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Book Review
Human papilloma virus (HPV) is the essential etiologic agent for intraepithelial and invasive squamous as well as glandular neoplasia of the cervix. Cervical carcinogenesis is regarded as the consequence of persistent high-risk HPV infection and co-factors.

It is clear that HPV infects epithelial reserve cells. These cells have a progenitor cell function. They are small cuboid cells with relatively large nuclei in basal layers of the columnar epithelium, the original squamous epithelium and metaplastic squamous epithelium (Figure 1). They are responsible for the regeneration of the epithelium and enable the metaplasia from columnar to squamous epithelium. After reserve cells are infected with HPV, they express non-structural viral proteins. If control of the viral genes in the reserve cells is lost, the dividing cell population expands and epithelial cell differentiation is delayed and is less complete.

It is widely agreed that HPV infection of the cervix is initiated when minor trauma (e.g., sexual intercourse) exposes the reserve cells of metaplastic squamous epithelium of the cervical transformation zone to the virus [1].

Squamous metaplasia is the transitioning of columnar epithelium to a stratified and more resilient squamous epithelium. Factors that induce this process include pH changes, changes in the sex steroid hormone balance, mechanical irritation, environmental conditions, and chronic inflammation.

But does the decades-old concept of microtrauma of the overlaying layers of metaplastic squamous epithelium as the point of viral entry hold up to morphological and functional scrutiny? No one has seen such epithelial defects under the microscope or colposcope [2]. From a functional point of view, it is precisely the squamous epithelium that protects the cervix from injury. Morphologically, the squamous epithelium consists of several layers. The squamous cells are very close to each other. There is little intercellular space between them and cell junctions are plentiful. While the squamous epithelium can withstand mechanical challenges, the columnar epithelium, whose function is not to provide mechanical protection but to secrete mucin, can be injured easily. The columnar epithelium is single-layered and the reserve cells less more accessible. Thus, it can be assumed that it is much easier for HPV to reach the reserve cells in this case.

The following hypothesis takes issue with the dogma of microtraumata of metaplastic squamous epithelium. It is hypothesized that the major pathway of cervical carcinogenesis starts with HPV infection of a distinct number of subcolumnar reserve cells of the columnar epithelium with and without microtraumata (Figure 1a), not with infection of the reserve cells of the metaplastic squamous epithelium.

After HPV infection the columnar epithelium of the cervix can present itself in many different ways. It can remain in its original form, be transformed into adenocarcinoma in situ (AIS), undergo normal metaplasia to become normal squamous epithelium (Figure 1b), or undergo an atypical metaplastic process producing a squamous intraepithelial lesion (SIL). More than one process may occur at the same time. With the influence of co-factors after varying lengths of latency, invasive cancer can develop.

This hypothesis provides explanations for the following findings:

1) Why do glandular, squamous and mixed lesions occur? The target cells for HPV, the subcolumnar reserve cells, can differentiate in both directions.

2) Why is malignant transformation of the original squamous epithelium of the cervix uncommon? [3]. The subcolumnar reserve cells are never located in the area of original squamous epithelium.

3) Why are recurrence rates low after excisional or destructive treatments of SIL and AIS? The predominant concentration of subcolumnar reserve cells is near the external os of the cervix [4]. This area is removed or destroyed during treatment.

4) Why are early age at first intercourse and multiparity risk factors for cervical cancer? Early age shows a physiological eversion of columnar epithelium onto the ectocervix (ectopy) and a most active transformation. Also pregnancy shows repeated eversion of columnar epithelium onto the ectocervix. Anatomically, the subcolumnar reserve cells can now be easily reached by HPV.
Scientific theories have to be continuously verified and falsified. The present “columnar epithelium hypothesis of cervical carcinogenesis” abstains from assuming traumatic epithelium defects of the metaplastic squamous epithelium to explain HPV infection of reserve cells and thus is more compatible with objective observations. Since the integration site of the HPV genomes into the cellular DNA of the host cell is unique, mapping of integration sites in cervical mixed lesions may allow this hypothesis to be tested [5].

References


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Future directions in the field of endometrial cancer research: the need to investigate the tumor microenvironment

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Summary

Endometrial cancer is the most commonly diagnosed gynecologic malignancy in the United States. In 2008, approximately 40,000 cases were newly diagnosed. Although the majority of these cancers are curable by means of hysterectomy and radiotherapy, a subset of endometrial tumors exhibits an aggressive phenotype characterized by lymphovascular invasion, high histological grade, and myometrial invasion, leading to poor prognosis. The mechanisms involved in this aggressive transformation are largely unknown, however, interactions between the primary tumor mass and the surrounding stroma likely play a role in this transformation. Despite the fact that research in other common malignancies has elucidated important associations between stromal protein expression and invasion, these mechanisms have been poorly explored in the area of endometrial cancer. In fact, few investigations have been conducted in the area of tumor microenvironment for endometrial tumors. Invasion and metastasis are two primary reasons for treatment failure related to endometrial cancer. Expression of stromal-derived proteins can potentially serve as biomarkers of aggressive disease as well as biomarkers for remission monitoring. In order to study how expression of these proteins relates to the prognosis of endometrial cancer, these proteins need to be explored in large sets of existing data and/or tissue banks. In this paper, we briefly review the role of three stromal related pathways, SDF-1alpha/CXCR4, HGF/c-Met, and VEGF-A in endometrial cancer prognosis as an overview of the literature. We report that the role of SDF-1alpha/CXCR4 and HGF/c-Met in endometrial cancer prognosis remains unclear, whereas the evidence pertaining to VEGF indicates that overexpression is involved in tumor growth and metastasis. Finally, we would like to highlight the need to explore stromal proteins as a potential tool for the detection of aggressive endometrial tumors and explore some of the molecular approaches that can be utilized in the exploration of the tumor environment.

Key words: Microenvironment; Tumor markers; Endometrial carcinoma.

Introduction

An increasing body of research indicates that stroma surrounding cancer cells plays an important role in the development and subsequent behavior of tumors [1]. Evidence shows that the interaction between neoplastic cells and the stroma is a critical factor in solid tumor growth [2]. The tumor microenvironment has been poorly investigated in endometrial cancer, the most common gynecologic malignancy in the US, affecting over 40,000 women annually. The Epidemiology and Genetics Research Program (EGRP) at the National Cancer Institute (NCI) recognized endometrial cancer as an under-investigated cancer at their 2005 workshop. Specifically, this group identified the lack of biomarkers for endometrial cancer development and progression as key challenges in the field [3].

In terms of prognosis, between 75 and 80% of endometrial cancer patients presenting with low-stage disease are successfully treated, however a subset of patients have a biologically aggressive disease characterized by lymphovascular invasion, high histological grade, and myometrial invasion [4]. Patients with these characteristics are at increased risk of recurrence following hysterectomy and signify a therapeutic challenge. The mechanisms that allow an aggressive endometrial cancer phenotype are largely unknown, although recent studies suggest the tumor microenvironment plays a role in this process. Research on the tumor microenvironment has been conducted for other malignancies, such as breast, prostate, and lung, however the endometrial cancer literature has lagged behind in this topic of research [5-7]. For example, the number of articles focusing on breast cancer and stroma returns more than 600 articles in Pubmed, whereas the same search for endometrial cancer produces only 68 articles. In the area of NIH funding, 45 breast cancer grants specifically studying tumor stroma are currently funded while no stroma-specific endometrial cancer projects were identified in the Computer Retrieval of Information on Scientific Projects database (CRISP) [8].

Furthermore, within the endometrial cancer literature, most of the research has focused on cancer initiating mutations, i.e. those involving oncogenic and tumor suppressor genes. Indeed, mutations in PTEN, k-ras, β-catenin, microsatellite instability, HER2/neu, and p53 comprise the majority of research related to endometrial cancer biology and prognosis. Using the keywords “endometrial cancer”, “oncogene”, and “tumor suppressor” yields over 500 journal articles whereas the keywords “endometrial cancer” and “stroma” yielded 68 journal articles. Hence,
the research related to this field has only recently acknowledged the stromal microenvironment and its contribution to endometrial cancer progression.

The tumor microenvironment includes both non-cellular and cellular components, namely the extracellular matrix (ECM) and stromal cells, respectively [9]. While the ECM provides structural support to the cell, stromal cells, including fibroblasts, endothelial cells, and inflammatory cells comprise a vast network of cells that supply the epithelium with paracrine factors which can enhance the progression of endometrial cancer. As endometrial epithelial cells continually acquire mutations, the ability of the local microenvironment to regulate cell growth becomes disrupted and results in an activated stroma, characterized by increased quantities of collagens, proteoglycans, and glycosaminoglycans [10]. Consequently, the activated stroma recruits additional inflammatory cells and fibroblasts which support the survival and proliferation of carcinoma cells due to abnormal paracrine signaling [11]. The reciprocal relationship between tumor cells and stromal cells allows for the continued growth and invasion of the primary tumor mass. The tumor microenvironment can also limit the access of therapeutics to the tumor, alter drug metabolism, and contribute to the development of drug resistance. Because of their role in all the stages of tumor development, stromal elements represent attractive therapeutic targets. Manipulating host-tumor interactions may be important in preventing or reverting malignant conversion, and re-establishing normal control mechanisms [2].

In this publication, we provide a brief overview of the keys cells of the stroma microenvironment related to endometrial cancer and highlight the importance of investigating this area in the future research studies. Moreover, the role of a few important pathways within each cellular context is presented. Finally, we briefly summarize molecular tools used in studying the stromal microenvironment in endometrial tumors.

**Cells of the microenvironment**

**Fibroblasts**

Fibroblastic cells are responsible for the remodeling of the ECM as well as producing paracrine growth factors that control cellular proliferation, survival, and death [12]. Importantly, fibroblasts are the predominant cell type in the stroma [13]. During the carcinogenic process fibroblasts migrate to the neoplastic lesion and begin to proliferate, increase collagen production, and express alpha-smooth muscle actin. These changes are collectively termed the desmoplastic response which is a hallmark of carcinoma-associated fibroblasts (CAF) [14]. Importantly, these changes are often accompanied by the recruitment of inflammatory cells which further promotes the dysregulated programming of tissues [12].

Fibroblastic-derived ligands and their cognate receptors have been studied in endometrial cancer, however the impact of these proteins on prognosis remains unclear. An important ligand/receptor pair is hepatocyte growth factor (HGF) and c-Met. This fibroblast-derived growth factor has mitogenic and motogenic effects on various cell types, yet, few studies have examined the prognostic role of these proteins in endometrial cancer. The association between overexpression of c-Met and poor prognosis has been reported in ovarian, breast, pancreatic, renal cell, and prostate cancers [15-19].

The only study to characterize this pathway in endometrial cancer patients was performed by Wagatsuma and colleagues [20]. Diffuse staining, defined as more than one-third of cancer cells showing positive staining of c-Met was significantly correlated with FIGO Stages III and IV and poorly differentiated histology compared to focal, or less than one-third of cancer cells showing positive c-Met staining. In terms of survival, diffuse c-Met expression was not indicative of worse survival, independent of FIGO stage, grade, myometrial invasion, and microvessel count. The importance of this pathway in other epithelial cancers suggests potential for these proteins to be involved in endometrial cancer progression. As only one study has analyzed this pathway in relation to endometrial cancer prognosis, more studies are needed to clarify this relationship. Additionally, the potential for these proteins to serve as therapeutic targets warrants further investigation into this system.

**Inflammatory cells**

The link between inflammation and cancer has been suggested frequently by epidemiology, basic sciences, and pathology disciplines. Although normal inflammation is essential to the host, perturbations in this system produce a microenvironment rich in cytokines and growth factors that promote cancer invasion [21, 22]. Inflammatory cells include macrophages, natural killer cells, dendritic cells, mast cells, and lymphocytes. In response to tissue injury, a network of chemical signals initiates the host response which is intended to heal the wounded tissue [22]. The initial step in the cascade of inflammatory events is the recruitment of leukocytes from the venous system, which is regulated by chemokines [22]. Following wound stimulation, chemokines are secreted by many cell types [23]. Leukocytes that express the appropriate receptors for chemokine ligands are attracted to high concentration areas of chemokines [24].

The main chemokines studied in endometrial cancer are SDF-1alpha (CXCL12) and its receptor, CXCR4. Four studies have studied the association between over-expression of SDF-1alpha/CXCR4 and prognosis, with contradictory findings. Using immunohistochemistry (IHC) Tsukamoto et al. reported that CXCR4 expression was significantly higher in tumors that invaded deep into the muscle layer of the endometrium compared to those tumors with superficial invasion. Muscular infiltration is an important prognostic factor in endometrial cancer as regional node metastases and distant organ metastases are significantly more likely to occur as the depth of muscular invasion increases [25].

Inflammatory cells comprise a vast network of cells that supply the epithelium with paracrine factors which can enhance the progression of endometrial cancer. As endometrial epithelial cells continually acquire mutations, the ability of the local microenvironment to regulate cell growth becomes disrupted and results in an activated stroma, characterized by increased quantities of collagens, proteoglycans, and glycosaminoglycans [10]. Consequently, the activated stroma recruits additional inflammatory cells and fibroblasts which support the survival and proliferation of carcinoma cells due to abnormal paracrine signaling [11]. The reciprocal relationship between tumor cells and stromal cells allows for the continued growth and invasion of the primary tumor mass. The tumor microenvironment can also limit the access of therapeutics to the tumor, alter drug metabolism, and contribute to the development of drug resistance. Because of their role in all the stages of tumor development, stromal elements represent attractive therapeutic targets. Manipulating host-tumor interactions may be important in preventing or reverting malignant conversion, and re-establishing normal control mechanisms [2].

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On the contrary, Mizokami and colleagues reported that SDF-1 alpha and CXCR4 expression in human endometrial cancer tissues was inversely related to histological grade, another established prognostic factor in endometrial cancer [26]. Similarly, Kodama et al. reported CXCR4 expression to be significantly lower in patients with endometrial tumors of high grade. Additionally, survival rates were significantly better in patients with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed with higher levels of CXCR4. The major conclusion from these two studies is that the CXCR4 protein is suppressed with higher levels of CXCR4.

Endothelial cells

Endothelial cells maintain tissue homeostasis during tissue repair and growth and are activated during carcinogenesis [12]. The formation of new blood vessels from the preexisting vasculature is necessary for invasive growth and metastasis of the primary tumor to distant sites, as blood vessels deliver nutrients and oxygen to tumor cells and provide a means of gas exchange and waste disposal [21, 27]. Endothelial cells secrete a number of soluble proangiogenic factors in response to cytokine production, growth factor secretion, and local conditions such as hypoxia. Cytokines and growth factors that induce angiogenic factor expression in tumor cells include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin-1beta (IL-1beta), and tumor necrosis factor-alpha (TNF-alpha) [28].

Vascular endothelial growth factor (VEGF) is among the most studied angiogenic factors in human cancers [29]. VEGF is responsible for increasing permeability of endothelial cells, thereby promoting the degradation of the basement membrane which is usually followed by endothelial cell proliferation [29]. Kamat et al. studied the association between VEGF-F, an isomer of the VEGF family, in 111 patients with endometrioid adenocarcinoma (type 1) by means of IHC [30]. High expression of VEGF-A in endometrial tumors was significantly associated with high FIGO stage [30]. Disease specific survival following endometrial cancer treatment was significantly lower in the univariate analyses among patients classified as high VEGF-A expressers; the relative risk of death was 19 times higher for high VEGF-A expressers compared to low expressers. When adjusted for known prognostic factors such as FIGO stage, grade, depth of myometrial invasion, high VEGF-A levels remained a significant prognostic factor of disease specific survival (p < 0.05).

Likewise, Hirai et al. reported an association between VEGF-A expression and established prognostic factors in postmenopausal endometrial cancer patients [31]. Specifically, positive VEGF-A expression was significantly associated with vascular invasion, myometrial invasion, lymphatic vessel invasion, and lymph node metastasis. Despite being associated with these risk factors, positive VEGF-A expression was not associated with 5-year disease-free survival or 10-year disease-free survival. Finally, in a population-based series of endometrial cancer cases (N = 316) with complete follow-up, Stefansson et al. reported that patients with a high expression of VEGF-A had significantly worse survival compared to those with low expression. Additionally, high VEGF-A expression was associated with the serous/clear cell histology, grade 3 tumors, and the presence of tumor necrosis [32]. The cumulative evidence related to VEGF-A in endometrial cancer suggests that this protein plays a significant role in aggressive endometrial cancers.

The need for further studies in the area of estrogen receptors

Exposures that increase circulating levels of estradiol-17 beta (E2) are known to increase the risk of developing type 1 endometrial tumors [33]. The molecular mechanisms of E2 signaling in endometrial cancer have not been fully clarified; however E2 is known to act with estrogen receptor (ER) to influence uterine growth and development [34, 35].

In addition to E2 stimulation of ER, stromal cells contribute to the activation of ER through two important mechanisms. In the first mechanism, stromal-derived pathways such as SDF-1 alpha/CXCR4 and HGF/c-Met activate downstream kinases, notably mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/AKT, which subsequently phosphorylate ER on the transcriptional activation function domain, AF-1 [36-39]. Ligand-independent stimulation of ER by MAPK and PI3K/Akt results in conformational changes in ER, recruitment of co-activators, and activation of target gene transcription, similar to estrogen activation of the receptor [40].

In the second mechanism, stromal cells surrounding the primary tumor cells contribute directly to the biosynthesis of estrogen. Estrogen metabolizing enzymes such as aromatase and the 17 beta-hydroxysteroid dehydrogenases (17 beta-HSDs) are abundantly expressed in stromal cells and convert androgen precursors and inactive estrogens into the metabolically active E2. Consequently, the intratumoral concentration of E2 increases which may further promote endometrial cancer progression through ER activation [35].

As ER interacts with stromal cells, this emphasizes the need to further investigate the endometrial tumor microenvironment, utilizing a broad spectrum of existing technologies for this research. Moreover, the role of stromal cells in ER-activation may be particularly important for patients with aromatase-positive stromal cells, as these patients have significantly worse survival compared...
to aromatase-negative stromal cells [41]. Aromatase inhibitors, although used infrequently for the adjuvant treatment of endometrial cancer, could potentially improve the outcomes for this subpopulation of patients.

**Approaches for studying the microenvironment**

Popular molecular techniques used for the detection of proteins in tissue and serum include immunohistochemistry (IHC), multianalyte technology, and gene expression profiling. IHC refers to the process of localizing proteins in cells of a tissue section exploiting the principle of antibodies binding specifically to antigens in biological tissues. Although IHC is crucial in complementing the information collected by histopathology, lack of reproducibility and standardization of IHC are major barriers to the widespread clinical application of this method in endometrial cancer. Moreover, the semi-quantitative nature of IHC does not lend itself to making informative predictions for survival and prognosis [42].

Another technique used for the detection of molecular abnormalities in cancer patients is multiplexed bead-based immunoassays, which can screen for hundreds of biomarkers simultaneously. In the area of endometrial cancer, this technology is used mainly in the context of serum, although fresh frozen tissue can also be used. At present, no serum biomarkers for the early detection of endometrial cancer or recurrence monitoring are routinely screened. A recent study performed at the University of Pittsburgh Cancer Institute distinguished prolactin as a potential marker for early detection of endometrial cancer based on its ability to differentiate endometrial cancer cases from normal controls [43]. Further studies utilizing multiplexed technology can potentially distinguish biomarkers that can predict recurrence following primary surgery, however the rationale for choosing biomarkers to study should be informed by biologically plausible mechanisms.

Finally, gene expression profiling is a powerful tool for distinguishing genes that are differentially expressed in normal vs neoplastic tissue. Few studies have implemented this approach in endometrial cancer, however this type of profiling can significantly add to the detection of abnormally expressed genes. Salvesen et al. recently investigated the genomic profile of aggressive endometrial cancers [44]. Their findings suggest aggressive endometrial cancers share a distinct transcriptional signature which can ultimately illuminate chemotherapy targets.

The major barriers to implementation of any molecular test are cost, availability of samples, and standardized protocols for the analysis of samples. Tissues that are collected and banked in tissue repositories are not routinely checked for many of the markers that could be of great diagnostic and prognostic value. Moreover, collection and banking of blood samples prior to treatment is seldom performed. The lack of standard collection of specimens has hindered the development of screening protocols in endometrial cancer.

**Conclusion**

Investigating the endometrial cancer microenvironment is very important, as it potentially facilitates the selective survival and growth of transformed cells. Furthermore, an improved understanding of stromal signaling pathways is likely to identify additional therapeutic targets for endometrial cancer, therefore it is critical to study the tumor microenvironment. To our knowledge, few studies have examined the endometrial cancer microenvironment. Factors such as tumor grade, FIGO stage, and histologic type comprise the traditional panel for determining the prognosis of endometrial cancer following hysterectomy, however these clinicopathologic features cannot reliably indicate which therapies are needed to prevent cancer recurrence. Adjuvant chemotherapy following hysterectomy may be necessary to prevent recurrence, but this knowledge relies on ascertaining the molecular abnormalities present in each individual case. Investigating the tumor microenvironment can potentially provide useful information for choosing the appropriate treatment regimen and for improving survival of patients. In endometrial cancer, no routine panel of molecular markers is examined following surgery yet this would greatly inform treatment protocols. Categorizing patients into meaningful risk strata would preclude overtreatment in low-risk patients while aggressive tumors would be treated with individualized therapies.

In this publication, three stromal-related pathways in the context of fibroblast, inflammatory, and endothelial cells have been reviewed. Although this paper is not an exhaustive review of all stromal markers and their significance in endometrial cancer, we have presented three pathways in order to highlight the importance of each pathway in endometrial cancer progression and characterize the approach for studying these proteins in endometrial cancers. The role of HGF and c-Met expression on endometrial cancer prognosis has only been examined in one study which argues for the need future investigations. In the case of SDF-1alpha and CXCRe4, the prognostic function of these proteins is unclear; the few studies that have examined this pathway present conflicting data. On the other hand, the evidence pertaining to VEGF indicates that overexpression is involved in tumor growth and metastasis and poorer prognosis in endometrial cancer.

Several challenges in studying endometrial cancer were identified by the EGRP report; namely, lack of endometrial cancer consortia prohibits researchers from examining risk factors and biomarkers in large cohorts of patients [3]. In the area of biomarkers, validation and replication of findings requires large datasets of cases with available tissue specimens. To overcome this limitation, partnering with established cancer consortia, for example, the Breast Cancer Family Registry would guide endometrial cancer investigators to setting up successful collaborative groups. Finally, the report identified a need for an interdisciplinary approach to studying endometrial cancer. Building collaborative networks among epidemiologists, physicians and other medical professionals has
great potential to develop scientifically feasible, well-designed studies that investigate the interplay of various factors involved in endometrial cancer [3]. Finally, future investigations need to consider finding newer cost-effective approaches for analyzing large numbers of samples, as high cost of these analyses and the need for highly specialized facilities is one of the key challenges to endometrial cancer investigation.

Summary

Better insight into molecular pathways involved in endometrial cancer may lead to the identification of novel biomarkers and targets for the development of diagnostic and therapeutic approaches for prevention and treatment of endometrial cancer. The tumor microenvironment is an under-studied area that could explain the differences in poor outcome following initial treatment. Obstacles in the area of biomarker development in endometrial cancer include the lack of standard protocols for sample collection at the time of surgery as well as cost. Developing a panel of markers to be immunohistochemically screened at the time of surgery would be advantageous for improving the current survival rates for endometrial cancer survivors. Moreover, developing serum biomarkers will be useful for screening women at risk of endometrial cancer.

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The clinical use of type 1 interferon in gynecology

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Summary

Interferons were initially described in terms of their role in blocking viral replication. They are important cytokines that act on various aspects of cell physiology. Importantly, they can affect cell proliferation or induce the differentiation of neoplastic cells. The exact way in which the interferon complex (IFN) acts on tumours is still unknown, although their use in clinical practice has been widely recommended, especially with tumours that are resistant to conventional treatments, or in situations where surgical removal might lead to a loss of organ function. IFN can be employed as a treatment for various diseases, including tumours. Indeed, interferon cytokines are the therapy of choice in certain situations. However, one of the difficulties yet to be overcome is the need for frequent administrations of the drug. We believe that the development of new formulations is needed to match the demand for its use in oncology treatment.

Key words: Gynecology; Oncology; Interferon; Clinical use.

Introduction

The interferon complex (IFN) is a family of glycoproteins synthesised from cells of different origin. It has various functions, including inhibition of viral replication, immunomodulation, stimulation of NK cells and monocytes, and anti-angiogenic and anti-proliferation action [1]. Interferons were discovered in 1957 by Isaacs and Lindenmann who described them as proteins that inhibit viral replication [2]. However, it has become clear that interferons do much more than simply inhibit viral replication. As with many other cytokines, they have a pleiotropic effect on many aspects of cell physiology, including cells growth, cell mobility, and cell function [3].

Type 1 interferons encompass a family of different proteins called IFN-α and IFN-β; they are produced by a wide variety of cells, including fibroblasts, epithelial cells, and leukocytes [4]. One of the most potent stimuli for the synthesis of type 1 interferon is viral infection, especially the double helix RNA produced during the replication of infected cells. The action of these cytokines generates a system of positive feedback that not only stimulates secretory cells, but also stimulates nearby cells, acting in a paracrine manner [5, 6].

IFN-α cytokines have 165 to 166 amino acids, share the same receptor, and exercise similar biological activities. Decades of research on IFN-α have revealed various biological effects, including anti-tumour and anti-viral activity, making it the cytokine type with the longest history of use in clinical oncology [7, 8]. IFN-α has already been used in the treatment of such diseases as hairy cell leukaemia (HCL) and chronic myelogenous leukaemia, and is now being used to treat certain T and B cell lymphomas, metastatic malignant melanoma, renal cell carcinoma, basal cell carcinoma, Kaposi’s sarcoma, papillomatous laryngitis, viral diseases like Hepatitis C, and gynaecological illnesses [9, 10]. Interferon is usually co-administered with other anti-cancer drugs in the hope of obtaining additional or complementary effects on anti-tumour growth, either through anti-proliferation or apoptosis or through an immune-mediated mechanism [11].

IFN-β is a glycoprotein composed of 166 amino acids produced by fibroblasts and synthesized in all parts of the organism. Its ubiquity renders it rather clinically effective, making it safe and tolerant to use [12]. IFN-β has been used as a treatment for various diseases and has been a notable success in treating remittent-recurrent multiple sclerosis [13].

After five decades of use in clinical trials and in animal tumour models, the mechanism by which type I interferon works and its therapeutic potential, according to patient response, remains an area of debate among researchers.

Mechanisms of Action

Interferons produce their effects by way of connecting to specific receptors on the cell surface, generating the activation of members of the JAK kinase family. JAK kinases phosphorylate the STAT (signal transducer and activator of transcription) family of transcription factors [14]. Type I interferon receptors Iα/β are members of the class II family of cytokine receptors. They are formed from two subunits made up of polypeptide chains called IFNAR-1, a glycoprotein of 557 amino acids with 21 amino acids in the transmembrane domain and 100 amino acids, acting in a paracrine manner [5, 6].
acids in the cytoplasmic domain, and IFNAR-2, which exists in three distinct forms: IFNAR-2a (short form), IFNAR-2b (soluble form), and IFNAR-2c (long form), containing 515 amino acids and acting as the principal functional receptor for IFN [15, 6].

IFN receptors are abundantly expressed by the majority of an organism’s cells [16]. The transmembrane domains of interferon receptors do not exhibit intrinsic enzymatic activity, although the cytoplasmatic domains of IFNAR-1 are associated in a non-covalent way with the tyrosine kinase (TYK) enzyme and those of IFNAR-2 with the Janus (JAK) kinase [17, 5]. This interaction of IFN with these receptors activates the JAK/TYK proteins and induces the phosphorylation of tyrosine residues in the receptor’s intracellular subunit. In addition to TYK, tyrosine phosphates are associated with the complex functioning in the cytoplasmic region of type I IFN receptors to regulate the association of TYK and JAK in IFN signalling [18, 19]. Phosphorylated tyrosine residues recruit STAT proteins, which include STAT1-α (91 kD) and its isoform STAT1-β (89 kD), STAT2 (113 kD), and p48 (48kD), known as the interferon regulatory factor (IRF). This combination of proteins, known as Interferon-stimulation gene factor 3, is present in the nucleus of cells activated by IFN [20-22].

Some tyrosine residues from the receptor’s intracellular subunit attach themselves to STAT proteins. After binding to the SH2 domain of STAT1, the receptor subunit binds the tyrosine residue in STAT2. The p48 protein then attaches itself to the heterodimeric STAT1/STAT2 molecule. The complex migrates to the nucleus, where it acts as a gene transcription factor binding the consensus sequence (GAAAN(N)GAAA) known as the interferon-stimulated response element (ISRE) [23, 24].

The immunological response of interferons

There are frequent discussions and studies examining systematic control over the appearance and progression of tumours. Findings in the literature reflect continuous efforts to determine the role of the immune system and the mechanisms that regulate it in the battle against tumours. It is well-established and accepted that the intrinsic cellular phenomena that instigate tumorigenesis stem from the growth of the tissue itself, not adhering to growth inhibition signals, and thereby evasion of death of cancer-causing cells, which then reproduce endlessly sustained by angiogenesis and invade tissues [25].

The immune system recognizes tumours when they start in the organism, but many tumours are not completely rejected, and the cancer progresses. There is a growing awareness that many immunological responses defined as anti-tumour effector mechanisms can actually work in different ways under differing organic conditions; they can become ineffective or even act in favour of the tumour [26].

One important role played by interferons in promoting protective immunological responses is their capacity to regulate the expression the major histocompatibility complex (MHC) proteins. The majority of tumour antigens that provoke immunological responses are cytosolic proteins synthesized endogenously that present themselves as peptides associated with class I MHC proteins. However, these antigens are recognized by MHC class I, CD8+ CTLs whose function is to destroy tumour cells [27, 28].

Interferons can influence cell proliferation or induce the differentiation of tumour cells. Anti-tumour effects may result in a direct action upon the proliferation or antigenic composition of tumour cells or in a modulation effect on cell populations. There may also be indirect effects, such as modulation of the immune response and inhibition of tumour-supporting angiogenesis. IFNs regulate gene expression and modulate the expression of proteins on the cell surface, influencing the differentiation and the speed of the cell’s proliferation [29].

Type I IFNs are capable of inducing apoptosis in various transformed cells and primary cells. The majority of the mechanisms used during apoptosis induced by type I IFN involve the activation of the caspase cascade [30-33], changes in the integrity of the mitochondrial membrane, and the subsequent release of cytochrome [34]. Apoptosis induced by IFN-α is associated with the activation of caspases 8, 9 and 3 [35], allowing for the induction of the expression of PKR proteins, which are important to the sensitivity of TNF-α and TNF cells relative to TRAIL-induced apoptosis [36].

Type I IFNs derive their anti-tumour effect by increasing populations of cytotoxic T cells, natural killer cells, and dendrite cells. Inhibition of angiogenesis can result from endothelial cell apoptosis, an important factor in the inhibition of tumorigenesis and metastasis formation [37]. INFs can increase the cytolytic activity of natural killer cells, and they stimulate the development of Th1 cells in humans. This effect mainly comes from type I IFN’s capacity to promote the expression, in T cells, of functional receptors for the main Th1’s cytokine inducer, the IL-2 [38].

Type I interferons also act on the humoral immunity by regulating three important functions of the B lymphocytes: development and proliferation, secretion of immunoglobulin (Ig), and Ig heavy chain switching [5]. By favouring the production of certain Ig isotypes, while inhibiting the production of others, the interferons facilitate interactions between the humeral and cellular immune systems, increasing the host’s defence against bacteria and viruses [39].

Type 1 IFN in the treatment of different pathologies

The exact mechanism by which IFN acts on tumours remains unknown. Our group undertook studies to try to clarify whether it acts directly on neoplastic cells, acts indirectly on immune response cells, or does both. Various studies have suggested that the apoptosis induced by IFN depends on the type of cell and is specific to each sort of interferon [40]. IFN-α might induce the apoptosis of certain malign haematopoietic cell lines, including
multiple myeloma and CML lines [41, 42], and IFN-β might induce the apoptosis of melanoma cells, ovarian carcinoma cells and multiple myeloma cells [43-45].

Using this data as a tool, the retroviral transduction of the codifying sequence of type I human IFN in two human melanoma cell lines resulted in cisplatin-induced apoptosis, which was associated with an IFN-dependent increase in p53 expression. This finding suggests that the transduction of IFN-α genes in human tumour cells could raise responsivity to cytotoxic agents, resulting not only in a more effective reduction of the tumour load, but also in the stimulation of an anti-tumour immunological response [46].

In breast cancer, IFN has been used in metastasis cases in which the disease was already considered incurable. Anti-estrogen therapy is the first line of treatment in cases in which the tumours are positive for hormone receptors. When the tumour becomes resistant to this therapy, one option is to use interferon [47]. The combination of tamoxifen and IFN-β in treating breast cancer can be more efficacious than the use of tamoxifen alone, and this effect can be observed regardless of whether the tumour expresses oestrogen receptors [48]. The use of chemotherapy followed by a combination of IFN-β, retinoides and tamoxifen has achieved good results in treating breast cancer with metastases [49].

Furthermore, the combination of IFN-α and retinoic acid can have an anti-proliferative effect, improving cell sensitivity to radiation therapy in the treatment of cervical cancer [50]. The efficacy of alternating recombinant intraperitoneal IFN-α 2 and cisplatin has been demonstrated in ovarian cancer patients with tumour masses with a diameter less than or equal to 5 mm, as a complete pathological remission in 50% of patients [51].

Interferon-α 2B has been used intralesionally in the treatment of a patient with vaginal invasive epidermal carcinoma. Papanicolaou stains and colposcopy showed complete regression of the vaginal lesion [52]. In treating NIC associated with HPV infection, positive results have been observed. After five years of treatment, cytology and histology were negative for NIC and HPV [53]. In another study, 16 patients with genital HPV combined with NIC I and II were treated with electroablation using diathermy loop excision and immunomodulation with human interferon β; this treatment achieved success in 13 patients, while the disease persisted in three cases [54].

It has recently been observed that there is a relationship between the mechanism of action of some chemotherapeutic agents, such as cyclophosphamide (CTX), and their action on immune system cells. This effect can now be explored to enable the design of more effective combination therapies using IFN-α [55].

