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Terminologia colposcopica: a personal perspective

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Introduction
According to the definitions of medical dictionaries [1, 2],
– terminology is a) science dealing with construction, investigation and arrangement of terms; b) vocabulary of a science or art;
– term is a word or combination of words commonly used to designate a specific entity;
– nomenclature is a classified system of terms and/or names of a specific field of science or organisms etc., e.g. binominal nomenclature (scientific classification of living organisms).
The anatomical nomenclature (nomina anatomica) is a classification formerly applied to anatomical terms but it has been superseded by terminologia anatomica; also called International Anatomical Terminology.
The mission of the Nomenclature Committee of the International Federation of Cervical Pathology and Colposcopy (IFCPC), as its name implies, is to classify colposcopic terms. The Nomenclature Committee has published the International Terminology of Colposcopy several times, with slight modifications in each. However, this terminology consists of not only the arrangements (classification) of colposcopic terms (nomenclature of colposcopy), but their definition and interpretation (terminology of colposcopy) as well as, i.e., its objectives are the same as that of the International Anatomical Terminology. Therefore one may wonder if the International Terminology of Colposcopy might be called “Terminologia Colposcopica”.
The aim of the terminologia colposcopica is to guide physicians in integrating colposcopy in identifying, screening, grading and managing lesions of the uterine cervix and vagina, and occasionally those of the vulva.

Definition of colposcopy
The colposcope is a binocular instrument used to study human tissue in vivo with magnification ranging from x5 to x25. This allows recognition of tissue changes not visible to the naked eye. The colposcope was devised by Hinselmann in Germany as an instrument applicable for vaginal examination, hence the name (colpos-vagina), to improve the visualisation of the vulva, vagina and uterine cervix [3]. Colposcopy per se is the recognition of colposcopic features, i.e., colposcopic patterns, signs (see below) and thereby detecting mucosal abnormalities.

Objectives of colposcopy
– To identify and reassure women with normal epithelium;
– to detect lesions (HPV infection, precancer, etc.);
– to be an important triage tool in the assessment of cervical abnormalities and/or high-risk HPV infections;
– to indicate and tailor biopsy or excision procedures;
– to exclude invasive cancer;
– to follow-up women treated for cervical and vaginal disease.
The objectives differ whether the colposcope is used in a triage setting (referral colposcopy) or routinely (integrated in the gynaecological exam), which is called routine colposcopy. In the former, the role of colposcopy is to serve as a guide for histological biopsy or to assess the severity of the lesion in the ‘see and treat’ policy [4]. In the latter, colposcopy is used to detect epithelial abnormalities with the potential of using colposcopy as a screening tool [5]. The screening potential of colposcopy is reflected in the concept of cervicography. Whichever the objective is, it is essential to understand the low sensitivity of colposcopy in distinguishing a) low-grade precursors from squamous cell metaplasia or HPV infection, b) CIN1 from CIN2 or small CIN3. Some colposcopic features (coarse patterns and signs), however, are highly suggestive of high-grade lesions or cancer (see below).

Referral colposcopy mostly includes biopsy tailored by the colposcope (colposcopically directed biopsy), but by definition, colposcopy per se does not involve biopsy.

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Assessment of colposcopy examination

Colposcopic examination can be:

- **satisfactory:** assessment of the cervix and the full transformation zone (TZ) is complete;
- **limited:** the cervix is assessable but the TZ is visualised only in part;
- **unsatisfactory:** the cervix is not visible, or severe inflammation, trauma, etc. precludes a full assessment of the uterine cervix.

Terms ‘adequate’ and ‘inadequate’ instead of satisfactory/unsatisfactory are also used in the literature, having the same meaning. However, the latter (old terms) might be preferred because a) they are widely used and familiar to clinicians, and b) they are included in the latest IFCPC terminology.

Colposcopy does have clinical implications even when the entire TZ cannot be assessed (limited colposcopy), particularly in routine colposcopy [6, 7]. The most important perhaps is reduction of the false-negative rate of cytology: the association of high-grade colposcopic features on the assessable part of the ectocervix with negative cytology is highly suggestive of false-negative cytology and invariably requires histology, i.e., cone biopsy or loop excision. In contrast, when negative cytology is associated with normal colposcopic findings even if the TZ is only partially visible, the risk of false-negative cytology is negligible and the woman can be assured confidently. In the presence of high-grade cytological findings (triage setting), colposcopically directed biopsy (punch biopsy) is not satisfactory unless the entire TZ is assessable. In this setting, if the TZ is not fully visible, one of the excision techniques (loop, cone biopsy) is indicated, which is tailored by the colposcopic finding, e.g., if there is no abnormality on the assessable TZ, high cone biopsy is needed as the lesion is in the endocervical canal and vice versa.

Specifying terms such as ‘limited colposcopy with normal ectocervix’ or ‘limited colposcopy with abnormal findings (with details)’ might be useful in reporting the results of colposcopic examination in such cases.

**Colposcopic Glossary**

*Definitions of colposcopic terms*

**Acetowhiteness**

Whitening following application of 3-5% acetic acid is a common phenomenon but its intensity, appearance and duration are different. The acetowhiteness is mainly due to transient denaturation of cellular proteins (nucleoproteins, cytokeratins) by the acetic acid (a non-coagulant fixative) [8, 9], which cross-links protein molecules and transiently converts the cytoplasm into an insoluble gel (transient dehydration), with preservation of the cell organelles [10, 11]. Following fixation of their nucleoproteins, cells become opaque. When rehydration of the cytoplasm occurs, nucleoproteins revert to their normal state [10].

This acetowhiteness depends on the number of epithelial cells and the amount of their cytoplasm and nuclear proteins (nuclear-to-cytoplasmic ratio). However, it is mostly proportioned to the amount of the nucleoproteins (nuclear density) precipitated by the acetic acid. The larger the amount of these nucleoproteins (e.g., numerous cells with large nuclei and small cytoplasm in the upper layers of the epithelium), the less the distance that the light penetrates the epithelium and the more intense is the whitening, which is also faster in appearance and lasts longer [11]. An increased amount of nucleoproteins is found in cancer, its precursors and immature squamous cells (metaplasia) as well as in the presence of viral DNA in the cells. In high-grade CIN, not only the number of cells with large nuclei and a small amount of cytoplasm is increased (high nuclear-cytoplasm ratio, high nuclear density) but there are extra proteins in the nuclei (polyplody, aneuploidy), explaining the intense acetowhiteness. Whitening may also occur in inflammation but it is not characteristic.

The acetic acid causes intra- and extracellular changes throughout the epithelium and even to a minor extent, also in the stroma of normal squamous epithelium. However, the cells remain relatively transparent, allowing visualisation of the vessels in the stroma, hence the pink color (MacLean). The single-layer columnar epithelium remains transparent and therefore pink-red after acetic acid application. In atrophic epithelium (postmenopausal women), the whitening is due to the vessels in the stroma, hence the pink colour (MacLean). The single-layer columnar epithelium remains transparent, allowing visualisation of the vessels in the stroma of normal squamous epithelium. However, the cells remain relatively transparent, allowing visualisation of the vessels in the stroma, hence the pink color (MacLean). The single-layer columnar epithelium remains transparent and therefore pink-red after acetic acid application.

The extent of light penetration in CIN3 is only in the superficial third of the dysplastic epithelium from where the light is reflected without reaching the stroma; consequently, the pink color changes to white (MacLean). The distance that the light penetrates the epithelium in CIN1 is longer than in CIN3, which also explains why the acetowhiteness is slower to appear, less intense and fades rapidly in low-grade lesions. Unlike CIN1, the whitening is more prominent in high-grade precursors as compared to metaplasia. The difference in whitening between CIN2 and CIN3 is indistinct.

Parameters of acetowhite changes include:

- intensity (pale, moderate, intense) and uniformity (homo- or heterogenous);
- rapidity of maximum white changing (slow [may require frequent application of acetic acid], quick);
- the length of time the epithelium retains its white appearance (short in duration, requires several applications to maintain colour change, moderate, long lasting).
Acetowhite epithelium

Acetowhite epithelium is a common term for tissues, which appear white following acetic acid application → acetowhitenning.

Atrophic epithelium

Atrophic epithelium is thin and consists mainly of parabasal cells (small cells with larger nuclei) with few intermediate cells and a single basal cell layer. Subepithelial vascularity is decreased. Due to its relatively higher nuclear density, more light is reflected than from the mature squamous epithelium, and because of its poor vascularity, the atrophic epithelium is less pink or rather gray, and its colour does not change following acetic acid application. As the parabasal cells contain less glycogen, the atrophic epithelium is usually iodine negative. The vessels are near the surface (thin epithelium), hence they are vulnerable and bleed easily.

Atypical vessels

Intrusion of the cancer cells into the stroma is invariably associated with accelerated cell proliferation and continuous unregulated production of angiogenic factors, resulting in an influx of more vessels to sustain cancer growth and expansion [12]. This in turn leads to irregular and forced vascular growth with newly formed vessels that have lost their consistent branching pattern because they are not capable of keeping up with the cell proliferation. These abnormally developed, non-branching vessels are called atypical vessels. Unlike normal vessels (finely branching capillaries), atypical vessels can paradoxically increase in size when they divide [13, 14], have abrupt or interrupted branching, and they may appear and disappear abruptly, i.e., they are irregular in width, shape and course. Their appearances vary considerably: commas, noodle-like, root-like, corkscrew, glyphs (like a pictogram), etc.

Border shape

Borders, margins, and edges are all synonyms that are used alternatively in the literature. Border shape depicts the border (interface) between two different tissues, and appears as a line (borderline or line of demarcation) through the colposcope. The borders can be inconspicuous (ill-defined, almost imperceptible – indistinct); distinct (easily recognisable – markedly distinct, well-defined); sharp; rolled (rolled edge); irregular (geographic); line-like (distinct and regular like a line around the lesion); sharp or strait. Inconspicuous margins, which are mostly geographic, usually but not invariably indicate minor changes, whereas line-like, sharp margins are suggestive of major changes. A marked rolled edge is characteristic of endophytic cancer and ulcer. A border may also appear within a lesion, which is called inner border, and most often occurs between high- and low-grade cervical intraepithelial neoplasia in the TZ.

Cervicalisation

The term cervicalisation implies the process of eversion of the endocervical lining canal onto the ectocervix. This term occurred in the old literature – it is no longer in use and has been replaced by ectopy → ectopy.

Cervicography

Pictures of the cervix (cervicograms) taken following acetic acid application by e.g., trained nurses (non-colposcopists) with a special camera (cerviscope) developed by Stafl in 1981 [15]. Cervicograms are sent for evaluation to expert colposcopists with feedback reports, including recommendations. Cervicography utilises the same principles as colposcopy and is used for screening cervical carcinoma. Its terminology is slightly different from that of colposcopy.

Cleft (crypt) openings

Colour tone sign

Colour tone sign depicts the colour of a colposcopic pattern and colour changes after acetic acid and Lugol solution application. Normal tissue patterns are pink, red with variation in tone, while the atrophic epithelium may be greyish. A red colour may also be due to haemorrhage. White lesions (leukoplakia) are mostly caused by hyperkeratosis. A dull oyster grey colour may result from microinvasion, while a yellow appearance commonly indicates necrosis. As for colour changing, see: acetowhitenning, acetowhite epithelium, iodine staining.

Colposcopic features

These are characteristic, special colposcopic findings important in practising colposcopy, i.e., in distinguishing normal and abnormal tissues to diagnose, screen, grade and localise lesions. Colposcopic features consist of colposcopic patterns (→) and signs (→) and comprise two tissue elements: a) the epithelium (thickness, architecture and density [content of nuclei]), b) composition and growth of the stroma (vessel formation, etc.). Each colposcopic feature is the summation and configuration of these morphological elements (Table 1).
Colposcopic findings
These are actually what are seen through the colposcope.

Colposcopic grading
Colposcopic grading implies grading the colposcopic patterns and signs, which has nothing to do with grading of the underlying lesion. However, with grading of colposcopic findings, one can suspect but not definitely diagnose the degree of abnormality → colposcopic indices.

Colposcopic indices
Colposcopic indices are scoring systems, assigning points to the variable colposcopic patterns and signs to overcome the subjective nature of colposcopy by standardising the colposcopic findings (e.g., Reid’s colposcopic index/colposcopic score). Related expressions: quantification of colposcopic images and grading of abnormal colposcopic findings.

Colposcopic patterns
These are characteristic individual colposcopic features, having special tissue basis (Table 1).

Colposcopic signs
These are common colposcopic features occurring in all colposcopic patterns (Table 1), i.e., they are not individual colposcopic features, rather part of them. Colposcopic signs are important additional features to colposcopic patterns in decision making.

Colposcopic variables
Less commonly used term for colposcopic features.

Congenital transformation zone (CTZ)
The CTZ is deviant squamous metaplasia histologically characterised by irregular epithelial projections into the stroma (dentate epithelial-stromal margin) with occasional keratinisation [16]. Its origin is obscure; it might be due to disordered squamous metaplastic maturation during late foetal life, with full or even excessive maturation in the surface and arrested maturation (immature metaplastic epithelium) in the deeper layers [16]. Slight keratosis or parakeratosis may occur (leukoplakia). CTZ is mostly seen in young women, indicating that full squamous cell maturation will finally take place later in life.

Colposcopically, CTZ presents with the same features as low-grade cervical intraepithelial neoplasia (acetowhite, punctation, mosaic, etc.) and is characteristically localised around the active TZ, occasionally extending to the vagina. Whether it is around the TZ or forms the distal circular part of it, is a matter of consideration. Interestingly, although it may be more intensely acetowhite than high-grade CIN, the maximum white change after acetic acid develops slowly. However, the whiteness is retained for a longer time as compared to high-grade CIN, i.e., CTZ may still be white when the CIN is fading [17]. Unlike CIN, it might also stain weakly with Lugol’s solution due to the incompletely glycogenated metaplastic cells, albeit the CTZ is iodine-negative in most cases.

Crypt openings → openings
Cuff (abnormal) openings → openings

Deciduosis
Deciduosis is “the presence of decidual tissue or of tissue resembling the endometrium of pregnancy in an ectopic site” [1]. In colposcopy, the term deciduosis comprises the changes of the uterine cervix (and vagina) during pregnancy. The most significant changes are:
– increased vascularity and interstitial fluid retention, resulting in cervical enlargement and softening;
– stromal (fibroblast) decidualisation, which is mostly focal and may appear as a plaque or pseudopolyp on the surface [18];
– enlargement and eversion of the glandular epithelium with increased mucus production and hypertrophy of villi, leading to an irregular surface;
– active (marked) squamous metaplasia, which mostly becomes apparent from the end of the first trimester [19].

Colposcopic findings include:
– enlarged TZ with irregular surface (course texture and deep in-foldings), polypoid appearance, decidual polyps and prominent gland openings (doughnut rims);
– accentuated capillary vessel patterns;
– acetowhiteness of squamous metaplasia;
– adherent mucus, making colposcopic examination difficult;
plush, purplish ectocervix, with dense iodine uptake [17];
gaping of the external os.
Due to progressive eversion of the endocervical mucosa, the new squamocolumnar junction moves downwards
towards the ectocervix during pregnancy. As a result, type 2, or even type 3 transformation zone may become type 1 in
the third trimester → transformation zone.

**Ectopy**

Ectopy is defined as columnar epithelium extending onto the ectocervix (synonyms: ectopia, cervical ectropion, cer-

**Erosion and ulceration**

Erosion is epithelial surface distraction whereby the underlying normal stroma (connective tissue) becomes visible. An ulcer is a local defect or excavation of the surface epithelium and the underlying stroma. Ulceration is the process of developing an ulcer.

In the past, the term erosion was used to denote ectopic columnar epithelium on the cervical surface. This is erro-

**Erythroplakia**

Erythroplakia is an out-of-date term formerly used for the TZ → transformation zone.

**Extension (localisation)**

Extension depicts the localisation of any lesion including various forms of HPV infection, etc. On the uterine cervix, the lesion can be within or outside the TZ, can overgrow the TZ and/or extend to the vagina. High-grade lesions tend to locate next to the neo-squamocolumnar junction. In the vagina, the lesion can reside on the anterior, posterior, or lat-

**Glandular (gland) openings → openings**

**Hyperkeratosis → keratosis**

**Inner border**

This a border between two abnormalities within one lesion → border shape.

**Intercapillary distance**

The distance between the punctuated dots in punctuation → punctuation.

**Iodine staining (iodine negativity)**

The application of Lugol’s solution/Lugol’s iodine is the iodine test, also called Schiller’s test. Only the cells rich in glycogen will take up iodine and stain deep brown. After Lugol’s iodine application, the squamous epithelium is dark brown in contrast to metaplasia that stains pale or partially depending on its maturation state; the more mature metaplasia stains the browner it becomes (partial iodine-positivity). It may have a speckled appearance. Columnar cells and epithelial abnormalities (precursor lesions, cancer) lack glycogen and remain unstained (iodine-negative). Atrophic epithelium takes up iodine and stains poorly.

Iodine negativity is a colposcopic term – part of the colour tone sign – which indicates no or minimal staining with Lugol’s iodine.

**Keratosis**

In the normal squamous epithelium covering the cervix and vagina, there are no keratinised cells in the superficial
layer, but this epithelium has the potential of developing keratinised cells as a protective mechanism under chronic stim-
ulation, e.g., uterine prolapse, chronic infection, etc. This is an abnormal differentiation of the covering epithelium and
because normally this stratified squamous epithelium lacks keratinised cells, it is called hyperkeratosis regardless of
thickness of keratinized cell layers. By definition, however, hyperkeratosis implies excessive formation of keratin (i.e.,
heavily keratinised squamous cells, squames, devoid of nuclei) covering the superficial epithelial layers. Hyperkeratosis
is usually associated with increasing thickness of the epithelium (acanthosis), which is another protective mechanism. Parakeratosis is also a protective reaction of the stratified squamous epithelium and is defined by the presence of layers of small keratinised squamous cells with small, pyknotic and hyperchromatic nuclei on the top of the superficial squamous cells. Albeit both hyper- and parakeratosis are protective phenomena associated with differentiation of the normal mature epithelium, it is not unusual that such epithelium covers abnormal lesions, including cancer.

Lesion size

The lesions may be small, moderate or large. Metric measurement for size does not seem appropriate as the lesion’s size is always proportional to the size of the uterine cervix. For this reason, a measure as percentage (< 25%, 25-50%, > 50%) of the visible cervical surface has been proposed. Large lesions usually cover multiple quadrants of the cervix, however, small lesions around the cervical os may also occupy even four quadrants, making measurement in quadrants inappropriate. Vaginal/vulvar lesions can be measured in centimetres.

Leukoplakia

Leukoplakia is defined as a dense white, elevated area (plaque) of the mucosal surface visible by the naked eye without magnification or acetic acid accentuation. It is often called hyperkeratosis. Acetic acid does not alter its appearance and it does not stain with Lugol’s iodine. Its surface can be smooth and glossy, rough or irregular. Leukoplakia is due to pronounced keratosis (hyper- and/or parakeratosis) which precludes visualisation of the underlying tissue. Leukoplakia can be caused by neoplastic changes, chronic irritation and reactive changes of the overlying normal epithelium, e.g., uterine prolapse → keratosis.

Location of the lesions → extension (localisation)

Lugol’solution/iodine → iodine staining (iodine negativity)

Metaplasia

In colposcopy, metaplasia is the process of replacement of the columnar cells by metaplastic cells, which differentiate into mature squamous cells (squamous metaplasia) in the TZ during adolescence, reproductive age and frequently the late foetal life. The process is relatively quick – mostly measured in days [12]. This is the most common protective mechanism of endocervical mucosa, physiologically initiated and promoted by the stimuli of the hostile vaginal environment (high acidity) to the columnar epithelium outside the cervical os. Other chronic stimuli (chemical, inflammation, hormonal, etc.) and destructive treatment (electrocautery, etc.) can also induce metaplasia [20]. The process is irreversible, and the evolving metaplastic epithelium cannot revert to columnar cells.

Squamous metaplasia starts from the proliferating reserve cells beneath the columnar epithelium (known as reserve cell hyperplasia [several layers of reserve cells]), tending to commence at the neo-SCJ junction. Reserve cell hyperplasia progresses to immature metaplasia and gradually with continuous maturation, end up with a mature squamous epithelium. The maturation of metaplastic squamous epithelium can be divided into three stages (pallor, attachment and fusion of the villi; stage 1-3) [21], but this distinction has no clinical significance. The process can be and mostly is arrested at any stage. Because of this, the colposcopic appearance of various stages is visible at the same time, and the maturation may resume at any time [20]. Metaplasia mostly takes place on the surface of the villi but in the crypts as well → metaplastic epithelium, columnar epithelium.

Metaplastic epithelium

Metaplastic epithelium constitutes a range from immature to mature squamous cells, normally situated exclusively in the TZ. These metaplastic cells have larger nuclei than columnar or mature squamous epithelial cells and their nuclei are uniform. The immature epithelium comprises few cell layers, which grow over time. At colposcopy, the surface of the metaplastic epithelium is smooth. Acetic acid application causes slight whitening, varying with the maturation of the metaplastic epithelium. The least mature epithelium demonstrates the most prominent whitening, mostly at the neo-SCJ, resulting in a distinct margin between columnar and metaplastic epithelium. However, the colouration is still weak and usually requires several acetic acid applications to become visualised [12]. The whitening of metaplasia is translucent, flocculent or snow white. Metaplastic epithelium stains either negative or only partially positive with Lugol’s iodine, and the colouration parallels its maturation state; the more mature being more brown → iodine staining (iodine negativity).

Feeding vessels supplying epithelial maturation occasionally grow from the stroma into metaplastic epithelium to maintain the blood supply of the proliferating cells. These capillary loops may appear as fine, uniform punctation at colposcopy. Some of the fine vessel loops may branch or grow laterally, causing fine mosaic patterns.
Mosaic
Mosaic is a vascular (colposcopic) pattern resulting from increased vessel formation with arborisation and/or laterally growing vessels that coalesce, surrounding and isolating surface epithelial cells into individual nests in a mosaic-like fashion (rectangular pattern). This is a progression from punctation due to further cell proliferation and production of angiogenic factors (→ punctation) [12]. Consequently, mosaic is commonly seen together with punctation, dots being adjacent to or within the mosaic area.

Mosaic patterns are categorised as fine and coarse. Fine mosaic is characterised by small, more or less regular nests with uniform surrounding vessels, smooth surface and inconspicuous border. In coarse mosaic, the nests are large and irregular in size and shape with non-uniform surrounding vessels. The surface is usually uneven with sharp borders. Mosaic lesions become acetowhite following acetic acid application, but, as with punctation, fine vascular changes are mainly transient, fading quickly (→ punctation). Fine mosaic occurs in metaplastic epithelium and in low-grade precursors but it is a nonspecific pattern, in contrast to coarse mosaic which is suggestive of a high-grade lesion.

In the literature, the term mosaicism is also commonly used to refer to mosaic vascular patterns. However, the term mosaic defined as “a pattern made up of numerous small pieces fitted together” [1] apparently is a better description. The term mosaicism is usually referred to in genetics, e.g., individuals with two distinct karyotypic cell lines.

Nabothian cyst
A Nabothian cyst (Nabothian follicle, Naboth’s ovula) is a grape-like retention cyst resulting from continuous mucus secretion of a cervical crypt with an occluded opening by squamous metaplastic epithelium. In its thin wall, branching vessels are clearly visible.

Openings
Openings are small apertures of the remaining crypts of the endocervical mucosa in mature metaplastic epithelium. Two forms can be distinguished: 1. Openings of mucosal invagination covered by columnar cells that might be partly visible with a colposcope. They are called crypt or cleft openings (synonym: gland or glandular openings) and are a normal component of the TZ. The orifice is smooth surrounded by a narrow acetowhite ring with a slightly raised surface; 2. Cuff (abnormal) openings develop in the presence of high-grade lesions in the crypts. Cuff openings are thick, more raised, dense acetowhite and frequently have a doughnut ring appearance.

Original columnar epithelium
The original columnar epithelium lines the endocervical canal, it is built up by single-layer, mucous-producing cells with small nuclei and a moderate amount of cytoplasm, mixed with a few ciliated cells. The endocervical lining has papillary projections (villi) separated by crypts (infolding columnar cell layer). Each villus has a core connective tissue with a loop of capillaries. The crypts are elongated and form a labyrinth extending deep (up to 1 cm) in the cervical stroma. They are often termed glands, but strictly speaking they are not glands as they have neither ducts nor acini. The vascularity is directly underneath the columnar cells (lamina propria), producing its red (pink-red) color. After menopause, the villi decrease in size or may disappear and columnar cells become atrophic. Close to the columnar cells occasionally another cell type, the reserve cells appear, which are very small with round nuclei and scarce cytoplasm.

Colposcopically, the columnar epithelium is red and has a characteristic grape-like or villous appearance, representing the papillary projections (villi) and crypts, with no white change after acetic acid application. Since glycogen is absent in the columnar cells, they do not stain with Lugol’s solution either (pale yellow, iodine negative). Sometimes, there are two or three subdivisions on the lips, appearing as cushions and called rugae that are an extension of the endocervical palmate folds or arbour vitae [22].

Original squamous epithelium
The original squamous epithelium (also called: native portio epithelium) covers the ectocervix and has multiple cell layers (basal, parabasal, intermediate and superficial) that vary in size depending on the effect of estrogens and progesterone [20]. Development of the superficial cells requires dominant estrogens effect. The basal and parabasal cells are immature with a relatively high nuclear-to-cytoplasmic ratio. With cell differentiation, the nuclei will decrease in size and the cytoplasm will become more abundant. The intermediate cells are of moderate size with round nuclei, while the superficial cells are the largest, having small (mostly pyknotic) nuclei. Both cell types have abundant cytoplasm rich in glycogen. In contrast to basal and to a certain extent to parabasal cells, intermediate and superficial cells do not undergo mitosis. Blood vessels are beneath the basement membrane supplying the dividing basal and parabasal cells. Not infrequently, however, these vessels can grow into the epithelium, but not up to the surface. The original squamous epithelium is non-keratinizing, i.e., devoid of a keratin layer on its surface, it is continuous with the stratified vaginal
epithelium, and is continuously remodelled by the sequence of proliferation-maturation-desquamation in the period [20] → keratosis.

Colposcopically, the original squamous epithelium is pink without any feature (no cleft openings, Nabothian cysts, remnants of columnar epithelium) and has a smooth surface. It does not stain white following acetic acid application but will stain mahogany brown with Lugol’s iodine (iodine-positive).

Parakeratosis → keratosis

Punctuation

Punctuation is a vascular (colposcopic) pattern in which the capillary loops are seen on end as punctate dots (stippled pattern). It is the result of increased capillary formation with capillary loops growing into the epithelium and approaching or reaching the uppermost epithelial layer. These loops may even extend above the epithelial surface (floating dots). As vascular growth continues, capillary loops become larger and irregular in size (irregularly sized surface dots) [12]. Along with the cell proliferation laterally, the distance between the loops (intercapillary distance) increases and they become irregularly spaced [13]. This is enhanced by the accelerated cell proliferation that tends to compress the small loops [14].

The increased capillary formation is due to high cell proliferation with production of angiogenetic factors stimulating feeding vessels to grow and intrude into the epithelium. High cell proliferation and turnover occurs in premalignant and frankly neoplastic lesions, but also during metaplastic and (rarely) in the healing process. Thus, albeit most common, punctuation is not specific for neoplastic processes.

Fine and coarse punctations are distinguished depending on the size of and the space between the punctate dots. The fine punctuation pattern is characterised by small, almost equally spaced, uniform dots with a short intercapillary distance, smooth surface and inconspicuous border. The vessels are fine. In coarse punctuation, the dots are large, irregular in size and unevenly spaced, occasionally appearing above the epithelial surface, and the intercapillary distance is increased. The vessels are coarse. The surface of the lesion is usually rough with a distinct border. Following acetic acid application, acetowhiten ing is commonly seen in both lesions. As compared with the saline application, the precipitated nuclear material by acetic acid may compress small capillary loops resulting in a lesser degree of punctuation.

Ridge sign → surface contour [23]

Ruga (pl. rugae) → original columnar epithelium

Saline colposcopy

Prior to acetic acid application, the cervix is cleaned and moistened with a saline-soaked cotton ball, which allows better assessment of vascular patterns, particularly with the use of a green filter.

Schiller test → iodine staining (iodine negativity)

Squamocolumnar junction (SCJ)

There are two squamocolumnar junctions: the original and the neo (new) squamocolumnar junction. The original is the junction between original squamous epithelium and columnar epithelium, while the neo (new) is the junction between metaplastic squamous and the columnar epithelium (→ transformation zone). In colposcopic practice, the term SCJ usually refers to the neo-SCJ. In the early intrauterine period, the original SCJ separates the columnar and squamous epithelium at the external cervical os, hence its name. Due to hormone (mostly estrogens) response, however, it moves onto the ectocervix (version of the columnar epithelium → ectropion) during late foetal life, in adolescence and during the reproductive age. After menopause, SCJ may be high up in the endocervical canal, precluding visualisation of the full TZ.

Surface contour

The surface contour depicts the surface appearance of a colposcopic pattern. The surface can be smooth, soft or coarse (rough) and irregular resulting from uneven cell proliferation. A coarse and irregular surface may have various appearances depending on the type and grade of the lesions: slightly uneven; micropapillary or papillary; raised, raised and papillary (e.g., low-grade lesions), raised and nodular; thick; thick, raised and glossy (leukoplakia); exophytic (e.g., condyloma acuminatum); cauliflower appearance (e.g., adenocarcinoma, adherent condylomas); raised and irregular like a mountain range (caused by stromal invasion and accelerated cell proliferation), raised with ridges (ridge sign – suggestive of high-grade lesions [23]); cerebroid (brain-like), and depressed (due to erosion and ulceration). The surface of the columnar epithelium is grapelike (vil lous) often with rugae. The surface (the lesion) can be homogenous or complex.
**Terminologia colposcopica: a personal perspective**

**Transformation zone (TZ)**

The TZ is the area of the ectopic columnar epithelium on the cervix, which as a rule is replaced by squamous metaplasia and is demarcated by the two (neo and original) SCJs, i.e., the area is situated between the original columnar (endocervical) and the original squamous (ectocervical) epithelium (→ squamocolumnar junctions). Components of the normal TZ include columnar and metaplastic epithelium, the latter in different stages of maturation, as well as cleft openings and Nabothian cysts.

There are three types of the TZ (types 1-3) [24]. Type 1 is completely ectocervical and fully visible, type 2 has an endocervical component but is fully assessable, while type 3 has an endocervical component and cannot be assessed entirely. All may be small or large. The TZ may extend onto the upper vagina.

The old terms: cervical ectopy, ectopia, and erythroplakia, formerly used interchangeably to denote TZ, are no longer used in modern colposcopic terminology.

The term atypical TZ denotes TZ with abnormal colposcopic findings as opposed to physiological TZ with physiological squamous metaplasia. The concept of the congenital transformation zone (→) is discussed separately.

**Vaginal adenosis**

Vaginal adenosis is the presence of columnar cells in the vagina. In women exposed to diethylstilbestrol (DES) in utero, not only the entire cervix but partly the upper vagina is covered by columnar epithelium due to premature arrest of the migration of the squamous epithelium. As a result, the original SCJ is located in the vagina. This is often associated with other abnormalities (multiple sulci, cock’s comb and pseudopolyps), collectively called cervicovaginal deformities. It should be remembered, however, that vaginal adenosis can occur with DES exposure. This condition is benign and undergoes spontaneous resolution in almost all women, mostly by the time of puberty. The colposcopic appearance resembles that of columnar and metaplastic epithelium and is frequently associated with circular sulci, pseudopolyps on the ectocervix and occasionally cervical protuberance of the cervix bulging into the vagina (cock’s comb) → squamo-columnar junction.

**Vulvoscopy**

Colposcopy of the vulva.

**Colposcopic classification**

Traditionally, colposcopic classification by IFCPC is focused on the colposcopic findings of the uterine cervix and vagina, i.e., mucosal tissues of the lower genitalia. The colposcopic findings of the vulva have not been covered. In addition, evaluation of the colposcopic examination, whether it is satisfactory or unsatisfactory, has been included. The latest IFCPC classification made a distinction between normal and abnormal colposcopic findings. However, colposcopic features suggestive of invasive cancer and miscellaneous findings were not included in the latter group in spite of the fact that all findings are not considered normal are abnormal. The use of terms ‘typical’ and ‘atypical’ colposcopic findings, substituting the ‘normal’ and ‘abnormal’ adjective is less common and not preferred.

Colposcopic findings may be classified either according to the types (patterns and signs) of colposcopic features, or based on the interpretation of the colposcopic findings. Colposcopic features comprise colposcopic patterns and signs (for definitions of colposcopic features, patterns and signs see Glossary).

1. The classification of colposcopic features (Table 1).

The main objective of this classification is to aid in learning the recognition of colposcopic features, i.e., colposcopic patterns and colposcopic signs. Colposcopically, all diseases of the uterine cervix and vagina are invariably made up by some of these colposcopic patterns and signs.

| Table 1. — Classification of cervical and vaginal (mucosal) colposcopic features (patterns and signs). |
| Colposcopic patterns                |
| Original squamous epithelium       |
| Atrophic squamous epithelium       |
| Original columnar epithelium       |
| Metaplastic squamous epithelium    |
| Openings                           |
| Nabothian cyst                     |
| Deciduosis                         |
| Punctuation (fine, coarse)         |
| Mosaic (fine, coarse)              |
| Atypical vessels                   |
| Acetowhite epithelium              |
| Leukoplakia                        |
| Erosion, ulcer                     |

| Colposcopic signs                   |
| Colour tone sign                   |
| Border shape                       |
| Surface contour                    |
| Extension (localisation) and size   |
2. Colposcopic classification (Table 2).

**Table 2. — Colposcopic classification.**

<table>
<thead>
<tr>
<th>Interpretation of colposcopic exam</th>
<th>Satisfactory (the transformation zone is fully assessable)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Limited (the transformation zone is partially assessable only)</td>
</tr>
<tr>
<td></td>
<td>Unsatisfactory (colposcopic assessment is precluded)</td>
</tr>
</tbody>
</table>

**Normal colposcopic findings**

- Original squamous epithelium
- Original columnar epithelium
- Transformation zone (type 1-3) (cervix only)
- Columnar epithelium
- Metaplastic epithelium
- Crypt openings
- Nabothian cysts
- Atrophic epithelium
- Deciduosis

**Non-specific colposcopic features**

- Fine acetowhiteness
- Fine punctuation
- Fine mosaic
- Leukoplakia (hyperkeratosis)
- Iodine negativity
- Erosion, ulceration
- Geographic border

**Colposcopic features suggestive of high-grade lesions**

- Dense acetowhiteness (oyster white)
- Dense punctuation
- Dense mosaic
- Sharp, straight outer border
- Sharp inner border

**Colposcopic features suggestive of cancer**

- Atypical vessels
- Additional features of frankly invasive cancer
  - Large lesion (> 50% of the visible uterine cervix)
  - Irregular surface (exophytic or endophytic growth)
  - Grey colour tone
  - Marked, rolled edge
  - Necrosis
  - Friable vessels (contact bleeding, haemorrhage)
  - Features of high-grade lesions

**Miscellaneous colposcopic findings**

- Congenital transformation zone
- Healing tissue
- Abnormal granulation
- Condyloma acuminatum
- Subtle and flat HPV infection (26)
- Endometriosis
  - Common cervical and vaginal infections (candida, trichomonas etc.)
  - Polyps
  - Radiotherapy reactions
  - Cyst and adenosis (vagina only)
  - etc.

**Location of cervical lesions**

- Within the transformation zone
- Outside the transformation zone
- Overgrowing the transformation zone
- Extending to the vagina
  - Upper lip, lower lip, upper and lower lip

**Location of vaginal lesions**

- Anterior, posterior, right or left vaginal wall
- Upper half, lower half
- Vaginal fornix
- Sub-urethral

Colposcopic classification is more complex as compared to sorting colposcopic features (Table 1). In addition to the classification of colposcopic findings based on their interpretation, it also includes assessment of the colposcopic examination per se, of the type of the TZ and the extension (location) of the abnormal lesions. Normal colposcopic findings result from tissue variants occurring physiologically at some point in a woman’s life. Doubtfully interpretable features,
called non-specific colposcopic features, include colposcopic patterns and signs that can be due to two or more different tissue changes, therefore their clinical implication is uncertain. In contrast, high-grade colposcopic features do suggest a high-grade precancer lesion or cancer but are by no means diagnostic. For diagnosis in such cases, biopsy and histopathology are mandatory. Miscellaneous colposcopic findings include colposcopic features of cervical and vaginal diseases other than precursors or invasive cancer. Some of the miscellaneous colposcopic findings are characteristic enough to be used as the basis of treatment decisions, e.g., polyps, condyloma acuminatum.

The major points of consideration in making colposcopic classifications as defined by IFCPC are the following:

– “the classification should be descriptive to allow colposcopists throughout the world to be able to describe lesions to each other and to undertake important collaborative research;
– nomenclature should be written in such a way that it can guide a colposcopist in training and aid the established colposcopist during the diagnostic process;
– the terminology should be pragmatic (…)” [25, 26].

These principles have not changed, and are equally valid today. Indeed, classifications are made for clinicians to speak the same language in talking to each other, in colposcopy reports and research, and to share ideas.

Colposcopy of vulvar diseases (vulvoscopy)

Colposcopy of the vulva is often called vulvoscopy. By definition, “vulvoscopy consists of careful observation and the possible use of a 5% acetic acid solution to the entire vulvar area to facilitate examination of the vulva by the colposcope” [27]. Theoretically, it may be beneficial in a) identifying subclinical lesions (i.e., lesions not visible to the naked eye), particularly after acetic acid application; b) delineating clinically evident diseases; and c) recognising details not evident clinically. In practice, however, unlike the vagina and the cervix, colposcopy of the vulva does not add much to the naked-eye examination. (28). In addition, acetic acid application may be painful, colouration with toluidine blue is unreliable and observation of the colposcopic features is mostly obscured by the dermal tissues. Moreover, biopsy remains the gold standard in diagnosing vulvar disease, irrespective of the colposcopic findings. Regarding colposcopic features of vulvar lesions, the same patterns and signs are applied as for the vagina and cervix.

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Conflict of interest statement

The author may have the following conflicts of interest: he is member of the Nomenclature Committee of the International Federation of Cervical Pathology and Colposcopy.

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Persistent high-risk human papillomavirus (HPV) infections as surrogate endpoints of progressive cervical disease. 
Potential new endpoint for efficacy studies with new-generation (non-HPV16/18) prophylactic HPV vaccines

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Summary
Recent data indicate that persistent HR-HPV infections represent a significantly increased risk of developing incident high-grade CIN and cervical cancer. Accordingly, 6-month (6M+) or 12-month (12M+) type-specific persistence of HR-HPV have been proposed as powerful surrogates of progressive disease. Because of substantial practical impact in future HPV vaccine trials using non-HPV16/18 vaccines, studies on HR-HPV persistence as a surrogate endpoint of progressive CIN have been subject to a comprehensive meta-analyses recently. The present communication was solicited to bring this important and timely topic to the awareness of the readers, in a format consisting of a review of the recent literature, supplemented with the author’s own experience from different studies. Based on a large number of relevant studies, there remains little doubt that persistence of HR-HPV for 6+ or 12+ months is associated with a significantly increased risk of developing incident high-grade CIN. However, some data also disclosed several important issues that need to be carefully considered and/or adequately resolved before adopting 6M+ or 12M+ HR-HPV persistence as a surrogate of progressive disease. These include i) definitions of HPV persistence, ii) HPV detection techniques and iii) testing intervals and iv) length of follow-up, as well as v) diagnosis of the surrogate endpoints, and vi) other study characteristics, including vii) the type of reference category used in calculating the risk estimates. All these issues are critically discussed in the present communication. Of major impact seems to be the reference category used to calculate these risk estimates, as evident from the NIS-LAMS cohort. Taken together, it is suggested that in all future studies using the 6M+ or 12M+ HR-HPV persistence as a surrogate endpoint of progressive disease, a “gold standard” should be used in calculating the risk estimates. In addition to deciding, 1) whether to use 6M+ or 12M+ persistence criteria, and 2) cytological, histological or combined surrogate endpoints (SIL, CIN1, CIN2, CIN/SIL), one should 3) use exclusively the HPV negative reference group in calculating the risk estimates for viral persistence endpoints. This is supported by the data from the recent meta-analysis as well as from the author’s combined NIS-LAMS cohort, both implicating that the most consistent association to progressive disease is obtained when women with persistent HR-HPV are compared with HPV-negative women. It is the conviction of this author that the two other reference categories (HPV transient and HPV mixed outcome) are far too heterogeneous and subject to potential misclassifications to give consistent and reproducible risk estimates for HR-HPV persistence as a surrogate endpoint of progressive CIN.

Key words: Human papillomavirus; High-risk types; Persistent infection; 6-mo and 12-mo persistence; Surrogate endpoint; Progressive disease; HPV vaccines; Efficacy trials; Study power; NIS cohort; LAMS study.

Introduction
Since the recognition of human papillomavirus (HPV) as the causal agent of cervical cancer (CC) and its precursor lesions (CIN), epidemiological data from different countries confirmed that the peak prevalence of cervical HPV infections occurs between 22-24 years of age, with constant decline with progressing age [1-7]. More recent studies on the natural history of HPV infections have further refined their dynamics in different populations. Accordingly, incident HR-HPV infections are clearly age-dependent, the 3-year cumulative incidence exceeding 50% among young women following the onset of their sexual activity [8-11].

On the other hand, clearance of the virus does not show such strict age-dependence [12], but continues at a rather constant rate from 30 years onwards when the clearance rates exceed the acquisition rates resulting in declining age-specific prevalence rates [13-15]. However, not all HPV infections will undergo spontaneous clearance; some of the acquired infections remain persistent [6, 8, 14, 15]. These persistent infections of the high-risk (HR) HPV types are
considered as prerequisite for developing a progressive disease intensely studied, e.g., for the necessary cofactors of 
HPV [12, 16-21]. Persistent infections and CIN are established from fewer than 10% of all new infections [8, 18, 19].
There is some evidence that HPV16 persists longer than the other HR-HPV types [18, 19]. Furthermore, prevalent infections persist longer in older women than in younger women [22], most probably because of increased probability of virus integration over time [23]. Time to progression from HPV infection to CIN2+ among HPV carriers is variable, but a majority of cases occur within the first three years [22-25]. Since persistent HR-HPV infection plays a key role in the development of CC, the detection of persistent HR-HPV infection represents a specific marker of an increased risk [19-25].

Indeed, several studies have demonstrated very high relative and absolute risks of CIN2+ and CC ascribable to type-specific persistent HR-HPV infections [18, 26-29]. This is particularly true with the women who acquire persistent HPV16 or HPV18 infection, but also applies to other HR-HPV types. The emerging data implicate, however, that HPV16 and HPV18 infections progress more rapidly than the other HR-HPV types [28-30]. Clearly, persistent HR-HPV infections represent a sign of increased risk of CC, and as such, 6-month (6M+) or 12-month (12M+) type-specific persistence of HR-HPV have been proposed as powerful surrogates of progressive disease, e.g., in ongoing HPV vaccine trials [19, 31, 32]. Recently, persistent HR-HPV infections have also been implicated as potential intermediate endpoints of high-grade CIN in CC screening [33, 34].

As shown by the published and ongoing vaccine trials [24], a relatively short-term vaccine efficacy study will have sufficient power to evaluate histological (CIN2+) endpoints associated with HPV16 and HPV18, but this is unlikely to be the case for the other HR-HPV types [19, 31, 32]. Indeed, a similarly powered study to evaluate CIN2+ endpoints for non-HPV16/18 types would need a significantly larger sample size (> 100,000 women) and significantly longer follow-up (up to 10 years), which would make such a study not feasible [19]. Thus, search for other (non-histological) endpoints as potential surrogate markers of disease progression is urgent. Indeed, persistent HR-HPV infection might be a potential candidate for being such a surrogate marker [35].

Because of its substantial practical impact related to the conduct of all future HPV vaccine trials using non-HPV16/18 vaccines, the data on HR-HPV persistence as a surrogate endpoint of progressive cervical disease were subject to comprehensive meta-analysis recently [35]. This exhaustive analysis disclosed several important issues that need to be carefully considered and/or adequately solved before implementation of 6M+ or 12M+ HR-HPV persistence as a surrogate of progressive disease in these future trials.

Unfortunately, no unanimous agreement has been reached as yet how to define persistent HR-HPV infection [35]. In some studies, HPV persistence is defined as two or more HPV DNA-positive tests [36, 37], while in some others, HPV persistence has been assessed using the time to clearance (i.e., duration of infection) [38-40], and yet in some others, as a proportion of HPV-positive visits [41, 42]. This issue is further complicated by differences in HPV detection methods, intervals in HPV testing, as well as whether a type-specific or non-type-specific HPV persistence is measured, and whether the analysis is restricted to HR-HPV types in general or to individual HR-HPV genotypes [35]. There is an urgent need to validate those multiple variables affecting the risk estimates of these virological endpoints [35-42]. It is also essential to reach a standardised definition of HPV persistence, to be uniformly applied in the future studies using HPV persistence as surrogate of disease progression.

Prompted by the rapid arousal of this topic into a sharp focus due to its major practical importance, we decided to explore the issue in our combined NIS and LAMS study cohort of over 15,000 women (to be described later), and recently analysed several potential (viral and other) surrogate markers of disease progression in two separate studies [35]. The first one [43] was completed before the meta-analysis of Koshiol et al.: [35] was published, while the second one [44] was designed to elucidate several of the key open issues that were raised in that meta-analysis.

The present communication was solicited to bring this important and timely topic to the awareness of the readers, in a format consisting of discussion of the core literature [35], supplemented with the experience of this author based on two multi-centre screening trials (NIS and LAMS studies) [43, 44]. We start with an address of the key information from a recent meta-analysis [35], followed by the description of the two approaches recently made by the author’s research group to tackle the issues that remain unsolved in the published literature. At the end, these key issues related to the acceptance of persistent HR-HPV as a surrogate endpoint in the future efficacy trials with new-generation non-HPV16/18 vaccines will be discussed. Recommendations are given how these assessments should be conducted to obtain (for 6M+ and/or 12M+ persistent HR-HPV infection) the most consistent risk estimates for the surrogate endpoint markers (CIN, SIL) of a progressive cervical disease.

