)

Summary

Objective: While gynaecological cancer patients who participate in Phase I clinical trials are not routinely considered for elective surgery because of a short life expectancy, this should not be overlooked in carefully selected responding patients. Methods/Results: We describe two cases of patients with different gynaecological cancers, who received treatment within separate phase I trials, and who then proceeded to surgical resection of their cancers, resulting in complete remission. Conclusion: Surgery, when feasible, should be taken into consideration as a potential management option, even when patients are receiving treatment within a phase I trial.

Key words: Phase I trial; Gynaecological cancers; Surgery.

Introduction

Phase I clinical trials are designed primarily to assess the tolerability and toxicity profile of an investigational medicinal product [1]. Patients who are referred for experimental therapies as part of a phase I trial have locally advanced or metastatic solid tumours and have generally exhausted all standard treatment options.

Clinicians managing patients in early clinical trials have to balance modest anti-tumour gains with the risk of drug-related toxicities. Surgery is usually only considered in patients with advanced or recurrent disease, where the benefits outweigh the risks and is generally carried out as a palliative procedure. For certain patients (including those with breast and colorectal cancers), several studies suggest that surgical approaches for carefully selected patients may be positively associated with increased long-term survival [2, 3]. Despite this, patients who participate in phase I trials are often deemed to have modest life expectancies and are not routinely considered eligible for elective surgery.

In this report, we highlight the importance of considering surgical interventions for patients undergoing experimental therapy within phase I trials, especially those who have had an anti-tumour response to such treatments. We describe two cases of patients with metastatic disease, who had surgery undertaken in this context.

Case Reports

Case 1

A 73-year-old female was diagnosed in January 2002 with FIGO Stage IIIC poorly differentiated serous ovarian adenocarcinoma. In addition, the patient was found to have an associated germline BRCA2 mutation. Her previous anti-cancer management included optimal surgical debulking followed by adjuvant carboplatin and paclitaxel chemotherapy completed in July 2002 with a complete Response Evaluation Criteria In Solid Tumors (RECIST) response. The patient had three recurrences of her cancer over the next five years, but her disease responded to treatment with single agent carboplatin on each occasion. During the third relapse, the carboplatin chemotherapy had to be discontinued prematurely due to a severe allergic reaction.

In October 2007, the patient was found to have RECIST progressive disease, with multiple liver metastases, which were not surgically resectable, as well as the development of ascites and CA125 tumour marker progression by Gynecologic Cancer InterGroup (GCIG) criteria. She subsequently commenced treatment with the poly (ADP-ribose) polymerase (PARP) inhibitor, olaparib (AZD2281; AstraZeneca) within a phase I trial (clinicaltrials.gov, NCT00516373). After 48 weeks of treatment with olaparib, her computed tomography (CT) scan showed a RECIST complete response and a CA125 response by GCIG criteria. Olaparib treatment was continued and the patient remained in complete cancer remission. Following two years and four months of treatment, an enlarged portocaval lymph node was noted on a surveillance CT scan (Figure 1). As this was the only site of relapse, she was referred for surgical resection of the enlarged node, which was undertaken in March 2010 (Figure 2). No other sites of disease were seen at surgery. Histopathology of the resected node confirmed metastatic high-grade serous papillary adenocarcinoma of ovarian origin. Treatment with olaparib was restarted four weeks after surgery and as of October 2010, the patient was still in RECIST complete remission.

Case 2

The second patient, a 37-year-old female, was diagnosed in December 2007 with Stage 1B moderately differentiated squamous cell carcinoma (SCC) of the cervix with involvement of her common iliac lymph nodes. Surgery was undertaken, prior to adjuvant chemo-radiotherapy with weekly cisplatin and brachytherapy. A CT scan in June 2008 confirmed RECIST progressive disease within the pelvis, but also revealed multiple lung metastases, making surgery inappropriate. In July 2008, the patient commenced treatment with an irreversible dual EGFR and HER2 inhibitor, afatinib (BIBW 2992; Boehringer Ingelheim) administered in combination with paclitaxel and bevacizumab within a phase I trial. A restaging CT scan after...
performed with clear histological margins (R0) in both

candidates for surgery as they both had an Eastern Coop-

Based on these criteria, our two patients were excellent

status, b) long disease-free interval, c) absence of other

resection for metastases include a) a good performance

benefit in selected cases [8], and this was also demon-

from cervical cancer at the appropriate time may be of

spread beyond the pelvis, such as multiple lung metas-

surgery, may be of benefit [7], but the presence of disease

selected cases, despite the inevitable morbidity of

may potentially be extended by optimal surgery in

patients with metastatic disease [10]. Both our patients

received optimal surgery and both remained in remission

in October 2010 after the surgical resection. The pro-

longed survival seen in both of these patients would not

have been anticipated with conventional treatment but is

based on the treatments which they received within their

phase I trial plus their surgery.

The phase I treatments included olaparib, which in

patients with ovarian cancer and BRCA1 and BRCA2

mutations has shown very promising results in early clin-

cal trials [11, 12], afatinib which has shown efficacy in

non small cell lung cancer harbouring EGFR activating

mutations [13], bevacizumab which in persistent or recur-

current cervical cancer as a second or third line treatment has

been shown to be well tolerated and active [14] and

weekly paclitaxel in cervical cancer, which has also

shown promising results [15].

In summary, we wish to emphasise that surgery, when

feasible, should be taken into consideration as a potential

management option even in patients with metastatic

gynaecological cancers, who are receiving treatment

within a phase I trial. As novel treatments for gynae-

ological cancer continue to produce promising results,

more cases of this type may be anticipated in the future.

Discussion

In general, patients with gynaecological cancers who

participate in phase I trials have a poor overall survival.

A retrospective review carried out at our phase I unit,

which included 142 patients treated between 2003 and

2008, showed an overall median survival of 11 months

[4]. Patients may occasionally respond to treatments

within phase I trials and in some cases, even merit con-

sideration for surgery, despite this not being appropriate

prior to trial entry.

These two cases illustrate this point in patients with

gynaecological Cancer in different ways. In ovarian

cancer, surgery for liver metastases may be of benefit in

a selected number of cases [5], but – as in Case 1 – the

presence of multiple liver metastases generally rules out

a surgical option. On the other hand, surgery for recurrent

extrahepatic disease, when limited in extent, can be ben-

eficial [6] and this was confirmed in Case 1. In cervical

cancer, total exenterative surgery for pelvic recurrence in

selected cases, despite the inevitable morbidity of

surgery, may be of benefit [7], but the presence of disease

spread beyond the pelvis, such as multiple lung metas-

as, in Case 2, contraindicates this approach. On the

other hand, surgery for limited pulmonary metastases

from cervical cancer at the appropriate time may be of

benefit in selected cases [8], and this was also demon-

strated in Case 2.

Favourable prognostic factors for improved survival in

patients with gynaecological cancers following surgical

resection for metastases include a) a good performance

status, b) long disease-free interval, c) absence of other

systemic disease and d) surgical complete resection [9].

Based on these criteria, our two patients were excellent

candidates for surgery as they both had an Eastern Coop-

erative Oncology Group (ECOG) performance status of

1, absence of other systemic disease, and surgery was

performed with clear histological margins (R0) in both

cases. Furthermore, the patient with ovarian carcinoma

was also considered to have a long disease-free interval

as her first disease relapse appeared more than one year

after the last cycle of her initial chemotherapy.

The literature data concerning surgery in metastatic

ovarian and cervical cancer clearly illustrate that survival

References


Address reprint requests to:
S.B. KAYE, M.D.
Drug Development Unit
The Royal Marsden NHS Foundation Trust
Section of Medicine
The Institute of Cancer Research
Sycamore House, Downs Road
Sutton, Surrey, SM2 5PT (UK)
e-mail: stan.kaye@rmh.nhs.uk
Peritonitis due to iatrogenic colpotomy after large loop excision of the transformation zone (LLETZ) in a patient with cervical intraepithelial neoplasia III: our experience of a rare case with review of the literature

M. Varras¹, C. Akrivis², A. Anastasiadis¹, G. Lekkas¹, G. Diakakis¹

¹Department of Obstetrics and Gynecology, “Elena Venizelou” General Maternity Hospital, Athens; ²Department of Obstetrics and Gynecology, “G. Chatzikosta” General State Hospital, Ioannina (Greece)

Summary

A case of peritonitis as an unusual complication of LLETZ (large loop excision of the transformation zone) for the treatment of CIN III associated with unrecognized iatrogenic posterior colpotomy is presented. After the procedure, the patient developed fever 38.3°C and diffused severe pelvic pain. The contributing factors, prevention and management of this complication are discussed. Also, the complications of cold knife cervical conization and LLETZ procedure are reviewed.

Key words: Cervical intraepithelial neoplasia; Treatment; Cervical cone biopsy; LLETZ; Complications; Peritonitis.

Introduction

Large loop excision of the transformation zone (LLETZ) is an effective method for the treatment of premalignant cervical disease. It has the advantage of being simultaneously diagnostic and therapeutic. It is often performed as an outpatient procedure and because there is only minimal tissue damage, is considered to provide an adequate sample for histological analysis [1, 2].

We present a case of peritonitis due to unrecognized iatrogenic posterior colpotomy, which occurred as a consequence of LLETZ. Our review of the literature yielded very few similar reports [3-5]. The purpose of this article is to point out the potential hazards of loop electrosurgical excision.