Using IFN-β in patients with pancreatic cancer over six days, a potent inhibitor effect has been observed on the proliferation of BxPC-3 (IC50, 14 IU/ml) and MiaPaCa-2 (IC50, 64 IU/ml) cells. The inhibitor effect of IFN-β was stronger than that of IFN-α in three cell lines and was modulated by the stimulation of apoptosis, although a cessation of the cell cycle was also induced.

The expression of type I IFN receptors was significantly higher in BxPC-3 cells (an IFN-sensitive cell line) than in Panc-1 or MiaPaCa-2 cells, and was located principally in the membrane. However in Panc-1 cells (the most resistant cell line), 60%–70% of the cells were negative for IFNAR-2c with cytoplasmic staining for IFNAR-2c. The anti-tumour activity of IFN-β, through the induction of apoptosis, is more potent than that of IFN-α in the pancreatic cancer cell lines [56]. In addition, in pancreatic tumours, treatment with IFN-α has yielded satisfactory results on cell lines cultivated in combination with 5-fluoracil (5-FU) and gemcitabine in patients receiving a range of doses of interferon over a period of 24 to 96 hours.

Antiproliferative and apoptotic effects were more evident in cells that expressed IFN receptors [57]. Beneficial effects have also been observed for the IFN-α/β and 5-FU combination in patients with advanced hepatocellular carcinoma (HCC) and tumour thrombosis in the principal portal veins. Thirty patients with advanced HCC, tumour thrombosis in the principal branch of the portal vein, and multiple nodules throughout the liver (grade 3 intrahepatic, 4 metastases) were evaluated. Some response was observed in ten patients (33.3%), including a complete response in six patients (20%) and a partial response in four patients (13.3%); there was no response in one patient (3.3%), and progressive disease in 19 patients (63.4%).

Interferon-α was one of the first agents to be used therapeutically to induce tumour regression in the treatment of Kaposi’s sarcoma (KS) associated with AIDS [58]. Following the introduction of anti-retroviral therapy and with the use of well-tolerated chemotherapeutic agents, complete tumour remission can be obtained. Local therapeutic options include excision, cryosurgery, radiation and alitretinoin gel. IFN-α induced a complete remission in 45% of patients with a CD4+ lymphocyte count that exceeds 400/microl [59].

Interferon IFN-β is known to cause cytostatic and cytolytic effects on human glyoma cells, and it is widely used in the treatment of gliomas. Clinical study has shown that vaccination with dendrite cells, including immunotherapy combined with IFN-α, is satisfactorily tolerated with an absence of toxicity, and improves the quality of life and survival of patients with malignant gliomas. The process of cell death observed in susceptible SK-MG-1 cells was accompanied by the morphological changes that are characteristic of apoptosis, caspase processing, and DNA fragmentation. IFN-β-induced apoptosis in human glioma cells by activating caspase-7 and DNase-gamma. Similar activations of caspases were found in some cells that were resistant to apoptosis. These findings can help to improve therapy with IFN-β in the future [60].

In one study, 30 patients with progressive metastatic renal cell carcinoma were treated with IFN-α, thalidomide and capetcytamine, with noteworthy anti-tumour activity. Eleven of these patients had previously received two or more systemic therapies; two patients had complete reactions, seven patients had partial reactions, and 11 patients presented with stable illness. The patients who responded completely to the therapy did not have a recur-
Cervical intraepithelial neoplasias (NICs) can present with spontaneous regression or progression to more serious lesions. Low-grade lesions (LSIL) present with a high index of spontaneous regression. Studies have shown that more than 90% of patients with LSIL show regression within 24 months [70, 71]. The 2006 consensus for the management of patients with intraepithelial neoplasia recommends follow-up with cytology every six months for LSIL patients, which should be complemented with a colposcopy in HIV-positive cases, with a return to routine annual follow-ups after two CIN-negative cytologies [72].

For high-grade lesions, NIC II and NIC III, the same consensus recommends that follow-up with only cytology and colposcopy is unacceptable; instead, treatment should be initiated, either using ablative (laser) or excisional (cold cone excision, laser cone excision, diathermic knife) methods, with the first methods being indicated only in cases of a satisfactory colposcopy. Excisional techniques have the advantage of permitting the histological study and evaluations of the margins, since the degree to which this is compromised is an important predictive factor for recurrence [72].

There is no consensus in the literature as to the possible complications during pregnancy resulting from previous cone excision or diathermic knife treatment. Some studies have not found an increase in the incidence of obstetric complications among pregnant women who had previously undergone these procedures [73]. Other studies have shown that these complications do exist and further are responsible for a significant increase in perinatal morbidity. Among the most common of complications are pre-term labour, low birth weight, and the premature rupture of the membranes [74-76]. A recently published meta-analysis proved that cone excision (cold and laser) is associated with an increase in the levels of pre-term labour, low fetal weight and a higher incidence of caesarean section [77]. Diathermy loop excision has been significantly associated with an increase in the levels of pre-term labour, low fetal weight, and premature rupture of the membranes.

The rising number of HPV infections and the appearance of CIN in young women underscore the importance of developing therapies that do not interfere with patients' future ability to reproduce [78]. Conservative treatment with intra-lesional interferon could be a therapeutic option for patients of childbearing age since it does not alter the anatomy of the cervix, the major factor that generates gestational complications.

**Clinical importance of interferon use in the treatment of cervical neoplasias**

Cervical intraepithelial neoplasias (NICs) can present with spontaneous regression or progression to more serious lesions. Low-grade lesions (LSIL) are those considered to have a high index of spontaneous regression. Studies have shown that more than 90% of patients with LSIL show regression within 24 months [70, 71]. The 2006 consensus for the management of patients with intraepithelial neoplasia recommends follow-up with cytology every six months for LSIL patients, which should be complemented with a colposcopy in HIV-positive cases, with a return to routine annual follow-ups after two CIN-negative cytologies [72].

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**New perspectives on the formulation of interferons**

The use of interferon to treat various chronic diseases, as well as tumours, points to the utility of this cytokine as a treatment of choice in a variety of situations. However, one of the barriers to be overcome is the need for frequent administrations of the drug. The use of “artifices” to provide a more prolonged action is being developed and used with some clinical success, with the classical example being the use of the pegylated IFN molecule to treat hepatitis C [79].

However, pegylated IFN cannot be used in the treatment of tumours where an intra-lesional injection of the drug is needed, which requires a slow excipient release of the drug within the tumour itself. The world pharmaceutical industry has done testing in various similar situations and developed liposomes, phospholipids, microparticles/nanoparticles, heat-shock proteins delivered via viral vectors, and different types of additives that might serve as pharmaceutical carriers for drugs and genes [80]. With tumours, liposomes have been selected to carry and release drugs and even monoclonal antibodies [81].

**Conclusion**

Interferons were first described as a substance capable of “interfering” with the process of viral replication, but various studies have found evidence that IFNs have other biological actions, among them an inhibiting action on cell growth. Type I interferon (α/β), either in isolation or combined with other drugs, has been used to treat many different tumours and has shown benefits stemming from its biological action.
This review has shown that type I IFN (α/β) achieves satisfactory results when used on various tumour types, and highlighted the development of new therapies, which have reduced collateral effects, thereby promoting an improvement in the prognosis and quality of life of these patients. Nevertheless, new strategies for combining and releasing this drug need to be developed to increase its use and hence improve clinical responses. It is a challenge for the pharmaceutical industry to develop formulations of this cytokine, combined with nanoparticles or liposomes that will allow for the drug’s slow release at the lesion centre and in so doing enhance IFN action.

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References


Vitamin D-1α-hydroxylase and vitamin D-24-hydroxylase in benign and malignant breast tissue

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Summary

Background: It is known that 1,25(OH)2D₃ can be metabolized to 1,24(OH)₂D₃ in breast tissue. This tissue-specific expression of 24-OHase may act as a pivotal link between vitamin D status (25(OH)D₃ level) and the anticancer effects of 1,25(OH)₂D₃. Different expressions of the enzymes of vitamin D metabolism are found in breast cancer cells and tissues, and alternative splicing may play a role in biological functions and may cause tissue-specific variations. We describe the expression of vitamin D-1α-hydroxylase and vitamin D-24-hydroxylase in benign and malignant breast tissues. We estimated that alternative splicing of the enzymes would lead to a catalytically dysfunctional product and may lead to a lower reduction of the target protein. Material and Methods: Expression of 1α-OHase and 24-OHase RNA and protein was assessed using a real-time polymerase chain reaction (RT-PCR) and on protein level by Western blot in benign and malignant breast tissue samples. Results: In breast cancer tissue the expression of 1α-OHase and 24-OHase were reduced significantly compared to benign breast tissue. Conclusion: The results described above do not support results of previous studies. Alternative splicing of 1α-OHase and 24-OHase may regulate the levels of active enzyme but is more likely due to different cell types in samples with the result of testing a variety of tissue samples not purified benign and malignant breast cancer cells. The significance of smaller variants in cells has not been clarified either, but it is known that they are not able to use 25(OH)D₃ as a substrate to generate 1,25(OH)₂D₃.

Key words: Vitamin D₃; 1α-hydroxylase; 24-hydroxylase; Breast tissue; Breast cancer.

Introduction

In Western countries breast cancer is the most common form of cancer and cause of death from cancer among women [1]. Many factors have been linked to the various breast cancer risks including vitamin D₃ synthesis in the skin due to exposure to sunlight or dietary intake [2, 3]. Several studies show that the status of vitamin D₃ might be inversely associated with risk of breast cancer [4, 5]. The biologically active metabolite of vitamin D₃ is the secosteroid 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃). There are two principal enzymes involved in the formation of 1,25(OH)₂D₃ from vitamin D₃: the hepatic vitamin D-25-hydroxylase (25-OHase) and the renal 25-hydroxyvitamin D-1α-hydroxylase (1α-OHase), encoded by the CYP27B1 gene for vitamin D₃ and 25-hydroxyvitamin D₃, respectively [6, 7]. Both 25(OH)D₃ and 1,25(OH)₂D₃ can be degraded through the catalysis of vitamin D-24-hydroxylase (24-OHase), encoded by the CYP24 gene [8].

In the kidney, 1α-OHase and 24-OHase form a classic feedback mechanism. Dietary intake and exposure to sunlight significantly influence the circulating concentration of 25(OH)D₃, which is considered to be a reliable indicator of vitamin D’s availability through diet and synthesis in the skin [9]. In contrast, the circulating concentration of 1,25(OH)₂D₃ is tightly regulated by renal 1α-OHase and therefore kept in a relatively narrow range. Several studies revealed an inverse correlation between plasma levels of 25(OH)D₃ and the risk of breast cancer [10-12]. The risk of breast cancer is more than five times higher for women with a plasma 25(OH)D₃ concentration of <50 nmol/l than that of those with a plasma concentration exceeding 150 nmol/l [12].

Liganded vitamin D receptor and the retinoid X receptor are bound to vitamin D-responsive elements by 1,25(OH)₂D₃ in the promoter region of CYP 24. Therefore, according to its role as a negative feedback enzyme, the expression of 24-OHase is induced [13]. Active 1,25(OH)₂D₃ is metabolized by 24-OHase to less active products, which compete with 1α-OHase for their common substrate, 25-OH D₃. Thus the risk of potentially harmful hypercalcemic side-effects of vitamin D are reduced [14].

Relatively low levels of catabolic 24OHase activity were found in extrarenal tissue [11, 15, 16]. Different splice variants of a given protein can display different biological functions and may cause tissue-specific variations in healthy cells. A number of studies show alternative splicing of 1α-OHase and 24-OHase in different human cells (e.g., breast cancer, endometrial and myelomonocytic cells [17-19]. These splice variants may lead to a catalytically dysfunctional protein.

In this study we will describe the expression of 1α-OHase and 24-OHase in human breast cancer as well as in benign breast tissue.
Material and Methods

Malign tumor and benign tissues were obtained from sections of Caucasian female patients who had undergone surgery for invasive breast cancer or benign tumors, e.g., fibro adenoma, at the University of Schleswig-Holstein, Campus Lubeck, with patient consent and local ethical consent. The mean age of primary tumor diagnosis (63 years, range 36-76 years) and histology were validated from histopathology reports and patient medical records. A sample of breast tissue was excised from the relevant frozen surgical sample, which had been stored in liquid nitrogen.

RNA Isolation

Total RNA from cell cultures and homogenized healthy and malignant breast tissue samples were extracted with TRIZOL (Invitrogen, Karlsruhe, Germany) according to the manufacturer’s instruction. The integrity of isolated RNA was verified by 1% agarose gel electrophoresis and the amount was quantified spectrophotometrically at OD260/280 nm.

Reverse transcription and real-time PCR

The synthesis of cDNA from 2 μg total RNA was performed using Super Script-II reverse transcriptase (Invitrogen, Karlsruhe, Germany). RT reaction mixture was diluted 1:5 and 2 μl of RT reaction mixture were used as the template for real-time PCR and 0.5 mmol/l primers (TBP forward 5’-CCA TCT ACA GAC TCT CAC AAC-3’, reverse 5’- CTG CGG TAC AAT CCC AGA ACT-3’; 24-OHase forward: 5’- GCA GCC TAG TGC AGA TTT -3’, reverse: 5’- ATT CAC CCA GAA CTG TTG -3’). After adding 12.5 μl of Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Karlsruhe, Germany), the volume was adjusted to 25 μl with nuclease-free water. The samples were amplified in the DNA Engine Opticon 2® (Biorad; Hercules, USA) System and PCR was performed by an initial denaturing step at 50°C for 2 min and 95°C for 2 min followed by 50 cycles with a denaturing step at 95°C for 15 sec, primer annealing at 57.3°C (24-OHase) and 60°C (TBP) for 15 sec, and an extension phase at 72°C for 15 sec for 24-OHase and Hprt.

A melting curve was generated after 50 cycles for the final PCR product of all genes investigated by decreasing the temperature to 65°C for 15 sec followed by a gradual increase in temperature to 95°C. During the gradual heating process the fluorescence was measured at increments of 0.2°C. The relative 1αOHase and 24OHase gene expressions of data between benign and malign tissue samples were obtained by normalizing to MCF7 cells as the calibrator and TBP as the housekeeping gene. The fold change was determined with the formula: efficiencies target gene (calibrator gene to investigate-sample gene to investigate) / efficiencies housekeeping gene (calibrator housekeeping gene-sample housekeeping). The experiments were performed in triplicates for each gene.

Statistical evaluation

Statistical analysis of real-time PCR results was performed using the Student’s t-test.

Western blot

Cells were maintained under standard cultivation conditions, washed twice with PBS and lysed in a sample buffer (125 mM tris, 30% glycerine, 8% SDS, pH 6.8). Frozen samples of benign and malignant breast tissues were homogenized with Ultra Turrax T25 in TBS buffer containing 0.1% Triton X-100 and 1 mM PMS and lysed in the aforementioned sample buffer; 10 μg of protein lysates were separated on 12.5% SDS PAGE under reducing conditions and transferred to a nitrocellulose membrane (Optitran BA-S 85, Schleicher Schuell, Dassel, Germany). To eliminate non specific binding sites, membranes were blocked overnight at 4°C with a blocking agent (PBST with 5% non fat powdered milk). Therefore, membranes were incubated with primary antibodies against human 24OHase (Santa Cruz Biotechnologies, Heidelberg, Germany) in a dilution of 1:5000 for tissue protein samples and 1:2500 for cell culture protein lysates over night at 4°C.

The secondary antibodies conjugated to horseradish peroxidase (donkey anti goat IgG, Calbiochem, Germany) were added in a dilution of 1:10000. The obtained signals were visualized using the enhanced chemiluminescence (ECL) detection system (Amersham Biosciences, Freiburg, Germany) and as loading control compared to β-actin.

Results

The expressions of 1α-OHase and 24-OHase mRNA in benign and malignant breast tissue were analyzed by quantitative real time PCR. Expression of mRNA was found to be lower in malignant breast tissue compared to benign tissue for both enzymes. The expression of 1α-OHase was decreased by 76% in malignant tissue (p < 0.001), and 24-OHase in malignant tissue had decreased compared to benign tissue by 58% to 42% (p < 0.001). At the mRNA level we found 24-OHase expression in the cancer was reduced in comparison to benign tissue by 83% (p < 0.001). Protein determination through Western blot continuously showed a distinctive band of 55 kDa in benign tissue. Further bands could not be detected in the benign tissue.

Discussion

CYP24 is one of the most sensitively regulated target genes for 1,25(OH)2D3 [20]. The correlation in expression among 1-alpha-Hydroxylase, VDR, and 24-OHase in benign breast tissue provided evidence for localized synthesis of 1,25(OH)2D3. An increased expression of 24-OHase in breast tumors was assumed to be a response to the enhanced localized production of 1,25(OH)2D3 [20].

The aim of this study was to analyze the metabolism 1α-OHase and 24-OHase on mRNA- and protein level in benign and malignant breast tissues. We found in both tissues a band in the Western blot at 55kDa, which matches the wildtype of 24-OHase and a band at 56kDa for the 1α-OHase.

Investigating malign cells we found, aside from a weaker main band, a higher number of additional bands. This could indicate the wild-type enzyme in lower concentrations; moreover it might indicate a higher number of splice variants which may not show any functional activity. Activity measurements should be performed to evaluate their function. Other groups observed lower synthesis as well as less reduction of 1,25(OH)2D3 in breast cancer [21, 22]. Wu et al. detected an Intron 2 containing splice variant for 24-OHase, which represents a catalyt-
Vitamin D-1α-hydroxylase and vitamin D-24-hydroxylase in benign and malign breast tissue

cally inactive enzyme. In addition, single nucleotide polymorphism (SNP) in an intron as well as different splice pattern between exons 9 and 11 in the 24-OHase gene was detected [22]. An intron-containing variant could reduce the expression of normal 24OHase enzymes as ncRNA with a regulatory effect. The intron 2 containing splice variant described by Ren et al. was detected in macrophages [19], which encodes for a non-functional 24OHase enzyme. The expression of further intron-containing ncRNA does not appear unlikely. The expression in macrophages of the inflammatory infiltrate of carcinoma would also explain why the reduced protein expression is limited to malignant mammary tissue.

The study group cloned a 2.5kB cDNA splice variant which was 200 bp shorter than the wild type and corresponded to a 36kDa-band. We found additional bands in our malign tissues that measure between 56 and 37 kDa. The bands were detected constantly, an indication for further splice variants. Splice variant peptide and wild-type may be in direct competition with each other for substrate binding.

In some cancer tissue samples further bands were detected. They could comply with the splice variants cloned by Ren et al. [19]. Furthermore, compared to malignant tissue, an increase in protein level can be found in benign tissue. The increased expression in mRNA level in the benign tissue was even clearer. The tissue we examined must be considered to be very heterogeneous.

The results comply with the research results of Anderson et al. [23] regarding mRNA levels in tissue which showed a reduced expression in mammary carcinoma on the mRNA level compared to the benign breast tissue.

Townsend et al. [24] well able to show activity measurements, contrary to our data, that the RNA in malignant tissue as well as the expression of the protein was highly regulated. It seems that our data show the heterogeneity of cells in malign and benign breast tissues, assuming that these results might not be caused by breast cells but also other cells like fibrocytes.

A stronger degradation of calcitriol could support carcinogenesis. In malign cell lines on the RNA level, a sig-
A significant increase of 24-OHase was detected, which is consistent with our results and in accordance with the research of Albertson et al. [25], who declared 24-OHase to be a potential oncogene via DNA array and positional cloning analyses of genomic amplification.

The examination regarding autocrine or paracrine effects of 1,25(OH)₂D₃ turns out to be more and more interesting in connection with protective and growth reducing attributes of the hormone. It is unknown if breast tissue has a reduced synthetic 1α-OHase activity in comparison to other cells, which would explain the higher potential of malignant degeneration. Among women with a higher UV-radiation, lower activity of 1α-OHase is partially neutralized by a higher supply of vitamin D₃ [26]. Whether a decreased enzyme expression can be explained by a decreased serum level of vitamin D₃, or in addition, whether a change in activity of the enzymes is explained by variants, has to be examined in continuative studies.

Figure 2. — Expression of (A) protein and (B) 24-OHase mRNA in human breast tissue samples. The expression of 24-OHase mRNA was analyzed by quantitative real-time PCR. Data are presented as relative mRNA fold change of malign compared to healthy breast tissues samples and were calculated according to the calibrator normalized method and standardized to the endogenous control TBP. For detection of 24-OHase protein, 10 μg of total protein extracts were separated by SDS-PAGE, transferred onto nitrocellulose membranes and blotted with specific antibody against human 24-OHase.
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Prognostic significance of intratumoral vascular endothelial growth factor as a marker of tumour angiogenesis in epithelial ovarian cancer

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Summary

Objectives: The aim of this study was to examine the prognostic significance of vascular endothelial growth factor (VEGF) in epithelial ovarian cancer (EOC). Methods: Surgical specimens of 105 patients with primary EOC FIGO Stages 1 to 4, who underwent surgical staging, were investigated. Expression of VEGF was evaluated by immunohistochemical staining using related monoclonal antibodies. The correlation of this data with survival and established prognostic factors such as histological grade, FIGO stage and residual tumour status was evaluated. Multivariate analysis and correlation tests were performed. Results: The results of VEGF expression were correlated with clinicopathological variables and overall survival. No correlation between the VEGF expression and clinicopathological factors was identified. However, VEGF expression was found to be significantly correlated to survival, and a prognostic factor independent of the stage of disease and residual tumour status (p < 0.0001). Conclusion: High intratumoral VEGF expression, a marker of angiogenesis, appeared to be an independent prognostic factor for overall survival in women with EOC. Angiogenic evaluation of patients with EOC may play a role in predicting a subgroup of patients with aggressive disease. These patients could be the target of front-line molecular targeted therapy with anti-angiogenic agents.

Key words: VEGF; Angiogenesis; Ovarian carcinoma; Prognosis.

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death from a gynaecological malignancy. Despite advances in chemotherapy and screening methods the death rate has changed little over the past few decades [1].

Due to the insidious onset and the paucity of symptoms two-thirds of cases will present with advanced disease (FIGO Stage 3 and 4). Established prognostic markers of ovarian cancer are tumour stage, residual tumour status, histological tumour type and grade [2]. However, these do not appear to offer any information to the potential sensitivity of molecular targeted therapies.

The molecular biology underlying EOC remains poorly understood. Currently much attention has been focused on the pathophysiology of tumour growth and development in EOC to predict patients at increased risk of recurrence and/or metastasis.

It is well recognised that tumour angiogenesis is critical for tumour growth beyond 2 mm and is associated with prognostic significance in a variety of solid malignancies such as breast, prostate, gastric and non-small cell lung cancer [3]. However, results from studies in EOC are conflicting and the clinicopathological significance has been debated [4-7]. Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein expressed in a wide variety of normal and transformed cell types [8]. VEGF is a key angiogenic factor and acts as a highly specific mitogen promoting endothelial cell migration and inhibiting apoptosis [9]. Studies have suggested a specific role for VEGF in various phases of ovarian carcinogenesis with effects on tumour growth and neovascularisation illustrated in animal models and in humans [10, 11]. Currently, interest has focused on the use of anti-angiogenic therapy in an attempt to inhibit the pro-tumour effects. Investigation of novel prognostic markers offers an insight into the mechanisms of tumour development and suggests potential avenues for the development of new therapeutic targeted therapies, through the use of monoclonal antibody therapies.

The future role of these therapies in cancer has yet to be established; a promising therapeutic strategy in solid tumours would be to target angiogenic factors such as VEGF-A [12-14].

Our study aims to determine intra-tumoral VEGF status in patients with EOC, and to investigate its relation to prognosis. The elucidation of the prognostic role of VEGF in patients with EOC may enable us to stratify patients with EOC into groups based upon the expression of VEGF, and to tailor treatment schedules, developing novel therapies to inhibit angiogenesis. These therapies are more likely to be most effective in tumours expressing high levels of VEGF.
Materials and Methods

Patients

VEGF expression was studied in 105 primary EOC samples, in patients who underwent surgical staging and tumour debulking at the Royal Free NHS Trust, London, UK between 1995 and 2000. Information on tumour grade, stage, presence or absence of residual disease after staging, and age at diagnosis was extracted from the case notes. Only patients considered to have primary EOC were included in this study. Survival was calculated from the operation date until death or December 2005 when any survivors were censored.

Tissue samples

Specimens were retrieved from formalin-fixed paraffin embedded tissue. Five micrometer sections were cut from the paraffin block. The paraffin blocks from each tumour were histologically confirmed by a single pathologist and were chosen after reviewing the haematoxylin and eosin slides of respective tumour by two observers including a pathologist.

Immunostaining with VEGF-A

Five micrometer sections were cut, and deparaffinised through graded alcohols. Immunohistochemical staining was carried out using commercially available monoclonal antibody to VEGF-A (R &D Systems, Abingdon UK; working dilution 1:40). Placental tissue was used as a positive control. For negative controls, the primary antibody step was omitted, and IgG2B was added to test for non-specific background staining. Sections were stained on three separate occasions to ensure reproducibility.

Scoring of cases

For the assessment of VEGF, areas with available tumour were inspected by two independent observers and the percentage of positive cells was recorded as number of fields at a magnification of x200. The presence of VEGF was quantified by counting the number of positive (brown) focal areas representing VEGF per cm² of tissue section and scoring them accordingly. Focal areas of positive tumour cells were counted in three separate fields by two observers who were blinded to all clinical data. In all cases there was less than 5% variation in results between sections and observers. The mean value was used as the final VEGF score for each case. A cut-off point was determined by using the median VEGF score of all cases [15].

Statistical analysis

The correlation between VEGF-A levels and other prognostic variables was statistically analysed by means of Pearson’s chi square test, Spearman rank correlation analysis and univariable analysis. Survival rates were examined using Kaplan-Meier plots of analysis. The statistical significance of differences between the survival rates of groups with different VEGF expression, i.e., high or low expression, was assessed by the log-rank test. The independent prognostic significance of variables was assessed by multivariate analysis, by means of a multivariable Cox regression model; p values of < 0.05 were considered statistically significant. All statistical analysis was carried out using SPSS version 15.0.

Results

Clinicopathological characteristics

The mean age of the patients at diagnosis was 58 years (range 22-82 years). Serous cystadenocarcinoma was the commonest histological subtype (55%) followed by undifferentiated (10%), endometrioid (11%), mucinous (14%), and clear cell (10%). All patients were treated surgically in the first instance. Of these 70% had their tumour optimally debulked (< 2 cm residual disease). Clinicopathological staging showed that the majority of patients had advanced stage disease (66% and 18% for Stage 3 and 4, respectively) whereas 16% had early-stage disease (10% and 6% for Stage 2 and 1, respectively). Seventy percent of the tumours were poorly differentiated (grade 3), 22% were moderately differentiated (grade 2), and 8% were well differentiated (grade 1). The results are summarised in Table 1.

Table 1. — Patient characteristics.

<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Number (n = 105)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58</td>
</tr>
<tr>
<td>Mean age</td>
<td>58</td>
</tr>
<tr>
<td>Range</td>
<td>22-82</td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
</tr>
<tr>
<td>Stage 1 (n = 6)</td>
<td>48</td>
</tr>
<tr>
<td>Stage 2 (n = 10)</td>
<td>57</td>
</tr>
<tr>
<td>Stage 3 (n = 70)</td>
<td>39</td>
</tr>
<tr>
<td>Stage 4 (n = 19)</td>
<td>32</td>
</tr>
<tr>
<td>Tumour grade</td>
<td></td>
</tr>
<tr>
<td>Grade 1 (n = 8)</td>
<td>47</td>
</tr>
<tr>
<td>Grade 2 (n = 23)</td>
<td>34</td>
</tr>
<tr>
<td>Grade 3 (n = 74)</td>
<td>59</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
</tr>
<tr>
<td>Serous (n = 58)</td>
<td>37</td>
</tr>
<tr>
<td>Endometrioid (n = 12)</td>
<td>69</td>
</tr>
<tr>
<td>Clear cell (n = 11)</td>
<td>41</td>
</tr>
<tr>
<td>Mucinous (n = 8)</td>
<td>55</td>
</tr>
<tr>
<td>Undifferentiated (n = 33)</td>
<td>33</td>
</tr>
<tr>
<td>Level of debulking</td>
<td></td>
</tr>
<tr>
<td>Residual tumour &gt; 2 cm</td>
<td>31 (30%)</td>
</tr>
<tr>
<td>Optimal debulking &lt; 2 cm</td>
<td>74 (70%)</td>
</tr>
</tbody>
</table>

Table 2. — Association of the degree of immunostaining for VEGF and clinicopathological variables.

<table>
<thead>
<tr>
<th>FIGO Stage</th>
<th>Low VEGF expression (%)</th>
<th>High VEGF expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 (n = 6)</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Stage 2 (n = 10)</td>
<td>57</td>
<td>43</td>
</tr>
<tr>
<td>Stage 3 (n = 70)</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>Tumour grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1 (n = 8)</td>
<td>47</td>
<td>53</td>
</tr>
<tr>
<td>Grade 2 (n = 23)</td>
<td>34</td>
<td>76</td>
</tr>
<tr>
<td>Grade 3 (n = 74)</td>
<td>59</td>
<td>41</td>
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<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous (n = 58)</td>
<td>37</td>
<td>63</td>
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<tr>
<td>Endometrioid (n = 12)</td>
<td>69</td>
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<tr>
<td>Clear cell (n = 11)</td>
<td>41</td>
<td>59</td>
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<tr>
<td>Mucinous (n = 8)</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>Undifferentiated (n = 33)</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Level of debulking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 cm (n = 74)</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>&gt; 2 cm (n = 31)</td>
<td>44</td>
<td>56</td>
</tr>
</tbody>
</table>

p > 0.05 for all above subgroup comparisons.

VEGF analysis

Patients were divided into two groups based on intratumoural VEGF expression, and the median value of the VEGF score, which was 3, was taken as the cut-off point. Therefore the groups were:

1) High VEGF expression; score of 3 or > 3.
2) Low VEGF expression score of < 3.
No relationship was observed between intratumoural VEGF levels to the age of the patient or to the postoperative residual tumour, the histological grade, the histological subtype or FIGO stage of the tumour. The results are summarised in Table 2.

Survival analysis revealed that patients who had low intratumoural VEGF expression had a median survival time of 36.7 months (SD 21.7) whereas the median survival of patients with a high VEGF expression was 10.8 months (SD 7.8). This difference in survival was shown to be statistically significant by the Cox proportional hazards model after tumour stage was accounted for ($p < 0.001$). A Kaplan Meier survival plot is shown in Figure 1.

Discussion

The central concept that tumour growth is angiogenesis dependent is well accepted today. Every increment of tumour growth requires an increment of vascular growth [3, 16, 17].

Angiogenesis has been suggested as a prognostic factor in various solid tumours but its clinicopathological significance in ovarian cancer and its exact role is still unclear. The results of various studies are contradictory. Hollingsworth et al. reported that microvessel counts correlated with progression-free and overall survival in advanced ovarian cancer [18]. Univariate analyses showed that high microvessel density (MVD) counts confer a worse prognosis for overall/progression-free survival whereas a low MVD was correlated with better survival. This however failed to show significance in multivariate analysis [19, 20].

Our results indicate that patients with tumours that express high levels of VEGF have a worse overall survival rate compared to those with low VEGF or no VEGF expression. A median survival advantage of approximately 25 months is seen with patients who have low VEGF expression ($p < 0.001$). This result was further substantiated when a multivariate Cox regression model was constructed in which established prognostic factors were accounted for. VEGF expression maintained statistical significance with regards to patient survival ($p < 0.001$). These results exhibit that immunohistochemical assessment of VEGF expression within a tumour can display further information about the potential for antiangiogenic activity, and its effect on tumour behaviour and subsequent prognosis for the patient. The literature to date has been unclear as to the role of VEGF expression within ovarian tumours. Some studies have shown that it is a significant independent prognostic factor. Paley et al. found that patients with early-stage disease (FIGO Stage 1 and 2) showed poorer prognosis with increased VEGF expression within the tumour [21]. Shen et al. showed that survival of patients with high VEGF expression was significantly worse than those with low VEGF expression, and in multivariate analysis VEGF expression together with stage was an independent prognostic indicator for overall survival [22]. Interestingly, there was no correlation with microvessel density contradicting previous work. Raspollini et al. showed that VEGF and MVD were both independent predictors of survival in advanced disease (FIGO Stages 3 and 4), and also correlated with the likelihood of response to chemotherapy [23]. In contrast, some authors showed no independent relationship with prognosis [24, 25].

In our study we showed that the prognostic effects of VEGF, which are seen in all disease stages, are independent of established clinicopathological variables, such as stage, grade and residual tumour status.

From our work we have illustrated that there were no associations between VEGF and any of the clinicopathological variables, including stage and grade of the tumour. This is in agreement with other published work [8, 18, 24, 26, 27]. Some studies have suggested that stage and grade are associated with VEGF expression, although these studies had a large proportion of early-stage disease [22]. Heterogeneous expression of VEGF, difficulties in maintaining a standardised scoring system for the quantitative assessment of VEGF, and small sample size, may be the factors accounting for the inconsistent results among the studies thus far [28].

Due to the central role of angiogenesis in cancer progression, inhibition of tumour angiogenesis could be a therapeutic tool to arrest tumour progression. Bevacizumab, a monoclonal antibody against VEGF-A, is the first anti-angiogenic drug in oncology, having been approved by the FDA for that purpose. Previous studies have shown that in tumours VEGF is over-expressed in solid tumours including breast and colorectal cancer. In EOC over-expression is associated with ascites formation, malignant progression and poor prognosis. Preclinical models of solid tumours show that anti-VEGF therapy causes slowing of tumour progression, resolution of malignant effusions, and synergy with cytotoxic agents [29, 30]. Randomised trials of bevacizumab have established its efficacy in colon, breast, lung and renal cancer [30]. Monk et al. have shown some clinical benefit from using bevacizumab in recurrent ovarian cancer [31]. There are currently ongoing trials with bevacizumab, VEGF Trap (VEGF receptor decoy), and other agents tar-
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References


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Getting the VEGF pathway [32]. The GOG 218 and ICON 7 trials are ongoing at present and are studying the addition of bevacizumab to front line chemotherapy.

Further evaluation and research is required in future studies to substantiate the finding that intratumoral expression of VEGF in patients may be of clinical relevance, and may play a role in predicting a subgroup of patients who are amenable to anti-angiogenic targeted therapy. This may help to stratify patients into those whom would benefit from first-line anti-angiogenic therapy, in addition to standard chemotherapy.