**Data on recent meta-analysis**

In this communication, the purpose is by no means to reiterate the comprehensive meta-analysis recently published by Koshiol et al.: [35], which the reader is referred to for further details. For introduction to the topic, however, it is appropriate to identify the key studies included in this meta-analysis as well as to summarise the core data extracted from these studies in a highly synthetic form.

Altogether, the authors went through a substantial amount of literature and identified 41 eligible studies that covered > 22,500 women analysed (in different designs) for the association between HPV persistence and cervical neoplasia.
Persistent high-risk human papillomavirus (HPV) infections as surrogate endpoints of progressive cervical disease. Potential new etc.

All abstracted data were reviewed twice by independent readers to ensure data accuracy. In many studies, several relative risks (RR) were given, but for the meta-analysis, these were selected through decision rules for analyses of the association of HPV persistence and CIN2-3/HSIL+ endpoint, to maintain the independence of observations [35]. Sensitivity analyses suggested that the results of this meta-analysis were robust to reasonable changes in the decision rules, and funnel plot asymmetry analyses showed little evidence of publication bias.

In this meta-analysis [35], the data extracted from these studies were presented as several figures and tables, summarising the key indicators of these studies [16, 18, 26, 42, 45-62]. For the purpose of the present communication, the key observations can be summarised in one table, synthesising the data most relevant to the present discussion (Table 1). There are several important observations that deserve to be addressed in some more detail here.

First of all, there remains little doubt that HPV persistence is strongly and consistently associated with incident CIN2-3/HSIL+ in practically all these studies [16, 18, 26, 42, 45-62]. This led the authors to emphasise the value of HPV DNA testing may be useful in the screening programs by identifying women who are at high risk of CC [35]. Any further discussion of this last subject, however, falls outside the scope of the present communication.

In the published studies included in this meta-analysis [35], several different surrogate endpoints of progressive disease were used, ranging from ASCUS Pap smear to biopsy-confirmed CIN2/3 [16, 18, 26, 42, 45-62]. As evident from Table 1, the strength of the association between HPV persistence and cervical neoplasia increased with increased disease were used, ranging from ASCUS Pap smear to biopsy-confirmed CIN2/3 [16, 18, 26, 42, 45-62].

Table 1. — Risk estimates for HR-HPV persistence as a predictor of surrogate endpoints (ASCUS, LSIL, CIN1+, CIN2-3, HSIL) using optional reference categories*.  

<table>
<thead>
<tr>
<th>Author (Reference No.)</th>
<th>Relative Risk</th>
<th>95% Confidence Interval Lower bound</th>
<th>Upper bound</th>
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<tr>
<td>Kjaer et al. [26]</td>
<td>4.9</td>
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<td>13.2</td>
<td>6.5</td>
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<td>14.1</td>
<td>2.3</td>
<td>84.5</td>
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</table>

*Meta-analysis by Koshiol et al. [35].
ing grade of cervical lesions. This is consistent with the view that CIN1 lesions (and their surrogates LSIL and/or ASCUS) in most of the cases represent only transient HPV infections, being very common among young sexually active women, and possess a high tendency for spontaneous regression accompanied by virus clearance [1, 2, 8, 14, 15, 17]. In contrast, long-term persistence of HR-HPV positivity is clearly associated with neoplastic transformation and thus clinically relevant as a surrogate endpoint of progressive disease [1, 2, 5-8, 11-17, 35].

Another observation of major importance is that the strength of association of HPV persistence and incident CIN2-3/HSIL+ varied widely and was partially dependent on the HPV referent group (Table 1) [35]. In settings like this [16, 18, 26, 42, 45-62], three different referent categories can be used in testing the strength of persistent HPV as predictor of progressive disease: 1) HPV-negative women, 2) women with mixed HPV outcome, and 3) women with transient HPV infections. These three referent categories will be discussed in more detail later. While viewing the data in Table 1, it is obvious that those studies comparing women with persistent HPV infections with those who were HPV-negative produced the highest and most consistent relative risks [16, 26, 45-51]. This is feasible because it is commonly agreed that the risk of developing high-grade CIN is very low among HPV-negative women [1, 2, 8-10]. On the other hand, comparing women with persistent HPV infections to those with transient infections produced the weakest relative risks [16, 26, 45-47, 50, 55-62]. This could implicate that the risk of high-grade CIN among women with HPV infections of shorter duration (transient) is by no means negligible. As the authors correctly point out, one cannot exclude the possibility that a substantial proportion of these women classified as transient HPV infections in these studies (i.e., duration < 6 months) do, in fact, represent persistent infections with an onset well before the baseline visit, and upon clearance soon after, were actually misclassified [35].

In overall evaluation, the second most consistent risk estimates were obtained in studies where HPV persistence is compared with mixed HPV outcome [16, 18, 26, 42, 45-47, 50, 52-54]. As the name implies, this reference category consists of women with mixed patterns of HPV outcomes, including short-term persisters with clearance, followed by reactivation etc., i.e., a pattern known as fluctuation [1, 2, 8]. As shown in long-term follow-up studies [2, 8], the majority of these fluctuators eventually turn out to clear their infections, which seems to make this reference category more close to a HPV-negative referent group as to the obtained risk estimates for HPV persistence as predictors of high-grade disease (Table 1).

An additional point is associated with the duration of HPV persistence which is closely linked with the HPV testing interval [35]. It was noted that the associations between HPV persistence and cervical neoplasia appeared stronger in studies with longer duration of HPV infection (> 12 months) and longer HPV testing intervals (> 6 months or > 12 months) [16, 18, 26, 42, 45-62]. This is not unexpected, because these variables could reflect a longer exposure to oncogenic HPV conferring a higher risk for developing high-grade CIN and CC. Similarly, testing HR-HPV+ at longer testing intervals also decreases the potential misclassification (of both exposure and outcome), because the majority of HPV infections associated with low-grade lesions will regress during the testing interval [2, 8, 11, 14, 15, 35].

Finally, the meta-analysis under discussion [35] clearly disclosed a major gap in our knowledge, i.e., the lack of studies providing data on type-specific persistence and its association with disease progression. Astonishing as it might sound, only few studies provide such data for HPV16 and HPV18. Although the associations between HPV16 and/or HPV18 persistence and high-grade CIN were consistently positive, there was a major heterogeneity in the risk estimates varying from 4.5 (95% CI, 0.24-85.1) (not significant) to 279.7 (95% CI, 16.0-4,894.5) [35]. Knowing that even a single detection of HPV16/18 appears to increase the risk of developing CIN3 and CC [2, 3, 10, 12, 28, 30, 63], it is of utmost importance in the future studies to focus on these associations at the HPV genotype level.

Based on a large number of relevant studies [16, 18, 26, 42, 45-62], it is easy to agree with the authors of this meta-analysis [35] stating that these data demonstrate that two HPV-positive visits are associated with increased risk of high-grade CIN. This comprehensive meta-analysis clearly confirmed that repeated HPV detection is associated with an increased risk of invasive CC, and its precursors, despite the differences in i) definitions of HPV persistence, ii) HPV detection techniques and iii) testing intervals, iv) diagnosis of surrogate endpoints, and v) other study characteristics, including the type of reference category [35]. Needless to say, several of these differences need further assessment and standardization, before consistent and reproducible risk estimates can be provided for the association of persistent HPV infection and development of cervical neoplasia.

Experience from the combined NIS and LAMS study cohort

Prompted by the rapidly increasing interest in this topic, we decided (in 2008) to explore this issue in our combined NIS and LAMS study cohort, comprising over 15,000 women, of whom almost 2,000 have been prospectively followed-up for detection of incident cervical disease. We recently analysed several potential (viral and other) surrogate markers of disease progression in two separate studies [43, 44]. The first one [43] was completed before the meta-analysis of Koshiol et al.: [35] was published, while the second one [44] was designed to elucidate several of the key open issues that were raised in that meta-analysis.
Aims

In the first of our two studies [43], we found the 6M+ and 12M+ persistence of HR-HPV was a powerful surrogate of developing an incident CIN, but second only to persistent HSIL Pap smear. It also became obvious that these risk estimates are critically dependent on which endpoints (SIL, CIN1+, CIN2+) are used, and particularly how the reference category is defined [43]. Our second analysis was the first study to directly compare the impact of the three optional reference categories; i) HPV-negative, ii) transient HPV, and iii) mixed HPV outcome on the strength of the association between 6M+ and 12M+ HR-HPV persistence and disease progression [44]. Instead of a single surrogate endpoint, we used four: i) SIL (cytology), ii) CIN1+ (biopsy), iii) CIN2+ (biopsy) and iv) combined CIN/SIL (cytology/biopsy) surrogate endpoints of progressive disease. In both studies, the combined cohort consisted of 1,865 women, prospectively followed-up in the NIS study [64] and in the LAMS study [65], derived from the total cohort of 15,301 women [43, 44].

Study design

Our two recent analyses [43, 44] are based on a combined cohort of the NIS and the LAMS studies, previously described in a series of original reports [64-69]. Both studies are international multi-centre trials testing optional screening tools in low-resource settings of three NIS (New Independent States of the Former Soviet Union) countries (Russia, Belarus and Latvia) [64] as well as in two Latin American countries (Brazil and Argentina), respectively [65]. The design and baseline data of both cohort studies have been previously detailed [64, 65], and described here only in brief so as to give the necessary background to the data discussed next.

The NIS Cohort

The material of the NIS study cohort comprises 3,187 consecutive women attending six different outpatient clinics in the three NIS countries between 1998-2002. These women derived from three different groups: i) women participating in cervical cancer screening (= SCR patients); ii) gynaecological outpatients (= GYN patients), and iii) patients attending STD clinics (= STD patients). The mean age of these women at enrolment was 32.6 (± 10.7 SD) years (median 30.6, range 15-85 years) [64]. The study design, baseline data and interim results have been detailed in a series of reports already cited [11, 14, 15, 17, 64]. All eligible women had Pap smears taken and were tested for HR-HPV using HC2 assay, and also with PCR (n = 1,500) and confirmative hybridisation. Patients with ASC-US or higher Pap had biopsy confirmation at baseline [64].

The LAMS study

The LAMS study is a combination of a population-based, cross-sectional study and a longitudinal cohort study of women enrolled in regions with different (low, intermediate, high) incidence of CC in Brazil and Argentina, as described in detail recently [65]. In the first phase the four clinics examined a total of 12,114 women between February 2002 and June 2003, enrolled in the original LAMS Study cohort. The mean age of these women at enrolment was 37.9 years (range 14-67; median 37.7 yrs). In this screening trial, eight different diagnostic tests were compared: cervical cytology (conventional and liquid-based cytology) was compared with 1) four optional screening tools suggested for low-resource settings: a) visual inspection with acetic acid (VIA), b) visual inspection with Lugol iodine (VILI), c) cervicography, d) screening colposcopy); and 2) with the new molecular diagnostic tools (HPV testing by HC2 assay), performed a) in samples collected by physicians, and b) in those taken by self-sampling devices [65-69]. Women testing positive with any of these techniques were examined by colposcopy at the second visit. In addition, a 5% random sample of all test-negative (PAP, VIA, VILI, HC2) women were referred for colposcopy to assess false negative exams, and 20% of baseline HC2-negative women were referred for new HC2 to assess incident HPV infections.

Prospective follow-up

In both studies, prospective follow-up (FU) is an essential component of the design [11, 14, 15, 17, 64-69]. In the NIS cohort, all women presenting with biopsy-confirmed low-grade lesions were assigned for prospective FU, while high-grade lesions were treated. Altogether, FU data are available from 887 women, divided into four sub-cohorts according to their baseline HPV/Pap smear status [11, 14, 15, 17, 64]. Altogether, 33 women with baseline CIN3 were excluded from the analysis, leaving 854 women in the final FU-cohort of the NIS study. FU visits were scheduled at 6-mo intervals, planned to cover 24 months. The mean FU time reached in this trial was 17.2 mo (SD, 11.6 mo; median, 16.6 mo; range 1-43 mo) [64].

In the LAMS study the same criteria were used to allocate the women into the FU and treatment groups [65]. A total of 1,011 women completed at least one FU visit including examination by Pap smear, VIA/VILI, colposcopy and biopsy, whenever abnormalities were detected. Also in the LAMS study, FU visits were scheduled at 6-mo intervals,
planned to cover 36 months. The mean FU time reached in this study was 21.7 mo (SD, 8.1 mo; median, 24.2 mo; range 1-54 mo). All high-grade lesions were promptly treated and followed-up for the same period, using repeated Pap test and colposcopy at 6-month intervals, and HC2 assay at 12-month intervals [65-69].

Outcomes and endpoints of cervical lesions and HR-HPV infections

For both recent analyses [43, 44], the data of the 854 women from the NIS Cohort and 1,011 women from the LAMS study were merged into the same file, and the combined cohort of 1,865 women was analysed for the four surrogate endpoints of disease progression, based on cytology or histology or both: 1) progression to SIL; 2) progression to CIN1+, 3) progression to CIN2+, and 4) progression to CIN/SIL. Progression to SIL (any degree) was based on detection of either LSIL or HSIL in any of the Pap smears taken during the FU of a baseline Pap-negative woman. Being an endpoint of progressive disease, women who subsequently cleared their incident SIL (the last visit status) were excluded from this sub-group [43, 44]. Similarly, baseline biopsy-negative women who developed CIN1+ in any of the consecutive biopsies taken during the FU were defined as progression to CIN1+. As a progression to CIN2+ was defined any case where biopsy-confirmed progression from baseline negative-, NCIN- or CIN1 biopsy was established in the subsequent FU-visits. Finally, a fourth category was built up, consisting of women in whom the progression was defined by both biopsy and cytology, i.e., progression to LSIL/HSIL or to CIN1+/CIN2+. Times to progression into SIL, CIN1+, CIN2+, and CIN/SIL were calculated from the baseline visit to the respective FU-visit when the progression event was first detected. Progression rates were calculated dividing the numbers of progression events by woman months at risk (WMR), and expressed per 1,000 WMR [43, 44].

Methods

Because have been detailed in a series of recent reports [11, 14, 15, 17, 64-69], the methods used in the NIS cohort and in the LAMS study are described here only as far as pertinent to elaborating the data discussed in this communication.

Detection of HPV DNA by Hybrid Capture 2 assay

In both studies, HPV testing was performed by Hybrid Capture 2 (HC2) assay using cervical swabs (collected by a physician) and self-sampling devices (tampons, in LAMS study only), as described previously [64-69]. The samples were analysed only for the presence of HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The usual limit of 1 pg/ml of HPV16 DNA was used as the positive control (CO), i.e., samples were classified as HR-HPV positive with the RLU/CO \( \geq 1.0 \) pg/ml cut-off.

Assessment of HPV persistence

Statistical analyses were performed using the SPSS and STATA software packages (PASW for Windows, Version 17.0.2., SPSS Inc., Chicago, USA and STATA/SE 11.0. Stata Corp., TX, USA). The incidence rates (of SIL, CIN1+, CIN2+, CIN/SIL) were expressed as cases/1,000 WMR, and their 95% confidence intervals (95%CI). Incidence rates in the NIS and LAMS cohorts were compared by calculating RR (rate ratio) statistics (with 95%CI).

In both studies [43, 44] the power of two viral endpoints was estimated: i) more than 6-mo persistent (6M+) HR-HPV infection, and ii) more than 12-mo persistent (12M+) infection, as predictors of progressive cervical disease, defined by intermediate surrogate endpoints, i.e., progression to SIL, CIN1, CIN2. In the second study, also CIN/SIL was included among these intermediate endpoints of progressive disease [44]. These two viral surrogates (6M+ and 12M+) are based on detection of HR-HPV infection in two and three, respectively, subsequent samples taken at > 6 and > 12 months apart during the FU. Because of the longitudinal nature of the combined NIS-LAMS cohort, the data file was transformed to a panel data suitable for analysis by a generalized estimating equation (GEE) model, clustered by women-ID (subject variable) and FU visit (within-subject variable) [70, 71]. GEE adjusts for the serial correlation within subjects by modeling the covariance structure within subjects. Because all dependent variables are binomial (presence/absence of SIL, CIN1, CIN2, CIN/SIL), the logit link function was used. The exchangeable working correlation structure with a robust variance estimator to account for within-subject correlation was selected as the best-fit covariance pattern, using the Quasi-likelihood Information Criterion (QIC) [70, 71]. Because HR-HPV persistence clearly depends on time since the previous sample, therefore, a time variable was included as a covariate in these GEE models.

In the longitudinal assessment of HR-HPV persistence, women who were HR-HPV-positive at a specific (baseline or FU) visit \( t \) were considered 1) 6M+ persistent, if their subsequent assessment \( t+1 \) was also HR-HPV positive, and 2) 12M+ persistent, if also the next visit \( t+2 \) sample was HR-HPV positive. However, also the exact sampling intervals were calculated for each individual woman, and the strict 6.0-month and 12.0-month cut-offs were applied to adjust the above defined 6M+ and 12M+ persistence categories at the level of individual women.
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In the first of these two analyses [43], no attention was paid to the reference category used in the above calculations, any women not fulfilling the 6M+ or 12M+ persistence criteria were used as a reference. In the second analysis [44], however, we were particularly interested in assessing the impact of the optional referent groups on the strength of the association between 6M+/12M+ HR-HPV persistence and the surrogate endpoints (SIL, CIN1, CIN2, CIN/SIL). Three optional reference categories exist: 1) women who cleared their HR-HPV after 6M+ or 12M+ persistence (= HPV transient reference); 2) women who remained HR-HPV negative throughout the whole FU period (= HPV negative reference); and 3) women whose HR-HPV infection ran a fluctuating (mixed) course, with HR-HPV positive test followed by temporary clearance, subsequent activation, etc. (= HPV mixed outcome reference). This last category excluded women whose HR-HPV was cleared at the last FU visit. In the GEE file, six new panels were created comparing the i) 6M+ and ii) 12M+ persistence criteria (= 1) with each of the three referent groups (= 0) as follows; 1) +/+ or +/+/+ (= 1) compared with -/-/- (- = 0) at all their (t+1,2,...,j) visits; 2) +/+ or +/+/+ (= 1) compared with -/-/-(- = 0) at all their (t+1,2,...,j) visits; 3) +/+ or +/+/+ (= 1) compared with -/-/- or -/-/-/- or -/-/-/- (- = 0) at their (t+1,2,...,j) visits. Relative risks (RR; 95% CI) were calculated for the associations of 6M+ and 12M+ persistence with the four intermediate surrogates (SIL, CIN1, CIN2, CIN/SIL) separately using the three reference categories. All statistical tests performed were two-sided and declared significant at a p value < 0.05 level [43, 44].

HPV persistence as a surrogate of progressive disease in the combined NIS-LAMS cohort

Combined reference category

In the first analysis [43], we calculated the risk estimates (relative risk, RR) for the potential surrogates as predictors of disease progression using three intermediate outcome events: SIL, CIN1+ and CIN2+ (Table 2). A wide variety of potential endpoints were tested, including those assessed at the baseline visit, those available at the 6-month and 12-month FU-visits, as well as several of those based on persistent viral events (HR-HPV assay) and persistent clinical abnormalities (Pap smear cut-off). In this study, no attempt was made to distinguish between the different reference categories in these calculations. HPV genotype-specific data are scanty and available from the NIS cohort only [43, 64].

Of the predictors available at baseline, testing HR-HPV positive with HC2 assay is the single most powerful risk factor for subsequent progression to SIL, CIN1+ and CIN2+, with the highest RR = 8.69 obtained for CIN2+ endpoint. This far exceeds the power of baseline ASCUS, LSIL and HSIL cytology, and these cytological endpoints are of no value in predicting CIN1+. This is in sharp contrast to these cytological endpoints assessed at the 6-month FU-visit, of which HSIL is by far the single most powerful predictor of progression to CIN2+, with RR =47.14 (95% CI 17.29-128.66), and less powerful for CIN1+ (RR = 9.67). ASCUS and LSIL are all significantly associated with the development of CIN1+ and CIN2+, with RR varying between 2.5 and 4.6. Importantly, ASCUS at the 6-month FU visit is a powerful predictor of SIL outcome, with RR = 17.98, which is among the highest of all these associations (Table 2).

The strength of all these cytological endpoints is substantially diminished when assessed at the 12-month FU-visit as compared with the 6-month visit. However, HSIL at the 12-month FU-visit is still a significant risk factor for CIN2+ progression, with RR = 21.48, which is second only to that of HSIL at the 6-month visit. In contrast to the declining power of these cytological endpoints, the strength of the virological endpoints, i.e., testing HR-HPV+ at FU visits, is markedly increasing. Indeed, testing HR-HPV+ at the 12-month visit is associated with progression to CIN2+ with RR=10.72 (95% CI 3.16-36.37), and to CIN1+ and SIL outcomes with RR > 4.0.

Of the virological endpoints of interest in the present discussion, the most powerful seems to be the 6M+ persistent HR-HPV as a predictor of incident CIN1+: RR = 18.61 (95% CI 2.53-136.50), being among the highest in this panel (Table 2). No additional benefit is obtained using the 12M+ HR-HPV persistence criteria in predicting any of the three outcomes. The limited number of cases in each strata hampers the calculations of these data for individual HPV genotypes. In Table 2, non computable (NC) denotes situations where none of the < 6M+ or < 12M+ persistors progressed to the relevant outcome event while 6M+ and 12M+ persistors did, and NC is for situations where no cases were in either of the outcome (progressed/not progressed) categories. Albeit formally not calculable in both situations, RR in the former can be considered to reach infinity.

Analogous to the 6M+ and 12M+ persistent HR-HPV, we made similar calculations for persistent Pap abnormalities, separately for ASCUS and SIL. As shown in Table 2, 6M+ persistent SIL is a powerful predictor of disease progression to CIN1+ (RR = 13.75) and CIN2+ (RR = 8.93), being superior in this respect to 6M+ persistent ASCUS. Interestingly, this power is practically lost when the 12M+ persistence criteria are used.

Specific Reference Categories

In the second study, prepared after the appearance of the above discussed meta-analysis [35], we conducted the same analysis using three different reference groups on women with 6M+ and 12M+ HR-HPV persistence [44]. Table 3 summarises the RR's for the 6M+ and 12M+ HR-HPV persistence to predict the four surrogate endpoints of progressive disease progression.
disease (SIL, CIN1+, CIN2+, CIN/SIL) in the GEE model, run separately for the three referent groups. A series of cytological surrogates (i.e., HSIL at 6- and 12-mo FU visit, 6M+ and 12M+ persistent SIL and ASCUS) were similarly evaluated as predictors of progressive disease, just to make a comparison with the virological surrogates.

The results presented in Table 3 are markedly different from those in Table 2. This is because the strength of the association of both 6M+ and 12M+ HR-HPV persistence with all four surrogate endpoints is critically dependent on the reference category used in these calculations. With few exceptions, the highest RRs are obtained when the HPV-negative reference category is used, irrespective of whether the surrogate endpoint is SIL, CIN2+ or CIN/SIL. As to the CIN1+ surrogate endpoint, however, RR for 12M+ persistence is the highest (RR = 10.2) when the HPV transient reference is used, and RR for 6M+ persistence is highest (RR = 21.6) using the HPV mixed-outcome reference. The difference between the three reference categories is most marked when RRs of both 6M+ and 12M+ persistence are compared in the SIL surrogate endpoint, followed by CIN2+ surrogate and CIN/SIL surrogate groups. In fact, RRs are far above 10 in all associations of 6M+ and 12M+ persistence with SIL, CIN2+ and CIN/SIL surrogates when the HPV-negative reference group is used, whereas these associations almost lose their significance in many occasions when the HPV transient- and HPV mixed-outcome referent groups are being used. With the HPV-negative reference group, the
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In Table 3, risk estimates for 6M+ and 12M+ HR-HPV persistence as surrogate endpoints of SIL, CIN1+, CIN2+ and CIN/SIL using optional reference categories in GEE models are presented. The strongest single association is obtained between 12M+ persistence and SIL surrogate (RR = 27.6), followed by the 6M+ persistence and SIL (RR = 20.9), 12M+ and CIN2+ (RR = 19.3) as well as 6M+ and CIN2+ (RR = 18.8). In contrast, when the HPV transient- and HPV mixed-outcomes are used as referent groups, RR=10 is exceeded in two associations only, i.e., 12M+ with CIN1+ and 6M+ with CIN1+, respectively (Table 3).

In addition to these virological endpoints, also most of the cytological markers seem to be significantly associated with the four surrogate endpoints (Table 3). Of all cytological predictors, HSIL at the 6-mo FU visit shows the strongest association with progression to CIN2+, with RR = 47.14 (95% CI 17.29-128.70), followed by HSIL and CIN2+ at 12-mo FU visit, with RR = 21.48 (95%CI 5.08-90.79). Of the 6M+ or 12M+ persistent Pap smear abnormalities, the single most powerful association is obtained between the 6M+ persistent SIL and CIN1+ (RR=13.75, 95%CI 2.97-63.51), while most of the others are not significant predictors of CIN1+, CIN2+ or CIN/SIL.

Considerations for the future

From the studies discussed above [43, 44] as well as from the recent meta-analysis [35], several important issues arise that deserve some discussion in this context. As mentioned above, these issues are related to definition of HPV persistence, definition of progressive disease, intervals of HPV testing, reference category used in calculations, as well as the HPV detection methods. Discussion of the latter falls outside the scope of this communication, however, the reader is being referred to comprehensive HPV textbooks addressing the technical aspects of HPV detection methods [1, 2]. Needless to emphasise that there is an urgent need to validate those multiple variables affecting the risk estimates of the discussed virological endpoints [35, 44], and it is also essential to reach a standardised definition of HPV persistence, to be uniformly applied in future studies using HPV persistence as a surrogate of disease progression.

Definition of HPV persistence

As recently pointed out [35, 44], no unanimous agreement has been reached as how to define persistent HR-HPV infection. In some studies, HPV persistence is defined as two or more HPV DNA-positive tests [36, 37], while in some others HPV persistence has been assessed using the time to clearance (i.e., duration of infection) [38-40], and yet in some others, as a proportion of HPV-positive visits [41, 42]. Certainly, this is among the first issues to be agreed on because of a major cause of misclassification (persistent or not) and as such a major source of biased estimates in all studies assessing the risk conferred by HR-HPV persistence for disease progression [16, 18, 26, 35, 42-44, 45-62]. Indeed, definition and predictors (determinants) of HPV persistence [17] are among the key issues in understanding the natural history of genital HPV infections, recognised already in the early prospective follow-up studies since the 1980s [8, 72-74]. When a prospective cohort of women with a cervical HPV lesion at baseline is prospectively followed-up for a prolonged period of time, at least six different disease outcomes can be distinguished, as recently
detailed in the textbook of this author [8]. These can be defined as follows: 1) early regression, 2) persistence, 3) fluctuation, 4) late regression, 5) progression, and 6) recurrent disease. As repeatedly emphasized [8, 75–77], the definition of these disease outcomes is based on clinical assessment only, i.e., colposcopy, Pap smear, and biopsy.

The issue becomes more complicated when the dynamics of viral events are being assessed in a longitudinal setting, as done more recently when robust HPV detection techniques became generally available since the late 1990s [11, 14, 15, 17]. As to these viral outcomes, the three most obvious ones are: 1) acquisition of new infection, 2) persistent infection, and 3) clearance of the infection. However, referring back to long-term prospective cohort studies [72–74], at least two other outcomes are well established: 1) persistently HPV DNA-negative, and 2) fluctuation. The former is straightforward, including women who remain persistently HPV DNA-negative in repeated HPV testing (e.g., at 6-mo intervals) for the entire follow-up time [11, 14, 15, 17]. The latter is far more complex, and not well understood even today, characterised by intermittent appearance, disappearance and reappearance of HPV DNA in repeated sampling of women in a longitudinal setting. Undoubtedly, many of the women included in the HPV mixed-outcome referent categories in the recent studies on HPV persistence [16, 18, 26, 35, 42–44, 45–62] represent women with this type of fluctuating HPV outcome.

While considering HPV persistence, there are actually several different issues that need to be considered. Apart from the strict definition of persistence as an event (phenomenon), we need to consider whether 1) this persistence is type-specific or non-type-specific (= any HPV type), 2) whether the analysis is restricted to HR-HPV types collectively or to individual HR-HPV genotypes [35]. These considerations are closely linked with HPV detection techniques, and certainly responsible for the major differences in the results reported in the studies included in the above discussed meta-analysis [35]. Importantly, the data are completely insufficient at this stage to draw any firm conclusions on HPV persistence at the genotype level as a surrogate of progressive disease, albeit well established when HR-HPV types are counted collectively [35, 43, 44].

At least equally important as to provide genotype-specific data, is the jointly agreed definition of HPV persistence as the viral event itself. The optimal should be to record exact times of persistence for each individual woman in such analysis, e.g., at a 1-month accuracy level. Unfortunately, this can only be done when the sampling interval is short. As compared with the current situation, much improvement could be achieved if the sampling could be done at 3-month intervals. In most settings (large-scale multi-centre trials), this is not a realistic goal, however, but the sampling intervals are necessarily longer, ranging from six months to one year or even longer. It is the conviction of this author that the misclassification (persistent/not persistent) increases rapidly in settings where the sampling intervals exceed one year. This is simply because (due to the reasons not well understood) many of the key events affecting HPV persistence (acquisition, clearance) do seem to take place within the time frame of around 12 months or even shorter [appropriate literature cited in 11, 14, 15, 17]. With the sampling interval of six months, these events can be traced with reasonable accuracy, albeit not flawless to define the exact duration of infections in all cases.

To increase the complexity, the context at which HPV persistence is detected also needs to be considered. Referring back to the six outcome patterns established in the early follow-up studies cited above [8, 72–77], it is evident that viral persistence can be associated with all of them, and not only among women classified as persistors in this overall outcome assessment, who represent a minority of the cohort after ten years of follow-up [8, 72–77]. To fully exploit the entire follow-up cohort in any such analysis for HPV persistence, all six outcome patterns need to be considered and further stratified to identify every single woman with the recorded HPV detection data fulfilling the criteria of e.g., 6-month or 12-month persistence. This applies equally well to 1) early regressors (those cleared after > 6 months of duration), to 2) persistors, and 3) fluctuators (with persistent episodes lasting > 6 months), as well as to 4) late regressors, 5) progressors, and 6) recurrent disease category, the latter being closely associated with viral persistence [78]. Because of its inherent study design, the Finnish HPV Cohort Study run between 1981–1998 did not include women who were baseline negative for clinical HPV lesions. Thus, another outcome category needs to be added in the above list; women with incident disease, which usually follows incident HPV infection with two to three months of delay [11, 15].

Because of the fact that the aim of all these efforts [35] is to assess, as reliably as possible, the real value of HR-HPV persistence as a surrogate endpoint of progressive disease, it is essential to consider only those cases which represent true viral persistence. Referring to the above listed seven patterns (incident event included), caution must be exerted while classifying women as persistors from some of these categories. By definition, HPV infection is not persistent if it is transient in outcome. Of the above seven outcome categories, viral persistence is explicit only in three of them: persistors, progressors and recurrent outcome. In all others (early regression, late regression, fluctuation, incident disease), HPV infection can be potentially transient in nature. Strictly speaking, women from these outcome patterns should not be automatically included among the 6M+ or 12M+ HPV persistence group (even if otherwise eligible), without definite confirmation of their final outcome. Because there is no means to verify this “final outcome” beyond the termination of patient monitoring, the simple most consistent approach of doing this is to assess whether HPV infection persists or is cleared at the last follow-up visit. If persistent, the woman is eligible for the HPV persistence group, otherwise she should be included in the reference group (HPV-transient reference). Because these issues are closely related to the definitions of the referent groups, they will be discussed in more detail in that specific section.
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Algorithm for HPV genotype-specific persistence

To translate the data on HPV persistence accumulated from our long-term cohort studies [8, 64, 65, 72-77] to the level of individual genotypes, an algorithm has recently been created to define HPV genotype-specific persistence [79]. Because pertinent to considerations as how to produce reproducible results, this new algorithm deserves some more treatise also in this context.

In longitudinal studies with repeated HPV testing results available using any of the genotyping techniques, the necessary first step is to define the genotype-specific outcome of HPV infection for each woman, by comparing the viral status at each FU visit to the baseline HPV status. As the first step, the six distinct main outcomes must be identified: 1) always HPV negative; 2) incident HPV infection; 3) genotype-specific persistence; 4) non-genotype-specific persistence; 5) fluctuation, and 6) virus clearance. Of these 1) and 2) are straightforward. 3) Genotype-specific persistence denotes for any case with two (or more) consecutive FU-samples positive for the same individual genotype (or genotypes in multiple-type infections). 4) Non-genotype specific persistence includes all cases with two (or more) consecutive samples being HPV positive, but not for the same individual genotype. 5) Fluctuation is a pattern where consecutive samples are intermittently HPV+ and HPV-, without any two consecutive samples positive for the same or different viral genotype. 6) In this primary categorization, virus clearance should include only the baseline HPV+ cases who have cleared their infection by or at the last FU visit.

As explained above, HPV infections in individual women can fall into more than one outcome category, and a secondary classification is necessary to maximally exploit the data of all outcomes. Accordingly, the patterns 2, 3, 4 and 5 listed above can be further stratified to the two persistent-outcome categories (type-specific and not type-specific) as follows. Among category 2 (incident infections), we can pick up all cases demonstrating genotype- and non-genotype-specific persistence of this incident infection. Similarly, the original categories 3 and 4 can be further stratified to genotype-specific and non-genotype specific persistence, whenever different or additional to these same categories in the first-line assessment. By definition, category 5 (fluctuation) does not include any viral persistence, but, importantly, the cases testing HPV-negative at the last FU visit should be classified as clearance, to indicate that these infections were transient in nature. The same might apply to category 2 as well, if the incident infection is cleared without persistence. Of the last category 6), the cases fulfilling the criteria of type-specific or non-type specific persistence to be included as such in this secondary classification can also be identified. As discussed later, in calculations comparing HPV persistence using the HPV transient outcome reference group, these women should be included in the latter [44].

As the final step, persistors from the first-line and second-line classifications must be combined to create a new variable (e.g., combined persistence), including both type-specific and non-type-specific persistors. Having completed this, the individual genotypes responsible for either type-specific or non-type-specific persistence at each FU visit are identified, called persisting genotype. Because of the multitude of individual genotypes and their combinations showing persistence, the last step would be the conversion of individual genotypes to HPV species, following the newly described phylogenetic classification of papillomaviruses [80]. By doing that, it can also be easily assessed whether or not the species that persists is the same as detected at the first HPV-positive visit (baseline species), which enables computing the species-specific persistence [79].

Intermediate endpoints of disease progression

The other variable with significant impact on the risk estimates for HPV persistence as a predictor of progressive disease is the intermediate (surrogate) endpoint used to define the progressive disease [35, 43, 44]. In most of the published studies, only CIN2/3 or CC has been used as an endpoint [26, 35, 45-50], whereas the lower grade endpoints (ASCUS, CIN1/LSIL) have been used more rarely [26, 45, 46]. One of the messages of the meta-analysis was that the strength of association between HPV persistence and cervical neoplasia seems to increase with the increasing grade of the endpoint [35].

This was confirmed only in part by our recent studies where several surrogate endpoints of progressive disease were tested, including both histological and cytological as well as their combination [43, 44]. As evident from Table 3, RRs are higher for the CIN2+ endpoint (RR > 18) than for the CIN1+ endpoint (RR = 6.6-9.6), but this is only true with the HPV negative referent group, and even then, RRs for the SIL endpoint seem to be slightly higher (RR > 20). The SIL endpoint in our study also includes all HSIL cases, however, contributing to the increased RR for this surrogate endpoint [44]. On the other hand, RRs with the combined CIN/SIL (RR = 11.7-16.42) endpoint are slightly downgraded by the inclusion of all CIN1 and LSIL cases among this surrogate endpoint [44].

As to the effect of the surrogate endpoints in referent groups other than the HPV negative group, there was no consistent grade-related trend (Table 3). The highest single RRs were obtained for the CIN1+ endpoint, but because of no events, RRs were not computable for CIN2+ (6M+ persistence). Noteworthy is the fact that both 6M+ and 12M+ viral persistence was significantly associated also with SIL and CIN/SIL endpoints, when either HPV transient or HPV mixed outcome referent groups were used. In these settings, RRs fall between 2 and 5.5, which seems to be in good agreement with the RRs (far below 10) in comparable studies included in the meta-analysis of Koshiol et al. [35].
HR-HPV cofactors for incident CIN1, CIN2 and CIN3 are different

One of the reasons for this obvious difference in the strength of association with HR-HPV persistence between the different surrogate endpoints (SIL, CIN1, CIN2, CIN3) might be true differences in their biological potential as surrogates of progressive disease [63]. Indeed, a novel approach to gain further insights in the genuine biological differences between CIN1, CIN2 and CIN3 is to assess whether the cofactors needed to promote the HR-HPV-driven disease progression differ at different stages of CIN. In other words, it is of interest to know if the HPV cofactors needed for progression from normal epithelium to i) CIN1 are different from those required for progression to ii) CIN2 and further iii) to CIN3, and whether any of these eventual differences in cofactors are related to the individual HPV genotypes [81-83]. We recently completed the first analysis of these cofactors in a prospective setting, using multinomial (polytomous) regression analysis for HR-HPV cofactors in increasing the risk of incident CIN1, CIN2 and CIN3 [63].

In the combined NIS-LAMS cohort of 1,865 women, 90 (4.8%), 39 (2.1%) and 14 (1.4%) cases progressed to CIN1, CIN2, and CIN3, respectively [63]. Baseline HR-HPV was the single most powerful predictor of incident CIN1, CIN2 and CIN3. When controlled for residual HPV confounding by analysing HR-HPV positive women only (n = 1,105), the risk profiles of incident CIN1, CIN2 and CIN3 were unique, i.e., completely different HPV cofactors were associated with progression to CIN1, CIN2 and CIN3 in univariate and multivariate analysis, irrespective of whether non-progression, CIN1 or CIN2 was used as the reference outcome. This study using polytomous logistic regression models in a prospective setting where residual confounding by HR-HPV was controlled, unequivocally demonstrates that different HPV cofactors are associated with incident CIN1, CIN2 and CIN3. These data substantiate the concept that each CIN grade represents a distinct biological entity, as also suggested by the extensive natural history data [8, 72-77]. This should have important implications in at least two fields: 1) lumping together of CIN2 and CIN3 in the histological classification of cervical cancer precursors should be revisited, and 2) one should reconsider using the combined CIN2/CIN3 endpoint in any studies assessing the risk factors of CIN/CC. The next urgent step is to assess whether these different cofactor profiles are linked with individual HR-HPV genotypes in this prospective cohort.

Extent of the follow-up time

Given the rarity of the incident CIN2+ and CIN3+, reliable incidence rates cannot be expected in studies where the follow-up times are too short, particularly if less than two years. This is well illustrated by our historical Kuopio cohort, where > 500 women with clinical HPV infection at baseline were followed-up for almost 20 years (1981-1998). Results from this study have been presented in several communications [72-77], and discussed in detail in our textbook [8]. Some of the lessons learned from this classical study deserve to be addressed here as well, because they are pertinent to the present discussion.

Soon after the onset of the Kuopio cohort in 1981, it became apparent that cervical HPV lesions in individual patients can adopt at least four outcomes: regression, persistence, progression, and recurrence [8]. It soon became evident also that the proportion of women classifiable into these four categories is clearly time-dependent and subject to continuous variation over time. During this long-term follow-up, two important patterns in the natural history of cervical HPV lesions emerged: 1) trends in regression, and 2) those in disease progression. Importantly, the effect of the follow-up time on these two outcomes is entirely different [8].

During the follow-up, it was observed that the rate of spontaneous regression increased in parallel with the follow-up time, from 24.8% at 25 months to 39.7% at 45 months, further to 54% at 57 months, and up to 63.8% after 83 months. Thereafter, the proportion of lesions undergoing regression increased very slowly, to 66.4% at 96 months, and reaching the 68.9% plateau after 123 months (i.e., 10 years) of the mean observation period. This is important while interpreting the different cohort studies, the majority of which have been run for a relatively short time only [84]. Importantly, a very long follow-up is needed to reach the plateau in the spontaneous regression rates, which approaches 70% in ten years [8, 72-77].

Even more important than spontaneous regression is, however, progression of HPV lesions to high-grade CIN. The Kuopio cohort was the first to demonstrate that disease progression follows a temporal pattern completely different from that of regression. In contrast to increasing regression rates over time, no such increase could be observed in progression rates [8, 72-77]. The hard fact seems to be that the progression rate levels off at around 14%-15% after the first 24 months of follow-up, and remains unchanged through the entire 10-year observation period [8]. In practical terms, this means that HPV lesions predestined to progression do so quite rapidly, almost invariably during the first two years of follow-up. It is important to remember, however, that all women in the Kuopio cohort were HPV-positive at baseline (i.e., clinical disease on Pap smear, colposcopy and biopsy), which certainly contributes to this relatively short time to progression. Certainly, progression to CIN among baseline HPV-negative women is 1) much more rare, and 2) takes a considerably longer time. Recent evidence also indicates that there are differences in progression times and progression rates between individual HR-HPV genotypes [28-34], and the same applies to the times of type-specific persistence, which seems to be subject to considerable variation as well [79, 85].

The most important implication of these historical data has been that practically all major cohort studies conducted for baseline HPV-positive women have started relying on this two-year observation period, during which practically all...
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Progressing cases can be detected. The same is true with the NIS and LAMS cohorts as well. In practical terms, ten years are needed to detect all cases that will eventually regress, but only two years to disclose the progression of events among these baseline HPV-positive women.

Reference groups used in calculating the risk estimates

In the above, reference was already made to those several issues that remain to be clarified, before reproducible risk estimates can be provided for the association between persistent HPV infection and progression to CIN [35, 43, 44]. Most of these have been addressed in the preceding sections of this communication. The last but not least of these open issues is the way how the risk estimates are calculated for the association of HR-HPV persistence and incident CIN [35, 44]. In the above-mentioned meta-analysis [35] as well as in our recent approach [44], it became apparent that one of the variables with the highest impact on these risk estimates is the type of the reference category used in these calculations. In calculating the risk estimates for persistent HR-HPV to predict incident CIN endpoints, three different reference categories can be used: 1) HPV negative women; 2) women with transient HR-HPV; and 3) HPV mixed-outcome group [35, 44] to be discussed in brief next.

HPV mixed outcome reference group

As the name implies, this is the most heterogeneous of these three reference groups, consisting of women in whom HPV infections can run a highly divergent course. Due to this reason, also the risk estimates for 6M+ and 12M+ persistence obtained using this reference group are low compared with those calculated using the HPV-negative reference category, but not markedly different from those obtained with the HPV transient outcome category [35, 44].

As discussed in context with our classical cohort [8, 72-77], at least six different outcome patterns can be recognised for HPV infections during long-term follow-up. When always negative women and those showing transient infections (= virus clearance) are excluded, this leaves all the other outcomes eligible for this mixed outcome reference category. In a recent approach [44], included in this category were all women who did not meet the criteria of HPV negative- or HPV transient groups. Most notably, this group includes women whose HPV infections demonstrated a fluctuating course, with HPV-positive test(s) followed by negative test(s) and subsequently by another positive test, or alternatively -/+/-+. Importantly, these women i) did not demonstrate 6M+ or 12M+ persistence, and ii) they did not have their HR-HPV infection cleared at the last visit, which make them distinct from the HPV transient group. Even today, this fluctuating course is still poorly understood [8], and it remains a potential source of several types of bias in these assessments. Indeed, we cannot exclude the possibility that the last-visit HPV+ sample represents the onset of persistence, which, however, remains undetected because the follow-up is terminated at this visit. In the same way, if a long HPV-negative phase extends over 2 to several visits in this process of fluctuation, there is a possibility of misclassifying these women as HPV-negative or even HPV transient, if this period overlaps the last follow-up visit.

On the other hand, some of the women with short-term fluctuation might eventually prove to be those with persistent infection in long-term (several years) follow-up [8]. In our analysis, this is indirectly supported by the fact that these women are at relatively high risk for incident CIN2+, as shown by the non-significant RR (2.04, 95% CI 0.75-5.54) for 12M+ persistence using this reference category (Table 3). On the other hand, the risk of developing CIN1+ seems low because 6M+ persistence, RR = 21.56 (95% CI 2.93-158.7), is higher than obtained in comparison with the HPV-negative referent group. The risk estimates for SIL and CIN/SIL endpoints are low (but significant), implicating that the risk of developing these endpoints is somewhat lower than among 6M+ and 12M+ HPV persistent women, but still substantial [44].

HPV transient reference group

In the discussed meta-analysis, studies where women with persistent HPV infections were compared to those with transient HPV infections usually reported the lowest RRs [35]. This was our experience as well [44]. Again, we need to pay some attention to the approach used to define these transient infections. In some studies, all infections that eventually regress during the follow-up are transient, whereas in some others, infections that cleared within less than six months (6M-) or 12 months (12M-) were defined as transient. In the former, such transient infections potentially estimate the effect of HPV persistence beyond short-term infections, whereas in the latter, they clearly denote short-term viral exposure. In our study [44], transient was defined as any HR-HPV infection that cleared during the prospective follow-up, i.e., tested HPV-negative at the last visit, irrespective the duration of infection. In doing so, we wanted to distinguish this group of transient infections from those women who have persistent (6M+ or 12M+) infection that did not clear during the follow-up. Using this strict definition (cleared/not cleared), we obtain RRs for 12M+ persistence as high as 10.18, whereas the RRs for 6M+ were either not computable (for CIN1+ and CIN2+ surrogate) or fall around 3 (for SIL) and 5 (for CIN/SIL) (Table 3).

Being substantially lower (but still statistically significant) than RRs obtained in comparison with the HPV negative reference group, these risk estimates in the persistent-transient comparison indicate that women in the latter represent a heterogeneous group, where: i) the risk of disease progression is far from zero in some women, but ii) very low in
some others. The former would include those who had their transient infection persisting but cleared at the end, whereas the latter represent women with true short-term transient infections. Furthermore, the possibility cannot be completely excluded that some of the women classified as transient HPV infections actually had persistent infections prior to the baseline HPV testing, and as such were actually misclassified. The way to control for that is to include only baseline HPV-negative women, which would significantly reduce the size of this reference group, however [44].