Case Report

A 23-year-old woman was found to have a cervical intraepithelial lesion (CIN) III diagnosed by colposcopy and guided cervical biopsies and was treated with large loop excision of the transformation zone (LLETZ) under general anesthesia in a private maternity hospital in Athens, Greece. The cervical tissue was removed in one piece measuring 2.5 x 3 cm in diameter. The next day, the patient was admitted to the Emergency Department of a State Hospital in Athens and hospitalized because of fever 38.3°C and diffused pelvic pain. Physical examination demonstrated mild tachypnea; the abdomen was distended, with guarding and rebound tenderness. Detailed inspection of the vagina and cervix was difficult but pus-like discharge was noted inside the vagina. Bimanual gynecological examination showed high tenderness cervical motion. Estimation of the uterus and the adnexa was difficult because of the resistance of her abdominal wall. The patient was hemodynamically stable, with good bladder function. Peristaltic sounds were diminished. Hematocrit was 40.1%, hemoglobin 13.9 gr/dl and white blood count 11,000/mm³, with 91.8% polymorphonuclear leucocytes and platelets 140,000/µl. General urinalysis was negative. Renal and liver function tests were normal. A computed tomography (CT) scan of the upper and lower abdomen showed hydronic levels at the small and large bowel without a picture of complete obstruction, and indentified the presence of gas within the uterine cavity (Figure 1).

An emergency laparotomy was performed. Diffused peritonitis with abdominal pus was found without great effects of adhesions and an opening of about 2 cm in the maximum diameter in the posterior vaginal wall was observed through the posterior space of Douglas (Figure 2). No bowel perforation was detected and the vermiform appendix macroscopically was normal. The abdomen and pelvis were washed with adequate amounts of fluid and the deficit in the posterior vaginal wall was easily repaired using interrupted absorbable sutures. Also, interposition of omentum was used to ensure the repair of the colpotomy (Figure 3). Cultures of the abdominal pus grew great colonies of E. coli and enterococcus falcalis. Postoperatively, intravenous broad-spectrum antimicrobial therapy was given: mitromidazole 500 mg/100ml three times daily and a combination of piperacillin sodium (4 g) with tazobactam sodium (0.5 g) four-times daily. In addition, heparin of low molecular weight was administered for all the postoperative days the patient remained in hospital. The patient’s postoperative course was uneventful. The histopathological examination of the surgical cervical specimen confirmed the existence of CIN III; the surgical borders were free of disease.

Discussion

Cold knife conization of cervix is used as a diagnostic and therapeutic modality for cervical intraepithelial neoplasia [6]. However, the LLETZ procedure is the procedure of choice for such cases. It is performed with a large loop of thin wire, which forms a diathermy electrode and...
Peritonitis due to iatrogenic colpotomy after large loop excision of the transformation zone (LLETZ) in a patient with etc.

allows a deep excision of the transformation zone with minimal tissue damage [1]. Most surgeons prefer to excise the tissue in one piece. Thus, the loop size is chosen according to the diameter of the transformation zone [1]. In 1984, Cartier first described LETZ (loop excision of transformation zone) as electrodiathermy loop excision using a small wire loop for accurate directed cervical biopsies [7]. Prendeville et al. in 1989, adapted this technique by designing larger fine wire loops and called it LLETZ (large LETZ). They reported a successful cure rate of 98% for CIN at a single treatment [8]. The equipment was cheap, simple and the technique was easy to learn [2]. The recurrence rates of LLETZ are 4%, similar to those of cold knife and laser conization [1, 9, 10].

Complications of cold knife conization are related with 12-20% morbidity [6]. Early complications are hemorrhage and infection, with bleeding occurring at a frequency of 4-21% of cases [6, 11]. Also, a retroperitoneal hematoma has been described, probably due to trauma to the vaginal artery caudal to its branching from the uterine artery [6]. Placement of hemostatic sutures at the conclusion of the conization procedure to ligate the descending cervical branch of the uterine artery has been recommended to decrease the frequency of postoperative bleeding [12-15]. Also, Monsel’s solution and vaginal pack without hemostatic sutures are effective in protecting cervical hemorrhage [16]. Cervical stenosis is a late complication of cold knife conization from damage to the cervical channel and causes dysmenorrhea, if partial stenosis occurs or hematometra if the stenosis is complete [6, 11]. The incidence of hematometra has been reported to be less than 1% [17]. In some of the reported cases the patients have resumed normal menstruation after surgery, but later developed constriction of the canal that resulted in amenorrhea. Group B streptococcal meningoencephalitis and retroperitoneal psoas abscess have also been reported [11, 18]. A possible pathogenetic mechanism for the development of retroperitoneal psoas abscess is an inadvertent opening of the retroperitoneal space during the cone biopsy causing direct spread of infection to this space [11]. Finally, bowel, bladder and ureteral injuries have been described [13-15]. In patients with cystocele or enterocoele, a dissection of the anterior and posterior vaginal mucosa might improve the exposure of the cervical and paracervical tissue, decreasing therefore the risk of bowel, bladder and ureteral injury [15].

The significant advantages of LLETZ compared to laser or cold knife conization are shorter operative time, less handling of the tissue, reduced bleeding and reduced discomfort for most of the procedure. Moreover, there is
no hazard to the surgeon’s eye-sight, and equipment breakdown occurs less often, leading to higher efficiency at a relative lower cost [1, 19-22]. The short-term complications of LLETZ include infection, vaginal discharge, and inadvertent injury to vaginal sidewalls [2]. The most common long-term complication is cervical stenosis [2]. To minimize the incidence of cervical stenosis, a loop depth of 8 mm, which is the maximum crypt depth, has been recommended. Patients with evidence of cervical canal involvement may require a separate pass of a smaller loop to excise the deeper aspect of the cervical canal effectively. The cone excision can thus be extended to various depths with some authors stating that this normally should not exceed more than 10-20 mm into the canal [23]. Rare complications of the LLETZ procedure are infections and infertility caused by cervical stenosis [1]. Also, cervical incompetence is included in the complications of the procedure [2]. Nannapaneni et al. [2] described an extremely rare complication of intraabdominal bleeding following LLETZ.

Peritonitis as a complication of cervical conization with cold knife or diathermy loop excision of the transformation zone for treatment of CIN has rarely been described [3-5] and the causes for this event are usually an incidental colpotomy due to anatomical particularity of the cervical outline, extensive cervical lesions or extensive cervical resection [5]. In addition, patient with a distorted cervix, previous cervical conization or pelvic irradiation are at increased risk [1]. In the patient described here an emergency laparotomy was performed based on her history of recent LLETZ and her clinical picture. We believe that contributory factors to the formation of colpotomy were the anatomical distinctiveness of the cervical outline and the extensive cervical lesion. The resection of a CIN lesion with a smaller size of loop or with the use of needle excision of the transformation zone (NETZ) should possibly have prevented this complication. Iatrogenic insertion of air, found in the CT scan, through the cervix within the endometrial cavity during the surgery the previous day is an option. Another possible option, but less common is the production of gas due to E. Coli (endometritis emphysymatosa).

Conclusions

LLETZ is an excellent technique for the treatment of CIN. Complications are usually few and mild, mainly minor bleeding and discomfort. Our case serves as a reminder that the LLETZ procedure, although simple and easily performed can result in major complications. Extremely rare complications are peritonitis and bowel obstruction. Surgeons should be aware of these possibilities because immediate identification is crucial for prevention of further damage. It is suggested that in cases with anatomical particularity of the cervical outline or extensive cervical lesions one should use a smaller loop-wire or make a needle excision of the transformation zone (NETZ) for the treatment of CIN.

References


Address reprint requests to:
M. VARRAS, M.D.
Platonos 33, Politia (Kifisia)
14563 Athens (Greece)
e-mail: mvarras@otenet.gr
Bilateral androblastoma (Sertoli-stromal cell tumor) of the ovary: a rare cause of virilization in a teenager

A. Warenik-Szymankiewicz, R. Słopień, M. Gaca, H. Kędzia, P. Kądziołka, T. Opala

1Department of Gynecological Endocrinology, University of Medical Sciences of Poznań
2Department of Anesthesiology in Obstetrics and Gynecology University of Medical Sciences of Poznań
3Department of Pathology University of Medical Sciences of Poznań
4Department of Mother’s and Child’s Health University of Medical Sciences of Poznań (Poland)

Summary
A case of a 17-year-old patient diagnosed with bilateral androblastoma of the ovary is presented. The patient was admitted because of secondary amenorrhea, hirsutism and acne. After clinical, ultrasonographic and hormonal examinations an androgen-producing ovarian tumor was suspected and consequently laparotomy with right ovarian excision was carried out. During surgery the right ovarian tumor was excised and exploration of the left ovary revealed an ovarian tumor with a diameter of 10 mm, which was then also excised. The pathologic diagnosis was a bilateral androblastoma of the ovary measuring 40 mm x 30 mm x 20 mm in the right ovary and 10 mm in diameter in the left ovary. We concluded that androblastomas, in spite of their low incidence, are a possibility that should always be considered in women of all ages presenting with signs of virilization.

Key words: Androblastoma; Virilization; Teenager.

Introduction
Androblastomas (Sertoli-stromal cell tumors) are a rare cause of female virilization. The tumors are known to contain Sertoli cells, Leydig cells, indifferent stromal cells or all three cell types in various proportions and varying degrees of differentiation [1]. They occur at all ages but are most commonly encountered in women during their early reproductive years [2]. They are usually unilateral, and bilateral androblastomas are present in only less than two percent of cases [3].

Case Report
A 17-year-old patient with an unremarkable medical history was admitted to our department because of secondary amenorrhea, hirsutism and acne. Menarche was at the age of 13, however menses were irregular (occurring every 2-6 months) with the last menstruation present at the age of 14. Physical examination revealed a normal body mass index, an increase in body hair distribution with regard to the uterus and ovaries.