In conclusion the expression of VEGF-A is an independent prognostic indicator in our series of patients, with all stages of ovarian cancer. The angiogenic evaluation of the tumour, by means of VEGF expression, may help in identifying a group of patients in which anti-angiogenic therapy is more effective, and could be used as a first-line agent, either alone or in combination with standard platinum-based chemotherapy.
Expression and significance of β-catenin, Glut-1 and PTEN in proliferative endometrium, endometrial intraepithelial neoplasia and endometrioid adenocarcinoma

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Summary

Objective: The aim of this study was to explore the potentiality of β-catenin, Glut-1 and PTEN proteins as markers for a diagnosis of endometrial intraepithelial neoplasia (EIN). Design: Ten proliferative endometrium, 83 endometrial hyperplasia (59 benign hyperplasia, 24 EIN) and 24 endometrioid adenocarcinoma sections were immunostained for β-catenin, Glut-1 and PTEN protein expression. Results: (1) Abnormal expression of β-catenin was detected in 10% of benign hyperplasia, 50% of EIN and 67% of endometrioid adenocarcinoma. (2) Overexpression of Glut-1 was present in 58% of EIN and 71% of endometrioid adenocarcinoma. (3) Complete loss of PTEN immunoreactivity was found in 20% of proliferative endometrium, 29% of benign hyperplasia, 38% of EIN and 63% of endometrioid adenocarcinoma. Conclusions: The abnormal expression of β-catenin and overexpression of Glut-1 may be useful markers in distinguishing benign hyperplasia from EIN, whereas lack of PTEN expression is not an appropriate marker for a diagnosis of EIN.

Key words: β-catenin; Glut-1; PTEN; Benign endometrial hyperplasia; EIN; Endometrioid adenocarcinoma.

Introduction

Endometrial hyperplasia is well accepted as the precursor of endometrial carcinoma, which represents the most common invasive gynecological malignancy in the world. Since the World Health Organization (WHO) endometrial hyperplasia scheme has poor reproducibility and lacks clinical predictive value, Mutter and colleagues have proposed EIN as the precursor of endometrioid adenocarcinoma [1-5]. It has been reported that this new concept has significant correlations with the risk of developing endometrial carcinoma [2, 5, 6].

Carcinogenesis is usually accompanied with abnormal expression of signaling molecules. Aberrant expression of β-catenin is found in many cancers including endometrioid adenocarcinoma and it occurs in a relatively early stage in cancer development [7, 8]. Glut-1 protein is absent in most types of normal epithelial cells, while overexpression of Glut-1 is associated with a wide spectrum of human malignancies [9,10]. PTEN protein is a tumor suppressor that is inactivated in a wide variety of human cancers. Mutation of the PTEN tumor suppressor gene has been found in 55% of EIN and 83% of endometrioid adenocarcinomas [11]. Our current study is to examine the expression of β-catenin, Glut-1 and PTEN in proliferative endometrium, EIN and endometrioid adenocarcinoma, and to explore the possibility of these proteins as EIN diagnostic markers.

Materials and Methods

Tissue samples. A hundred and seventeen endometrial tissue specimens were collected from archives (from 2001 to 2006) of the Department of Pathology, Zhongnan Hospital of Wuhan University, including ten proliferative endometrium, 83 endometrial hyperplasia and 24 endometrioid adenocarcinoma samples. According to the EIN diagnostic and classification criteria [11-13], 24 of 83 endometrial hyperplasias were histopathologically reclassified as EIN and 59 cases as benign hyperplasia (Figure 1A, 1B).

Immunohistochemistry. Immunohistochemical staining was performed by the streptavidin-peroxidase method according to the manufacturer’s instructions. The primary monoclonal antibodies (against β-catenin and PTEN) and the polyclonal antibody (against Glut-1) were purchased from Maixin-Bio, China. The samples of normal colonic mucosa, perineurium of peripheral nerve and endometrial stroma cells were used as positive controls for β-catenin, Glut-1, PTEN expression, respectively. Sections incubated with PBS, instead of the corresponding primary antibodies, were used as negative controls. The average ratio of positive cells was assessed under microscope (x400).

Evaluation of immunohistochemical staining. β-catenin expression was divided into two groups according to the immunoreactivity: (1) Normal membranous expression, the staining reaction of > 90% cells was localized on the cell membrane. (2) Abnormal expression, including cytosolic expression (30-90% of cells showed a cytoplasmic staining), and/or nuclear expression (nuclear staining was seen in more than 20% of cells), or negative [7, 8]. Glut-1 positive staining showed linear membranous staining, particularly at cell-cell borders. Glut-1 expression was graded into four groups according to the percentage of the positively stained cells: (1) -, negative; (2) 1+, rare if estimated 1-5% of detected cells were positive; (3) 2+, focal if confluent foci of positive cells were separated by a significant nonstained area; (4) 3+, diffuse if all the cells were positive. The - and 1+ were categorized as low expression and 2+ and 3+ were scored as overexpression [9, 10]. Brown staining in the cytoplasm was accepted as PTEN immunoreactivity. PTEN expression was classified as three grades: (1) -, negative (expression complete loss), if none were stained positive; (2) 1+, partial loss, if < 50% were stained positive; (3) 2+, expression present, if > 50% were stained positive [14, 15].

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Expression and significance of β-catenin, Glut-1 and PTEN in proliferative endometrium, endometrial intraepithelial neoplasia etc.

Figure 1. — Morphology of EIN lesions. (A) Comparison of EIN and normal proliferative endometrium. The black line is an interface between an EIN lesion and the proliferative background (hematoxylin-eosin x 40). (B) Crowded glands displaying middle cytopathic atypia. The black arrows indicate the residual normal glands (hematoxylin-eosin x 100).

Figure 2. — Expression of β-catenin (x 200). (A) β-catenin expression on the cell membrane of normal proliferative endometrium. (B) Cytoplasmic and nuclear expression of β-catenin in an EIN lesion.

Figure 3. — Overexpression of Glut-1 in (A) an EIN lesion and (B) endometrioid adenocarcinoma (x 200). The black arrow in (A) indicates undetectable Glut-1 expression in the residual normal gland.
Statistical analysis. SPSS 11.0 statistics program was used for data analysis with the chi-square test and \( p < 0.05 \) was accepted as statistically significant.

Results

Normal membranous expression of \( \beta \)-catenin, shown in Figure 2A, was present in all ten proliferative endometrium tissues (Table 1). The abnormal expressions of \( \beta \)-catenin, shown in Figure 2B, were present in six of 59 (10%) benign hyperplasias, 12 of 24 (50%) EINs and 16 of 24 (67%) endometrioid adenocarcinomas, respectively (Table 1). The frequency of abnormal \( \beta \)-catenin expression in EIN and endometrioid adenocarcinoma were much higher than that in benign hyperplasia \( (p < 0.01) \), but the difference was not statistically significant between EIN and endometrioid adenocarcinoma.

Glut-1 expression was undetectable in the ten proliferative endometrium samples. Benign hyperplasia exhibited a low level of expression. High level of Glut-1 protein was present in 14 of 24 (58%) EINs (Figure 3A) and 17 of 24 (71%) endometrioid adenocarcinomas (Figure 3B), but the difference was not significant between EIN and endometrioid adenocarcinoma.

Complete loss of PTEN immunoreactivity was found in two of ten (20%) proliferative endometrium samples, 17 of 59 (29%) benign hyperplasias, nine of 24 (38%) EINs (Figure 4A) and 15 of 24 (63%) endometrioid adenocarcinomas. Partial loss of PTEN expression was observed in six of 59 (10%) benign hyperplasias, six of 24 (25%) EINs and five of 24 (21%) endometrioid adenocarcinomas (Figure 4B). The frequency of complete loss of PTEN expression in endometrioid adenocarcinoma was significantly higher than that in proliferative endometrium and benign hyperplasia \( (p < 0.05) \). However, there was no significant difference regarding the complete loss of PTEN expression between EIN and other categories examined (Table 2).

Discussion

Based on the WHO classification criteria, namely the glandular complexity and nuclear atypicality, endometrial hyperplasia has been classified into four categories: simple, complex, simple atypical and complex atypical hyperplasia \([1, 2]\). The overall risk of progression of hyperplasia to cancer is 5-10%, but different types have different progression risk \([13]\). Complex atypical hyperplasia is the most significantly correlated with endometrioid adenocarcinoma. Although this classification system has the benefit of unifying the terminology, it shows poor reproducibility and limited information for appropriate therapeutic options \([3, 4, 13, 16]\). The new concept of EIN is based on the genetics of endometrial precancers.
together with morphometrical and clinical studies [6], according to which, endometrial hyperplasia is categorized into benign hyperplasia and EIN [2, 12, 13]. Benign hyperplasia is caused by an abnormal hormonal state, while EIN is monoclonal hyperplasia at the beginning and becomes a precancerous lesion at advanced stage [13]. The EIN classification has been reported to be more objective, reproducible, clinically relevant and precise in predicting the progression of hyperplasia to cancer [6], thus the identification of diagnostic markers of EIN will be informative for clinical trials.

β-catenin normally expresses on the cell membrane, while aberrant expression of β-catenin has been found in the cytoplasm and/or nucleus [7, 8]. Aberrant expression of β-catenin is associated with many cancers and their precursors including endometrioid adenocarcinoma [8, 17-19]. In our study, the occurrence of abnormal β-catenin expression in EIN (50%) and endometrioid adenocarcinoma (67%) was significantly higher than that in benign hyperplasia (10%) (p < 0.01). This result proves that the aberrant expression of β-catenin is an early event and suggests that it plays an important role in carcinogenesis of endometrioid adenocarcinoma.

The expression of Glut-1 is absent in most types of normal epithelial cells and the overexpression is commonly found in a wide spectrum of human malignancies, such as colon/esophagus/thyroid/lung/ovary/breast cancers [9,10]. Furthermore, investigators believe that expression of Glut-1 is a potential marker for malignant transformation [20]. Since EIN is a precursor of endometrioid adenocarcinoma, representing approximately 80% of sporadic endometrial cancer, we compared the expression of Glut-1 in proliferative endometrium, benign hyperplasia, EIN and endometrioid adenocarcinoma. We demonstrated that detection of Glut-1 in proliferative endometrium and benign hyperplasia was negative or low, whereas Glut-1 in EIN and endometrioid adenocarcinoma was overexpressed (Table 2). This expression pattern of Glut-1 is in agreement with data in the literature [9, 10, 21]. Our findings indicate that Glut-1 overexpression may be an early molecular event and play an important role in tumorigenesis of endometrioid adenocarcinoma, and that Glut-1 may be a useful marker in distinguishing benign hyperplasia from EIN and endometrioid adenocarcinoma.

Mutations of PTEN, reflected in the loss of PTEN protein expression, has been found in 55% of EINs and 83% of endometrioid adenocarcinomas [11]. It has been proposed that altered PTEN expression may be a diagnostic marker for the earliest endometrial precancers [11]. However, there have been conflicting reports describing either no difference among the cases of EIN, endometrial carcinoma and proliferative endometrium regarding PTEN expression [15], or normal PTEN expression in EIN whereas normal proliferative, anovulatory endometrium containing PTEN-null glands [16]. In the present study, we showed that there was no difference in complete and partial loss of PTEN expression between EIN and proliferative endometrium, or benign hyperplasia, or endometrioid adenocarcinoma (Table 2). These results suggest that PTEN is a relatively insensitive and nonspecific marker for EIN diagnosis and its routine clinical value is limited. Lack of PTEN protein expression may be an early event in endometrial carcinogenesis, but it is not an appropriate diagnostic marker for EIN.

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References


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Occurrence of malignancy in endometrial polyps during postmenopause

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Summary

Objective: To evaluate the occurrence of endometrial polyp malignancy in pre- and postmenopausal women with or without symptoms. Materials and Methods: A retrospective study was performed on 351 patients with endometrial polyps diagnosed by hysteroscopy. Results: Histological findings of biopsies obtained by operative hysteroscopy confirmed the presence of a simple endometrial polyp in 179 cases, polyps with typical simple hyperplasia in 42 cases, polyps with typical complex hyperplasia in 24 cases, polyps with atypical complex hyperplasia in three cases; carcinomatous polyps in seven cases; atrophic polyps in 17 cases; functional polyps in 56 cases; and inadequate sample in 23 cases. All seven patients with adenocarcinoma were symptomatic; six out of seven patients with adenocarcinoma were in postmenopause and one was in premenopause. The association between menopausal status and symptoms, and the presence of a malignant lesion was statistically significant (p < 0.001). Conclusions: This study revealed that prevalence of endometrial polyp malignant transformation was ≤ 2.84% in postmenopausal and symptomatic patients.

Key words: Endometrial polyps; Postmenopause; Adenocarcinoma.

Introduction

Endometrial polyp is a sessile or pedunculated lesion (single in 80% of the cases and multiple in 20% of the cases), originating from the endometrium, and it may possibly include glands, stroma and blood vessels. Microscopically, a polyp is a proliferative endometrial proliferative lesion, red/yellowish in colour, and showing either a compact or a jelly-like appearance, translucent with haemorrhagic and/or necrotic areas. Its prevalence has been estimated to be approximately around 25% [1-5].

Polyps histologically include a hyperplastic, atrophic and functional pattern. An endometrial polyp can be seen as a pre-neoplastic lesion showing cellular atypia associated with simple or complex hyperplasia. A polyp can be considered as the beginning of a malignant tumour when two main criteria are accomplished: 1) absence of pathological findings among the cells of the polyp base and 2) absence of malignant cells in the surrounding tissue [5, 6]. Clinical outcome usually includes abnormal uterine bleeding and infertility [7, 8]. The gold standard in the diagnosis of endometrial polyps is hysteroscopy [8-11]; transvaginal ultrasound (TVS) and sonohysterography can be considered as accurate diagnostic tools [9, 12-19]. The risk of malignant change has been evaluated to be between 0.5% and 4.8% [1, 20-24].

The aim of this study was to assess the risk of malignant changes in endometrial polyps, the relationship between pre- and postmenopausal status, and the symptoms.

Materials and Methods

Three hundred and fifty-one patients underwent surgical hysteroscopy from January 2003 to May 2008. A preoperative diagnosis of endometrial polyps was made by either ultrasound (US) and/or hysteroscopy and/or sonohysterography. Patient age ranged between 21 and 86 years (medium age 52).

One hundred ninety-nine patients had regular menses, whereas 152 patients had been amenorrhoeic for at least 12 months, even with FSH > 40 mIU/ml. TVS was performed using a Hitachi H21 scanner until 2005, then a Voluson 730 expert scanner, with an endovaginal 7.5 MHz probe. Patients showing no US findings were submitted to sonohysterography and/or diagnostic hysteroscopy. On B-mode TVS the presence of an endometrial polyp was suggested when a focal echogenic area was identified within the endometrium. Color-Doppler TVS was considered positive for a polyp finding when a single arterial vessel penetrating the endometrium from the myometrium was identified. We also identified pedunculated patterns, stated as anterior, posterior, or lateral according to the relationship with the uterine wall [11].

The sonohysterography technique was performed through insertion of a speculum in the vagina, disinfection of the cervix and placement of a catheter through the cervical canal using Collins forceps when necessary. After removing the speculum, a vaginal US probe was placed and at the same time sterile saline solution was inserted through a 20 cc syringe connected to the catheter. The advantages of using a liquid solution were: 1) uterine cavity dilatation; 2) optimising the study of the wall profile and the uterine cavity content.

Hysteroscopy was performed using a rigid optical fibre tool (Karl Storz Endoscope).

The uterine cavity was dilated with carbon dioxide. Hysteroscopy was performed under spinal anaesthesia, using a Storz resectoscope with a continuous flow system with a pressurised solution of mannitol-sorbitol for distension of the uterine cavity. Biopsies were sent for histological evaluation. All patients underwent endometrial biopsy during operative hysteroscopy.

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Histological findings were classified as endometrial polyps, polyps with simple hyperplasia without atypia, polyps with complex hyperplasia without atypia, polyps with simple hyperplasia and atypia, polyps with complex hyperplasia and atypia or endometrial carcinoma.

Statistical analysis was performed by bivariate analysis to study possible statistically significant differences between the two groups of women in pre- or postmenopause and the presence or absence of symptoms. We used the chi-square test for comparison between the examined and expected frequencies for neoplasia development. In order to analyse the association between a polyp and malignant lesion we used a logistic regression analysis. The quantity of the relationship of the variables between a polyp and malignant lesion we used a logistic regression analysis. The quantity of the relationship of the variables has been reported as odds ratio (OR), and values lower than 1 indicate an increased risk of developing a malignant lesion in postmenopause rather than in premenopause; values lower than 1 indicate a lower risk of developing a malignant lesion in postmenopause rather than in premenopause.

Results

One hundred and seventy-nine out of 351 patients submitted to operative hysteroscopy had a simple endometrial polyp, 66 an endometrial polyp with typical simple or complex hyperplasia, three an endometrial polyp with atypical complex hyperplasia, 56 a functional endometrial polyp, 17 an atrophic endometrial polyp, seven an endometrial polyp with adenocarcinoma, and 23 an inadequate sample. All the results had histological confirmation of the surgical diagnosis (Table 1).

Table 2 shows histological results for the pre- and postmenopausal groups.

Table 1. — Histological diagnosis of biopsies.

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial polyp</td>
<td>179 (50.99%)</td>
</tr>
<tr>
<td>Endometrial polyps with typical simple and complex hyperplasia</td>
<td>66 (18.82%)</td>
</tr>
<tr>
<td>Endometrial polyps with atypical complex hyperplasia</td>
<td>3 (0.85%)</td>
</tr>
<tr>
<td>Endometrial polyps with proliferative endometrium</td>
<td>50 (14.24%)</td>
</tr>
<tr>
<td>Endometrial polyps with atrophic endometrium</td>
<td>17 (4.85%)</td>
</tr>
<tr>
<td>Endometrial polyps with adenocarcinoma</td>
<td>7 (1.99%)</td>
</tr>
<tr>
<td>Inadequate sample</td>
<td>23 (6.55%)</td>
</tr>
<tr>
<td>Total</td>
<td>351 (100%)</td>
</tr>
</tbody>
</table>

Table 2. — Correlation between histological diagnosis and symptomatic and asymptomatic patients.

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Symptomatic No. (%)</th>
<th>Non-symptomatic No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial polyp</td>
<td>77 (42.8%)</td>
<td>102 (57.2%)</td>
<td>179</td>
</tr>
<tr>
<td>Endometrial polyps with typical simple and complex hyperplasia</td>
<td>46 (69.8%)</td>
<td>20 (30.2%)</td>
<td>66</td>
</tr>
<tr>
<td>Endometrial polyps with atypical complex hyperplasia</td>
<td>3 (100%)</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Endometrial polyps with proliferative endometrium</td>
<td>39 (77.1%)</td>
<td>11 (22.9%)</td>
<td>50</td>
</tr>
<tr>
<td>Endometrial polyps with adenocarcinoma</td>
<td>7 (100%)</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Endometrial polyps with atrophic endometrium</td>
<td>12 (72.7%)</td>
<td>5 (27.3%)</td>
<td>17</td>
</tr>
<tr>
<td>Inadequate sample</td>
<td>19 (81.8%)</td>
<td>4 (18.2%)</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 3. — Correlation between histological diagnosis and pre- and postmenopausal patients.

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Premenopausal No. (%)</th>
<th>Postmenopausal No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial polyp</td>
<td>73 (40.78%)</td>
<td>106 (59.22%)</td>
</tr>
<tr>
<td>Endometrial polyps with typical simple and complex hyperplasia</td>
<td>57 (86.36%)</td>
<td>9 (13.64%)</td>
</tr>
<tr>
<td>Endometrial polyps with atypical complex hyperplasia</td>
<td>3 (100%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Endometrial polyps with proliferative endometrium</td>
<td>50 (100%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Endometrial polyps with adenocarcinoma</td>
<td>1 (14.28%)</td>
<td>6 (85.72%)</td>
</tr>
<tr>
<td>Endometrial polyps with atrophic endometrium</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Inadequate sample</td>
<td>12 (52.17%)</td>
<td>11 (47.83%)</td>
</tr>
</tbody>
</table>

Table 4. — Malignant lesion in pre- and postmenopause and symptomatic and asymptomatic patients.

<table>
<thead>
<tr>
<th>No.</th>
<th>Malignant lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>199</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>126</td>
<td>1 (0.8%)</td>
</tr>
<tr>
<td>73</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>152</td>
<td>9 (10.8%)</td>
</tr>
<tr>
<td>83</td>
<td>9 (10.8%)</td>
</tr>
<tr>
<td>69</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Seventy-seven out of 179 patients with endometrial polyps were symptomatic, and 102 were asymptomatic. Forty-six out of 66 patients with typical hyperplasia were symptomatic and 20 were asymptomatic. Three patients with atypical hyperplasia were symptomatic. Six patients with secretive endometrium were symptomatic. Thirty-nine out of 50 patients with proliferative endometrium were symptomatic and 11 were asymptomatic. Seven patients with adenocarcinoma were symptomatic. Twelve out of 17 patients with atrophic endometrium were symptomatic and five were asymptomatic. Nineteen out of 23 patients with inadequate sample were symptomatic and four were asymptomatic. The difference between the two groups was significant ($p < 0.01$) Table 2.

Table 3 shows different histological diagnoses between pre- and postmenopausal patients.

Seventy-three out of 202 premenopausal patients had a simple endometrial polyp, 57 had an endometrial polyp with typical, simple or complex hyperplasia, three an endometrial polyp with atypical complex hyperplasia, 56 a functional endometrial polyp, one an endometrial polyp with adenocarcinoma, and 12 an inadequate sample.

One hundred and six out of 149 postmenopausal patients had a simple endometrial polyp, nine out of 149 an endometrial polyp with typical simple or complex hyperplasia, three an endometrial polyp with atypical complex hyperplasia, 56 a functional endometrial polyp, one an endometrial polyp with adenocarcinoma, and 12 an inadequate sample. The statistical difference between the two groups of pre- and postmenopausal patients ($p < 0.05$) are shown in Table 3. Table 4 shows an association between malignant lesions in pre- and postmenopause and the presence or absence of symptoms.
One case resulted positive in symptomatic/premenopausal patients (0.8%) and nine cases in the symptomatic/postmenopausal group, that is 10.8% (25.25; p < 0.001). No malignant lesion was detected in either pre- or postmenopausal asymptomatic patients.

Discussion

The possibility that an endometrial polyp is associated with cancer or is considered as a neoplastic lesion itself is extremely low (0.5% to 4.8%) [1].

The controversies in the rate of carcinoma in endometrial polyps may be related to the experience of the sonographer, to the characteristics of the population selected in the different studies, or to the presence of other risk factors such as hypertension, weight, HRT or tamoxifen [18]. In our study, the rate of neoplastic lesions was 2.84%.

The low prevalence of malignant degeneration of endometrial polyps makes the research of risk factors or predictive factors able to identify new cases needing a surgical approach very challenging.

In this study risk factors were considered only for patient age, menopausal status, and the presence of abnormal uterine bleeding. Age seemed to be a relevant risk factor.

Malignancy was found in women over 45, increasing over age 60. A 43-year-old woman was the only single case. According to Hileto et al. [24], our findings suggest a significant relationship between malignant or premalignant lesions, and symptoms, either in postmenopause (AUB) or in premenopause (metrorrhagia and/or spotting), (p < 0.01). Other authors revealed that the presence of abnormal uterine bleeding, either in menopause or in perimenopause, was not found to be a risk factor that relates to malignant changes [1, 18, 23]. On the other hand, endometrial polyps showing a carcinomatous pattern were found only in symptomatic women [8, 25].

Diverse authors have considered malignant changes in endometrial polyps, Ben-Arie et al. [18], in a retrospective study including 430 cases, found adenocarcinoma confined in a polyp in 3.0% of cases. Orvieto et al. [22] reported adenocarcinoma in one out of 146 endometrial polyps examined. Bakour et al. [20] found a percentage of 3.2% of carcinomas confined in a polyp, taking into consideration women with abnormal uterine bleeding. Savelli et al. [1] found four adenocarcinomas (0.8%) in 509 cases. Antunes et al. in a retrospective study performed on 475 women who had an endometrial polyp diagnosis, revealed 13 adenocarcinomas (2.74%), considering age as one of the most considerable risk factors [20]. All polyps should be removed and sent for histological evaluation when a patient is menopausal and symptomatic; premenopausal and asymptomatic patients should undergo regular TVS checks. Patients showing a thickening of the endometrium revealed by TVS should undergo sonohysterography.

Conclusion

The prevalence of malignant changes in endometrial polyps was ≤ 2.84%. Abnormal uterine bleeding (menorrhagia, AUB) could be a sign of either malignancy or premalignancy; this is also supported by the results of our data, in which the symptoms resulted to be statistically significant (p < 0.01). The anamnestic data concerning age and premenopausal or postmenopausal status can be considered as risk factors for malignant changes. The association between menopausal status and symptoms and the presence of malignant changes in endometrial polyps were statistically significant (p < 0.001).

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The effects of genital *Schistosoma haematobium* on human papillomavirus and the development of cervical neoplasia after five years in a Zimbabwean population

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Summary

**Background**: High-risk human papillomavirus (HPV) is responsible for cervical cancer and genital Schistosoma haematobium infection has been hypothesized to be an additional co-factor or even an independent risk factor for cervical neoplasia. The present study aimed to investigate the impact of schistosomiasis on HPV persistence and development of cell atypia in a group of rural Zimbabwean women with confirmed high-risk HPV. **Methods**: A five-year follow-up was done among women previously included in a study on genital schistosomiasis. Women who had high-risk HPV at baseline were invited after 5 years for examination of cell atypia, genital schistosomiasis, and high-risk HPV. Both vaginal lavage samples (low-cost) and cervix brush samples (high-cost) were obtained for further analysis. **Results**: Thirty-seven women were re-examined. Genital *Schistosoma haematobium* of a minimum of five years' duration was associated with the development high-grade squamous intraepithelial neoplasia, but not with persistent high-risk HPV. There was a high concordance between the brush and vaginal lavage (96.3% agreement, kappa 0.93); however, the number of β-globin negative vaginal lavage samples was unacceptably high. **Conclusions**: Findings warrant an exploration in a larger longitudinal study where a vaginal swab should be explored.

Key words: Electrosurgery; LEEP; Vulvar intraepithelial neoplasia; VIN.

Introduction

Infection with human papillomavirus (HPV) is a widespread sexually transmitted infection, with at least 50% of sexually active adults in the world experiencing a genital HPV infection in their lifetime [1]. High-risk and probably-high-risk HPVs are likely the sole cause of cervical cancer although other agents such as HIV may influence development [2-4]. *Schistosoma (S.) haematobium* is a parasitic disease usually acquired in childhood and maintained by regular contact with fresh water in endemic areas. It is widely transmitted on the African continent and has been hypothesised to cause leukoplasia, papillomatous tumours and cancer of the genital tract [5-12]. Moreover, the clinical appearance of genital schistosomiasis may be mistaken for malignancy and may be accompanied by contact bleeding and a friable uneven mucosal surface [5, 10, 13]. Schistosomiasis may play a role in the development of genital malignancy directly or indirectly by increasing the susceptibility to HPV [14-16].

The continued presence and reinfection of HPV in women with genital schistosomiasis would indicate that the parasite plays a role in the development of cervical cancer. Here we report the findings from a 5-year follow-up where we aimed to explore a possible impact of schistosomiasis on HPV persistence and the development of cell atypia in a cohort of women with high-risk HPV. The group also provided the opportunity to compare two sampling procedures in rural Africa.

Methods

**Study group**

As reported previously the baseline study on HPV in 236 rural Zimbabwean women was nested in a study on genital schistosomiasis [13, 17]. The study was performed before anti-retroviral therapy and mass anti-schistosomal therapy had become available. The study was conducted in the rural North-western part of Zimbabwe in a subsistence farming area (Mupfure) where 557 women between the ages of 15 and 49 were included [13, 17]. Due to economic and logistic constraints baseline specimens were only analysed five years after for sexually transmitted diseases. After the Zimbabwean land reform we drove more than 13,000 km in order to find the 54 women who had high-risk HPV. At baseline women had been asked for surnames, maiden names, the name and address of a relative, and the name of their eldest child. They were searched for by the same staff as five years prior and clinical investigations were offered in the same clinic, by the same clinician [13].

**Sample taking and clinical definitions**

The baseline and the 5-year consultations and gynaecological investigations were done in the exact same ways, as has been
described previously [13]. Briefly, investigations were commenced by cervico-vaginal lavage. Saline (5 ml) was sprayed on the vaginal wall and cervix twice, whereupon it was drawn back into a syringe and deposited into cryotubes. Papanicolaou (Pap) smears were taken in all women. Biopsies were taken where necessary, as advised by the pathologist. Urine samples (not done in the 5-year survey), biopsies and Pap smears were examined for S. haematobium ova [18]. A single terminal-spined ovum gave a positive diagnosis. Leukoplakia was defined as white plaque on the mucosal surface, visible with or without acetic acid. Papilloma was defined as a sessile mass either on the mucosa or vulva, whitish in colour, often with a cauliflower appearance. Homogenous yellow sandy patches were sandy looking areas with no distinct grains using 15-times magnification [13, 19] whereas grainy sandy patches portrayed oblong (approx. 0.05 by 0.2 mm) grains situated in the mucosa. S. haematobium ova found in cytology (Pap smears) was the strongest predictor for the genital sandy patch types and grainy sandy patches were pathognomonic for S. haematobium in the genitals. In the 5-year survey a cervical sample was taken using a Cervex-brush® (Rovers, Oss, The Netherlands); blood specimens were not taken in this survey.

Sample preparation and HPV detection

The cervical lavage was centrifuged for 2 min at 12,000 rpm and the supernatant discarded. The pellet was resuspended in 500 μl TE (10 mM Tris, 1 mM EDTA, pH 8.0). This suspension was stored at −80°C for 1 hr; 100 μl of this suspension was taken, boiled for 10 min, and centrifuged for 5 min at 12,000 rpm. The Cervex brushes were transported and stored in 60% ethanol in 50 ml tubes until further use. After vigorous vortexing the samples were centrifuged, the supernatant discarded and the cell pellet resuspended in 1 ml ethanol and transferred to a cryotube. The brush samples were washed once with TE, resuspended in 150 μl TE with 0.2 μg/ml proteinase K, and incubated overnight at 37°C. Proteinase K was inactivated by boiling for 10 min.

The presence of DNA was confirmed by β-globin PCR using primers PC03/04 [20]. HPV detection was performed using the GP5+/6+ HPV PCR [21]. Detection of PCR products was performed in an enzyme immunoassay (EIA) format as described by Jacobs et al. [22]. After detection of HPV with a high-risk HPV probe cocktail, typing analysis was performed on all HPV positive samples for high-risk HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82. These probes represent all high-risk or probable high-risk types [3].

Human immunodeficiency virus detection

Due to the logistic constraints of analysing and giving adequate pre- and post-test counselling (transport problems, privacy issues) we chose not to re-test women for HIV in the 5-year survey. The last HIV results were three years old. Sera from all women had been tested for HIV with Recombigen (Organon Technika, Boxtel, The Netherlands); blood specimens were denied the permission by heads of their household or who came too late were given the opportunity to see a local gynaecologist at a time of their choice. All Pap smears were followed up as recommended by the pathologist. All applicable cases were offered full treatment and follow-up, including surgery where necessary, with an independent Zimbabwean gynaecologist.

Statistical analysis

Chi-square, Fisher’s exact test (for numbers below 5) and odds ratio (OR) with 95% confidence interval (95% CI) were used when comparing prevalence in two groups of subjects. A p value below 0.05 was considered statistically significant. An unweighted kappa statistic was used to determine the percentage of correlation between the two sampling methods beyond that expected by chance.

Results

Baseline HPV and schistosomiasis results have been presented previously [13, 17]. Briefly, HPV was found in 81 of the 236 tested cases (34%) and high-risk HPV in 54/236 (22%). Figure 1 shows that 37/54 women with high-risk HPV came for the 5-year survey (69%). Eleven had passed away, eight of them had HIV and none had high-grade squamous intraepithelial dysplasia (HSIL) previously. Genital schistosomiasis was found in 49% (270/557) of the women and prevalences were not different in the different age groups, whereas there was a significant decrease in high-risk HPV prevalence with age.

Seventeen of the 37 (46%) women had HPV after five years, 9/17 (52%) had the same subtype as baseline, and 12/17 (71%) were found to have a new type (either together with the old type (n = 4) or alone (n = 8)). In this community types 33, 35 and 45 (50%) were the most persistent and newly acquired HPV types, whereas types 16 and 18 constituted 20%. High-risk HPV was associated with genital schistosomiasis at baseline (age adjusted OR 1.9, 95% CI 1.1-3.6, p = 0.032). Together, high- and low-risk HPV were almost associated with genital schistosomiasis (age adjusted OR 1.6, 95% CI 0.95-
HPV co-infection at follow-up was found in 10/21 (48%) of the women with genital schistosomiasis, and 7/16 (44%) of women without genital schistosomiasis (not significant). New HPV infection was found in 6/17 genital schistosomiasis-positive, versus 6/15 genital schistosomiasis-negative. Persistent HPV subtype infection was found in 6/21 genital schistosomiasis-positive women and in 3/16 genital schistosomiasis-negative women. HIV was associated with HPV persistence (p = 0.047). New HPV types were found more often in HIV-positive women but the difference was not significant (age adjusted OR 4.0 95% CI (0.83-20), p = 0.085).

Before the last survey two women had hysterectomies for unknown reasons. Both had cervical schistosomiasis at baseline, one of which had a ‘malignant-looking-lesion’ (but no atypical Pap smear), while the other had HGSIL. At baseline 21/37 (56%) of the women with high-risk HPV had genital schistosomiasis and HPV was found in all who had HGSIL. After five years genital schistosomiasis was found in 6/7 of those who had developed HGSIL. Running a multivariate analysis controlling for HIV and age, HPV was significantly associated with the development of HGSIL (adjusted OR 3.9, 95% CI 1.3-11.8, p = 0.018). HGSIL was found more often in women with genital schistosomiasis (adjusted OR 7.1, 95% CI 0.5-92.1, p = 0.136), but the figures are low and the difference is non-significant.