**HPV negative reference group**

As pointed out, of all influential variables, the reference group has the most dramatic impact on the risk estimates for the association between 6M+ and 12M+ viral persistence and disease progression [35, 44]. This was clearly shown in the analysis as well, where RRs were of different magnitude (RR = 10-27) as compared with the others (RR = 2-5), when the HPV negative referent group was used in calculating these estimates (Table 3). This applies to all other surrogates except CIN1+, in which the highest RRs were obtained using the two other reference categories. These data are fully consistent with the results of the studies included in the recent meta-analysis [35], where the use of HPV negative women as the reference group resulted in the highest RRs. This is feasibly explained by the fact that the risk of incident CIN2-3/HSIL+ approaches zero among HPV negative women [26, 35, 45-50, 85]. This seems to apply equally well to SIL and CIN/SIL endpoints [85], as shown by RRs up to 27 when women with 6M+/12M+ persistent HR-HPV are compared with HPV negative women in the NIS-LAMS cohort (Table 3) [44].

**Conclusions**

The present communication addressed the issues to be considered before adoption of viral endpoints (6M+ and/or 12M+ HR-HPV persistence) instead of the conventional histological endpoint (CIN2+) as new surrogates of progressive disease, e.g., in future efficacy trials with the new generation prophylactic non-HPV16/18 vaccines [19, 31, 32]. The data reviewed in this discussion implicate that persistent HR-HPV infections (6M+ and 12M+) are powerful predictors consistently associated with progressive cervical disease defined by surrogate intermediate endpoints SIL, CIN1+, CIN2+ and CIN/SIL. However, there seems to be substantial variation in the risk estimates of these associations, which seem to depend on several variables, as discussed in more detail above.

One of the questions is, whether the 6M+ or 12M+ HR-HPV persistence endpoint should be selected, i.e., are there major differences in the risk estimates between these two? Indeed, there seems to be some variation in these risk estimates, depending of the length of HR-HPV persistence [43, 44]. In our study, RRs calculated for 12M+ persistence were higher than those obtained for 6M+ persistence criteria, with few exceptions. This difference seems to persist, irrespective of which of the three reference categories was used. The only major exception was the substantially higher risk (RR = 21.6) for 6M+ persistence to associate with CIN1+ as compared with that (RR = 5.7) of 12M+ persistence, when HPV mixed outcome was used as the reference group [44]. Importantly, there were no exceptions to this rule when the data were calculated using the HPV negative reference category, where 12M+ persistence invariably gave higher RRs (Table 3). These observations are in full agreement with the data reported in the meta-analysis, where the associations between HPV persistence and CIN/CC appeared stronger in studies with longer duration of HPV infection (12M+) [35]. To this author, this observation sounds the only logical one considering the basic biology of HPV infections [8, 12, 16, 17], indicating that a longer duration of HR-HPV infections means longer exposure to viral oncogenes and increases the likelihood of developing progressive disease [85].

Taken together, it is suggested that in all future studies (whether vaccine efficacy trials or screening trials), using the 6M+ or 12M+ HR-HPV persistence as a surrogate endpoint of progressive disease, a “gold standard” should be used in calculating the risk estimates for this association. In addition to deciding, 1) whether to use 6M+ or 12M+ persistence criteria, and 2) cytological, histological or combined surrogate endpoints (SIL, CIN1, CIN2, CIN/SIL), one should 3) start using exclusively the HPV negative reference group in calculating the risk estimates for viral persistence endpoints. This is supported by the data from the recent meta-analysis [35] as well as from the author’s combined NIS-LAMS cohort [43, 44], both implicating that the most consistent association to progressive disease is obtained when women with persistent HR-HPV are compared with HPV-negative women. It is the conviction of this author that the two other reference categories (HPV transient and HPV mixed outcome) are far too heterogeneous and subject to potential misclassifications to give consistent and reproducible risk estimates for HR-HPV persistence as surrogate endpoint of progressive CIN.

**Acknowledgements**

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Persistent high-risk human papillomavirus (HPV) infections as surrogate endpoints of progressive cervical disease. Potential new etc.


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Clinicopathologic analysis of extramammary Paget’s disease

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Summary
Purpose: To retrospectively analyze the clinicopathologic characteristics of patients with extramammary Paget’s disease who were surgically treated in a single institution. Method: The charts of 14 patients with extramammary Paget’s disease were retrospectively reviewed, and the clinicopathologic data were collected and analyzed. Results: From January 1990 to July 2009, 14 patients were treated at our institution. Most patients (11/14 patients) had delayed diagnosis. Two patients (14.3%) had associated malignant neoplasms. Eight of 14 patients (57.1%) had positive surgical margins; of these patients, five patients had no evidence of recurrence. In the six patients with negative surgical margins, two patients (33.3%) developed recurrence. Conclusions: The diagnosis of extramammary Paget’s disease is commonly delayed. Because of the possible association with other malignancies before or after the diagnosis of extramammary Paget’s disease, thorough examinations are recommended. Disease recurrence is common regardless of the surgical margin status, so long-term monitoring of patients is recommended.

Key words: Extramammary Paget’s disease; Retrospective; Clinicopathologic analysis.

Introduction
Sir James Paget described a nipple skin lesion associated with invasive ductal carcinoma of the breast in 1874 [1]; similarly, skin lesions on the vulva were first described by Dubrennilh in 1901 [2]. Extramammary Paget’s disease (EMPD) occurs mainly among elderly, postmenopausal women and is a rare (1%-2%) neoplastic vulgar lesion [3, 4]. Clinically, EMPD is most commonly accompanied by pruritus (70%) [5, 6]. Due to the non-specific clinical presentation, such as a pink, eczematoid area with hyperkeratosis, these lesions are often mistaken for eczema or contact dermatitis, so the diagnosis and treatment are often delayed [5]. Because the tumor cells generally spread in situ in the epidermis, surgical excision is the standard treatment; however, the tumor usually extends well beyond the gross lesion based on histopathologic examination, and recurrence is common (21%-61%) [6, 9]. Thus, extensive resection with a sufficient tumor-free surgical margin is needed [10, 13]. Also, because there is an associated underlying skin or visceral adenocarcinoma in 20%-55% of cases, thorough examinations to identify other regional rectal, urothelial, or vulvar malignancies has been recommended at the time of diagnosis [7, 9, 12-16].

Because EMPD is such a rare condition, it has not been possible to estimate the true incidence or frequency with which it becomes clinically malignant or co-exists with a visceral adenocarcinoma. In addition, the clinical importance of positive surgical margins is unclear. The aim of this study was to retrospectively analyze the clinicopathologic characteristics of patients with EMPD who were surgically treated in a single institution.

Materials and Methods
After obtaining approval from our Institutional Review Board (IRB), our medical database was reviewed to identify all women with EMPD. Fourteen women who were surgically treated in the Division of Gynecologic Oncology at our institution between January 1990 and July 2009 were identified. The charts of these patients were retrospectively reviewed, and the data were collected regarding patient demographics, previous treatment, symptoms, disease location, surgical margin status, depth of invasion, associated malignancies, and status of recurrence. Disease recurrence was defined as a new lesion in a period > 6 months after surgery. Duration of follow-up was calculated as a period from pathologic diagnosis to the last visit. The relationship between microscopic margin status and disease recurrence was investigated.

Results
Fourteen patients with EMPD of the vulva were treated at our institution during the study period. The mean age of the patients was 54.3 years (range 29-72 years). The mean gravidity of the patients was 2.57 (range, 0-6). The topography of the lesion was the right labium [4], left labium [6], and bilateral labia [4]. The most common symptom at the time of diagnosis was vulvar pruritus (7/14 patients). Except for three patients who were asymptomatic and diagnosed incidentally, most patients (11/14 patients) had a delay from initial symptoms to pathologic diagnosis for a long time, with a median delay in diagnosis of 61.8 months (range, 4 months to 10 years). Two patients (14.3%) were treated with unilateral vulvectomies, five patients (35.7%) had wide local excisions (WLEs), and seven patients (50%) underwent radical vulvectomies. Also, except for four patients with primary repairs, ten patients underwent reconstructive procedures by a plastic surgeon (skin graft [1], fasciocutaneous flap [8], and gracilis myocutaneous flap [11]). Two patients (14.3%) had associations with malignant neoplasms; one

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Discussion

EMPD is a rare disease which primarily affects elderly, postmenopausal women. Although we identified two cases involving 29-year-old patients, 11 of the other 12 patients were postmenopausal, with mean age of 58.6 years. Of note, our patients had a delay from initial symptoms to pathologic diagnosis of EMPD because of the non-specificity of their symptoms. Except for three patients who were asymptomatic and diagnosed incidentally, most patients (11/14) had delayed diagnosis for a long time, with a median of 61.8 months (range, 4 months to 10 years). Most patients had a history of using topical corticosteroids for the treatment of pruritus. Late diagnosis also occurred because many patients preferred not to visit a gynecologist due to personal taboos or a fear of malignancy [14].

Skin biopsy should be performed on all patients with pruritic eczematous lesions of apocrine gland-bearing areas that have failed to respond to standard topical treatment [13]. The differential diagnosis should include contact dermatitis, psoriasis, fungal infections, seborrheic dermatitis, lichen sclerosis, anogenital intraepithelial neoplasia, melanoma, histiocytosis, and mycosis fungoides [17]. Based on histopathologic examination, Paget’s cells are large round cells with abundant pale cytoplasm and large vesicular nuclei, which may be central or laterally compressed. Mitotic figures are unusual. The lesions may be distributed singly or in groups within the epidermis and epithelia of adnexal structures [13].

Immunohistochemistry has been used to diagnose Paget’s disease and to identify the likely cell of origin. Paget’s cells typically stain for markers of apocrine and eccrine derivation, including low molecular weight cytokeratin (CK), gross cystic disease fluid protein (GCDFP-15), periodic acid-Schiff (PAS), and carcinoembryonic antigen (CEA); staining for S100 is negative [13].

Several studies have reported a high frequency (20%-55%) of associated underlying skin or visceral adenocarcinomas [7, 9, 12-16, 18-21]; more than 14.3% occurred in our study. Taking into consideration that EMPD occurs in elderly and postmenopausal women, patients with EMPD have an increased risk of developing a second malignancy [5]. Thorough examinations to detect other

patient had invasive vulvar adenocarcinoma, and one patient who had a history of breast cancer and underwent surgery > 10 years earlier was diagnosed with recurrent breast cancer during the 6-month follow-up period. Most patients (13/14 patients [92.8%]) had Paget cells confined to the epidermis; one patient had Paget cells infiltrating into the subcutaneous tissue with inguinofemoral lymph node metastasis and underwent postoperative adjuvant radiation treatment. The patient with underlying vulvar adenocarcinoma also underwent inguinofemoral lymph node dissection and postoperative adjuvant radiation treatment. Eight of 14 patients (57.1%) had positive surgical margins; of these patients, five patients who were followed regularly until recently had no evidence of recurrence after a median follow-up of 43 months. Of the six patients with negative surgical margins, two (33.3%) developed recurrences and received radiation treatment.
The standard treatment for EMPD is surgical excision. Traditionally, radical vulvectomy has been done because of the high recurrence rate and the risk of an underlying adenocarcinoma. However, this type of surgery is associated with significant disfigurement and a persistent local recurrence rate due to the characteristics of EMPD, such as multicentricity and ill-defined margins [6, 7, 9, 15, 22, 23]. Therefore, many gynecologists advocate WLE of the visible lesion with resection to the fascia and a 2-3 cm grossly normal skin margin is adequate. In the current study, two patients (14.3%) were treated with unilateral vulvectomy, five patients (35.7%) had WLEs, and seven patients (50%) underwent radical vulvectomy with or without reconstructive procedures by a plastic surgeon. A high proportion of the radical vulvectomies in the current study may have been associated with the topography of the disease, the size of the lesion, and the fear of recurrence.

Some investigators have reported a reduction in local disease recurrence by up to 50% following surgical excision of vulvar EMPD with the use of intraoperative frozen section analysis [24], while others have reported that frozen section analysis of surgical margins can be misleading in EMPD with false negative rates of 35% [25]. Indeed, permanent margin status is not predictive of local recurrence [6, 12, 25]. Although we did not use frozen section analysis in our study, the patients with positive surgical margins have had no evidence of local recurrence until recently after a median follow-up of 43 months. In the patients with negative surgical margins, two patients (33.3%) developed recurrences at 33 and 67 months postoperatively. These data suggest that patients with EMPD require long-term follow-up and careful examination of any suspicious vulvar lesions.

A limitation of our study was that number of patients was small due to the rarity of the disease. However, our report has highlighted the current state of clinicopathology, management, and prognosis of EMPD. The therapeutic strategies offering both a low rate of recurrence and minimal tissue destruction requires further investigation.

References

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Is the combination of mitomycin C, bleomycin and methotrexate effective as a neoadjuvant treatment for cervical cancer in women?


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Summary

Objective: To determine the effectiveness of the combination of mitomycin C, bleomycin and methotrexate as a neoadjuvant treatment in preparation for surgical treatment of cervical cancer. Methods and Materials: Twenty-seven patients with carcinoma of the uterine cervix (stages exophytic IB2 and IIB-IIIB) who had not previously undergone any treatment received mitomycin C, bleomycin and methotrexate in five sessions, once every four weeks. Results: The objective response rate was approximately 81%, including 16 complete responses and six partial responses. Significant toxic effects were not observed. Responsive patients underwent surgery and remained without evidence of disease for the next 20 years. Unresponsive patients did not fare well and passed away within five years after treatment. Conclusion: Our data suggest that this strategy may be effective for advanced cases, enabling patients to receive surgical treatment.

Key words: Bleomycin; Mitomycin; Methotrexate; Cervical cancer; Chemotherapy.

Introduction

Uterine cervix cancer (in its diverse histological types and clinical forms) is among the most frequently occurring female tumors in developing countries [1]. The average after-treatment life span of five years is inversely proportional to the clinicopathological stage of the tumor. Thus, patients with tumors in Stages I and IB have a remaining life span of five years in 88% and 98.5% of cases, respectively, and the lymphatic nodules are compromised in 20% to 30% of the cases. In Stages II and IIIB, the percentage of patients with 5-year survival decreases to 65% and then drops further to 14% to 15% when tumors are in Stage IV. Lymphatic nodules are involved in 30% to 50% of cases in Stage II and in 60% or more of cases in Stages III and IV [2].

Surgery and radiotherapy are adequate and effective treatments for tumors in the early stages (I and II). However, the remaining after-treatment life span is directly related to the volume of the tumor and, above all, to the extent to which the lymphatic nodules are compromised [3, 4]. In fact, good results have also been achieved for tumors in Stages I and II, especially for those of smaller size, when they were submitted to partial or total hysterectomy with or without radiotherapy [5]. The exception is the exophytic cervical tumor in Stage IB2; it is usually voluminous, making the surgical approach difficult [2].

Satisfactory results have not been obtained for tumors in Stages III and IV submitted to surgical therapy and/or radiotherapy. In this situation, the prescribed treatment includes adjuvant chemotheraphy. This, however, is a palliative therapy chiefly applied to recurrent tumors not amenable to surgical or radiotherapeutic treatment or to metastatic tumors, or as surgical and/or radiotherapeutic post-treatment [6]. Despite advances in modern chemotherapy, results have not been satisfactory when using this technique [7]. Hence, uterine cervical carcinoma may be considered to have low sensitivity to chemotherapeutic agents [6]. Previous therapeutic failures might be partially attributed to the non-ideal conditions in which adjuvant chemotherapy has been employed. For example, in relapse cases, areas previously submitted to radical surgery hinder the diffusion of antiblastic agents at ideal pharmacological concentrations and cause a large percentage of cells to enter the resting phase due to vascular deficit and hypoxia [8, 9].

Antineoplastic chemotherapy can employ mitomycin C, whose mechanism includes cross-linking of several types of DNA, the promotion of DNA degradation and the inhibition of DNA synthesis [10]. Bleomycin is isolated from Streptomyces verticillus. Like all antitumoral antibiotics, it is a bacterial product that can inhibit the proliferation or function of cancer cells by preventing the incorporation of thymidine into DNA, thus inhibiting synthesis [11]. Bleomycin appears to exert a specific clinical effect on the epidermoid type of carcinoma. Methotrexate inhibits folic acid reduction during DNA synthesis in cell replication [12]. Chemotherapeutic agents act in different phases of the cell cycle; thus, the use of different combinations of drugs not only allows the expansion of the chemotherapeutic scheme but also makes it possible to reduce the doses of each agent. The desired effects can therefore be increased while toxicity is decreased [6].

There are few data regarding neoadjuvant chemotherapeutic treatments with the combination of mitomycin C, bleomycin and methotrexate and possibly followed by...
surgery in women with uterine cervical carcinoma. Thus, this study aimed to evaluate the effectiveness and adverse reactions of this treatment with a 20-year follow-up.

Methods and Materials

This was an open-label, prospective study with 27 volunteers selected from 60 women with uterine cervical neoplasia between May 1980 and May 1985. All of them had either exophytic tumors in Stage IB2 or tumors in Stages IIB-IIIB. These were the inclusion criteria: absence of distant metastasis and of previous treatment for another type of cancer as well as contraindication for chemotherapeutic agents (cardiovascular disease and anemia of unknown etiology). Furthermore, women over the age of 75 were not included. Before starting therapy, all women were informed of the protocol, and they signed an informed consent prior to participate. The study project was approved by the ethics committee of the institution. Participants were submitted to a neoadjuvant chemotherapeutic scheme. Ages ranged from 27 years to 68 years.

To measure the diameter of the cervix before and after treatment, we developed a device to measure the largest diameter of the uterine cervix. The device had two 15 cm-long aluminum stems with fixed articulations between them, and at the point where the two stems came together, there was a semicircular scale, also made of aluminum. The scale had marks ranging from 2-10 cm at 2-cm intervals, and it was fastened to the stems by three screws and three brass washers to allow opening and closing of the stems. Measurements that had been previously obtained were classified as < 6 cm and > 6 cm. Two patients were in the < 6 cm category prior to treatment, and all others had a cervix measuring over 6 cm in diameter. Six patients had tumors in Stage IB, three in Stage IIA, nine in Stage IIB, two in Stage IIIA, and seven in Stage IIIB.

The chemotherapeutic scheme herein proposed as a neoadjuvant consisted of at least three courses and at most five courses lasting one day each. There were four to six week intervals between the courses. The scheme follows below: bleomycin: 30 mg/m² in intravenous infusion with physiological saline solution given immediately after the administration of bleomycin; mitomycin C: 20 mg in intravenous bolus given at the end of the mycin C.

Of the 27 volunteers, five patients. No cases of progressive disease were found. Thirteen patients presented adverse reactions, mostly nausea, vomiting (50%) and altered values of hemoglobin and leucopenia (50%). These reactions did not limit the application of the proposed treatment. Five patients (18.5%) suffered from discrete alopecia, and one patient had injection-site necrosis resulting from overflow of mitomycin C.

Table 1 shows the values of the objective response with respect to the clinical observations prior to treatment, involvement of parametria, clinical stages, histological types and degrees, and cervical diameter prior to therapeutic program.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Objective Response Number of patients</th>
<th>No Response Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical observations</td>
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<td></td>
</tr>
<tr>
<td>Tumor restricted to cervix</td>
<td>17/19 89.47</td>
<td>2/19 10.53</td>
</tr>
<tr>
<td>Cervical + vaginal tumor</td>
<td>5/8 63.00</td>
<td>3/8 37.00</td>
</tr>
<tr>
<td>Involvement of the parametria</td>
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<td></td>
</tr>
<tr>
<td>Right</td>
<td>6/7 85.71</td>
<td>1/7 14.29</td>
</tr>
<tr>
<td>Left</td>
<td>2/3 66.67</td>
<td>1/3 33.33</td>
</tr>
<tr>
<td>Right + Left</td>
<td>4/5 80.00</td>
<td>1/5 20.00</td>
</tr>
<tr>
<td>Free</td>
<td>9/11 81.82</td>
<td>2/11 18.18</td>
</tr>
<tr>
<td>Clinical Stages</td>
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<td></td>
</tr>
<tr>
<td>IA</td>
<td>6/6 100.00</td>
<td>0 0</td>
</tr>
<tr>
<td>IIA</td>
<td>2/3 66.67</td>
<td>1/3 33.33</td>
</tr>
<tr>
<td>IIB</td>
<td>9/9 100.00</td>
<td>0 0</td>
</tr>
<tr>
<td>IIIA</td>
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<td>1/2 50.00</td>
</tr>
<tr>
<td>IIIB</td>
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<td>3/7 42.86</td>
</tr>
<tr>
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<tr>
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<td>5/24 20.83</td>
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<tr>
<td>Adenocarcinoma</td>
<td>3/3 100.00</td>
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</tr>
<tr>
<td>Histological degrees</td>
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<td></td>
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<tr>
<td>I</td>
<td>3/3 100.00</td>
<td>0 0</td>
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<tr>
<td>II</td>
<td>10/12 83.33</td>
<td>2/12 16.67</td>
</tr>
<tr>
<td>III</td>
<td>9/12 75.00</td>
<td>3/12 25.0</td>
</tr>
<tr>
<td>Cervical diameter prior to treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 6 cm</td>
<td>20/25 80.00</td>
<td>5/25 20.0</td>
</tr>
<tr>
<td>≤ 6 cm</td>
<td>2/2 100.00</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Statistical analysis

Responses were categorized as complete response (CR; total regression of the tumor), partial response (PR; regression of the cervix diameter was equal to or greater than 50% of the initial measurement), stable disease (SD; regression of the cervix diameter was less than 50% of its initial measurement), progressive disease (PD; the cervix diameter lengthened).

Descriptions of the observed toxic effects and their consequences, if any, as well as abnormalities in the radiological and lab exams at the end of treatment programs were included.

Results

There was an objective response in 81.4% of the cases (16 complete responses and 6 partial responses). The disease stabilized toward the end of treatment in the other five patients. No cases of progressive disease were found.

Discussion

Diverse clinical studies give evidence of the value and efficacy of neoadjuvant chemotherapeutic schemes for the treatment of uterine cervix carcinoma, emphasizing benefits such as the early treatment of systemic micro metastases, reduction of primary tumor size, and enhanced possibility of surgical and/or radiotherapeutic complementation [13, 14]. This study showed that the combination of mitomycin C, bleomycin and methotrexate was effective and had no serious side-effects. Also,
more radical therapeutic complementation following chemotherapy was beneficial for the patients.

Chemotherapy as a first-rate treatment for advanced uterine cervical carcinoma was first suggested by Friedlander et al. [15] and shortly afterwards by Kim et al. [16]. Those studies were an important step forward not only in the treatment of this type of cancer but also in the role of chemotherapy. The reported responses were 67% and 100% when using a scheme that included cisplatin, vinblastine and bleomycin. As the use of neoadjuvant chemotherapy expanded, patients with tumors previously considered to be inoperable were able to undergo successful surgery after chemotherapy [13-16].

Most schemes utilized in neoadjuvant chemotherapy are based on combinations of agents that include cisplatin, and the objective responses to these treatments are in the 60%-100% range [15-17]. None of the previous studies have reported serious toxicity, although severe myelosuppression was observed by Rustin et al. [17], while Kim et al. [16] had to modify the dosage due to serious neutropenia.

Finally, our research suggests that better histological differentiation of tumors can increase the chance of a response to the proposed treatment. We therefore believe that the proposed neoadjuvant chemotherapeutic treatment might be an alternative for women with uterine cervical carcinoma, as it improves the indication for surgery and/or radiotherapy as a follow-up treatment without serious adverse effects.

References


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Cancer in pregnancy:
maternal and fetal implications on decision-making

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Introduction

Cancer complicates one out of 1,000 pregnancies. No standardized therapeutic interventions have been reported for these patients. Methods: Fifteen patients with cancer during pregnancy were diagnosed between 6.5 and 36 weeks of gestational age between January 1991 and December 2007. Results: Among the 15 cases one patient with early diagnosis (11 weeks) asked for interruption of pregnancy, two patients rejected chemotherapy in order to avoid fetal effects, seven patients underwent surgery during the first or second trimester, and two patients agreed to start the treatment only after delivery. Standard platinum-based chemotherapy (cisDDP) was postponed in six patients to the second trimester (administered after surgery in 2 cases). Chemotherapy was started between 18.3 and 29.6 weeks (median 22.3 weeks). One patient had pPROM (22.3 weeks) after chemotherapy with cisDDP. Ten patients were delivered by elective cesarean section and three by vaginal delivery. Mean gestational age at delivery was 33.5 weeks (range 32.1-40.0); mean weight at birth was 2,550 g (range 1,250-3,450). None of the newborns showed congenital malformations, and all had normal Apgar scores. Anemia occurred in two newborns. At a median follow-up of 56 months (range 2-198 months) all children were well and healthy. Eleven out of 15 mothers are alive and well, and one is alive with disease. An advanced neoplasm was diagnosed in three patients who died. Conclusion: When platinum-based chemotherapy is administered during the 2nd-3rd trimester, adverse effects in newborns are comparable to those in the general population. Deliberate treatment delay to achieve fetal viability or to improve fetal outcome may be reasonable for patients with early-stage cancer.

Key words: Cancer; Pregnancy; Fetal outcome; Chemotherapy; Surgery; Radiotherapy.

Case Reports

Clinical findings

In this retrospective analysis, 13 patients with a diagnosis of cancer during pregnancy were selected between January 1991 and December 2007 regardless of whether or not they received specific medication. All cases were managed and treated at the Gynecologic Oncology Unit and the Obstetrics Department, University of Brescia.

All patients were followed throughout their pregnancies by obstetricians and gynecologic oncologists experienced in high-risk pregnancies and neoplastic diseases. Patients were kept fully informed and treatment strategies were planned and carried out taking into consideration each patient’s decisions. All patients were carefully counselled about treatment options.

Treatment decision-making was based on several items: gestational age, fetal risks concerned with treatment, stage and prognosis of the disease, the patient’s medical condition, and the patient’s desire for pregnancy.

All patient information connected with this study was collected from the medical records. We selected data regarding the disease, such as histopathologic diagnosis, stage, timing and kind of treatment, and data regarding the pregnancy, with particular care about complications.
Diagnosis of cancer was made during physical and ultrasonographic examination planned for pregnancy or, in four cases, in relation with symptoms. Biopsies confirmed the neoplastic lesions in all cases.

Four patients were diagnosed in the first trimester, six in the second, and three in the third trimester of pregnancy.

The mean maternal age at diagnosis was 36 years (range: 22 to 42). Gestational age at diagnosis ranged from 8.3 to 37 weeks (mean 19 weeks) (Table 1).

Fetal outcome was assessed with birth weight, Apgar score (a 5-min Apgar score of 7-10 was considered normal) [6] and neonatal complications and pediatrician assessment of possible congenital malformations from hospital records. Prematurity was defined as delivery occurring at a gestational age of less than 37 weeks [7, 8] (Table 2).

Children were extensively followed-up. Physical and neurological development and clinical history were investigated. School performance and secondary sexual development were documented for the older children.

Clinical features of pregnant patients are listed in Table 1.

Cervical carcinoma

Six cases of cervical cancer were observed:

Patient 2: presented a diagnosis of squamous cervical carcinoma, Stage IB2 at 11 gestational weeks (GW). The woman’s preference was to terminate the pregnancy although an alternative option was offered and discussed. Afterward she underwent neoadjuvant chemotherapy (paclitaxel and cisplatin) followed by radical surgery.

Patient 6: presented a diagnosis of squamous cervical carcinoma, Stage IB2 at 20.2 GW. Eight days after the administration of the first course of chemotherapy, 90 mg of cisDDP (50 mg/m²), she was admitted to the hospital with extremely preterm premature rupture of the membranes (pPROM), which led to miscarriage within a few hours. Microbiological vaginal culture was negative. Chemotherapy was restarted seven days later, with the addition of paclitaxel (175 mg/m²). Chemotherapy was followed by radical surgery and adjuvant pelvic radiation for positive pelvic nodes. The patient was alive without evidence of disease two years after diagnosis.

Patient 8: at 20.5 GW was diagnosed with squamous cervical carcinoma Stage IIA. She received neoadjuvant chemotherapy with cisDDP 200 mg/m², VCR 4 mg, in four courses starting at 23.5 GW. Cesarean section was performed at 32 GW, 22 days after the last chemotherapy cycle. Concomitant with the cesarean section radical hysterectomy with pelvic lymphadenectomy was performed. The patient was alive without evidence of disease 13 years after diagnosis.

Patient 11: presented a diagnosis of squamous cervical carcinoma Stage IB2 at 24.5 GW. She underwent neoadjuvant chemotherapy with cisDDP 180 mg/m², in three courses, starting at 27 GW. Cesarean section was performed at 36 GW, 14 days after the last chemotherapy cycle. In the same surgical session radical hysterectomy with pelvic lymphadenectomy was performed. The patient is alive without evidence of disease three years after diagnosis.

Patient 13: presented a diagnosis of cervical cancer at 36 GW. Histological examination revealed an endocervical mucinous adenocarcinoma, Stage IB2. Cesarean section was performed at 40 GW; 14 days later she underwent neoadjuvant chemotherapy followed by radical surgery. After one year she was alive and well.

Patient 14: presented a Stage IIB cervical cancer at 30 GW. After a cycle of corticosteroid for fetal lung maturity induction, a cesarean section was performed. As a consequence of pelvic lymph nodal spread chemoradiation treatment was planned after surgery. The patient died of disease 32 months after treatment.

Ovarian cancer

Five cases of ovarian cancer occurred during pregnancy:

Patient 1: underwent right salpingo-oophorectomy by laparotomy at 6.5 GW for an adnexal mass which was later diagnosed as granulosa cell tumor Stage IIB. The patient decided to continue the pregnancy and to delay any chemotherapeutical treatment after delivery. After a cesarean section the patient received five courses of cisDDP, vinblastin and bleomycin. Fifteen years later the patient developed abdominal recurrence and, after surgical debulking, she is still in treatment with chemotherapy.

Patient 3: a persistent right ovarian mass was detected at 14 GW and subsequently removed by laparoscopic salpingo-oophorectomy. Stage IC mixed epithelial ovarian tumor of mucinous and endometrioid origin was detected. Adjuvant chemotherapy was considered postponable. The patient had a late preterm labor and delivered vaginally at 34.4 GW. After five months she was alive without evidence of disease.

### Table 1. — Clinical features of pregnant patients.

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age yrs</th>
<th>Gestational age at diagnosis (weeks)</th>
<th>Cancer</th>
<th>Stage</th>
<th>Gestational age at treatment (weeks)</th>
<th>Treatment</th>
<th>CT</th>
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**Table 2. — Fetal outcome.**

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<th>Type of delivery</th>
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<th>Toxicity</th>
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<th>Age at follow-up</th>
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AS: Apgar Score - VD: Vaginal delivery - CS: Cesarean section

**Patient 5:** a Stage IIIA papillary-serous ovarian borderline tumor, with omental invasive implants, was diagnosed at 13 GW by laparoscopic right salpingo-oophorectomy and surgical staging. Chemotherapy with cisDDP 450 mg/m² in six courses was started at 18.3 GW. Cesarean section was performed at 35.6 GW. Two years later the patient underwent laparoscopic left salpingo-oophorectomy for a 4 cm ovarian mass. Histopathological analysis revealed a papillary-serous ovarian borderline tumor. The patient, to date, is alive after 12 years with no evidence of disease.

**Patient 9:** an ovarian mass was detected by routine pelvic ultrasound examination and the patient underwent laparoscopic left-oophorectomy at 7 GW. A Stage IC endometrioid adenocarcinoma was diagnosed. Adjuvant chemotherapy started at 19 GW with cisDDP 175mg/m² in five courses. Delivery was performed at 34.3 GW by cesarean section concurrently with hysterectomy, residual oophorectomy, omentectomy, lymphadenectomy, multiple biopsies and peritoneal washings. The patient is in complete remission at 18 months.

**Patient 15:** the patient presented a 8 cm vascularized atypical adnexal mass at 22 GW. Laparotomic unilateral salpingo-oophorectomy with frozen section was performed. Following the intraoperative diagnosis of malignant intracystic mucinous carcinoma of the ovary, surgical staging was performed. The definitive diagnosis was: Stage IA well differentiated mucinous carcinoma of the ovary. The patient delivered at term a healthy baby. CT scan was negative a month after delivery. The patient is in follow-up with no evidence of disease.

**Colonrectal carcinoma**

**Patient 7:** presented at diagnosis a Stage IV colorectal carcinoma with multiple peritoneal metastases at 18.4 GW. For the advanced disease, with an expected low survival rate, no chemotherapy was administered. The patient underwent palliative surgery at 22.5 GW to avoid intestinal obstruction. She had a preterm labor and delivered vaginally at 33.2 weeks of gestation. She died a few months afterward.

**Brain tumor**

**Patient 4:** presented with a persistent headache in early pregnancy with no other apparent symptoms. After a few weeks MRI revealed a cerebral mass. She underwent neurosurgical resection at 16 GW. Histological analysis diagnosed a focal astrocytoma. The patient delivered at 37.5 weeks by cesarean section. After one year she was alive and well without disease.

**Liver carcinoma**

**Patient 10:** was diagnosed with a primary neoplasia of the liver at 26.1 GW during diagnostic procedures for liver dysfunction. MRI showed advanced disease, biopically confirmed as Stage IV liver cancer. Considering the high mortality rate due to the extent of hepatic resection required, surgery was not recommended. Cesarean section was performed at 34 GW. She died four months after delivery.

**Urethral carcinoma**

**Patient 12:** presented with persistent hematuria, dysuria and recurrent urinary tract infections. Physical examination revealed an urethral neoplasia. Biopsies revealed clear cell adenocarcinoma. Neoadjuvant chemotherapy with cisDDP started at 30 GW, and 135 mg/m² total were administered throughout three courses. Cesarean section was performed at 33.2 GW. Subsequent surgery was performed, including an en bloc total cystectomy, removal of the anterior wall of vagina and of the distal and proximal urethra, pelvic lymphadenectomy and bladder reconstruction. After one year she was alive without disease.

**Fetal outcome**

Among the seven cases in which surgery was performed during pregnancy (patients 1, 3, 4, 5, 9 and 15), six underwent abdominal surgery, three of which were within the 14 GW. The aim of intervention was fulfilled in all seven cases, and no obstetrical complications arose. In six cases (patients 5, 6, 8, 9, 11, 12) the fetus was exposed to chemotherapeutic agents. No obstetrical complication was observed except a spontaneous abortion (pROM). In patients treated with chemotherapy, delivery was planned after a mean of 19 days after the last cycle.

Apgar scores at one min ranged from 5-9 and at 5 min from 8-10. Newborn weight ranged from 1,250 to 3,450 g, all of which were within normal range for gestational age. No congenital malformation in any newborn was diagnosed. Hearing-evoked potentials and neurologic follow-up were normal. Anemia occurred in two newborns which was successfully treated with blood transfusion. No other effects have been observed in the infants.
The mental health of children exposed to chemotherapeutic agents in utero were investigated and none presented any behavioral or cognitive disorder.

At a median follow-up of 56 months (range 2-198 months) all children were well and healthy. In five children the neurological development at an older age (6-7 years old) was normal, with a normal scholar performance.

No case of placental or fetal metastatic involvement originating from maternal cancer was observed.

Discussion

The management of cancer in pregnancy implies many clinical and ethical issues. First of all, the typical standard management for any specific malignancy is often not applicable since it would directly affect the pregnancy. For abdominal tumors, the presence of a pregnant uterus constitutes a technical problem both for an adequate surgery and radiation therapy. However, even if chemotherapy is technically feasible at any gestational age, concerns often arise regarding possible adverse effects on the fetus. Therefore, clinicians should carefully consider several aspects such as stage, site of tumoral spread, maternal and fetal prognosis and, obviously, gestational age. The decision should consider the patient’s condition and treatment options. Therapeutic indications should refer primarily to the necessity for treatment, which is mainly related to prognosis, with careful evaluation of the options, which depend mostly on the gestational age [9]. The clinical management is to be evaluated case by case trying to balance the risks and severity of fetal adverse effects with the benefits linked to an adequate treatment. The timing of delivery should be carefully planned, evaluating which treatments can be performed during pregnancy and which are not feasible and therefore postponed.

As far as surgery is concerned, two different aspects should be taken into consideration: surgery itself and anesthesia. While the latter is a general issue, regardless of the kind of intervention, the former brings a number of problems related to the type and extent the treatment requires and may differ from case to case.

Surgical interventions may present some risk to the fetus, especially laparotomic surgery for abdominal disease. During the first trimester abdominal surgery appears to be somewhat more hazardous [10]. However, the bigger volume of the uterus in second and third trimesters may constitute a technical problem for surgical procedures. Complications in surgery during pregnancy are usually related to maternal anemia, lower tolerance to hypoxemia and reduction in functional residual capacity. During surgery the fetus is exposed to the transplacental effects of anesthetic agents [11]: however, with the modern anesthetic techniques many problems can be very well handled with minimal risk to the fetus. Surgery may create a stress event directly on the mother and the fetus and it can trigger preterm labor, so it is preferable, whenever possible, to wait until the third trimester. Extraperitoneal surgery seems to interfere minimally with pregnancy [10]. For women affected by breast cancer during the first and second trimesters, radical mastectomy and axillary dissection may avoid the need for radiation; during the third trimester treatment options can be based on conservative breast surgery followed by radiation after delivery. Surgical treatment can be performed on pregnant women affected by more rare tumors of the brain, thyroid, bladder or kidney and colorectal cancers [12]. Surgical treatment for ovarian cancer can be performed by open surgery or the laparoscopic technique [13, 14]; tumor mass excision, unilateral or bilateral salpingo-oophorectomy are usually feasible.

We have reported seven cases of surgery during pregnancy. In one case (brain tumor, patient 4), pregnancy did not alter the surgical procedure in any way. In another case (colon cancer, patient 7), the management was not affected by pregnancy since a palliative intervention was required. In the five cases of ovarian disease (patients 1, 3, 5, 9 and 15), surgery was modulated by balancing the need for an adequate staging and the desire to continue the pregnancy. Therefore laparoscopic or laparotomic removal of the affected ovary with accurate exploration of abdominal organs, multiple random peritoneal biopsies and cytological analysis of peritoneal washings was performed, postponing any other procedure such as hysterectomy or lymphadenectomy after delivery. No surgery-related complications were reported in our cases.

During pregnancy several changes occur in the physiology of several organs, which in turn lead to modifications in pharmacodynamics of many drugs. The increased blood volume and increased renal clearance might decrease active drug concentrations; while the faster hepatic function and changes in the gastrointestinal system may also affect drug absorption and peak concentrations [15].

Many chemotherapeutic agents are listed in the Food and Drug Administration pregnancy category D, because there are data on pregnant women indicating potential risk to the fetus [16]. The teratogenic properties of many drugs depend on the timing of exposure, the dose and the characteristics of placental transfer. Placental pharmacokinetics leads to potential toxicity to the fetus. Recent pharmacogenomic studies confirm that the presence of MRP-related proteins in the syncytiotrophoblast play an important role in fetal “chemoprotection” from antiblastic drugs, like cisplatin and vincristine [15-17]. The placenta, in fact, retains the capacity to bioinactivate pharmacologically active molecules and secrete them in the maternal circulation. The placenta presents a crucial role as a barrier to cytotoxic agents and an active filter for the fetal blood, thus the time of delivery must be chosen carefully, allowing some time to pass before proceeding to the cesarean section [17, 18].

Malformations are related to the gestational age at exposure. The incidence of fetal malformations for first-trimester chemotherapy exposure with a variety of agents ranges from 14% to 19%. Organogenesis is complete after 12 weeks with the exception of the brain and gonads. When exposure occurs in the second or third trimester the incidence of fetal malformations drops to 1.3%. Cytotoxic drugs administered in the second and
third trimesters are not teratogenic [19, 20], but may lead to intrateral growth restriction (IUGR), prematurity and stillbirth [21]. Cisplatin is the most important agent for many gynaecologic cancers. Sensorineural hearing loss was reported by Raffles in a child born at 26 weeks, after exposure to this drug six days before delivery [22] but many reports suggest that most of children exposed in utero to cisplatin during the second or the third trimester did not present any malformation [23-26].

We have reported six cases of chemotherapy administered during pregnancy. We considered it safe to postpone the first administration of chemotherapy from 18 GW and on. In two cases (cases 5 and 9) when cancer was diagnosed during the first trimester, we postponed the treatment, respectively, for five and 12 weeks in order to reach 18 GW. In the other four cases (cases 6, 8, 11 and 12) cancer was diagnosed in the second and third trimesters, therefore the treatment was started immediately. Drugs, doses and schedules employed were adequate, according to type and stage of the disease.

Another patient (case 1) agreed to receive chemotherapy only after delivery, postponing the treatment at 26 weeks, even though we suggest starting as soon as 18 GW. The effects of radiation exposure, including radiation therapy, are principally related to the dose received, the field of irradiation and the week at the time of exposure [27]. Exposure during the preimplantation phase from day 0 to day 14 is likely to cause miscarriage during organogenesis from weeks three to eight in a wide range of congenital malformations and the greatest growth restriction. Exposure during the fetal stage from weeks eight or nine to 40 leads to growth restriction [28]. Daly’s review of medical exposure suggests that radiation exposure during organogenesis is predominantly associated with malformation of the fetus [29]. Risk as a function of dose is quantified in the American Association of Physicists in Medicine report on fetal dose from radiotherapy: ideal dose to the fetus should be kept below 0.05 Gy [30]. The site of the tumor is obviously one of the most important factors in pregnancy. The greatest determinant of dose is the distance from the field edge, with dose falling roughly exponentially with distance: pelvic fields would result in abortion. It is possible, however, to treat non-pelvic fields and allow the pregnancy to continue without increased risk of deterministic effects by ensuring that the dose to the fetus is below 0.1 Gy [31]. Although the risk of carcinogenesis cannot be excluded, the risk below this dose is extremely low.

In our series no patient had indications for radiation therapy during pregnancy.

**Conclusion**

Malignant disease during pregnancy raises a conflict between optimal maternal therapy and fetal well-being. Each patient should be evaluated individually, considering both the aggressiveness of the cancer and the gestational age when the therapy is applied. Many authors today suggest that treatment of cancer in pregnant women should adhere to the same criteria as in non-pregnant patients with the required modifications due to the pregnancy. However it has been suggested that therapeutic abortion should be offered to all patients who develop cancer during the first trimester.

Our data suggest that pregnancy is not adversely affected by treatment. Modern techniques in surgery and anesthesiology allow a safe and generally adequate surgical approach, when it is required. Surgery, even in the first trimester, can be safe and should be considered as diagnostic or, if necessary, primary treatment.

Chemotherapy should be performed with fetal surveillance and monitoring. Exposure to antiblastic drugs during the first trimester increases the risk of spontaneous abortion, fetal death, and major malformation [15] while during the second and third trimester, increases the risk of IUGR and low birth weight. Exposure to antiblastic drugs in utero does not affect neonatal morbidity or mortality even if further follow-up is required to determine any potential long-term effects.

There is little doubt that gestational age exerts an influence on outcome of prenatal births and infants, when compared with those born at term, having higher rates of mortality and neonatal morbidity. In our series the delivery timing was planned in relation to gestational age, stage of the disease and its curability. The time of delivery, planned after 32 GW, took place in a perinatal center experienced in high-risk pregnancies. Delivery was delayed by two to three weeks after chemotherapy to allow the bone marrow to recover. No cases of malformation or small for gestational age at the delivery were reported.

Deliberate delay of treatment to achieve fetal viability or to improve fetal outcome would be reasonable for patients with early-stage cancer with a good prognosis, whereas treatment delay in advanced cancer raises concerns about maternal morbidity [9].

Pregnant woman with cancer must be informed about the lack of evidence regarding long-term consequences of chemotherapy exposure.

**References**


Cancer in pregnancy: maternal and fetal implications on decision-making


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Advantages of radio frequency (RF) cone biopsy compared to large loop excision of the transformation zone (LLETZ) in patients with high-grade squamous intraepithelial lesions: a retrospective study

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Summary

Objective: The aim of this study was to compare radio wave cone biopsy to the LLETZ method in patients with high-grade squamous intraepithelial lesions. Method-Results: This was a retrospective study of 186 patients diagnosed with HGSIL who underwent cone biopsy either with the LLETZ method (82/186) or with the radio wave method (104/186) in the 2nd Obstetrics and Gynecology Department, University of Athens, Aretaieion Hospital, Athens, Greece during the period January 1999 to December 2008. The mean age of the patients was 31 years (range 23 to 53 years). The volume of cone ranged from 1.2 x 2 cm up to 3 x 3.6 cm in both techniques. Histopathological analysis revealed focal or extensive high-grade squamous intraepithelial neoplasia extending into the underlying endocervical glands in 128/186 patients. Concomitant low-grade squamous intraepithelial lesions were observed in 160/186 patients and colloidal atypia was observed in 172/186 patients. The endocervical margins were free of disease in 172/186 cases. In seven cases the neoplastic lesions were at least 0.1 cm from the margin and in seven cases they extended to the margin. In all cases a degree of tissue coagulative change was observed, but not extensive to the point of obscuring the diagnosis. Conclusion: 4.0 MHz radio wave surgery is an excellent alternative in the treatment of HGSIL. Clear surgical margins due to decreased heat and tissue damage, controlled hemostasis, faster healing, and patient and doctor satisfaction are notable advantages.

Key words: Yolk sac tumor; Omentum.

Introduction

High-grade squamous intraepithelial lesions (HGSIL) include moderate to severe dysplasia, precancerous lesions, and carcinoma in-situ (preinvasive cancer that involves only the cervical epithelial layer) [1]. High-grade lesions develop most often in women between the ages of 30 and 40, but can occur at other ages as well [1, 2]. Nevertheless, HGSIL on a Pap smear may be associated with malignancy of the cervix. Therefore, a proper diagnostic evaluation is essential [2]. Colposcopic evaluation is necessary when a HGSIL Pap smear is discovered. A biopsy may also be done to determine the amount of abnormality. Possible treatment for HGSIL includes the loop electrosurgical excision procedure (LEEP), cryotherapy, conization (also called cone biopsy) and laser therapy [3, 4].