During surgery the right ovarian tumor was excised and exploration of the left ovary revealed a left ovarian tumor with a diameter of 10 mm, which was then also excised. The pathologic diagnosis was a bilateral androblastoma of the ovary measuring 40 mm x 30 mm x 20 mm in the right ovary and 10 mm in diameter in the left ovary.

Both tumors were localized in the cortical part of the ovaries. The larger one was partially covered by an ovarian capsule and loose connective tissue. Sectioning revealed a solid tumor with a brown-yellow appearance. Upon microscopic examination Sertoli cells were observed forming solid tubular structures with focal hollow tubules and solid islands. Leydig cells were also found in abundant amounts - forming distinct areas of focal groups inbetween the Sertoli cells. The tumors were both well differentiated without mitotic activity, therefore classifying them both as Stage IA (Figures 1 and 2).

Postoperatively the patient presented with no complications. Two days after the operation the level of testosterone was 0.21 ng/ml [0.06-0.82]. The patient was then followed up within one month, at which time she reported the occurrence of spontaneous menstruation and a discreet improvement in her hirsutism and acne severity. In the analytical control the level of testosterone was 0.36 ng/ml [0.06-0.82]. TVS showed no abnormalities with regard to the uterus and ovaries.

Discussion
In this case the main clinical features and reasons for consultation of the patient were secondary amenorrhea and symptoms of severe hyperandrogenism. Secondary amenorrhea, when it presents in young women, may be due to variety of causes such as hypothalamic insufficiency, hyperprolactinemia, hyperandrogenic syndromes, and may also be due to primary ovarian insufficiency. Hyperandrogenic symptoms may have an ovarian or suprarenal origin. Among ovarian causes polycystic ovarian syndrome is the predominant one. Tumors of the ovary associated with hyperandrogenism include primitive tumors of the sexual cords and stroma (granulosa cell...
However in spite of their low incidence, they are a possibility that should always be considered in women of all ages presenting with signs of virilization.

References


Address reprint requests to:
R. SLOPIEŃ, M.D.
Department of Gynecological Endocrinology
Ul. Polna 33
60-535 Poznań (Poland)
e-mail: asrs@wp.pl
Malignant lymphoma of the vagina successfully treated with rituximab, adryamicin, cyclophosphamide, vincristine sulfate, and prednisolone

K. Nasu, M. Okamoto, M. Nishida, N. Takai, H. Narahara

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

Summary

Purpose: Primary malignant lymphoma of the vagina is extremely rare. The most common histologic subtype is diffuse large B-cell lymphoma (DLBCL). We report a case of vaginal DLBCL successfully treated with chemotherapy consisting of rituximab, adryamicin, cyclophosphamide, vincristine sulfate, and prednisolone (R-CHOP), followed by pelvic irradiation. Case: A 44-year-old Japanese woman was admitted complaining of atypical genital bleeding and puruloid vaginal discharge. Gynecological examination showed an ulceration of the vaginal wall and a hard mass the size of a goose egg beneath the left vaginal wall, which had infiltrated to the left pelvic wall. The pathological diagnosis based on a punch biopsy taken from the vaginal tumor was non-Hodgkin’s lymphoma. Based on immunohistochemical study, the tumor was subclassified as activated B-cell type DLBCL. The patient was diagnosed with Ann Arbor Stage IEA DLBCL and Stage III vaginal cancer, according to the International Federation of Gynecologists and Obstetricians (FIGO) classification system. She was successfully treated by six courses of R-CHOP, followed by radiation therapy. The patient is well without evidence of disease 13 months following the initial treatment. Conclusion: Little attention has been paid to the use of rituximab in addition to conventional chemotherapy and the importance of clinical and morphological subgrouping of DLBCL arising in the vagina. The present case indicates that the effects of rituximab on the prognosis of vaginal DLBCL must be evaluated, and that clinical use of immunophenotypic subgrouping should be considered for vaginal DLBCL.

Key words: Malignant lymphoma; Vagina; Chemotherapy; Radiation.

Introduction

Secondary involvement of the female genital organs can be seen in up to 40% of disseminated malignant lymphomas, but primary malignant lymphoma of the vagina is extremely rare [1-3]. Chorlton et al. [1] reviewed 9,500 cases of lymphomas in women and found only four cases of primary vaginal malignant lymphomas, i.e., an incidence of one in 2,375 cases.

Clinical symptoms of vaginal malignant lymphomas usually include vaginal bleeding (70%), perineal discomfort (40%), and persistent vaginal discharge (20%). However, patients with vaginal malignant lymphoma may also present with a clinically detectable vaginal or pelvic mass [3], or with symptoms of abdominal pain, introtial mass, dyspareunia, or urinary frequency [2, 3] and 20% of cases apparently remain asymptomatic [1]. Vaginal malignant lymphomas produce an ill-defined, very firm thickening or induration of the vaginal wall and extend toward the rectum, bladder, or pelvic walls [1-3]. At presentation, contiguous structures and/or regional lymph nodes are commonly involved [3].

Most are non-Hodgkin’s lymphomas (NHL). The most common histologic subtype is diffuse large B-cell lymphoma (DLBCL) with immunohistochemistry staining positive for CD20 [3, 4-8]. Guarini et al. [4] reported that 52.9% of cases of vaginal NHL were classified as high-grade and that 90.9% of cases were diagnosed as in Stages IE and IIE. However, in many reports older lymphoma classifications were used and immunophenotypic data were not provided.

In this case report, we present an additional case of vaginal DLBCL successfully treated with chemotherapy consisting of rituximab, adryamicin, cyclophosphamide, vincristine sulfate, and prednisolone (R-CHOP), followed by pelvic irradiation. We also discuss the importance of subclassification of vaginal DLBCL with immunohistochemistry.

Case Report

A 44-year-old Japanese woman (gravida 2, para 2) was admitted complaining of atypical genital bleeding and puruloid vaginal discharge. The patient did not have fever, weight loss, or night sweats. Gynecological examination showed an ulceration of the vaginal wall and a hard mass the size of a goose egg beneath the left vaginal wall, which had infiltrated to the left pelvic wall. Cytology of the cervix, endometrium, and ulcerated vaginal wall were negative. Bilateral inguinal and femoral lymph nodes were not evident. The pathological diagnosis of a punch biopsy taken from the vaginal tumor was NHL (Figure 1). As shown in Table 1, immunohistochemical study subclassified the tumor cells as activated B-cell (ABC) type DLBCL. No distant metastasis was detected by chest X-ray, intravenous pyelogram, cystoscopy, or colon fiberscopy. Magnetic resonance imaging and contrast-enhanced computed tomography (CT) revealed a large soft tissue mass with central necrosis involving the posterior vaginal wall (Figure 2). PET-CT revealed an increased 18F-fluorodeoxyglucose focal uptake in the vaginal mass. Distant metastases or lymph node involve-
Table 1: Immunocytochemical analyses of DLBCL of the vagina.

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>CD5</th>
<th>CD10</th>
<th>CD20</th>
<th>Bcl-2</th>
<th>Bcl-6</th>
<th>MUM-1</th>
<th>CD79a</th>
<th>Ki-67</th>
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<td>Guarini et al. (1999) [4]</td>
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<td>Mahendran (2008) [7]</td>
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<tr>
<td>Ikuta et al. (2010) [8]</td>
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<tr>
<td>Nasu et al. (present case)</td>
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Discussion

Because of its rarity, there is no established treatment protocol for primary NHL of the vagina. The mainstays of treatment for NHL have been chemotherapy and/or radiation therapy, and the majority of cases of vaginal DLBCL presented in the literature were also treated with chemotherapy and radiation therapy [5]. Standard chemotherapy treatment includes a CHOP regimen for at least three cycles, with a minimum of six cycles for bulky disease, followed by radiation therapy. It has been reported that DLBCL of the vagina responds very well to this protocol, with a 70-90% cure rate, especially in the early stages [5]. However, the use of novel treatments, including monoclonal antibodies, is being assessed and becoming a standard of treatment [9]. The addition of the anti-CD20 monoclonal antibody, rituximab, to CHOP (R-CHOP) has led to a marked improvement in the survival of patients with DLBCL [10]. However, as shown in Table 2, there have been only five cases of vaginal DLBCL treated with R-CHOP previously reported in the literature [7, 11, 12].

DLBCL is heterogeneous both clinically and morphologically. Patients with DLBCL have highly variable clinical courses: although most patients respond initially to chemotherapy, fewer than half of the patients achieve a durable remission [13]. Although a combination of clinical parameters of the International Prognostic Index (IPI) is currently used to assess a patient’s risk profile, these prognostic variables are considered to be proxies for the underlying cellular and molecular variation within DLBCL [14]. DLBCL can be divided into two prognostically significant subgroups, germinal center B-cell-like (GCB) and ABC-like [15]. Initially, a third subgroup (simply termed type 3) was also defined. This was based on a collection of cases that could not be classified into either the GCB- or ABC-subgroup and does not represent a distinct subgroup [16]. The GCB group has a significantly better survival than the ABC group, with the 5-year survival rates being 15-30% and 50-60%, respectively [17]. Although the type 3 group is heterogeneous and not well defined, it is suggested to have a poor outcome similar to the ABC group [15].