At baseline the homogenous yellow sandy patch (HYSP) type was significantly associated with baseline high-risk HPV [13]. A few tests were run to see if the relationship could be elucidated five years after. Interestingly, there was now a tendency towards significant association between baseline HYSP and squamous intraepithelial dysplasia (p = 0.078). It is not known whether HYSP may be in the causal pathway for high-risk HPV or cervical cancer. However, the Spearman Rank correlation was well below 0.7. Furthermore, neither baseline nor current HYSP were associated with new or remaining high-risk HPV (separately or together). We therefore chose to run these phenomena in a multivariate analysis against squamous intraepithelial dysplasia. High-risk HPV remained the only significant predictor (adjusted OR 5.1 95% CI (1.5-18), p = 0.010), whereas HYSP was no longer associated with squamous intraepithelial dysplasia (adjusted OR 4.8 95% CI (0.4-53). The figures are low, however, and must be interpreted with caution. Leukoplakia and papillomatous tumours (separately or together) were neither associated with baseline nor with 5-year genital schistosomiasis.

Forty-three women were enrolled for comparison of the sampling by lavage and Cervex brush, an adequate specimen was obtained in all, either by brush or lavage or by both; 13/45 (28.9%) lavage samples were β-globin negative whereas only 3/45 (7.0%) Cervex brush samples were β-globin negative. HPV could be detected in 17/40 (42.5%) of the Cervex-brush samples. In lavage 17/32 (43.8%) were HPV positive. Conclusive HPV results from both samples could be obtained in 27 women. In 26 of the 27 women, brush and lavage results were the same (96.3% agreement, kappa 0.93). In all 12 women who were HPV positive in both samples, at least one HPV type occurred in both samples. Because of the high concordance between the two samples, a conclusive result from one sample was sufficient to assess the HPV types.

Discussion

Schistosomiasis is highly endemic in Zimbabwe and in many of the countries where cervical cancer prevalences are high [23, 24]. It is treatable and control or eradication are deemed possible [25]. Five years after baseline all women with squamous intraepithelial neoplasia in this rural Zimbabwean area had HPV. However S. haematobium ova was also found in almost all the women who developed high-grade dysplasia. Moreover, homogenous yellow sandy patches, found in genital S. haematobium infection, were found more frequently in women with cervical intraepithelial neoplasia than in those without [13].

Yet we neither found an association between S. haematobium infection and the persistence nor the acquisition of HPV in this small population. Confirming other studies, HPV prevalence was significantly higher in HIV-positive than in HIV-negative women [4, 17, 26-28]. Multivariate and bivariate analyses after controlling for high-risk HPV, however, showed that neither HIV nor genital schistosomiasis were significantly associated with cervical intraepithelial neoplasia.

The small study population limits the statistical power and results must be interpreted with caution. As a further limitation to this study it should be noted that women were tested for high-risk HPV only twice over a fairly long time span. Infection with an HPV type induces an immune response against that particular HPV type in most infected women, eventually resulting in clearance of the infection. Clearance of the virus is thought to take between six and 12 months resulting in protection against re-infection, if not life-long, at least, until the immune response has waned [29, 30]. However, the duration of this natural immunity has not been explored. Therefore, after five years clearance and re-infection by the same genotype cannot be entirely precluded.

Previously it has been shown that persistent high-risk HPV infection is necessary for the development and maintenance of high-grade cervical lesions [29-31]. The knowledge of the impact of HIV co-infection on HPV persistence is limited although the relationship between HIV and HPV is widely accepted [32]. HIV seropositivity and higher levels of immunosuppression have been found to be important determinants of persistent HPV infection and HIV seropositive women [32].

There is a paucity of studies both on HPV and genital schistosomiasis in rural Africa [17]. With the implementation of the HPV vaccine programmes and anti-schistosomal mass treatment programmes it is important to look for package implementation and research [33]. Gynaecological investigations in rural Africa may be cumbersome and prohibitively expensive. Hence we must
explore simple diagnostic procedures and even self-sampling. Our study showed a high concordance between samples obtained by the Cervex brush and a vaginal lavage. A similar concordance has previously been observed by us between Cervex brush samples and vaginal swabs [34]. However, for unknown reasons, the number of β-globin negative vaginal lavage samples was unacceptably high. Since Cervex brushes are not available in this low-resource area, a vaginal swab may be a good alternative to vaginal lavage, as has been shown in South Africa [35].

In conclusion HPV-mediated suppressed immune competence may facilitate persistent HPV infection, which in turn may induce the development of high-grade cervical lesions. Our study suggests that genital schistosomiasis may also play a role in this process, but larger studies are needed to confirm the findings.

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References

The effects of genital Schistosoma haematobium on human papillomavirus and the development of cervical neoplasia after five etc.


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mailto: News@SLS.org
Recent trends in incidence, mortality and survival after cancer of the female breast and reproductive organs. Umbria, Italy: 1978-2005

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Summary

This study analyzed the incidence, mortality and survival after cancer of the female breast and reproductive organs in the Umbria region of Italy with the aim of generating hypotheses to explain trends. Mortality data were supplied by ISTAT (1978-1993) and ReNCaM (1994-2005) and incidence (1994-2005) and survival (at 12/31/2007) data by RTUP. Joinpoint regression was applied to evaluate temporal trends of the age-adjusted incidence and mortality rates. Mortality, incidence and relative survival rates were compared with national and international data. The incidence of breast cancer increased up to 2001 and afterwards significantly decreased; mortality rates significantly decreased after 1994. Uterine corpus incidence was practically stable, and decreased over the study period; mortality from all uterine subsites significantly decreased from 1978 onwards. Trends in ovarian cancer incidence and mortality (after 1985) were constant. Trends in occurrence of breast and cervical cancer were linked to population screening of Umbrian women, noting a low compliance by younger females with cervical cancer screening and emphasizing the opportunity of starting breast cancer screening at a younger age. Trends in the incidence of cancer of the uterus and ovary, though unsteady, were probably related to modifications in risk factor exposure. Survival was better for breast and cervical cancers than in the 1978-1982 period and might be due to early diagnosis and progress in therapy.

Key words: Gynecological cancers; Incidence; Mortality; Survival.

Introduction

In 2004 the estimated number of new cases of breast cancer in the European Union was about 275,000, with annual deaths close to 88,000. Gynaecological cancers presented a frequency of about 51,000 incident cases of uterine cancer, 43,000 of ovarian cancer and 30,000 of cervical cancer; deaths were respectively 12, 28 and 13.5 thousand [1].

In Italy the estimated number of new cases of breast cancer was 37,302, constituting about 25% of all female cancers. There were 1.6% uterine cervix cancers, 2.9% ovarian cancers, and 3.9% endometrial cancers [2, 3].

Currently in Umbria, a region in central Italy with about 450,000 resident women, cases of female breast cancer are over 600 annually, with about 35 cases of uterine cervix cancer, over 120 endometrial cancers, and close to 80 ovarian cancers [4].

Cervical cancer screening was offered in the 1980s to Umbrian women aged 25-64 and at present covers most of the targeted female population. Screening for early detection of breast cancer was introduced in the early 1990s on a voluntary basis, and in the late 1990s as active mass screening for females aged 50-69. The procedure was first started in only some local health districts but now covers over 65% of the target population in the region [5].

The Umbrian Population Cancer Registry (RTUP), a regional cancer registry, was established in the early 1990s, therefore data on incidence and survival are available for the period 1994-2005 [5]. Furthermore for the 1978-1982 period, an ad hoc survey was carried out in the region to determine the incidence of cancer [6]. The Registry also collects regional mortality data from municipal offices and death certificates from local health districts, and annually publishes general mortality statistics, updated to the previous year.

In this paper we describe findings of cancer incidence, mortality and survival in this region, with the aim of generating hypotheses to explain trends. Explanations will be proposed for trends, focusing on the role of local health service intervention.

Materials and Methods

Mortality data were supplied by the National Institute of Statistics (ISTAT) from 1978 until 1993. For 1994-2005 period, they were supplied by the regional Nominative Causes of Death Registry, (ReNCaM), based on the Registry population Offices of Umbrian municipalities that are linked with death certificates.

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collected by Local Health Districts and later used for national surveys by ISTAT. No major or systematic difference seemed to appear when ISTAT and ReNCaM-based mortality data were compared. Since ReNCaM data are easier to access than ISTAT mortality data, they allow the inclusion of recent years in the analysis. Causes of death were classified according to the 10th International Classification of Diseases (WHO, 1992) [7]: breast cancer as C50, cervical cancer as C53, endometrial cancer as C54, total uterus cancer as C55 and ovarian cancer as C56.

Incident cases were taken from the Umbrian Population Cancer Registry database from 01/01/1994 to 31/12/2005. All cases were collected, coded, stored and analyzed in accordance with standard methods recommended for cancer registries [8], using the ICD X [7]. Incidence rates referring to 1978-1982 period, are in relation to cases resulting from the ad hoc survey carried out in the 1980s [6]. Age-adjusted mortality and incidence rates were calculated for each site. Site-specific trends for standardized rates were analyzed by “joinpoint regression” [9], using SEER software [10]. 1978-1982 incidence data are reported in figures as observed rates, as the joinpoint analysis was applied only to 1994-2005 rates. Mortality rates were calculated for all combined subsites of uterine cancers, because death certificates frequently do not specify the subsite and only death certificates for recent years can be linked with the cancer Registry, and even then not always.

The Umbria population in the 1991 census was used as the standard in the joinpoint analyses, in an attempt to reduce bias due to the exceeding difference in age structure. Mortality or incidence trend was approximated since it is described by straight segments but was allowed to change over the study period (i.e. segments have different slopes). The grid search method detected segments best describing data. A year when a change in trend was detected over the study period is called a “joinpoint” and significant joinpoints are retained in the final model detected segments best describing data. The maximum number of joinpoints for each analysis was three. The expected annual percent changes (EAPCs) are reported to describe linear trends by period.


### Results

Table 1 reports incident cases for the different sites and periods, and relative rates per 100,000, age-adjusted by Umbrian and world populations. Table 2 reports the corresponding mortality data. The mortality/incidence ratio was, in the last period, 0.23 for breast cancer, 0.41 for total uterus and 0.66 for ovarian cancer. Clearly breast cancer presented higher incidence and mortality rates. In the last period the 72% rate of deaths from unspecified uterine subsites was still high.

Results of the joinpoint analyses by cancer site, applied to mortality and incidence rates, are reported in Figures 1 and 2.

### Table 1. — Annual number of incident cases and age-adjusted (U=Umbria, W=world population as standard) incidence rates for the selected sites and periods.

<table>
<thead>
<tr>
<th>Site</th>
<th>Period</th>
<th># cases</th>
<th>U-rate (s.e.)</th>
<th>W-rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>1978-82</td>
<td>367</td>
<td>91.2 (2.1)</td>
<td>55.8</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>508</td>
<td>109.0 (4.9)</td>
<td>66.3</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>600</td>
<td>120.3 (5.1)</td>
<td>72.5</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>649</td>
<td>132.2 (5.5)</td>
<td>83.0</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>636</td>
<td>126.1 (5.1)</td>
<td>76.7</td>
</tr>
<tr>
<td>Uterus cervix</td>
<td>1978-82</td>
<td>55</td>
<td>13.6 (0.8)</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>39</td>
<td>8.6 (1.4)</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>39</td>
<td>8.2 (1.3)</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>39</td>
<td>8.1 (1.3)</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>37</td>
<td>7.3 (1.2)</td>
<td>4.9</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td>1978-82</td>
<td>84</td>
<td>20.5 (1.0)</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>112</td>
<td>23.5 (2.3)</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>119</td>
<td>24.2 (2.3)</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>117</td>
<td>24.8 (2.3)</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>124</td>
<td>24.0 (2.2)</td>
<td>13.1</td>
</tr>
<tr>
<td>Uterus unsp.</td>
<td>1978-82</td>
<td>55</td>
<td>4.1 (0.4)</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>2</td>
<td>0.5 (0.3)</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>5</td>
<td>0.9 (0.4)</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>4</td>
<td>0.7 (0.4)</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>2</td>
<td>0.3 (0.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Ovary</td>
<td>1978-82</td>
<td>51</td>
<td>12.7 (0.8)</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>83</td>
<td>17.6 (2.0)</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>93</td>
<td>19.0 (2.0)</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>81</td>
<td>17.3 (1.9)</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>87</td>
<td>16.7 (1.9)</td>
<td>9.8</td>
</tr>
</tbody>
</table>

### Table 2. — Annual number of deaths and age-adjusted (U=Umbria, W=world population as standard) mortality rates for the selected sites and periods.

<table>
<thead>
<tr>
<th>Site</th>
<th>Period</th>
<th># deaths</th>
<th>U-rate (s.e.)</th>
<th>W-rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>1978-82</td>
<td>124</td>
<td>31.0 (1.2)</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>170</td>
<td>34.6 (2.7)</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>170</td>
<td>31.8 (2.5)</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>180</td>
<td>32.5 (2.5)</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>164</td>
<td>28.4 (2.3)</td>
<td>14.6</td>
</tr>
<tr>
<td>Uterus cervix</td>
<td>1978-82</td>
<td>6</td>
<td>1.3 (0.2)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>8</td>
<td>1.7 (0.6)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>14</td>
<td>2.5 (0.7)</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>8</td>
<td>1.4 (0.5)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>7</td>
<td>1.1 (0.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td>1978-82</td>
<td>4</td>
<td>1.0 (0.2)</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>8</td>
<td>1.6 (0.6)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>10</td>
<td>1.7 (0.6)</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>10</td>
<td>1.7 (0.6)</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>5</td>
<td>0.9 (0.4)</td>
<td>0.5</td>
</tr>
<tr>
<td>Uterus unsp.</td>
<td>1978-82</td>
<td>16</td>
<td>11.9 (0.7)</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>26</td>
<td>5.1 (1.0)</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>24</td>
<td>4.0 (0.9)</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>18</td>
<td>2.9 (0.7)</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>31</td>
<td>4.8 (0.9)</td>
<td>2.3</td>
</tr>
<tr>
<td>Ovary</td>
<td>1978-82</td>
<td>22</td>
<td>5.4 (0.5)</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>1994-96</td>
<td>49</td>
<td>9.4 (1.4)</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>1997-99</td>
<td>52</td>
<td>9.4 (1.4)</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>2000-02</td>
<td>46</td>
<td>8.5 (1.3)</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>2003-05</td>
<td>62</td>
<td>10.8 (1.4)</td>
<td>5.1</td>
</tr>
</tbody>
</table>

For breast cancer, significant joinpoints were found in incidence and mortality (Figure 1). Mortality rates increased up to 1994 (0.93% per year, n.s.) and signifi-
cantly decreased thereafter by 1.70%; incidence rates, from 1994 to 2005, increased on average by 0.90 (n.s.) each year till 2001 and afterwards significantly decreased by 1.74%. The increase in incidence rates in the initial period seemed to start from 1982.

For cancer of the total uterus, incidence and mortality decreased over the study periods. For incidence the decrement was 0.49 (n.s.), and the mortality rates significantly dropped by 3.20 per year. In this site, the decrease in incidence seemed to start from 1979.

Incidence rates from ovarian cancer showed a small, non-significant decrease of 0.58; the mortality trend, from 1978 to 2005, presented a significant joinpoint. Rates sharply increased (up to 1985 by 11.10% per year) and afterwards remained constant (0.10%, n.s.) (Figure 2). In the 1978-1982 period incidence rates were lower than in the most recent period.

Figure 2 also shows non-significant trends in the incidence rates of cervical and uterine cancers. The former slightly increased by 0.15%, the latter non-significantly decreased by 1.83% and this trend seemed to start in the 1980s.

Relative survival trends are different for the selected cancer sites. A constant improvement in survival was found for breast and cervical cancers, while no clear modifications emerged for uterine cancers. Survival improvement was rather modest for ovarian cancer (Figure 3).

A comparison with Italian registry incidence rates is reported in Figure 4. The breast cancer ranking shows a north-south gradient: the rate in the Ferrara Province was nearly double the rate in the Salerno Province. The Umbrian register is ranked sixteenth in the 22 registries. Incidence rates of cervical and uterine cancers presented the same variability. The Umbrian registry is twentieth in the rank of cervical cancer rates, and ninth in uterine cancer rates. The variability of ovarian cancer rates is less wide, ranging from 11.9 per 100,000 inhabitants in the Modena Province to 7.6 in Naples; the Umbrian rate is sixth.

The comparison with European cancer mortality rates, referred to the year 2000 is reported in Table 3. Adjusted mortality rates for the Umbria region were quite similar to the Italian for ovarian cancer and lower for breast and total uterine cancers. Compared with several European countries, the Italian rates were among the lowest for all examined sites.

Five-year adjusted relative survival rates in Umbria, compared with other Italian cancer registries, were very similar to the Italian average (Table 4). As in the rest of Italy, the highest survival rate was found for breast cancer (83%) and the lowest for ovarian cancer which was 38% in the Umbria region. The highest rate of 60% was found in the Trento Province.
Discussion

The analysis of incidence, mortality and survival trends is an important tool for monitoring cancer control and assessing primary or secondary prevention interventions. The decrease in breast cancer mortality in recent years confirms the observed trend in the Umbria region of Italy.
Figure 4. — Age-adjusted incidence (world population) for the selected cancer sites in Italy, 1998-2002 (note: the incidence rate scales in the graphs are different).
and in the European Union [3, 16, 17]. In Italy, data from cancer registries did not show a decrease until 2002 [3], while the present data, which cover a more recent period, indicated a significant decrease. The forecast variation in breast cancer incidence in Italy indicated a steady trend in the 2000-2010 period [18].

Relative survival rates increased constantly and in the most recent period rose to 90% at 5-year follow-up.

The increasing incidence trend until 2001 followed by a significant decrease, a drop in mortality and improvement in survival, were clearly typical consequences of early diagnosis and then of mass screening; treatment improvements also contributed to reduce mortality and improve survival [19]. The comparison with Italian registry data suggests that despite widespread screening, the incidence is very low. However in Umbria, the incidence increased in women aged up to 50 which might suggest starting screening at a younger age.

The difficulty in considering the different uterus sites separately when analyzing mortality, hinders interpretation of trends [20, 21]. An attempt to specify the subsite using incidence data from the Umbrian Cancer Registry yielded unreliable results because of the small proportion of linked cases.

Although diffusion of Pap testing for cervical cancer may have led to some improvements in stage at diagnosis, it mostly reduced incidence by removing premalignant lesions before progression [22, 23]. In Umbria the incidence of cervical cancer increased in women aged from 25 to 44, it decreased until 59, and then increased up to 74 years of age. The all-age annual number of cases was under 40. Mortality rates showed the same trend with a delay of ten years. These observations seem to indicate that screening compliance, in the first target years of the female population, was probably low enough.

As for breast cancer, the relative survival increase for the uterine cervix could also be due to population screening throughout the Umbria region, as also results from the very small annual number of incident cases which, on the other hand, make survival rates unstable.

The incidence of uterine cancers doubled in women aged from 50 to 55 years, showing a peak at 64-69 years, confirming the common pattern in European countries which shows the disease is less common in premenopausal women than in those aged over 50-55. This is also linked, on one hand, to the increasing number of women reproducing at a late age and to the long-lasting protective effect of estrogen-progestin oral contraceptives, and, on the other, to use of exogenous hormones that increase the risk of endometrial cancer [24, 25].

In the Umbria region mortality from uterine cancer was very unsteady while mortality from total uterine cancer constantly increased until 1984 [4]. Relative survival rates remained practically constant over the study period and, in the Umbria region, this seemed to be the effect of improvements in therapy.

Ovarian cancer mortality rates showed a very high variability. Improvements in the diagnostic definition of abdominal cancers probably contributed to the increasing trend from 1985 onward [16]. This fact seems to be confirmed by the evident increase in incidence rates starting from the 1980s that were practically constant in the 1994-2005 period. It will be interesting to evaluate this trend considering prescriptions in the Umbria region of menopausal hormone therapy, even if its relationship with ovarian cancer is still not clear [26-28]. At present these data are unavailable. Furthermore the number of gynecologists who prescribe alternative therapeutic approaches for menopausal symptoms seems to be on the increase [29-31]. Modifications of personal and behavioral risk factors like consumption of beverages such as coffee and black tea or smoking [32, 33] are difficult to evaluate in a population study.

The relative ovarian cancer survival rates also slightly increased from 1978 to 2005, which could depend, among other things, on different criteria for classifying borderline cystoadenal carcinomas that, if considered malignant, increase survival rates.

In conclusion, it seems that in the Umbria region widespread population screening for breast and cervical cancers, together with advances in therapy, has led to a constant increase in survival, while survival for uterine and ovarian cancers has not improved much.

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References

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The value of proliferation indexes in breast cancer

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Summary

Introduction: During the past several years, the Ki-67 antigen has gathered great interest in its role as a prognostic marker. Nevertheless, despite the large number of published papers, the role of Ki-67 in clinical practice remains controversial. Aim: To evaluate the association between Ki-67 immunoreactivity and other clinicohistopathological parameters. Methods: We retrospectively analyzed the archival pathology tissues of 356 patients, diagnosed and treated in our department, from 2002 to 2006. Statistical analysis was used to examine the association between Ki-67 expression and other clinicopathological factors. Results: The expression of Ki-67 was correlated with the mitotic count, tumor grade and size, and p53, HER2, and EGFR expression. Furthermore, Ki-67 expression was significantly related with nodal status and inversely associated with hormonal expression. Moreover, invasive carcinomas appeared to have greater proliferation values than in situ carcinomas, while invasive ductal carcinomas were correlated with higher Ki-67 expression compared to lobular cancers. Conclusion: The expression of Ki-67 appears to be a valuable method of proliferation measurement that could prove helpful in clinical practice. Further research is warranted in order to standardize the methodology and to reach uniformity in regard with the optimal cut-off value.

Key words:

Introduction

Breast cancer is the most common female cancer and the second frequent cause of cancer death in women [1]. Adjuvant chemotherapy has been shown to improve survival in patients with breast cancer but has potentially serious side-effects, and is costly [2]. The need for reliable and validated prognostic factors that could help select those patients that would most likely benefit from adjuvant therapy, triggered the onset of various studies [3]. During the past several years, increasing evidence has accumulated that proliferation markers may identify subgroups of patients who will stand to benefit most from adjuvant antineoplastic therapy [4]. Several different methods have been developed to estimate the proliferation rate of tumors, based on their capacity to detect specific phases of the cell cycle [5]. Mitotic count estimates are the oldest and one of the most reliable forms of assessing cellular proliferation [6]. Besides the mitotic activation index, some studies have investigated the value of immunochemistry to detect antigens related to cell cycle G1, S, G2, and M phases. Among them, the Ki-67 antigen, a nonhistone nuclear protein that is expressed in all continuously cycling cells of mid-G1, S, and G2 phase and in mitosis, has gathered great interest in its role as a prognostic marker [7]. Nevertheless, despite the large number of published papers, the role of Ki-67 in clinical practice remains controversial [8, 9]. This is probably attributable to the lack of clear evidence regarding its prognostic value and the absence of standardized methodology and cut-off values.

In this retrospective study we examined the association between Ki-67 immunoreactivity and other clinicohistopathological parameters and discuss the clinical implications of our findings.

Methods

We retrospectively analyzed the archival pathology tissues of 356 patients with breast cancer diagnosed and treated in the First Department of Surgery, Hippokration Hospital, Athens Medical School, between 2002 and 2006. The study protocol was approved by the hospital’s ethics committee.

The following factors were evaluated: patient age at diagnosis, tumor size, grade, ER, PR, and HER2 status as well as p53 and Ki-67 expression. Furthermore, in a subgroup of patients we were able to collect data regarding the mitotic activity of the tumor (234 patients) and the expression of the epidermal growth factor receptor (EGFR) (127 patients).

Immunohistochemistry was carried out on 5 μm tissue sections from paraffin blocks using the avidin-biotin immunoperoxidase method. Briefly, paraffin was removed from the slides by heating them at 60°C for 10 min, followed by three washes in xylene. After gradual hydration through graded alcohol, the slides were incubated for 30 min in 0.3% hydrogen peroxide in methanol to quench endogenous peroxidase activity. The sections were incubated in citrate buffer (pH 6.0) for two cycles of 5 min in a microwave oven for antigen retrieval. The following monoclonal antibodies were used: primary antibodies directed against estrogen receptor (NCL-ER-6F11), progesterone receptor (NCL-PGR), c-erbB-2 oncprotein internal

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domain (NCL-CB11), p53 (NCL-CMI) [Novocastra, Newcastle upon Tyne, UK] and Ki-67 (clone MIB-1), and EGFR (clone H11) [Dako, Glostrup, Denmark] were applied in a dilution of 1/50, 1/50, 1/100, 1/300, 1/800, 1/50 and 1/50, respectively. For statistical analysis purposes, the sections were scored as either negative or positive. Staining for p53, ER, PR, and EGFR was graded as positive if 10% or more of the tumor cells were stained. The HER2 membrane staining was scored as 0, 1+, 2+ or 3+. Tumors with 0, 1+ or 2+ staining were defined as negative and with 3+ as positive. Mitotic activity was evaluated as the number of mitotic figures per 10 high-power fields (HPF) [mitotic activity index (MAI)]. For clinical analysis, three mitotic activity index subgroups were considered: low (MAI < 10/HPF), medium (10 ≤ MAI ≤ 15) and high (MAI > 15). Tumor grading was performed according to the Ellis and Elston grading system.

Statistics

A standard statistical software package SPSS (SPSS Inc, Chicago IL) was used in the analysis. After taking into consideration the absence of a universally established threshold for Ki-67 values, we used the receiver operating characteristic (ROC) analysis, to discriminate tumors with high and low proliferation rates (as measured by the MAI). The ROC curve was generated by plotting sensitivity of all possible cut-off points for Ki-67 expression on the y axis as a function of 1-specificity on the x axis. Descriptive statistics were calculated for all variables. The chi-square test or Fisher’s exact test, as appropriate, was used to examine the association between Ki-67 immunoreactivity and the expression of EGFR, p53, ER, PR and HER2, as well as other clinicopathological factors such as tumor grade, stage and mitotic activity. The one-sample Kolmogorov-Smirnov test was used to test if a variable was normally distributed. Due to the absence of normal distribution the Mann-Whitney test or Kruskal-Wallis test were used to analyze the correlations between continuous and categorical variables. p values less than 0.05 were considered statistically significant.

Results

We retrospectively analyzed the medical archives of 356 patients with breast cancer. The mean age at diagnosis was 56 years (SD± 14 years). The tumor characteristics are presented in Table 1.

According to ROC analysis the best cut-off point for balancing the sensitivity and specificity of a test is the point on the curve closest to the 0, 1 point. Hence in this study, the highest validity was achieved at a cut-off value of 27.5% (Figure 1). Nevertheless, due to practicality and after consideration of the ROC curve, a threshold of 27% was used. By using this threshold the expression of Ki-67 was correlated with mitotic count, tumor grade and size and the p53, HER2 and EGFR expression. Furthermore Ki-67 expression was significantly related with nodal status and inversely associated with hormonal expression. Moreover, invasive ductal carcinomas were correlated with higher Ki-67 expression compared to lobular cancers. All the above correlations remained significant even after the Ki-67 expression was considered as a continuous variable, while also invasive carcinomas appeared to have greater proliferation values than in situ carcinomas.

Table 1. — Tumor characteristics.

<table>
<thead>
<tr>
<th>Tumor size (mean ± SD)</th>
<th>2.5 ± 1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological type I, number (%)</td>
<td></td>
</tr>
<tr>
<td>In situ</td>
<td>22 (6.2%)</td>
</tr>
<tr>
<td>Invasive</td>
<td>164 (46.1%)</td>
</tr>
<tr>
<td>Both</td>
<td>169 (47.5%)</td>
</tr>
<tr>
<td>Histological type II, number (%)</td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>301 (84.6%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>35 (9.8%)</td>
</tr>
<tr>
<td>Ductal/lobular</td>
<td>4 (1.1%)</td>
</tr>
<tr>
<td>Colloid</td>
<td>3 (0.8%)</td>
</tr>
<tr>
<td>Tubular</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>Medullary</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Papillary</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>Micropapillary</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>Adenoid cystic</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>T stage, number (%)</td>
<td></td>
</tr>
<tr>
<td>Tis</td>
<td>22 (6.2%)</td>
</tr>
<tr>
<td>T1</td>
<td>151 (42.4%)</td>
</tr>
<tr>
<td>T2</td>
<td>136 (38.2%)</td>
</tr>
<tr>
<td>T3</td>
<td>25 (7%)</td>
</tr>
<tr>
<td>T4</td>
<td>3 (0.8%)</td>
</tr>
<tr>
<td>N stage, number (%)</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>132 (37.1%)</td>
</tr>
<tr>
<td>N1</td>
<td>97 (27.2%)</td>
</tr>
<tr>
<td>N2</td>
<td>34 (9.6%)</td>
</tr>
<tr>
<td>N3</td>
<td>23 (6.5%)</td>
</tr>
<tr>
<td>Tumor grade, number (%)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>12 (3.4%)</td>
</tr>
<tr>
<td>II</td>
<td>134 (37.6%)</td>
</tr>
<tr>
<td>III</td>
<td>165 (46.3%)</td>
</tr>
<tr>
<td>TNM stage, number (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>22 (6.2%)</td>
</tr>
<tr>
<td>I</td>
<td>81 (22.8%)</td>
</tr>
<tr>
<td>IIA</td>
<td>77 (21.6%)</td>
</tr>
<tr>
<td>IIB</td>
<td>54 (15.2%)</td>
</tr>
<tr>
<td>IIIA</td>
<td>41 (11.5%)</td>
</tr>
<tr>
<td>IIIB</td>
<td>3 (0.8%)</td>
</tr>
<tr>
<td>IIC</td>
<td>23 (6.5%)</td>
</tr>
<tr>
<td>Positive tumors for [number (%)]</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td>292 (82%)</td>
</tr>
<tr>
<td>PR</td>
<td>243 (68.3%)</td>
</tr>
<tr>
<td>HER2</td>
<td>108 (30.3%)</td>
</tr>
<tr>
<td>P53</td>
<td>147 (41.3%)</td>
</tr>
<tr>
<td>Ki-67 index (mean ± SD)</td>
<td>27 ± 20</td>
</tr>
<tr>
<td>Tumors with Ki-67 index [number (%)] ≤ 27</td>
<td>216 (60.7%)</td>
</tr>
</tbody>
</table>

No correlation was found with patient age and gender. The relationships between Ki-67 and the clinicopathologic factors are presented in Table 2.

Discussion

Proliferation plays an important role in the development of mammary carcinomas. Various methods have been validated as measures of proliferation, including the mitotic count, the detection of cells undergoing DNA synthesis using flow cytometry or the thymidine labelling index or the bromodeoxyuridine labelling assays and the use of immunohistochemical staining of antigens associated with proliferation such as Ki-67, Ki-S1 and topoiso-
The value of proliferation indexes in breast cancer

With the exclusion of mitotic counting the determination of the proliferative index by means of Ki-67 immunohistochemistry has gained increasing attention due to its reliability and relative simplicity [11]. This study demonstrated that Ki-67 expression was related with tumor size, grade and nodal status, all well-known prognostic variables in breast cancer [12]. Furthermore the expression of Ki-67 was highly correlated with mitotic count. The latter is a well established proliferation index that is used in clinical practice in several countries, including ours, and is also incorporated into tumor grading systems; a feature which explains the association of high Ki-67 expression with increasing histologic grade [13, 14].

In agreement with previous studies, we showed that the Ki-67 index was higher in ductal infiltrating carcinomas than in lobular infiltrating carcinomas [15, 16]. Based on histological and clinical data many researchers advocate that lobular infiltrating carcinomas are biologically distinct from ductal infiltrating carcinomas, and they are characterized by hormone receptor-positivity, HER2 negativity and slow proliferation rate [17, 18]. Moreover, the cell proliferation rate was significantly lower in in situ than in infiltrating carcinomas; a finding compatible with the lower malignant potential of the former. However no differences were detected between invasive ductal cancers and invasive ductal cancers associated with ductal carcinoma in situ.

Another noteworthy finding of this study was that Ki-67 expression had an inverse association with hormone receptor status. Consistent with our observation, previous researchers have shown the presence of dissociation between steroid receptor expression and proliferation in about 73% of epithelial cells in breast cancer [19]; moreover it has been reported that ER and Ki-67 expression might be involved in different pathways during breast cancer development [20]. Furthermore, in keeping with the aggressive biologic behavior of tumors with high proliferation rates, Ki-67 expression was related with unfavorable prognostic markers such as the p53, the HER2 and the EGFR [21, 22].