All treatments directed toward neoplastic conditions of the cervix should be based on a biopsy and not a Pap smear alone. Treatment for a precancerous lesion of the cervix depends on a number of factors which include whether the lesion is low or high grade, whether the woman wants to have children in the future, the woman’s age and general health, and the preference of the woman and her doctor. A woman with a low-grade lesion may not need further treatment, especially if the abnormal area was removed during biopsy, but she should have a Pap test and pelvic exam regularly by a physician with expertise in this area. Treatment for precancerous lesions may cause cramping or other pain, bleeding or a watery discharge. Follow-up consists of regular Pap smears and if necessary colposcopy for an extended period of time.

Radio frequency (RF) is an alternative in the treatment of such lesions with the advantage of free surgical margins. High frequency radiofrequency energy has a strong affinity for water. Targeted tissue/cell readily absorbs energy due to high water content. Intracellular pressure increases as water molecules expand. Votizalation results in cell conversion to vapor. The process emits low-temperature steam which aids in coagulation. Cell-specific interaction enables meticulous dissection with tissue preservation.

We present the results of a comparison made in our Department between two methods of cone biopsy (LEEP and RF).

Materials and Method

A retrospective study was carried out on 186 patients diagnosed with HGSIL who underwent cone biopsy either with the LLETZ method (82/186) or with the RF method (104/186) in the 2nd Obstetrics and Gynecology Department, University of Athens, Aretaieion Hospital, Athens, Greece during the period January 1999 to December 2008. The indications for LEEP were Pap smears indicating HGSIL or repeated cervical smears with LGSIL; 167/186 patients also had cervical biopsies after colposcopy which were positive for HGSIL. We searched our databases regarding demographic data, histopathologic findings
(free margins or not), postoperative complications (infection, vaginal bleeding, pain, dyspareunia), possible recurrences during the follow-up period, and patient and doctor satisfaction.

All the procedures were done with general anesthesia. Patients were placed in the lithotomy position on the examining table. A proper-size speculum was used and the cervix was painted with Lugol solution. The described RF procedure was achieved by using Ellman Surgitron electrosurgical equipment. The radio wave unit converts electrical current into controlled energy in the RF of the electromagnetic spectrum. A fully rectified current is used to produce a pure, continuous flow of high-frequency current. This filtration and current produce the least high-frequency interference. Such a procedure should not be used in the presence of flammable anesthetics, liquids or skin preparations.

A limitation of our study is the relatively small number of patients who participated in our study. Moreover, the fact that the two methods were performed by different doctors might be a further limitation. Finally, a longer follow-up period may be necessary to achieve safer conclusions regarding the recurrence rates.

Discussion

Our retrospective study compares two different methods of cone biopsy. The advantages of the RF cone biopsy is the fact that it is characterized by less blood loss which leads to a more clear operating field and increased visibility. The procedure could be performed with less tissue distraction and so faster healing and quicker recovery could be achieved. It should be mentioned that the low level of the tissue distraction and the controlled direction of the RF current lead to less postoperative pain and chance of infection. In the other group of patients extensive areas of carbonization and epithelial distortion at the margins of the excision were noted. These facts in combination with the small learning curve offer great satisfaction to surgeons working with this modern technique. The above-mentioned findings are similar to the current literature in the field [5-15].

On the other hand, special attention should be paid in choosing optimal power settings and the correct electrode, and ensuring movement with care not to pass too slowly through the tissue in order to prevent increased tissue damage. A contraindication of radio wave procedures is the fact that the method could not be used on patients with older, nonshielded pacemakers due to the high-frequency interference. Such a procedure should not be used in the presence of flammable anesthetics, liquids or skin preparations.

A limitation of our study is the relatively small number of patients who participated in our study. Moreover, the fact that the two methods were performed by different doctors might be a further limitation. Finally, a longer follow-up period may be necessary to achieve safer conclusions regarding the recurrence rates.

Conclusion

RF surgery using 4.0 MHz provides many benefits in the treatment of HGSIL. Clear surgical margins due to decreased heat and tissue damage, controlled hemostasis, faster healing, patient and doctor satisfaction are notable advantages. For this reason, it is proposed to be an excellent alternative in the treatment of HGSIL.
References


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Role of lymphadenectomy in endometrioid endometrial cancer

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Summary

Objective: To assess the risk factors associated with node involvement. Study design: In the period 1990-2008 a total of 265 endometrial cancers were treated in the Institut Universitari Dexeus. We analysed the rate of myometrial invasion, tumour grade, histological type and node involvement. Results: Overall, 86% of tumours were endometrioid, 5.3% papillary serous, 4.9% mixed and 2.6% endometrial stroma sarcoma. Among those with endometrioid histology, lymphadenectomy was not performed (NL) in 85 cases (37.2%), whereas pelvic lymphadenectomy (PL) or pelvic and aortic lymphadenectomy (PAL) was carried out in 84 (36.8%) and 22 cases (9.6%), respectively. In NL patients the overall disease-free survival (DFS) rate at five years was 92.8%. In the PL group, node involvement was observed in 2.4% of cases and the five-year DFS rate was 92.3%. Among PAL patients, 18.6% showed node involvement (72.7% positive pelvic nodes and 63.6% aortic). Aortic involvement was present in 5.9% of cases when there was no pelvic disease, whereas in the presence of positive pelvic nodes the rate of aortic involvement was 50%. The DFS rate at five years was 93.6%. Referring to the risk factors, when infiltration was > 50% of the myometrium, lymph node involvement occurred in 37% of cases and G3 tumors in 45.5%. Conclusions: Node involvement is more commonly observed in cases with > 50% myometrial invasion and G3, accounting for 25% of cases that can be considered as at-risk patients. When node involvement is present it is equally distributed between the pelvic and aortic levels. As node involvement is a predictive factor for distant metastasis, the 25% of patients considered to be at risk should undergo pelvic and aortic lymphadenectomy.

Key words: Endometrial cancer; Lymphadenectomy; Management; Paraortic lymph node; Pelvic lymph node.

Introduction

Endometrial cancer (EC) is the most common gynaecological cancer and its incidence is rising. The standard treatment for EC is hysterectomy with bilateral salpingo-oophorectomy and pelvic and aortic lymphadenectomy, although patients in the early stages can be treated with hysterectomy alone. However, despite adequate surgical intervention the cancer reoccurs in some patients. Some authors propose that lymphadenectomy should only be performed in high-risk patients, as the remainder will not benefit from this intervention and will show higher morbidity rates due to the technique [1-4]. Others suggest starting with pelvic lymphadenectomy and, in the event that this is positive, following up with aortic lymphadenectomy. It has also been argued that only suspicious nodes should be removed, as more than 50% of cases are identifiable macroscopically, with only 5% of hidden metastasis [5, 6]. At all events there is no consensus as to the scope of the lymphadenectomy which should be performed, and prospective findings from the PORTEC and GOG studies [2, 7] appear not to support the need for this intervention. More recently the ASTEC study group [8] have demonstrated that lymphadenectomy has no effect on survival rates.

Given the controversy over the role of lymphadenectomy in endometrial cancer the present study sought to analyse the risk factors for node involvement and determine the usefulness of this procedure in treating patients with endometrial cancer.

Material and Methods

We conducted a retrospective study of 300 patients diagnosed and treated for endometrial cancer during the period 1990-2008. Thirty-five were excluded: 12 patients presented a concomitant ovarian neoplasm and in 23 there was insufficient clinical data. The final sample comprised 265 patients. The mean age of patients was 58.8 years (SD 11.6, range 29-95). Of the total, 86% (228) showed endometrioid histology and 14% (37) non-endometrioid. Surgical staging included lymphadenectomy in 161 (60.7%) patients, being pelvic in 89 cases and both pelvic and aortic in 72 cases. During the first period the indication for aortic lymphadenectomy was positive pelvic nodes. Since 2002 pelvic and aortic lymphadenectomy have been performed in high-risk patients. In this study the surgeon made the final decision whether or not to perform lymphadenectomy. A preoperative biopsy was performed to assess invasion and histological grade.

The surgical procedure began by collecting peritoneal fluid or with a peritoneal wash for cytological analysis. This was followed by extrafacial hysterectomy, with the surgical sample being sent for intraoperative biopsy. Pelvic lymphadenectomy included the dissection of all the nodes of the common, external and internal iliac vessels, as well as all the fatty and lymph tis-
sue above and to the side of the obturator nerve. Aortic lymphadenectomy included the dissection of all nodes and fatty tissue around the aorta and vena cava, from the bifurcation of the aorta to the level of the left renal vein. The mean number of pelvic nodes obtained per patient was 17 (range 2-33), while the mean number of aortic nodes was 7 (range 1-24).

Patients were classified into two groups according to clinical parameters, tumour grade, myometrial invasion, lymphovascular space involvement and histological type. Low-risk patients were: endometrioid histological type, < 50% myometrial invasion, G1 and G2. High-risk patients were: endometrioid tumours with > 50% myometrial invasion or all G3 or lymphovascular space involvement and all tumours non-endometrioid.

Complementary radiotherapy was administered to high risk patients, always if deemed appropriate by the radiotherapist. The variables analysed were: myometrial invasion, histological grade, histological type, pelvic and/or aortic node involvement, adjuvant radiotherapy and chemotherapy, reoccurrence and metastasis.

Endometrioid adenocarcinomas were analysed. We also separately analysed those patients in whom a lymphadenectomy had not been performed, those who had undergone a pelvic lymphadenectomy and those who had had both a pelvic and aortic lymphadenectomy.

**Statistical analysis**

Quantitative variables were compared using either the t test or the Mann-Whitney U test depending on the assumptions fulfilled. Qualitative variables were compared using either Pearson’s chi-square test or Fisher’s exact test. The rate of disease-free survival was estimated via Kaplan-Meier survival models. All tests were bilateral and significance was set at $\alpha = 0.05$.

**Results**

A total of 265 patients were analysed. Myometrial invasion was absent in 27.7% of cases, whereas in 54.2% there was < 50% invasion, in 16.2% there was > 50% and in only 1.9% of patients was serosal infiltration observed. With respect to tumour grade, 55% were G1, 29.1% were G2 and 11.7% G3. After surgery 214 patients were classified as Stage I, 15 as Stage II, 27 as Stage III and two as Stage IV.

**Tumours with endometrioid histology**

There were 228 cases with endometrioid histology. Pelvic lymphadenectomy was performed in 84 patients (36.84%) and both pelvic and aortic lymphadenectomy in 59 (25.87%). Among the latter the pelvic lymphadenectomy was complete in 49 cases, and only pelvic sampling in ten patients. The aortic lymphadenectomy was complete in 25 cases, with sampling alone being performed in the remaining 34 patients. The number of pelvic and aortic nodes obtained was, respectively, 17.3 (SD 6.3, range 2-33) and 7 (SD 4.5, range 1-24). Radiotherapy was administered to 23.7% of patients. Eight (3.5%) patients recurred, but none of the patients with subsequent recurrence had previously received radiotherapy.

Eleven (4.8%) cases of distant metastasis were detected over a mean follow-up of four years.

Ten patients received chemotherapy, four of which had positive nodes (five were classified as IIIC) and two presented a positive peritoneal wash. Only one of these patients showed distant metastasis, this being a woman with 12 positive nodes (4 pelvic and 8 aortic). The remaining patients with distant metastasis had not received chemotherapy.

Both pelvic and aortic lymphadenectomy were performed in 59 patients, their mean age being 56.81 years (SD 10.4, range 29-74). Node involvement was observed in 11 (18.6%) of these patients: this was at the pelvic level in 72.7% of cases, at the aortic level in 63.6% and solely at the aortic level in 27.3%. Furthermore, when pelvic nodes were negative there was only aortic involvement in 5.9% of cases, whereas when the pelvic lymphadenectomy was positive there was also aortic involvement in 50% of patients.

We then analysed node involvement in relation to known risk factors such as myometrial invasion and tumour grade. This showed that in the absence of myometrial invasion, or when this was < 50%, there was node involvement in 2.8% of G1/G2 tumours and 33.3% of G3 tumours. However, with > 50% myometrial invasion or serosal infiltration the rate of node involvement was 45.4% in G2 tumours and 60% in G3 ($p < 0.05$) (Table 1).

Table 1.— Rate of node involvement according to degree of myometrial invasion and tumour grade in patients with endometrioid tumours who underwent pelvic and aortic lymphadenectomy.

<table>
<thead>
<tr>
<th>Tumour grade (%)</th>
<th>G1 (%)</th>
<th>G2 (%)</th>
<th>G3 (%)</th>
<th>Total N+ with respect to degree of myometrial invasion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50% invasion</td>
<td>0</td>
<td>0</td>
<td>33.3</td>
<td>9.7</td>
</tr>
<tr>
<td>&gt; 50% invasion</td>
<td>0</td>
<td>40</td>
<td>50</td>
<td>37.7</td>
</tr>
<tr>
<td>Serous</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Total N+ according to tumour grade (%)</td>
<td>4.5</td>
<td>19.2</td>
<td>45.4</td>
<td>$p &lt; 0.05$</td>
</tr>
</tbody>
</table>

The mean age of patients with positive nodes (N+) was 60.6 years (SD 12.4) compared with 55.9 (SD 9.8) for those without node involvement ($p < 0.05$). Radiotherapy was administered to 45.8% of these patients.

After a mean follow-up of 62.9 months two cases of recurrence (3.38%) were detected at six and 122 months. Both these patients were N- (4.1%). Distant metastasis was observed in three cases (5.1%), at 23, 57 and 74 months after diagnosis; these cases corresponded to 18.2% of N+ patients but only 2.1% of N- patients ($p < 0.05$).

Overall disease-free survival (DFS) rate at five years was 93.6%. Broken down by group the DFS rate was 50% for patients with both pelvic and aortic N+, 66.77% in patients with pelvic N+ but aortic N-, and 93.5% for patients with a pelvic N- but aortic N+, as well as in those cases where both were N- (Figure 1, Table 2).

**Pelvic lymphadenectomy alone** was performed in 84 patients. Node involvement was detected in two of these...
cases (2.4%), the remainder being negative. The mean age of N+ patients was 69 years (SD 4.2) compared with 57 (SD 9.4) for N- patients. Radiotherapy was administered to 20 of these patients (23.8%). There were five cases (6%) of recurrence, none of whom showed node involvement. Distant metastasis appeared in two cases (2.3%), both free of node involvement. The overall five-year DFS rate was 92.3% (Table 2).

No lymphadenectomy was performed in 85 cases (37.2%). Radiotherapy was administered to 8.3% of these patients. One patient (1.2%) with < 50% invasion (G2) had a recurrence. There were six cases of metastasis (7%). Four of these patients were considered to be high risk but the pathology results were deferred, and it was decided not to perform further surgery as three of them were aged over 80. The mean age of patients with metastasis was 74.1 years. The five-year DFS rate was 92.8% (Table 2).

Discussion

In 1988 the International Federation of Gynaecology and Obstetrics (FIGO) [9] stated that correct staging of endometrial cancer required both pelvic and paraaortic lymphadenectomy. Since then it has been proposed that radiotherapy is not necessary when the lymphadenectomy shows there is no node involvement. However, there is little clinical evidence regarding the benefits of lymphadenectomy, a procedure that also increases morbidity [3]. Although large series have reported an improvement in survival rates following lymphadenectomy [10, 11],

Table 2. — Clinical characteristics of patients according to the type of lymphadenectomy performed.

<table>
<thead>
<tr>
<th></th>
<th>Endometrioid</th>
<th>Pelvic lymphadenectomy</th>
<th>Paraaortic lymphadenectomy</th>
<th>No lymphadenectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>228</td>
<td>59</td>
<td>84</td>
<td>85</td>
</tr>
<tr>
<td>Age</td>
<td>58.4±11.4</td>
<td>56.8±10.4</td>
<td>57.3±9.5</td>
<td>60.5±13.3</td>
</tr>
<tr>
<td>Pelvic nodes</td>
<td>17.4±6.4</td>
<td>18.4±6.4</td>
<td>16.6±6.3</td>
<td>ns</td>
</tr>
<tr>
<td>Aortic nodes</td>
<td>7.0±4.6</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>190 (84.4%)</td>
<td>38 (65.5%)</td>
<td>74 (88.1%)</td>
<td>78 (94%)</td>
</tr>
<tr>
<td>II</td>
<td>12 (5.3%)</td>
<td>6 (10.3%)</td>
<td>5 (6.0%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>III</td>
<td>21 (9.3%)</td>
<td>14 (24.1%)</td>
<td>4 (4.8%)</td>
<td>3 (3.6%)</td>
</tr>
<tr>
<td>IV</td>
<td>2 (0.9%)</td>
<td>0 (0.0%)</td>
<td>1 (1.2%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>54 (23.7%)</td>
<td>27 (45.8%)</td>
<td>20 (23.8%)</td>
<td>7 (8.2%)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>10 (4.4%)</td>
<td>8 (13.6%)</td>
<td>1 (1.2%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Myometrial invasion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63 (28.0%)</td>
<td>10 (16.9%)</td>
<td>12 (14.5%)</td>
<td>41 (49.4%)</td>
</tr>
<tr>
<td>&lt; 50%</td>
<td>126 (56.0%)</td>
<td>31 (52.5%)</td>
<td>60 (72.3%)</td>
<td>35 (42.2%)</td>
</tr>
<tr>
<td>&gt; 50%</td>
<td>32 (14.2%)</td>
<td>16 (27.1%)</td>
<td>10 (12.0%)</td>
<td>6 (7.2%)</td>
</tr>
<tr>
<td>Serous</td>
<td>4 (1.8%)</td>
<td>2 (3.4%)</td>
<td>1 (1.2%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>133 (59.4%)</td>
<td>22 (37.3%)</td>
<td>53 (63.9%)</td>
<td>58 (70.7%)</td>
</tr>
<tr>
<td>2</td>
<td>70 (31.3%)</td>
<td>26 (44.1%)</td>
<td>23 (27.7%)</td>
<td>21 (25.6%)</td>
</tr>
<tr>
<td>&gt; 50%</td>
<td>21 (9.4%)</td>
<td>11 (18.6%)</td>
<td>7 (8.7%)</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td>Node involvement</td>
<td>13 (5.7%)</td>
<td>11 (18.6%)</td>
<td>2 (2.4%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Pelvic node involvement</td>
<td>10 (4.4%)</td>
<td>8 (13.6%)</td>
<td>2 (2.4%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Aortic node involvement</td>
<td>7 (3.1%)</td>
<td>7 (11.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrences</td>
<td>8 (3.5%)</td>
<td>2 (3.4%)</td>
<td>5 (6.0%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>11 (4.8%)</td>
<td>3 (5.4%)</td>
<td>2 (2.4%)</td>
<td>6 (7.1%)</td>
</tr>
<tr>
<td>Progression</td>
<td>18 (7.9%)</td>
<td>5 (8.5%)</td>
<td>6 (7.1%)</td>
<td>7 (8.2%)</td>
</tr>
<tr>
<td>Disease-free survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rate at 60 months</td>
<td>92.7%</td>
<td>93.6%</td>
<td>92.3%</td>
<td>92.5%</td>
</tr>
</tbody>
</table>

ns: non significant.
with some considering that its scope is a determining factor [12-16], the ASTEC study [8] found no impact on survival. Nevertheless, the authors considered that lymphadenectomy was not contraindicated since it enables a better classification of high-risk patients, thus helping to identify those susceptible to treatment [17]. These results are complemented by the findings of an Italian multi-centre study [18] which randomised 500 patients and also reported no differences in survival rates between the groups. However, both these studies were criticised by Höckel and Dornhöfer [19], who pointed out that the disease was only treated at the pelvic level (pelvic lymphadenectomy and pelvic radiotherapy). Given that in around 30% of cases lymph metastasis occur only at the paraaortic level, and that when the pelvic lymphadenectomy is positive the aortic lymphadenectomy is also positive in 50% of patients, then in approximately 75% of patients with node metastasis these would be outside the pelvic area and, therefore, uncontrolled [19, 20]. Both studies demonstrated effective local control, as there was a 50% reduction in recurrence rates. Therefore, the results did not translate into improved survival. Adjuvant therapy with paclitaxel-carboplatin may be the best option in these cases. Another criticism of this study was that in the standard surgery group the surgeon could remove the pelvic nodes if it was considered of benefit to the woman. This concession contradicts the stated aim of the study, which was to assess the therapeutic effect of lymphadenectomy. Moreover, the fact that 43% of patients included were low risk dilutes the possible therapeutic effect of lymphadenectomy, and it should also be noted that half the patients had 12 or fewer nodes [20, 21]. Other studies have shown that 12 nodes is the minimum number for correct staging [21, 22], although there is no consensus regarding the most suitable number. Finally, the sample size of this study was also criticised due to the low proportion of N+ patients [20].

In our series none of the patients with a recurrence had received radiotherapy, thus supporting the notion of better local control following this treatment. In recent years, since publication of the PORTEC 2 trial [23] which demonstrated that brachytherapy offered the same degree of local control as external radiotherapy but with less morbidity, the majority of patients have received radiotherapy in the form of brachytherapy. In terms of the type of surgery performed it can be seen that the subgroup of patients with an endometrioid tumour and in whom a lymphadenectomy was not carried out constituted a lower risk subgroup; consequently, radiotherapy was only administered to 8.3% of these patients, although even so the rate of recurrence was only 1.2%. When pelvic lymphadenectomy is compared with pelvic and aortic lymphadenectomy it can be seen that the latter corresponds to a subgroup of higher risk patients in whom radiotherapy was more often administered (52.5% vs 23.8% for patients undergoing only a pelvic lymphadenectomy), this also being reflected in a lower rate of recurrence (3.38% vs 5.9%).

If lymphadenectomy is considered to enable a better selection of at-risk patients, the question which remains to be answered concerns its scope. In their series Mariani et al. [24] only performed lymphadenectomy with high-risk patients. None of their patients with a G1 or G2 endometrioid tumour < 2 cm in diameter and < 50% myometrial invasion had node involvement, the five-year survival rate being 100%. This represents 27% of all endometrial cancers [24]. Among high-risk patients there was node involvement in 22%, this being N+ pelvic and N+ aortic in 51% of cases, only N+ pelvic in 33% and only N+ aortic in 16%. Thus, 67% of patients with node dissemination had positive aortic nodes. Moreover, although the appropriate scope of aortic lymphadenectomy had previously been unclear [25, 26] Mariani et al. showed that when the aortic result was positive, 77% of nodes were above the mesenteric artery; they thus recommended that paraaortic lymphadenectomy should reach the level of the left renal vein. In our series, 63.6% of patients with positive nodes had aortic involvement, and this was solely aortic in 27.3% of cases. This raises the question as to whether aortic lymphadenectomy should always be performed. Some authors opt for pelvic lymphadenectomy in high-risk patients, and only perform aortic lymphadenectomy when there are positive pelvic nodes. Our data showed that when the pelvic lymphadenectomy was negative, there was aortic involvement in 5.9% of cases. However, 50% of patients with a positive pelvic lymphadenectomy also had aortic involvement. As regards the 84 patients in whom only a pelvic lymphadenectomy was performed it should therefore be assumed that when this was negative (82 cases) the rate of aortic involvement was 5.9%. This corresponds to 4.8 theoretical patients with aortic node involvement. We believe that this number is sufficiently high to suggest that this involvement should not go undetected. At all events it is useful to know the type of any node involvement, since when there is a positive result the likelihood of metastasis is 18.2%, compared with only 2.1% in the absence of nodes. This is also linked to differences in survival, and thus lymphadenectomy is detecting a subgroup of patients at risk of metastasis, on whom efforts should be focused in terms of complementary treatment.

One issue that is considered to be critical [27] is how to select low- and high-risk patients preoperatively. Although Mariani et al. [24] report excellent outcomes with preoperative biopsy other authors [28-30] consider that preoperative analysis is less reproducible. One prospective, randomised study found the preoperative and definitive analyses to be correlated in only 67% of cases when assessing myometrial invasion and in only 58% for histological grade, there being over staging in 18% of patients [30]. This prospective study confirmed previous reports [28-30] regarding the limited agreement between preoperative and definitive analyses. In our centre we demonstrated a positive predictive value of 47% for the absence of invasion, 93% for invasion < 50%, and 92% for invasion > 50%. Therefore, preoperative biopsy based on frozen section is useful to establish whether there is invasion of less than or more than 50%, but it is not very
accurate in terms of diagnosing the type of invasion [31]. This may cease to be a problem under the new FIGO 2009 classification, which does not distinguish between Stage Ia and Ib; Ia now refers to cases of myometrial invasion < 50% and Ib to cases with > 50% [32].

At all events, common sense should be applied. The high rate of comorbidities makes surgery more difficult. In these cases the removal of any suspicious nodes should be mandatory, as around 50% of them will be positive [5, 6]. In the future it is possible that the combination of imaging techniques with determination of the sentinel node will reduce the need for lymphadenectomy without preventing correct staging [33].

Taking the data as a whole we believe that the role of lymphadenectomy remains unclear, although there is evidence to suggest that it helps to target complementary treatment in those patients who might most benefit, as well as selecting subgroups of very high-risk patients. In conclusion, we consider that lymphadenectomy should be performed in high-risk patients, and that it should be a full pelvic and aortic procedure to the level of the left renal vein.

References

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Different patterns of p16 immunoreactivity in cervical biopsies: correlation to lesion grade and HPV detection, with a review of the literature

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Summary

p16 is one extensively studied marker in gynecological pathology. However, its routine application in the diagnosis of squamous intraepithelial lesions of the uterine cervix may present difficulties for the general pathologist. The aim of the present study was to examine a series of 100 cervical biopsies/LEEP specimens, with detailed HPV-typing, for patterns of p16 immunoreactivity and possible correlations with morphology and HPV types. Four patterns of immunopositivity were recognized, according to the distribution of positively stained cells, and these correlated to lesion grade. A review of the pertinent literature concerning p16 immunoreactivity in squamous intraepithelial lesions and nonneoplastic epithelia of the uterine cervix is included in an effort to summarize the existing data and the remaining questions at both the practical and theoretical level.

Key words: Cervix; CIN; HPV; Immunohistochemistry; p16; Patterns; Review; SIL.

Introduction

The role of human papilloma virus (HPV) in squamous intraepithelial lesions of the uterine cervix has been investigated extensively in molecular studies, which revealed multiple interactions between HPV oncoproteins and their cellular targets. These result in alterations of cell cycle control and apoptosis [1-4]. Several associated markers have been investigated for their potential utility in assisting the histopathologic classification of preinvasive lesions and in facilitating the distinction from non-HPV-induced alterations [5-8].

One extensively studied marker is p16, a cyclin-dependent kinase inhibitor, which affects pRb-mediated regulation of the G1/S transition [9-13]. p16 is strongly expressed in some normal tissues [14], while inactivation or overexpression has been reported in human neoplasms [15-17]. HPV-related precursor lesions are often associated with increased p16 expression. This is considered a result of functional inactivation of pRb by high-risk (HR) HPV E7 protein, affecting a negative transcriptional feedback loop [10-12]. A dramatic enhancement of p16 RNA level has been observed in vitro after immortalization by HPV16 or HPV18 [18] and correlation has been reported between HR-HPV oncogene expression and high scores of p16 positivity [19]. However, despite the presence of high levels of p16 in these lesions, its suppressor function is not normally exerted.

After a few initial reports concerning p16 status in cervical cancers and precancerous lesions [11, 20], several investigators have examined immunohistochemically the expression of p16 in cervical squamous intraepithelial lesions and its possible correlation with HR-HPV types and/or lesion “progression” [6, 7, 21-50]. Different criteria have been used for p16 immunoreactivity evaluation, with some authors reporting any type of immunostaining, some focusing only on diffuse immunoreactivity, and others reporting nuclear and cytoplasmic staining separately, as presented in the following.

In routine evaluation of cervical biopsies, often assisted by p16 immunostaining, we observed different patterns of reactivity which often could not be easily categorized. This led us to a different approach of staining patterns. The aim of the present study was to examine a series of cervical biopsies/LEEP specimens with detailed HPV-typing for patterns of p16 immunoreactivity and possible correlations with morphology and HPV types. A review of the pertinent literature concerning p16 immunoreactivity in biopsy specimens of cervical squamous intraepithelial lesions and nonneoplastic epithelia is included in the following discussion in an effort to summarize the existing data and the remaining questions at both the practical and theoretical level.

Materials and Methods

The study included 100 specimens from 100 different patients. These specimens included 77 punch biopsies and 23 loop electrosurgical excision procedure (LEEP)/conization specimens retrieved from the archives of the Department of Pathology, School of Medicine, University of Thessalia, Larissa.
Pathology, University Hospital of Larissa, Thessalia, Greece. The samples were fixed in 10% buffered formalin solution, embedded in paraffin blocks and cut at 3 μm sections.

The corresponding archived H&E slides were reviewed for the purpose of the study by two pathologists independently. In those cases where there was interobserver variation, a final consensus diagnosis was reached jointly. A prerequisite for every cervical biopsy to be included in the study was the availability of HPV testing with a PCR-based technique in order to verify the presence and/or the type(s) of HPV in the sample. We included 25 high-grade squamous intraepithelial lesions and 55 low-grade squamous intraepithelial lesions. These cases were classified according to previously published criteria [51, 52].

Additionally, we included in the study 20 cervical biopsies from 20 different patients without any diagnostic histopathologic abnormality. These specimens had only minor cytopathologic alterations and most of the cases had negative HPV testing results. Nonetheless, for a variety of unrelated lesions colposcopic biopsies had been obtained.

**Immunohistochemistry (IHC) for p16**

IHC for p16 was performed on deparaffinized 3 μm sections in a commercially available automated immunostainer (Bond Max, Vision Biosystems, Australia). For antigen retrieval, Bond Epitope Retrieval Solution 2 (30 min, Vision BioSystems, Mount Waverley, Australia) was used. A monoclonal anti-p16 antibody (6H12, Novoceastra, Newcastle upon Tyne, UK) was used at 1:100 dilution and binding of the primary antibody was assessed by the Bond Polymer Refine Detection (Vision Biosystems, Newcastle upon Tyne, UK), with DAB as a chromogen. A light hematoxylin counterstaining was used.

Negative control slides were processed similarly by omitting the primary antibody. Positive control slides were selected from known high-grade squamous intraepithelial lesions associated with high-risk HPV infection.

**Assessment of immunohistochemical staining**

All slides were initially evaluated by two pathologists, whose evaluations were conducted blindly and independently. During a subsequent joint evaluation, a final consensus immunoreactivity evaluation was obtained and used for further analysis.

The reaction was evaluated as positive if nuclear and/or cytoplasmic immunostaining was clearly demonstrated. Weak “blush” staining was not considered positive. After preliminary analysis of the findings, the pathologists involved in the evaluation of the immunohistochemical staining realized that the visualized immunoreactivity differences among various cases were best appreciated by categorizing the observed staining into four different patterns according to the extent of immunoreactivity: A, A-low, B, and C. These are presented schematically in Figure 1. Pattern A included occasional positive cells, dispersed or in small groups, usually above the parabasal layer. Pattern A-low was distinguished from pattern A by the presence of occasional positive cells, observed mainly in the lower epithelial layers, dispersed or in small groups. Pattern B consisted of diffuse positivity in the horizontal plane, which involved the basal, parabasal and intermediate layers, without extending to the upper third of the epithelium. Pattern C consisted of diffuse positivity in all epithelial layers.

For certain comparisons pattern A and pattern A-low were considered together as focal staining, while patterns B and C were considered together as diffuse staining for further analysis.

**HPV detection and typing**

DNA extraction was performed by using the Qiamp DNA mini kit (QIAGEN, Hilden, Germany), as described by the manufacturer. The quality of extracted DNA was assessed by spectrophotometry and DNA integrity for each sample was assessed by PCR amplification of the β-globin gene by aPCO4, GCβ primers.

DNA was amplified under standard conditions with the L1 consensus HPV PGMY09/PGMY11 primer set, giving a PCR product of 450 bp. Digestion of PCR products by restriction enzymes (Ddel, BamHI, Rsal, PstI, HindII, HaeIII, Sau3AI; New England Biolabs) and subsequent agarose gel electrophoresis allowed HPV genotyping.

Samples that were negative for PCR by the PGMY09/PGMY11 primers were checked for high and low risk HPV types (16, 18, 31, 33/6, 11, respectively), by commercial kits (Maxim Biotech, CA, U.S.A.). All the results were confirmed by Innolipa HPV genotyping kit (Innogenetics, Gent, Belgium).

**Statistical evaluation**

Statistical analyses were performed using the Statistical Package SPSS 13.0 for Windows (Chicago, USA); p values < 0.05 were considered indicative of statistical significance.

**Results**

Twenty-five high-grade squamous intraepithelial lesions, 55 low-grade squamous intraepithelial lesions, and 20 biopsies without diagnostic histopathologic abnormalities were included in the study (Table 1). The age of the patients based on the pathology reports varied from 17 to 58 years (mean 37.5 years).

Four patterns of immunopositivity were recognized according to the distribution of positively stained cells, as described in the previous section, and these correlated to lesion grade (Table 1). Nuclear as well as cytoplasmic immunoreactivity was usually observed. The different patterns are presented schematically in Figure 1, while representative examples of immunopositivity are presented in Figures 2-3. Overall, p16 positivity correlated to the presence of a squamous intraepithelial lesion (p < 0.001) and to the detection of HPV (p < 0.001). Sensitivity of p16 immunopositivity for the detection of SIL was 81.2% and specificity 85%.

Table 1.— Immunoreactivity patterns of p16 in different groups of lesions.

<table>
<thead>
<tr>
<th></th>
<th>Pattern A</th>
<th>Pattern B</th>
<th>Pattern A-low</th>
<th>Pattern C</th>
<th>Negative Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGSIL</td>
<td>11</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>LGSIL</td>
<td>1</td>
<td>7</td>
<td>18</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Specimens negative for SIL</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

*HPV(+).

![Figure 1. — Patterns of p16 immunoreactivity presented schematically.](image)
High-grade squamous intraepithelial lesions (HGSIL)

Twenty-five lesions diagnosed as HGSIL were included in the study. All of them had a positive HPV test (100%). High-risk HPV types were detected in all cases, HPV 16 and HPV 31 being the most common types (Table 2).

Immunoreactivity for p16 (Figure 2a) was detected in 24 biopsies diagnosed as HGSIL (96%). Only patterns B and C were encountered.

Low-grade squamous intraepithelial lesions (LGSIL)

Fifty-five lesions diagnosed as LGSIL were included in the study. Fifty-one (92.7%) had a positive HPV test. HPV16 and HPV6/11 were the most common types, followed by HPV53, HPV33 and HPV45 (Table 2). Among HPV-positives, 70% of the cases tested for HPV type were associated with high- or probable high-risk virus types. Four cases with a negative HPV test exhibited p16 immunopositivity.

Immunoreactivity for p16 was detected in 41 biopsies diagnosed as LGSIL (74.5%). Pattern A-low was the most common (Table 1, Figure 3b), while pattern C was observed in only one case. This latter case and two of the cases exhibiting pattern B positivity were characterized by markedly increased nuclear dimensions in the upper epithelial layers in comparison to other cases. The percentage of high-risk or probable high-risk HPV associated lesions positive for p16 was 71.4% (25/35). This was not significantly different from immunopositivity observed in low-risk HPV associated lesions.

In cases with an A-low pattern of immunoreactivity HPV6/11 were the most common, followed by HPV16 and HPV53. In cases with pattern A immunoreactivity HPV16 was the most common. Cases with diffuse immunoreactivity (patterns B and C) were mostly associated with HPV types 31, 6/11, 58.

Negative specimens

Twenty biopsies considered negative for an HPV-associated squamous intraepithelial lesion on histopathologic examination, even on review, were included in the study. Five of these biopsies had a positive HPV test (25%).
HPV types detected included 16, 18, 61, 33 and 53. The immunohistochemical stain for p16 was positive in only three of these cases (15%), with an A-low pattern of positivity in two cases and pattern B in one case associated with HPV 53. Careful review of this latter case showed that its histopathologic characteristics could be considered borderline for the diagnosis of a squamous intraepithelial lesion.

Discussion

Our study, in concurrence with previously published studies, showed that p16 immunoreactivity is increased in cervical squamous intraepithelial lesions. High-grade lesions were characterized in all positive cases by diffuse immunoreactivity in the dysplastic cervical epithelium. Both these findings are in agreement with previous studies which are summarized in Tables 3 and 4.

P16 decelerates the cell cycle; it functions as a tumor suppressor by modulating the responses to hyperproliferative signals [53, 54] and has a role in cellular senescence [55]. The expression of p16 is altered in several human tumors by deletions, mutations, or methylation [15-17, 56], while germline mutation carriers are predisposed to a high risk of pancreatic and breast cancers [57]. Gene alterations have been also described in cervical carcinoma cases [58-62]. However, the increased expression observed in HPV-related intraepithelial squamous lesions is mainly attributed to the presence of a feedback loop, which depends on the status of pRb, and the well-known potential of HR-HPV E7 proteins to inactivate the latter [13, 19, 63-64]. pRb inactivation is a main action of E7 protein, but the interactions are probably influenced by several factors, as presented in the following.

E7 proteins of different HPV types differ in their efficiency for pRb binding and degradation [13, 62-63]. As a consequence, HPV type would be expected to influence the action of the feedback loop that results in increased p16 expression. Integration of the viral genome with associated loss of the inhibitory E2 action [65], might be another important factor in this cascade of events. In addition, alterations of the CDKN2A gene or its promoter(s) might occur in some intraepithelial lesions [59, 66]. Finally, other factors, related or not to the feedback mechanism, may affect the degree of overexpression [10]. As a result, despite the repeatedly reported correlation of p16 immunopositivity with detection of HPV and with detection of SIL, expectations concerning the discovery of a marker showing a positive immunoreaction in every squamous intraepithelial lesion or in every HR-HPV related lesion would probably not be fulfilled, especially considering the additional role of technical factors. A review of previously published studies summarized in Table 3, together with the results of our present study, reveal exactly these limitations, although they also point to spe-
detection of SIL was 81.2% and specificity 85%, while study sensitivity of p16 immunopositivity for the predictive value varied from 75.7% to 94.1%. In the presence of low-grade lesions to 100%. It is of note that, despite the case of certain diagnostic dilemmas.

Table 3. — Positive p16 immunostaining in high- and low-grade squamous intraepithelial lesions reported in the literature.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Antibody used</th>
<th>Evaluation of staining</th>
<th>HGSIL positivity</th>
<th>LSIL positivity</th>
<th>Non-neoplastic epithelia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sano et al. 1998 [11]</td>
<td>JC8</td>
<td>&gt; 5% cells</td>
<td>37/37 (100%)</td>
<td>20/20 (100%)</td>
<td>negative</td>
</tr>
<tr>
<td>Keating et al. 2001 [6]</td>
<td>G175-405 (Pharmingen)</td>
<td>C</td>
<td>34/37 (91.9%)</td>
<td>21/24 (87.5%)</td>
<td>3/24 (12.5%)</td>
</tr>
<tr>
<td>Klaes et al. 2002 [21]</td>
<td>E6H4</td>
<td>Diffuse staining</td>
<td>53/53 (100%)</td>
<td>15/17 (88.2%)</td>
<td>7/58 (12.1%)</td>
</tr>
<tr>
<td>Tsuda et al. 2003 [22]</td>
<td>Polyclonal (Pharmingen)</td>
<td>N ≥ 5% cells</td>
<td>1/9 (11.1%)</td>
<td>4/33 (12.1%)</td>
<td></td>
</tr>
<tr>
<td>Agoff et al. 2003 [23]</td>
<td>E6H4 (MTM)</td>
<td>N and C ≥ 5% cells</td>
<td>163/193 (84.5%)</td>
<td>43/76 (56.6%)</td>
<td>24/208 (11.5%)</td>
</tr>
<tr>
<td>Yoshida et al. 2004 [24]</td>
<td>JC8 (Neomarkers)</td>
<td>N and C</td>
<td>36/37 (97.3%)</td>
<td>3/8 (37.5%)</td>
<td>3/38 SM (7.9%)</td>
</tr>
<tr>
<td>Wang et al. 2004 [25]</td>
<td>E6H4 (MTM)</td>
<td>Any immuno-reactivity</td>
<td>36/38 (94.7%)</td>
<td>54/73 (72%)</td>
<td>24/149 (16.1%)</td>
</tr>
<tr>
<td>Branca et al. 2004 [26]</td>
<td>Polyclonal (Abcam)</td>
<td>N and/or C</td>
<td>95/117 (81.2%)</td>
<td>7/20 (35%)</td>
<td>0/9 (0%)</td>
</tr>
<tr>
<td>Negri et al. 2004 [27]</td>
<td>ClNec p16 Histology Kit (DakoCytomation)</td>
<td>N and C ≥ 5% cells in lower third</td>
<td>31/31 (100%)</td>
<td>71/96 (74.7%)</td>
<td>ND</td>
</tr>
<tr>
<td>Tringler et al. 2004 [28]</td>
<td>i6P04 (Neomarkers)</td>
<td>N and C</td>
<td>46/46 (100%)</td>
<td>13/18 (72.2%)</td>
<td>7/108 (6.5%)</td>
</tr>
<tr>
<td>Volgareva et al. 2004 [29]</td>
<td>E6H4 (MTM)</td>
<td>N and/or C</td>
<td>28/62 (45.2%)</td>
<td>19/51 (37.2%)</td>
<td>1/31 (3.2%)</td>
</tr>
<tr>
<td>Lorentzato et al. 2005 [7]</td>
<td>p16INK4A (Dako)</td>
<td>Any immuno-reactivity</td>
<td>40/43 (93%)</td>
<td>20/29 (68.9%)</td>
<td>1/27 (3.7%)</td>
</tr>
<tr>
<td>Guimarães et al. 2005 [30]</td>
<td>p16/4sh4 (Labvision)</td>
<td>N and C ≥ 1% cells</td>
<td>13/18 (72.2%)</td>
<td>15/26 (57.6%)</td>
<td>ND</td>
</tr>
<tr>
<td>Murphy et al. 2005 [31]</td>
<td>p16 (Pharmingen)</td>
<td>N or C</td>
<td>78/79 (98.7%)</td>
<td>38/38 (100%)</td>
<td>0/20 (0%)</td>
</tr>
<tr>
<td>Drey et al. 2005 [32]</td>
<td>JC8 (Biocare Medical)</td>
<td>N and/or C</td>
<td>74/77 (96.1%)</td>
<td>20/27 (74.1%)</td>
<td>6/85 (7.0%)</td>
</tr>
<tr>
<td>Kalof et al. 2005 [33]</td>
<td>ClNec p16 Histology Kit (DakoCytomation)</td>
<td>N and C</td>
<td>17/17 (100%)</td>
<td>24/25 (96%)</td>
<td>Variable weak C-positivity in the lower half</td>
</tr>
<tr>
<td>Qiao et al. 2005 [34]</td>
<td>G175-405 (Pharmingen)</td>
<td>C Continuous</td>
<td>16/16 (100%)</td>
<td>ND</td>
<td>0/15 (0%)</td>
</tr>
<tr>
<td>Lin et al. 2005 [35]</td>
<td>E6H4 (MTM)</td>
<td>&gt; 5% cells</td>
<td>29/30 (96.7%)</td>
<td>0/20 (0%)</td>
<td></td>
</tr>
<tr>
<td>Benevolo et al. 2006 [36]</td>
<td>E6H4 (DakoCytomation)</td>
<td>N,C</td>
<td>20/21 (95.2%)</td>
<td>17/154 (37.2%)</td>
<td>0/17 (0%)</td>
</tr>
<tr>
<td>Ishikawa et al. 2006 [37]</td>
<td>E6H4 (MTM)</td>
<td>Moderate and strong</td>
<td>77/88 (87.5%)</td>
<td>13/53 (24.5%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>Yildiz et al. 2007 [38]</td>
<td>ClNec p16 Histology Kit (DakoCytomation)</td>
<td>N and/or C</td>
<td>20/20 (100%)</td>
<td>12/15 (80%)</td>
<td>ND</td>
</tr>
<tr>
<td>Hariri and Oster 2007 [39]</td>
<td>p16 Histology Kit (Dako)</td>
<td>N and C ≥ 5% cells in each layer</td>
<td>49/49 (100%)</td>
<td>65/91 (71.4%)</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Van Nickerk et al. 2007 [40]</td>
<td>E6H4 (DakoCytomation)</td>
<td>N and C ≥ 5% cells in each layer</td>
<td>124/128 (96.9%)</td>
<td>32/56 (57.1%)</td>
<td>50/218 (22.9%)</td>
</tr>
<tr>
<td>Regauer and Reich 2007 [41]</td>
<td>E6H4 (MTM)</td>
<td>Moderate to strong N and C</td>
<td>48/48 (100%)</td>
<td>3/30 (10%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>Iaconis et al. 2007 [42]</td>
<td>P16 (MTM)</td>
<td>Moderate to strong N and C</td>
<td>36/36 (100%)</td>
<td>1/23 (4.3%)</td>
<td></td>
</tr>
<tr>
<td>Kong et al. 2007 [43]</td>
<td>E6H4 (DakoCytomation)</td>
<td>N and/or C ≥ 5% cells</td>
<td>16/16 (100%)</td>
<td>11/12 (91.7%)</td>
<td>2/30 (6.7%)</td>
</tr>
<tr>
<td>Eleuterio et al. 2007 [44]</td>
<td>E6H4 (DakoCytomation)</td>
<td>Moderate or diffuse N and C ≥ 10% cells</td>
<td>12/13 (92.3%)</td>
<td>4/26 (15.4%)</td>
<td>0/57 (0%)</td>
</tr>
<tr>
<td>Fochi et al. 2007 [45]</td>
<td>Ab7 16PO7 (Neomarkers)</td>
<td>C and N ≥ 5% cells</td>
<td>65/65 (100%)</td>
<td>80/88 (90.9%)</td>
<td>9/114 (7.9%)</td>
</tr>
<tr>
<td>Silva et al. 2007 [46]</td>
<td>P16 (Cell Marque)</td>
<td>N and C</td>
<td>14/14 (100%)</td>
<td>26/34 (76.5%)</td>
<td>0/14 (0%)</td>
</tr>
<tr>
<td>Redman et al. 2008 [47]</td>
<td>JC8 (Dako)</td>
<td>N and C &gt; 5% cells</td>
<td>ND</td>
<td>30/31 (97%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>Ozoglu et al. 2008 [48]</td>
<td>E6H4</td>
<td>N,C</td>
<td>22/22 (100%)</td>
<td>6/13 (46.2%)</td>
<td>3/25 (12%)</td>
</tr>
<tr>
<td>Pinto et al. 2008 [49]</td>
<td>G175-405 (Pharmingen)</td>
<td>N and C</td>
<td>51/61 (84%)e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>H612 (Novocastra)</td>
<td>N and/or C</td>
<td>24/25 (96%)</td>
<td>41/55 (74.5%)</td>
<td>3/20 (15%)</td>
</tr>
</tbody>
</table>

N: nuclear; C: cytoplasmic; SM: squamous metaplasia; ND: no data.