The subclassification of DLBCL by CD10, Bcl-6, and interferon regulatory factor (IRF) 4/MUM1 expression has been proposed [18]. Cases with CD10 expression in >30% of cells are regarded as GC type as well as cases that are CD10-, Bcl-6+, IRF4/MUM1-. All other cases are regarded as of non-GC type [18]. Hans et al. [18] reported that the 5-year overall survival for the GCB group was 76% compared with only 34% for the non-GCB group, which is similar to that reported using a cDNA microarray [15]. The addition of other markers such as Bcl-2 and cyclin D2 may lead to an improvement in the immunophenotypic subgrouping of DLBCL [19]. It has been demonstrated that the expression of Bcl-6 or CD10...
was associated with better overall survival, whereas expression of MUM1, Bcl-2, or cyclin D2 was associated with worse overall survival [18]. The addition of the anti-CD20 antibody, rituximab, to the conventional chemotherapy was reported to have eliminated the negative impact of the expression of Bcl-2 and the positive impact of Bcl-6 on clinical outcome [10, 20, 21]. However, the use of immunohistochemical panels to assign prognostic groups does not currently have a role in routine clinical practice [17], especially in DLBCLs arising in the vagina. As shown in Table 1, there have been only four cases of vaginal DLBCL with detailed immunophenotypic subgrouping.

In summary, we presented an additional case of vaginal DLBCL that was subgrouped as ABC type and successfully treated by chemotherapy, R-CHOP, followed by pelvic irradiation. As discussed above, little attention has been paid to the use of rituximab in addition to conventional chemotherapy or to the importance of clinical and morphological subgrouping of DLBCL arising in the vagina. It is important to evaluate the effects of rituximab on the prognosis of vaginal DLBCL. In addition, clinical use of immunophenotypic subgrouping should be considered for vaginal DLBCL.

References


Address reprint requests to:
K. NASU, M.D., Ph.D.
Department of Obstetrics and Gynecology
Faculty of Medicine
Oita University, Idaigaoka 1-1
Hasama-machi, Yufu-shi
Oita 879-5593 (Japan)
e-mail: nasu@oita-u.ac.jp
Malignant mixed Müllerian tumor of the fallopian tube: a case report

T. Watanabe¹, T. Sugino², S. Furukawa¹, S. Soeda¹, H. Nishiyama¹, K. Fujimori¹

¹Department of Obstetrics and Gynecology, ²Department of Pathology, Fukushima Medical University School of Medicine, Fukushima (Japan)

Introduction

Primary carcinomas of the Fallopian tube are rare, accounting for less than 2% of gynecological malignancies [1]. Adenocarcinoma is the most common histology in Fallopian tube malignancies [2], whereas sarcoma, particularly malignant mixed Müllerian tumor (MMMT), is extremely uncommon, with only about 70 cases reported in the English literature to date [3, 4]. Of these cases, approximately 30 cases displayed heterologous sarcomatous elements, such as rhabdomyosarcoma, chondrosarcoma and osteosarcoma [3, 5]. We report herein a case of MMMT in the Fallopian tube with heterologous chondrosarcomatous elements.

Case Report

A 60-year-old woman (gravida 2, para 2) presented complaining of abdominal enlargement and pain. She had a history of breast cancer with total mastectomy and axillary lymph node dissection at 36 years of age. Family history included the deaths of her mother and younger sister from ovarian cancer. Echography and computed tomography suggested the presence of a large mass in the right adnexal region. Serum levels of CA72-4 were mildly elevated. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, pelvic and paraaortic lymph node dissection, and resection of intrapelvic metastases. The tumor formed a large polypoid mass within the right Fallopian tube and had penetrated the wall to the paraovarian space. Microscopic examination revealed two components of poorly differentiated adenocarcinoma and high-grade sarcoma with chondromatous differentiation. The patient received six courses of adjuvant chemotherapy with ifosfamide and cisplatin and is currently in remission.

Summary

Malignant mixed Müllerian tumor (MMMT) of the female genital tract is uncommon and extremely rare in the Fallopian tube. We describe a case of primary MMMT of the Fallopian tube with carcinomatous and heterologous mesenchymal components in a 60-year-old woman. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, pelvic and paraaortic lymph node dissection, and resection of intrapelvic metastases. The tumor comprised two elements (Figure 2a): adenocarcinoma forming irregular papillary and tubular structures, and spindle cell sarcoma resembling fibrosarcoma and focal heterologous mesenchymal malignancy with cartilaginous differentiation. Tumor within the lumen mostly comprised adenocarcinoma (Figure 2b), whereas the extraluminal tumor included sarcomatous elements with chondrosarcoma in addition to the epithelial tumor (Figure 2c, d). Transitional features from carcinoma to sarcoma were observed (Figure 2e). The final clinicopathological diagnosis was MMMT with heterologous chondrosarcoma arising in the Fallopian tube. The tumor was staged as FIGO IIc.

Postoperatively, serum levels of CA72-4 fell within normal ranges. The patient received six courses of adjuvant chemotherapy with ifosfamide and cisplatin and is currently in remission. Although MMMT in the Fallopian tube shows poor prognosis, primary cytoreductive surgery with platinum-based combination chemotherapy may improve survival.

Key words: Malignant mixed Müllerian tumor; Fallopian tube; Heterologous element.
Discussion

The great majority of Fallopian tube tumors are diagnosed after surgery by anatomopathological evaluation based on the criteria established by Hu et al. and modified by Sedlis as follows: 1) main tumor in the tube and arising from the endosalpinx; 2) histological pattern reproducing the epithelium of the mucosa and usually showing a papillary pattern; 3) if the Fallopian tube wall is found to be totally invaded, a transition zone between benign and malignant epithelium should be able to be

Figure 2. — Microscopic findings of the tumor. a) The tumor in the right Fallopian tube (A) has penetrated the wall (arrow) to the paraovarian region (B). b) The main component of tumor A is poorly differentiated adenocarcinoma. c) Tumor B comprises epithelial and mesenchymal components. d) The tumor has a heterologous element with chondromatous differentiation (*). e) Transition from carcinoma to sarcoma is seen. Bars: a, 10 mm; b, 0.2 mm; c, 1 mm; d, 0.5 mm; e, 0.2 mm. a-e: hematoxylin and eosin staining.
demonstrated; and 4) the ovary and endometrium are either normal or contain less tumor than the tube [6, 7].

MMMT of the Fallopian tube grossly resembles Fallopian tube carcinoma, with a dilated tube that contains an intraluminal papillary mass.

The Fallopian tube is the least common site for MMMT in the female genital system, accounting for less than 4% of reported cases [8]. To date, only about 70 cases of primary MMMT of the Fallopian tube have been reported, including about 30 reports describing tumors with heterologous mesenchymal elements. Patients were in the fifth or sixth decade of life, with a mean age of 58 years [9]. Symptoms of MMMT in the Fallopian tube resemble those of Fallopian tube carcinoma. Patients present with abdominal pain, atypical genital bleeding, or abdominal distension [8, 9]. A discrepancy between cytological abnormalities in cervical or endometrial smears and negative findings on colposcopy, cervical biopsy, and endometrial curettage or hysteroscopy could prompt further diagnostic exploration, but diagnosis is not usually made until the time of surgery [10].

Histologically, MMMT consists of both carcinomatous elements with predominantly glandular differentiation assuming an endometrioid, clear cell, papillary serous, or rarely squamous pattern and sarcomatous elements. About half of the reported patients had well differentiated adenocarcinoma and the remaining half had poorly differentiated adenocarcinoma. The sarcomatous portions can exhibit differentiation towards mesenchymal tubal tissue layers such as smooth muscle or stroma and are therefore designated as consisting of homologous components. In contrast, the predominant presence of structures that are foreign to the Fallopian tube such as bone or non-smooth muscle fibers, cartilage and their polymorphic precursor cells lead to the classification of the heterologous component of the sarcomatous portion of MMMT.

Treatment for this disease is identical to that for epithelial ovarian cancer. Exploratory laparotomy is necessary to remove the primary tumor, stage the disease, and resect metastases. The overall 5-year survival rate for patients with tubal carcinoma is 44% [11]. However, the survival rate of MMMT is very poor, and most patients die of the disease within two years [9]. Extratubal spread is the most important prognostic factor for survival in adenocarcinoma of the Fallopian tube. The presence or absence of heterologous elements does not appear related to outcome. A single positive observation is the markedly better probability of survival for women with tumors confined to the muscularis [12].

Postoperatively, platinum-based combination chemotherapy has been performed for patients with MMMT of the Fallopian tube. VAC or CYVADIC therapy was also administered in several cases [9]. In a prospective phase II GOG study, overall 5-year survival was 62% for patients with Stage I or II uterine MMMT who received ifosfamide and cisplatin [13]. Complete response rate for paclitaxel and carboplatin therapy was 4/5 (80%) in patients with advanced or recurrent MMMT of the uterus [14]. Some papers have indicated that adjuvant radiation therapy can improve survival for patients with uterine MMMT [13]. Although some reports have described beneficial effects of platinum-based chemotherapy and radiotherapy for tubal MMMT, no standard adjuvant therapy has been devised due to the very small number of cases encountered.

Although the pathogenesis of MMMT is somewhat unclear, three main theories to explain the histological features found in this type of tumor have received strong support. First, the collision theory suggests that the carcinoma and sarcoma represent two independent neoplasms. Second, the combination theory suggests that both components are derived from a single stem cell that undergoes divergent differentiation early in the evolution of the tumor. Third, the composition theory suggests that the stromal component of MMMT is not truly neoplastic, but actually a reactive response to the presence of the malignant epithelial component. Recently, several lines of evidence have supported a monoclonal origin of MMMT with subsequent divergent differentiation. Immunohistochemical studies of MMMT have suggested a common epithelial origin [15]. Furthermore, the epithelial and mesenchymal components frequently share patterns of X-inactivation, allelic loss, and TP53 mutation [16-18]. Clinically, the carcinoma component is more frequently found in metastatic deposits, leading most clinicians to approach this tumor as a poorly differentiated carcinoma rather than a sarcoma [15, 19]. In the present case, a transition area from carcinoma to sarcoma was histologically observed in this tumor and may add weight to the combination theory.