The recommendation for the staining thresholds and cut-offs of the level of Ki-67 expression has been mostly arbitrary. Previous investigations have used 10%, 20% and 25% as the cut-off, whereas others have chosen the

| Table 2. Relationships between Ki-67 and other clinicopathologic factors. |
|------------------|----------------|----------------|----------------|----------------|
| Ki-67 index      | Values %       | ≤ 27 | > 27 | p       |
| MAI Low          | 14.5           | 27 (21.3%) | 3 (2.8%) | < 0.05 |
| Medium           | 21.6           | 72 (56.7%) | 19 (17.8%) |          |
| High             | 42.8           | 28 (22%) | 85 (79.4%) |          |
| Tumor size       | 2.3            | 2.8 | < 0.05 |
| Histological type I, low | 18*           | 18 (8.4%) | 4 (2.9%) |          |
| Invasive         | 29.2           | 94 (43.7%) | 70 (50.4%) |          |
| Both             | 26.4           | 103 (47.9%) | 65 (46.8%) |          |
| Histological type II, ductal | 28.5          | 174 (86.6%) | 127 (94.8%) | < 0.05 |
| Lobular T stage, | 18             | 27 (13.4%) | 7 (5.2%) |          |
| T1               | 23.4           | 103 (54.5%) | 47 (37.6%) | < 0.05 |
| T2-T4            | 31.6           | 86 (45.5%) | 78 (62.4%) | < 0.05 |
| N stage          | 25.4           | 86 (49.7%) | 46 (40.7%) | < 0.05 |
| N0               | 30.3           | 57 (32.9%) | 40 (35.4%) |          |
| N1               | 26.5           | 22 (12.7%) | 12 (10.6%) |          |
| N2               | 34.3           | 8 (4.6%) | 15 (13.3%) |          |
| Grade 1          | 10.5           | 12 (6.4%) | 0 (0%) | < 0.05 |
| 2                | 16.9           | 115 (61.5%) | 19 (15.3%) |          |
| 3                | 37.6           | 60 (32.1%) | 105 (54.7%) |          |
| TNM 0            | 18             | 18 (9.8%) | 4 (3.4%) | < 0.05 |
| 1                | 21.9           | 58 (31.5%) | 23 (19.7%) |          |
| II A             | 29.1           | 47 (25.5%) | 30 (25.6%) |          |
| II B             | 35             | 25 (13.6%) | 29 (24.8%) |          |
| III A            | 27.7           | 27 (14.7%) | 14 (12%) |          |
| III B            | 26.6           | 1 (0.5%) | 2 (1.7%) |          |
| III C            | 34.3           | 8 (4.3%) | 15 (12.8%) |          |
| ER+              | 23.3           | 198 (91.7%) | 93 (66.9%) | < 0.05 |
| ER-              | 44.8           | 18 (8.3%) | 46 (33.1%) |          |
| PR+              | 23.6           | 164 (75.9%) | 78 (56.1%) | < 0.05 |
| PR-              | 34.9           | 52 (24.1%) | 61 (43.9%) |          |
| HER2+            | 33.6           | 50 (23.4%) | 58 (41.7%) | < 0.05 |
| HER2-            | 24.4           | 164 (76.6%) | 81 (58.3%) | < 0.05 |
| P53+             | 33             | 70 (33%) | 77 (56.2%) | < 0.05 |
| P53-             | 23             | 142 (67%) | 60 (43.8%) |          |
| EGFR+            | 41.6           | 4 (5.7%) | 11 (19.3%) | < 0.05 |
| EGFR-            | 27.5           | 66 (94.3%) | 46 (80.7%) |          |

*By using Ki-67 as a continuous variable, analysis showed a significant association with histological type I.
median of positively stained cells [23–25]. In a study by Spy- 
ratos et al., the choice of the optimal threshold depen-
ded on the clinical objective [26]. As an exclusion cri-
terion from chemotherapeutic protocols the optimal cut-off of value was 10%. On the other hand, if Ki-67 was used to identify patients sensitive to chemotherapy proto-
cols, a cut-off at 25% appeared to be more suitable. In 
this study, we evaluated the relationship between Ki-67 
expression and other histopathological parameters by 
treating the former both as a continuous and as a catego-
rical variable. Our analysis showed that the optimal cut-
off value for discriminating tumors with high and low 
proliferation was 27%. As described previously, this 
threshold was significantly associated with all the well-
known prognostic factors for breast cancer.

In summary, Ki-67 correlated well with established 
prognostic factors such as hormone receptor, HER2/neu 
status and tumor staging. It appears to be a valuable 
method of proliferation measurement that could prove 
helpful in identifying patients with unfavorable prog-
nosis. Nevertheless, further research is warranted in 
order to standardize the methodology and to reach uniform-
ity in regard to the optimal cut-off value.

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nostic factor”. Cancer, 100, 455.


Epidemiological modelling of risk factors of human papilloma virus in women with positive cytology in the county of Csongrád

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Summary

This study was carried out to determine the prevalence and risk factors of genital HPV infection in women diagnosed with non-negative cytology in Southeastern Hungary. Cervical samples were collected for cytology and HPV testing from women seen at gynaecological outpatient clinics and diagnosed with non-negative cytology. The observed overall average HPV infection rate was found to be 61%. A smoking habit was the only risk factor in the logistic regression analysis that related significantly to exposure to HPV infection. Thus, prevention strategies should focus on the regular clinical cytological screening of HPV-infected patients and on the reduction of smoking.

Key words: HPV infection; Smoking; Non-negative cytology; Prevention of cervical cancer; Southeastern Hungary.

Introduction

Cervical carcinoma, one of the most frequent malignancies in women worldwide, is a major human cancer of which the viral etiology should be considered [1]. Most epidemiological studies have been concerned with genital HPV infections, which are transmitted primarily through sexual contact [2, 3]. A strong positive trend has been found between increasing numbers of sexual partners and the prevalence of genital HPV infections [4]. Various cohort studies have assessed the risk of genital cancer-linked HPV infections progressing to cervical intraepithelial neoplasia and subsequently to invasive cancer [4]. From the epidemiological data alone, it can be concluded that more than 90% of cervical cancers overall may be attributable to HPV infections [1]. The present study was carried out to determine the prevalence and risk factors of genital HPV infection in women diagnosed with non-negative cytology in the county of Csongrád in Southeastern Hungary.

Materials and Methods

A cross-sectional survey was performed at the Department of Obstetrics and Gynaecology at the University of Szeged. Cervical samples were collected for cytology and HPV testing from women seen at gynaecological outpatient clinics and diagnosed with non-negative cytology. Both the Bethesda [5] and the Papanicolaou classification (Pap smear) were used for cytology evaluation. Colposcopy and routine gynaecological examinations were performed in every case.

Results

A total of 72 women diagnosed with positive cytology were examined for the prevalence of HPV. The observed overall average HPV infection rate was found to be 61% (44/72). High-risk HPV positivity was detected in 38 samples (86%). There were five condyloma cases in the HPV-infected group (11%) and one condyloma case (3%) in the HPV-negative group.

Analyses were carried out with the STATA software package (version 9.0). The Fisher exact test and Student’s t-test were performed. Logistic regression analysis was applied to obtain an overview of the risk of HPV infection.

The difference between the mean ages of the HPV-infected patients (n = 44; mean age: 30.2 years, SD: 9.3 years) and the non-infected women (n = 28; mean age: 31.2 SD: 7.5 years) was statistically non-significant.

The smoking habit proved to be the only risk factor in the logistic regression analysis that related significantly to exposure to HPV infection (p < 0.05). In the age groups younger than 24 years and older than

HPV DNA determinations were carried out by means of the HPV hybrid capture assay (DIGENE™ HPV). Virus types were classified into two categories: high-risk HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and low-risk (6, 11, 42, 43 and 44) types [6].

In a recent meta-analysis study, the high-risk HPV positive range rate in women with positive cytology was found to be 76% (range 55-89%) [7]. Thus, an envisaged sample size of 72 patients was estimated through use of the formula of Hsieh [8] on the assumption of a prevalence of infection of 60% at a significance level of 5% with a power of 90%. Data concerning age, occupation, lifestyle, sexual practices and health status were extracted from the patient register.

Analyses were carried out with the STATA software package (version 9.0). The Fisher exact test and Student’s t-test were performed. Logistic regression analysis was applied to obtain an overview of the risk of HPV infection.
24 years old, 58% (30/51) and 67% (14/21) of the women, respectively, were infected with HPV. This difference was statistically again non-significant.

Discussion

In the second half of the 1990s, HPV testing was generally applied in Hungary for the clinical screening of women of fertile age [9]. However, with regard to the results of international studies on large numbers of patients, and from cost-benefit considerations, this practice was later modified. HPV testing could be performed only when suggested by the results of cytological examinations carried out because of the possibility of HPV infection.

A previous cohort study in Northeastern Hungary reported a prevalence of 35% in a female population of cases with positive cytology [10]. In our study the corresponding prevalence was 61%. The prevalence of HPV infection in Southeastern Hungary also turned to be significantly higher than reported from elsewhere in Hungary.

The high-risk HPV-positive cases included 43% who smoked. The smoking habit has likewise proved to be significantly related to HPV infection in several previous studies [9, 11].

Thus, prevention strategies should focus on the regular clinical cytological screening of HPV-infected patients and attempts to reduce smoking.

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Reference


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Verification of the accuracy of cervical cytology reports in women referred for colposcopy

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Summary

Objective: To verify the accuracy of cervical cytology in correlation with colposcopic and histological findings. Design/Setting/Population/Methods: In this retrospective chart review study 545 women, referred to the outpatient clinic for colposcopy, were included in the study. During the 4-year study period, two consultants performed the coloscopies and further necessary procedures, whereas patient charts were reviewed by two of the co-authors. Results: The median age of the study population was 35 years (range: 16-65). Thirty-four percent of the cases were new and 11% of the women were referred after receiving their first ever cervical smear. Ninety-two percent (503/545) of the colposcopies were satisfactory. Concordance between colposcopic findings and the histology report was 87%, whereas concordance between cytology and histology reports was as low as 60% for HPV-related lesions, 72% for LGSIL and 86% for HGSIL. “See and Treat” was offered to 53 (10%) women and 48 (90.5%) of them had high-grade lesions on histology justifying treatment at the first visit. Conclusions: The concurrent use of cytology and colposcopy provides better chances for earlier detection of lesions demanding intervention; 80%-90% of patients with severe dyskaryotic smears will have a histology report confirming CIN III. See and treat management can be decided sometimes, if supported by the colposcopic findings, and an audit should confirm accuracy to, at least, 90% of cases.

Key words: CIN; Cervical screening; Smear test; LLETZ; HPV.

Introduction

Papanicolaou’s seminal publication in the 1940s, which showed that exfoliated cervical cells could be reliably harvested and spread, fixed and stained on a glass slide, laid the foundations of cervical cancer screening [1]. In 1968, Junger and Wilson published a list of ten criteria against which screening strategies could be judged [2]. Our understanding of cervical carcinogenesis has progressed immensely during the last 30 years, leading to the realization that the HPV family of viruses is an essential factor in the causation of the disease. The gradual increase in the use of exfoliative cytology in the late 1960s and 1970s naturally resulted in an increase in detecting abnormal cells. Cervical cytology is the most widely used screening tool, despite the fact that it has a false-negative rate of 25-50% [3, 4]. Other disadvantages include cost, need for expertise, need for repeat visits for reports, etc. Colposcopy of the cervix is currently one of the second-line investigative modalities in the management of cervical disease [5-8]. As shown repeatedly, colposcopy can be used as a first-line screening tool as well [5]. However, cost of equipment, technical difficulties and the expertise required, have prevented its widespread use as a screening tool. Combining colposcopy with cytology increases the diagnostic accuracy [9]. Treatments such as laser and large loop excision of the transformation zone (LLETZ) have since been introduced. The incidence of cervical cancer following treatment of CIN III is now less than 1% and the consequent mortality rate is reported to be lower than 0.5% [10].

Materials and Methods

This is a retrospective analysis using data from the colposcopy clinic of our department identifying those patients (n = 545) who underwent colposcopic examination from 2003 to 2006. The following data, which were based on spontaneous screening material, were retrieved for each woman: age, colposcopic impression, procedure performed, biopsy result. The age of these women was between 18 to 65 years old (mean 35) and 24% of them (n = 130, mean 32.5) were smokers (Table 1). Over the study period 17 women less than 20 years old underwent colposcopy examination due to abnormal smears (16-19 years). Punch biopsies were performed on 343 women (63%). See and treat management was offered to 53 (10%) women (Figure 1). The colposcopies were satisfactory in 92% (n = 503) of cases (Table 2). The percentage of new cases over these four years was 34% (n = 186, mean 46.5). For 11% of the study group (n = 61, mean = 15) the referral smear was their first ever smear test (Table 1).

Results

The majority of smear test results of the women in our study (273) were low-grade squamous intraepithelial neoplasias (LGSIL) (50%), followed by 163 high-grade squamous intraepithelial neoplasias (HGSIL) (30%). Only 10% (20%) had low grade or inadequate smears. Over the study period of four years (2003-2006) the correlation between colposcopic opinion and pathology result was 87% (Table 1, Figure 2). Thirty percent of the pathology reports came back as chronic cervicitis ± HPV infection, 33% as CIN I and 30% as high grade lesion CIN II-III (Figure 3, Table 3). Only 9% of reports came back as normal. Two cases of invasive carcinoma and

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other pathologies like warts and VIN were also identified. See and treat management was offered to 53 (10%) women and 48 (90.5%) of them had high grade lesions on histology justifying treatment at the first visit (Table 4). Seventeen women less than 20 years old underwent colposcopy examination due to abnormal smears (16-19 years). Two of them had CIN I and one CIN II. The majority of these women (14/17) were normal or had minor pathology (Figure 3). Mean time interval between smear and colposcopy was 31 days and mean time interval between colposcopy and pathology report was 22 days.

Discussion

The aim of a cervical cancer screening program is to screen for precancerous changes in the cervical epithelium which can be treated, thereby reducing the incidence of cervical cancer. Although cervical cancer screening has never been subjected to a randomized trial, few argue against its success in promoting women’s health. It has been estimated from systematic reviews that routine primary cervical screening carries a 50-70% sensitivity to detect CIN III [11].

The findings of HGSIL are a reason for referral for urgent colposcopic assessment. HGSIL smears usually correlate with a high-grade CIN lesion, which eventually require treatment. Of patients with HGSIL 80%-90% will have a histology report confirming CIN III [12]. CIN is identified, over a 10-year period, in up to 10% of women following treatment for CIN, this representing both persistent and recurrent disease [13, 14].

“See and treat” was offered to 53 women in our study and 90.5% of them had high grade lesions on histology justifying treatment at the first visit. This small overtreatment rate is acceptable, since the high correlation rate between colposcopic opinion and histology clearly demonstrates a high standard of colposcopic acumen. Hence, a see and treat management could be decided sometimes on the grounds also of the colposcopic findings and an audit should confirm accuracy to at least 90% of cases [15]. Actually a lot of women prefer to have the
Also treatment that fails to remove the whole of the lesion may result in patients having more than one focus of residual CIN and therefore the adequacy of secondary ablation is much harder to assess. The subsequent detection of additional foci of disease is therefore not necessarily indicative of treatment failure.

Conclusion

Cervical cancer screening is a multidisciplinary activity in which the various components of a program must work with the same goals, protocols and definitions in order to be objective. A cervical biopsy is part of the screening process. It follows a referral, the need for which has been indicated by the cervical smear report. A biopsy has several functions. It confirms, alters or refutes the suggested diagnosis and, in some cases, it adequately treats the lesion. When a well organized screening program exists, it is expected that cytology reports will be available to the colposcopist (when examining the woman) and to the histopathologist (when diagnosing biopsies). Similarly, clinical reports must be available to the histopathologist. In this way, professionals will have access to all the necessary information and be able to exercise their judgment and make appropriate recommendations.

Table 3.

<table>
<thead>
<tr>
<th>Biopsies Results LLETZ see and treat</th>
<th>Normal cervicitis n HPV</th>
<th>CIN I</th>
<th>CIN II-III</th>
<th>Invasive carcinoma</th>
<th>Other pathologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>6 (6%)</td>
<td>37</td>
<td>33</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(38%)</td>
<td>(33.5%)</td>
<td>(31.5%)</td>
<td>4 warts</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>9 (8%)</td>
<td>35</td>
<td>33</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(33%)</td>
<td>(31%)</td>
<td>(30.5%)</td>
<td>4 warts</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>9 (10%)</td>
<td>20</td>
<td>30</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(22.5%)</td>
<td>(34%)</td>
<td>(32.5%)</td>
<td>1 warts</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>11 (11%)</td>
<td>26</td>
<td>35</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(25.5%)</td>
<td>(34%)</td>
<td>(29.5%)</td>
<td>2 warts</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35 (mean 118 (30%) 131 (mean 29.5%) 33 33%) 30 30%) 11 warts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.

<table>
<thead>
<tr>
<th>See and treat</th>
<th>Correlation of “see and treat” colposcopies with pathology reports</th>
<th>Women less than 20 years old who had colposcopy</th>
<th>CIN III</th>
<th>Minor pathology (cervicitis, HPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>13 (92%)</td>
<td>4</td>
<td>1 CIN I</td>
<td>3</td>
</tr>
<tr>
<td>2004</td>
<td>17 (88%)</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2005</td>
<td>10 (90%)</td>
<td>5</td>
<td>1 CIN II</td>
<td>4</td>
</tr>
<tr>
<td>2006</td>
<td>13 (92%)</td>
<td>6</td>
<td>1 CIN I</td>
<td>4</td>
</tr>
<tr>
<td>53 mean</td>
<td>48 mean</td>
<td>17 mean</td>
<td>3 (mean 1)</td>
<td>13 (mean 4)</td>
</tr>
<tr>
<td>13 (10%)</td>
<td>12 (90.5%)</td>
<td>4 (3%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References


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Loop electrosurgical excision procedure in Greek patients with vulvar intraepithelial neoplasia

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Summary

Objective: The aim of our study was to evaluate the therapeutic effectiveness of loop electrosurgical excision procedure (LEEP) in Greek patients with vulvar intraepithelial neoplasia (VIN). Materials and Methods: Between January 2002 and January 2009, 55 women with histologically confirmed VIN usual type were included in our study. For the LEEP procedure we used a high frequency electrosurgical unit with at least 80 W output. The tissue was removed to the second surgical plane. Statistical analyses were performed using the SPSS-13 for Windows. Results: Complete response rate at 12-month follow-up was 100%. Complete response rate at 48 months of follow-up was 80%. Recurrence rate at 48 months of follow-up was 20%. Conclusion: LEEP may constitute a valuable excisional method for the treatment of VIN. It provides an interpretable specimen of the whole lesion within a few minutes. It needs a short period of training and has low cost.

Key words: Electrosurgery; LEEP; Vulvar intraepithelial neoplasia; VIN.

Introduction

Vulvar intraepithelial neoplasia (VIN) is a premalignant lesion involving mostly the non-hairy skin of the vulva. There are two distinct types, the usual VIN type (warty, basaloid and mixed) and the differentiated VIN type [1]. The two types differ in morphology, biology and clinical features [1, 2].

The usual VIN type is related to human papilloma virus (HPV). It occurs predominantly in younger patients and tends to be a multifocal and multicentric disease [1, 2]. It carries the same demographic risk factors as those observed in women with cervical intraepithelial neoplasia (CIN) [3]. VIN is caused by high-risk HPV types (primarily 16, 18 and 31) and coexists with other multicentric intraepithelial lesions in the lower genital tract [4-7]. It is seen adjacent to approximately 30% of squamous cell carcinomas (SCC) of the vulva (basaloid and warty type) [1, 2].

The differentiated VIN type is not related to HPV. It occurs less commonly, mainly in older women, and is often observed in association with keratinizing SCC [1, 2]. VIN 3 has a certain invasive potential both in treated (3.3%) and untreated (9%) patients. The progression to invasion may occur many years after VIN 3 has been diagnosed [8, 9] and spontaneous regression may occur (1.2%) [8].

Treatment protocols use either surgical procedures (partial superficial vulvectomy, loop electrosurgical excision procedure (LEEP) and laser CO2 surgery) or topical medical therapies (5% 5-fluorouracil, 5% imiquimod) [4].

The aim of our study was to evaluate the therapeutic effectiveness of LEEP in Greek patients with VIN.

Material and Methods

Between January 2002 and January 2007, 55 women with histologically confirmed usual type VIN were referred to the 2nd Department of Gynecology of St. Savvas Anticancer–Oncologic Hospital of Athens. Only patients with lesions ≥ 2 cm² in total extent were included in the study. Women with the differentiated VIN type or with recurrent VIN, and pregnant women were excluded from the study.

The total extent of disease was measured prior to LEEP by recording the length and width of all lesional tissues and calculating the total area involved. Prior biopsy sites were not included in the measurements.

For local anesthesia two hours before the operation we used an eutectic mixture of lidocaine and prilocaine on the vulva and one suppository diclophenac per rectum. A few minutes before the operation 2% lidocaine solution diluted with normal saline (1:10) was used to infiltrate the subepithelial papillary dermis beneath and about 1 cm adjacent to the treatment fields.

In all women the lesional tissue was treated with LEEP-6 mm away from the lesion margins (5 mm for free-lesion margins and 1 mm for thermal effect). The tissue was removed to the second surgical plane (reticular dermis). Depth was controlled by performing procedures at high magnification under colposcopic guidance.

For the LEEP procedure we used a high frequency electrosurgical unit with at least 80 W output. For electroexcision we used a 2.5 cm rectangular loop electrode and we selected the blend cut mode with 30 W power output. We created an 18 mm rectangular loop from a stainless steel wire or transformed the semicircular shape of the common loops into a rectangular form with a width up to 2.5 cm. For electrofulguration we used a 5 mm ball electrode and we selected blend coagulation mode with 50 W power output.

All patients were advised to have only protected intercourse during the first six weeks following the procedure and to return for follow-up at six weeks (or earlier if recurrence was noted). The post-treatment follow-up protocol included physical and colposcopic assessment at three, six, nine and 12 months for the first year and yearly thereafter.
Complete response was defined as no clinical and colposcopic evidence of any vulvar lesion. Partial response was defined as reduction of total VIN area by more than 50%. Recurrence was defined as development of new lesional epithelium in complete responders.

The study was approved by the Ethical Committee of the Hospital. Written informed consent was obtained from each woman. Statistical analyses were performed using the SPSS-13 for Windows.

Results

The median age at diagnosis of VIN was 42 years (range 25-52 years). The median follow-up was 48 months (range 12-84 months). The median operating time was 10 min (range 5-15 min) depending on multifocal and extent of the lesion. The median healing time was six weeks (range 4-8 weeks) depending on the depth of injury (2nd-3rd surgical plane) and extent of the wound.

All tissue specimens had free surgical margins. In our study population we had ten VIN 1, 35 VIN 2 and ten VIN 3 cases.

Complete response rate at 12-month follow-up was 100%. Partial response rate, at 12 months of follow-up was 0%. Recurrence rate at 12 months of follow-up was 0%.

Complete response rate at 48 months follow-up was 80%. Recurrence rate at 48 months of follow-up was 20%. None of the treated patients progressed to invasive cancer during a mean follow-up of 48 months. These data are shown in Tables 1 and 2.

Table 1. — Response at 12-month follow-up (n = 55).

<table>
<thead>
<tr>
<th>VIN</th>
<th>Complete response</th>
<th>Partial response</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIN 1</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VIN 2</td>
<td>35 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VIN 3</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>55 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 2. — Response at 48-month follow-up (n = 55).

<table>
<thead>
<tr>
<th>VIN</th>
<th>Complete response</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIN 1</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VIN 2</td>
<td>27 (77.14%)</td>
<td>8 (22.86%)</td>
</tr>
<tr>
<td>VIN 3</td>
<td>7 (70%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>44 (80%)</td>
<td>11 (20%)</td>
</tr>
</tbody>
</table>

Discussion

VIN 3 has a certain invasive potential both in untreated (9%) and treated (3.3%) patients. The progression to invasion may occur many years after VIN 3 has been diagnosed [8, 9]. The mean time to progression is 55 months (range 4-216 months) [8]. For that reason patients with VIN 3 should be followed carefully for a very long period of time. Spontaneous regression may occur (1.2%) [8]. In our study none of the treated patients progressed to invasive cancer, during a mean follow-up of 48 months, which might be related to the small number of patients and the close follow-up in our study population.

The choice of therapy for high-grade VIN has been dominated by the premalignant nature of the disease. Although extensive surgery is no longer the advised treatment, standard therapy for patients with VIN still comprises surgical removal of all visible lesions to relieve symptoms and to prevent the development of invasive disease [8]. Currently there is not enough available evidence to support the removal of all involved vulvar skin [8].

In cases of VIN, treatment should be directed towards preservation of the normal anatomy and function of the vulva [10]. Repeat local resections preserve them better than primary extensive surgery [11, 12]. Symptomatic relief is best achieved by local excision instead of a skinning vulvectomy [11, 12].

There are potential advantages of LEEP for treating VIN lesions, particularly those of limited size. These include: low cost of the equipment, avoidance of the operating room, avoidance of the general anesthesia and provision of a specimen [3, 13]. LEEP may be more accurate than laser CO2 in uncovering foci of early invasion (LEEP uses excision rather than ablation) [3, 13]. In our study all tissue specimens had free surgical margins. The operating time ranged between 5-15 min depending on multifocal and extent of the lesion. From our experience we believe that every gynecologist is capable of performing LEEP on VIN after five to ten supervised applications with a high index of confidence.

The period of tissue repair and degree of postoperative pain were largely related to the overall extent and site of LEEP, rather than the depth of penetration of treated areas. Patients with treated areas ≥ 6 cm² and/or perianal skin included in the treatment field, experienced the longest periods of repair (48 days) and the most severe degree of discomfort [3]. In our study the median healing time was six weeks (range 4-8 weeks) depending on the depth of injury (2nd-3rd surgical plane) and the overall extent of the wound. Keeping in mind the postoperative discomfort, especially over the perianal area (< 1.5 cm from the anus), it is preferable to save islands of normal skin for acceptable healing in extensive lesions. Large VIN lesions can be removed in segments in two appointments (1-2 months apart) [13].

There is no indication that recurrences of VIN 3 depend on the type of surgery used, except cryosurgery, which has a high failure rate [8, 14]. Cure rates are influenced principally by factors other than the specific cytodestructive method [3]. Included among these are later activation of latent HPV DNA, contamination from adjacent HPV positive lesions, skin appendage involvement, positive surgical margins, and immunosuppressed states [15]. However, recurrences do occur significantly more often after involved surgical margins than after free surgical margins [8]. LEEP and laser CO2 produce similar therapeutic success rates in patients with VIN [3, 14]. In our study all tissue specimens had free surgical margins and the recurrence rate at 48-month follow-up was 20%.

The negative effect of vulvar surgery for the patient is great and irreversible. It has been shown that half of the
women suffer from sexual dysfunction and psychological problems following vulvectomy (radical or simple) [16, 17]. It has also been suggested that postoperative sexual functioning and somatopsychic reactions after treatment for VIN 3 correlated with the magnitude of the excision [16, 17]. In our study none of the patients complained of post-treatment sexual dysfunction.

The main complications in our study population were spot bleeding (10%), instant pain of low intensity (only when the clitoris was operated) and postoperative discomfort (70%) for one to two hours. The newly formed epithelium after a mean period of seven weeks presents excellent topography with little or no disfigurement. In extensive lesions multiple applications of LEEP resulted in a low degree of vitiligo (the thermal effect extended to the 3rd surgical plane).

It is clear that current treatments for VIN are suboptimal and continue to represent a clinical challenge. The best approach is individualized management based on clinical presentation, extent of disease and patient preference.

In conclusion, LEEP may constitute a valuable excisional method for the treatment of VIN. It provides an interpretable specimen of the whole lesion within a few minutes, needs a short period of training, and has low cost.

References


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Preservation of ovarian function by ovarian transposition prior to concurrent chemotherapy and pelvic radiation for cervical cancer. A case report and review of the literature

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Summary

Background: For over 45 years, ovarian transposition has been proposed for patients with cervical cancer to preserve ovarian function prior to pelvic radiation. We report a case of preservation of ovarian function and regular normal menstrual cycles after pelvic cisplatin-based chemoradiation and perform a literature review. Case: A 29-year-old female with cervical cancer underwent laparoscopic ovarian transposition prior to cisplatin-based chemoradiation. At 3-year follow-up after completion of her chemoradiation treatment indicated that she was still free of any disease. She is experiencing normal menstrual cycles at regular monthly intervals. Conclusion: The present case shows that it is possible to retain ovarian function and menstrual cycles by ovarian transposition prior to pelvic chemoradiation. This provides an option for cervical cancer patients who desire preservation of ovarian function.

Key words: Ovarian function; Ovarian transposition; Pelvic chemoradiation; Cervical cancer.

Introduction

For over 45 years, ovarian transposition has been proposed for patients with cervical cancer to preserve ovarian function prior to pelvic radiation.

We report the case of a 29-year-old female with cervical cancer who underwent laparoscopic ovarian transposition prior to cisplatin-based chemoradiation. A 3-year follow-up after completion of her chemoradiation treatment followed by brachytherapy indicated that she is still free of any disease. She is experiencing normal menstrual cycles at regular monthly intervals.

Case Report

A 29-year-old woman, gravida 2, para 1, presented with menorrhagia and was diagnosed with cervical adenocarcinoma. FIGO (International Federation of Gynecology and Obstetrics) Stage IB1. Pelvic computerized tomography (CT) revealed an enlarged left pelvic lymph node of 1.5 cm. However, pelvic magnetic resonance imaging (MRI) detected no suspicious lymph node and the cervical tumor could not be identified. Since this young patient wished to retain her fertility, a trachelectomy was planned. This intervention was only to be performed if no positive lymph nodes were detected perioperatively. A right pelvic node was identified as positive; as a consequence, the trachelectomy was aborted. In preparation for pelvic radiation, an ovariopexy was done. Final pathologic examination of the lymphadenectomy specimen showed two positive nodes out of 29. The right sentinel node and one of the non-sentinel pelvic lymph nodes situated on the left side contained metastases. The patient was referred to our institution to receive concurrent chemotherapy and pelvic radiation. A total of 45 Gy in 25 fractions was delivered to the pelvis and high-dose rate (HDR) brachytherapy was used to deliver an additional dose of 24 Gy in three fractions to point A. The radiation treatment was delivered concurrently with weekly cisplatin at a dose of 40 mg/m². The patient had a complete response to treatment. During follow-up, she mentioned that she experienced normal monthly menstrual cycles, starting one month after finishing treatment. Early follicular-phase serum hormone assessment showed a follicle stimulating hormone (FSH) level of 17.6 U/l, luteinizing hormone (LH) level of 6.7 U/l, and estradiol level of 140 pmol/l. She has now been followed-up for three years and is free of any disease. She still does not present any menopausal symptoms.

Discussion

We have reported the case of a young woman who maintained normal menstrual cycles following an ovariopexy and concurrent cisplatinum-based chemotherapy and pelvic radiation for cervical cancer. The standard treatment for early-stage cervical cancer is usually pelvic lymphadenectomy followed by a modified radical hysterectomy. Pelvic radiation with a curative intent is an alternative. Landoni et al. [1] conducted a randomized control trial comparing radical surgery to radiotherapy for patients with Stage IB-IIA cervical cancer. The results were similar for both treatment arms in terms of 5-year overall survival and disease-free survival.

Since cervical cancer is not a hormone-dependant cancer and the risk of ovarian metastases is negligible,
the ovaries are left in situ during surgery. Therefore, young women undergoing surgical treatment for early cervical cancer do not become menopausal. For selected cases of patients with early-stage cervical cancer who desire to preserve reproductive function, a radical vaginal or abdominal trachelectomy with pelvic lymphadenectomy is an alternative. The eligibility criteria for radical vaginal trachelectomy include the following: women younger than 40 years with a strong desire to preserve fertility, no clinical evidence of impaired fertility, lesion sizes < 2 cm, FIGO Stages IA-IB1, absence of upper endocervical canal involvement and negative regional lymph nodes. Abdominal radical trachelectomy may be an option for selected women with Stage IB1 lesions and a clinical diameter of 2-4 cm wishing to preserve their fertility who would be excluded from the radical vaginal approach [2]. In the present case report, the trachelectomy was aborted because pelvic lymph node metastases were found at the time of staging of the nodes.

This patient was referred for definitive chemotherapy and radiation treatment. Pelvic radiation to a dose of 45 Gy usually induces ovarian failure. When this is the case, long-term hormone replacement therapy is indicated for young women and is continued until at least the age at which they would normally have reached menopause. Ovarian function can be preserved by ovarian transposition prior to pelvic radiation. In addition to preserving ovarian function, ovarian transposition could potentially preserve fertility in patients in whom the uterus and at least one of the ovaries have been preserved. For over 45 years, ovarian transposition has been proposed for patients irradiated for cervical cancer [3]. Several techniques of uni- or bilateral ovariopey are described in the literature. Ovarian transposition by laparoscopy is an attractive alternative to laparotomy. It offers the advantages of smaller incisions, a reduction in scar tissue, fewer postoperative complications such as wound infections or hernias, and a shorter hospital stay [4, 5] and can be done in an outpatient setting.

Huang et al. [6] reported a series of patients on whom they performed bilateral ovarian transposition without any significant complications. Laparoscopic ovarian transposition was done prior to pelvic irradiation in premenopausal patients with a gynecologic malignancy. In 14 cases, the ovaries were transposed superiorly to an anterolateral position, 3-4 cm above the umbilical line. None of the surgery required conversion to laparotomy and no complications were observed. Of the seven patients who were under 39 years old, only one experienced ovarian failure. This patient received chemoradiation while the others underwent pelvic radiation only.

The potential complications of ovarian transposition include injury to the ureter or to the ovarian vessels, torsion of the ovarian vascular pedicle, bleeding, functional ovarian cysts and cancer recurrence in transposed ovaries [7-9].

The rate of ovarian function preservation associated with ovarian transposition has been studied by several authors [8]. Chambers et al. [10] reported that lateral ovarian transposition in patients irradiated for cervical cancer resulted in preservation of normal ovarian function in up to 70% of patients. Fenney et al. [11] assessed the effectiveness of lateral ovarian transposition in preserving normal ovarian function in women with Stage I-II A cervical cancer who were treated primarily by radical hysterectomy and pelvic lymphadenectomy. Lateral ovarian transposition was performed at the time of radical hysterectomy in 132 patients and 28 received postoperative pelvic radiation therapy. Only 3/104 (2.9%) patients who underwent lateral ovarian transposition without postoperative pelvic radiotherapy experienced menopausal symptoms, while ovarian failure occurred in 14/28 (50%) patients who received postoperative pelvic radiation therapy. The risk of ovarian failure with pelvic radiation therapy after lateral ovarian transposition was significant. The chance of maintaining normal ovarian function depends on the dose delivered to the ovaries and on the patient’s age. The human oocyte is very sensitive to radiation, with an estimated LD50 of less than 2 Gy [12]. The number of primordial oocytes present at the time of treatment, together with the biologic dose of radiotherapy received by the ovaries will determine the fertile window and influence the age of ovarian failure [13]. Wallace et al. [12] reported a model to predict the age of ovarian failure after treatment with a known dose of radiotherapy received by the ovaries. They showed that the estimated sterilizing dose decreases with increasing age, as the remaining population of primordial oocytes declines.

Our patient maintained regular menses, meaning that besides normal ovarian function, her endometrium was still functional. After pelvic radiation, the basal layer of the endometrium is usually completely destroyed. There is limited literature about endometrial activity after a curative dose of pelvic radiotherapy, but as a general rule, the degree of damage depends on the total dose delivered. A dose of 40 Gy is considered sufficient to destroy the normal endometrium. However, cases have been reported of residual functional endometria even after curative radiotherapy of up to 80 Gy [14].