* In the first biopsy; ** Atrophy, with or without atypia; *** HPV(+); † Including cases suspicious for HPV presence; ‡ Mainly HSIL and diagnostically challenging cases.

cific applications of p16 IHC, which can be invaluable in the case of certain diagnostic dilemmas.

Table 3 summarizes the results of previously published studies, the antibodies and the evaluation criteria used. Positivity varied from 10% for low-grade squamous intraepithelial lesions to 100%. It is of note that, despite the undoubted influence of technical problems and geographic differences in HPV-type distribution, with increasing numbers of cases in each study there often appears a small group of HGStILs that do not show any immunoreactivity. In the three largest series [23, 40, 45] reporting more than 200 cases (SILs and controls) each, sensitivity of p16 for the detection of SIL varied from 76.6% to 94.8%, with a value of 83.7% calculated in the total number of their cases, while specificity varied from 77.1% to 92.1%, with a value of 84.6% calculated for the total number of cases. In the same studies the positive predictive value varied from 75.7% to 94.1%. In the present study sensitivity of p16 immunopositivity for the detection of SIL was 81.2% and specificity 85%, while the positive predictive value was 95.6%. The results point towards the use of p16 immunostain as a surrogate test in conjunction with histopathologic evaluation. Addition of a consecutive p16-stained slide to the HE-stained slides has been shown to significantly improve interobserver agreement for both punch and cone biopsies [21, 67].

By focusing only on diffuse immunopositivity, differently defined by different groups, the percentage of positively stained lesions varies, as summarized in Table 4. It has been suggested that this type of immunoreactivity is associated with integration of the viral genome, but there is still no direct proof of this [33]. The alternative explanation of monoclonality associated with other (epi)genetic alterations that might lead to p16 overexpression also lacks support. In our material diffuse positivity was observed in all p16-positive high-grade lesions, its sensitivity for HSIL being 96% and its specificity 88%. In the group of low-grade lesions there was no significant difference in HR-HPV detection between cases with or without diffuse positivity. Although this might be partly due to the...
Different patterns of p16 immunoreactivity in cervical biopsies: correlation to lesion grade and HPV detection etc.

Table 4. — Percentage of high- and low-grade squamous intraepithelial lesions showing diffuse p16-immunopositivity as reported in the literature.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Evaluation of diffuse staining</th>
<th>HGSI (%)</th>
<th>LGSI (%)</th>
<th>Non-neoplastic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sano et al. [11]</td>
<td>&gt; 80%</td>
<td>100</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>Keating et al. [6]</td>
<td>Continuous in the horizontal plane</td>
<td>70.3</td>
<td>37.5</td>
<td>0</td>
</tr>
<tr>
<td>Klaes et al. [21]</td>
<td>Diffuse staining</td>
<td>100</td>
<td>88.2</td>
<td>12.1</td>
</tr>
<tr>
<td>Agoff et al. [23]</td>
<td>&gt; 75%</td>
<td>57</td>
<td>11.8</td>
<td>3.8</td>
</tr>
<tr>
<td>Yoshida et al. [24]</td>
<td>&gt; 30%</td>
<td>86.5</td>
<td>0</td>
<td>2.6</td>
</tr>
<tr>
<td>Wang et al. [25]</td>
<td>Diffuse staining</td>
<td>81.6</td>
<td>36</td>
<td>5.2</td>
</tr>
<tr>
<td>Branca et al. [26]</td>
<td>Diffuse intense</td>
<td>5.1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Tringler et al. [28]</td>
<td>&gt; 80%</td>
<td>80.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Volgareva et al. [29]</td>
<td>&gt; 25%</td>
<td>6.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Guinannes et al. [30]</td>
<td>&gt; 30%</td>
<td>33.3</td>
<td>26.9</td>
<td>ND</td>
</tr>
<tr>
<td>Murphy et al. [31]</td>
<td>&gt; 50%</td>
<td>55.7</td>
<td>60.5</td>
<td>0</td>
</tr>
<tr>
<td>Dray et al. [32]</td>
<td>Diffuse, at least parabasal</td>
<td>94.8</td>
<td>25.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Kalof et al. [33]</td>
<td>Diffuse, 2/3 to full thickness</td>
<td>88.2</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Yildiz et al. [38]</td>
<td>Full thickness</td>
<td>50</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Hariri and Oster [39]</td>
<td>Continuous basal and parabasal</td>
<td>100</td>
<td>71.4</td>
<td>6</td>
</tr>
<tr>
<td>Regauer and Reich [41]</td>
<td>Diffuse staining</td>
<td>100</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Kong et al. [43]</td>
<td>&gt; 80%</td>
<td>100</td>
<td>58.3</td>
<td>0</td>
</tr>
<tr>
<td>Focchi et al. [45]</td>
<td>&gt; 25%</td>
<td>100</td>
<td>90.9</td>
<td>0</td>
</tr>
<tr>
<td>Shi et al. [46]</td>
<td>&gt; 50%</td>
<td>92.8</td>
<td>64.7</td>
<td>0</td>
</tr>
<tr>
<td>Redman et al. [47]</td>
<td>&gt; 80%</td>
<td>ND</td>
<td>23.4</td>
<td>0</td>
</tr>
<tr>
<td>Ozgel et al. [48]</td>
<td>&gt; &gt;25%</td>
<td>77.3</td>
<td>15.4</td>
<td>0</td>
</tr>
<tr>
<td>Present study</td>
<td>Patterns B+C</td>
<td>96</td>
<td>14.5</td>
<td>5</td>
</tr>
</tbody>
</table>

*In the first biopsy; †All associated with high-risk or unknown HPV types; ‡HPV(+).

Table 5. — Positive p16 immunostaining in HR-HPV positive LGSIIL tested by PCR or Hybrid Capture.

<table>
<thead>
<tr>
<th>Authors</th>
<th>p16 positivity</th>
<th>Method of HR-HPV detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agoff et al. [23]</td>
<td>6/7 (85.7%)</td>
<td>HC2</td>
</tr>
<tr>
<td>Wang et al. [25]</td>
<td>36/44 (81.8%)</td>
<td>PCR</td>
</tr>
<tr>
<td>Kalof et al. [33]</td>
<td>17/18 (94.4%)</td>
<td>PCR</td>
</tr>
<tr>
<td>Benevolo et al. [36]</td>
<td>14/19 (73.6%)</td>
<td>PCR</td>
</tr>
<tr>
<td>Ishikawa et al. [37]</td>
<td>12/37 (32.4%)</td>
<td>PCR</td>
</tr>
<tr>
<td>Van Nickerk et al. [40]</td>
<td>28/42 (66.6%)</td>
<td>HC2</td>
</tr>
<tr>
<td>Ordi et al. [50]</td>
<td>39/50 (78.6%)</td>
<td>HC2</td>
</tr>
<tr>
<td>Present study</td>
<td>25/35 (71.4%)</td>
<td>PCR</td>
</tr>
</tbody>
</table>

HC2: Hybrid Capture II HPV test.

small number of cases, it is noteworthy that in several studies presented in Table 5 a significant percentage of LGSIILs associated with HR-HPV, as detected by PCR or HC2, does not exhibit any p16 immunopositivity.

Another aspect of p16 immunostaining is the possible correlation with lesion “progression”. It has been suggested [6] that certain phases of a given HR-HPV-associated neoplastic process may have different indices of p16 expression. Increased p16 immunopositivity has been reported to correlate with progression to high-grade lesions [25, 27, 30]. In a more recent study evaluating diffuse p16 immunostaining the negative predictive value in predicting the outcome of CIN1 cases was as high as 96% [39]. In a study including conization specimens with coexisting CIN1 and CIN3 areas, all CIN1 were p16 positive [68]. p16 staining did not predict persistence or clearance of HR-HPV after treatment for CIN in a study by Branca et al. [26]. In our material follow-up information was limited and correlation to outcome was not possible.

An interesting finding of our study was the difference in HPV-type distribution between cases showing pattern A positivity and those showing pattern A-low, that is between two patterns of sporadic/focal positivity. To the best of our knowledge, this distinction has not been made in previous studies, although staining patterns corresponding to our patterns B and C have been described by different groups of investigators [34, 42, 43]. The above difference might reflect an earlier/increased sporadic expression of E7 in certain lesions, but also underlines a relative lack of recent studies correlating basic biological events and their morphologic appearances.

In summary, the results of our study support the use of p16 as an adjunctive test, in conjunction with careful morphologic evaluation. Although p16 immunohistochemistry has emerged in the last few years as a helpful, inexpensive test that might be used instead of HPV testing in diagnostically problematic biopsies, it lacks in most large studies 100% sensitivity or specificity. Awareness of its patterns of positivity and its limitations might allow for a more proper use in certain clinicopathological settings, aided by standardization of staining and evaluation protocols.

References


Different patterns of p16 immunoreactivity in cervical biopsies: correlation to lesion grade and HPV detection etc.


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Mezourlo, Larissa (Greece)
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Detection of human papillomavirus E6/E7 mRNA in women with high-risk HPV types 16, 18, 31, 33 and 45 which are associated with the development of human cervical cancer

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Summary

Purpose of investigation: The aim of our study was to increase the clinical meaningfulness of the virological data through mRNA E6/E7 oncoprotein identification, and to find a correlation between codon 72 polymorphism of the p53 gene and integration of HPV in host cell genomes. Methods: We analyzed 80 cervical samples from women with HPV DNA types 16, 18, 31, 33 and 45. Transcripts of HPV were detected by the NucliSense EasyQ HPV assay and genotyping of the TP53 polymorphism was conducted using a TaqMan assay. Results: Twenty percent of 80 tested samples were positive for mRNA Papillomavirus. The frequency of Arg/Pro heterozygotes in controls was over-represented compared with mRNA positive samples while there were no significant differences in the distribution of Pro/Pro and Arg/Arg alleles. Conclusion: The introduction of HPV mRNA testing in clinical analysis improved diagnostic accuracy of HPV infections. Our data suggest that a structural difference at codon 72 of the p53 gene may not be a sufficient risk factor for cervical carcinogenesis.

Key words: HR-HPV; E6/E7 mRNA; p53 polymorphisms; Cervical cancer.

Introduction

Human papilloma viruses (HPVs) are the cause of a range of proliferative lesions upon infection of epithelial cells [1].

More than 130 epitheliotropic genotypes belong to the heterogeneous group of papillomaviruses, 16 of these DNA viruses are considered “high-risk” types and are linked with the development of malignant diseases [2]. HPV types 16 and 18 are associated with more than 70% of all cervical cancers [3].

The integration of the viral genome into the host genome disrupts E2, the negative regulator of early gene E6 and E7 transcription, and this results in an overexpression of these oncoproteins [4, 5].

The overexpression of E6 and E7 leads to the accumulation of DNA damage and the development of cervical cancer [6]. These oncoproteins bind and functionally inactivate tumor suppressor protein p53 and members of the retinoblastoma (Rb) tumor suppressor family (pRb, p107 and p130), resulting in the dysregulation of critical cellular functions such as cell-cycle control, apoptosis, senescence, DNA repair and maintenance of genomic stability [7-10].

Normally, p53 promotes cell cycle arrest or initiates apoptosis responding to cellular stress. E6 promotes p53 accelerated proteosomal degradation via ubiquitination [11]. The absence of p53 stops cell cycle arrest in response to genetic insult, promoting the accumulation of harmful mutations that may contribute to malignant progression. The oncoprotein E7 promotes cell cycle advancement and entry into the S-phase. The best characterized target of E7 in this regard is the retinoblastoma tumor suppressor protein (pRB), which is linked and destabilized by E7 via ubiquitin-mediated proteosomal degradation [12]. The substitution of arginine (Arg) by proline (Pro) at codon 72 in the p53 gene seems to be a polymorphism that alters the primary structure of the p53 protein [13]. Recently, biochemical and functional differences between the two p53 forms have been identified [14, 15]. It was demonstrated experimentally that the Arg form of the p53 protein was more susceptible than the Pro form to binding and degradation by the HPV-E6 oncoprotein [16]. However, similar studies on cervical and other human cancers have produced contradictory results [17, 18].

The aim of this study was to increase the clinical meaningfulness of the virological data through mRNA E6/E7 oncoprotein identification by using the real-time NASBA method, and to find a possible correlation between codon 72 polymorphism of the p53 gene and the integration of HPV in host cell genomes.

Materials and Methods

In the study we analyzed 80 cervical samples of women aged between 18 and 60 years, with HPV DNA types 16, 18, 31, 33 and 45 from the city of Messina and province. In particular these were taken from patients who, according to the Bethesda System Cytology classification, 57% (46 patients) belonged to category HSIL (high-grade squamous intraepithelial lesion), 25% (20 patients) to category LSIL (low-grade squamous intraepithelial lesions) and 18% (14 patients) had normal cytology. The largest percentage of patients were positive for HPV DNA type 16 (54%; 43 patients), while for other high-risk HPV types...
Detection of human papillomavirus E6/E7 mRNA in women with high-risk HPV types 16, 18, 31, 33 and 45 which are associated etc.

Results

The NucliSENS test allowed us to identify the viral mRNA-positive patients and, depending on that, those presenting an integration of the virus into the host genome. Twenty percent (16 specimens) of the 80 tested samples were positive for papillomavirus mRNA. Particularly 12.5% (10 specimens) of mRNA positive samples showed HPV DNA type 16 and the remaining 7.5% (6 specimens) showed HPV DNA type 31 while none of the samples positive for types 18, 33 and 45 had viral mRNA detected. No multiple infections were detected in patients positive to the NucliSENS test while different genotypes studied, type 31 was present in 28% (22 patients) of the samples, type 18 in 14% (11 patients) and only 2% (2 patients) of specimens were positive for HPV types 33 and 45. Cervical specimens were tested with the NucliSENS EasyQ HPV assay (bioMérieux Italia S.p.A) according to the manufacturer's instructions. This is a real-time nucleic acid amplification and detection assay for the qualitative determination of E6/E7 mRNA from five carcinogenic HPV types 16, 18, 31, 33 and 45 in cervical scrapes [19, 20]. We have also investigated the p53 codon 72 polymorphism in high-risk HPV positive samples. None of the patients with negative results to HPV DNA testing and negative to NucliSENS EasyQ HPV testing. From whole blood, we performed the extraction of nucleic acids using the Puregene Blood Kit (Gentra, Milan Italy). Genotyping of the TP53 codon 72 (rs1042522) polymorphisms was conducted using a TaqMan assay with an ABI PRISM 7900 sequence detection system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The polymerase chain reaction (PCR) contained 10 ng of genomic DNA, 1x TaqMan Genotyping Master Mix, and 1x of assay mix (C_2403545_10). PCR was performed using 96 well plates on a thermal cycler (ABI 9700; Applied Biosystems). Reaction conditions were 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Discussion

It is widely known that persistent infection by high-risk types of papillomaviruses has been associated with the development of human cervical cancer. Epidemiological and experimental evidence has demonstrated that progression to malignancy requires the loss of expression of the viral E2 gene in cancer cells and the over-expression of E6 and E7 genes in the integrated HPV genome. It follows that the detection of E6/E7 mRNA indicates HPV oncogenic activity and may be used as a clinically predictive marker to identify women at risk of developing high-grade cervical dysplastic lesions and cervical carcinoma. HPV testing is today widely accepted for reflex testing in case of abnormal Pap smear and screening with HPV may be implemented in the future.

HPV infection is necessary [21] but not sufficient to cause cervical cancer. The prevalence of HPV DNA in cervical smear specimens is very high, and most infections are transient. For these reasons the potential use of HPV DNA testing for early detection of cervical cancer is limited and would only marginally reduce the follow-up colposcopy and histology [22]. Testing for HPV oncogenic activity, rather than for the presence of HPV DNA, may therefore be a more relevant clinical indicator of the development of cervical lesions and cervical cancer [23, 24]. The NucliSENS EasyQ HPV test tries to establish direct detection of the expression of oncogenic risk factors E6 and E7 from the five most prevalent HPV types in cervical cancer worldwide (HPV 16, 18, 31, 33 and 45). NucliSENS EasyQ HPV is a high medical value test for physicians that can significantly contribute to improved patient management due to its capacity to not only detect E6/E7 oncogenic activity, but also provide individual genotypic information for these five high-risk HPV genotypes in cervical specimens. Our data revealed that a significant percentage of patients with normal cytology showed integration of the viral genome, thus highlighting how the introduction of HPV mRNA testing in clinical analysis improved diagnostic accuracy of HPV infections, distinguishing one clinical infection by a transient infection with high risk of clinically active tumorigenic transformation. The oncogenic activity of high-risk HPVs is explained in part by the ability of the viral E6 oncoprotein to target p53 for degradation and thus to inhibit p53-mediated transcription [25]. Several studies
report the p53 dysfunction caused by HPVs depend on the status of a polymorphism at codon 72, Arg or Pro. Although our study was conducted on a heterogeneous group of specimens, data suggested that a structural difference at codon 72 of the p53 gene may not be an obvious and sufficient risk factor for cervical carcinogenesis. In any case the real effect of the p53 codon 72 polymorphism on HPV-associated cervical neoplasia needs to be investigated along with other in vivo and in vitro research.

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References


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The effect of hyaluronic acid (Cicatridine) on healing and regeneration of the uterine cervix and vagina and vulvar dystrophy therapy

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³Gynaecological Oncology and Obstetrics Center, Poznan University of Medical Sciences in Poznan (Poland)

Summary

Procedures aimed at the treatment of precancerous lesions and ectopia on the uterine cervix are frequently linked to lesions of anatomical structures. The application of hyaluronic acid (Cicatridine vaginal ovules) promotes accelerated healing of the uterine cervix and acquisition of a normal shape in the uterine cervix canal. Local application of hyaluronic acid in the vagina following radiotherapy due to cancer in the uterine cervix or endometrium favourably affects the healing of post-irradiation lesions in the vagina and improves quality of life. Over 90% of patients responded positively to the application of hyaluronic acid in the form of a cream on dystrophic lesions in the vulva. Hyaluronic acid aids the healing process of post-procedural wounds in the uterine cervix, following radiotherapy applied due to cancer of the uterine cervix, endometrium and in vulvar dystrophy.

Key words: Hyaluronic acid; Uterine cervix; Dysplasia of uterine cervix; Radiotherapy; Endometrial carcinoma; Cancer of uterine cervix; Dystrophy.

Introduction

Uterine cervix treatment of precancerous lesions or ectopia are linked to surgical, thermal or electric trauma. A significant proportion of females after radiotherapy due to cervical or endometrial cancer suffer post-irradiation reactions in the vagina, involving the thinning and an increasing fragility of the vaginal walls, vasodilation and atrophy. This may lead to a narrowing of the vaginal walls, its adhesion, ulcerations and even to the formation of vesico-vaginal and recto-vaginal fistulas [1, 2]. At the same time, irradiation linked to surgical castration induced by the procedure reduces several functions, thus affecting the quality of life [3-5].

Reparative and remodelling processes within the uterine cervix manifest a variable duration, depending on the type of therapeutic intervention. During menopause, mainly due to oestrogen deficiency, a significant proportion of women also manifest vulvar lesions. The amount of adipose tissue becomes reduced, collagen fibres disappear, the epithelium becomes thinner and its sublayer demonstrates traits of chronic inflammation, lichen sclerosus. At the time morphotic elements of the vulva undergo atrophy: the small pudendal lip and clitoris develop dystrophic lesions. Occasionally, the epithelium is white, thickened and cracked, which is characteristic of a hypertrophic dystrophy [6].

Hyaluronic acid, the main component of Cicatridine, acts on two levels – water retention in the tissues and through binding to proteins and the formation of a proteoglycan network. Thus it improves tissue hydration and the import of nutrients to the tissue. This results in a normal tissue turgor, its improved elasticity and in epithelial regeneration in atrophy and dystrophy of vaginal mucosa. Through its presence in the extra-cellular matrix and due to its modulating potential as well as due to its promotion of capillary sprouting, hyaluronic acid favourably affects healing processes, including the formation of a normal epithelium [7-9].

The study was aimed at evaluating Cicatridine, applied in the form of ovules (1 ovule contains: 0.005 g sodium salt of hyaluronic acid, 0.06 g – Centella asiatica oil extract (Hydrocotyle asiatica L., Hydrocotyle repanda Pers., Gotu kola), 0.06 g – calendula oil extract, 0.06 g – aloe oil extract, 0.002 g – tea-tree essential oil) in the course of healing and reparation processes following surgical procedures on the uterine cervix, and following radiotherapy applied due to cancer of the uterine cervix, endometrium and in vulvar dystrophy.

Patients and Methods

The total number of 213 women, including 109 patients following procedures involving the uterine cervix due either to CIN I - CIN III (following electrocoagulation) or ectopy of the uterine cervix (following electrocoagulation and cryotherapy), 53 women following radiotherapy due to cancer of the uterine cervix or cancer of the endometrium and 51 women in whom earlier vulvar dystrophy was histologically confirmed.

A control group consisted of 86 patients following procedures on the uterine cervix, following radiotherapy or showing vulvar dystrophy, in whom the studied preparation was not applied. The analysed material is listed in Table 1.

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In patients following procedures on the uterine cervix aimed at accelerated re-epithelization, the application of hyaluronic acid in the form of vaginal ovules was started 24 hours after the operation.

In patients following radiotherapy, indications for the treatment involved the detection of inflammatory/necrotic lesions in the vagina, adhesions of vaginal walls and complaints associated with coitus after the completion of brachytherapy. Vaginal ovules of the preparation were applied once daily, in the evening for ten subsequent days and, then, for a period of one month, every second day and, in cases showing improvement, for a further month every third day.

In women with vulvar dystrophy cream containing hyaluronic acid was applied for a month; it was applied to affected sites twice a day. In cases of improvement the procedure was recommended to be continued for a further three months and, then, for two months, applying it at night. Patients in the control group applied no therapy following procedures on the uterine cervix and radiotherapy. Women in the control group and those affected by vulvar dystrophy included patients who following diagnosis did not want to participate in the therapy and decided to follow hygienic procedures only.

Results of the treatment were evaluated after six weeks and three months, and women treated with vaginal ovules of the preparation were evaluated by visual examination of the uterine cervix and/or vagina and patient feeling. In women with vulvar dystrophy treated with hyaluronic acid, evaluation was conducted three and six months after the completion of the therapy by visual inspection and comfort of the patients.

Statistical evaluation was performed by statistical software, using the Wilcoxon and the Mann-Whitney U tests.

Results

Most of the examined women (99-90.83%) in whom Cicatridine vaginal ovules were applied following operations on the uterine cervix due to CIN I-CIN III and ectopy of the uterine cervix after six weeks demonstrated a healed cervix with no deformations or endometriotic foci and in none of the cases did the cervical canal outlet demonstrate any narrowing.

In the control group only 28 women (66.67%) achieved a similar result. Following three months, 106 women of the treated group (97.25%) had a healed cervix while in the control group the fraction of patients was only slightly lower (90.48%) (Table 2).

In patients following radiotherapy treated with vaginal ovules of the preparation a healed vagina was noted in 12 women (22.64%) and improvement was detected in 45 women (84.91%). In the control group no healing or improvement could be detected in any of the patients during the three-month observation period (Table 3).

In the group of 51 women with vulvar dystrophy a positive response to treatment with cream containing the examined substance was noted in 46 patients (90.2%) and in a significant fraction (64.71%) the group consisted of healthy, asymptomatic patients. In patients not exposed to cream, hygienic management resulted in a response in 65% women but in such cases just improvement, not total elimination of the symptoms could be noted (Table 4).

Discussion

Procedures on the uterine cervix, performed due to pre-cancerous lesions (CIN) or ectopy, are linked to a more or less extensive tissue lesion, associated with various complaints, including bleeding, spotting, excessive discharge, fibrosis, endometriosis, narrowing of the uterine cervical canal and a psychological discomfort reflecting the fear that sexual contact may negatively affect the healing process.
The effect of hyaluronic acid (Cicatridine) on healing and regeneration of the uterine cervix and vagina etc.

Table 3. — Women administered the examined preparation vs the control group following radiotherapy due to cancer of the uterine cervix or cancer of the endometrium.

<table>
<thead>
<tr>
<th></th>
<th>Examined group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Healing 3 months</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Patients following radiotherapy due to cancer of the uterine cervix</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Patients following radiotherapy due to endometrial cancer</td>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 4. — Results of observation in patients affected by vulvar dystrophy (preparation vs placebo).

<table>
<thead>
<tr>
<th></th>
<th>Examined group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Healthy asymptomatic women</td>
<td>33</td>
<td>64.71*</td>
</tr>
<tr>
<td>Improvement</td>
<td>13</td>
<td>25.49*</td>
</tr>
<tr>
<td>Stabilization</td>
<td>1</td>
<td>1.96*</td>
</tr>
<tr>
<td>Deterioration</td>
<td>4</td>
<td>7.64</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100.00</td>
</tr>
<tr>
<td>Positive response</td>
<td>46</td>
<td>90.20*</td>
</tr>
</tbody>
</table>

* p < 0.05

The results of our earlier studies, on a lower number of patients, indicated that locally applied Cicatridine preparation accelerated repair processes to a variable extent, depending on the type of procedure on the uterine cervix [10]. In a higher number of women following procedures on the uterine cervix we have confirmed that hyaluronic acid accelerates the healing processes and prevents narrowing of the canal in the uterine cervix. It is quite possible that this involves the same mechanism which prevents development of adhesions after application of hyaluronic acid following abdominal operations in an animal model [11].

Radiotherapy, used following oncological surgery in the treatment of uterine cervix cancer or cancer of the endometrium, induces port-irradiation injury overlapping with castration-linked hypo-oestrogenism. This represents a significant problem of deteriorated life quality while the decreasing pH in the vagina is linked to reduced sexual satisfaction [1-4, 12].

Studies of Katz [1] and those of Lorenz et al. [2] demonstrated that irradiation induced dryness and narrowing of the vagina and in 26% of females it resulted in late post-irradiation reactions, which had a strong negative impact on sexual function, and in this way decreasing the quality of life.

Application of vaginal ovules with the studied preparation has induced healing of the vaginal lesions in over 20% of the patients (Table 3) and an improved vaginal outlook and subjective perception in over 80% of the patients. Such activity has been confirmed by other authors both in animal models and in the course of second-look operations, which have demonstrated the anti-adhesion effects of hyaluronic acid [11, 13].

In the therapy of vaginal dystrophy we see a continuous introduction of novel therapeutic methods. Ayhan et al. [6] obtained a satisfactory clinical response to the application of testosterone and corticoids in such patients but they were not accompanied by histological improvement. In the last ten years the photodynamic method of treating vulvar pathology has been introduced, which proved to be effective following a variable period of application (on average after 6 months) but the disease relapsed [14, 15].

In conclusion, intense application of Cicatridine is non-invasive, easy to implement and inexpensive, and in our group of 51 patients it resulted in a favourable effect of the treatment in over 90% of the patients.

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Clinical analysis of borderline ovarian tumors

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Summary

Purpose: The goal of this study was to evaluate the incidence and clinical features of borderline ovarian tumors (BOTS). Methods: We retrospectively performed chart reviews of 22 patients with BOTS who were diagnosed and treated in the university medical center from 1998 to 2009 inclusively. Results: BOTS among ovarian pathology in our hospital were detected in 22 patients (1.79%). The mean age was 50 years, range (20-90). Post surgical FIGO staging was Stage I = 86.4%, and Stage II = 13.6%. The most common histologic subtype was mucinous (59%). Five patients (22.7%) had a unilocular cyst at ultrasonography. Conservative surgery was performed in 31.8%. One patient of them had normal spontaneous delivery after term pregnancy. Two patients had a recurrence. One patient with recurrent disease underwent transformation to invasive cancer and died 35 months after the initial diagnosis. Conclusion: Clinicians should warn patients about the early relapse of BOTS and these patients may need careful follow-up due to the possibility of recurrences.

Key words: Borderline ovarian tumors; Conservative surgery; Recurrence.

Introduction

Epithelial ovarian tumors of low malignant potential were first described by Taylor in 1929 as a group of patients with ‘semimalignant’ or hyperplastic ovarian tumors without histological evidence of stromal invasion but with peritoneal implants [1]. He noted that these patients had a good prognosis (as tumors with histologic features and biological behavior) between benign and frankly malignant epithelial ovarian neoplasms. In 1971, this group of tumors was accepted by the International Federation of Gynecology and Obstetrics (FIGO) as carcinoma of low malignant potential [1], and in 1973 by the World Health Organization (WHO) as borderline tumors [2]. The 2003 WHO classification defined the borderline tumor as more than two of the tumors lacking stromal invasion, with budding, multilayered epithelium, mitotic activity and nuclear atypia [3]. Borderline ovarian tumors (BOTS) account for 14-15% of primary ovarian neoplasms [4]. Histologic types include serous 55% – the most common – mucinous 40%, mixed 2%, endometrioid 2%, clear-cell < 1% and transitional-cell (or Brenner) tumors < 1%. The latter three histological types are very uncommon – 5% [1]. In up to 10-15% of the BOTS isolated tumor cells or small clusters of tumor cells in the stroma can be detected. One or more such groups may be present. If none of these foci exceed 10 mm2 or 3-5 mm in the largest diameter the lesion is defined as microinvasion [5]. Peritoneal implants were classified into two types as invasive and noninvasive. BOTS are more common in reproductive-age women (mean age 30-50) and have a much better prognosis than invasive tumors. However, they can be present with metastatic disease and can recur. Recurrences can occur as long as ten or 15 years after the primary tumor, and may be in the form of invasive carcinoma. Because of the generally benign behavior of these tumors, their management has become progressively more conservative, allowing women to maintain their fertility. The response to chemotherapy, hormonal therapy, and radiotherapy was poor. The five-year survival for women with Stage I BOTS is about 95% to 97%, but because of late recurrence the ten-year survival is only 70% to 95%. The five-year survival for Stage II-III patients is 65% to 87% [1]. Approximately 80% of patients during diagnosis present with a FIGO Stage I tumor and recurrences are encountered in approximately 10% of all patients. Recurrences can be seen as late as 10-39 years after initial diagnosis, and 0.6% to 19% of patients have recurrence as an invasive carcinoma [6]. In BOTS, pelvic ultrasound and serum tumor marker CA 125 are widely used in diagnosis. Preoperative CA 125 was increased in 56% of patients with a borderline tumor [7]. Carcinoembryonic antigen (CEA) and CA 19-9 for mucinous tumors have been proposed. Preoperative serum CEA and CA 19-9 were increased in 32% and 45% of borderline mucinous tumors, respectively [7]. The purpose of this study was to evaluate frequency of occurrence, diagnosis, treatment, and clinical outcome for 22 patients with ovarian borderline tumors and compare the findings with the recent literature.

Materials and Methods

We retrospectively performed a chart review of 22 patients with ovarian borderline tumors who were diagnosed and treated in one university hospital in Korea from 1998 to 2009 inclusively. The incidence of BOTS among ovarian pathology in our hospital during this period was 1.79%. Histological classification and staging of all tumors were determined according to the 2003 WHO classification and the FIGO classification [3, 7]. All patients underwent primary surgical treatment in order to achieve surgical reduction and to establish the stage of the disease. All patients received intraoperative frozen section. The conservative surgery with staging procedure was performed in...
young patients who desired to preserve fertility. All patients had preoperative serum CA 125, CA 19-9 and CEA measured and underwent transvaginal sonography. For CA 125 the upper limit of normal is 35 U/ml and the upper limit of normal CEA values is 4.9 ng/ml. For CA 19-9, 37 U/ml was used as the upper limit of normal, being the 97th percentile in healthy women. Adjunctive chemotherapy was needed in patients with advanced Stage I or progression to invasive cancer. Secondary cytoreductive surgery was performed on patients who had a recurrence. In all statistical analyses, a $p$ value of $<0.05$ was considered statistically significant. Disease-free and overall survival rates were calculated from the date of surgery to the time of recurrence, last follow-up, or death. Disease-free and overall survival curves for patients were constructed according to the Kaplan-Meier method and statistical differences between the curves were calculated with the log-rank test. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 12.0.

**Results**

Mean age for all 22 patients at the time of diagnosis was 50 years (range 20-90 years). Median follow-up was 42.8 months (range 15-122 months). Thirteen patients had mucinous tumors (59%), five tumors were serous (23%), three tumors were Brenner (13.5%), and there was one mixed tumor (4.5%). Patients included in the study were in Stage I (19/22; 86.4%) and II (3/22; 13.6%). Two patients had an intraepithelial carcinoma lesion and another two patients had microinvasion (9%). CA 125 levels were elevated in six patients (27.3%), CA 19-9 levels were elevated in four patients (18.2%) and CEA levels were elevated in one patient (4.5%). Preoperative CA 125 was more frequently elevated in patients with serous tumors (2/5; 40%) than mucinous tumors (4/13; 30.8%; $p = 0.575$). Preoperative CA19-9 and CEA were only elevated in patients with mucinous tumors (4/13; 25%; $p = 0.336$ and 1/13; 7.7%; $p = 0.867$). However the difference in elevation of tumor markers between histologic type was not statistically significant. Among the 22 women with borderline tumors, 19 (86.4%) underwent laparotomy and three (13.6%) underwent laparoscopy. The type of surgical procedure undertaken varied widely. Conservative surgery (unilateral salpingo-oophorectomy (USO)) was performed in seven (31.8%) patients with disease apparently confined to the ovary, who were young and wanted to preserve fertility. Eleven (50%) patients received surgical staging procedures, six patients received complete surgical staging including lymphadenectomy, and five received limited surgical staging without lymphadenectomy. The analysis of sonography findings of ovarian borderline tumors correlated with histologic types is done. We observed a higher number of multilocular cysts (84.6%) in patients with mucinous BOT than other histologic types. Five (22.7%) BOTS were unilocular, and 17 (77.3%) were multilocular. Seventeen (77.3%) BOTS showed papillae, and five (22.7%) did not. Many BOTS showed fluid/solid mixed echogenic tumors (68.2%). The percentage of pure solid and pure fluid tumors was 13.6 and 18.2%, respectively. The size of tumors ranged from 2-30 cm (mean 13.5 cm). The mean size of the mucinous tumors (15.6 cm) was the largest compared to other histologic types. However the difference in transvaginal ultrasound (TVS) findings of ovarian borderline tumors correlating with histologic types was not statistically significant. One patient with a mucinous BOT had a normal spontaneous delivery at term pregnancy. Tumor recurrence was recorded in two patients (9.0%) with mucinous tumors. During follow-up one patient died due to recurrent disease, 35 months after the initial diagnosis (4.5%). She underwent recurrence two times, 15 and 25 months after the primary surgery. The secondary recurrence progressed to carcinoma, and her age at the time of primary surgery was 37 years. The initial pathology was endocervical type mucinous BOT with intraepithelial carcinoma. The patient received USO and a staging operation (appendectomy, pelvic lymph node dissection, partial omentectomy) as initial surgical procedures. Another patient survived after recurrence. This 24-year-old patient had a mucinous BOT with intraepithelial carcinoma at the time of initial diagnosis. She showed multiple recurrences of the contralateral ovary, right iliac bone and lung 23 months after primary surgery (USO). She has now been alive for 36 months with subclinical lung and iliac bone lesions after the second surgery (USO and partial omentectomy). The cumulative survival rate was 62%/25 months, 31%/50 months and 23%/75 months. The disease-free survival rate was 57%/25 months, 29%/50 months and 23%/75 months.

**Discussion**

Borderline ovarian tumors tend to occur in a younger age group than do invasive ovarian tumors, more than half of patients are premenopausal [6, 7]. In our study, the mean age at diagnosis was 50 years. This is as same as the average age in the literature; 86.4% of the patients were FIGO Stage I, and Stage III and IV disease were not encountered. Up to 90% of BOTS were Stage I disease. These patients had a 5-year disease-free survival of almost 100% and an excellent overall prognosis [6]. Generally accepted prognostic factors include the extent of residual disease after primary surgery, FIGO stage, histologic type and age. Stage I, serous type and age less than 40 years are the low-risk group. In this series, the most common histologic type was mucinous (59%) which is in contrast with Makarewicz’s report on 114 cases [8]. In Korea, approximately 300 cases of borderline ovarian tumors are diagnosed annually, and the majority are histologically mucinous types (70%) followed by serous types (28%) [9-11]. In our study, each incidence of microinvasion or intraepithelial carcinoma was 9% (2 patients each). All of mucinous borderline intraepithelial carcinomas (BIECa) recurred. BIECa may imply a difference in prognosis even in the absence of stromal invasion [12]. Borderline tumors represent intermediate stages of mucinous tumorigenesis and are sometimes difficult to distinguish from BIECs, which can recur in 6% of cases [13]. Microinvasion has been studied separately in muci-
Clinical analysis of borderline ovarian tumors

The natural history of mucinous borderline tumors is poorly understood. However, this treatment has been abandoned in favor of more conservative surgery to preserve subsequent fertility in young patients with BOTS. In our conservative surgery group, two of six patients (33.3%) developed recurrence. All of the recurrent patients had mucinous tumors (2/13:15.4%). One patient died due to progression to carcinoma (1/13:7.7%). The natural history of mucinous borderline tumors is poorly understood. However, in a prospective study of 339 cases of BOTS they observed seven progressions (2.0%) into invasive carcinoma, five in serous tumors (2.4%), and two in mucinous tumors (1.6%) [18]. Also, recurrence of Stage I mucinous BIECa has been reported in only 12 of 226 (5.3%) [13, 19]. Both BOTS and BIECa have excellent prognosis, and conservative surgery is the treatment of choice in these cases. Nonetheless, such patients should be completely staged and the tumor should be extensively sampled to rule out stromal invasion because experience with these tumors is still scarce. Furthermore, mucinous borderline tumors are further resistant to anticancer drugs compared with mucinous carcinoma, which is the same experience as our patients. The potential limitations of our study are that it was a retrospective study with a small number of cases. Thus, we cannot consider any clear recommendations for better diagnosis and management of BOTS.

Conclusion

Large prospective multicenter studies with a large number of patients and studies related to K-ras mutation of mucinous tumors are needed in the near future. However we should remember, BOTS may show atypical behavior as two groups – one with an excellent prognosis of 80-90% with a 10-year survival rate and a second with a much poorer outcome. Mucinous BIECa may result in poor outcomes similar to our cases. In Korea’s situation mucinous borderline tumors are prevalent, and especially patients with mucinous BIECa may need a warning about early recurrence and close follow-up for a long time.

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Screening for cancer of the cervix with simultaneous Pap smear and colposcopy.
– The efficacy of Pap smear and colposcopy –

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Summary

Objective: Some Japanese institutes have been performing a population screening program for cervix cancer involving the simultaneous use of Pap smear and colposcopy. This program may be a good model for evaluating the efficacy of Pap smears and colposcopy. Methods & Materials: The subjects included 2,000 women who underwent primary screening at the Kanagawa Health Service Association. Results: 1) The incidence of ACF (atypical colposcopic findings) was 3.6%, whereas that of abnormal Pap smears (ASC-US and above) was 1.1%; 2) Of 88 women who showed abnormal findings on Pap smear and/or colposcopy, only three cases appeared abnormal in both methods, i.e., the two methods were complementary; 3) Colposcopy was more useful for detecting mild dysplasia than the Pap smear. However, colposcopy may possibly detect benign reparatory lesions; 4) The incidence of unsatisfactory colposcopic findings (UCF) was high (24.2%), whereas no unsatisfactory cases were found by Pap smear. Conclusions: The sensitivity of the Pap smear for detecting mild dysplasia is low, whereas that of colposcopy is high. However, colposcopy may not be suitable for primary screening due to its high UCF. The low sensitivity of Pap smears may be improved by repetition or adding ancillary HPV testing.

Key words: Pap smear; Colposcopy; Cervical cancer; Screening.

Introduction

It has been clarified that cancer of the cervix originates from human papillomavirus (HPV) infection [1]. Accordingly, the introduction of HPV testing has been suggested as the useful strategy for screening of this cancer [2, 3].

The standard screening method for this type of cancer is use of the Pap smear as the primary screening method, and colposcopy for the detailed exam. The Kanagawa Health Service Association, however, has a long history of using Pap smears and colposcopy simultaneously for primary screening. This program may be a good model for evaluating the two methods.

The present study evaluated the role and efficacy of the Pap smear and colposcopy in primary screening for cancer of the cervix.

Materials and Methods

Materials

The subjects included 2,000 consecutive women who underwent screening at the Central Clinic of the Kanagawa Health Service Association between February 10 and September 15, 2009. The screening programs are based on governmental or company regulations or individual application. The cytology sampling and colposcopy were performed by the first author.

Methods

– Pap smear

The cell samples were obtained using a cotton tip (Osaki applicator, Osaki Medical Co. Ltd, Nagoya, Japan) and cytobrush plus (Medscand Medical and CooperSurgical Co., Trumbull, USA) rinsed with physiological saline for the vaginal portio and the cervical canal, respectively, and the cells from the two samples were separately placed onto each half of a slide, and the tips of the instruments were rotated without making the cell-free area on the slide. The slide samples were immediately placed into 95% ethyl alcohol for fixation. Then, the samples were sent to the Cytology Center of our Institution and processed using routine Papanicolaou staining and diagnostic procedures.

The cytologic diagnosis was based on the Bethesda System for reporting cervical cytology [4, 5].

– Colposcopy

The colposcopic diagnosis was based on the Barcelona 2002 colposcopic classification [6]. Additionally, normal colposcopic findings (NCF) were divided into two subcategories, NCF I and NCF II. Abnormal colposcopic findings (ACF) were divided into four groups, according to their subgroupings for white epithelium (W), punctation (P) and mosaic (M), which were divided into three categories, as listed below.

NCF
1) NCF I: NCF with a squamocolumnar junction (SCJ) localized outside of the external os.
2) NCF II: NCF with a SCJ localized within the cervical canal that was confirmed by opening the canal with a forceps.

ACF
1) ACF1: W1, M1, P1, in which squamous metaplasia was suspected.
2) ACF2: W2, M2, P2, in which mild or moderate dysplasia was suspected.
3) ACF3: W3, M3, P3, in which severe dysplasia or carcinoma in situ was suspected.
4) ACF4: atypical vessels (aV) associated with W, M, P, in which microinvasive cancer was suspected.

Conclusions:
The sensitivity of the Pap smear for detecting mild dysplasia is low, whereas that of colposcopy is high. However, colposcopy may not be suitable for primary screening due to its high UCF. The low sensitivity of Pap smears may be improved by repetition or adding ancillary HPV testing.

Key words: Pap smear; Colposcopy; Cervical cancer; Screening.
W was quantitatively subgrouped based on thickness, i.e., color (bluish, pure, or ivory white) and surface texture (smooth or coarse). M was based on the presence of a regular or irregular vessel network and vessel diameter. P was based on the distance between Ps and P shape.

Suggestions for women who demonstrated abnormal Pap smear or colposcopy findings.

Women with ASC-US were advised to undergo a second cytology examination within six months, and those with ACF1 were instructed to undergo a second colposcopy within one year. Those with LSIL or ACF2 or worse findings were advised to undergo a detailed examination involving cytology, colposcopy, and colposcopy-guided biopsy.

Results

Thirty-three women had undergone hysterectomy (calculated from the 335th subject, and the incidence of hysterectomy was 2.0%). Therefore, 1,967 women underwent the "pure" screening for cervical cancer.

Incidence of unsatisfactory Pap smear and colposcopy findings

Incidence of unsatisfactory colposcopic findings (UCF), NCF I, and NCF II in the 1,967 women who underwent colposcopy was 24.2%, 13.3%, and 59.0%, respectively (Figure 1, mean age ± SD: 50.4 ± 12.7).

When subgrouped according to delivery history and delivery modality, the incidence of women who had undergone vaginal delivery including cesarean section (CS) in one of multiple deliveries, those who had only undergone CS and those who had no history of delivery was 67.0% (n = 1,340), mean age ± SD: 55.2 ± 8.5, 5.0% (n = 99, 50.1 ± 8.1), and 28.1% (n = 561, 39.4 ± 8.6), respectively. Incidence of UCF in these three subgroups was 20.3% (Figure 2), 53.6% (Figure 3), and 38.2% (Figure 4), respectively.

In Pap smears, no unsatisfactory cases were encountered in this series.

Abnormal findings

Colposcopy

Incidence of ACF1, ACF2, and ACF3 in colposcopy was 2.8% (n = 56), 0.7% (n = 13) and 0.1% (n = 1), respectively, and the overall incidence was 3.6% (Figure 1). No ACF4 or worse findings were found. Among the subgroups of delivery modality, the incidence of ACF in the women who underwent vaginal delivery, CS, and those with no delivery history was 1.0% (ACF1: 0.9% and ACF3: 0.1%) (Figure 2), 2.1% (only ACF1) (Figure 3), and 9.8% (ACF1: 7.5% and ACF2: 2.3%) (Figure 4), respectively.
Screening for cancer of the cervix with simultaneous Pap smear and colposcopy. – The efficacy of Pap smear and colposcopy

– Pap smear

The incidence of an abnormal Pap smear was 1.1%, including incidences of 0.7% (n = 14) for ASC-US, 0.1% (n = 1) for AGC, and 0.3% (n = 6) for LSIL. There were no cases with HSIL or worse findings in this series.

Of the colposcopy findings of 14 cases with ASC-US and one with AGC, 11 were classified as NCF, two as UCF, and two as ACF1. In six LSIL cases the colposcopy findings were classified as NCF in 4, UCF in one, and ACF1 in one case.