In conclusion, the present report underlines a case of advanced Fallopian tube MMMT with heterologous chondrosarcoma elements. We performed optimal debulking surgery and ifosfamide and cisplatin therapy in this case, and the patient has remained free of disease as of more than one year after diagnosis. Although MMMT of the Fallopian tube is extremely aggressive and historically shows very poor prognosis, complete surgical resection with platinum-based combination chemotherapy may improve survival.

References


Pseudomyxoma peritonei - case report

O. Nikolic1, S. Djurdjevic2, S. Stojanovic1, M. Basta Nikolic1, M. Mocko Kacanski3, S. Secen4

1Department of Radiology, Clinical Center of Vojvodina
2Department of Gynecology and Obstetrics, Clinical Center of Vojvodina
3Department of Histopathology, Medical Faculty, University of Novi Sad
4Department of Abdominal Surgery, Clinical Center of Vojvodina, Novi Sad (Serbia)

Summary
The syndrome pseudomyxoma peritonei is rare, present in only 2/10,000 laparotomies. We report the case of a 58-year-old woman with a primary tumor of the appendix, and secondary involvement of other structures and organs of the abdominal cavity. In our case, we performed maximal surgical reduction of the tumor, with remaining implants on diaphragmatic domes and liver, as we did not have technical conditions to safely perform prolonged surgery which would have included a surgical procedure on the liver and administration of intraoperative chemotherapy. The patient underwent six series of parenteral chemotherapy, but refused the second-look surgery. Even though our patient did not receive intraperitoneal chemotherapy, maximal surgical tumor reduction, and refused second-look surgery, she is still alive and without any major complaints two years after the surgery.

Key words: Pseudomyxoma peritonei; Appendix; Ovary; Diagnosis; Treatment.

Introduction
Pseudomyxoma peritonei is characterized by the presence of gelatinous ascites and mucinous implants on the peritoneum and omentum. It is present in 2/10,000 laparotomies and more often in women (ratio 3:1) [1]. In multiorgan involvement it is not possible to determine the organ of primary origin only by imaging and intraoperative findings. The most reliable method is immunohistochemical staining.

Case Report
A female patient (58 years old) came for a gynecological examination for lower abdominal discomfort. Endovaginal ultrasound (US) examination revealed an enlarged left ovary (diameter 55 mm) with tumor mass consisting of solid-cystic components and papillary proliferations suspicious for malignancy. There was ascites in the Douglas pouch. The uterus and right ovary had a normal morphology. During preparation for elective surgery, apart from standard laboratory blood and urine analysis, chest X-ray, ECG and internist examination, abdominal and pelvic computed tomography (CT) were performed. Moreover, blood concentrations of tumor marker CA 19.9 and CEA were elevated (CA 19.9: 263.4 IU/ml, CEA: 146.8 ng/ml). CT examination showed: 1) a left ovarian cystic mass with internal septa and solid components (size 32 x 55 x 48 mm); 2) moderate ascites in the Douglas pouch; 3) diffuse omental implants - omental caking; 4) a cystic mass (4 cm) with ring postcontrast enhancement in the ileocecal region; and 5) sub-diaphragmatic, perihepatic and perisplenic peritoneal implants.

After preoperative preparation, surgery was performed. The operation revealed: 1) a mucinous malignant left ovarian tumor (ex tempore histopathology - malignant); 2) peritoneal tumor implants in the Douglas pouch, left paracolic region, bladder peritoneum; 3) tumor of the appendix; 4) diffuse great omentum carcinomatosis; and 5) gelatinous tumor tissue in the region of the diaphragm domes, spleen and liver, which indicated the syndrome of pseudomyxoma peritonei. During surgery total abdominal hysterectomy with bilateral salpingo-oophorectomy, resection of the bladder and Douglas pouch peritoneum with the removal of tumor implants, appendectomy, total omentectomy and splenectomy were performed. Postoperative recovery was satisfactory, and the patient was discharged ten days after the surgery. Histopathology examination included the following methods: paraffin blocks of specimen fixed in pulverized formalin were made, then cut to the thickness of 5 µm and finally dyed with the methods H&E, PAS, CK 7, CK 20, estrogen receptors (ER) and CA125. In the specimen, all organs showed the same biphasic histological appearance - part of the material

Figure 1. — Cystic appendiceal lesion, omental caking, perihepatic, perisplenic peritoneal implants and ascites, left ovarian cyst.
In such cases of multiple organ involvement, it is of great importance to apply immunohistochemical methods of staining, mostly CK-7 and CK-20 which aid in differentiation of organs from which a mucoproducive lesion had arisen. Expression of CK-7 indicates that the lesion originates from ovarian tissue, while expression of CK20, i.e. membranous and cytoplasmatic positive reaction with antibodies to CK-20, indicates that the lesion originates from the digestive system [7]. In cases of pseudomyxoma peritonei syndrome maximal surgical reduction of tumor including total peritonectomy is performed. Additional intraperitoneal chemotherapy, which gives the best chances for success, is also administered. In such cases, appendectomy should always be performed, as the appendix in this syndrome is very often malignantly altered. From a histological point of view, several similar but different histological entities should be distinguished: disseminated peritoneal adenomucinosis (DPAM), peritoneal mucinous carcinomatosis (PMCA), and peritoneal mucinous carcinomatosis (PMCA-I/D) with a different microscopic appearance [8]. In our case, we performed maximal surgical reduction of the tumor, with remaining implants on the diaphragmatic domes and liver, as we did not have technical conditions to safely perform prolonged surgery which would have included a surgical procedure on the liver and administration of intraoperative chemotherapy. After completing parenteral chemotherapy and a control CT examination, second-look surgery should have been performed with the aim of reducing any eventual residual tumor tissue on the diaphragm domes and liver, as part of a protocol in such cases [9, 10]. The patient underwent six series of parenteral chemotherapy, but refused the second-look surgery. Even though our patient did not receive intraperitoneal chemotherapy and maximal surgical tumor reduction, she is still alive and without major complaints two years after the surgery.

Discussion

Mucinous lesions can be found in different locations in the body, but the most common are bowel (appendix) and ovarian tumors [2, 3]. These lesions can be seen in benign, intermediate and malignant variants, but the most serious complication is clinically called peritoneal pseudomyxoma. It is simple if the tumor lesion is limited to the single organ no matter if the pseudomyxoma has developed i.e., diagnosis of the localization of the primary neoplastic lesion is made easier. A basic problem arises when more organs, which can all have primary mucinous lesions, are involved, or in other words if, as in our patient, tumor with a substantial production of mucus has spread to many organs [4-6]. In our case, the left ovary, appendix, omentum and peritoneum were involved, while implants were also found in appendice epiploicae.

Figure 2. — Omentum, uterus, adnexa, spleen and appendix.

Figure 3. — CK20 strong positivity of epithelium.

In such cases of multiple organ involvement, it is of a great importance to apply immunohistochemical methods of staining, mostly CK-7 and CK-20 which aid in differentiation of organs from which a mucoproducive lesion had arisen. Expression of CK-7 indicates that the lesion originates from ovarian tissue, while expression of CK20, i.e. membranous and cytoplasmatic positive reaction with antibodies to CK-20, indicates that the lesion originates from the digestive system [7]. In cases of pseudomyxoma peritonei syndrome maximal surgical reduction of tumor including total peritonectomy is performed. Additional intraperitoneal chemotherapy, which gives the best chances for success, is also administered. In such cases, appendectomy should always be performed, as the appendix in this syndrome is very often malignantly altered. From a histological point of view, several similar but different histological entities should be distinguished: disseminated peritoneal adenomucinosis (DPAM), peritoneal mucinous carcinomatosis (PMCA), and peritoneal mucinous carcinomatosis (PMCA-I/D) with a different microscopic appearance [8]. In our case, we performed maximal surgical reduction of the tumor, with remaining implants on the diaphragmatic domes and liver, as we did not have technical conditions to safely perform prolonged surgery which would have included a surgical procedure on the liver and administration of intraoperative chemotherapy. After completing parenteral chemotherapy and a control CT examination, second-look surgery should have been performed with the aim of reducing any eventual residual tumor tissue on the diaphragm domes and liver, as part of a protocol in such cases [9, 10]. The patient underwent six series of parenteral chemotherapy, but refused the second-look surgery. Even though our patient did not receive intraperitoneal chemotherapy and maximal surgical tumor reduction, she is still alive and without major complaints two years after the surgery. Moreover, control CT examinations have not shown any
signs of disease progression (peritoneal implants were the same in size and number, and no ascites was present). Regarding the fact that for our patient maximal tumor resection and second-look surgery were not performed, and that control CT showed no progression of the disease, while the general condition of the patient 24 months after the surgery was rather good, we expect that the prognosis of the disease in this case will correlate with the available references. Ronnett reports that the 5- and 10-year survival rates for all patients with carcinomatosis (PMCA-I/D+PMCA) are 26% and 9%, respectively. The mean and median survival times for all patients with carcinomatosis were 35 and 22 months [6].