Endocavitary brachytherapy adds to the risk of endometrial atrophy and cervical stenosis [15]. In 1998, in a retrospective study, Morice et al. [15] reported that they assessed fertility outcome after ovarian transposition with uterine conservation and pelvic radiation therapy. In this series, all patients were treated surgically and received adjuvant pelvic radiation (no chemotherapy). A total of 37 patients were divided into two groups. Group 1 consisted of 27 patients treated for clear-cell adenocarcinoma of the vagina and/or the cervix. In this group, 20 patients were treated with adjuvant brachytherapy alone (60 Gy) while the other seven received a combination of EBRT and brachytherapy. The other group (Group 2) consisted of nine patients with an ovarian pure dysergimina and one with a parauterine soft tissue sarcoma. These patients received only EBRT. A significant difference in pregnancy rates was observed between the two groups (15% vs 80%) and menstrual disorders were more
frequently found in Group 1. These differences could be explained by the fact that many patients from Group 1 had morphological and/or functional anomalies of the genital tract following exposure to diethylstilboestrol. Furthermore, all patients in this group received brachytherapy.

Image-guided brachytherapy is gaining in popularity for the treatment of gynecological cancer. This approach has the potential to allow part of the endometrium to be spared in patients with early-stage disease.

The literature on preservation of ovarian function with chemoradiation is scarce. Farber et al. [16] reported a case of laparoscopic ovarian transposition in a 28-year-old female with rectal cancer. The patient received concurrent fluorouracil (5-FU), 350 mg/m² for five days during week 1 and 5 of her pelvic radiation. The radiation treatment consisted of a total dose of 45 Gy to the pelvis (including the rectum and its lymphatic drainage) in 25 daily fractions. Both ovaries received less than 0.8 Gy. In addition, the patient received adjuvant chemotherapy for six months. Upon follow-up 13 months after treatment, the patient was found to have regular menstrual cycles. Her FSH, LH and progesterone levels were within normal ranges.

The literature on cisplatin-based chemoradiation and preservation of ovarian function is also very scant. Cisplatin is probably associated with ovarian damage (up to date: ovarian failure due to anticancer drugs and radiation). A review of the literature on the effect of Cisplatin and ovarian function produces very little data. The published studies are based on multi-drug regimes in which a regimen containing cisplatin induced transient amenorrhea in the majority of patients. In 1994, Maneshi et al. [17] investigated menstrual and hormonal patterns in ten fertile women with locally advanced cervical cancer treated with neoadjuvant chemotherapy. Chemotherapy consisted of two cycles of high-dose cisplatin (40 mg/m², days 1 to 4) and bleomycin (15 mg/m², days 1 and 8) separated by an interval of 21 days. Five patients became amenorrheic after one course (postmenopausal levels of FSH 40 mU/l were observed) and one more after two courses of chemotherapy. The duration of gonadal dysfunction could not be determined in this study since all patients were further treated by surgery or radiotherapy. The authors suggested that this might be transient and that a review of the hormone profiles during and after chemotherapy indicated that recovery of ovarian function may be expected seven to nine weeks after the completion of chemotherapy.

Meir [18] studied the rate of ovarian failure in 168 young female patients post-chemotherapy. All 168 cancer patients were treated with combination chemotherapy. The overall ovarian failure rate observed was 34%. The chemotherapy agents were divided into five drug groups: alkylating agents, cisplatinum, plant alkaloids, antimetabolites and antibiotics. The alkylating agents were found to be associated with the highest risk of ovarian failure (OR = 3.98). Cisplatin caused ovarian failure with an odds ratio of 1.77, although the difference was not statistically significant.

Cases of pregnancies after pelvic irradiation for genital cancer have been published [15]. A successful spontaneous pregnancy in a patient with rectal carcinoma treated with pelvic radiotherapy and concurrent chemotherapy was recently reported [19]. Patients with cervical cancer who receive postoperative pelvic irradiation are traditionally treated with a four-field box technique. With this conventional radiation technique, the target volume (i.e., the external and internal iliac nodes as well as the vaginal cuff with an adequate security margin) is covered with an anterior-posterior (AP) and a posterior-anterior (PA) and two lateral opposed beams. The cranial border of this field is either L5/S1 or L4/L5. Transposed ovaries should be moved laterally and superiority above the L4/L5 vertebral interspace, preferably at the level of L1 and L2.

Since a considerable portion of the small bowel is irradiated with the box technique, small bowel sequelae are the most important acute and chronic toxicities of pelvic radiation. In an attempt to reduce the toxicity of postoperative pelvic radiation therapy, several authors looked at the role of intensity modulated radiation therapy (IMRT), and all reported a reduction in irradiated small-bowel volumes when using this technique [20, 21]. The authors also documented better rectal and bladder sparing when IMRT was used compared to standard external beam radiation. Typically, IMRT involves the use of more than four beams, which are directed from different angles. IMRT results in a larger amount of pelvic tissue being irradiated at a low pelvic dose but a smaller percentage of the organs being treated at toxic-dose level (i.e., above 30 Gy). Although IMRT has the potential to spare the small bowel, rectum and bladder, if left in place or laterally transposed (Figure 1), the ovaries will receive at least 20
Preservation of ovarian function by ovarian transposition prior to concurrent chemotherapy and pelvic radiation for cervical etc. 197

Gy, which is sufficient to induce ovarian suppression. In our opinion, the growing number of patients being treated with pelvic IMRT justifies investigating the need for superior transposition of the ovaries.

To our knowledge, this is the first case report in the literature describing preservation of ovarian function after concurrent chemotherapy and pelvic radiation for cervical cancer. Nevertheless, because the patient’s early follicular FSH level has increased to above 10 IU/l [22], some ovarian damage has occurred. Therefore, she should be encouraged to have children as soon as possible and to proceed expeditiously to IVF [23], if necessary. If she is not yet in a position to start a family, she should be advised to have IVF oocyte vitrification. Using this technique, Chian et al. [24] have reported an oocyte survival rate of 85%, a clinical pregnancy rate per cycle of 45% and a live-birth rate of 40%.

One cannot make conclusions based on a single case, but this observation shows that it is possible to retain ovarian function and menstrual cycles by ovarian transposition before pelvic chemoradiation.

The emergence of image-guided brachytherapy may permit part of the endometrium to be spared, thus allowing the possibility for fertility to be preserved. These opportunities warrant further investigation.

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External iliac artery ligation due to late postoperative rupture after radical lymphadenectomy for advanced ovarian cancer - two case reports

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Summary
According to the present guidelines for advanced epithelial ovarian cancer (EOC), bulky lymph nodes should be removed as part of the routine surgical staging and the primary goal being removal of all macroscopic tumor residuals. Furthermore, EOC-patients with bulky lymph node relapse can potentially benefit from lymphadenectomy in terms of recurrence and overall survival. We present two cases of severe postoperative hemorrhage due to external iliac artery rupture ten and 12 days after radical bulky lymph node removal in primary and recurrent EOC-patients. Both cases were successfully managed by ligation of the two arms of the external iliac artery achieving immediate hemostasis. No crossover bypass was required to maintain lower extremity perfusion. Late rupture of the iliac vessels is a rare complication of systematic lymphadenectomy in EOC. This complication can be managed by unilateral external iliac artery ligation without mandatory subsequent graft interposition or crossover bypass.

Key words: Hemorrhage; Ovarian cancer; Bulky lymph nodes; External iliacal artery.

Introduction
Radical surgery with primary goal of maximal tumor reduction remains the cornerstone of the clinical management of advanced epithelial ovarian cancer (EOC).
Lymph node dissection is part of the clinical staging according the FIGO-classification, while various guidelines recommend systematic pelvic and paraaortic lymphadenectomy in patients without macroscopic residual disease [1-3]. Additionally, there is some indirect evidence to suggest that EOC-patients with bulky lymph node relapse can potentially benefit from secondary systematic lymphadenectomy in terms of progression free survival and overall survival, with hereby associated acceptable complications rates [4].
Lymphocele formation and intraoperative bleeding due to vessel injury are in general the two most common complications of lymphadenectomy [3]. We present two unusual cases of late severe abdominal bleeding one day prior to hospital discharge, respectively on the 11th and 12th postoperative days. Both patients had advanced EOC after systematic pelvic and paraaortic bulky lymph node dissection. Both cases were due to spontaneous rupture of the one external iliac artery and were successfully treated with emergent ligation of the vessel.

Case Reports
Case 1
A 58-year-old woman was referred to our institution for surgical cytoreduction. She initially presented with abdominal distention and stool irregularities. Computed tomography (CT)-scan examination and sonography revealed massive ascites, multiple retroperitoneal bulky nodes and peritoneal carcinomatosis as well as a large pelvic mass 15 cm in diameter. The patient did not have any significant comorbidity, except well controlled hypertension. She underwent a midline laparotomy with en bloc resection of the uterus, ovaries, rectum, Douglas- and bladder-peritoneum. In the upper abdomen, we performed an extensive peritonectomy of the right and left sides of the diaphragm, the omental bursa, the right Gerotta’s fascia as well as an omentectomy. Since complete macroscopic tumor resection was achieved, we proceeded with systematic pelvic and paraaortic lymphadenectomy and removal of all retroperitoneal bulky nodes (Figure 1). No major intraoperative injuries of the retroperitoneal vessels occurred. The patient recovered quickly after an initial 3-day period in the intensive care unit. On the 5th postoperative day a negative barium enema showed an intact rectal anastomosis. The final histological examination revealed a FIGO IIIc serous cystadenocarcinoma of both ovaries with the tumor stage pT3b N1 (51/51) G3.

On postoperative day 11 the patient collapsed and complained of acute dyspnea. The abdomen was suddenly distended and the oxygen saturation fell to 35%. A CT-angiogram revealed bilateral pulmonary embolism of the caudal lobes as well as an active bleeding of the left external iliac artery just distally of the iliac bifurcation with massive hemoperitoneum. The patient underwent emergent laparotomy. Four liters of blood was drained out of the abdominal cavity. The left external iliac artery was completely and spontaneously ruptured just proximal to the iliac bifurcation, most likely secondary to arterial wall
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Discussion

Radical surgery in advanced ovarian cancer is widely accepted since postoperative tumor residual disease is the most relevant prognostic factor in ovarian cancer [5]. Multivisceral techniques such as diaphragmatic resection or stripping, splenectomy, distal pancreatectomy, and liver resection are being routinely employed in many centers during cytoreductive surgery for primary ovarian cancer [6]. Furthermore, even if the therapeutic impact of systematic lymphadenectomy in primary epithelial ovarian cancer is still under debate in multicenter prospective randomized studies, systematic pelvic and paraaortic lymphadenectomy is currently recommended in cases of complete tumor resection because of the high incidence of lymph node metastases, especially in the high paraaortic region [2].

Despite the continuously increasing trend towards radical operations, current aspects of modern perioperative patient care imply early patient mobilization and early discharge by means of a “fast track surgery” protocol. “Fast track surgery” shows no differences in patient morbidity while significantly reducing patient discomfort and duration of hospitalization. Reported experiences on gynecologic oncology services have demonstrated that early patient discharge was possible in most cases and was associated with a low rate of readmission [7].

Lymphoceles formation is considered to be the most common postoperative complication of the lymphadenectomy procedure, with an average incidence of 22-48.5% [8].

Intraoperative bleeding also constitutes a common complication, especially in the event of bulky disease fixated to the great retroperitoneal vessels. High levels of operative skills are required not only to perform complete and adequate lymph node dissection, but also to successfully treat any iatrogenic injury.

In a prospective observational study of ovarian-cancer patients with pelvic/aortic lymph node relapse, Benedetti Panici et al. reported the following results after lymphadenectomy performed on 29 patients: four (14%) severe complications in terms of intraoperative hemorrhage (two), caused by lumbar and common iliac vein bleeding. One pulmonary embolism and one bowel occlusion. Nine patients (31%) had mild complications such as lymph cyst (two), chylous ascites (two), pneumonia (one), wound infection (one), leg edema (one), and deep vein thrombosis (two) [4].

In the present article we report two rare cases of late postoperative bleeding due to spontaneous rupture of the external iliac artery 11 and 12 days after radical cytoreductive surgery and lymphadenectomy for epithelial ovarian cancer. Although the iliac vessels are considered to be the most common sites of iatrogenic operative injury during pelvic cancer surgery [9, 10], to our knowledge these are the first cases of such late postoperative bleeding complication reported after surgery for ovarian cancer.

Due to the fact that the rupture of the vessel was com-
Complete and the hemorrhage was massive both patients had to be treated immediately surgically with no option of embolization or stenting. Extraordinary was also the fact that in both patients no bypass operation was necessary and both patients recovered well without any signs of ischemic dysfunction or pain. A possible explanation for this can be the fact that due to the chronic compression of the pelvic vessels by large bulky nodes and tumor, both patients had developed sufficient collateral arteries, which were able to maintain the arterial supply of the leg.

We conclude that after extensive lymphadenectomy techniques in advanced ovarian cancer, especially in cases of bulky lymph nodes, serious and life-threatening bleeding complications can be expected even two weeks after the procedure. The external iliac artery can be ligated in emergency situations and, in contrast to many reported experiences, an interposition graft or crossover bypass can potentially be avoided in palliative patients due to the collateralization induced by the chronic arterial compression through the bulky nodes or the tumor.

References


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A novel technique for surgical reconstruction of the perineal floor following anteroposterior exenteration of the pelvis - case report and review of the literature

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Introduction

Pelvic exenteration is the only potentially curative surgical procedure for patients with recurrent cervical, vaginal, vulvar or rectal cancers, especially following adjuvant chemotherapy or radiotherapy. Morbidity rates, however, remain high, which is significantly attributed to complications of the pelvic floor reconstruction techniques. We describe a novel reconstruction technique of the pelvic floor, involving a combination of an oblique rectus abdominis myocutaneous flap and a synthetic absorbable mesh as a pelvic sling for additional support, in a 63-year-old female patient with recurrent vulvar carcinoma. Combining the use of myocutaneous flaps and prosthetic mesh material can provide an effective alternative solution to the complications arising from pelvic floor reconstruction of large defects after exenteration procedures, especially in previously irradiated settings. Further studies are necessary to define the long-term outcomes and indications of these techniques, as well as the optimal combination between the available myocutaneous flaps and prosthetic materials.

Case Report

A 63-year-old female patient was referred to our hospital in January 2008 for treatment of a locally recurrent vulvar carcinoma, which was infiltrating both the urogenital and the anal perineal triangles. The patient had originally been diagnosed with vulvar carcinoma in 1995. She was then treated with vulvar excision and inguinal and femoral lymph node dissec-
tion. Pathology of the specimen revealed a moderately to well differentiated squamous cell vulvar carcinoma, with negative resection margins and negative for metastases lymph nodes. The patient did not receive any adjuvant treatment and remained free of disease for 11 years. In 2006 she presented with a local recurrence of her vulvar carcinoma. She subsequently underwent an additional wide local excision with negative resection margins followed by adjuvant radiotherapy. One year later, in December 2007 the patient presented once more with a local recurrence of her disease, which was at this time complicated by the presence of a rectovaginal fistula (Figure 1). After a complete clinical and radiological workup it was decided to submit the patient to salvage surgical treatment through an antero-posterior exenteration, since her disease was found to be restricted in the pelvis.

The operation was carried out via a hypogastric and epigastric midline incision. After entering the peritoneal cavity, the resectability of the tumour as well as the macroscopic absence of distant metastases was assessed and confirmed. A laterally extended endopelvic resection was carried out consisting of a composite exenteration of the pelvic visceral compartments en bloc with the endopelvic parietal structures. Special attention was paid to performing a multimesovisceral excision, by including total mesorectal and mesometrial excision and removal of the uretovesical compartment (Figure 2). The resection was completed through an additional perineal incision, which included the pelvic floor skin, from the pubic symphysis to the ischial tuberosity. Anorectal function was restored through a permanent left end colostomy and urethrovesical function was
A novel technique for surgical reconstruction of the perineal floor following anteroposterior exenteration of the pelvis - case etc.

Restored with the formation of an autotopic, right, incontinent neobladder, by use of an ileal conduit in which both the ureters were implanted.

Subsequently, attention was drawn to the reconstruction of the pelvic floor defect. In view of the fact that the patient had been previously subjected to regional lymphadenectomy and extensive radiotherapy of the region, she presented with widespread, longstanding lymphedema of the thighs. This immediately posed questions as to the suitability of a gracilis muscular flap. Moreover, considering the size of the pelvic defect, even preoperatively the decision was to use an oblique rectus abdominis flap for the pelvic floor reconstruction (Figure 3). The stoma sites had been marked preoperatively, and it was decided to utilise the left rectus abdominis muscle, since the patient already had a right paramedial incision from a previous open cholecystectomy. The flap was elevated from the subcutaneous adipose tissue of the anterior abdominal wall and its size was estimated to sufficiently cover the pelvic floor defect. The skin incisions were made, outlining the flap and they were carried down through the subcutaneous tissue to the oblique fascia. The flap was then dissected from the fascia and the deep inferior epigastric vessels were dissected with a fascial and muscle wrap to create a myocutaneous flap with a muscle band extending down to the pubic symphysis. This rectus abdominis wrap enveloping the inferior epigastric vessels serves as protection against extensive stretching and eventual compromise of the vascular pedicle during flap translocation to the perineum. The flap was ultimately inverted into the pelvis and was fashioned to match the pelvic wall defect.

In addition to this, an absorbable Vicryl mesh was utilized so as to reconstruct the pelvic peritoneal sling, with the aim of preventing the small bowel from coming in contact with the newly created pelvic floor. The mesh was anchored with sutures to the sacral promontory, the lateral pelvic wall and the symphysis pubis in order to close the pelvic brim, and a closed suction drain was left in the pelvic cavity. Both the abdominal and perineal skin incisions were closed primarily. Figure 4 demonstrates the final result of the abdominal and perineal trauma at the completion of the operation.

Pathological examination of the resected specimen revealed a moderately differentiated squamous cell vulvar carcinoma, 5 cm in diameter, which was infiltrating the outer urethra, the vaginal wall, and the anal canal wall as well as the perineum, with negative resection margins and lymph nodes. The uterus and ovaries as well as the urinary bladder were closely adherent to the vulvar carcinoma, but they were not invaded by it.

The patient recovered well from the surgery with no complications and was discharged three weeks later. However, she later on developed hepatic metastases and eventually succumbed to her disease six months after the operation.

Discussion

Pelvic exenteration is a radical surgical procedure most commonly used in cases of advanced or recurrent cancer, in which less radical options are not technically possible or would not be sufficient to remove the entire tumour. This procedure is performed for many types of cancer including genitourinary and colorectal cancers.
Considerable morbidity has been associated with the resultant large pelvic defect left after pelvic exenteration. Complications include pelvic abscess and/or fistula formation, intestinal obstruction and perineal wound problems. Therefore, a reliable pelvic floor reconstruction is critical after pelvic exenteration. Primary repair of the pelvic floor is most often unfeasible, but it is also imprudent, especially in previously irradiated tissues [6, 7]. Many different methods have been advocated to obliterate the large pelvic dead space left after pelvic exenteration. These include use of synthetic absorbable mesh, omentum or other autologous tissue. The use of well vascularised myocutaneous flaps has been shown to significantly reduce the rate of pelvic wound complications. The advantages of myocutaneous flap reconstruction include reduction of the pelvic dead space, interposition of well-vascularised, non-irradiated tissue and replacement of the resected skin [7]. Frequently used myocutaneous flaps are those based on the gracilis, gluteus maximus and rectus abdominis muscles, either with a vertical or an oblique flap [4, 8, 9-11]. In our patient, a choice was made to use the oblique rectus abdominis myocutaneous flap (ORAM), taking into consideration the anticipated great size of the pelvic floor defect. Moreover, the lymphedema of the patient’s thighs made the gracilis myocutaneous unsuitable in this case, although it is usually favoured whenever bilateral abdominal stomata are planned [3]. This ORAM flap is easy to rotate, it can be fashioned so as to fill the pelvic dead space completely and it can augment the perineal wound with well vascularized epithelium [11]. Its use has been associated with a favourable outcome as to the severity of postoperative complications. That is, it has been shown that the complications typically following ORAM flap reconstruction are less often life threatening, life altering or fatal [11].

However, even in the best case scenarios, the effort to reconstruct the pelvic floor after radiotherapy is rarely without complications. One of the major considerations of the surgical techniques utilised for pelvic floor reconstruction after pelvic exenteration or abdominoperineal resection is to provide optimal support for intraabdominal organs. In this perspective the use of prostheses in pelvic floor reconstruction has been investigated. Many different surgical procedures have been designed to prevent the small bowel from coming in contact with the pelvic floor. Most often they are used to prevent radiation – induced bowel injury associated with pelvic postoperative radiotherapy and in pelvic prolapse surgery. The idea is to create an artificial sling which can close the pelvic brim, and recreate the peritoneal pelvic layer. The mesh can serve as additional supportive tissue or as a way of reinforcing inadequate or unsuitable tissue, it can induce new supportive tissue growth and it can be combined with other surgical techniques, whenever there are concerns that these will prove inadequate [5]. The prosthetic materials used today are either synthetic (absorbable, non-absorbable or mixed) or biological (autologous, allograft or xenograft donor tissue). Non-absorbable mesh reconstruction has been more or less abandoned due to the high rate of complications (mesh erosion, enteric fistula formation, etc). The two most common types of absorbable mesh slings are Polygalactin 910 (Vicryl; Ethicon, Sommerville, NJ, USA) and Polyglycolic acid (Dexon; Davis & Geck Co, Danbury, CO, USA) [12-14]. Bioprosthetic materials have also been used, such as autologous fascia lata from the lateral thigh, human acellular dermal matrix [6] and xenograft donor tissue, such as porcine small intestinal submucosa mesh [15].

There have been a few reports of simultaneous use of both a myocutaneous flap and prosthetic mesh in reconstructing the pelvic floor after pelvic exenteration [2, 6]. To our knowledge, this is the first case in which a biodegradable synthetic mesh has been used to create a pelvic sling in combination with ORAM flap reconstruction. The surgeon’s choice of the absorbable Vicryl mesh was based on the fact that the time period of 90 to 120 days, which is necessary for the mesh to dissolve completely, would be more than sufficient to ensure the ORAM flap viability by preventing the small bowel to fall back in the pelvis. Furthermore, the mesh can promote host connective and epithelial tissue growth and differentiation, resulting in the formation of a novel natural pelvic sling lasting long after the mesh has been absorbed.

In conclusion, the combined utilisation of myocutaneous flaps and prosthetic mesh material can provide an effective alternative solution to the complications arising from pelvic floor reconstruction of large defects after exenteration procedures, especially in previously irradiated settings. Defining the most advantageous technique for pelvic floor reconstruction after anteroposterior exenteration for recurrent carcinoma, however, will necessitate additional studies to evaluate the long-term outcomes of this technique compared with traditional reconstructive surgical procedures. The indications, contraindications and possible complications of this technique should be determined in a series of patients, and the role of various prosthetic materials and different myocutaneous flaps should be thoroughly explored to provide patients undergoing pelvic exenteration with the optimum reconstructive option.

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Usefulness of routine intraoperative staging of suspicious adnexal tumours: illustration by two cases of adult granulosa cell tumour

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Summary

Granulosa cell tumours (GCTs) account for less than 3% of all ovarian malignancies but are among the most common sex cord-stromal tumours. They may develop at any age. Symptoms related to oestrogen production by the tumour may occur. Because GCTs are uncommon and cannot be diagnosed preoperatively, their management is challenging. Surgery with salpingo-oophorectomy and painstaking staging is mandatory. Adjuvant chemotherapy is required in some patients.

We report two cases of adult GCTs that illustrate the usefulness of extensive abdominal exploration in every patient with a suspicious ovarian mass, to obviate the need for a second staging procedure. With this strategy, the prognosis is excellent, although the possibility of late recurrences requires prolonged follow-up.

Key words: Ovary; Neoplasms; Gonadal tissue; Sex cord-gonadal stromal tumours; Granulosa cell tumour; Conservative treatment.

Introduction

Granulosa cell tumours (GCTs) account for only 2% to 3% of all ovarian malignancies, although they are the most common sex cord-stromal tumours [1]. Adult GCTs account for 95% of cases and are seen in women older than 50 years, whereas the juvenile form (5% of cases) usually occurs in women younger than 30 years [2].

Because GCTs are uncommon and cannot be diagnosed preoperatively, their management is challenging. We report two cases of adult GCTs in which extensive exploration during initial surgery, despite negative frozen-section findings, obviated the need for repeat surgery after the definitive histological diagnosis was established.

Case Reports

Case 1

A 57-year-old woman was referred for the management of a uterine myoma and polyp. A sonogram of the pelvis showed a 47-mm tissue mass in the left ovary. Computed tomography (CT) of the abdomen and pelvis disclosed a mass along the left side of the uterus described as a “suspicious heterogeneous ovarian hypertrophy”. There were no other abnormalities. The serum CA125 level was normal. Total laparoscopic hysterectomy was scheduled.

Laparoscopy showed a 50-mm ovarian cyst with no extracystic vegetation. Painstaking examination of the abdomen and pelvis found no other gross abnormalities. An initial peritoneal fluid sample was obtained for cytological examination, and the left adnexa was removed. Frozen-section histology suggested a benign tumour. According to her initial pathology, total hysterectomy and contralateral salpingo-oophorectomy were performed. However, the definitive histological diagnosis was adult GCT, of the diffuse or sarcoma-like subtype, FIGO Stage IA, with no concomitant endometrial malignancy. Given that extensive exploration during surgery found no gross abnormalities, repeat staging surgery was not performed. Follow-up involved a physical examination and sonogram at 6-month intervals. At last follow-up five years after surgery there was no evidence of a recurrence.

Case 2

This 42-year-old woman presented with a long-standing history of hirsutism, which had not been evaluated. A sonogram of the pelvis disclosed a solid, 26-mm, highly vascular mass in the right ovary. Magnetic resonance imaging (MRI) of the pelvis showed a solid lesion consistent with a benign hormone-secreting tumour. Serum levels of CA125 and carcinoembryonic antigen were normal, and serum androgen levels elevated.

Laparoscopy showed a 20-mm mass in the right ovary consistent with a benign lesion. No other abnormalities were found by careful examination of the abdomen and pelvis, allowing initial peritoneal fluid cytology and unilateral salpingo-oophorectomy. The definitive histological diagnosis was adult GCT with a well-defined capsule and very few mitoses. The FIGO stage was IA.

Laparoscopy showed a 20-mm mass in the right ovary consistent with a benign lesion. No other abnormalities were found by careful examination of the abdomen and pelvis, allowing initial peritoneal fluid cytology and unilateral salpingo-oophorectomy. The definitive histological diagnosis was adult GCT with a well-defined capsule and very few mitoses. The FIGO stage was IA.

Given the early disease stage and absence of gross abnormalities by laparoscopic examination of the peritoneum and omentum, repeat surgery for staging was not performed. At last follow-up three years later, there was no evidence of recurrence.

Discussion

GCTs are the most common hormone-secreting ovarian tumours and often cause symptoms related to hyperoestrogenism. Abnormal uterine bleeding or gynecomastia may suggest the diagnosis. The patient may report abdominal distension or pelvic pain, which may reflect either a large or ruptured tumour or intracystic bleeding.
which is a common event [2]. The diagnosis may be made fortuitously, as in our first patient, whose tumour probably did not produce hormones.

Endometrial abnormalities are common in patients with GCTs [1]. In a case-series of 54 patients, uncomplicated endometrial hyperplasia was found in 32% of cases, endometrial atypias in 13%, and endometrial cancer in 9% [3]. Similarly, 4%-20% of patients in other studies had endometrial adenocarcinoma [1]. Of our two patients, one (Case 1) had a benign intrauterine polyp. No patient had evidence of malignant endometrial disease. Investigations to identify concomitant endometrial disease must be performed before surgery, most notably when a conservative procedure seems appropriate. Similarly, breast cancer may be present, particularly in patients with juvenile GCTs, and appropriate investigations must therefore be performed [1].

Sonography of the pelvis shows a suspicious mass whose features are not those of an epithelial tumour. There is a large, multiloculated, cystic mass with solid zones of variable size [4]. Bilateral involvement is rare. Colour Doppler shows an abundant vascular supply and may disclose haemorrhagic foci [2].

Tumour marker assays may contribute to the diagnosis by showing normal CA125 levels with elevated inhibin A and B levels. Inhibin A and B assays may be useful for monitoring the response to treatment and detecting recurrences. Unfortunately, they were not measured before or after surgery in our patients.

Surgery is the cornerstone of the treatment of GCTs. Simple salpingo-oophorectomy is usually performed, as the tumour is generally unilateral. Conservative surgery is possible in women of childbearing potential. Non-conservative total hysterectomy should be performed in postmenopausal women and in patients with advanced tumours. Peritoneal staging is mandatory and must include examination of the entire abdominal and pelvic cavity, biopsies of abnormalities or routine biopsies, and omentectomy. Repeat surgery for staging was not performed in our patients, as extensive exploration found no abnormalities during the initial procedure. It would have been preferable to obtain routine biopsies, according to the procedure for suspicious adnexal masses. The role for lymph node dissection is not clearly defined in the literature. Lymph node dissection has been recommended, despite the lack of evidence for improved outcomes [1]. Lymph node dissection seems appropriate in patients with advanced GCTs. Therefore, lymph node dissection was not performed in our patients, as this procedure would have required a second major surgical procedure and has not been proved beneficial in early-stage GCT. Given the risk of concomitant endometrial disease, hysteroscopy and endometrial biopsies are mandatory.

No data are available in the literature regarding frozen-section histological examination of GCTs. In our experience, frozen-section histology fails to contribute to the intraoperative diagnosis. Therefore, regardless of frozen-section histological findings, patients with suspicious masses should undergo optimal staging during the initial procedure, in order to obviate the need for repeat surgery. GCTs have a smooth outer surface. The capsule is ruptured in 10% of cases of adult GCT. The cut section reveals solid and cystic areas that are yellowish in colour, with a few haemorrhagic foci.

In patients with factors of adverse prognostic significance, surgery is followed by adjuvant chemotherapy with a bleomycin-etoposide-cisplatin (BEP) type combination [5]. Taxanes have been found effective in the treatment of sex-cord tumours and have less expected toxicity than BEP. Combining a taxane with cisplatin may hold promise as a future treatment strategy [6].

GCTs are radiosensitive. However, the only available data on radiation therapy were obtained in patients receiving palliative treatment and responses were short-lived [7].

Hormone therapy may have a role. In one patient, a third recurrence of progesterone receptor-positive GCT was treated with tamoxifen, which ensured a complete response at 22 months with no recurrence during the 5-year follow-up [8].

GCTs are often diagnosed early and consequently carry an excellent prognosis, with long-term survival rates of 75% to 90% for all stages combined [1]. These tumours grow slowly and usually have long recurrence times (mean, 6 years) [8]. Oestradiol and inhibin assays can be used to monitor patients with hormone-secreting GCTs. The most widely reported prognostic factors are FIGO stage, tumour size, intraperitoneal tumour rupture, bilateral involvement, incomplete surgical excision, and age [9-11]. However, FIGO stage may be the only independent prognostic factor [10, 12]. Cytological features of adverse prognostic significance include more than 5-10 mitoses, presence of cellular atypias, and poor differentiation [3]. Unilateral juvenile GCTs carry a better prognosis than adult GCTs.

Conclusion

Although GCTs constitute the most common sex cord-stromal tumours, the diagnosis is usually made only by the definitive histological examination. A rigorous management strategy including routine examination of the abdominal and pelvic cavity, peritoneal cytology, painstaking staging, and unilateral salpingo-oophorectomy can obviate the need for repeat staging surgery in these patients who are often young. Together with the early diagnosis, this strategy is associated with a good prognosis. However, the risk of late recurrences mandates prolonged follow-up.

References


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A rare case of ovarian Burkitt lymphoma associated tumor lysis syndrome

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Summary

A 21-year-old woman who presented with pelvic mass, fever and cough was admitted. Ultrasonography revealed a large solid mass and serum CA125 was increased. A bilateral salpingo-oophorectomy was performed and pathological diagnosis showed Burkitt lymphoma of bilateral ovaries. Adjuvant chemotherapy was administrated after surgery. However, on the next day, the patient had an unexpectedly high fever, sigh-like breathing, dilated pupils, and died despite rescue. This is the first report on the post-treatment tumor lysis syndrome with ovarian Burkitt's lymphoma. Identifying patients at risk and initiating therapy early are essential to avoid serious complications associated with tumor lysis syndrome.

Key words: Tumour lysis syndrome; Ovarian cancer; Burkitt lymphoma; Adjuvant chemotherapy.

Introduction

Tumor lysis syndrome (TLS) is a serious and potentially life threatening complication, which almost always occurs following the treatment of chemosensitive tumors, such as hematological malignancies and various solid tumors. These tumors all have a large tumor burden, a rapid tumor growth rate, and high sensitivity to cytotoxic therapy [1]. The typical clinical manifestations of TLS include nausea, vomiting, fever, chest tightness, cardiopalmus, renal colic, oliguria, and joint discomfort, but some patients may be asymptomatic. The laboratory abnormalities include hyperkalemia, hyperuricemia, hyperphosphatemia, and hypocalcemia. TLS can be very dangerous because it may induce acute renal failure and even death. Nevertheless, the optimal treatment time of TLS could more often than not be missed when the symptoms are atypical and the laboratory evidence insufficient. We report a rare case of TLS based on the ovarian Burkitt lymphoma, which calls the attention of clinicians to the high risk factors of TLS in ovarian Burkitt lymphoma.

Case Report

A 21-year-old woman, gravida 0 and para 0, was admitted to our hospital. She presented with a pelvic mass, fever, and cough. Physical examination showed that her body temperature was 37.3°C, heart rate 90 bpm, blood pressure 125/84 mmHg, and respiratory rate 18 bpm. She had no pallor, no jaundice, weakened vocal fremitus, dull percussion note, decreased breath sounds, and no cardiac murmur. Neither the liver nor the spleen was palpable. The central pelvic mass was about 20 cm in diameter.

During the patient’s stay in the hospital, she was treated with antibiotics, but her body temperature fluctuated between 37ºC and 39ºC, and her pleural fluid and ascites increased quickly as shown on X-ray examination. Thoracentesis was performed once and abdominal paracentesis three times to determine the cytoscopy and biochemical analysis of puncture fluid and to relieve the compression. Bloody hydrothorax (650 ml) and dark brown, slabby peritoneal fluid (500 ml, 4300 ml and 3900 ml, respectively) were collected. Fluid smear showed mesothelial cells in obvious proliferation, pus cells, few lymphocytes, and no malignant cells. All results of bacterial cultures including aerobic-formers, anaerobes, mycobacteria and fungus of puncture fluid and blood were negative. Results of blood routine examination for three times showed that the total white blood cell count was abnormally high and increasing with time (11.1 × 1012, 12.4 × 1012, and 20.2 × 1012, respectively), and so was the percentage of neutrophilic granulocytes (69.1%, 81.5%, and 82.5%, respectively). By contrast, the percentage of lymphocytes decreased gradually (15.8%, 12.8%, and 8.9%, respectively). The levels of serum lactate dehydrogenase (LDH) (565 U/l) and peritoneal fluid LDH (534 U/l) were elevated, and so was that of serum CA125 (517 U/l). Ultrasonography (US) of the abdomen and pelvis revealed a solid mass in the bilateral adnexal regions that measured 15 × 11 × 16 cm³. There were dark fluid regions and abundant blood flow signals in the mass.