– Cases with both abnormal Pap smear and colposcopy findings

Only three cases presented abnormal findings for both cytology and colposcopy, although 88 cases presented abnormal findings in one of the two methods. All three cases were classified as ACF1 on colposcopy, and two were found to be ASC-US, and one was found to be LSIL on Pap smear. The latter case was confirmed to be moderate dysplasia after a detailed exam. The other two cases are being followed-up.

– Diagnosis at the detailed examination

Cases that were classified as ACF1 on colposcopy were subjected to the standard follow-up procedure without a detailed examination. However, one case that was also classified as LSIL on Pap smear, was subjected to a detailed exam, and moderate dysplasia was diagnosed, as mentioned above. In 13 cases classified as ACF2 and one classified as ACF3, eight cases of mild dysplasia were diagnosed, and the other six are currently being examined. All of these cases were found to be negative by cytology.

No lesion was found in 15 cases that were classified as either ASC-US or AGC excluding five that are currently being investigated, and in six cases classified as LSIL, one case of moderate dysplasia and two of mild dysplasia were found, and the other three are currently being examined. The two cases of mild dysplasia were grouped in NCF by colposcopy at the primary screening. However, a tiny ACF1 lesion was found on the external os during a second colposcopy performed as part of the detailed examination, and colposcopy-guided biopsy confirmed mild dysplasia.

Discussion

Cancer of the cervix originates at the squamocolumnar junction (SCJ), where layers of squamous cells and columnar cells come into contact with each other. Therefore, the SCJ should be visualized on colposcopy, and cellular samples should be correctly obtained on Pap smear when screening is performed. The result of colposcopy is categorized as unsatisfactory colposcopic findings (UCF), if the SCJ is not visible. The incidence of UCF was 24.2% in total and 20.3% in women with a history of vaginal delivery, whereas in women with a history of CS and those who had not undergone a previous delivery it was 63.6% and 28.2%, respectively. In other words, at least one out of every four women show unsatisfactory findings on colposcopy. The incidence of UCF was high in the present series, although it is generally considered to range from 10-15%. This may be due to the increasing size of the older age group in the Japanese population. The mean age of the present series was 50.4 ± 12.7. The colposcopy results suggest that colposcopy is not suitable for primary screening for cervical cancer. In contrast, there were no unsatisfactory Pap smear results in this series. The incidence of ASC-US, which is described to be 5% or less in the Bethesda system(4), was 0.7%.

The incidence of abnormal findings on colposcopy was 3.6%, and those in women who had undergone vaginal delivery and CS were 1.0% and 2.3%, respectively, whereas that in those who had no history of delivery was 9.8%. Therefore, the screening procedure for women who had no history of delivery should be performed carefully. In contrast, the incidence of abnormal cytology was 1.1%, which is reasonable for primary screening in Japan. However, abnormal colposcopy findings occurred more frequently than abnormal Pap smear findings. The detailed examination revealed that eight cases of mild dysplasia were found through colposcopy, whereas only two cases were found through Pap smear. One case of moderate dysplasia was detected with both methods, although only three cases were found to be abnormal by both methods. In the two cases of mild dysplasia detected by cytology tiny lesions were found on the external os at the secondary colposcopy performed during the detailed examination. Therefore, the two methods act complementarily in the detection of cervical lesions, although it is possible that colposcopy is more able to detect mild dysplasia than the Pap smear. Similar results have been reported by Kuramoto et al. [7]. They screened 12,138 women at the Tumor Clinic of Kitasato University Hospital and found abnormal findings with cytology and/or colposcopy in 1,918 women excluding those with cancer. Sensitivity (class III a and above) for mild and moderate dysplasia of the Pap smear was 28% (n = 228) and 61% (n = 100), whereas that for severe dysplasia and carcinoma in situ (CIS) was 92.6% (n = 68) and 96.3% (n = 98), respectively. In contrast, sensitivity of colposcopy was all above 85% regardless of the severity of the lesion. We should realize that the ability of the Pap smear to detect less severe lesions like mild or moderate dysplasia is low, whereas its ability to detect more serious lesions such as severe dysplasia and CIS is high. It has also been found that 74.9% (n = 646) of 862 cases with positive colposcopy and negative cytology findings were diagnosed with reserve cell proliferation or squamous metaplasia [7]. Consequently, we should understand that ACF in colposcopy is not always related to neoplastic lesions with the ability to progress to cervical cancer. Our procedure for an ACF1 lesion is follow-up without biopsy; otherwise, the incidence of the detailed examination would be elevated by a further 2.8%, although more mild dysplasias may also be detected.

Primary screening for cervical cancer using both Pap
smears and colposcopy has been performed in some institutes in Japan as well as Central and Eastern Europe including Hungary [8], whereas using cytology has only been performed in the USA and Western Europe. Recently, the high sensitivity of HPV testing for detecting cervical lesions has been reported, and it is recommended that the HPV test should be added as an ancillary test when cytology results present findings of ASC-US [2, 5]. In addition, other reports have recommended simultaneous cytology and HPV testing [2] or HPV testing followed by cytology for positive HPV [3]. In other words, the use of colposcopy tends to be firmly restricted. This may be due to the high cost of colposcopy in Western countries compared with that in Japan.

Colposcopy produces a high incidence of unsatisfactory cases, whereas it is better at detecting milder dysplasia than the Pap smear. Therefore, we should be prepared to accept a low incidence of mild lesions in exchange for being able to detect severe lesions if screening is performed using only cytology. Repeating the Pap smear test may compensate for this unfavorable characteristic. Wright [9] reports that the sensitivity of the Pap smear for detecting CIN2 (moderate dysplasia) or worse lesions was 56.4% when ASC-US or worse findings appeared to be positive and 42.2% when those of LSIL or worse were positive, although these results are much lower than those of our series [7]. Conversely, he noted that using HPV testing in addition to cytology elevated the detection sensitivity to 97.4% [9]. HPV testing is a promising ancillary method that could be used to compensate for the low-detection sensitivity of cytology as a replacement for colposcopy.

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PCNA and Ki-67 in endometrial hyperplasias and evaluation of the potential of malignancy

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Summary

Objective: The aim of this study was to investigate malignancy potential in endometrial hyperplasias and association with PCNA and Ki-67. Methods: Hysterectomy or probe curettage materials of 62 patients (20 simple hyperplasias (SH), six SH with atypical changes, five complex hyperplasias (CH), 11 CH with atypical changes, ten proliferative endometrium (PE) and ten secretory endometrium) were included in our study. Immunohistochemical staining for PCNA and Ki-67 protein was performed on formalin-fixed and paraffin-embedded tissue samples. Results: Immunoreactivity of PCNA was found to be significantly higher in atypical CH as compared to all other groups (p < 0.05). Also immunoreactivity of PCNA was significantly lower in SH as compared to atyp-ical CH, and PE (p < 0.05). Average values showed that Ki-67 immunoreactivity is highest for atypical CH, and PE. Immunoreactiv-ity of Ki-67 was found to be significantly higher in atypical CH as compared to other groups except PE (p < 0.05). Conclusion: PCNA immunoreactivity can be useful in patients with endometrial CH showing mild or moderate atypical changes in terms of preferring more conservative treatment modalities in those with low PCNA index. Also we suggest that Ki-67 could be insufficient to determine the potential of malignancy.

Key words: Endometrial hyperplasia; Endometrial carcinoma; PCNA; Ki-67.

Introduction

Endometrial hyperplasia is the precursor lesion of endometrial carcinoma, which is the most frequent cancer of the female genital system in many countries [1]. It is generally believed that endometrial hyperplasias have the potential of premalignancy. Prospective studies to definitively determine the rate of progression to cancer of endometrial hyperplasias can only be performed in limited number of patients [2]. Simple hyperplasia has been shown to progress into cancer with a rate of 1%, complex hyperplasia with 3%, simple atypical hyperplasia with 8%, and complex atypical hyperplasia with 29%. The period of progression to cancer for hyperplasias without atypical changes is reported to be ten years on average, and four years for hyperplasias with atypical changes [2, 3].

Chronic anovulation (adolescence, polycystic ovarian syndrome, perimenopausal period), hyper production of endogenous oestrogen (tumors with granulosa cells, techema, adrenocortical hyperplasia, obesity), and exogenous estrogen intake are meant to be the risk factors in endometrial hyperplasia etiology [4].

Ki-67 is a non histone protein and it is located on the 10⁰ chromosome; 90% of Ki-67 is in the nucleolus and 10% is in the nucleoplasm. Ki-67 antigen is very quickly catabolized because it contains large amounts of serine, thyronine, proline and glutamic acid. Ki-67 antigen’s half life is very short, approximately one hour. Ki-67 antigen exists in the proliferative phase cells. The tumors with high Ki-67 antigen expression are more agressive, have poor prognosis, more vascular invasion, and metastasize more than cells without Ki-67 antigen expression [5, 6].

Picartz et al. showed the growth fraction determined by Ki-67 in normal and neoplastic endometrial tissues in 1999 [7]. Thus, the cells that are immunoreactive to Ki-67 are thought to represent the growth fraction. In 1984 Gerdes et al. determined in detailed cell cyle analysis the presence of Ki-67 antigen in the cell nucleus in G1, S and G2 phases as well as mitosis through the cell cycle. On the other hand, they showed that Ki-67 antigen was not synthesized in the cells in the G0 phase [8]. Also, it was indicated that the Ki-67 index was an independent prognostic factor in breast cancer survival and recurrence [9].

Previously to those findings, in 1978, Miyachi et al. determined an antigen and its reactive antibody in the proliferating cell nucleus of the serum of patients with systemic lupus erythematosus. They named it “proliferating cell nuclear antigen” (PCNA) which was later shown to have a similar peptide structure with “cyclin” [10].

PCNA is an aspartic acid rich in glutamic acid which is a 36 kDa nuclear polypeptide located on the 20⁰ chromosome and is 262 amino acids in length. It is the cofactor of DNA polymerase, responsible for DNA replication and it is located in the nucleus. PCNA is synthesized through cell cycling and regulates the cycle. The synthesis rate is correlated with cell proliferation and DNA synthesis. Although the proliferation potential of PCNA is directly correlated in normal cells, similar findings are not always observed in neoplastic cells [11, 12].
The immunohistochemical evaluation of the alteration of PCNA indicates both active DNA replication and DNA damage that might lead to carcinogenesis [13]. Immunohistochemical PCNA analysis has been done in carcinomas including the breast, and hepatocellular and gastric carcinomas previously [14].

Oncogenes have been studied to determine the malignancy potential of endometrial hyperplasias. Investigation of cell cycle-related antigens with immunohistochemical methods has become an important field in research for the development of malignancies. Proliferating cell nuclear antigen (PCNA) and Ki-67 are the two most emphasized cell cycle-related antigens [15].

Based on the above-mentioned data, we tried to investigate the malignancy potential in endometrial hyperplasias and an association with PCNA and Ki-67.

Materials and Method

Study Design

This study was performed on 62 patients who underwent hysterectomy for any reason or endometrial tissue sampling with probe curettage, and diagnosed with endometrial hyperplasia, proliferative or secretory endometrium with pathological examination in Dr. Zekai Tahir Burak Women’s Health Education and Research Hospital, Ankara, Turkey. The subjects with polycystic ovarian syndrome, any malignant disease such as breast, colon, etc., diabetes mellitus, known cardiovascular and metabolic diseases, body mass index > 28 kg/m², on medication such as thyroid hormone, oral contraceptives or HRT were excluded.

Hematoxylin-eosin sections were re-evaluated to confirm the histopathological diagnosis. Classification accepted by the World Health Organization (WHO) and International Society of Gynecological Pathologists (ISGP) was used in the pathological definition and classification of the endometrial hyperplasias. The patients with endometrial hyperplasias were classified as having simple hyperplasia without atypia (n = 20), complex hyperplasia without atypia (n = 5), simple atypical hyperplasia (n = 6) and complex atypical hyperplasia (n = 11), as well as control groups with proliferative endometrium (n = 10) and secretory endometrium (n = 10) after pathological evaluation.

The study protocol was approved by the ethical committee. All patients were clearly informed about the aim of the study, and written informed consent to the protocol was obtained from all patients.

Immunohistochemical staining

Immunohistochemical analyses for PCNA and Ki-67 were performed on formalin-fixed, paraffin-embedded archival tissue using the streptavidin–biotin–peroxidase technique. For all patients, a 4 µm histologic section was deparaffinized in xylene and rehydrated in descending dilutions of ethanol. For antigen retrieval, slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked by 60 min of incubation with 0.3% hydrogen peroxide. Slides were incubated with PCNA (1/100 dilution, Clone PC 10, DAKO) and Ki-67 (1/100 dilution, Clone MB-1, DAKO). Sections were incubated with a streptavidin–biotin–peroxidase kit (Ultra Vision Large Volume Detection System Anti-Polyvalent, HRP, LabVision, USA), and after incubation the reaction product was detected using diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer’s hematoxylin, and mounted with mounting medium. Only nuclear PCNA and Ki-67 expression were accepted as specific. Appropriate positive and negative controls were stained for each antibody. For Ki-67, in microscopic analysis, the percentage of positive nuclei in 1000 consecutive cells of the most evenly stained areas of the tumor were counted. For PCNA, a total of 100 cells were counted at x10 magnification, and the percentage of the number of nuclei stained with PCNA was indicated as PCNA-LI.

Statistical analysis

For statistical analyses, SPSS for Windows Version 11.0 statistical software (SPSS Inc., Chicago, IL) was used. The groups were compared with Anova one-way variance analysis and the Tukey post-hoc test. A p value of 0.05 was taken as the threshold level for statistical significance.

Results

The mean age of the patients was 44.31 ± 7.54 years and the gravidity of the patients was 2.28 ± 1.43. According to pathological classification, 20 patients out of 62 included to our study were diagnosed with simple hyperplasia, six with simple hyperplasia with atypical changes, five with complex hyperplasias, 11 with complex hyperplasias with atypical changes, and ten with proliferative endometrium and ten with secretory endometrium.

Regarding immunohistochemical staining, PCNA immune reactivity was found to be the highest for complex atypical hyperplasia (9.91%) and the lowest for secretory endometrium (4.22%).

Immunoreactivity of PCNA was significantly higher in complex atypical hyperplasia (9.91%) as compared to simple hyperplasia (9.02%), simple hyperplasia with atypical changes (6.88%), complex hyperplasia (7.72%), proliferative endometrium (8.66%) and secretory endometrium (4.22%) (p < 0.05). On the other hand, the PCNA-LI index was found to be significantly lower in secretory endometrium as compared to all other groups (p < 0.05). According to statistical analyses, PCNA immune reactivity was significantly lower in simple hyperplasia as compared to simple hyperplasia with no atypical changes, complex atypical hyperplasia, and proliferative endometrium (p < 0.05) (Figure 1).

The mean percentual grades of Ki-67 expression were 26.2% for simple hyperplasia without atypia, 15.75% for complex hyperplasia without atypia, 23.32% for simple atypical hyperplasia, 41.88% for complex atypical hyperplasia patients, as well as 42.05% for proliferative endometrium and 14.95% for secretory endometrium (control groups). Comparing the mean values of Ki-67 immune reactivity in the study groups, the highest immunoreactivity values were observed in complex atypical hyperplasia and proliferative endometrium. According to statistical analyses, immune reactivity of Ki-67 was found to be significantly higher in complex atypical hyperplasia compared to all other groups (p < 0.05). However, no statistically significant difference could be found in proliferative endometrium (Figure 2).
Discussion

It was generally believed that the majority of hyperplasias had precancerous potential [2]. However, this concept of continuity changed in time, and more importance is now given to the existence of atypical cytological changes. Currently, it is believed that the most important factor regarding progression of endometrial hyperplasia to endometrial carcinoma is the existence of atypical cytological changes [3].

Determining the malignancy potential of endometrial hyperplasias, it is important to avoid unnecessarily excessive treatment, particularly in young patients, and studies with that purpose are still kept up to date [2, 3]. Immunohistochemical measurement of the proliferative activity of cells has been widely used to assess the biological behavior of human tumors [15]. Ito and colleagues [16] investigated the immune reactivity of PCNA in various endometrial lesions and they found no significant difference between the PCNA index of the groups they classified as simple hyperplasia (n = 4), complex hyperplasia (n = 14), and atypical hyperplasia (n = 10). Furthermore, no significant difference was found between the PCNA index of endometrial hyperplasias [16]. Yu and colleagues [17] studied the PCNA index in cases with proliferative endometrium, secretory endometrium, and invasive adenocarcinoma and the highest immunoreactivity was found in proliferative endometrium. The PCNA index was higher in atypical hyperplasias as compared to simple hyperplasia. In addition, PCNA staining was observed in a patchy fashion in atypical hyperplasias, especially in regions where atypical cytological characteristics were present. They concluded that according to this finding, atypical hyperplasia developed in a patchy fashion [17]. Different from Yu et al.’s. study, we classified atypical hyperplasias as complex and simple, and also found PCNA immunoreactivity significantly higher in complex atypical hyperplasia as compared to simple hyperplasia as compared to other groups. Cinef and colleagues [18] studied PCNA immunoreactivity in simple endometrial hyperplasias and complex endometrial hyperplasias without typical nuclear changes. They found a significantly higher PCNA index in complex hyperplasia as compared to simple hyperplasia.

Terlikowski et al. [19] studied PCNA immune reactivity in simple hyperplasia, simple atypical hyperplasia, complex hyperplasia, and complex atypical hyperplasia in a study they performed in 2001. They found the PCNA index in a average of 23% of simple hyperplasia, 28% of simple atypical hyperplasia, 35% of complex hyperplasia, and 39% of complex atypical hyperplasia. According to these findings, they suggested that the PCNA index was a reliable index for the determination of differentiation of premalignant/malignant endometrial changes. They suggested that performing PCNA analysis would be beneficial, especially in patients under 40 years of age, diagnosed with endometrial hyperplasia, while more conservative treatment methods could be selected for those with lower proliferation indices [19]. Therefore, our findings are similar to those in this study. However, in contrast to previous findings, we have found significantly lower PCNA immunoreactivity in simple atypical hyperplasia as compared to simple hyperplasia without atypical changes and complex atypical hyperplasia. Thus, we suggest that PCNA immunoreactivity can be useful in patients under 40 years of age with complex endometrial hyperplasia showing mild or moderate atypical changes. More conservative treatment modalities would be preferred in patients with a low PCNA index, however, we can evaluate this as a negative factor for those with high PCNA indices. Furthermore, studies with greater numbers of patients should be performed, and certain cut-off values should be determined for the use of PCNA index in practice. Findings of lower PCNA immunoreactivity in simple atypical hyperplasia as compared to other hyperplasia groups indicate that the precancerous potential of this pathology should be reviewed.
Another cell cycle-related antigen, Ki-67, has been shown to be an independent prognostic factor for survival and recurrence in many recent studies [20-22]. Risberg and colleagues [23] analyzed Ki-67 expression in proliferative endometrium, secretory endometrium, simple hyperplasia, complex hyperplasia, and atypical hyperplasia and found the highest expression in proliferative endometrium. However, no statistically significant differences in the Ki-67 index of endometrial hyperplasias were found [23]. In the study of Ambros [24], the highest Ki-67 index was found in proliferative endometrium with an average of 23.2%. They obtained the mean values of 9.8% in simple hyperplasia, 12.7% in complex hyperplasia, and 10% in atypical hyperplasia [24]. Ioffe and colleagues [25] similarly found the highest Ki-67 index in proliferative endometrium (38.4%).

Our findings are concordant with these three studies. In our study, the significantly higher values of Ki-67 expression in complex atypical hyperplasia and absence of any significant difference between proliferative endometrium may indicate that Ki-67 could be insufficient to determine the potential of malignancy. Although Ki-67 has been shown to be an independent prognostic factor in terms of survival and recurrence in prostate carcinoma and breast carcinoma, any finding indicating that Ki-67 is capable of showing the relation of endometrial lesions with malignancy has not been found in the few performed studies. Our findings also support this view.

In conclusion, PCNA immunoreactivity can be useful in patients with complex endometrial hyperplasia showing mild or moderate atypical changes in terms of preferring more conservative treatment modalities in those with a low PCNA index. We also suggest that Ki-67 could be insufficient to determine the potential of malignancy. However, the main limitation of this study is its sample size. With our small sample size the data cannot exactly determine any specific importance of PCNA and Ki-67. Further larger series investigations are needed to clarify this subject.

References


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Hormone therapy for postmenopausal endometrial cancer survivors: a survey among Greek obstetricians-gynaecologists

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Summary

Purpose of investigation: In this study we evaluated the prescription attitude of Greek obstetricians-gynaecologists towards hormone replacement therapy (HRT) for endometrial cancer survivors. Methods: An anonymous questionnaire was sent to 900 members of the Hellenic Society of Obstetrics and Gynaecology, presenting a hypothetical case of an endometrial cancer survivor with indications for HRT, followed by a series of relevant questions. Results: Three hundred and three valid responses were received and analysed according to age, gender and practice setting. HRT would be prescribed by 30.4% of gynaecologists; as far as type of regimen is concerned, 67.4% would prescribe tibolone, 22.8% estrogen-only and 9.8% estrogen plus progestagen. In contrast, 69.6% would not prescribe HRT due to the fear of endometrial cancer recurrence (88.2%), development of breast cancer (2.8%) or both (4.7%); among them, 28.4% would prescribe central nervous system (CNS) medications, selective estrogen receptor modulators (SERMs), phyto-oestrogens or bisphosphonates, as alternates. Conclusions: One out of three Greek gynaecologists would prescribe HRT to endometrial cancer survivors. Alternative therapies, mainly CNS medications, would be suggested by the opposers. Key words: Endometrial cancer survivors; Hormone replacement therapy; Tibolone; Estrogen replacement therapy; Estrogen-progestogen combination; Prescription attitude.

Introduction

Endometrial cancer (EC) is the most common gynaecologic cancer. It is most frequently diagnosed in postmenopausal women (median age 63 years) [1], even though 20-25% of cases occur before menopause [2]. The majority of the cases are of early stage. Treatment of EC in premenopausal women (total hysterectomy with bilateral salpingo-oophorectomy and, in selected cases, radio- or chemotherapy) results in iatrogenic menopause and estrogen deficiency states, such as hot flushes or osteoporosis. Since the 5-year survival rate for Stage I EC is over 80% [1], the women are expected to live many years in a postmenopausal state. Hormone replacement therapy (HRT) might improve their quality of life, targeting climacteric symptoms and osteoporosis. As the most common form of EC is the endometrioid type, which is estrogen-dependent, the medical community, until the 1990s, considered that HRT was contraindicated in EC survivors [3]. Despite this belief, data from clinical studies have failed to show an increased risk of EC recurrence or mortality in case of HRT use by EC survivors [4-11].

The aim of the present study was to investigate the attitude of Greek obstetricians-gynaecologists towards prescription of HRT or an alternative therapy as treatment for menopausal symptoms in EC survivors.

Materials and Methods

A questionnaire was sent to 900 obstetricians-gynaecologists, members of the Hellenic Society of Obstetrics and Gynaecology, out of a total of 2,700 at the time of the study (April 2009). The selection was random from the society’s register (every third registered member was selected). The questionnaire was anonymous and its first part included demographic data: age, gender and type of practice [academic center, National Health System (NHS) hospital, private practice]. The second part included a hypothetical case of a patient with a history of EC followed by a series of relevant questions. This study was part of a more extended one concerning cases of cervical, endometrial, ovarian and breast cancer survivors.

The following case was presented: a 52-year-old female Caucasian, para 2 was treated at the age of 49 (being premenopausal) with abdominal hysterectomy plus bilateral salpingo-oophorectomy for a well differentiated EC of endometrioid type (FIGO Stage IA, grade 1). Since then (i.e., three years ago), clinical laboratory and imaging follow-up were negative for recurrence. The woman was complaining of menopausal symptoms (hot flushes, night sweats, vaginal dryness and libido impairment), while a bone densitometry revealed osteopenia. The gynecologists were asked (1) whether they would prescribe HRT (closed-type answer: “yes” or “no”), (2) if yes, which hormonal regimen they would prefer to prescribe (closed-type answer: “estrogen-only”, “estrogen/progestogen combination”, “tibolone” or “selective estrogen receptor modulators (SERMs)”) and (3) if not, why (open-type answer) and (4) which alternative therapy the gynecologists would suggest.

The questionnaire was based on a similar one by Rozenberg et al. [12] although modifications were taken place. The chi-square test was used to define differences among the groups. Data were analysed by the use of SPSS for Windows, version 11 (SPSS Inc., IL, USA).
Results

A total of 303 responses to the questionnaire were collected, all with valid answers (overall response rate: 33%). Regarding the type of practice, 11.6% (n = 35) were working in an academic center, 23.7% (n = 72) in an NHS hospital and 64.7% (n = 196) in private practice. A percentage of 81.5 (n = 247) were males and the remaining 18.5% (n = 56) females. Finally, 48.9% (n = 148) of the responders were younger than 48 years of age and classified as “younger gynaecologists” and 51.1% (n = 155) as “older”. The cut-off point of 48 years was chosen as it represented the mean age of the responders.

In the first question “Would you prescribe HRT in an EC survivor?”, 30.4% answered “yes” (n = 92) and 69.6% “no” (n = 211). Gynaecologists working in an academic center answered “yes” in a greater proportion (30.4%) than their colleagues working in an NHS hospital (15.3%) or in private practice (33.0%) (p < 0.001). As far as age is concerned, “younger gynaecologists” were willing to prescribe HRT in a greater proportion (40.5%) than their “older colleagues” (19.5%) (p < 0.001). Finally, there was no significant difference as far as gender was concerned.

In the second question “If yes, which hormonal regimen would you prescribe?” 67.4% would prescribe “tibolone” (n = 62), 22.8% “estrogen-only” (n = 21) and 9.8% “estrogen plus progestagen” (n = 9) (p < 0.05). There were no significant differences regarding age, gender or type of practice.

In the third question, “If no, why?”, among those who were not willing to prescribe HRT (n = 211), 88.2% would do so because of the fear of EC recurrence (n = 186), 2.8% because of the fear of development of breast cancer (n = 6), 4.7% for both reasons (n = 10) whereas (4.3%) did not answer (n = 9). There were no significant differences regarding age, gender or type of practice.

In the fourth question, “If not, which alternative treatment would you suggest?”, the majority (71.6%) of the participants, regardless of age, gender or type of practice, were not willing to prescribe any medication at all (academic center: 70.6%, NHS hospital: 86.9% and private practice: 64.7%, p = NS). This unwillingness was apparent regardless of age group (“younger”: 68.2% vs “older”: 68.9%, p = NS) or gender (males: 70.5% females: 73.2%, p = NS). A minority would offer other treatment options, such as central nervous system (CNS) medications (21.3%), phyto-estrogens, biphosphonates, or SERMs (in total 7.1%).

Discussion

According to the results of this study, two out of three Greek obstetricians-gynaecologists are reluctant to prescribe HRT to EC survivors. Tibolone is the preferred regimen by the majority of gynaecologists who are in favor of HRT. The fear of EC recurrence is stated by the vast majority as the main reason for not prescribing HRT. Finally, the gynaecologists who avoid prescribing HRT in women with a history of EC prefer to prescribe no regimens at all or alternative medications, such as CNS drugs for the treatment of vasomotor symptoms.

Until the 1990s, HRT was contraindicated for menopausal symptoms in women treated for EC, as this neoplasia is usually estrogen-dependent [13]. This attitude was based on the theoretical risk that oestrogens may trigger carcinogenesis in patients with EC, despite the fact that data from well-designed randomized trials were inconclusive. Given the lack of evidence for detrimental effects of HRT on EC survivors, the American College of Obstetricians and Gynecologists (ACOG) issued a Committee Opinion in 2000, stating that the decision to use HRT in these women should be individualised on the basis of potential benefits and risks [14].

A series of studies have addressed the issue of possible beneficial effect of HRT in EC survivors. In 1986, Creasman et al., in a case-control study, were the first to report that the administration of conjugated oestrogens in 47 women with Stage I disease had favourable effects [4]. Four other retrospective studies (three cohort [5, 6, 9] and one case-control [8]) reported on patients with a history of EC Stage I-III and found that the prescription of conjugated estrogens with or without progestagens did not increase the rate of recurrence or death. All these studies are limited by their retrospective design, small sample size and short follow-up period. In addition, in a case-control study [10], women with a previous history of EC, Stage I-II, received conjugated estrogens with or without medroxy-progesterone and showed no increase in recurrence rate or mortality. Finally, a randomized, double-blind, prospective trial of estrogen vs placebo [11], in surgical Stage I-II women, cannot conclusively refute or support the safety of estrogen treatment with regard to risk of EC recurrence.

To the best of our knowledge, there is only one similar study concerning the prescription attitude of Belgian gynaecologists, which showed that two out of three professionals would prescribe HRT to a woman with a history of early stage EC, no signs of recurrence and indication of early stage EC, no signs of recurrence and indication of HRT [12]. On the contrary, in our study, only one out of three Greek gynaecologists was willing to prescribe hormonal regimens in EC survivors. It is noteworthy that the aforementioned Belgian study was published before the results of the Women’s Health Initiative study (WHI) [15] and Million Women Study (MWS) [16], which dramatically changed the prescription attitude. Although there are no previous published data in the Greek literature, it seems reasonable to postulate that the results of the WHI and MWS studies had a negative impact on the prescription attitude. In addition, the absence of official guidelines on HRT prescription issued by the Hellenic Society of Obstetricians and Gynaecologists might play a role in refraining gynaecologists from prescribing HRT due to the fear of malpractice. Moreover, in Greece there is a widely held misconception among women of a possible association between use of exogenous sex hormones and cancer.

Another finding of our study is the fact that tibolone was the preferred hormonal regimen for women with a
history of early Stage EC. This could be attributed to the knowledge that tibolone is being converted to a Δ4 androstenedione metabolite in the endometrium, which has no estrogenic activity [17, 18]. Nevertheless, the MWS reported significantly increased risk for EC in users of tibolone at an incidence rate of six cases per 1,000 women over a period of five years [16].

Interestingly, gynaecologists working at academic centers as well as younger colleagues most usually prescribe HRT in comparison to other groups of colleagues. This could be explained by the fact that gynaecologists of academic centers more closely follow the rapid progress of science on this issue; in a similar way, younger gynaecologists are, usually, better informed as they have recently completed their training.

The limitations of our study include the facts that the questionnaire was sent only to a subgroup of Greek gynaecologists (33%) and the response rate was rather low (33%). Nevertheless, the randomly selected subgroup (every third registered member was selected) weakens the first limitation.

In conclusion, the majority of obstetricians-gynaecologists practicing in Greece would not prescribe HRT for relief of menopausal symptoms in EC survivors due to the theoretical risk of disease relapse. Tibolone is the preferable regimen among those who are willing to prescribe hormonal therapy. The majority of the gynaecologists who would not prescribe hormonal therapy suggest either no medication at all or the use of CNS regimens.

References

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Leptin receptor expression in neoplastic and normal ovarian and endometrial tissue

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Introduction
Leptin receptor expression is decisive for much of neoplastic cell growth. Leptin acts as a mitogenic factor in normal, as well as in cancer cells [1-3]. There are two identifiable leptin receptors (ObR), the short (ObRa) and the long (ObRb) isoforms. Few data regarding leptin receptors exist on female reproductive system tumors in humans [4]. Leptin induces the proliferation of cancer endometrial cells and increases their aggressiveness, as was shown in the drug matrigel in the laboratory [5]. Petridou et al. found a strong positive correlation between leptin levels and endometrial cancer in 84 women with histologically proven endometrial carcinoma [6]. In the study of Koda et al. both expression of leptin and leptin receptors (long isoform), was found in 30% and 57% of the sample, respectively. Even though no statistically significant correlation was established, there was a tendency of cross-correlation of leptin with local spread and moderately differentiated tumors [4].

The purpose of this study was to investigate the expression of leptin receptors (Ra & Rb isoforms) in benign and malignant ovarian and endometrial tissue.

Materials and Methods
Histological samples from 37 patients aged 37-72 years were collected between the years 2004 and 2007. During scheduled surgery, two samples, 1 cm each, were collected from pathologic and physiologic tissue, respectively. The samples revealed 16 cancers of the ovaries, 15 cancers of the endometrium and six uterine fibromyomas. Patients with previous new-adjunct chemotherapy/radiotherapy were excluded. The examination of preparations for the expression of leptin and its receptors was made with the method of RT-PCR. T. Results: A BMI > 30 was correlated with increased expression of leptin receptors. Both Ra and Rb receptors were expressed in normal and neoplastic tissues. A statistically significant difference in leptin receptor expression was detected between normal and neoplastic tissue, with expression being around 5-fold higher in neoplastic tissue. Conclusion: Endometrial neoplasms and long leptin isoform receptor expression were associated with an increased BMI. A role of long isoform in endometrial carcinogenesis is proposed.

Statistical Analysis
Coding and processing: Initially the variables were coded and derivative variables were also created, e.g., BMI was dichotomized and epidemiologic characteristics of samples were constructed as well as for histological characteristics and serologic markers. The Student’s t-test was used for comparisons of parametric variables and the Mann-Whitney U test for ordinal ones. Normality was tested with the Smirnov-Kolmogorov test. Percentiles were used to present non quantitative data. The level of importance was set at 0.05. Statistics were processed with the program SPSS for Windows, v. 13.0.

Results
Subgroups of patients were studied according to BMI distribution, histological type, age, as well as stage and grade of differentiation. The results showed expression of leptin and its receptors (Ra > Rb) in the preparations examined. Mean patient age was 54.80 ± 10.35 and BMI was 31.49 ± 6.43. Mean leptin Ra and Rb concentrations were 0.98 ± 0.72 and 0.02 ± 0.03, respectively (Table 1).
The majority of neoplasms were adenocarcinomas and serous neoplasms. All adenocarcinomas came from the endometrium, while serous carcinomas were mainly in the ovaries (Table 2). Most neoplasms were of low differentiation (grade 3). Uterine neoplasms were statistically significantly correlated with higher BMI values, compared with ovarian ones (Table 3). A statistically significant difference was traced in normal tissue Rb concentrations depending on BMI. A BMI > 30 was correlated with increased expression was observed between obese and non-obese women as regards BMI. A BMI > 30 was correlated with increased expression of leptin receptors (Table 4). A statistically significant difference in the expression of Rb was acquired the same size order as Ra and the difference was minimized (Table 6).

**Discussion**

According to the results of the present study, there was a statistically significant difference in the expression of leptin receptors between normal and neoplastic tissue of the endometrium and ovaries, while endometrial neoplasms were associated with a higher BMI. The knowledge that obesity is combined with increased danger of carcinogenesis has been well established, and obesity treatment has been proposed as a cancer prevention measure [7]. Although obesity is known to be related with endometrial cancer, data on ovarian cancer are not explicit. Recent studies lead to the conclusion that also ovarian neoplasms are associated with obesity. Nevertheless, compared with the endometrium, the relative risk is smaller (1.14 for the ovary compared with 2.89 for endometrial neoplasms) [8, 9].

Gynecological cancers have been associated with leptin blood levels and the density of leptin tissue receptors. Both in normal and neoplastic cells, leptin promotes development, metastasis and infiltration while it enhances angiogenesis. However the role of leptin and its receptors in the development of reproductive system carcinomas remains unclear [6].

In the present study the concentrations of short type, as well long type receptors were calculated, both in normal and neoplastic tissues. There is some indication of receptor over-expression in endometrial cancers. Koda et al. did not detect any receptors in normal endometrial tissue with the immunohistochemistry method, while they traced ObR-b presence in 30% of neoplastic preparations [4]. Yuan et al. found no difference in long isoform concentrations between normal and neoplastic tissues, while there was a reduction in short isoform concentrations observed. The concentration of ObR-a was found higher than ObR-b. Whereas no statistical difference was observed between obese and non-obese women as regards ObR-a, the ObR-b concentrations were significantly elevated in obese individuals [10]. Rb expression exhibits variation according to menses and it is the prominent receptor in the endometrium [11]. Sharma et al. found that leptin induces enzyme phosphorylation, leading to increased infiltration capacity. Inhibiting the relevant pathways resulted in inhibition of cell infiltration induced by leptin [5]. Carino et al. also found that the effects of leptin on proangiogenic molecules involved in endometrial cancer were more evident in malignant versus benign cells [12]. According to the above findings, it could be speculated that some difference in receptor expression in favor of Rb could be responsible for endometrial carcinogenesis. The increase in expression of Rb appears to be

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**Table 1.** — Epidemiologic characteristics, serologic and tissue markers of women’s samples.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Min - Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (n = 31)</td>
<td>54.80 ± 10.35</td>
<td>32-75</td>
</tr>
<tr>
<td>BMI (n = 28)</td>
<td>31.49 ± 6.43</td>
<td>18.94-50.20</td>
</tr>
<tr>
<td>Ra (n = 22)</td>
<td>0.98 ± 0.72</td>
<td>0.05-2.50</td>
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<tr>
<td>Rb (n = 19)</td>
<td>0.02 ± 0.03</td>
<td>0.00-0.43</td>
</tr>
</tbody>
</table>

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**Table 2.** — Histological classification of neoplasms.

<table>
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<th>Serous Cancer</th>
<th>Adenocarcinoma</th>
<th>Endometrioid</th>
<th>Fibro-myo-ma</th>
<th>Borderline</th>
<th>Other</th>
</tr>
</thead>
<tbody>
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<td>2</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Ovarian origin</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total (n = 35)</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

---

**Table 3.** — Body mass index (BMI) and ovarian/uterine neoplasms.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI - Ovary (n = 13)</td>
<td>28.73 ± 4.90</td>
<td>0.035</td>
</tr>
<tr>
<td>BMI - Uterus (n = 14)</td>
<td>33.98 ± 7.03</td>
<td></td>
</tr>
</tbody>
</table>

---

**Table 4.** — Differences in the expression of leptin receptors, depending on the size of obesity.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>BMI &gt; 30</th>
<th>No.</th>
<th>BMI ≤ 30</th>
<th>No.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb</td>
<td>11</td>
<td>0.01 - 0.06 - 0.15</td>
<td>5 0.002 - 0.004 - 0.009</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Rb-n*</td>
<td>14</td>
<td>0.00 - 0.01 - 0.04</td>
<td>7 0.000 - 0.001 - 0.004</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

* Normal tissue.

**Table 5.** — Differences in expression of leptin receptors in normal and neoplastic tissue.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Neoplastic tissue</th>
<th>Normal tissue</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb</td>
<td>19</td>
<td>0.004 - 0.024 - 0.083</td>
<td>26 0.001 - 0.008 - 0.022</td>
</tr>
<tr>
<td>Ra</td>
<td>22</td>
<td>0.36 - 0.97 - 1.25</td>
<td>29 0.06 - 0.17 - 0.44</td>
</tr>
</tbody>
</table>

**Table 6.** — Differences in expression of leptin receptors in neoplastic endometrial and ovarian tissue.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Neoplastic ovarian tissue</th>
<th>Neoplastic endometrial tissue</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rb</td>
<td>9 0.005 - 0.02 - 0.05</td>
<td>10 0.008 - 0.07 - 0.35</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ra</td>
<td>22 0.66 - 1.06 - 1.45</td>
<td>12 0.29 - 0.53 - 1.45</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s. = non significant.
more rapid than the increase in Ra expression. This event might be of clinical importance because of the different roles the two receptor types play in cellular proliferation. Even though a protective role is preserved for ObR-a, this effect seems to be neutralized, probably due to the ObR-b influence as a result of their higher concentration. As a consequence, the rapid increase of ObR-b together with some lowering in ObR-a concentration might account for carcinogenesis.

Conclusions

Endometrial neoplasms were associated with increased BMI and obesity was associated with increased expression of long leptin isoform receptors in normal tissue. In neoplastic tissue an increase in ObR-b was observed, approaching the same size order of ObR-a. These results support an important role of long isoforms in endometrial carcinogenesis.

References

Accessory polar renal artery encountered in transperitoneal systemic laparoscopic paraaortic lymphadenectomy

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Summary

Objective: To increase vigilance among gynecologic surgeons for the presence of accessory polar renal artery (APRA) encountered with transperitoneal systemic laparoscopic paraaortic lymphadenectomy (LPAL). Methods: A retrospective review was conducted on 156 women who underwent LPAL for various gynecologic malignancies between November 2003 and December 2009. Results: The median age was 52 years (range, 23-82 years), and the number of previous abdominal surgeries, respectively, of the women were 2, 0-7, 24.1 kg/m² (range, 17.4-35.0 kg/m²), and 0 (range, 0-3). During the study period, we found four women with APRA. There were three cases of right lower APRAs arising from the abdominal aorta, caudal to the inferior mesenteric artery (IMA), terminating at the parenchyma of the lower pole of the right kidney. In the other case, the APRA arose from the abdominal aorta superior to the IMA. There were no vascular complications, such as transection or ligation of the APRA. Conclusion: It is important for the gynecologic oncologic surgeon to have knowledge of retroperitoneal vascular anatomy, experience in laparoscopic surgery, and an accurate surgical technique to avoid vascular injury during LPAL.

Key words: Accessory polar renal artery; Gynecology; Laparoscopy; Lymphadenectomy; Paraaortic lymph node.

Introduction

Each kidney is generally supplied by a single renal artery that originates from the aorta at the level of the second lumbar vertebra, but there are variations in renal vascular anatomy. In the past, such vascular anomalies and variations were of concern mostly to renal transplant surgeons and vascular surgeons involved in procedures such as urologic and renal transplant vasculoplasty, vascular reconstruction, repair of abdominal aortic aneurysms, and renal artery stenosis. However, with the recent advancement of paraaortic lymphadenectomy in the field of gynecologic oncology, the importance of retroperitoneal and vascular anatomy is getting more attention from gynecologic surgeons as well. Considering the current widespread use of laparoscopic surgery in gynecologic oncology, an awareness of renal vascular anatomy is important, especially for arterial variations. This knowledge is essential in the prevention of inadvertent transaction or ligation of variant renal arteries, which can lead to renal segmental ischemia or even renal infarction [1-3].

The purpose of this study was to analyze the incidence and clinical significance, to increase vigilance of an accessory polar renal artery (APRA) encountered by transperitoneal systemic laparoscopic paraaortic lymphadenectomy (LPAL) in order to minimize untoward vascular complications for gynecological oncologic surgeons.

Methods

All procedures were performed by a single gynecologic surgical team in a single institution. A retrospective chart review was conducted on 156 women who underwent LPAL for various gynecologic malignancies in Kangbuk Samsung Hospital between November 2003 and December 2009. We reviewed clinical charts and analyzed data on patient age, parity, body mass index (BMI), history of previous abdominal surgery, number of patients per final diagnosis, FIGO stage of gynecologic malignancy, and any operative complications. All the patients provided written informed consent regarding any complications and the possibility of conversion to laparotomy. The history of previous abdominal surgery and BMI did not affect our decision to perform surgery.

Indications for LPAL up to the level of the left renal vein were any operable endometrial cancer regardless of stage and grade [4, 5]. For patients with ovarian cancer, fallopian tube cancer, or primary peritoneal cancer, LPAL was performed when optimal debulking surgery was expected from laparotomy [6]. With cervical cancer, LPAL to the level of the left renal vein was performed in FIGO Stage IB1 with larger than 2 cm in size, IB2, and IIA and also when pelvic or paraaortic lymph node metastasis was confirmed on frozen section analysis in FIGO Stage IB1 with the tumor size less than 2 cm. LPAL at the level of the inferior mesenteric artery (IMA) instead of the left renal vein was performed in patients with cervical cancer FIGO Stage IB1 with tumor less than 2 cm in size and no evidence of lymph node metastasis during surgery on frozen section analysis. For borderline ovarian tumors, patients underwent LPAL when frozen section analysis showed papillary serous cell types or a suspicion of ovarian cancer.
Results

A total of 156 women underwent LPAL during the study period. The median age of the women was 52 years (range, 23-81 years) and the median parity was two (range, 0-7). The median BMI was 24.1 kg/m² (range, 17.4-35.0 kg/m²) and the median number of previous abdominal surgeries was 0 (range, 0-3). Table 1 shows the patient distribution of each gynecologic malignancy and FIGO stage. Four patients had an APRA. Three had a right lower APRA arising from the abdominal aorta inferior to the IMA, terminating at the parenchyma of the lower pole of the right kidney. In the other patient, the polar artery arose from the abdominal aorta superior to the IMA (Figure 1). The base-line characteristics, name of laparoscopic procedures, final histopathologic diagnosis, and follow-up of these four patients are shown in Table 2. There were no vascular complications with the APRA such as transection or ligation. In all patients with APRA, multidetector-row computed tomography (MDCT) angiogram was performed postoperatively to identify other associated vascular anomalies (Figure 2).

Figure 1. — Pictures of APRAs (white arrows) taken during LPAL ① inferior vena cava, ② abdominal aorta, ③ left renal vein, ④ inferior mesenteric artery, ⑤ right common iliac artery, ⑥ left common iliac artery, ⑦ right kidney, ⑧ right ureter.

Figure 2. — Postoperative MDCT angiogram of patient B. Note the two different right renal arteries arising from the abdominal aorta supplying the upper and lower poles of the right kidney. The lower vessel is a right lower accessory polar artery measuring 4 mm in diameter, which is narrower than the upper main renal artery measuring 10 mm in diameter.
Accessory polar renal artery encountered in transperitoneal systemic laparoscopic paraaortic lymphadenectomy

Discussion

Arterial or venous abnormalities of the kidneys are relatively common and have been reported in up to 25-40%. The most frequent variation among these anomalies is the APRA, which has also been called the "aberrant," "anomalous," "supernumerary," "lower polar," and "accessory polar," artery because there is no universal agreement regarding its nomenclature [1, 7]. Here we use the term APRA to avoid confusion. The incidence of APRA varies widely. APRA occurs unilaterally or bilaterally as single or double vessels with a frequency of 9-31.3% [8]. Khamanarong et al. reported an overall incidence of 7.32% and 3.56% for upper and lower polar arteries, respectively [2]. The overall incidence of single aberrant vessels is similar between the right and the left kidney, but Kappor et al. reported that it was more common on the right than the left using multispiral computed tomographic angiography (MSCTA), which is more sensitive than conventional methods of detecting aberrant vessels less than 2 mm in diameter [9]. Klemm et al. reported that 13 APRAs were found in 9/86 patients (10.4%) who underwent laparoscopic infrarenal paraaortic lymphadenectomy. In that study with 13 subjects, eight cases were right lower APRAs and five cases were left lower APRAs [10]. Moreover, there is a significant difference in the incidence and location of APRAs that exist between sex and race [11]. Hence, in our Korean women study, only four women (2.56%) with APRAs were detected.