References

Borderline clear cell adenofibroma of the ovary associated with ovarian endometriosis: a case report

Th. Vasilakaki¹, E. Skafida¹, E. Arkoumani¹, X. Grammatoglou¹, N. Firfiris², K. Manoloudaki²

¹Department of Pathology, Tzanion General Hospital of Piraeus
²Department of Anesthesiology, General Hospital of Larissa (Greece)

Summary

Clear cell tumours of the ovary are relatively uncommon. Most of them are clear cell carcinomas. Benign and borderline clear cell tumours are extremely rare and almost always fibromatous. We report a case of a 34-year-old woman. Ultrasound and computed tomography showed a right ovarian mass 8 cm in diameter. The patient underwent right salpingo-oophorectomy. Microscopic examination revealed an enlarged right ovary which was replaced by a tumour measuring 8 x 5.5 x 3 cm and the cut surfaces had a fine honeycomb appearance with cysts in different sizes embedded in firm stroma. Microscopic examination revealed glands and cysts that were different in size and shape within an abundant stromal component without evidence of stromal invasion. Many cysts and glands were lined by a single layer of flattened, cuboidal or hobnail cells with mild to moderate cytologic atypia and prominent nucleoli. Psammomatous calcifications were occasionally indentified. Features of endometriosis were also present adjacent to the tumour. Lesional cells were positive for Ker 7 and CA125. Staining for p53 was focally positive. Based on the above characteristic morphologic and immunohistochemical findings a diagnosis of borderline clear cell adenofibroma was made. The patient was free of recurrence four years after surgery.

Key words: Clear cell tumour; Ovary; Adenofibroma; Endometriosis.

Introduction

Ovarian adenofibromas consist of epithelial and stromal elements with the latter predominating. The epithelium is usually of the serous type. Adenofibromas with endometrioid, clear cell, mucinous and mixed epithelium are unusual [1, 2]. Benign and borderline clear cell adenofibromas are extremely rare. Clear cell adenofibromas of borderline malignancy constitute less than 1% of borderline tumours of the ovary and to date only a few cases have been reported in the world literature [3, 4].

Case Report

A 34-year-old woman presented at our hospital with a month history of lower abdominal pain. There was no family history of malignancy. Laboratory investigation including complete blood count, biochemical examination and tumour markers (CEA, CA19-9, CA125) were normal. Cervical cytology was negative. Ultrasound and abdominal and pelvic computed tomography showed a right ovarian mass 8 cm in diameter and the patient underwent right salpingo-oophorectomy. Gross examination revealed an enlarged right ovary which was replaced by a tumour measuring 8 x 5.5 x 3 cm and the cut surfaces had a fine honeycomb appearance with cysts in different sizes embedded in firm stroma. Microscopic examination revealed glands and cysts that were different in size and shape within an abundant stromal component without evidence of stromal invasion (Figure 1). The stromal component was predominantly fibrotic with an interlacing pattern. Foci of calcifications were occasional indentified. Many cysts and glands were lined by a single layer of flattened, cuboidal or hobnail cells with mild to moderate cytologic atypia and prominent nucleoli (Figures 2, 3, 4). In the tumour clear cells predominated but papillary structures were not seen. Vascular or lymphatic space invasion and necrosis were absent. Features of endometriosis were also present adjacent to the tumour (Figure 6). No significant pathologic findings were present in the fallopian tube.

Immunohistochemical study showed that the lesional cells were positive for Ker 7 and CA125 and negative for CEA and Ker 20 (Figure 5). Stain for p53 was focally positive. Based on the above characteristic morphologic and immunohistochemical findings a diagnosis of borderline clear cell adenofibroma was made. The patient was free of recurrence four years after surgery.

Discussion

Clear cell tumours of the ovary are relatively uncommon. Three main subtypes are recognised: clear cell adenofibroma, clear cell adenofibroma of borderline malignancy and clear cell carcinoma. Most clear cell tumours are clear cell carcinomas which comprise 2% to 3% of all epithelial ovarian neoplasms [3-5]. Borderline clear cell tumours are extremely rare and composed of glands or cysts lined by one or more layers of predominantly clear or hobnail cells showing moderate to marked nuclear atypia set in a dense fibrous stroma. The stromal component is cellular and resembles an ovarian fibroma. Mitotic activity (≤ 3 mitosis/HPF) may be found. Capsular stromal or lymphovascular invasion should always be absent [2-4, 6].

Patients with borderline clear cell tumours may have a similar non specific clinical presentation. They can be asymptomatic until the tumour grows to a certain size and can be palpable on routine examination as a pelvic mass or can be presenting with abdominal enlargement, pelvic pain and vaginal bleeding [2, 3].

Borderline clear cell adenofibromas can be distinguished from typical clear cell carcinomas based on...
Borderline clear cell adenofibroma of the ovary associated with ovarian endometriosis: a case report

Figure 1. — Borderline clear cell adenofibroma (H&E x 100).

Figure 2. — Tumour cells with clear or oxyphilic cytoplasm and moderate nuclear atypia (H&E x 100).

Figure 3. — Tumour cells with clear or oxyphilic cytoplasm and moderate nuclear atypia (H&E x 200).

Figure 4. — Foci of calcifications (H&E x 100).

Figure 5. — Lesional cells immunoreactive with Ker 7 (Ker 7 x 100).

Figure 6. — Features of endometriosis adjacent to the tumour (H&E x 100).
nuclear features of the epithelium and on the characteristics of the stroma. In a clear cell carcinoma the fibrous connective tissue is usually entirely non specific, does not resemble ovarian stroma and lacks an increased periglandular cellularity [2, 7].

With the exception of two cases borderline clear cell adenofibromatous tumours have a benign course following removal of the ovary [3, 4, 8].

Recently Momotani et al. reported a case of ovarian clear cell adenofibroma of borderline malignancy associated with high levels of CA19-9. Postoperatively the serum CA19-9 level decreased to the normal limit [9].

The pathogenesis of ovarian clear cell tumours has yet to be fully elucidated [2, 4].

Clear cell tumours of the ovary are frequently associated with ovarian endometriosis [2, 4, 10, 11]. It has been suggested that clear cell tumours develop from endometriosis but there has been little molecular evidence supporting this speculation [12, 13].

Recently ovarian clear cell tumours, including benign, borderline and malignant lesions showed immunohistochemical expression of hepatocyte nuclear factor-1beta (HNF-1beta) in the nucleus while other types of ovarian epithelial tumours (endometrioid, serous, mucinous and Brenner tumours) rarely expressed it [4, 13, 14]. HNF-1beta is also expressed in ovarian endometriosis of atypical type or of a reactive nature.

Early differentiation into the clear cell lineage takes place in the endometriotic epithelium, and clonal expansion of such cells is probably responsible for the development of clear cell tumours of the ovary [13].

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Address reprint requests to:
Th. VASILAKAKI, M.D,
5 Zappa Street
14565 St. Stephanos
Athens (Greece)
e-mail: thvasilakaki@yahoo.gr
Tumor of the mesosalpinx: case report of a female adnexal tumor of probable Wolffian origin

X. Tianmin¹, C. Weiqim¹, C. Mianhua¹, L. Xiaociu¹, G. Hongwen², Y. Min²

¹Department of Gynecology and Obstetrics, ²Department of Pathology, Second Hospital of Jilin University, Changchun City, Jilin (China)

Summary
We report a rare case of a 45-year-old woman who underwent laparoscopy for a right mesosalpinx mass. Pathologic examination showed a female adnexal tumor of probable Wolffian origin (FATWO). FATWO represents a rare gynecologic tumor and its clinical and pathological features are often ignored. Immunohistochemistry plays the most part in the diagnosis of FATWO. Through this report, we aimed to call attention to this disease in order to better understand the correct treatment and surgical possibilities, and to evaluate and perform the prognosis properly.

Key words: Tumor of the mesosalpinx; Female adnexal tumors of probable Wolffian origin (FATWO); Immunohistochemistry.

Introduction
Ovarian tumors are a common female tumor; however, a mesosalpinx tumor is relatively rare, and often misdiagnosed as an ovarian tumor. In most cases, it is only diagnosed definitely during surgery. Moreover, its clinical and pathological features, as well as management criteria, are often ignored. By reviewing the diagnosis and therapy process of a case of mesosalpinx tumor as well as the relative literature, the aim of the study was to call attention to this disease and to understand the correct treatment, and surgical possibilities, and to carefully follow-up.

Case Report
A 45-year-old patient with regular menstruation, gravida 1, para 1, had a physical exam five months before coming to our hospital. A pelvic cavity tumor was found and the ultrasonic diagnosis indicated that the size of tumor in the right adnexa area was about 3.0 x 2.5 cm, which was not treated. She had been rechecked in different hospitals several times, and the growth of tumor was not significant. Due to abdominal discomfort, she came to our hospital to be rechecked ten days prior, and the diagnosis indicated that the tumor had grown slightly and was solid. The patient was worried that the tumor might continue to grow. The gynecological examination results were: normal vulva, smooth vagina, soft, wet mucous membrane, a small amount of white secretion and free of any peculiar smell. The cervix was smooth and the anterior uterus was about 5 x 6 x 5 cm and free of tenderness. In the right adnexa, an approximately 5.0 x 3.0 cm solid mass could be touched, with a clear boundary, acceptable movement, and obvious tenderness. In the left adnexa area there was no obvious abnormality by touch. The color Doppler ultrasound reported anterior uterus normal in size, a clear uterine cavity line, inner membrane about 0.6 cm thick, and the uterine wall echo uneven.