The patient underwent exploratory laparotomy, which revealed enlarged bilateral ovaries (left 8 × 15 × 10 cm, right 7 × 5 × 6 cm) with a solid profile and multifocal necrosis. No other masses or lymphadenopathy were noted. Frozen section suggested a malignant ovarian tumor, a suspected dysgerminoma or hematopoietic tumor. Although the patient was very young, we performed bilateral salpingo-oophorectomy with her parent’s consent because her condition was critical. Final histologic examination of paraffin sections revealed Burkitt lymphoma of the bilateral ovaries. Immunohistochemical analysis demonstrated CD20 positive and CD3 negative, expressed by the neoplastic lymphoid cells.

The postoperative course was not uneventful. During the first three days after surgery, the patient had an electrolyte disturbance and high LDH levels (Table 1). With supportive and symptomatic treatments, the general condition of the patient was stable. On the fourth day, however, US showed hydrothorax again, and adjuvant chemotherapy was administrated. Cisplatin (70 mg) was transfused into the patient’s abdominal cavity and highly agglutinative staphylococcin (4000 ul) was...
injected into her pleural cavity. Quite unexpectedly though, on the morning of the fifth day, the patient had a high fever (39.7°C), sigh-like breathing, dilated pupils, and died in spite of rescue.

Discussion

Tumor lysis syndrome is a group of symptoms characterized with metabolic derangements that are caused by the massive and abrupt release of cellular components into the blood and followed by rapid spontaneous or induced lysis of malignant tumor cells. The increased concentrations of uric acid, potassium, calcium, and urea can result in all kinds of clinical symptoms of tumor lysis syndrome.

According to the definition system developed by Cairo and Bishop [2], TLS can be categorized as laboratory TLS (LTLS) and clinical TLS (CTLS). LTLS is diagnosed if two or more serum values, including potassium, calcium, phosphate or uric acid, are abnormal or increase or decrease by 25% from the baseline values which are determined within three days before treatments or seven days after treatments. CTLS is diagnosed when LTLS is present besides one or more clinical complications, including renal insufficiency, cardiac arrhythmias/sudden death, and seizures.

The patient described in the present paper underwent electrolyte disturbance but did not have TLS after surgery. However, she very soon developed TLS and died after chemotherapy. The laboratory evidence and clinical complications confirmed the existence of both LTLS and CTLS when she died. However, no obvious signs of TLS were observed before it occurred, and her condition worsened abruptly and she died shortly after. It is very likely that the patient’s lasting lower level of potassium, normal level of urea nitrogen and creatinine, and her seemingly good general condition after chemotherapy misled us into underestimating the danger of her developing TLS.

As can be seen from this case, the key to TLS management is the recognition of TLS. New guidelines have been published to address the importance of recognition of risk factors, monitoring of at-risk patients, and appropriate interventions, which was the key to preventing or managing TLS [3]. More attention should be paid to high risk factors of TLS when evidence is insufficient. Previous studies showed that high-risk factors related to the development of tumor lysis syndrome included rapid growth rate, large size, sensitivity to chemotherapy, baseline azotemia and increased LDH levels [4]. This patient had risk factors, such as a large pelvic mass, elevated LDH level, Burkitt lymphoma, and elevated body temperature, although the laboratory values of her serum potassium, calcium, uric acid and urea nitrogen did not meet the diagnostic standards. Therefore, we suggest that some protective measures should be considered for patients who do not seem to have LTLS, CTLS, or either but have risk factors of TLS before they receive chemotherapy or any other active therapies. In addition, close monitoring of electrolyte levels in patients undergoing chemotherapy is also important and necessary. Taken together, identifying patients at risk and initiating therapy early are essential to avoid serious complications associated with TLS.

Table 1. — The laboratory values of liver function, kidney function and the serum electrolytes.

<table>
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<tr>
<td>ALT (0-50 U/l)</td>
<td>13</td>
<td>19</td>
<td>24</td>
<td>24</td>
<td>65</td>
<td>242</td>
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<tr>
<td>AST (0-50 U/l)</td>
<td>24</td>
<td>33</td>
<td>14.7</td>
<td>10.3</td>
<td>191</td>
<td>1055</td>
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<tr>
<td>TBIL (2-24 umol/l)</td>
<td>18.4</td>
<td>13.5</td>
<td>2.23</td>
<td>2.36</td>
<td>3.77</td>
<td>8.2</td>
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<tr>
<td>LDH (109-346 U/l)</td>
<td>565</td>
<td>585</td>
<td>129.3</td>
<td>132.8</td>
<td>129.4</td>
<td>97.9</td>
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<tr>
<td>UN (1.78-7.14 mmol/l)</td>
<td>5.35</td>
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<td>3.25</td>
<td>2.23</td>
<td>6.22</td>
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<td>CREA (30.6-97.3 umol/l)</td>
<td>48</td>
<td>40</td>
<td>31</td>
<td>32</td>
<td>53</td>
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<td>K (3.5-5.5 mmol/l)</td>
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<td>3.96</td>
<td>3.69</td>
<td>2.23</td>
<td>2.36</td>
<td>3.77</td>
<td>8.2</td>
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<tr>
<td>Na (135-145 mmol/l)</td>
<td>134.7</td>
<td>136</td>
<td>128.6</td>
<td>129.3</td>
<td>132.8</td>
<td>132.4</td>
<td>97.9</td>
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<tr>
<td>Cl (96-108 mmol/l)</td>
<td>96.8</td>
<td>94.9</td>
<td>84.2</td>
<td>86.6</td>
<td>89.1</td>
<td>89.6</td>
<td>123.1</td>
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<tr>
<td>Ca (2.1-2.6 mmol/l)</td>
<td>2.11</td>
<td>2.24</td>
<td>1.95</td>
<td>1.57</td>
<td>1.8</td>
<td>1.92</td>
<td>0.82(0.98-1.45)</td>
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<tr>
<td>Mg (0.7-1.1 mmol/l)</td>
<td>0.92</td>
<td>0.88</td>
<td>0.83</td>
<td>0.79</td>
<td>0.77</td>
<td>0.71</td>
<td>0.61(0.51-0.73)</td>
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ALT: alanine aminotransferase; AST: aspartate amino transferase; TBIL: total bilirubin; LDH: lactate dehydrogenase; UN: urea nitrogen; CREA: creatinine.

*Showed the calibrations of referenced values. # Refers to low-abnormality. Bold italic figures refer to high-abnormality.

References


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Primary adenocarcinoma of the rectovaginal septum arising in pregnancy in the absence of endometriosis

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Summary

A case of primary adenocarcinoma of the rectovaginal septum (PARVS) is reported with clinical and pathological findings. A 37-year-old Caucasian woman with a history of sterility and small posterior leiomyoma, a few months after a cesarean section, was admitted because of vaginal spotting, abdominal pain and constipation. Her previous history did not reveal exposure to diethylstilbestrol (DES). Pelvic computed tomography showed a heterogeneous pelvic mass in the Douglas pouch, measuring 9 cm in diameter, located in the rectovaginal septum, involving the rectal and vaginal wall. Histological examination of neoplastic tissue revealed solid sheet structures, occasional tubular lumen, extensive necrotic areas and clear cells. The neoplastic elements showed immunoreactivity for Mullerian markers (cytokeratin 7, CA-125 and vimentin). Because, the present case of PARVS cannot be due to DES exposure, the clear appearance of the neoplastic elements could represent only one differentiation of Mullerian rests. Moreover, because no foci of endometriosis were identified in several sections of the neoplasm, uterine and cervical wall, and tissues nearby the neoplasm could represent a rare subtype of PARVS arising in the absence of endometriosis.

Key words: Adenocarcinoma; Rectovaginal septum; Immunohistochemical staining.

Introduction

Primary adenocarcinoma of the rectovaginal septum (PARVS) is a very rare neoplasm, with only about 20 cases reported in the literature [1]. The majority of cases are associated with benign endometriosis [2-8].

PARVS without endometriosis is even rarer, because only four previous cases have been described in the literature [1]. In this paper, another case of PARVS arising in the absence of endometriosis is reported. Immunohistochemical study and a review of the literature were performed to clarify the clinicopathological features of this rare malignancy. Moreover, peculiar histological findings which were not observed previously are discussed.

Case Report

A 37-year-old Caucasian woman with a history of sterility and small posterior leiomyoma a few months after a cesarean section was admitted because of vaginal spotting, abdominal pain and constipation. Rectal and vaginal examination revealed a pelvic mass emerging on both the posterior vaginal and anterior rectal surfaces as ulcerated lesions.

Her medical history revealed ovarian stimulation treatment for infertility the two previous years. The patient denied previous hormone therapy and intrauterine exposure to diethylstilbestrol (DES).

Preoperative tests showed high serum CA-125 and CA 19.9 levels. Pelvic computed tomography (CT) showed a heterogeneous pelvic mass in the Douglas pouch, measuring 9 cm in diameter. The lesion, located in the rectovaginal septum, showed central necrotic areas and involved the rectal and vaginal wall with no clear anatomic planes being identifiable (Figure 1A). The uterus, the remaining pelvic organs and the peritoneum were free of disease.

A part of the lesion emerging on the vaginal mucosa was biopsied and microscopic examination revealed infiltration of a poorly differentiated adenocarcinoma. An en bloc resection of the tumor, including the proximal vagina, a low colorectal resection with end-to-end anastomosis, and a hysterectomy with bilateral salpingo-oophorectomy were performed.

After one year from diagnosis and after six cycles of combined chemotherapy with carboplatin and taxol, the patient was alive with hepatic and pulmonary metastases. No recurrence, instead, was observed in the pelvic region.

Pathological findings

On macroscopic examination, the surgical specimen showed a large white mass with central necrotic areas, located in the rectovaginal septum. The lesion had infiltrated the rectal and vaginal walls and emerged on both the posterior vaginal and anterior rectal surfaces as ulcerated lesions, measuring 1 cm in diameter (Figure 1B). No endometriosis was found either in the rectovaginal septum or in the genital tract organs.

Neoplastic tissue was fixed in 10% buffered formalin, processed and embedded in paraffin. Five-micron sections were stained with hematoxylin and eosin. Using the avidin-biotin peroxidase complex method, antisera direct to the following antigens were applied: pancytokeratin (Clone: AE1/ AE3, Dilution: 1:1000; DAKO) cytokeratin 7 (CK7) (Clone: OV-TL12/30, Dilution: 1:100; DBS Pleasanton), Ca-125 (Clone: OC125, Dilution: 1:100, DAKO), Monoclonal Carcinoembryonic antigen (CEA) (Clone: II-7, Dilution: 1:500, DAKO), Vimentin (Clone: Ab-2V9, Dilution: 1:10000, Neomarkers) estrogen receptors (ER) (Clone: SP1, Dilution: 1:100, Neo-markers) and progesterone receptors (PGR) (Clone: PGR636, Dilution: 1:200, DAKO).

On microscopic examination, the neoplasm was characterized by solid sheet structures, occasional tubular lumen and exten-
sive necrotic areas. In many areas, there were clear cells, characterized by moderate-severe cytological atypia, with bizarre multinucleated cells (Figure 1C) and up to seven mitoses per high power field were identified in the most heavily affected areas.

On immunohistochemical analysis, the neoplastic cells were positive for pancytokeratin, cytokeratin 7 (CK7), CA-125, vimentin and negative for ER, PGR and CEA.

The final pathologic diagnosis was primary adenocarcinoma of the rectovaginal septum arising in the absence of endometriosis.

Discussion

Primary rectovaginal septal masses can be primary malignant or benign lesions that have developed in the connective tissue of the rectovaginal space. Examples of benign lesions, observed in the rectovaginal septum, include neurilemmoma (schwannoma) [9] or endometriosis [10], while malignant masses include extragastrointestinal stromal tumor [11], extra-osseous Ewing’s sarcoma [12] and leiomyosarcoma [13].

PARVS represents another malignant lesion of the rectovaginal septum. This lesion is a very rare, with only about 20 cases reported in the literature [1]. The majority of PARVS are associated with benign endometriosis [2-8]. PARVS without endometriosis is rarer, because only four previous cases have been described in the literature [1].

Like all masses of the rectovaginal septum, clinically PARVS can be characterized by uterine prolapse [14], urinary retention [15], vaginal or rectal bleeding, abdominal pain and dyspareunia [16].

Colonoscopy shows indirect signs of external compression and, sometimes, negative biopsies [16].

Techniques of medical imaging, such as CT, can reveal the presence of a neoplasm of the rectovaginal septum, but this technique often fails to make a diagnosis because of the rarity of the lesion and because a mass in the rectovaginal septum can be mistaken for a uterine or ovarian tumor [16].

In our case, the lesion already observed during pregnancy, had been misinterpreted as a leiomyoma.

The diagnosis of PARVS can be established by CT-guided biopsy [1] or by a laparotomy with a large biopsy [15], revealing the features of epithelial malignant lesions.

Histological types of PARVS described in the literature are serous papillary adenocarcinoma [2-4, 17], adenocanthoma [18] and clear cell adenocarcinoma [16, 19].

Pathological diagnosis of PARVS can pose particular problems on biopsy examination because this neoplasm must be differentiated from carcinomas of other pelvic organs and metastasis from primary neoplasms elsewhere. Thus, immunohistochemical analysis plays an essential role in making the correct diagnosis of PARVS, revealing Mullerian differentiation. Therefore, markers, such as CK 7, vimentin, and CA-125 are vital in distinguishing this rare entity from rectal adenocarcinoma and cervical and vaginal squamous carcinoma.

In addition, the clear cell appearance and age of our
patient could have brought about a diagnosis of primary vaginal clear cell carcinoma. In our view, this diagnosis can be excluded because the neoplasm principally involved the rectovaginal space and infiltration of the vaginal wall represents only a late event [20, 21].

Since no foci of endometriosis were identified in several sections of the neoplasm, uterine and cervical wall, and tissues nearby the neoplasm, in our case the final pathological diagnosis could be primary adenocarcinoma of the rectovaginal septum, arising in the absence of endometriosis.

However, the absence of foci of endometriosis does not represent any evidence that the present case of PARVS did not develop from rectovaginal endometriosis.

In accordance with some authors, we believe that neoplastic overgrowth could cause destruction of the endometriosis [15].

Moreover, because our patient denied intrauterine exposure to DES, the present case cannot be considered a subtype of PARVS related to DES use, and clear cytoplasm of its neoplastic elements could represent only one differentiation of Mullerian rests due to pregnancy.

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References


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Primary serous papillary carcinoma of the peritoneum mimicking pelvic actinomycosis: a case report and brief literature review

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Summary

Primary serous papillary carcinoma of the peritoneum (PSPCP) is a rare tumor that diffusely involves the peritoneal surface [1]. Primary peritoneal carcinoma (PPC), is histologically identical to epithelial ovarian carcinoma (EOC); however, it is differentiated from EOC based on the absence of gross ovarian involvement, and if present, only minimal microscopic invasion of the ovarian stroma. Both EOC and PPC can present with carcinomatosis. PSPCP has previously been referred to by many names including mesothelioma, papillary carcinoma of peritoneum, serous surface papillary carcinoma, and extraperitoneal serous carcinoma, reflecting the controversy surrounding its histogenesis and clinical behavior [2, 3]. We report the case of a 59-year-old woman with serous peritoneal carcinoma mimicking actinomycosis.

Introduction

Primary serous papillary carcinoma of the peritoneum (PSPCP) is a rare tumor that diffusely involves the peritoneal surface [1]. Primary peritoneal carcinoma (PPC), is histologically identical to epithelial ovarian carcinoma (EOC); however, it is differentiated from EOC based on the absence of gross ovarian involvement, and if present, only minimal microscopic invasion of the ovarian stroma. Both EOC and PPC can present with carcinomatosis. PSPCP has previously been referred to by many names including mesothelioma, papillary carcinoma of peritoneum, serous surface papillary carcinoma, and extraperitoneal serous carcinoma, reflecting the controversy surrounding its histogenesis and clinical behavior [2, 3]. We report the case of a 59-year-old woman with serous peritoneal carcinoma mimicking actinomycosis.

Case Report

A 59-year-old woman presented with complaints of lower left abdominal pain for about two months. The patient had a normal appetite and there was no history of chronic disease such as diabetes mellitus or tuberculosis. The patient had a history of a right salpingo-oophorectomy. Physical examination revealed tenderness in the lower left abdomen but no palpable mass. The body temperature was 37.7°C. Laboratory tests revealed a white blood cell count of 9,100/µl, C-reactive protein (CRP) level of 128.43 mg/l, and erythrocyte sedimentation rate (ESR) of more than 120 mm/hr. The serum levels of CA 125, CA 19.9 and CEA were 649.0 kU/l, 5.7 kU/l and 158.7 kU/l.

An ultrasound (US) was performed which showed an inflammatory or cancerous mass that appeared to originate from the left adnexa. Abdominal-pelvic computed tomography (CT) and pelvic magnetic resonance imaging (MRI) revealed about a 10 cm poorly defined mass composed of solid and cystic portions. The mass infiltrated adjacent tissues including the rectosigmoid colon, distal ascending colon and small bowel. The wall of the small bowel and colon were thickened. CT and MRI findings suggested an inflammatory mass such as an actinomycosis. However, a tumor of the ovary or gastrointestinal tract with perforation or inflammation, could not be ruled out (Figure 1). Colonoscopy and gastroscopy were within normal limits.

The patient received antibiotic therapy (ampibactam, 50 mg/kg, 24-hr continuous infusion) for one week. The mass did not respond to the antibiotic therapy; the presence of a malignant tumor could not be excluded, and laparotomy was performed. The laparotomy revealed about a 10 cm sized mass with severe adhesion to the omentum, bowel and peritoneum. After adhesiolysis, mass excision was performed. The laparotomy revealed about a 10 cm sized mass with severe adhesion to the omentum, bowel and peritoneum. After adhesiolysis, mass excision was performed. Frozen biopsy results were consistent with serous carcinoma – possibly originating from the ovary. On section, the cut surface showed multiple growing papillary masses. Subtotal hysterectomy, left salpingo-oophorectomy and omentectomy were performed. The appendix was not visible. There was no sign of seeding on the surface of the liver, spleen and pancreas.

On microscopic examination, a diffusely infiltrating tumor was seen with an aggressive papillary growth pattern with a typical slit-like appearance. The tumor cells were highly malignant with irregular, pleomorphic nuclei and prominent nucleoli. The tumor closely invaded the soft tissue adjacent to the left ovary and salpinx, the former of which was not grossly enlarged. However, no actual tumor invasion of the ovary or salpinx was confirmed microscopically. The uterus was free of tumor as well. Multiple biopsies from the omentum, abdominal and pelvic peritoneum showed widespread tumor infiltration with the same histological features and confirmed the diagnosis of primary peritoneal carcinoma, or primary serous papillary carcinoma of the peritoneum (Figure 2). The patient received adjuvant chemotherapy with taxol and carboplatin.
Discussion

PSPCP is a rare type of peritoneal neoplasm with a reported incidence of up to 7.5% of all cases clinically suspected to be epithelial ovarian carcinoma (EOC) [4]. The clinical presentation results from local tumor effects involving multiple organs. The most common presenting complaints are ascites, an abdominal mass and pleural effusion. The symptoms may be vague and mild until the disease is advanced [5, 6]. Because the symptoms of PSPCP are similar to those of EOC, the preoperative assessment is the same as it is for woman suspected of having ovarian cancer.

Surgery is important for both the diagnosis and treatment of PSPCP. If the ovaries appear normal with widespread disease elsewhere in the abdomen, PSPCP becomes the leading diagnostic possibility. However, the distinction between PPC and EOC may only be definitive after histological examination, where evaluation of the extent of ovarian invasion by the tumor is performed; surface involvement of the ovaries is present in approximately 96% of cases. Given the similarities between PSPCP and EOC, treatment of PPC is the same as for serous ovarian papillary carcinoma.

The patient presented with findings suggestive of inflammation such as pelvic actinomycosis. The history of mild fever and left lower abdominal pain supported this diagnosis. Abdomino-pelvic CT and pelvic MRI findings suggested an inflammatory mass such as a pelvic actinomycosis. The common findings on abdomino-pelvic CT for PSPCP include: ascites, omental involvement from lace-like omental infiltration to frank omental masses, irregular parietal peritoneum thickening, and mural thickening of the sigmoid colon. The differential diagnosis of diffuse peritoneal involvement includes: metastatic peritoneal carcinomatosis mainly from ovarian cancer, benign disease such as tuberculosis, a malignant neoplasm of the GIT, pseudomyxoma peritonei, and malignant peritoneal mesothelioma [7-9].

Recently, Vasileios et al. described the imaging features of PSPCP on CT and MRI. The MRI findings were similar to the findings of CT imaging including: ascites, peritoneal thickening and enhancement, and peritoneal nodules or a mass. However, the MRI made it possible to identify the ovaries, which were not detected by CT. Imaging of the ovaries is important to exclude ovarian cancer. MRI has been reported to be a very sensitive modality for the assessment of peritoneal disease [10].

The usual CT findings of pelvic actinomycosis include: an abscess, an infiltrative mass with dense and non-homogenous contrast enhancement, and thickening of the bowel [11]. Pelvic MRI findings of pelvic actinomycosis showed the presence of a mass of relatively low signal intensity on T2 weighted sequences in association with...
extensive pelvic infiltration. The co-existence of infiltrative changes in the uterus, bladder, and the visualization of a tract in the posterior cervix, were all helpful findings supporting the clinical suspicion of pelvic actinomycosis [12].

In conclusion, PSPCP is a rare entity. It has characteristics similar to other peritoneal diseases. The correlation of clinical presentation with radiology findings, surgical staging, and histological analysis is essential for determining the correct diagnosis. We treated a patient with PSPCP where the initial CT and MRI findings suggested actinomycosis.

References

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Cervical cancer metastasis to the scalp: case report and literature review

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Summary

Background: Distant metastasis in carcinoma of the uterine cervix is a rare manifestation, with scalp metastasis being an exceptional event. Case: We describe a 48-year-old woman with Stage IIb, squamous cell carcinoma of the cervix who was initially treated with radical hysterectomy and lymphadenectomy including paraaortic nodes. The patient presented eight months later with swelling over the top of the scalp. The scalp was involved in the disease as the sole anatomic site of distant metastasis. Conclusion: A search of the literature revealed only seven cases of such distant metastatic involvement of the scalp from cervical cancer. More frequent reports and better understanding on these rare events may give new insight to clear strategies to prevent scalp metastases.

Key words: Uterine cervical cancer; Scalp metastasis; Chemoradiotherapy.

Introduction

Spread of cervical cancer usually occurs through direct local extension and distantly by invading lymphatics. Hematogenous spread is rather uncommon, although such metastases may occur to abdominal and thoracic viscera or to bone, in decreasing order of frequency [1]. Scalp involvement is extremely rare, with only six indexed cases in published reports (Table 1). We report the case of a patient with cervical cancer who presented with metastasis to this very unusual site.

Case Report

A 48-year-old premenopausal woman was diagnosed as having squamous cell carcinoma of the cervix. Her lesion was typed as FIGO Stage IIb, and she was treated by radical hysterectomy and pelvic lymphadenectomy including paraaortic nodes. At gross examination the tumor measured 4.0 x 2.5 x 3.0 cm and extended into the bilateral parametrium. The microscopic report showed invasive squamous cell carcinoma, nonkeratinizing small cell type, nuclear grade 2. The paracervical soft tissue, uterine corpus, fallopian tubes, ovaries and all the resection margins (including the vaginal cuff) were histologically uninvolved. Thirty-one of the 41 lymph nodes dissected harbored tumor metastases. The patient was offered concomitant postoperative adjuvant chemoradiotherapy, considering the risk factors identified in the specimen. She was treated with four cycles of combination chemotherapy with paclitaxel (175 mg/m²) and carboplatin (AUC 5) with concurrent external radiotherapy 45 Gy in 20 fractions over a 4-week time period.

The patient was on regular follow-up and did well for eight months when she reported a 2-week history of a rapidly growing, painful swelling with erythema of overlying skin on the head. There was no history of vomiting or symptoms suggestive of raised intracranial pressure. Examination showed a 2 x 2 cm firm, tender, noncompressible, nonpulsatile swelling over the midline frontal scalp fixed to the underlying skull. As shown in Figure 1, magnetic resonance imaging (MRI) of her cranium confirmed the presence of the scalp mass with involvement of both tables of the occipital skull with osteolytic change. There was no evidence of direct invasion into the brain parenchyma or superior sagittal sinus. Systemic evaluation including whole-body computed tomography (CT) and tumor scintigram did not show any other foci of metastases. Fine-needle aspiration cytology showed metastatic squamous cell carcinoma with extensive necrosis. The cytological features were similar to the cervical primary mass. The patient was started on a course of palliative radiotherapy (total dose = 45 Gy/15 fractions/3 weeks). She observed significant relief from pain and there was partial regression of swellings, but declined any chemotherapy.

Figure 1. — T2-weighted post-gadolinium magnetic resonance coronal image showing midline scalp swelling with osteolytic change to both tables of the skull (arrow). Enhanced subdural space is seen. Inset: scintigram shows clearly enhanced uptake in scalp swelling (arrow).
Discussion

This report described a case of squamous cell carcinoma of the uterine cervix in a patient who presented with isolated scalp metastasis. Cervical cancer commonly spreads through direct local extension, and when distant spread occurs, it is usually because of lymphatic invasion. Hematogenous spread in cancer is a late process and usually is seen to spread to liver, lungs and bones, especially in poorly differentiated subtypes [1, 2]. As summarized in Table 1, a review of the reported cases suggests that occurrence of scalp metastases does not appear to be related to initial stage of presentation. It is detected in patients treated for early as well as advanced stages of disease (Table 1). The location of the scalp involved and number of lesions in the scalp were varied and nonuniform [2-6]. Except for a report from Park et al. [6], these patients had the scalp as the sole site of metastasis. In contrast to the five prior cases of scalp metastases of cervical squamous cell carcinoma reported in published communications, Abhishek et al. [2] documented the first case of such an occurrence in cervical adenocarcinoma.

We present the seventh case of scalp metastasis following radical hysterectomy and concurrent chemoradiotherapy for FIGO Stage Ib cervical cancer with histopathology showing a small cell non-keratinizing squamous cell carcinoma. Although our patient had involvement of pelvic lymph nodes including paraaortic nodes, the lack of mediastinal or supraclavicular lymph node involvement makes retrograde lymphatic spread very unlikely for explaining isolated hematogenous involvement of the scalp from distant malignancies. Abhishek et al. [2] attributed the presence of rich vascularity, warmth and immobility to explain metastases in the scalp region. Another hypothesis that has been used to explain this kind of spread involves interaction between tumor cells and endothelial cell receptors in the target organs. This possibility was first described by Nicolson et al. [7] to explain the tendency of cancer cells to metastasize to specific and rare organs.

In conclusion, isolated metastatic involvement of the scalp from a primary lesion in the cervix, under local control, is an extremely rare feature of distant metastatic presentation. The prognosis of patients with bone metastasis is dismal and most die within several months after discovery of a metastatic lesion [3]. Due to poor prognosis the policy of treatment should be directed toward maintaining quality of life, rather than drastic treatment trials. Although the patient is still under intensive therapy, the dismal prognosis of this event prompted us to report. With growing technical advances and knowledge, we can expect more frequent reports and better understanding of the mechanisms and natural history of these rare manifestations, thereby leading to clear strategies to prevent scalp metastases.

References


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Ovarian metastasis following gallbladder carcinoma: a case report

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Summary

Background: Mucinous ovarian cancer raises problems of differential diagnoses because it is often difficult to distinguish the primary from the metastatic form. Most metastatic ovarian tumors originate from the gastrointestinal tract, mainly colorectal, gastric, pancreatic; the gallbladder is a very rare source of ovarian metastases. Case: We report a case of ovarian metastases from a gallbladder cancer, incidentally diagnosed more than 2.5 years earlier during a laparoscopic intervention for biliary lithiasis. Conclusion: The interest of this case lies in the long progression-free survival, the venous thromboembolism syndrome that preceded by a few months the diagnosis of the ovarian mass and the discrepancy between the radiologic and the laparoscopic stage assessment.

Key words: Gallbladder carcinoma; Ovarian metastasis; Trousseau syndrome.

Introduction

Gallbladder cancer is rare. About 3,000 deaths from extra-hepatic biliary tract neoplasms were recorded in Italy in 2002, representing 1.3% of all cancer deaths in men and 2.7% in women [1]. In Western countries, the incidence is higher in women (about 1.5/100,000/year) and in people aged over 65 years [2]. Because symptoms are not very specific and can be confounded with biliary lithiasis, gallbladder cancer is often identified incidentally during laparoscopic resection for gallstones or it is diagnosed at a very advanced stage, when pain and/or jaundice appear. Prognosis is largely influenced by the stage at diagnosis, being very poor for Stage IV tumors (median survival: 3-6 months) and favorable for in situ tumors (about 80% of patients are alive at 5 years). Although the definition of Stages II and III have been amended in the sixth edition (2002) of the TNM-AJCC Cancer Staging system with respect to the fifth edition (1997), in general we can say that long-term outcome depends on the depth of invasion of the gallbladder wall, and that prognosis is worsened by the presence of lymph node metastases, and by venous, lymphatic or perineural invasion [3]. Neither adjuvant chemotherapy nor adjuvant radiotherapy has significant advantages in terms of survival.

Ovarian metastases from biliary carcinoma are rare [4-6]. Most are synchronous diseases, i.e., a pelvic mass that leads to the diagnosis of primary gallbladder cancer. The case we describe is intriguing because the ovarian mass became evident 32 months after resection of the gallbladder tumor and it was difficult to distinguish from a primary ovarian malignancy.

Case Report

A 55-year-old woman was admitted to the Department of Medical Oncology of the University of Naples Federico II (Italy) in July 2008 after a total body computed tomography (CT)-scan revealed ascites, two liver nodules, multiple subcutaneous nodules within the abdominal wall, multiple abdominal enlarged lymph nodes and a huge pelvic mass involving both ovaries (Figure 1A, B). Serum levels of tumor markers were elevated (CEA = 42 ng/ml; CA 19.9 = 10,000 U/ml; CA 125 = 281 U/ml).

The pathological anamnèsis revealed that the patient had received an incidental diagnosis of the gall bladder adenocarcinoma in November 2005 during a laparoscopic resection of the gallbladder for biliary lithiasis. A second operation, performed in December 2005 to verify R0 resection, revealed no residual neoplasm on the liver bed (IV and V hepatic segment), but a lymph node behind the principal biliary duct proved to be metastatic (final stage was pT2,N1; TNM Stage IIB, AICC Cancer Staging Manual, sixth edition, 2002). The patient received adjuvant chemotherapy (5-fluourouracil plus lederfolin) from February to June 2006.

Between April and June 2008, the patient experienced recurrent episodes of venous thrombosis in the left jugular vein, the parva saphena of the left leg and in the left brachial vein, and was, thus, on therapy with warfarin sodium.

In August 2008, abdominal laparoscopy was carried out for staging and histological definition of the neoplasm: a large mass originating from the right ovary occupied all the pelvis, up to the umbilical region; about 150 cc of bloody ascites were aspirated; there were no macroscopic tumor deposits on the parietal peritoneum, abdominal wall, liver, stomach, or colorectal surface and no enlarged lymph nodes were observed. The anterior peritoneum wall and the right ovary were biopsied, and the histological analysis indicated a “mucinous, borderline, G2 adenocarcinoma of the ovary wall”; the peritoneum was free from neoplasia. At immunohistochemistry, sections were cytookeratin-7 positive, cytookeratin-20 negative and weakly CEA-positive. Cytology of the ascites revealed no tumor cells. The same pathologist re-analyzed the paraffin-embedded tissues obtained during surgery in 2005 and confirmed the diagnosis of adenocarcinoma infiltrating the gallbladder wall (Figure 2A) and one
regional lymph node; at immunohistochemistry, neoplastic cells were CK7+, CK20- and focally CEA+.

Because the bilateral ovarian mass was judged operable at laparoscopy, in September 2008, the patient underwent radical hysterectomy, resection of the bilateral ovarian masses, omentectomy and regional lymphadenectomy. Exploration of the abdominal cavity and the liver surface did not reveal any macroscopic neoplastic deposit; no residual disease after surgery was present (macroscopically R0). Histological diagnosis was bilateral ovarian mucinous adenocarcinoma infiltrating the capsules of both ovaries, the Douglas cavum, and three lymph nodes near the left iliac artery; a cluster of signet-ring cells was present on the right ovary (Figure 2B). The bilateral tumor, the neoplastic cells on the surface of the ovaries, the focal signet-ring cell area and the previous gallbladder adenocarcinoma suggested a diagnosis of metastasis from the biliary-tract tumor.

Paraffin-embedded tissues of the gallbladder, regional lymph nodes (resected in 2005) and ovarian mass (resected in 2008) were examined at the Department of Pathology at the Massachusetts General Hospital, Boston, USA, and the results of the consultation confirmed a metastatic mucinous ovarian adenocarcinoma form gallbladder tumor.

On October 2008, the patient became symptomatic with abdominal pain, fever, dyspnea, cough, anorexia and fatigue. She then underwent restaging, and a total-body CT scan showed multiple enlarged lymph nodes in both the mediastinum and abdominal cavity; one nodule (25 mm diameter) in the left breast; two metastatic nodules (20 and 27 mm, respectively) at the VI hepatic segment; a mass (16 x 14 mm) behind the gastric antrum; multiple subcutaneous and infra-muscular nodules in the abdominal wall (maximum diameter, 29 mm); and partial thrombosis of the pulmonary arteries, the left vena brachiocephalica, the sub-renal tract of the inferior vena cava, the left vena iliaca communis and the right vena femoralis. Esophagogastroduodenoscopy did not show pathologic involvement of the gastric mucosa.