Why polar vessels develop is not clearly understood, but these vessels are clinically important because they might represent segmental arteries supplying a particular area of the kidney. APRAs are end arteries with no anastomoses or collateral circulation. Therefore, intraoperative, inadvertent transection, ligation, or occlusion of these arteries can cause segmental ischemia or infarction. This might need to be managed with segmental resection of the kidney because irreversible damage can occur in the renal parenchyma unless revascularization is performed within two hours [1]. However, the safe warm ischemia time is only 30 minutes in the literature on partial nephrectomy [12]. If renal infarction is diagnosed postoperatively during the evaluation of fever or flank pain, continued monitoring of kidney function and blood pressure is necessary because it can lead to postoperative hypertension [1, 2, 10, 11, 13]. Moreover, APRAs tend to be longer and narrower than the main renal arteries, resulting in lower perfusion pressure and higher resistance across the artery. This can lead to renovascular hypertension even without vascular injury [3]. APRAs most often pass anterior to the ureter, and such a retroreteral variant is likely to be associated with ureteropelvic junction obstruction and hydronephrosis [7]. There is a higher chance of accompanying ovarian and ureteral abnormalities if APRAs exist bilaterally [7]. Paraaortic lymphadenectomy is an indispensable surg-

Table 1. — Patient distribution of each gynaecologic malignancy and FIGO stage.

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB1</td>
<td>27</td>
</tr>
<tr>
<td>IB2</td>
<td>31</td>
</tr>
<tr>
<td>IIA2</td>
<td>4</td>
</tr>
<tr>
<td>IIB</td>
<td>15</td>
</tr>
<tr>
<td>IA</td>
<td>27</td>
</tr>
<tr>
<td>IB</td>
<td>5</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
</tr>
<tr>
<td>IIIA</td>
<td>1</td>
</tr>
<tr>
<td>IIIC1</td>
<td>3</td>
</tr>
<tr>
<td>IIIC2</td>
<td>6</td>
</tr>
<tr>
<td>IA</td>
<td>2</td>
</tr>
<tr>
<td>IB</td>
<td>3</td>
</tr>
<tr>
<td>IC</td>
<td>7</td>
</tr>
<tr>
<td>IIIA</td>
<td>2</td>
</tr>
<tr>
<td>IIIC</td>
<td>10</td>
</tr>
<tr>
<td>IIIC</td>
<td>3</td>
</tr>
<tr>
<td>IA</td>
<td>1</td>
</tr>
<tr>
<td>IB</td>
<td>1</td>
</tr>
<tr>
<td>IC</td>
<td>1</td>
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<td>IIIC</td>
<td>1</td>
</tr>
<tr>
<td>IIIA</td>
<td>1</td>
</tr>
<tr>
<td>IIIB</td>
<td>2</td>
</tr>
<tr>
<td>IIIC</td>
<td>10</td>
</tr>
<tr>
<td>IIIC</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. — Clinical characteristics of the patients with accessory polar renal artery.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Final diagnosis</th>
<th>Operative procedures</th>
<th>Postoperative treatment</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 52</td>
<td>Endometrial adenocarcinoma, Stage I A</td>
<td>LAVH with BSO, LPL, LPAL, LA, Peritoneal washing cytology</td>
<td>None</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>B 61</td>
<td>Primary peritoneal cancer, Stage II C</td>
<td>Extrafascial LAVH with BSO, LPL, LPAL, LO, LA, multiple peritoneal biopsy, peritoneal washing cytology</td>
<td>Taxol-carboplatin chemotherapy</td>
<td>AWD in second line chemotherapy</td>
<td></td>
</tr>
<tr>
<td>C 35</td>
<td>Invasive SCC of the uterine cervix Stage I A</td>
<td>LRHV, LPL, LPAL, LA, LBOT</td>
<td>Concurrent chemoradiation (weekly cisplatin)</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>D 54</td>
<td>Endometrial adenocarcinoma, Stage III A</td>
<td>LAMRVH with BSO, LPL, LPAL, LA, Peritoneal washing cytology</td>
<td>Concurrent chemoradiation (with taxol-carboplatin)</td>
<td>NED</td>
<td></td>
</tr>
</tbody>
</table>

LAVH: laparoscopically assisted vaginal hysterectomy; BSO: bilateral salpingo-oophorectomy; LPL: laparoscopic pelvic lymphadenectomy; LPAL: laparoscopic paraaortic lymphadenectomy; LA: laparoscopic appendectomy; LO: laparoscopic omentectomy; LAMRVH: laparoscopic assisted modified radical vaginal hysterectomy; LRHV: laparoscopic radical vaginal hysterectomy; SCC: squamous cell carcinoma; LBOT: laparoscopic bilateral ovarian transposition; NED: no evidence of disease; AWD: alive with disease.
cal procedure in deciding the surgical stage and prognosis of gynecological malignancies. As long as gynecologic surgeons perform these procedures, encounters with these anomalies will be inevitable. In our experience, the IMA tends to branch from the left of the midline of the abdominal aorta to supply the hindgut, which makes the LPAL procedure seem easier to perform on the right side than the left. However, unexpected hemorrhage can occur if there is vascular injury to the APRA on the right side. If the vascular injury is identified during surgery, segmental loss of renal parenchyma can be prevented by anastomosis or vessel reimplantation with the help of a vascular surgeon before ischemia develops. However, if the diagnosis is made afterwards, it is hard to salvage that portion of kidney because these vessels are end arteries without collateral circulation. Moreover, vascular injury complicated by severe intraoperative hemorrhage can lead to more technically challenging and therefore less accurate surgery [13]. Therefore, both novice and experienced trainees should take care to avoid vascular injuries associated with an ARPA.

Conclusions

To avoid vascular injury during LPAL, it is important for the gynecological oncologic surgeon to have knowledge of retroperitoneal vascular anatomy, experience in laparoscopic surgery and accurate surgical technique to ensure adequate exposure, careful dissection, and surgical stage and prognosis of gynecological malignancies. As long as gynecologic surgeons perform these procedures, encounters with these anomalies will be inevitable. In our experience, the IMA tends to branch from the left of the midline of the abdominal aorta to supply the hindgut, which makes the LPAL procedure seem easier to perform on the right side than the left. However, unexpected hemorrhage can occur if there is vascular injury to the APRA on the right side. If the vascular injury is identified during surgery, segmental loss of the renal parenchyma can be prevented by anastomosis or vessel reimplantation with the help of a vascular surgeon before ischemia develops. However, if the diagnosis is made afterwards, it is hard to salvage that portion of kidney because these vessels are end arteries without adequate oncologic outcome.

References


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Case Reports

Uterine müllerian adenosarcoma with sarcomatous overgrowth and lung metastasis in a 25-year-old woman

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Summary

Uterine müllerian adenosarcoma with sarcomatous overgrowth (MASO), uncommon in premenopausal women, is a rare variant of uterine adenosarcomas characterized by a sarcomatous portion constituting >25% of the tumor. Uterine MASO often appears as a benign, protruding cervical polyp. However, in contrast to typical müllerian adenosarcomas (MAs), MASO is a highly aggressive tumor, frequently associated with a fatal outcome. Though very rare in premenopausal women, because of the high aggressiveness and malignant potential, uterine MASO should be considered, even in women of a young age with benign-appearing polypoid masses, and treated aggressively at the time of initial diagnosis without delay. We present herein a case of uterine MASO in a 25-year-old woman with lung metastasis who was lost to follow-up for one month after the initial diagnosis had been established.

Key words: Uterus; Adenosarcoma; Sarcomatous overgrowth.

Introduction

An aggressive variant of adenosarcoma, müllerian adenosarcoma with sarcomatous overgrowth (MASO) has a benign glandular component and a malignant sarcomatous component that constitutes > 25% of the tumor [1]. It is unusual to diagnose MASO in the premenopausal age group and the most common presenting symptom is abnormal vaginal bleeding, and these tumors are often considered benign [1].

MASO has more malignant characteristics than classic adenosarcoma, and is frequently associated with postoperative recurrence or metastasis with a fatal outcome [1-4]. Because of the rarity of such tumors in the young age group, the clinical suspicion for MASO is very low, often resulting in a delay in diagnosis. Clinicians and pathologists should keep in mind the possibility and characteristics of this gynecologic malignancy.

We present herein a case of uterine MASO in a 25-year-old woman with lung metastasis who was lost to follow-up for one month after the initial diagnosis had been established.

Case Report

A 25-year-old previously healthy nulligravida presented with two months of irregular vaginal bleeding at a local clinic. The medical and surgical histories were unremarkable. On pelvic examination, a 3 cm polypoid mass with a stalk was seen within the cervical os; on ultrasound, a 2.8 x 2.0 x 1.5 cm hypochogenic endometrial polypoid mass was demonstrated. The patient underwent a hysteroscopic polypectomy with dilation and curettage. The pathologic evaluation confirmed uterine MASO. It was recommended that she be transferred to our hospital for treatment. The initial pelvic magnetic resonance imaging (MRI) showed a 1.6 x 1.4 x 1.1 cm irregular mass within the endometrial cavity that invaded into approximately one-half of the myometrium. There was no abnormal hypermetabolic lesion except for uterus in PET computed tomography (CT). She declined the recommended surgery at that time. She sought evaluation at our hospital again with aggravated vaginal bleeding and abdominal distention after a one month loss to follow-up. Pelvic (MRI) showed a 4.3 x 2.8 x 1.7 cm enlarged mass invading > 2/3 of the myometrium (Figure 1). PET CT and chest CT demonstrated metastases to the left hilar (2 cm) and infrahilar (1.5 cm) lymph nodes and both lungs.

She underwent debulking surgery consisting of a laparoscopically-assisted vaginal hysterectomy, bilateral salpingectomy, pelvic lymph node dissection, paraaortic lymph node dissection, partial omentectomy, and incidental appendectomy. Both ovaries were grossly normal in appearance, thus the ovaries were preserved. As the resectability of the lung metastases was thought not to be technically feasible, the lung masses were left in situ. A meticulous survey of the gastrointestinal tract, paracolic gutters, liver, spleen, kidneys, and the undersurface of the diaphragm revealed no additional lesions.

Grossly, the cut surface of the uterine body showed a yellow to brownish mass with focal necrotic area, measuring 5 x 6 cm in size (Figure 2). Microscopically, the endometrium showed protruding biphasic tumor tissue consisting of benign epithelial cells and neoplastic stromal components constituting > 25% of the tumor. The epithelial component was comprised of well differentiated neoplastic epithelial cells. The stromal component showed round or polygonal shaped atypical cells with prominent nucleoli and mitosis (Figures 3 and 4). The tumor tissue infiltrated > 1/2 of the myometrium without penetration of the parametrium. The other pelvic organs and lymph nodes were free of lesions and the washing cytology was negative. The FIGO stage was IVb. Immunohistochemical stains demonstrated that the epithelial cells lining the glands were positive for cytokeratin. The cytoplasm of the stromal cells had a positive reaction for vimentin and p53, but negative for cytokeratin and desmin.

After surgery, the patient received six cycles of combination chemotherapy (intravenous taxol [175 mg/m²] and carboplatin...
After three cycles of chemotherapy, the chest CT showed improvement of the lung and left hilar lymph node metastases. With completion of six cycles of combination chemotherapy, aggravation of the left hilar left node metastases was seen on chest CT; however, no evidence of recurrent tumor or metastasis on pelvic CT was noted. She was subsequently treated with second-line combination chemotherapy (6 cycles of intravenous ifosfamide [1500 mg/m²] and carboplatin [AUC 5]) and radiotherapy (200 cGY/day [total 6000 cGY]) to the right hilar area for three months. Decreased metastatic involvement to the left hilar lymph nodes and no evidence of lung metastasis were seen following systemic chemotherapy and mediastinal radiotherapy. One month later, however, metastasis to the mediastinal and left supraclavicular lymph node was noted on chest CT and PET CT. The disease progressed rapidly and metastasized to the right proximal humerus and left proximal femur. Five months after the last treatment, her cause of death was the disseminated disease.

**Discussion**

The spectrum of mixed müllerian tumors ranges from adenofibromas (with benign epithelial and stromal components) and adenosarcomas (with benign epithelial components and malignant stromal components) to carci-
Uterine müllerian adenosarcoma with sarcomatous overgrowth and lung metastasis in a 25-year-old woman

radiation therapy and chemotherapy. If distant metastatic disease is encountered, the patients should be offered systemic chemotherapy. The chemotherapeutic agents consist of either doxorubicin or a combination of cisplatin and ifosfamide with mesna [2]. Recently, Toyoshima et al. [9] reported that a combination of paclitaxel and carboplatin for treatment of advanced uterine MMMTs resulted in a complete response rate of 80% and a median progression-free interval of 18 months. Considering the results of our case with refractory pulmonary metastasis and no pelvic recurrence, surgical debulking including respectable metastatic lesion at the initial treatment followed by combination chemotherapy or chemoradia-
tion could have led to a more favorable outcome although there are no substantial recommendations for adjuvant treatment. However, studies addressing the optimal time to initiate postoperative adjuvant therapy in relation to the interval to tumor recurrence are needed to establish recom-

mendations regarding the timeliness of adjuvant therapy and its effect on survival, tumor progression, and the disease-free period [10].

In conclusion, the patient presented herein was an extremely rare case, and one of the youngest patients diagnosed with MASO and distant metastasis and fatal progression. Because MASO of the uterus has a highly aggressive malignant potential, gynecologists and pathol-
ologists should be aware of the consequences associated with a delay in the diagnosis and/or initiation of therapy for MASO. And, this entity, although rare, should be in the differential diagnosis of young women presenting with abnormal menstrual bleeding in the presence of a pelvic mass.

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[6] Clement P.B., Scully R.E.: “Mullerian adenosarcoma of the uterus: a clinicopathologic analysis of 100 cases with a review of the liter-


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Fallopian tube primary cancer: report of five cases and review of the literature

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Summary

Objective: The aim of this retrospective study was to analyze the clinical characteristics, management and prognosis of five patients with fallopian tube primary cancer (FTPC) who were diagnosed and treated in our departments. A review of the current literature is also presented. Materials and Methods: Between January 2000 and August 2009, five cases with histologically confirmed FTPC were diagnosed in our departments and were then evaluated retrospectively. All patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy. Results: We had two patients in Stage IA (40%), two patients in Stage IC (40%) and one patient in Stage IIIA (20%). All patients received adjuvant chemotherapy with platinum-based combinations and two of them received additional radiotherapy. Conclusion: FTPC, compared with ovarian primary cancer (OPC), is more likely to present at an early stage and have an overall more favourable outcome. More extensive clinical research must be performed to have definite aetiologic, diagnostic and management modalities.

Key words: Fallopian tube cancer; Treatment; Chemotherapy; Prognosis.

Introduction

Fallopian tube primary cancer (FTPC) is a very rare disease, accounting for approximately of 0.14-1.8% of all female genital tract malignancies [1, 2]. The true incidence is probably underestimated because advanced cases may be incorrectly diagnosed as ovarian primary cancer [3]. Carcinomas are more than 95% of all FTPC, whereas malignant mixed mullerian tumours are exceptional [4, 5].

The aetiology of FTPC is largely unknown, but may be similar to the aetiology of ovarian primary cancer (OPC) [2, 6]. Hormonal, reproductive and possibly genetic factors might increase the risk for FTPC [7]. History of pregnancy, high parity and use of oral contraceptives significantly decreases the risk for FTPC [2, 7].

FTPC most commonly occurs in postmenopausal women, with a mean age of 55 years [8, 9]. The most common symptoms and signs in women with FTPC are abdominal pain (30-49%), vaginal bleeding or discharge (50-60%) and palpable pelvic mass (12-61%) [6, 10]. The rate of preoperative diagnosis is low and even the intraoperative diagnosis is missed in up to 50% of patients [11, 12]. Even when such disease is resected, it is often impossible on histopathological examination to determine the origin of the tumour [7, 13].

The aim of this retrospective study was to analyze the clinical characteristics, management and prognosis of five patients with FTPC who were diagnosed and treated in our departments, together with a review of the current literature.

Material and Methods

Between January 2000 and August 2009, five cases with histologically confirmed FTPC were diagnosed in the Department of Obstetrics and Gynaecology of the University of Patras Medical School and the 2nd Department of Gynecology of St. Savvas Anticancer - Oncologic Hospital of Athens. These cases were evaluated retrospectively.

All patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy. Lymph node sampling of pelvic and paraortic lymph nodes was performed in one case. A cytologic test of the peritoneal fluid was performed in all patients. Staging procedures were performed by a gynaecologic oncologist.

Tissue specimens were stained with haematoxylin-eosin. Cases were identified according to FTPC diagnostic criteria established by Hu et al. and modified by Seldis [9, 14]. Staging was determined using the surgical staging system for FTPC established by the International Federation of Obstetrics and Gynecology (FIGO) [15]. Tumour histologic classification was performed using the criteria of the World Health Organization (WHO).

Results

The median age at diagnosis of FTPC was 64.6 years (range 54-77 years). The median follow-up was 49 months (range 22-70 months).

The most common symptoms and signs were abdominal pain (60%), vaginal bleeding or discharge (40%) and palpable pelvic mass (40%). None of the women with FTPC were diagnosed preoperatively. The median preoperative serum CA-125 level was 370.1 (range 9.4-153.8). It was elevated in three patients (60%) and normal in two patients (40%) (Table 1).

In our study, we had two patients had fallopian tube adenocarcinoma, one patient fallopian tube malignant mixed Mullerian tumour, one patient synchronous fallop-

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ian tube adenocarcinoma and endometrioid endometrial cancer and one patient with synchronous fallopian tube adenocarcinoma and ovarian cancer.

According to the FIGO classification, two patients were in Stage IA (40%), two patients in Stage IC (40%) and one patient was in Stage IIIA (20%). All patients received adjuvant chemotherapy with platinum-based combinations and two patients received additional radiotherapy. These data are shown in Table 2.

During a mean follow-up of 49 months, one patient with Stage IC disease died 36 months after surgery and four patients are well with no evidence of relapse.

Discussion

FTPC is a rare entity. More than 90% of FTPC is papillary serous adenocarcinoma [4, 8]. Other cell types include endometrioid and clear cell carcinoma. Rare types include sarcoma, germ cell tumours and lymphoma [4, 5, 8]. In our study four patients had adenocarcinoma and one patient malignant mixed mullerian tumour.

FTPC is usually diagnosed in early stages [8, 16]. The nonspecific nature of the signs and symptoms in patients with FTPC still makes preoperative diagnosis exceptional [12, 16]. The relatively early occurrence is probably the principal explanation for the typical stage distribution seen in patients with FTPC, with an overrepresentation of early-stage patients compared with ovarian carcinoma [16]. In our study two patients were in Stage IA, two patients Stage IC and one patient Stage IIIA.

The ultrasound (US) appearance of FTPC can be nonspecific, mimicking other pelvic diseases such as tuboovarian abscess, ovarian tumour and ectopic pregnancy [8]. The distinction between FTPC and OPC by transvaginal colour and pulsed Doppler depends on the stage and spread of the tumour [17]. In early stages it is usually possible to distinguish them, but in advanced stages the distinction is not possible [17, 18]. The diagnostic accuracy may be improved by the introduction of 3D power Doppler sonography [18]. Magnetic resonance imaging (MRI) is superior to computed tomography (CT) and US in detecting local tumour infiltration of the bladder, pelvic fat, vagina, pelvic sidewalls and bowel [8, 19]. None of our study women with FTPC were diagnosed preoperatively.

Serum CA-125 is a useful tumour marker for diagnosis, assessment of response to treatment and detection of tumour recurrence during follow-up [20]. Although CA-125 is not diagnostic of FTPC, more than 80% patients have elevated preoperative serum CA-125 levels and 87% of tumour tissues stain positively for CA-125 [20, 21]. Preoperative serum CA-125 level is an independent prognostic factor of disease-free and overall survival in patients with FTPC [20]. Postoperative serum CA-125 levels have been associated with response to chemotherapy [20]. Postoperative serum CA-125 level is an early and sensitive marker for tumour progression during follow-up of patients with FTPC [20]. In our study we had preoperative serum CA-125 levels elevated in three patients (60%).

FTPC is an aggressive malignancy with a tendency to metastasize even in apparently early stages of the disease. The pattern of spread of FTPC is similar to that of OPC, principally by the transcoblic exfoliation of cells that implant throughout the peritoneal cavity [22]. In approximately 80% of patients with advanced disease, metastases are confined to the peritoneal cavity [22]. Tumour spread may also occur by means of contiguous invasion, transmural migration, haematogenous dissemination and through lymphatic spread [23]. Metastases to the pelvic and paraaortic lymph nodes have been documented in at least 33% of the patients with all stages of disease [24].

Surgery is the treatment of choice for FTPC. Surgical principles are the same as those used for OPC. Patients with FTPC should undergo total abdominal hysterectomy with bilateral salpingo-oophorectomy and comprehensive surgical staging including peritoneal washing, omentectomy, peritoneal biopsies, pelvic and paraaortic lymph node sampling [3, 16, 25, 26, 27]. Considering the strong tendency for lymphatic spread of the tumour, a systematic pelvic and paraaortic lymphadenectomy should be preferred to lymph node sampling [27]. Aggressive cytoreductive surgery, with removal of as much tumour as possible, is warranted in patients with advanced disease [26, 27]. In our study all patients underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy and total omentectomy. Lymph node sam-

<table>
<thead>
<tr>
<th>Table 1. Clinical features.</th>
<th>Patients</th>
<th>Percentage (%)</th>
</tr>
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<tbody>
<tr>
<td>Age at diagnosis &lt; 60</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>≥ 60</td>
<td>3</td>
<td>60%</td>
</tr>
<tr>
<td>Symptoms &amp; signs Abdominal pain</td>
<td>3</td>
<td>60%</td>
</tr>
<tr>
<td>Vaginal bleeding or discharge</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>Palpable pelvic mass</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>Preoperative CA-125 levels ≤ 35</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>&gt; 35</td>
<td>3</td>
<td>60%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Histopathologic findings - treatment.</th>
<th>Patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>4</td>
<td>80%</td>
</tr>
<tr>
<td>II</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>III</td>
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<td>20%</td>
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<tr>
<td>IV</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Histological type Adenocarcinoma</td>
<td>4</td>
<td>80%</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>Grade Grade 1</td>
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<tr>
<td>Grade 2</td>
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</tr>
<tr>
<td>Grade 3</td>
<td>3</td>
<td>60%</td>
</tr>
<tr>
<td>Surgery Yes</td>
<td>5</td>
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</tr>
<tr>
<td>No</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Chemotherapy Yes</td>
<td>5</td>
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</tr>
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<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Radiotherapy Yes</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>60%</td>
</tr>
</tbody>
</table>
pling of pelvic and paraortic lymph nodes was performed in only one case.

Because of its rarity, the optimal therapeutic strategy for FTPC has not been well defined [26]. Based on the propensity for microscopic distant spread and the relatively high risk of recurrence despite complete surgical resection, chemotherapy seems to have a strong rationale as adjuvant treatment for patients with early stage FTPC [7, 26]. Single agent chemotherapy does not seem to be effective, while platinum-based combination chemotherapy is the most commonly used adjuvant therapy for FTPC patients [7, 26, 27]. Patients with Stage IA and IB may not require adjuvant chemotherapy, as for patients with OPC [7]. All other patients must be treated with platinum-based combinations [7, 26, 27]. However, very few data are currently available regarding chemotherapy for advanced stage FTPC [7, 26, 27]. Pelvic radiotherapy in FTPC has not been shown to improve survival and the role of whole abdomen radiotherapy is still uncertain [7, 28, 29]. In view of its low efficacy and high rate of serious complications, the use of postoperative radiotherapy in the treatment of patients with PFTC is no longer recommended [7]. In our study five patients received adjuvant chemotherapy with platinum-based combinations and two patients received additional radiotherapy.

Stage of FTPC at the time of diagnosis and residual disease after initial surgery, are the most important prognostic factors [25, 30]. FTPC compared with OPC is more likely to present at an early stage and have an overall more favourable outcome [31]. The reported 5-year survival rate for patients with Stage I is about 95%, for patients with Stage II about 75%, while for patients with Stage III it is about 69% and for patients with Stage IV about 45% [32]. The improved survival for FTPC compared with OPC is most pronounced for patients with advanced stage disease [31]. More extensive clinical research must be performed to have definite etiologic, diagnostic and management modalities.

To conclude, FTPC, compared with OPC, is more likely to present at an early stage and have an overall more favorable outcome. More extensive clinical research must be performed in order to have definite etiologic, diagnostic and management modalities.

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Presentation of a patient with pT2bN1M0 small cell carcinoma of the uterine cervix who obtained long-term survival with maintenance chemotherapy, and literature-based discussion

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Summary

Background: Small cell carcinoma of the uterine cervix is a rare cervical carcinoma that advances early and is associated with a poor prognosis [1]. Due to the small number of cases, treatment strategies have not yet been established. We experienced one case of small cell carcinoma of the uterine cervix, who underwent postoperative concurrent chemoradiotherapy (CCRT) followed by maintenance chemotherapy because of parametrial invasion and pelvic lymph node metastasis, and obtained a long-term survival. We present this case together with a literature-based discussion.

Case Report

The patient was 26 years old, had conceived on two occasions without giving birth, and her height and weight were 150 cm and 51 kg, respectively. The patient had been aware of her atypical genital bleeding since the spring of 2006. When visiting her former doctor in November 2006, the patient showed a thumb-tip-sized hemorrhagic tumor mass on the anterior lip of the vaginal portion of the uterine cervix. A cytodiagnosis was performed, and as the mass was suspected to be squamous cell carcinoma. The patient was referred to the authors’ department for close investigation and treatment of the cervical lesion. There was no noteworthy medical history. Pelvic examination found the uterus to be normal-sized and a thumb-tip-sized hemorrhagic tumor mass on the anterior lip of the vaginal portion of the uterine cervix. The patient was diagnosed with Stage Ib1 cervical carcinoma because of no invasion in the bilateral parametrium. Neither peripheral blood nor biochemical tests showed abnormalities. Tumor marker tests were as follows: SCC, 1.3 ng/ml; CEA, 1.3 ng/ml; CA125, 11.5 U/ml; neuron specific enolase (NSE), 8.4 ng/ml (normal range: 0 to 12.0 ng/ml); and pro-gastrin-related peptide, 22.4 pg/ml (normal range: 0 to 46.0 pg/ml). The cytodiagnosis of the uterine cervix revealed high-N/C small ovoid cells aligned in an Indian file as well as pair cells (Figure 1). The cytodiagnosis suggested small cell carcinoma. Histopathology diagnosis of the cervical tumor revealed that high-N/C small tumor cells with poor cytoplasm had proliferated to form a solid mass. Thus, the patient was diagnosed with small cell carcinoma. Positron emission tomography (PET)-CT showed accumulation of 18F-fluorodeoxyglucose in the cervical tumor and the left external iliac lymph nodes.

The patient underwent radical hysterectomy, bilateral salpingo-oophorectomy, pelvic lymphadenectomy, and paraaortic lymph node biopsy with a diagnosis of Stage Ib1 cervical carcinoma in December 2006. Figure 2 shows the removed specimens. Postoperative histopathology diagnosis, like the preoperative biopsy, revealed that high-N/C small tumor cells with poor cytoplasm had proliferated to form a solid mass (Figure 3A). There was also marked invasion to the lymphatic vessels. On immunostaining, NSE and Grimelius stains were positive, while chromogranin A was negative, and cytokeratin was negative. From these findings, the patient was finally diagnosed with small cell carcinoma of the uterine cervix. The small cell carcinoma had metastasized to the left external iliac nodes, the left cardinal ligament, and the vaginal stump (Figure 3B-D). With a diagnosis of pT2bN1M0, the patient underwent CCRT as a postoperative therapy. For the radiotherapy, a total of 45 Gy radia-
tion was delivered to the whole pelvis and paraaortic regions, and also three fractions of 18 Gy radiation (6 Gy × 3 times) were delivered to the vaginal stump. For the chemotherapy, once-weekly administration of 30 mg/m² nedaplatin was performed a total of six times. For the maintenance chemotherapy, from May 2007 ten courses of PE therapy (CDDP, 15 mg/body; VP-16, 100 mg/body × 3) were performed, consisting of three 4-week courses followed by seven 3-month courses. No recurrent signs have been observed for 39 months after the first operation.

Discussion

Small cell carcinoma of the uterine cervix is a rare disease accounting for 0.5 to 6% of the malignant tumors of the uterine cervix [1], and is known as a carcinoma that advances early and is associated with a poor prognosis. Atypical genital bleeding is often observed as a symptom [2, 3]. In some case reports, this carcinoma was observed in patients aged from 33 to 46, tending to occur in young people, compared with usual cervical carcinomas; in other case reports, it developed in patients aged from 22 to 26 [1-7]. Reported causes include association with HPV18, deletion of the short arm of chromosome 3, deletion of the chromosome 9p21 region, and deletion of the p53 gene [1, 2].

Cytodiagnosis of the uterine cervix is generally considered difficult because of the small cells and their low atypism [1, 3]. In fact, this case was diagnosed with small squamous cell carcinoma at first. Cytodiagnosis of small cell carcinoma requires carrying out screening while considering small cell carcinoma. However, this is considered to be difficult because of the small nuclei. In recent years, cytodagnosis with liquid specimens has become widespread, and immunostaining and other techniques using liquid specimens may increase diagnostic precision.

Concerning tumor marker tests, NSE is generally positive in two-thirds of the cases [8], while SCC, CEA, and SLX are known to be positive at the rate of 30% or less. In the present case, NSE was negative. However, NSE is known to become positive at recurrence, which makes NSE important as a recurrence marker. In cases with no significant tumor markers, PET-CT testing, which enables systemic scanning, may be very effective.

Immunostaining is a useful histopathology diagnosis [2, 9]. According to some reports, NSE and Chromogranin A stainings are positive in 70-88% and 40-50%, respectively. In addition, Grimelius and CD56 stainings are also useful as immunostaining, and Virswanathan et al. [4] reported that chromogranin, synaptophysin, and CD56 stainings were positive in 31.4%, 37.6%, and 29.4% of 51 patients with small cell carcinoma or neuroendocrine carcinoma, respectively.

Small cell carcinoma of the uterine cervix is known to advance early and to cause early distant metastasis; pelvic lymph node metastasis is observed at diagnosis in many patients, and occurs generally at a rate of 40-86% [10]. Sheets et al. [11] report that pelvic lymph node metastasis and vascular invasion were observed in eight (57%) and seven (50%) of the 14 operated patients with small cell carcinoma of the uterine cervix, respectively.

Small cell carcinoma of the uterine cervix is associated with a poor prognosis. Recurrence is generally seen in 80% of cases within 8-16 months after diagnosis, and is also often observed in non-irradiated fields [4, 6]. The five-year survival rate ranges generally from 17-67% for all extensive stages, but was reported to be 0% in patients showing FIGO Stage II or higher or positive pelvic lymph node metastasis [4, 5, 12-14].

Standard therapy for small cell carcinoma of the uterine cervix has not yet been established because it has not been examined in large case groups [5-7, 13]. According to the literature, early-stage small cell carcinoma of the uterine cervix should be treated by surgery followed by chemotherapy or CCRT [7, 13, 15]. Regarding the procedure of hysterectomy, Sevin et al. [16] report radical hysterectomy to be desirable. However, Kasamatsu et al. [17] describe that although providing benefits for patients who show shallow cervical stromal invasion without pelvic lymph node metastasis, radical hysterectomy is less useful for other patients. Bifulco et al. [18] emphasize that postoperative chemotherapy should be carried out in such a way that it has a greater effect than operative procedures. Many reports show that VAC (vincristine, doxorubicin, and cyclophosphamide) or PE (CDDP, VP-16) are
Presentation of a patient with pT2bN1M0 small cell carcinoma of the uterine cervix who obtained long-term survival with etc.

Efficacious as chemotherapy for small cell carcinoma of the uterine cervix, and many literature reports refer to regimens for small cell lung carcinoma [3, 5]. Hoskins et al. [15] performed a historical control study to examine the efficacy of CCRT in 31 cases of small cell carcinoma. The first group of 17 patients had undergone PE and whole-pelvis irradiation, and some had received additional irradiation to the paraaortic lymph nodes. The second group of 14 patients had further undergone TC therapy and irradiation to the paraaortic lymph nodes. There were no significant differences in the therapeutic effect between the first and second protocols. With both protocols, however, the three-year survival rate for CCRT was 60%, which was higher than any previously reported value. Lee et al. [7], in their report on early-stage, or Ib-IIa, small cell carcinoma of the uterine cervix, describe that between postoperative-chemotherapy and CCRT groups there were no significant differences in the five-year survival rate (52% vs 45.5%, \( p = 0.37 \)) or in patient backgrounds, concluding that CCRT cannot lead to improvement in prognosis. Lee et al. [7] reported that a neoadjuvant-chemotherapy (NAC) group had poorer prognosis than a non-NAC group, suggesting that neoadjuvant chemotherapy may need to be carefully selected. On the other hand, Bermudes et al. [19] reported that they found 50% or more tumor regression in 84.7% of bulky-disease patients treated with three courses of PVB (cisplatin, vincristine, and bleomycin) as NAC, and observed their survival time to change with post-NAC tumor diameter. This suggests that if chemotherapy with a high response rate is established, NAC should be examined for efficacy.

We decided to treat the present case with CCRT after radical hysterectomy. Although TC, VAC, PE, and other drugs were considered for use in combination with the radiotherapy, nedaplatin was selected in order to reduce side-effects. It was decided to perform PE therapy as maintenance chemotherapy because a randomized study on small cell lung carcinoma showed the response rate of PE therapy to be 78% [20]. Since there are no reports examining maintenance chemotherapy for small cell carcinoma of the uterine cervix, only the previously-examined maintenance chemotherapy for small cell lung carcinoma served as a reference, and the usefulness of maintenance chemotherapy has not yet been established. However, Hanna et al. [21] reported on the usefulness of maintenance chemotherapy: 4-time treatment with etoposide, ifosfamide, and cisplatin, and subsequent three-month oral administration of etoposide resulted in exten-
sion of the recurrence-free survival time in patients with small cell lung carcinoma. Thus, it was planned to apply PE therapy as maintenance chemotherapy in the present case, a high-risk patient showing parametrial invasion and pelvic lymph node metastasis. It has been reported that the three-year survival rate is 0% in patients with Stage IIb or higher small cell carcinoma of the uterine cervix [13]. Considering that we have observed no recurrence in the present case, PE therapy may become regarded as useful maintenance chemotherapy, but could not be suggested by only the results of the present case.

Concerning prognostic factors of small cell carcinoma of the uterine cervix, Chan et al. [13] carried out a multivariate analysis of prognostic factors in 23 cases of small cell carcinoma of the uterine cervix, and found smoking and clinical extensive stage to be independent significant poor-prognosis factors. In particular, they reported smoking to be a poor-prognosis factor in Stage I-IIa, cases. The present case was a smoker, and this needs to be pointed out. Boruta 2nd et al. [3] report pelvic lymph node metastasis to be a significant poor-prognosis factors. Hoskins et al. [15] regard not only clinical extensive stage but image-based extensive stage to be the unique prognostic factor. This may be because many patients diagnosed with FIGO Stage I-II small cell carcinomas of the uterine cervix have already had paraaortic lymph node metastasis or distant metastasis. Lee et al. [7] report FIGO stage to be a unique poor-prognosis factor. Thus, only the FIGO stage can be considered a definite poor-prognosis factor, however, further examination may be necessary in the future. The present case has lived with no disease for 39 months, although is expected to have a very poor prognosis due to her postoperative extensive Stage of IIb, positive pelvic lymph node metastasis, and smoking history.

Small cell carcinoma of the uterine cervix is a rare cervical carcinoma that is associated with a poor prognosis. While the rareness of this carcinoma makes it difficult to plan clinical studies, it is desirable that a standard therapy be established. It is important to separate small cell carcinoma of the uterine cervix from other cervical carcinomas, and to establish more efficacious therapy for this carcinoma. We believe that there is a need to firstly bring together cases of small cell carcinoma of the uterine cervix on a national level, and to examine these cases in a retrospective manner.

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A case of primary ovarian adenomyoma mimicking ovarian malignancy

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¹Department of Pathology, ²Department of General Surgery, ³Department of Obstetrics and Gynecology, The Catholic University of Korea (Korea)

Summary
Adenomyoma is a benign tumor composed of smooth muscle and benign endometrium. These tumors typically originate within the uterus. An extrauterine adenomyoma is an extremely rare entity. After an extensive literature search, only four cases of primary ovarian adenomyoma appear to have thus far been reported. Here, we report a case of ovarian adenomyoma in a 39-year-old woman mimicking malignant neoplasm of the ovary, along with a brief literature review.

Key words: Ovarian tumor; Adenomyoma.

Introduction
Smooth muscle tumors of the ovary are rare and adenomyomas presenting outside the uterus are extremely uncommon. Adenomyomas, benign tumors composed of smooth muscle and non-neoplastic endometrium, typically originate within the uterus. Thus far, only four cases of primary ovarian adenomyoma have been reported [1-4]. Owing to the extremely low prevalence of this tumor type, we have only very limited data regarding its clinical features and presentation. Recently, we encountered a case of a 39-year-old woman with unilateral ovarian adenomyoma mimicking an ovarian malignant neoplasm.

We report this case and also include a brief literature review.

Case Report
In April 2009, a 39-year-old woman with 0-0-0-0 parity visited our hospital with a chief complaint of diffuse low abdominal pain that had persisted for two weeks. At presentation, her general condition was relatively good, and no special findings were reported on the physical examination, with the exception of some abdominal tenderness. The patient’s medical and family history were unremarkable. No pelvic examination was possible because the patient had not yet experienced sexual intercourse. A rectal examination was conducted and a huge palpable mass was detected in the left adnexal area. Upon abdominal computed tomography (CT), a huge left ovarian tumor measuring over 10 cm was detected; it was heterogeneous, harbored solid and cystic regions, and was interpreted as malignant. Additionally, an 8 x 7 cm sized subserosal myoma originated from the right fundal area of the uterus and a moderate quantity of ascites were noted (Figure 1). Pelvic magnetic resonance imaging (MRI) detected a 13 x 10 x 8 cm sized multiseptated large ovarian tumor of the left ovary, which was suspected to be malignant or borderline malignant (Figures 2A, 2B). No other specific findings, such as metastasis or invasion of adjacent tissue, were detected. Routine blood testing, biochemical testing, urine analysis, and electrocardiography were normal, with the exception of mild leukocytosis. The results of tumor marker analysis were as follows: CA 125 587.6 U/ml, CA 19-9 44.32 U/ml, CEA 0.739 ng/ml, CA 15-3 8.16 U/ml and β-hCG 0.554 mIU/ml. We performed gastroendoscopy and colonoscopy, and both were also negative. All of these findings were suggestive of a primary ovarian malignant neoplasm. Based on the diagnosis of ovarian cancer, the patient underwent an explo-laparotomy. Laparotomy revealed a huge ovarian tumor harboring a cystic portion with hemorrhage and a hard solid portion. The tumor mass originated from the left ovary and adhered to the left tube, colon, omentum, and adjacent tissue. The surface of the tumor was in a partially ruptured state and approximately 400 cc of a brownish turbid intrabdominal fluid was noted. Other internal

Figure 1. — CT scan imaging of the abdomen and pelvis shows a large multiseptated tumor originating from the left ovary, measuring 13 x 10 x 8 cm.
organs, including the right ovary and tube were grossly free, except for the known uterine myoma. Our presumptive diagnosis was ovarian malignancy, and we planned to conduct surgical staging. However, a histologic evaluation of the ovarian tumor during laparotomy resulted in its identification as a benign ovarian tumor composed of an endometrial cyst and ovarian fibroma. The surgical staging was cancelled and we closed the patient’s abdomen after carrying out the myomectomy.

Grossly, the ovary measured 12.5 x 8.5 x 5.5 cm, weighed 280 g, and was firm to soft in consistency. The cut of the firm area was whitish, solid, and evidenced a whirling pattern. We noted multiple large to cystic spaces filled with serosanguineous fluid and blood clots in the soft area, varying in diameter from 5 cm to 0.2 cm (Figure 3). Microscopically, the mass consisted of bundles of increased spindle cells possessing cigar-shaped nuclei with irregularly dispersed proliferative endometrial glands, stromal cells, and hemosiderin-laden macrophages within the stroma and endometrial glandular lumen (Figures 4A, 4B). The increased smooth muscle bundles exhibited multiple hyalinization foci. The endometrial glands evidenced focal stratification, but no nuclear atypia and mitotic activity were noted. Necrosis was absent in the bundles of smooth muscle and endometrial glands. The results of immunohistochemistry for smooth muscle actin, desmin, and vimentin were positive on smooth muscle cells (Figure 6).

Based on the pathologic and immunohistochemical results, the intraoperative diagnosis of endometrial cyst and ovarian fibroma was denied, and the patient was diagnosed with primary ovarian adenomyoma. The postoperative course was uneventful. The patient was discharged from hospital on postoperative day 5 with no problems. No adjuvant treatment was administered. Tumor marker levels were checked on postoperative day 1, and were as follows: CA125 193.8 U/ml and CA19-9 16.28 U/ml. The tumor marker levels were normalized, and no abnormal findings have been noted after six weeks of follow-up.

Discussion

Adenomyomas, benign tumors composed of smooth muscle and non-neoplastic endometrium, typically originate within the uterus. An adenomyoma presenting outside the uterus is a relatively uncommon occurrence. To the best of our knowledge, only four cases of ovarian adenomyoma have been reported thus far, and each of these cases exhibited somewhat different features. The first reported primary ovarian adenomyoma was detected in an endometriotic cyst on the right ovary of a 36-year-old...
A case of primary ovarian adenomyoma mimicking ovarian malignancy

Figure 4. — The ovary evidences an increased number of smooth muscle bundles (A, H&E, 200x). Bundles of smooth muscle were increased and contain interspersed endometrial glands and stroma (B, H&E, 200x).

Figure 5. — Smooth muscle bundles immunohistochemically positive for smooth muscle actin (A, 200X) and desmin (B, 200x).
The endometrial glands and stroma, but can be differentiated
and harbors large to small multicystic spaces lined by
The endometriosis component cannot be differentiated,
cells are negative for smooth muscle actin and desmin.
The storiform pattern. By immunohistochemical testing, these
consists of the spindle fibroblast-like cells arranged in a
leiomyoma, and thecoma. In mixed tumors of fibroma
of ovarian fibroma and endometriosis, endometriosis,
diagnosis of ovarian adenomyoma includes mixed tumor
establishment of a diagnosis [4, 7, 9]. The differential
diagnosis between ovarian adenomyomas and the other ovar-
ian solid tumors appears difficult [8]. Therefore, differen-
tial diagnosis still requires histopathologic examination,
and immunohistochemistry can also prove useful in the
establishment of a diagnosis [4, 7, 9]. The differential
diagnosis of ovarian adenomyoma includes mixed tumor
of ovarian fibroma and endometriosis, endometriosis,
leiomyoma, and thecoma. In mixed tumors of fibroma
and endometriosis of the ovary, the fibroma component
consists of the spindle fibroblast-like cells arranged in a
storiform pattern. By immunohistochemical testing, these
cells are negative for smooth muscle actin and desmin.
The endometriosis component cannot be differentiated,
and harbors large to small multicystic spaces lined by
endometrial glands and stroma, but can be differentiated
by the presence of a prominent smooth muscle region.
Leiomyoma can be excluded due to the presence of endometrial glands and stroma. Thecoma can be ruled out
because it is grossly yellowish and harbors no fat-contain-
ing cells, endometrial glands, or stroma. Additionally,
thecoma is negative for smooth muscle actin.

In this study, we have described the case of a 39-year-
old woman with primary ovarian adenomyoma mimicking
ovarian malignancy. In such cases, there is an even
possibility of overtreatment if an accurate intraoperative
histological test is conducted. The case described in this
study demonstrates that further study will be required to
identify clearly the characteristics of this rare tumor.

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Cervical adenocarcinoma with clear cell morphology. Report of six cases and literature review

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Summary
Clear cell cervical adenocarcinoma (CCA) is a rather rare malignancy of the genital tract. We report six cases of CCA, diagnosed in our laboratory during a 15-year period: five patients with sporadic primary CCA and one young patient with CCA and a history of in utero exposure to DES. The possible DES exposure, clinicopathological findings as well as the differential diagnosis and the prognosis of such patients are presented in a mini-review of the literature.

Key words: Cervix; Adenocarcinoma; Clear cell cancer.

Introduction
Adenocarcinomas of the uterine cervix comprise a heterogeneous group of neoplasms that account for 15-25% of cervical carcinomas and display a variety of clinical features and histological patterns [1].

Clear cell adenocarcinoma (CCA) of the cervix is a rare subtype with main pathological characteristics of clear cell morphology, distinct pathogenesis and characteristic clinicopathological features [2].

The first case report of a CCA was made by Meyer in 1903 [3]. However, according to the recently established criteria [4], the morphology and location of this first reported CCA is more consistent with a mesonephric tumour.

This misconception that clear cell and mesonephric carcinomas of the cervix and vagina are one and the same tumour is responsible for the wrong classification of most of the reported cases and the lack of reliable statistical data.


Clear cell adenocarcinomas develop either as spontaneous tumours in older women (mean age 47 years), or in young women (mean age 19 years) after in utero exposure to nonsteroidal estrogens, particularly diethylstilbestrol (DES) [6]. Between these two subtypes of CCA there are differences in pathogenesis, certain clinical characteristics as well as the prognosis [6]. In older women, CCA develops in the endocervix and the prognosis is similar to the usual infiltrating squamous cell cervical carcinomas. The pathogenesis is unclear and endometriosis is strongly implicated [7,8]. On the other hand, in young women with DES-associated CCA the tumours are located in the ectocervix, there is an association with vaginal adenosis, and the prognosis is considered to be excellent, with 10-year survival reaching 85% [5, 9]. More than 60% of the reported cases of CCA in the later decades of the 20th century could be linked to DES exposure [6], whereas DES-unrelated cases account for 5% of cervical adenocarcinomas [10].