In the right adnexa area, there was a 4.7 x 3.5 cm mixed echo and the shape was relatively regular and boundary relatively clear; the echo contained separations. There was no obvious mass in the left adnexa. Color Doppler flow imaging detected no abnormality. Serum tumor markers CA125, CA 19-9, CEA and AFP were all normal. The diagnosis was a right ovarian tumor. Laparoscopy exploration was carried out under anesthesia. During exploration, the uterus, fallopian tubes, and ovaries were macroscopically normal and there was no ascites. A solid mass of 4.0 x 3.0 x 3.0 cm was seen inside the right mesosalpinx; it was smooth, yellow and white, and fragile. After excising the mesosalpinx tumor completely, intraoperative fast-frozen pathological diagnosis was performed. In consideration of the adnexal tumor of Wolffian origin, excision of the right oviduct was also performed after consulting with the patient and her family. After surgery, the paraffin section report showed mesonephric remnants of tumor in the right mesosalpinx (Wolffian adnexal tumor). Results of immunohistochemical staining were: CK (AE1/AE3) (+), α-inhibin (+), vimentin (+), calretinin (+), PR (+), CD10 (–), CK7 (–), ER (–). (Figure 1 and 2/A-F).

Discussion
In 1973 Kariminejad and Scully described a series of neoplasms found in the uterine adnexa which they considered to be of probable Wolffian (mesonephric) duct origin and named them accordingly as female adnexal tumors of probable Wolffian origin (FATWO) [1]. In 2003, the World Health Organization officially nominated FATWO as a Wolffian adnexal tumor (WAT) [2]. There have been subsequent reports of similar tumors occurring in the ovary [3-5]. FATWO are very rare tumors. Most cases are benign but have the potential to recur and metastasize. There is limited knowledge about the optimal treatment for the neoplasms.

The Wolffian duct is the primordium of the urogenital system. It begins to develop into the primordial urogenital system in the fourth week of the embryo [6]. However, the female mesonephric duct degenerates gradually, and there are degenerated Wolffian remnants from the hilum of the ovary, along the mesosalpinx and uterine side, to the outer one-third of the vagina. The general situation is
that the tumors are mostly single sided and solid, the section is off white, hard feeling, there are occasionally bleeding necrotic areas, local calcification sometimes, and consequently a feeling of grittiness [7]. The ultrastructural features are the same as those of the Wolffian duct. The features under microscope are: cribriform pattern, forming of tubular cavities with different sizes and shapes, and sometimes capsules; gland tubules are arranged densely, forming densely arranged solid images, crooked gland tubules, branches are matched, and gland tubules covered with cubic or columnar epithelial cells. There is a diffused, solid cell mass, and the majority of tumor cells are free of nuclear atypia and karyoplastic phase [8]. Immunohistochemical staining shows CK7, CK19, inhibin, vimentin, calretinin, CD19 positive, and CMA negative [9, 10]. Pathologically, it can be differentiated from the supporting cell tumor, supporting interstitial cell tumor (dependent on pathological change of locations and shows hormone secretion symptoms), well differentiated adenocarcinoma of the endometrium (obvious cell atypia and nuclear mitotic figures, ER and PR positive), granulosa cell tumor (nuclear groove, cytoplasmic cavity, and endocrine changes), clear cell adenocarcinoma (solid area aggregated with clear cells, mastoid structure, obvious hobnail cells, and lumen mucilage), etc. In our case, under microscope, the tumor tissues closely formed a tubular structure with clear cytoplasm clear basilar membrane-like matter outside of the tubular wall and, the size of tumor cells was alike, regular round or oval nuclei, unobvious nucleolus visible, and rare nuclear mitotic figures. The possible hormone-dependency of FATWO has never been reported and only a single case of an estrogen and progesterone secreting FATWO has been reported [11].

The age of onset of the disease is 15-81 years old, and age 50 on average. The clinical features are: (a) free of obvious clinical symptoms; the majority of patients find pelvic cavity tumors during physical exams, and a minority have abdominal pain, abdominal swelling or abdominal tumor; (b) rare; in 1973, Kariminejad and Scully reported a case for the first time and since then about 50 cases have been reported in total [7]; (c) high rate of misdiagnosis; it is often misdiagnosed as an ovarian tumor; B-mode ultrasound is the regular inspection method, and there is no specific diagnostic method before operation; (d) for the majority of cases the biological behavior is benign with a positive prognosis; however, there is still a malign trend such as relapse, metastasis, etc.; in over 50 cases of WAT reported internationally, eight cases encountered relapse and metastasis, and two among them had metastasized when diagnosed [12, 13]; (e) for the therapy, conservative surgery is usually adopted (excision of oviduct/adnexa on the affected side). In our case, before surgery, the patient was misdiagnosed as having an ovarian tumor. Laparoscopy exploration was carried out and only after performing tumor excision and fast-frozen pathological diagnosis was the mesosalpinx adnexal tumor accurately diagnosed. Currently, postoperation fol-
low-up has been carried out for 15 months, and the patient has recovered well, and remains free of signs of relapse and metastasis.

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References

Malignant fibrous histiocytoma of the ovary: a case report

A. Stefanovic1,2, J. Stojnic1,2, K. Jeremic1,2, M. Jevtovic2, L. Arsenijevic2, N. Zecevic3
J. Atanackovic2

1Clinic of Obstetrics and Gynecology, Clinical Center of Serbia; 2Medical School, University of Belgrade; 3Clinic of Obstetrics and Gynecology “Narodni front”, Belgrade (Serbia)

Summary

Malignant fibrous histiocytoma (MFH) is the most common soft-tissue sarcoma of late adult life occurring predominantly in the extremities and the retroperitoneum. MFH of the ovary is very rare, with only six cases previously reported. A 67-year-old woman with a right pelvic tumor highly suspicious of ovarian carcinoma was submitted to exploratory laparotomy. Total abdominal hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, pelvic and paraaortic lymphadenectomy with right hemicolectomy were performed. Since a storiform-pleomorphic type of MFH was diagnosed from histopathological and immunohistochemical findings, chemotherapy was proposed as the postoperative treatment. Despite extensive surgery with negative surgical margins, the patient had recurrence of the tumor within four months, and was submitted to secondary surgery. A combination of chemo- and radiotherapy was performed postoperatively, but the patient developed respiratory problems and died one year later from the primary diagnosis.

Key words: Malignant fibrous histiocytoma; Ovary; Storiform-pleomorphic type; Prognosis.

Introduction

Malignant fibrous histiocytoma (MFH) is the most common type of soft tissue sarcoma (about 20-25%) in adults and tends to occur in the deep soft tissue of the extremities and retroperitoneum [1].

Malignant fibrous histiocytoma was first described as a separate entity in the category of soft tissue sarcomas by O’Brien and Stout as “malignant fibrous xanthoma” in 1964 [2]. The origin of the tumor cells is still unclear and a matter of ongoing debate, but the term is reserved for a small number of undifferentiated high-grade pleomorphic sarcomas. MFH is very diverse with five distinct subtypes: storiform-pleomorphic, myxoid, inflammatory, giant cell and angiomatoid [3].

Primary MFH of the ovary is extremely rare, with only six previously reported cases [4].

The management of MFH is controversial because of the heterogeneous nature of the disease. Surgical resection of all macroscopic disease is independently associated with improved disease-specific survival, and adjuvant chemotherapy and radiation could be acceptable alternatives if the surgical margins are tumor-free [4].

The prognosis is usually poor in the cases of intraabdominal and ovarian localization because the tumor is usually diagnosed in advanced stage, with a high percentage of local recurrences and systemic metastatic disease with surgical therapy as the only reliable method [5].

We report an usual case of a woman with a right ovarian tumor infiltrating the ileum and cecum, diagnosed after exploratory laparotomy as MFH.

Case Report

A 67-year-old, gravida 4, para 1, abortus 3 woman presented with abdominal distention and pain, and mild anemia together with a suspicious right adnexal and iliac mass. She was referred to our Oncology Unit in November 2005. The complaints had started two months before and had aggravated gradually, especially the abdominal pain and distention. Her personal medical history revealed arterial hypertension and chronic compensatory myocardopathy. Her past surgical history included operative resection of an uterine leiomyoma 30 years before.

A 11.5 x 5.5 x 6.5 cm, solid, heterogeneous mass with irregular margins was discovered in the right adnexal region with a small amount of ascites seen on sonography. A 12 x 5.5 x 6.8 cm, lobulated heterogeneous mass in the right adnexa was found infiltrating the ileum, cecum and ascending colon with suspected breakthrough into the cecal lumen, but no tumor implantation on the peritoneum and omentum was detected by computed tomography (CT) scan.

The serum level of CA 125 was 77.2 IU/ml (0-35 IU/ml), while other markers - CEA, CA 19-9, CA 15-3, and alpha feto protein were within reference range. Laboratory tests revealed moderate anemia with a normal platelet count (361000/mm3) and white blood cell count of 113000/mm3; all the other parameters were normal. The RTG scan of the thorax showed no pathological findings, whereas abdominal ultrasound (US) plus pelvic pathology detected a cystic formation with a diameter of 10 mm in the right hepatic section.

The initial diagnosis was suspicious for advanced right ovarian carcinoma. The patient underwent exploratory laparotomy due to a right adnexal and retrouterine mass highly suspicious of malignancy. Exploratory laparotomy was performed and a large, solid, lobulated, necrotic, grey-yellowish tumor about 120 mm in the largest diameter was found occupying the right adnexal and iliac space infiltrating the cecum and ascending colon, approximately 160 mm in length, and the uterine surface with a small amount of ascites. The left ovary and the left uterine tube were normal and all seemed completely independent of the mass. On gross examination the surface of the liver, spleen, and peri-
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The peritoneal surface was without implants or deposits. There was about 100 ml of serous ascetic fluid, and specimens of peritoneal washings for cytology were also taken.