In November 2008, the patient started chemotherapy with gemcitabine + oxaliplatin. After four cycles of chemotherapy, performance status (ECOG scale) increased from 2 to 0; and a CT-scan showed complete resolution of the thrombosis of the pulmonary arteries and the inferior vena cava, disappearance of the nodule in the left breast, and substantial reduction in the size of all the mediastinic and abdominal lymph nodes and nodules in the abdominal wall, and a minor response of liver metastases, with no new lesions.

Discussion

In Western countries, about 10% of ovarian neoplasms are metastatic tumors, mostly originating in the gastrointestinal tract (colon, appendix, stomach or pancreas). The differential diagnosis of mucinous ovarian carcinoma remains an intriguing challenge for the pathologist, especially when the ovarian mass is metachronous. Both microscopy and immunohistochemistry are useful but not determinant in distinguishing primary from metastatic disease. Cytokeratine expression is routinely performed because CK7 staining is present in virtually all primary ovarian tumors, and CK20 is very frequently expressed in colorectal and gastric tissue. However, CK7 positivity is
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also present in many gastrointestinal tumors, and about 75% of primary mucinous ovarian cancers have patchy CK20 staining [7]. Pathological anamnesis and gross features of the tumor mass may also aid diagnosis: bilaterality, advanced stage and surface tumor deposits are characteristics of metastatic disease, whereas unilateral, large ovarian masses suggest a primary tumor. Benign or borderline areas generally indicate a primary ovarian neoplasm, but these features are found in about 30% of intestinal carcinomas [7].

The long-term outcome for patients radically resected for a pT2N1 gallbladder cancer is unfavorable, median survival being less than 20 months [8]. Several retrospective analyses suggest that postoperative radiotherapy can prolong survival of patients with T2 gallbladder carcinoma invading the subserosal layer". J. Am. Coll. Surg., 2001, 192, 600.

Migrant superficial thrombophlebitis (historically called “Trousseau Syndrome”) is a paraneoplastic phenomenon mostly associated with pancreatic or lung tumors. About 5-10% of cases with unprovoked venous thromboembolism subsequently receive a diagnosis of a previously undetected tumor [10]. Interestingly, in our patient this syndrome preceded by a few months the diagnosis of the large ovarian mass subsequently identified as metastases from a gallbladder tumor, whereas deep venous thrombosis, requiring permanent anticoagulant treatment, occurred during advanced disease.

In conclusion, it is important to suspect metastatic disease when assessing a mucinous ovarian mass, and in case of multiple recurrent venous thrombophlebitis.

References

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Figure 2. — Adenocarcinoma infiltrating the muscle wall of the gallbladder (hematoxylin and eosin x 110) [2A]; mucinous adenocarcinoma extensively infiltrating the ovarian parenchyma (hematoxylin and eosin x 206). Note the focal mucinous differentiation at the inner left corner [2B].
Granular cell tumor of the female genital system. 
Clinical and pathologic characteristics of five cases 
and literature review

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Introduction
Granular cell tumor is a rare neoplasm of neurogenic-Schwann cell origin [1, 2], also known as Abrikossoff tumor because it was first described by Abrikossoff in 1926 [3]. It presents as a painless tumor, solitary (90% of the cases), in the soft tissues of the head and neck region (35%) in the skin and subcutis (30%) and in the viscera, mainly the respiratory tract (35% of the cases).

It affects mainly adults and females (male/female ratio is 1/3).

In 7-10% of the cases this rare neoplasm develops in the female genital system, mainly in the skin of the labia majora and the breast [4, 5]. The mean age of patients is 32 years (range 16-68) [4]. The tumor is considered as benign and the rate of recurrence after removal is rare (1-2%) [6].

We present the clinical and pathological characteristics of a series of granular cell tumors studied in Pathology Laboratory during the period 1998-2008, located in the vulva (four cases) and the breast (one case). Additional immunohistochemical investigation was performed at new sections from the paraffin blocks.

Case Reports
Four patients, 35-58 years of age presented with solitary painless tumors in the subcutis of the labia majora (left 3/4) and underwent an excisional biopsy.

The pathological examination revealed granular cell tumors measuring from 0.8-1.7 cm which were totally excised. No recurrence or metastasis occurred at a follow-up period of one to three years.

The fifth case, a 42-year-old patient, presented with a painless breast tumor which mammogram ultrasound (US) and breast examination showed to be consistent with a soft tissue lesion. The fine needle biopsy examination was inconclusive.

The patient underwent an excisional biopsy after a frozen section biopsy negative for cancer. Pathological examination revealed a granular cell tumor of the breast subcutis. No involvement of the breast tissue was observed. No recurrence or metastasis was noted in the follow-up period.

Pathological findings

In hematoxylin-eosin stained sections from all our cases the tumors presented similar morphology. Tumor cells were arranged in bundles, fascicles, cords and sheets, which were polygonal or spindle shaped with uniform round nuclei. Most distinctive was the cytoplasm which was abundant, granular and eosinophilic (Figure 1).

No mitotic activity or necrosis was observed. The covering epidermis was intact and showed prominent hyperplastic changes in 4/5 cases. The rete pegs, in 3/5 cases extended up to the tumor cells and deeply in the dermis, presenting a differential diagnostic problem from squamous cell carcinoma (Figure 2).

Immunohistochemical investigation by Ventana Automatic System showed a positive immunoreaction of the neoplastic cells to S100 (polyclonal Dako Ab) and focal reaction to NSE (polyclonal Dako Ab) and negative to cytokeratins, CK 5/6 (monoclonal, Dako) and 34bE12 (monoclonal, Cell Marque).

Cytoplasmic granules also stained positive to periodic acid Schiff (PAS) cytochemical stain and were resistant to diastase digestion.

Discussion
Granular cell tumor is described by various names such as granular cell myoblastoma, Abrikossoff tumor, granular cell shwannoma, granular neurogenic tumor, newborn epulis and myoblastic myoma.

Granular cell tumor is a typically smaller than 3 cm solitary tumor located in the dermis or subcutis and less commonly in the submucosa or the smooth muscle [7, 8]. It presents a slow growth rate and when superficial no ulceration of the epidermis is reported [7, 8]. The usual presentation is that of a slow growing, nontender nodule in any body site. Approximately, 25% of such lesions...
Granular cell tumor of the female genital system. Clinical and pathologic characteristics of five cases and literature review

occur on the tongue, 15% on the skin, 5% in the mammary glands, and 5-16% in the vulva [7-11]. It may develop in the viscera as well, with a predilection to lung tissues.

Among the genital organs, the labia majora of the vulva is the predominant site, and rarely it arises at the clitoris [12]. Grossly it is a pale white-yellowish nodule, usually well-circumscribed with solid fleshy cut surface.

The pathological examination usually reveals large polyhedral neoplastic cells with eosinophilic granular cytoplasm, small vesicular or hyperchromatic round to oval nuclei separated by fibrous septa and collagen [2, 8, 13]. The granularity of such tumor cells may be the expression of the accumulation of secondary lysosomes in the cytoplasm [2, 8, 13]. The lesions are usually positive for S100, vimentin, CD68 and neural-specific enolase but negative for CD57 and epithelial (EMA), melanocytic (Melan A), smooth muscle, dendritic cell and endothelial markers. The positivity for calretinin, S100 protein and neurospecific enolase excludes a histiocytic or a myoblastic origin and confirms the neural origin of this tumor [2, 8, 13].

Clinicians and pathologists should be aware of such an entity. The differential diagnosis includes schwannomas, basal or squamous cell carcinomas, melanomas and fibrous histiocytomas. In the differential diagnosis verruca simplex, condyloma acuminate, verruciform xanthoma, vulvar intraepithelial neoplasia, bowenoid papulosis, erythroplasia of Queyrat and verrucous carcinoma should be considered [10, 14].

The treatment of choice is conservative excision in all such tumors followed by a strict follow-up [1, 10]. All the tumors in our patients were completely excised with clear margins. Radiation and chemotherapy are not necessary for further treatment. Recurrence rates could reach 2-8%, however no recurrence or malignancy was observed in our patients.

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Borderline tumour arising in a transposed ovary in utero: a rare complication of the Estes operation

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Summary

Ovarian transposition into the uterine cavity to restore fertility, the so-called Estes operation, has rarely been performed worldwide. Malignant degeneration of such transposed ovaries has never been reported in the literature. We present a case of a borderline tumour arising in a transposed ovary after an Estes operation.

Key words: Estes operation; Borderline tumour; Transposed ovary.

Introduction

Borderline ovarian tumours comprise 10-15% of all ovarian malignant tumours. In contrast to ovarian carcinoma, borderline tumours occur more often in younger patients and carry a favourable prognosis [1]. Surgical treatment of borderline tumours may be either radical or conservative depending on the wish of the patient to preserve her fertility.

In the pre-IVF era patients with severely damaged or resected fallopian tubes were deemed childless. In an attempt to restore fertility, several centres have performed the Estes operation in such patients [2]. During this procedure one ovary is transposed into the uterine cavity through an incision in the uterine horn. In the literature, there are no reports of malignancy arising in such transposed ovaries. We report a case in which a borderline ovarian tumour occurred after an Estes operation.

Case Report

A 69-year-old postmenopausal woman was referred to our clinic because of vaginal bleeding. Her medical history revealed a unilateral salpingectomy in 1966 because of an ectopic pregnancy. In 1967 the remaining fallopian tube was partially resected because of a second ectopic pregnancy. In 1968 a salpingo-oophorectomy was performed but without success. In 1970 an Estes procedure was performed in Italy. The procedure included fixation of the uterus to the sacral bone, removal of one ovary and transposition of the other ovary into the uterine cavity through a cornual incision. Unfortunately, no pregnancy occurred.

Initial work-up of the bleeding included pelvic ultrasound that showed an endometrial thickness of 10 mm and a predominantly solid tumour measuring 6.6 cm that was connected to the uterus. Cervical cytology revealed no abnormalities. The serum CA125 was 27.9 U/ml and the serum CEA 3.7 mg/l. An attempt to perform an endometrial biopsy failed because the cervix was obstructed. A tumour in the transposed ovary was suspected and therefore an exploratory laparotomy was performed. After opening the abdomen, a normal size uterus was seen and a tumour apparently arising from the transposed ovary was growing into the uterine wall. Total hysterectomy and unilateral salpingo-oophorectomy was performed. Frozen section analysis of the uterus showed no signs of endometrial pathology. In the transposed ovary however, a borderline mucinous cystadenoma was found. Therefore, a staging procedure was performed. Final histopathology revealed a borderline mucinous cystadenoma of the ovary (Figure 1). All the other tissues that were removed (uterus, omentum, peritoneal biopsies, peritoneal cytology, pelvic nodes) were free from tumour so the case was staged as FIGO IA. The postoperative course was uneventful. Currently, the patient is alive and well three years postoperatively with no signs of recurrent disease.

Discussion

The Estes procedure was first performed by Estes in 1904 [3]. The original method consisted of bilateral salpingectomy, unilateral oophorectomy and transposition of the remaining ovary that was still attached to the
infundibulopelvic ligament into the uterine cavity through an incision in the uterine horn [2]. The indication for the procedure was infertility due to severe fallopian tube damage or resection. Later, a modification was proposed by Tuffier by making a larger area of ovarian surface protruding into the uterus [3]. Estes reported 27 patients who underwent the procedure and four of them achieved a pregnancy (15%) [4].

In the literature, only a few case histories mention late complications which include the formation of benign cysts and the formation of considerable adhesions [5]. No case of ovarian malignancy has been reported. This is the first case report of a borderline tumour arising in a transposed ovary after an Estes operation. Transposition of ovaries is sometimes performed in young patients who are surgically treated for early cervical carcinoma and who have a reasonable chance on postoperative pelvic radiotherapy. Although malignancies have been reported in such transposed ovaries they were usually metastatic tumours [6].

Because of the great anatomic distortion which can occur after the Estes operation, a tumour arising in a transposed ovary may be difficult to diagnose. The symptoms may be misleading and the clinical and ultrasound examination may be difficult to interpret. In such cases an exploratory laparotomy is advised with the possibility to perform frozen-section analysis to determine the type and extent of the surgical procedure.

References

Minimal deviation adenocarcinoma of the cervix in a patient with a high-grade cervical squamous intraepithelial lesion: case report and review of the literature

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**Introduction**

Minimal deviation adenocarcinoma, otherwise known as adenoma malignum, is a rare and particularly well differentiated type of cervical adenocarcinoma, and is often misdiagnosed because of its benign-looking histological features. Adenoma malignum represents only 1-3% of all cervical adenocarcinomas. The Papanicolaou smear as well as punch biopsies can fail in the detection of adenoma malignum. We present the case of a 55-year-old woman diagnosed as having microinvasive minimal deviation of the adenocarcinoma cervix, after conisation for a high-grade cervical squamous intraepithelial lesion. The patient was referred for an abnormal pap smear to our colposcopy clinic where the punch biopsies performed failed to diagnose the disease. The consequent cone biopsy because of CIN3, provided us with a definite diagnosis of adenoma malignum. Subsequently, the patient underwent a radical hysterectomy and pelvic lymph node dissection. The histologic examination was normal. Coexistence of a squamous intraepithelial lesion with adenoma malignum is extremely uncommon.

**Key words:** Minimal deviation adenocarcinoma; Adenoma malignum; Cervix; HPV; High-grade squamous intraepithelial lesion; Conisation.

Currently, it has been determined that human papillomavirus (HPV) is the main causal agent in the development of squamous cervical cancer. On the other hand the prevalence of HPV in cervical adenocarcinoma significantly varies from study to study and the pathogenetic role of HPV infection in the development of cervical adenocarcinoma remains unclear. HPV DNA has seldom been detected in minimal deviation adenocarcinoma according to relative studies [7, 8]. We report a case of minimal deviation adenocarcinoma accompanied by a high-grade cervical squamous intraepithelial lesion (CIN3).

**Case Report**

A 55-year-old woman (gravid 4, para 2) was referred to our outpatient colposcopy clinic because of an abnormal pap smear that showed a low-grade squamous intraepithelial lesion (CIN1). The patient reported vaginal bleeding (spotting) for the previous four months and her menopause had begun five years before. Otherwise, she was in good health with no medical history of chronic disease and she was not under any medication. There was not any family history of cervical, breast or ovarian neoplasia and she had received no hormone therapy in the past. Colposcopy by an experienced colposcopist was performed and a high-grade squamous intraepithelial lesion (CIN2) was diagnosed. Punch biopsies were taken. The histologic report of the punch biopsies was a high-grade squamous intraepithelial lesion (CIN3). Consequently, a cone biopsy and endocervical curettage were performed. The histologic finding of the cone biopsy was a high-grade squamous intraepithelial lesion (CIN3) together with a well differentiated microinvasive malpighian minimal deviation adenocarcinoma of 1 mm...
maximum dimension. The resection margins were clear. Ultrasound scan of the pelvis and abdomen was normal. Computed tomography of the pelvis and abdomen did not reveal any abnormal findings. Tumour markers (CEA, CA19-9, CA125, CA15-3, β-hCG, SCC) were within normal range. After the evaluation of gastrointestinal and respiratory systems by imaging and endoscopic studies, no evidence of tumour tissue or metastases was found. The patient subsequently underwent a radical hysterectomy and bilateral pelvic lymph node dissection. The uterus weighed 103 g when it was freshly resected and measured 9.5 x 6.5 x 4.0 cm. No gross abnormality was seen, except for the cone biopsy defect in the cervix, and the endometrium was unremarkable. The ovaries and fallopian tubes had a normal appearance which was confirmed by histologic evaluation. Neither residual minimal deviation adenocarcinoma nor a squamous intraepithelial lesion was observed in the cervix at the histologic microscopic examination. During the surgical approach no evidence of malignancy was observed in the parametrium or the pelvic lymph nodes, which was verified by the histologic report. The patient had an uneventful four-day post surgical course and follow-up was planned.

Discussion

The main clinical symptoms of advanced minimal deviation adenocarcinoma are profuse watery discharge and an enlargement of the cervix with erosion and hardening [9]. Abnormal cervical glandular cells are evident in the vast majority of advanced minimal deviation adenocarcinoma cases [10] and a preoperative cytological diagnosis was reported in 83.3% of cases [11]. Minimal deviation adenocarcinoma accounts for only 1-3% of all cervical adenocarcinomas [9, 11]. In the present case of microinvasive minimal deviation adenocarcinoma there was no clinical symptom and the vaginal bleeding (spotting) was attributed to the high-grade squamous intraepithelial lesion. It has been reported that preoperative punch biopsy failed to confirm a diagnosis of advanced minimal deviation adenocarcinoma [11]. In our case the pap smear and punch biopsy were also inadequate to identify microinvasive minimal deviation adenocarcinoma with the cone biopsy eventually establishing the diagnosis. The management of a squamous cervical lesion with cone biopsy led us to define the diagnosis of this rare form of cervical adenocarcinoma. We can conclude that this rare coexistence of adenoma malignum with a squamous cervical lesion was the salient fact that led us to a diagnosis of adenoma malignum.

Tsuda et al. [12] reported an interobserver variation in the histologic diagnosis of minimal deviation adenocarcinoma. It was suggested that any disagreement was caused by the absence of the consensus criteria for the differential diagnosis and by different observer interpretations regarding cellular atypia and invasion. The most reliable criteria for assessing the malignant nature of adenoma malignum are the disorganised arrangement of the glands that extend beyond the level of the normal endocervical glands and the presence of occasional mitoses in the glandular cells [13].

In our case report the microscopic examination showed a minute focus of a malpighian carcinoma of high differentiation. This microinvasive carcinoma was considered to originate from a gland which was completely replaced by carcinoma. Several abnormally shaped glands deeply protruded into the endocervical stroma accompanied by a fibroblastic type reaction. The glandular epithelium of a few glands presented small to moderate cellular atypia.

This type of cervical neoplasia, particularly the mucinous form, may occur simultaneously or before the development of an ovarian tumour, which is frequently a mucinous neoplasm or a rare sex cord stromal tumour. Thus, it is imperative to remove the ovaries not only to reduce the possibility of metastasis, but to prevent the development of such ovarian tumours. Adenoma malignum is strongly connected with Peutz-Jeghers syndrome [14] but this association was not established in our case. The pathogenesis of adenoma malignum may not be related to HPV infection [8, 15]. Pirog et al. [9] reported that two cases of minimal deviation adenocarcinoma were negative for HPV. An et al. [16] in a population-based study reported that in a total of four cases with minimal deviation adenocarcinoma, which constituted 1% of cervical adenocarcinomas, three were HPV negative whereas one was HPV positive with no cervical intraepithelial neoplasia. However, the present case demonstrates a well defined high-grade squamous intraepithelial lesion (CIN3) which can definitely be attributed to HPV, in a patient with adenoma malignum. Accepting the theory that minimal deviation adenocarcinoma is unrelated to HPV we conclude that the present case is a rare coexistence of adenoma malignum and CIN3. On the other hand we can argue that HPV can be related to adenoma malignum, as with other types of cervical adenocarcinoma [16], but we need more data to clarify the possible interrelation. It should be mentioned that a molecular study of nine cases of adenoma malignum revealed the loss of heterozygosity on the chromosome 19p13.3 [17]. Additionally, a serine threonine kinase gene, STK11, has been identified in 55% of mucinous minimal deviation adenocarcinomas, and may play an important role in the disease aetiology [14].

The therapy of adenoma malignum should be the same as for ordinary adenocarcinoma [13], while the prognosis is unsettled. An unfavourable prognosis is reported since the vast majority of such neoplasms are discovered in the later stages. Minimal deviation adenocarcinomas do not show any evident lesion despite the deep infiltration, resulting in delayed therapy and a poor prognosis. It is impressive that in some cases, adenoma malignum is not detected, even in hysterectomy specimens, prior to the growth of recurrent disease [18]. Nevertheless, some studies have suggested that survival rates and distribution of metastases are similar to those of a well differentiated usual adenocarcinoma [18]. In our case an opportune diagnosis allowed us to apply the appropriate treatment similar to the therapy that we would have applied if we had to deal with an ordinary well-differentiated microinvasive adenocarcinoma.
Conclusion

The diagnosis of minimal invasive adenocarcinoma of the cervix remains challenging. A deep biopsy (> 5 mm) is crucial for a precise diagnosis to be made especially when the disease is suspected by cytological examination. In the present case, cytology and punch biopsy failed to recognise the disease while the specimen from conisation provided us with a definite diagnosis. It is significant that the indication for conisation was the high-grade squamous intraepithelial lesion. Furthermore, close follow-up of known cases, in order to gain more information about the pathophysiology of the disease and the performance of the available therapeutic modalities, is essential.

References


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Dissecting cotyledonoid leiomyoma: a rare cause of chronic intractable menorrhagia (not amenable to medical treatment).

Case report

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Summary

Sternberg tumour, a cause of intractable menorrhagia, is quite rare with potential alarming macroscopic features leading to over-aggressive treatment. In this case it presented following many years of intractable menorrhagia which was resistant to long-term medical management and endometrial ablation techniques. Incidence with the previous use of endometrial ablation techniques has not been justified given the present literature. The histological diagnosis was arrived at with difficulty given the similarity with other types of cotyledonid leiomyomas.

Key words: Menorrhagia; Sternberg tumour; Leiomyomas.

Introduction

Menorrhagia accounts for a significant percentage of gynaecology referrals. Objectively, it is taken as the loss of 80 ml of blood or more in each menstruation [1]. In the UK, 5% of women between age 30 and 49 years consult their general practitioners yearly with menorrhagia [2]. Many cases are amenable to medical management. However, a significant percentage will need surgical intervention to achieve symptom resolution. In the UK, one in five women have a hysterectomy before the age of 60 years with menorrhagia being the main presenting problem in 50% of cases. This trend is also seen in one in three women in the US [3-5]. Leiomyomas are a known cause of menorrhagia. Ten percent of women with menorrhagia and 40% of women with severe menorrhagia have been found to have fibroids [6]. There are different types or variants. Care has to be taken to confirm the benign state of the lesion. Dissecting cotyledonoid leiomyoma is a rare benign variant which is quite alarming macroscopically and potentially leads to unnecessary treatment, particularly with few cases having been reported in the literature. It is a variant of leiomyoma which was initially described by Roth et al., and sometimes described as a Sternberg tumour [7].

Case Report

A 37-year-old parous woman had a chronic history of menorrhagia for the past 13 years. Significant past medical and surgical history included three previous caesarean sections, tubal ligation and large loop excision of the transformation zone for CIN II in association with human papilloma virus.

Initial investigations including a hematologic profile and pelvic ultrasound (US) scan were normal. A diagnosis of dysfunctional uterine bleeding was made. Cyclic progestogens resolved her symptoms for a short period of time.

Thereafter the patient was managed consecutively intermittently with a cocktail of medications which included a combination of tranexamic and mefenamic acid, combined oral contraceptive pills and the Mirena IUS. There was inadequate symptom resolution with these interventions.

Two endometrial ablation procedures were performed six months apart. The first procedure resolved the patient’s symptoms for a few months. After due counseling while avoiding a hysterectomy, she requested a second ablation procedure with which symptom resolution was achieved for a year. Following this, she presented again with the same symptoms and underwent a uterine scan 20 months after the second ablation. This revealed a bulky 14 weeks in size uterus.

This was the first time a uterine anomaly was found as previous scans had all been normal. A decision for subtotal laparoscopic hysterectomy was made following adequate counseling as she wanted a permanent solution to her recurring menstrual problems.

Intraoperatively a bulky uterus with spongy fungating placental-like tissue was seen protruding from both the anterior and posterior uterine surface near the left cornu extending into the broad ligament. There were smaller discrete deposits over the posterior serosal surface of the uterus. Due to the alarming nature of the incidental finding, a total abdominal hysterectomy was made following adequate counseling as she wanted a permanent solution to her recurring menstrual problems.

There was ill-defined diffuse myometrial neoplastic smooth muscle proliferation extending into the serosa. In addition, there were confluent leiomyomatous nodules with interspersed islands of both hydropic and myxoid change. The histological diagnosis was dissecting cotyledonoid leiomyoma with no evidence of metaplasia or neoplasia. Other tissues (cervix, tubes, ovaries, omentum) were found to be normal. However on review, as some of the features were similar to those of low-grade myxoid leiomyosarcoma, it was thought expedient for the patient to be kept under surveillance in an out-patient setting.
Discussion

There were few cases of dissecting cotyledonoid leiomyoma identified during the literature search. It is a rare benign tumour which is quite alarming macroscopically, potentially leading to unnecessary treatment. It is a variant of leiomyoma which was initially described by Roth et al. [7] and sometimes described as a Sternberg tumour and has been found in women of reproductive and perimenopausal age with presenting features including menstrual problems or presence of a pelvic mass [8, 9].

These tumours tend to arise in the subserosal myometrium of the lateral aspect of the uterus extending to the broad ligament and pelvic cavity. The exophytic component has spongy characteristics and bulbous protuberances over the surface that appears red and congested, resembling placental tissue while perinodular hydropic degeneration occurs as a result of extensive hydropic degeneration. Cellular atypia, mitoses and coagulative tumour necrosis are typically absent. Follow-up usually reveals a benign course.

Other variants of this tumour that have been described include:
1) cotyledonoid leiomyoma: similar to the dissecting cotyledonoid leiomyoma with the difference being the lack of a parent intramural component [10];
2) intramural dissecting leiomyoma: lack of a extraterine component as seen in the dissecting cotyledonoid variant [10];
3) cotyledonoid hydropic intravenous leiomyomatosis: the difference here is the presence of intravascular leiomyomatosis [11-13]. Vascular invasion may extend into the extraterine pelvic veins, sometimes as far as the right heart [12, 13]. Pulmonary metastases have been reported. Rate of recurrence and progressive growth is higher in this variant [14-16].

As these tumours are rather rare, not much is known about the lifespan, i.e. length of time to become overt macroscopically. This was an issue in our case as previous scans were essentially normal.

Macroscopic assessments done both at a previous caesarean section (13 years earlier) and hysteroscopies (done prior to endometrial ablations) 28 and 22 months prior to the hysterectomy had also been normal. Endometrial curettings obtained at the procedures had shown secretory endometrium with no evidence of the presence of the Sternberg tumour. The assumption is that the condition may have developed over a 22-month period.

As it is a rare condition, little is known about the pathophysiology or any associations. However as the patient had undergone two ablation procedures, a question was raised as to any contributory association. Could cellular damage from ablation techniques contribute to the development of this rare tumour? A study by Fadare et al. showed that there were no noteworthy histologic changes on examination of uterine leiomyomas exposed to the NovaSuresystem (Hologic Corporation, Marlborough, MA, USA) [16]. The probability of cellular changes as a result of the radiofrequency waves used in the procedure was the basis of this investigation. Given their results, they suggested that any necrosis found in leiomyomas obtained from patients who have undergone recent endometrial ablation was unrelated to the ablation procedure [17].

Endometrial ablation techniques have been found to be generally safe. This is based on several studies which have shown results of 20-year follow-up data on 1st generation techniques and 7-year follow-up data on 2nd generation techniques of which NovaSure trademark was one [18]. However, McCausland et al. showed the presence of intrauterine scarring, central haematometra, cornual haematometra, postablation tubal sterilization syndrome, retrograde menstruation and potential delay in the diagnosis of endometrial cancer as long-term complications. Nonetheless, no neoplastic or metaplastic changes were identified [19].

In summary, this is a rare condition with associated menstrual or pelvic features and could pose a challenge both intraoperatively or while instituting further management. Accurate histological assessment is needed to differentiate the variants involved, differing prognostic implications and appropriate institute management while avoiding over-treatment. More research is needed into the condition and any identifiable causal associations.

References


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Primary melanoma of the vagina: a case report

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Summary

Primary malignant vaginal melanoma is a rare vaginal tumour accompanied by an extremely high risk of local recurrence, distant metastasis and a small survival rate. Due to the fact that vaginal melanoma is quite uncommon there is lack of powerful prospective studies in the literature, thus the treatment choice remains controversial. An 85-year-old woman with a primary malignant vaginal melanoma located on the left lateral aspect of the distal vagina, with the greatest diameter almost 5 cm, was referred to our clinic. There was not any sign of local or distant metastasis identified. According to the most recently published data in the international literature, we decided along with the patient to perform conservative dissection of the tumor with free surgical margins as the appropriate mode of therapy. Radiotherapy, chemotherapy or radical extirpation cannot increase the survival time, even if there is a local or distant spread of melanoma.

Key words: Melanoma; Vaginal tumour; Vaginal malignant melanoma; Gynaecological malignancies.

Introduction

Malignant melanoma is a melanocyte tumour of the skin and mucosal membranes. The melanocytes are cells that embryologically derive from neural crest cells. Although 3% of healthy women can have melanocytes in the basal portion of vaginal epidermis, some of these cells are located in the vaginal mucosa, where the vaginal melanoma arises from [1].

Malignant melanomas are rare in areas of the body not exposed to ultraviolet radiation. Primary vaginal malignant melanoma is a very uncommon neoplasm, with less than 300 cases in the English literature [2]. A review of 84,836 cases of melanoma from the National Cancer Data Base that occurred between 1985 and 1994 showed that 91.2% of the melanomas were cutaneous, 5.2% were ocular, 1.3% were mucosal, and 2.2% involved unknown primaries [3]. In women, 1.6% of melanomas are genital; the vulva is the most frequently involved site (70%), followed by the vagina (21%) and cervix (9%). Malignant melanoma of the vagina is extremely rare and accounts for less than 0.3% of all melanomas in women and less than 3% of primary vaginal tumours [4].

Primary vaginal malignant melanoma usually appears in the 6th and 7th decade of life, mainly with vaginal bleeding, a palpable mass and vaginal discharge [5]. Vaginal melanoma is a highly lethal disease and has the worst prognosis of all vaginal malignancies, with a 5-year survival rate of 8.4% due to the ability of the tumour to have early metastases haematogenously, mainly in the liver, lungs, skin and brain [6].

Several procedures have been used for the therapy of this disease such as local excision, radical surgery, irradiation and chemotherapy. However due to the rarity of the disease, no prospective studies or validated treatment recommendations exist.

Case Report

An 85-year-old woman (gravida 0, para 0), 40 years after menopause, who complained of vaginal discharge and vaginal bleeding for a period of one-month, was referred to our gynaecological outpatient clinic. Detailed gynaecological examination revealed a hard nodule in the left lateral aspect of the distal vagina. This tumour had a dark brown colour, an irregular surface, bled easily on contact and the greatest diameter was almost 5 cm. The physical examination did not identify any superficial inguinoemorial lymph node with any increased diameter. The patient had never visited any gynaecologist for routine examination or Papanicolau smear. Her medical history was negative apart from a 10-year diagnosed heart arrhythmia, for which she took warfarin tablets. There was no family history of malignancy. Punch biopsies were taken from the tumour. The histological report for the recognised lesion revealed vaginal carcinoma with morphological and immunostochemical features (S 100p+, Melan-A+, HMB 45+) compatible with malignant melanoma of the vagina.

The patient was scheduled for wide local excision. Routine preoperative laboratory tests were normal. Tumoural markers CA 19-9, CA 125, CA 15-3, CEA, AFP were negative. Cardiological evaluation was normal apart from the already known arrhythmia. Chest X-ray was normal without any metastatic lesions or pleural fluid. The ultrasound scan of the upper and lower abdominal region was negative for liver metastases, an abdominal mass and fluid. Also computerised tomography of the pelvis, abdomen, chest and brain was negative for local metastasis. Dermatological evaluation was performed and no skin metastases were found. Under general anaesthesia we dissected the tumor with a surgical knife and two hard specimens of the tumour with a size of 3 x 2 x 1 cm and 2 x 1 x 0.5 cm were sent for histopathological examination. Wide excision in terms of a wide local removal of the vaginal wall around the lesion was performed. Then we thoroughly examined the vaginal canal and cervix and no other macroscopic lesions were found. Multiple blind biopsies were also taken from sites with no obvious gross disease which were proven to be normal. The uterine corpus, parametrium and adnexa were examined under general anaesthesia and no palpable masses were found.
Discussion

Primary growth of malignant melanoma in other organs apart from the skin is extremely uncommon. Malignant vaginal melanoma is a disease of postmenopausal women and the mean age of presentation is 60 years [7]. The most common location of melanoma is the lowest one third of the vagina. Primary malignant vaginal melanoma is a rare malignancy associated with high risk of recurrence, distant metastasis and a short 5-year survival time which is 14% [8]. This type of vaginal cancer is more aggressive in comparison to vulvar or cutaneous malignant melanoma.

The most important prognostic factors which appear to be correlated with survival rates, are tumour size and distant or regional metastases. Patients with tumour size ≤ 3 cm have a greater 5-year survival time than women with tumour size > 3 cm, as in our case report.

Several reports suggest that wide local excision followed by pelvic radiotherapy is the appropriate way to control the disease while radical surgery with adjuvant radiotherapy is reserved as a second-line therapy [9-11]. Some investigators underline the beneficial effect of radiotherapy, pelvic lymph node dissection and chemotherapy alone or in combination with surgical dissection of tumour in cases of primary vaginal melanoma [5, 12-14]. Due to the high rate of local recurrence, distant metastases and the small overall survival benefit with any mode of treatment, local resection is the preferred therapy since it causes less discomfort and has no side-effects for the patient while it increases the overall survival rate. Recent data suggest that surgery alone, especially wide local excision, is the most effective treatment modality in terms of controlling the tumour locally. Few researchers support aggressive surgical therapy, such as anterior and posterior exenteration [15]. Currently, it is generally accepted that conservative local resection with tumour-free surgical margins, as we did in our case, increases overall survival and protects from metastases and recurrence [1, 2, 8, 16]. In our case we applied this type of therapy after taking into consideration the current literature and the tolerance of the patient.

In conclusion, we can say that because of the nature of primary vaginal melanoma, which is very aggressive and gives rise to local and distant metastases, we can choose conservative dissection of the tumour with free surgical margins. Radiotherapy, chemotherapy or radical extirpation do not increase survival time even if there is spread to the lymph nodes, liver, lung or brain. The majority of patients with vaginal melanoma will die of their disease and surgical therapy is associated with increased overall survival. The aim of an operative intervention (wide local excision) should be a complete resection of the gross disease. Multicentre trials have to be conducted in order to enroll enough patients to study this rare disease more effectively. Prospective data are required to help us clarify the value of each of our treatment options. Due to their biological aggressiveness noncutaneous melanomas should be studied distinctively as a group suitable for clinical trials of adjuvant therapy.

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.

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