We report six cases of CCA diagnosed in our laboratory during a 15-year period: five patients with sporadic primary CCA and one young patient with CCA and a history of in utero exposure to DES. Furthermore, we performed a mini-review the current literature in the field, and the problems in the differential diagnosis from other lesions with clear cell morphology are discussed.

Material and Method
During the last 15-year period (January 1995-December 2009) in the Pathology Laboratory of Aretaieion University Hospital, Athens Medical School, 61 cases of infiltrating cervical carcinomas were examined including: 40 cases of squamous cell carcinoma (65.5%), 17 cases of endocervical adenocarcinomas (27.8%), and four cases of mixed type squamous and glandular carcinomas (6.5%). We retrospectively reviewed these cases and six cases with clear cell morphology were identified. Four were classified as pure adenocarcinomas and two as mixed cell carcinomas with squamous and glandular elements. The clinicopathological features were re-assessed and additional information was obtained from the files of the 2nd Department of Obstetrics and Gynecology of our hospital. Nuclear and histological grading was performed on each neoplasm based on tumour architecture and the guidelines of Christopherson et al. [11]. Histochemistry (PAS, PAS-diastase, mucicarmine) and...
immunohistochemistry for study of vimentin (V9 clone, ThermoScientific), cytokeratin 8/18 (K8.8+DC10, ThermoScientific), CEA (COL-1 clone, Neomarkers Fremont, CA), HMFG1 (1.10F3 clone), ER (6F.11, Novocastra), PgR (SP2, Neomarkers) were additionally performed in cases not studied previously. Moreover, we performed a mini-review on the field by using a Medline search for relative articles with the key word the term clear cell cervical carcinoma.

Clinical and pathological findings

One patient was a 32-year-old woman with a history of in utero DES exposure and HPV infection of the vulva. All the other patients were postmenopausal, aged 54-65 years old. A review of their records failed to reveal any history of in utero DES exposure. Abnormal vaginal bleeding was the presenting symptom in all patients. Pretreatment cytologic examination was positive for squamous cell carcinoma in two cases, suspicious for adenocarcinoma in two cases, and inconclusive in two cases.

All patients underwent radical hysterectomy, with the exception of the younger patient where the right ovary was preserved and translocated out of the pelvis to avoid menopausal symptoms.

In all cases the cervical tumours grossly presented as exophytic polypoid masses varying in size from 1.2-3 cm, occupying the transformation zone and extending to the endocervical canal. There was extensive infiltration of the cervical wall, but not extension to the pericervical tissues, the uterine cavity, the adnexa or the lymph nodes (Figures 1 and 2).

Microscopically, all tumours presented similar morphology consisting of tubules, cysts and solid sheets of large cells with clear cytoplasm and rather small cubical nuclei (Figure 3). Intracellular periodic acid-Schiff positive and diastase digestible material (glycogen) were also present in varying amounts. Mitotic figures were rare. In two cases, a synchronous development of an infiltrating squamous cell carcinoma was observed.

No hobnail cells, mesonephric remnants or cervical endometriosis were identified in our cases. Immunohistochemistry showed that the tumour cells were positive for CEA, cytokeratin and HMFG1 focally and negative for vimentin, ER and PgR.

The characteristic pathology and the immunophenotype established the diagnosis of clear cell adenocarcinomas. All the tumours were classified as FIGO Stage I and histological grade 2. The follow-up of our patients ranged from six months to four years. In four of the older patients recurrence was observed during the study period. However, no death from the disease was reported during the follow-up period.
Discussions

CCA was first observed in young women exposed to diethylstilbestrol (DES) in utero and a registry for clear cell tumours of the genital tract in young females was established reporting the main characteristics of this tumour in 1974 [5].

The age distribution of CCA is characteristic and has a bimodal peak, one around 20 years of age and the other in the 5th-6th decade of life [6]. In older patients primary CCA is rare and the pathogenesis is obscure. Some cases are associated with DES exposure while in most no such association is reported and endometriosis is implicated in the pathogenesis of the tumour [7, 8]. The most common complaint is vaginal bleeding, and on examination a polypoid, exophytic, or fungating cervical tumour is visible [10]. About two-thirds of the reported cases are FIGO Stage IB and the remaining Stage II or higher [10]. Depth of stromal invasion, FIGO stage, and pelvic nodal status are key prognostic indicators [10].

The microscopic patterns of clear cell adenocarcinoma are solid, tubulocystic, and papillary [12-14]. This growth pattern is important prognostically. A most favourable outcome is associated with a tubulocystic pattern, followed by papillary and solid patterns [12-14]. The cells comprising the tumour have abundant clear cytoplasm due to the accumulation of glycogen, as proven by histochemistry [12-14]. It should be emphasised that the clear cell morphology is caused by dissolution of intracellular proteoglycans due to histological preparations of the tissues, and is observed in many human neoplasms such as adenocarcinomas of the female genital system, neoplasms of the lungs and the kidney [15-17]. In our study, no histological or immunohistochemical differences between the six tumours were observed.

The differential diagnosis of this cervical tumour must be made from benign and malignant lesions such as florid microglandular hyperplasia, mesonephric hyperplasia and mesonephric tumours, Arias-Stella change and metastatic tumours with clear cell morphology (from uterus, ovaries or kidney) [15-18]. The difficulty in the differential diagnosis underlines the problems in the correct classification of CCA [4, 5, 7-9]. Histological features most helpful in the distinction from benign lesions include lack of a grossly visible mass, absence of a desmoplastic stromal reaction, lack of an infiltrative pattern, absence of cytologic atypia, low nuclear-cytoplasmic ratio, and lack of mitotic activity [10]. Clinical data and history are helpful in the differential diagnosis of CCA from metastatic tumours with clear cells while the immunophenotype of CCA, the presence of glucagon and the absence of mucin distinguish CCA from other primary cervical adenocarcinomas [10]. Atypical tuboendometrial glands of the cervix is a common co- finding of such carcinomas and for this reason, strong evidence exists that atypical cervical ectropion of the tuboendometrial type are precursors of clear cell adenocarcinoma [19-20]. The histologic grading of CCA has proven difficult. However, in a previous study including 23 cases, the grading of neoplasms did not correlate with survival [9].

The proposed therapeutic approach is similar to the common infiltrating cervical carcinomas, with special consideration of fertility-preserving techniques for young patients [10, 21]. In the majority of the reported series, when the lesions were smaller than 2 cm and well differentiated, the prognosis was excellent and there were no deaths from the tumour [10].

In conclusion, CCA of the cervix is a distinct tumour that must be diagnosed and classified correctly in order to be properly and timely treated.

References


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Difficulties in diagnosing and treating phyllodes tumor of the breast - case report

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Summary

A phyllodes tumor is a rare breast neoplasm, and in the majority of cases is benign. Its diagnosis is difficult because many characteristics of this neoplasm are also typical for other changes within the breast, especially for fibroadenoma. Palpable examination and imaging diagnostics are obviously very important in the process of establishing a diagnosis. However, histopathological examination is the most important one. Currently, the treatment process boils down to surgical removal of this tumor. It is essential to keep a sufficient margin of healthy tissues, which reduces the risk of local recurrence. In the described case the patient was admitted to hospital due to single tumors in both breasts. Mammosonography allowed us to pre-exclude changes of a malignant character. The right breast tumor was removed during mammotomy. Histopathological examination showed a phyllodes tumor, which is why the mass on the other side was removed surgically. In the period of an 18-month-observation no local recurrence was revealed.

Key words: Phyllodes tumor; Differentiation; Surgical treatment.

Introduction

A phyllodes tumor is a rare breast neoplasm. It accounts for only 0.3-0.9% of all breast tumors [1]. It occurs at all ages. However, the highest incidence of disease applies to patients between 45 and 49 years of age [2]. No dependencies between its occurrence and hormonal changes of the pre- and postmenopausal period or hormonal treatment have been determined. In the majority of cases this tumor is revealed incidentally because it gives no symptoms for a long time. Usually it is painless, hard, well limited and has mobile changes that can grow quickly. In some cases, when the tumor is large, the neoplastic process can include skin and the areola of the nipple. This is caused by pressure on the skin covering the tumor, which leads to ischemia and secondary ulcerating changes.

From the histopathological point of view, the following types of phyllodes tumors have been distinguished: benign, border and malignant. Malignant tumors account for 25-35% of all phyllodes tumors [3]. The presence of atypical cells, mitotic activity, hypertrophy of the stroma and the character of the margin of the change were assumed to be the criteria of such division [4].

Despite the fact that the morphological and histopathological structure is well known, the differentiation process, especially with fibroadenoma is still a problem. History taking, subject examination, breast ultrasonography and mammography can reveal a similar diagnosis in both cases. A fine-needle biopsy is considered to be essential for establishing diagnosis. Sometimes an unambiguous diagnosis is difficult to establish on this basis. Usually this is due to the lack of epithelium or stoma in the sample [2]. In differential diagnoses the following should also be taken into account: breast gland inflammation, abscess, inflammatory cancer, radiological scars, fibrocystic degeneration or fat necrosis [5].

A typical treatment process includes surgical removal of the change with a 1-2 cm margin of healthy tissues, which helps to reduce the risk of local recurrence. Mastectomy is only performed in extraordinary cases as it would cause significant breast deformation. It is advisable only in case of very large tumors when the possibility of obtaining a sufficient margin of healthy tissues is limited [6]. A phyllodes tumor spreads in a hematogenous way [7]. The most frequent metastases include lungs (76.6%), bones (28%), brain (9%) and less frequently the mediastinum and liver [7]. Metastases to lymph nodes apply to 5% of patients which is why removal of axillary lymph nodes is performed rarely [1]. Complications include infections of postoperative wounds and more rarely abscess or hematoma [2].

Radiotherapy does not play a significant role in standard treatment. It can be useful in the instances of local recurrence, remote metastasis and postoperationally as neoplasm prevention. Efficiency of chemotherapy has not been proved either. Hormonal therapy is not efficient either although 20-40% of phyllodes tumors have estrogen receptors and nearly all of them have progesterone receptors [1].

Local recurrence happens in 15-20%. The risk equals 21% for a benign type and 43% for a malignant type. It is estimated that remote metastases account for 5% of incidences and 33% of cases are preceded by local recurrence [1]. Totally a 5-year-survival concerns 90% of patients [8].
The scope of changes that can develop in breasts with which a phyllodes tumor should be differentiated is large. That is why this paper is aimed at demonstrating a case which underlines how important a detailed diagnostic process is in order to choose the correct treatment.

**Case Report**

A 22-year-old patient was admitted to the Clinic of Gynecological Surgery at the University of Medical Sciences in Poznan due to a tumor in the right breast for further diagnosis and treatment. On admittance, the patient did not report any ailments. During the physical examination, a mobile and slightly painful tumor 2-3 cm in diameter was detected in the right breast and a little bit smaller and mobile change in the left breast. The surrounding lymph nodes were not enlarged. Results of laboratory tests were normal. The ultrasonographic examination that was carried out on the day of admission revealed a solid, hypoechogenic area of 2.3 cm in diameter in the upper internal quadrant of the right breast and a tumor of the same character of 1.5 cm diameter on the boundary of quadrants of the left breast. The image of the armpits was bilaterally unchanged. Results obtained allowed us to establish an initial diagnosis indicating bilateral breast tumors at a low-risk of malignant changes, which qualified the patient for removal of the tumor by means of mammotome biopsy.

The procedure was carried out on the same day under ultrasound and proceeded without any complications. The mass in the right breast was removed entirely and the samples drawn were passed on to for histopathological examination. The next day after removing a pressure dressing and checking the wound the patient in generally good condition was discharged with a recommendation of a control check by her attending physician.

Seven and 14 days after the procedure the patient underwent control examinations. Examinations showed regular healing of the wound, and no bleeding was detected. The following diagnosis was taken on the basis of the histopathological examination: “phyllodes tumor-benign form”. Another consultation was recommended in order to remove the mass in the left breast. One month later tumorectomy of the left breast was carried out. The sample of the entirely removed mass was passed on for histopathological examination. The next procedures were normal and the breast will allow therapeutic mistakes to be avoided and the correct treatment to be chosen.

Six months later the patient underwent the first ultrasound breast control. The examination showed no focal changes in the site of the tumor and medium intense changes of a mastopathic character in the right breast, as well as a visible scar of 7.6 cm as a result of the left breast tumorectomy. The armpits were found regular. Further control mammography/sonography carried out 12 and 18 months after the procedure revealed no pathological changes.

**Discussion**

Phyllodes tumor is a rare but clinically significant breast neoplasm. The features of the tumor on examination or mammography/sonography are misleading and can cause many problems in the diagnostics and treatment process. This is due to the fact that they are not only specific for phyllodes tumors. Thus the most important problem in the diagnostic process is the differentiation with other breast tumors. The biggest similarity was observed in relation to a fibroadenoma [1, 5, 6]. This is the main problem as far as the diagnostic process of phyllodes tumors is concerned, as proved by numerous clinical reports [1, 2]. The case presented here seems to support the essence of this problem. The patient did not report any ailments and clinical indexes did not correlate with the histopathological results obtained later. Like fibroids, phyllodes tumors are palpably solid structures, well limited and mobile [2, 5]. Also, radiologically the change is well limited and ultrasonographically it may respond to a hypoechogenic area with outbreaks of cystic degeneration [2]. There is another problem related to the diagnostic process of this tumor, namely the determination of the degree of malignancy. It is considered that neither clinical tests nor imaging results can make such a distinction possible or define the risk of recurrence or metastases [1].

The majority of authors claim that the most important criteria differentiating benign tumors from malignant ones include: high mitotic index, stromal hyperplasia, pleomorphism of cell nucleus and infiltration of the margins. However, these are the criteria available after the removal of the tumor and histopathological examination [4]. Some papers report on the significance of the size of the tumor in the process of determining its character [3]. It is suggested that tumors of 10 cm in diameter and more involve a higher malignancy variant and a higher risk of local recurrence [3]. In the case of our patient the changes were 2-3 cm in size and after two years of treatment no recurrence has been reported. This may prove the relation between the size of the tumor and its malignancy and local recurrence. However, not all authors are of that opinion [1]. As was described in the introduction, surgical removal of the mass is the most appropriate solution.

The effectiveness of this treatment differs from one case to another because this type of tumor is likely to recur in the original location or near it. Mammographic/sonographic imaging in our patient suggested a benign change. That is why the tumor from the right breast was removed by means of mammotome biopsy. Despite the fact that a non-standard treatment was used, no recurrence was reported. The frequency of recurrence depends mainly on histopathological criteria and reaches 15-20% [1]. In their paper Guerrero, Ballad and Grau determined the risk of recurrence taking into account malignancy grade of tumors - 21% risk in the instance of benign changes and 43% in the instance of malignant changes [1]. The risk of recurrence arises also when the tumor is removed without a margin of healthy tissue. The majority of authors agree that the most suitable margin should be 1-2 cm [1, 6, 8]. The average recurrence time described in the literature amounted to 12 months [3]. This indicates that it is very difficult to forecast whether surgery will be successful. The risk of local recurrence depends on many factors that are difficult to determine.

In conclusion a phyllodes tumor is a significant diagnostic and therapeutic problem due to its low clinical specificity, which is why special attention should be drawn to differentiate this neoplasm from other changes. This will allow therapeutic mistakes to be avoided and the correct treatment to be chosen.
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Fibula metastasis as the presenting feature of vaginal cancer

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Summary

Background: Metastatic bone involvement in vaginal carcinoma has not been reported in the literature. Case: A 74-year-old woman was referred for a painful fibula to the orthopedic surgeon. A work-up revealed an isolated metastatic bone lesion in the right fibula of a primary squamous carcinoma of the vagina. Rather surprisingly this lesion had been missed during all previously regular clinical gynecological examinations. Palliative therapy including bone resection and radiotherapy of the metastatic lesion were given. Conclusion: This case highlights: (1) the unique presentation of a vaginal cancer by pain in the lower leg secondary to a metastasis in the fibula; (2) that a speculum examination can mask a mid-vaginal lesion; (3) the importance of aggressive treatment of a solitary bone metastasis in order to provide effective palliation.

Key words: Vaginal; Bone; Metastasis, Squamous, Cancer.

Introduction

Identifiable bone metastases are rare events in gynecological cancer. However the true incidence is unknown. Apparently low rates may be due to the fact that skeletal metastases generate symptoms mimicking benign conditions. As investigations become more accurate and tumor specific it is possible that these figures are going to rise. Currently the incidence of bone metastases in squamous cell carcinomas of the cervix is 15-29% [1]. Metastases of endometrial and ovarian cancer to the bone have a clinical incidence of respectively 5-6% and 2-4% [2, 3]. Vulvar bone metastases vary between 0.8% to 3.4% [4]. An English literature search with the terms: vaginal cancer, metastases and bone failed to identify any article on bone metastases in vaginal cancer.

Primary vaginal malignancies account only for 1-2% of all gynecological cancers [5, 6]. The majority of vaginal cancer patients present with vaginal discharge (62%) [5]. Less frequent symptoms are positive cytology (16%), tumor mass (13%), pain (4%), dysuria (2%) or incidental symptoms (3%) [5]. Bone metastasis has not been reported as an initial symptom. Seven out of ten patients are diagnosed in early stages (Stage I and II, respectively 49% and 22%); while 29% will be diagnosed in advanced stages (Stage III and IV, respectively 11% and 18%) [5]. These figures suggest that vaginal cancers may be missed at clinical examination. Other factors that may contribute to the relatively late presentation include patient reticence in reporting symptoms and failure to examine the patient vaginally. It is also possible that a standard speculum examination for a cervical smear may hide a vaginal cancer. If during removal of the speculum, visual inspection of the vagina is not performed a lesion lying in the anterior or posterior wall of the vagina may be overlooked.

Vaginal cancers have a tendency to spread into the surrounding tissues by direct infiltration and disseminate widely throughout the vaginal via the 'sub-mucosal' lymphatic plexus rather than by hematogenous pathways. Preoperative examinations routinely do not include a bone scan. FIGO staging is based on clinical examination and not on surgicopathologic findings.

This report describes a case of a bone metastasis in a patient with vaginal carcinoma presenting with pain in the right fibula due to a pathologic fracture.

Case

A 74-year-old woman with a painful swelling in the lower one-third of the right leg of a few weeks duration presented to her primary care physician. Initially this was treated with a non-steroidal anti-inflammatory medication. This management gave no improvement. A conventional X-ray was done revealing a bone fracture of the right fibula. Consequently she was referred to an orthopedic surgeon. Past medical history included an infected wound on the right foot four years earlier. One month prior to the bone swelling she had reported blood stained vaginal discharge. The patient was referred to a gynecologist. Clinical examination was recorded to be within normal limits and a cervical smear was reported as negative. Clinical examination by the orthopedic surgeon showed an expanded right lower leg which was very painful on touch. A bone scan revealed an increased uptake in the lower right leg. Additional imaging with magnetic resonance imaging (MRI) scan showed bone metastasis with central necrosis in the distal right fibula. A computerized tomography (CT) guided biopsy was performed and pathological examinations revealed an invasive squamous carcinoma. The gynecological examination was repeated suggesting a vaginal lesion. However, due to the narrow introitus no proper speculum could be placed and thus only a pediatric speculum and no adequate internal examination could be performed. CT revealed a vaginal mass, without any signs of intra-abdominal or retroperitoneal disease. No other metastases were identified. Surgical resection of the bone metastases was planned. A gynecological examination under anesthetic
revealed a circular mid vaginal tumor over a length of 5 cm. After installing a normal size speculum, necrotic tissue could be visualized in the middle of the vagina. The macroscopic appearance of the cervix and the upper vagina were normal. On rectovaginal examination both parametria were involved by spread of the cancer, the left parametrium completely and the right parametrium midway to the pelvic side wall. A biopsy of the cancer was taken and microscopic examination showed a moderate to poorly differentiated squamous cell carcinoma of the vagina. The distal part of the right fibula except for the last 2 cm was resected and an allograft was installed. Macroscopically the lesion was 6.7 cm in diameter and microscopically represented metastasis of a poorly differentiated squamous carcinoma, compatible with the vaginal tumor. The cancer was staged as a vaginal cancer FIGO Stage IV. An uneventful recovery was made. Further management consisted of radiotherapy to the right lower leg. Systemic treatments were discussed including biphosphates, after consulting the patient no further therapy was given. During a period of two months the patient had a minimal of pain and according to herself a reasonable quality of life. Thereafter she presented with a shortness of breath and a chest X-ray revealed multiple small lesions suggestive of metastases. These lesions were not present at the initial chest X-ray. Supporting palliative therapy was given. The patient slowly deteriorated over a period of two months. She succumbed six months after the initial diagnosis.

Discussion

Osseous metastases as a presenting symptom in gynecological tumors is extremely rare. In Table 1 the percentage of bone metastases in gynecological tumors are shown. If bone metastases do occur then the vertebrae are the most common site and the pelvic bones, femur, ribs, sternum, mandibula and skull are the less frequent sites. Combined autopsy and radiological series showed that the incidence of bony lesions in the bones of the forearm, hands, legs and feet vary between 1-4%, and none of them was a single lesion [2, 7]. The most common primary tumor site for metastatic bone lesions in the leg and foot are the colon, rectum, lung and kidney [8].

The present case is exceptional and peculiar in various ways. First of all this is the first report of bone metastasis in vaginal cancer. Secondly it was a single bony lesion and thirdly it was in a fibula.

There are two possible factors that could explain why bone metastases are a rare event in gynecological cancers. First of all gynecological tumors in general tend to spread locally and through the lymphatic system rather than by the hematogenous route. Only the latter route can cause spread to the limbs [9]. An explanation for bone metastasis in the lower legs could be the retrograde venous flow of tumor emboli [9], which is similar to skin metastasis development [10]. The second important fact that could contribute to the low incidence is the fact that skeletal metastases can be asymptomatic and only detected during autopsy or imaging [2].

Every new symptom in a cancer patient should be approached with care [11]. Bone pain in a cancer patient is a metastatic lesion until proven otherwise. Further examinations including plain X-ray or whole body bone scan should be done immediately in order to deny or confirm a lesion. CT and/or MRI are only to be used once the diagnosis is established in order to describe the extent of tumor involvement in soft tissue and bone marrow. It is tempting to suggest that skeletal pain is due to more general conditions like inflammation, trauma or arthritis. It is therefore important, as stated previously, that technical examination are performed in the follow-up of gynecological cancer patients based on symptoms and not on routine examinations [12].

The fact that vaginal and vulval cancers still have a delay in diagnosis of several months to years is intriguing. These cancers generally have clear symptoms and can be seen by the naked eye. Clinical gynecological examination should include a direct visualization of the cervix together with the entire vagina and an internal examination.

In general, distant metastases have to be regarded as an ominous sign [10]. The average duration of survival for patients with bone metastases is ten months or less [2, 3]. Decisions regarding therapeutic or palliative management can only be made if the complete extent of the disease is known. Bone metastases are often just a tip of the iceberg and in case of widespread disease palliative therapy with symptom management is the rule. In the literature there are only a handful of reports of single bone metastasis in patients with gynecological malignancies [13, 14], some having long-term survival [15, 16]. However this is probably an overestimation as many clinicians will only report on a success story of long-term survival and leave the short-term survival unknown. Nevertheless, aggressive treatment in a patient with a good clinical condition and a single bone metastasis should be considered.

Palliative treatment with radiation alone or in combination with orthopedic surgery is very effective in controlling symptoms. Furthermore biphosphonates should be considered to prevent or reduce skeletal complications [16, 17]. In animal models of bone metastases early biphosphonate administration followed by radiation led to improved remineralization and restabilization of osteolytic lesions [18]. The role of systemic treatment for symptomatic bone metastases is not well described.

The presence of bone metastases in vaginal cancer has not been reported previously. Based on the relatively rareness of vaginal cancer and the fact that this is the first report of bone metastasis in vaginal cancer patient, one cannot recommend the use of a bone scan in the preoperative setting nor in follow-up. It is important to consider

| Table 1.— The percentage of bone metastases in gynecological tumors. |
|-----------------------------|------------------|
| Vulva cancer: 0.8-3.4% |
| Vagina: the present report is the first case |
| Cervical cancer: 0.8-23% |
| Uterine cancer: 5-6% |
| Ovarian - and tubal cancer: 1-4% |
osseous metastases as a possible diagnosis in a patient with skeletal pain not responding to conservative measures in general and in cancer patients in particular.

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Peripheral primitive neuroectodermal tumor (PNET) of the vulva: a case report

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Summary

Introduction: Ewing’s sarcoma/peripheral primitive neuroectodermal tumor (ES-PNET) is a high-grade malignant neoplasm that often develops in the skeletal system. Primary extraskeletal ES-PNET is an uncommon condition that rarely affects the female genital tract. Tumors in the ovary, cervix, and uterine corpus and vulva are occasionally reported. Reports on the Ewing family of tumors involving the vulva are extremely rare in the relevant literature. Only a few cases of vulvar ES-PNET have so far been reported. Case presentation: A 14-year-old adolescent girl presented to the clinic with a 4-month history of a left vulvar mass. The mass was excised under general anesthesia, and re-resection was performed three weeks later to obtain negative microscopic margins. The patient received chemotherapy and radiotherapy; however, she died of pulmonary metastasis within nine months of the initial surgery. Conclusion: In summary, we describe a rare case of vulvar ES-PNET with distinct rosette-like structures in a 14-year-old adolescent girl with a very poor prognosis.

Key words: PNET, Vulva.

Introduction

Ewing’s sarcoma/peripheral primitive neuroectodermal tumor (ES/PNETs) is a high-grade malignant neoplasm that often develops in the skeletal system. It is considered to be a neurally derived neoplasm in the central nervous system, autonomic parasympathetic ganglia, and peripheral nerve tracts [1]. The scope of the term has expanded to encompass peripherally located tumors that are histologically similar and called peripheral PNETs (pPNETs). In general, these tumors affect the gastrointestinal tract (the stomach, intestines, and pancreas) and the lungs. PNETs that occur elsewhere in the female genital tract – including the vagina, endometrium and vulva are rare [2].

Only a few cases of vulvar ES/PNET have so far been reported. Here, we describe another case of vaginal ES/PNET with distinct rosette-like structures in a 14-year-old adolescent girl.

Case Report

A 14-year-old adolescent girl presented to the clinic with a nine-month history of a left vulvar mass. Her physical examination revealed a painless and mobile lesion. Clinically, it was thought to represent a Bartholin’s gland cyst or lipoma. The patient did not have any medical or surgical illness and there was no malignancy in her family history.

The mass was excised under general anesthesia. Pathological examination revealed a malignant lesion composed of a monomorphic population of small round blue cells. Within the cellular aggregates, a diffuse growth pattern was observed without any evidence of keratinization or glandular differentiation. Periodic acid-Schiff stain was negative for tumor cells. Three to five mitoses were counted per single high-power field. Rosette-like structures were observed. The tumor cells were immunohistochemically focally positive for CD99 in a membranous staining pattern.

Immunoperoxidase stains for vimentin and synaptophysin were diffuse and focally positive respectively, and stains for chromogranin, desmin, SMA, S100, CD 34, HMB 45 and pan- cytokeratin were all negative. The diagnosis of ES/PNET was made based on the microscopic and immunohistochemical data.

Discussion

ES/PNET is a high-grade malignant neoplasm that is often found in the skeletal system. Although Ewing’s sarcoma typically develops in bones, extraosseous tumors resembling Ewing’s sarcoma were first described in 1969 by Teft et al. [1]. Those who first used the term “primitive neuroectodermal tumor” (PNET) in 1973 were Hart and Earle and they used it to describe a group of small round cell tumors appearing to have developed from neuroectodermal cells [2]. The first series was reported by Angervall and Enzinger in 1975 [3].
Primary extraskeletal ES/PNETs are rarely observed and the most frequent extraskeletal sites include the chest wall, lower extremities, and the paravertebral region, while the less frequent sites are the pelvis and hip region, the retroperitoneum, and the upper extremities. Mostly observed among young patients, ES/PNETs had a peak incidence in the 1920s showing a slightly male predominance [4].

Extraskeletal ES/PNETs of the female genital tract are also uncommon and tumors are occasionally reported in the ovary [5] cervix [6], uterine corpus [7] and vulva. Reports on the Ewing family of tumors (EFTs) involving the vulva are extremely rare in the relevant literature. The reports on vulvar ES/PNET in the literature have been limited to a few cases so far [8, 9].

Immunohistochemical, karyotypic, and reverse transcription-polymerase chain reaction analyses can be used to diagnose the tumors belonging to the ES/PNET family. CD99, a monoclonal antibody to the cell surface protein MIC2, whose gene is located on the pseudoautosomal region of the X and Y chromosomes is the most useful immunohistochemical marker to diagnose PNET [10]. Cytogenetic and molecular genetic identification of the ES/PNET-associated translocation is the “gold standard” since nearly 90% of ES/PNETs are characterized by the translocation t(11, 22)(q24, q12) that leads to the fusion of the EWS gene on chromosome 22 to the FLI-1 gene on chromosome 11 [11]. Due to its high cost, and very limited availability two years ago, we could not use this method in the present case. In cases where molecular genetic evaluation could not be applied, immunohistochemical detection of CD99 antigen expression was shown to be valuable in diagnosis. The tumor in our case showed strong positivity for CD99 antigen, which has been proven to be a very useful and sensitive diagnostic marker to identify extraosseous ES/PNET.

Though translocation t(11, 22)(q24, q12) was not feasible in the present case, we were assisted in diagnosing ES/PNET by immunohistochemical studies including CD99 positivity, along with the evidence of neuroectodermal differentiation (numerous Homer-Wright rosettes).

EFTs are usually aggressive, showing a poor prognosis [12], which quickly lead to metastatic disease and death. In fact it is difficult to ascertain whether vulvar and vaginal tumors behave similarly to those neoplasms developing at more usual sites because there are too few cases reported, many of which have limited or no follow-up. Due to their rarity of these tumors, optimal methods to treat these tumors have not yet been established and it is also impossible to provide any survival rates based on limited data. Even admittedly limited data suggest that EFTs in the vulva or vagina could have more favorable outcomes opposed to those involving more usual sites as in the current case they may present with poor prognosis and may be aggressive even if the patient has undergone surgery and received chemotherapy and radiotherapy, and may be lethal within months.

Due to the rarity of vulvar ES/PNET cases, information about the diagnosis, treatment, follow-up and prognosis are insufficient. Also the low number of the cases precludes accurate standardization of therapies.

In conclusion, vulvar PNETs are extremely rare tumors. Due to their rarity, optimal methods to treat these tumors have not yet been established and it is also impossible to provide any survival rates based on limited data. Even admittedly limited data suggest that EFTs in the vulva or vagina could have more favorable outcomes opposed to those involving more usual sites as in the current case they may present with poor prognosis and may be aggressive even if the patient has undergone surgery and received chemotherapy and radiotherapy, and may be lethal within months. Each and every vulvar PNET case should be reported to ensure data accumulation.

References


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A case of uterine cervical carcinosarcoma recurrence who obtained a clinically complete response by ifosfamide, doxorubicin and cisplatin

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Summary
Cervical carcinosarcoma (CS) is a rare gynecologic tumor. The histogenesis, clinical features, and optimal treatment remain unclear. We report a case of cervical CS recurrence to the right lung, which had complete response by treating with ifosfamide, doxorubicin and cisplatin (IAP). A 61-year-old woman underwent semi-radical hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy for CS of the uterine cervix. Eleven months later, the patient presented with left pulmonary metastasis. She refused debulking surgery and had chemotherapy with IAP. After four cycles of chemotherapy, the metastatic tumor completely disappeared. Unfortunately, a re-occurrence tumor was seen in the same lung area six months after IAP. Eventually, she died 39 months after surgery.

Key words: Uterine cervix; Carcinosarcoma; Recurrence; Chemotherapy; Clinically complete response.

Introduction
Carcinosarcoma (CS) is a comparatively rare gynecologic neoplasm which arises mainly in the ovary or uterine endometrium. CS represents around 3% of all uterine malignancies [1]. Because of the rare occurrence of this tumor, its histogenesis, clinical features, and optimal treatment remain unclear. Cervical CS is much rarer in gynecologic malignancies. We report a case of cervical CS recurrence to the right lung in which a clinically complete response was obtained by treatment with ifosfamide, doxorubicin and cisplatin (IAP).

Case Report
A 61-year-old postmenopausal woman, gravida 2, para 2, presented to our hospital with irregular vaginal bleeding of three months duration. Vaginal exploration showed a cervical polyploid mass occupying the vagina. The cervical mass was 6 × 5 cm on magnetic resonance imaging (MRI) T2 image, arising from the cervix, with no invasion of the vaginal wall. Neither remote metastasis nor hydronephrosis was noted on computed tomography (CT) and pyelography. The blood count and serum biochemical data and tumor markers such as CA125, CA19-9, SCC, CEA and LDH were within normal limits. Biopsy revealed cervical heterologous CS and FIGO Stage Ib2. The patient underwent semi-radical hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy (Figures 1-a and 1-b). However, she did not have any adjuvant therapy since there was no stromal invasion and nodal metastasis according to the postoperative pathological examination.

Eleven months later, chest X-ray and CT scan showed a solitary metastasis in the area of the left lung and fine needle biopsy showed recurrence of the cervical CS (Figure 2, 3-a). The patient refused debulking surgery and had chemotherapy IAP. After four cycles of IAP, the metastatic tumor completely disappeared. Additional four cycles of IAP were given as consolidation therapy (Figure 3-b).

Unfortunately, a re-occurrence tumor was seen in the same left lung area six months after chemotherapy. Because of the dose limit of doxorubicin due to cardiac toxicity, the chemotherapy regimen was changed to paclitaxel and carboplatin (TC). Although partial remission was obtained by TC, she finally died 39 months after surgery.

Discussion
Uterine CS is a comparatively rare malignant neoplasm. The commonest site of occurrence is the body of the uterus. Cervical CS is extremely rare. The first case of cervical CS was described by Ferriera in 1951 [2]. There are only about 50 cases of cervix CS documented in the literature [3]. Most of these occur in postmenopausal women and form a polypoid mass, and the commonest clinical features are abnormal vaginal bleeding. Despite complete removal of tumor, the prognosis of CS is very poor and the metastasis rate is high [4]. Cervical CS is conventionally classified into homologous or heterologous as the CS originating in the corpus [5, 6]. The prognosis of heterologous CS may be better than that of the homologous [7, 8]. Complete surgical resection of the tumor most likely provides the best outcome of long-term survival. Radiotherapy to the pelvis reduces the risk of local recurrences, but there has been no survival advantage associated with postoperative radiotherapy [9, 10]. There are several negative opinions about adjuvant chemotherapy for uterine CS, whereas many more clinicians consider that chemotherapy for this tumor may be indispensable [3, 5]. Localized CS treated by surgery alone carries a high risk of local recurrence and metastatic disease. Of patients with clinical Stage I–II uterine CS...
53% developed recurrent disease within five years after total hysterectomy with surgical staging [11]. The single-agent doxorubicin or cisplatin or ifosfamide has activity against CS [12-15]. And the single-agent paclitaxel has had “moderate activity” [16]. The overall treatment response rate was 56% by chemotherapy with ifosfamide, doxorubicin and cisplatin [17]. The combination is superior to single-agent ifosfamide, doxorubicin or cisplatin in terms of response in patients with advanced, persistent and recurrent uterine CS [18-20]. That is why we chose ifosfamide, doxorubicin and cisplatin for this patient. However, toxicity of IAP combination treatment is not trivial. These drugs have myelotoxicity, doxorubicin cardiac toxicity, and cisplatin nephrotoxicity. Toxicity of this case was not severe; each was all within grade 2 in the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) ver. 3.0.

Future directions in the treatment of recurrent or metastatic CS of the uterus remain unclear. Also, the standard therapeutic method is not established at present. In this case, IAP therapy was very effective with mild toxicity and a clinically complete response. In other words it should be considered that IAP therapy can affect recurrence of CS of the heterologous tissue. However, no firm conclusions can be drawn because of the exceedingly rarity of such tumors.

References


Figure 1. A) Gross view of the surgical specimen. A polypoid tumor showing exophytic growth, measuring 9 × 9 × 4.5 cm. B) Histological section reveals malignant epithelium forming a glandular pattern. The background stroma also shows a sarcomatous pattern (original magnification × 40).

Figure 2. Microscopic findings. Left lower lobe lung metastasis by transbronchial needle aspiration cytology (TBAC). Many malignant spindle cells can be seen (original magnification × 400).
A case of uterine cervical carcinosarcoma recurrence who obtained a clinically complete response by ifosfamide, doxorubicin etc.

Figure 3. — A) Pretreatment CT scan showing 3 cm metastasis of the left lower lobe lung. B) CT scan showing complete disappearance of the left lower lobe lung metastasis.

There was no evidence of recurrence nor metastasis after the 6 courses of ifosfamide, doxorubicin and cisplatin.


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Vaginal primary malignant melanoma: report of four cases and review of the literature

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Summary
Objective: The aim of this retrospective study was to analyse the clinical characteristics, management and prognosis of four patients with vaginal primary malignant melanoma (VPMM) who were diagnosed and treated in our departments together with a review of the current literature. Materials and Methods: Between January 1997 and September 2009, four cases with histologically confirmed VPMM were evaluated retrospectively. All patients underwent wide local excision. Results: One patient was in Stage I (25%), two patients in Stage II (50%) and one patient in Stage IV (25%). Among them, one patient received additional radiotherapy and three patients received additional immunotherapy with interferon. Conclusion: The prognosis of VPMM is very poor, despite the treatment modality, because most cases are diagnosed at late stage.

Key words: Vaginal primary malignant melanoma; Treatment; Radiotherapy; Chemotherapy; Immunotherapy; Prognosis.

Introduction
Vaginal primary malignant melanoma (VPMM) is a very rare, but very aggressive tumour [1, 2]. The estimated incidence of VPMM is about 0.026/100,000 women per year [2, 3].

The aetiology of VPMM is largely unknown and does not have any known risk factors. It originates from melanocytes that are present in the vaginal mucosa [4, 5]. VPMM most commonly occurs in postmenopausal women in their sixth and seventh decades [6, 7]. The most common symptoms and signs in women with VPMM are vaginal bleeding (80%), vaginal discharge (25%), palpable vaginal mass (15%) and pain (10%) [6-9].

The aim of this retrospective study was to analyse the clinical characteristics, management and prognosis of four patients with VPMM who were diagnosed and treated in our departments, together with a review of the current literature.

Material and Methods
Between January 1997 and September 2009, four cases with histologically confirmed VPMM were diagnosed in the Department of Obstetrics and Gynaecology of the University of Patras Medical School and the 2nd Department of Gynaecology of St. Savvas Anticancer - Oncologic Hospital of Athens. These cases were evaluated retrospectively.

All patients underwent wide local excision. All staging procedures were performed by a gynaecologic oncologist.

All tissue specimens were stained with haematoxylin-eosin. The histologic diagnosis was confirmed by positive immunostaining. Tumour cells were positive for S-100 protein, Melan A and HMB-45.

Staging was determined using the surgical staging system for vaginal cancer established by the International Federation of Obstetrics and Gynaecology (FIGO). Tumour histologic classification was performed using the criteria of the World Health Organization (WHO).

Results
The median age at diagnosis of VPMM was 71.7 years (range 65-82 years). The median follow-up was 26 months (range 14-46 months).

The most common symptoms and signs were vaginal bleeding (100%), vaginal discharge (50%) and palpable vaginal mass (50%). These data are shown in Table 1.

According to the FIGO classification, we had one patient in Stage I, two patients in Stage II and one patient in Stage IV. Among them, one patient received additional radiotherapy and three patients received additional immunotherapy with interferon (Table 2).

During a mean follow-up of 26 months, one patient with Stage I and one patient with Stage IV died and two patients with Stage II are well with no evidence of relapse.

Discussion
Malignant melanoma is a tumour of the melanocytes of the skin and mucosal membranes. The histogenesis of VPMM is not known. However, it is thought to arise from melanocytes located aberrantly in the epithelium of the vagina [5]. Melanocytes can be found in the basal portion of the vaginal epidermis in 3% of healthy women [10]. Active junctional changes are thought to be the initial stages of development in malignant melanomas of the mucous membranes [11].

VPMM is a very rare disease with fewer than 250 cases reported in the English literature [1, 2]. The estimated incidence of VPMM is about 0.026/100,000 women per year [2, 3]. It accounts for 0.3-0.8% of all malignant

Summary
Objective: The aim of this retrospective study was to analyse the clinical characteristics, management and prognosis of four patients with vaginal primary malignant melanoma (VPMM) who were diagnosed and treated in our departments together with a review of the current literature. Materials and Methods: Between January 1997 and September 2009, four cases with histologically confirmed VPMM were evaluated retrospectively. All patients underwent wide local excision. Results: One patient was in Stage I (25%), two patients in Stage II (50%) and one patient in Stage IV (25%). Among them, one patient received additional radiotherapy and three patients received additional immunotherapy with interferon. Conclusion: The prognosis of VPMM is very poor, despite the treatment modality, because most cases are diagnosed at late stage.
Vaginal primary malignant melanoma: report of four cases and review of the literature

Table 1. — Clinical features.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60</td>
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<td>0%</td>
</tr>
<tr>
<td>≥ 60</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Symptoms &amp; signs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>Palpable vaginal mass</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>Pain</td>
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<td>0%</td>
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</tbody>
</table>

Table 2. — Histopathologic findings - treatment.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
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</thead>
<tbody>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
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</tr>
<tr>
<td>III</td>
<td>0</td>
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</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
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</tr>
<tr>
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<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>75%</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
<td>100%</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3</td>
<td>75%</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>25%</td>
</tr>
<tr>
<td>Biochemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
<td>100%</td>
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</table>

An appropriate and effective treatment protocol for VPMM has not been defined yet. There are several treatment options, but none of them has proved to be a standard approach [14]. Since VPMM is very rare, treatment options are often extrapolated from the knowledge gathered from CMM [8]. Treatment of VPMM with radiation and chemotherapy has been equally disappointing and surgery remains the primary treatment of choice [8, 14].

The spectrum of surgical therapy ranges from conservative surgery (vaginectomy, pelvic exenteration) [6, 14]. If local excision with clear margins is possible, the role of radical surgery as primary treatment for VPMM is unjustified [6]. Wide local excision followed by radiotherapy is appropriate for many patients with VPMM [14]. Nevertheless, if local excision is not possible, pelvic exenteration may be reasonable [6]. In our study all women underwent wide local excision.

The role of elective lymph node sampling remains controversial [4, 6, 8, 14]. Elective lymph node sampling has no survival benefit and leads to significant morbidity [14, 21]. Sentinel node biopsy has recently gained popularity [14, 22]. Since the rate of lymph node metastasis is low, lymph node dissection is not recommended in VPMM [14]. In our study none of the women underwent elective lymph node sampling or lymph node dissection.

Radiotherapy can be applied as primary treatment for patients who are unable or unwilling to have surgery [6, 14, 23, 24]. Radiotherapy can also be applied preoperatively as adjuvant treatment to reduce tumour size and enable more conservative surgery and postoperatively as adjuvant treatment for patients with incomplete tumour resection or with pelvic metastases [6, 14, 23, 24]. In our study one woman (25%) underwent postoperative radiotherapy. The role of chemotherapy in patients with advanced stage VPMM has not been established [25]. Dacarbazine (DTIC) has been the standard of care for many years in patients with advanced stage CMM, with response rates of 7.5% to 12.1% [26]. In our study none of the women underwent chemotherapy.

An appropriate and effective treatment protocol for VPMM has not been defined yet. There are several treatment options, but none of them has proved to be a standard approach [14]. Since VPMM is very rare, treatment options are often extrapolated from the knowledge gathered from CMM [8]. Treatment of VPMM with radiation and chemotherapy has been equally disappointing and surgery remains the primary treatment of choice [8, 14].
Despite the treatment modality, 5-year survival in all patients with VPMM ranges from 8.4% to 17.5% [5, 7, 14]. Tumour size (< 3 cm) is the most important prognostic factor, whereas tumor thickness is only a weak predictor of survival [7]. Recurrences of VPMM are most often seen locally in the pelvis or as distant metastases in the lungs, liver, bones, and brain [7, 8, 17]. The prognosis of VPMM is very poor, despite the treatment modality, because most cases are diagnosed at late stage [27]. In conclusion, the prognosis of VPMM is very poor despite the treatment modality because most cases are diagnosed at late stage.

References


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HANDBOOK OF WOMEN’S HEALTH
edited by Jo Ann Rosenfeld.
"Women’s health" is at its second edition.

The importance of this book lies firstly in pointing out how medical scientific research has always aimed at studying and evaluating middle-aged men. These results have then been applied to women of any age – ranging from childhood to old age – often leading to a distorted interpretation of results.

Women have a different biological role than men and many psychophysical situations do not exist in men or cannot be compared to those in women.

Underlining the need to use methods that specifically aim at assessing women’s health – as described in chapter 1 – allows research and medicine to take a major step forwards and also to deal with diagnosis errors and incorrect approaches to interpreting female clinical features.

As detailed in the list of contents, the book embraces the entire sphere of female physiology and pathology, at the same time bearing in mind psychological aspects and the importance of social context.

The chapters are all written very clearly, allowing anyone – from the student to the expert – to fully benefit from consultation of the manual.

The full range of issues covered provides an all-embracing knowledge about women’s health.

The chapters are written with expertise and always provide useful in-depth information which makes it easier to understand the contents.

The issue examined in chapter 5, section I, is particularly valuable: the study of psychosocial health in women throughout their life. Usually, this topic is not considered by doctors when assessing symptoms in their patients for purposes of correct diagnosis, which should – in turn – be the starting point for correct treatment.

If the patient’s past experiences are not taken into account, symptoms are often misinterpreted and, as a result, therapy may be inadequate. For this reason, the issue treated in this chapter is significant, especially if linked to chapters 21 and 22 of section V.

To conclude, I believe this text brings a significant progress in understanding the female universe as regards women’s physical and psychic health.

CONTENTS
Introduction Jo Ann Rosenfeld. Preventive health care for older women Jeanette E. Sooth-Paul, Deborah Bostock and Cheryl E. Woodson. Nutrition Gwendolyn Murphy, Victoria S.