Total abdominal hysterectomy, bilateral salpingo-oophorectomy, total omentectomy, pelvic and paraaortic lymphadenectomy with right hemicolecotomy along with permanent cutaneous ileostomy were performed.

The histopathological examination of the specimen revealed highly undifferentiated sarcoma or carcinoma sarcoma of the right ovary highly resembling MFH. In part of the ileum, the tumor was infiltrating the whole diameter of the bowel wall, with subsequent penetration into the lumen (from the serosa layer to mucosal layer). All 11/11 iliae lymphatic nodes were negative.

The histopathological report showed a poorly differentiated almost anaplastic solid tumor, with high mitotic activity (63/10 HPF) and atypical mitoses, together with increased cellularity and cytological pleomorphism. A heterogeneous growth pattern consisted predominantly of an epitheloid arrangement of large pleomorphic cells with eosinophilic cytoplasm and multinucleated, hyperchromatic nuclei, together with areas of atypical spindle shaped cells in a storiform growth pattern admixed with the giant cells (Figure 1).

Nuclear pleomorphism was evident, and giant multinuclear tumor cells with a bizarre appearance were scattered throughout the tumor (Figure 2). An inflammatory component was evident around the tumor, with persistent lymphovascular space invasion in perineural spaces. Tumor necrosis was a common finding in the specimen.

Additional immunohistological staining was warranted to obtain a definitive histopathological diagnosis. Positive staining was recorded for EMA (+), vimentin (+), CD 68 (+), S-100 (+) only in intertriginous cells, CD 3 (+), inflammatory component, while other markers were negative (AE 1/AE 3 (-), CK 7 (-), CK 20 (-), CD 34 (-), CD 117 (-), SMA (-), desmin 8 (-), CD10 (-), CD 20 (-), CD 21 (-), CD 30 (-), CD 35 (-) and Ki index 55%).

The definitive pathology diagnosis showed a pleomorphic undifferentiated sarcoma involving the right adnexa with no normal ovarian tissue identified. The staining pattern was consistent with a pleomorphic sarcoma (vimentin (+), S-100 (+), CD 34 (-) and suggestive of ovarian stromal origin CD 56 (+). The definitive histopathological diagnosis showed a storiform pleomorphic MFH with inflammatory component. The Coindre score was 8/9 with a G-3 degree of histological malignancy.

The postoperative course was expected to have no complications. Three months following the first laparotomy, while receiving chemotherapy (gemcitabine and docetaxel), the patient complained of bloody vaginal discharge, abdominal distention with pain predominantly in the epigastrium, and fatigue. An 86 x 70 mm, irregular mass, predominantly cystic in the right pelvic cavity was diagnosed by US. In the right iliac region there was a hetero-dense mass 7 x 5 cm with predominantly expansive growth and close contact with the anterior wall of the rectum and posterior wall of the urinary bladder; no susceptible enlarged lymph nodes were identified by CT scans of the pelvis and abdomen. Recurrence of the tumor in the pelvis and abdomen was treated surgically.

Complete resection of the mass and small bowel (15 cm in length) and terminal end-to-end bowel anastomosis were performed. Postoperative chemotherapy with subsequent radiotherapy was administered. A year after the initial diagnosis was established the patient experienced respiratory difficulties and intermittent chest pain, and subsequently was diagnosed with widespread lung metastasis. Unfortunately, despite aggressive treatment which included two surgeries and adjuvant therapy, the patient died of progressive disease two months later.

Discussion

Today the term malignant fibrous histiocytoma is reserved for a small number of undifferentiated high-grade pleomorphic sarcomas [6]. It accounts for about 20-25% of soft tissue sarcomas, occurring most commonly in the lower extremities (70-75%), followed by the
retroperitoneum in males over 40 years of age (peaking in the 5th and 6th decades). Histogenesis of the tumor is still uncertain and remains controversial. It is thought to originate from undifferentiated primitive mesenchyme cells which are capable of multidirectional differentiation [7, 8].

Occurrence of the tumor has been reported in almost all parts of the body including the ovaries. These sarcomas have rarely been documented in the lung, kidney, bladder, stomach, small intestines, ovaries, liver and other soft tissues [9]. The ovary as a primary site of MFH is very rare with only six cases previously reported, including all five subtypes. Intraabdominal MFH is rare, as is MFH of the Mullerian tract, though cases involving the vagina and paravaginal space have been described [10].

The clinical manifestations are dependent on size of the tumor and are very unspecific. The lesions can grow to a large size due to their intraabdominal (retroperitoneal) location before the onset of symptoms which contributes to the delay in diagnosis of the disease. Preoperative diagnosis of MFH is very difficult and additionally, even at laparotomy, is hardly possible. In most cases the definitive diagnosis is histopathological or immunohistohemical. MHF has no specific or characteristic finding on US or CT scans and MRI. Also, no tumor markers are useful or specific in the case of MFH. Therefore, the pathological diagnosis is a necessity [11]. Pathological diagnosis of soft tissue sarcoma is occasionally difficult as in our case and immunohistochimistry must be employed together with the clinical findings at laparotomy. The initial diagnosis of all six cases of primary ovarian MFH was ovarian carcinoma. Two of the reported cases showed arising MFH from a benign dermoid cyst and one case was associated with an appendicular carcinoid lesion [10]. Surgery was a basic treatment for all cases with additional chemotherapy in four of six cases including cisplatinum, cyclophosphamide, gemcitabine, and docetaxel.

MFH is an aggressive tumor with a high potential of demonstrating metastases to other body parts and with high rates of local recurrence. Metastatic rate varies with histologic subtype: storiform/pleomorphic (20-65%), giant cell (50%), myxoid (23-30%) and inflammatory (25-30%). The incidence of local recurrence of all soft tissue sarcomas except myxofibrosarcoma is reported to be 40% [12]. Patients with MFH have had poor outcomes because of high affinity for local recurrence and hematogenous spread [13].

The American Joint Committee on cancer staging system (in the absence of metastatic disease) uses the histologic grade to define stage, with additional contributions from tumor size and depth. High-grade histology for soft tissue sarcoma is connected with negative prognostic factors for those patients, regardless of the grading system. Patients with high-grade tumors with poor differentiation, cellular pleomorphism, coagulative necrosis, and numerous bizarre mitoses are at considerable risk for metastatic disease, and as many as 50% of these patients die from the disease [1].

Tumor grade, as in most soft tissue sarcoma subtypes, predicts the risk of developing distant metastases, but not local failure. Mortality is associated with histological tumor grade, but also to quality of surgical margins [13]. Radical surgical treatment is still the only therapy with curing possibilities. In most cases resection must be extended to the adjacent organs as well, in order to guarantee radical removal. The primary standard therapy is complete excision with a tumor-free resection margin if possible. Adjuvant treatment with radiotherapy and chemotherapy are brought into question [14].

When surgical resection was the only treating tool, 42% of 200 cases of MFH developed metastases within two years involving the lungs (82%), lymph nodes (32%), liver (15%) or bone (15%), with a 2-year survival rate of 60%. The rate of local recurrence is 44% [15]. The 5-year survival rate after undergoing surgery is 67.2% in contrast to 14% with a 5-year survival rate of patients with abdominal MFH [16].

Pezzi et al. in 1992 [17] reported a 5-year disease-free survival of 50.6% among a series of 227 patients who received only surgery (26%) or a combination of surgery with radiation therapy (73%). Tumor size and histological grade are the most important prognostic signs for MHF. The five-year survival rate was reported as 82% if the primary tumor was smaller than 5 cm, while if it was larger than 5 cm to 10 cm, the overall survival was 68%.

The Soft Tissue and Bone Sarcoma Group of the European Organization for Research and Treatment of Cancer has been investigating for more than two decades the role of different chemotherapy protocols for advanced and metastatic soft tissue sarcomas including MHF. The conclusion is that the most active single agent is doxorubicin with response rates of 20-25% and with no multi-agent regimen yet proven superior in survival [18, 19].

Factors predictive of poor outcomes in MFH are proposed to be high-tumor grade, tumor size more than 10 cm, the presence of tissue necrosis on histological examination, identification of 19p chromosomal aberrations and expression of proliferating cell nuclear antigen [10, 20]. Two reports stated that generally large tumor size (more than 5 cm for non-myxoid types, and more than 10 cm myxoid), high grade, deep infiltration beyond the subcutaneous layer and positive resection margins are all poor prognostic factors [5, 15].

In general, prognosis is poor with a 60% survival rate after two years and a recurrence rate of 50-82% in cases of retroperitoneal MFH. The most frequent sites of metastatic spread are the lungs, liver, bone and bone marrow [21]. The number of reported cases of MFH of the ovary is insufficient to form any therapy protocols and references regarding prognosis, thus the only available data concern MHF of other localizations in the body.

Complete surgery with negative resection margins is the treatment of choice in cases of MFH of ovarian origin. As postoperative supplementary treatment, radiation therapy and chemotherapy were involved. Since there are no sufficient reports regarding supplementary therapy for MFH of intraabdominal localization, further investigations are necessary.
References


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Address reprint requests to:
A. STEFANOVIC, M.D.
Belgrade Medical School
Clinic for Gynecology and Obstetrics
Visegradska 26
11000 Belgrade (Serbia)
e-mail: ststefan@eunet.rs
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All questions concerning the Academy may be sent to:
PETER BOSZE, M.D. - P.O. Box 46 - Budapest 1301 (Hungary)
Phone: +36 1 4290317 - Fax: +36 1 2752172 - E-mail: eagc@cme.hu

www.cme.hu

Administrative Office:
1301 Budapest, P.O. Box 46 - Hungary
Fax (36 1) 4290318 - E-mail: eagc@cme.hu