

Pitfalls in the diagnosis of gynaecological malignancy

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Abstract

Gynaecological epithelial tumours are commonly encountered in surgical pathology departments. Pathologists may have major problems in the interpretation of certain tumours. In this session, the emphasis is on the pitfalls in the diagnosis of pre-malignant and early invasive carcinomas of the vulva and cervix, together with the difficulty and confusion in the diagnosis of the endometrial hyperplasia and grade 1 endometrioid endometrial carcinoma.

Squamous vulval intraepithelial neoplasia (VIN) and early invasive carcinoma

VIN

The traditional three-tiered grading of VIN-U was revised by the ISSVD committee in 2004, with the recommendation that the category of VIN 1 be discontinued. VIN 1 is considered poorly reproducible, and usually due to either reactive change or HPV effect. There is no evidence that VIN 1 is a precursor to cancer. VIN-U, therefore refers to VIN 2 and VIN 3, now combined into single category, high-grade VIN. VIN-D, referred to as 'atypical hyperplasia' by some researchers, is a highly differentiated form of VIN 3.

Two morphological patterns of VIN-U are recognized, although the distinction has no clear prognostic significance. In the most common type, the architecture is 'warty', or 'condylomatous' with papilliferous epithelial hyperplasia, hyperkeratosis and parakeratosis, and prominence of the granular layer. The nuclei are enlarged and pleomorphic with binucleated and multinucleated cells, and frequent mitoses. Koilocytotic cells are prominent, consistent with HPV effect. The cells usually have abundant eosinophilic cytoplasm with well-defined cell borders. Individual cell keratinization may be seen.

In the second pattern, 'basaloid' VIN, there is a proliferation of small, relatively uniform cells resembling epithelial parabasal cells. The epithelium is hyperplastic with smooth bulbous contours and a flattened surface. The cells are relatively uniform and show little maturation. The nuclear:cytoplasmic ratio is high and frequent mitoses are characteristic. The cytoplasmic boundaries are poorly defined. Koilocytosis may be present superficially but is a minor feature. These two patterns of VIN-U, warty/condylomatous and basaloid are not mutually exclusive and, not infrequently, co-exist; the ISSVD terminology classifies such cases as VIN, mixed (warty/basaloid) type. Generally, however, VIN-U is classified according to the predominant pattern present.

VIN-D is a more subtle lesion, demonstrating differentiation of the epithelium and lacking significant architectural disarray. The epithelium is typically hyperplastic with parakeratosis, but may be atrophic. There are usually prominent irregular, elongated

and anastomotic rete ridges. The hallmark feature is the presence of large, overly mature keratinocytes with abundant eosinophilic cytoplasm and prominent intercellular bridges. Nuclei are vesicular with one or more conspicuous nucleoli. While atypical cells may be present throughout the epithelium, these cells are most prominent in the basal and parabasal layers, and in the rete ridges. Whorls of differentiated cells may be present within rete ridges, often associated with keratin pearls. The adjacent basal cells are usually hyperchromatic with irregular angulated nuclei. Mitoses are confined to the basal and parabasal layers and inflammation is often seen within the superficial stroma and attacking the abnormal epidermis.

Early/superficially invasive SCC

The WHO defines depth of tumour invasion as the measurement of the tumour from the epithelial–stromal junction of the adjacent most superficial dermal papilla to the deepest point of invasion. This measurement is more accurate than tumour thickness, defined as the measurement from the overlying surface, (or granular layer if keratinized), to the deepest point of invasion. Tumour thickness may vary depending on the presence of ulceration, epithelial hyperplasia or VIN.

Repeated attempts have been made to identify those early invasive lesions that have a negligible risk of nodal spread, and may be treated by local resection without groin node dissection. The term ‘micro-invasive’ is no longer used, having been superseded by The TNM/FIGO staging system which defines a stage 1A lesion as tumour confined to the vulva, or vulva and perineum, 2 cm or less in greatest dimension and with stromal invasion no greater than 1 mm. This definition includes cases with lymphovascular space invasion, although this rarely occurs. Tumours conforming to this definition have 5 and 10 year tumour recurrence-free survival of 100% and 94.7%, respectively. Stage 1A tumours are generally treated by wide local excision with 1 cm lateral and deep margins, without inguino-femoral lymph node dissection.

Frankly invasive SCC with a depth of invasion of up to 3 mm have associated lymph node metastases in approximately 10% of cases. This figure rises to 40% in those with tumour infiltrating to a depth of 4 mm or more. Invasive SCC is usually treated by radical local excision and groin node dissection. Intermediate tumours with stromal invasion of between 1 and 2 mm have a small but undefined risk of groin node metastases, and further studies are required to direct management of this sub-group.

Identification of early invasive carcinoma represents one of the most problematic areas in vulval pathology, and one that has significant prognostic and management implications. Invasive carcinoma is difficult to distinguish from VIN clinically, and extensive sampling of specimens is necessary to identify occult invasion. Histologically, invasion is associated with changes of both the architecture and cytology of the invading epithelium, and reactive changes in the stroma that is invaded, although in some cases the latter may be minimal.

The earliest evidence of invasion is the presence of buds or irregular tongues of atypical cells penetrating the basement membrane of the epithelium. The cells within the invasive focus show maturation, manifesting as an increase in the amount and the eosinophilia of the cytoplasm. Dyskeratotic cells, squamous pearls and foci of keratinization may be present. Nuclear changes, including more prominent nucleoli,

may be evident. Additional features suspicious of early invasion include anastomosis of slender elongated rete ridges, and scalloping of the epithelium at the epithelial–stromal junction with loss of peripheral palisading of basal cells. The presence of single cells ‘streaming’ into the stroma is diagnostic of early invasion. Progressively invasive nests usually have irregular outlines. There is frequently, although not invariably, a stromal reaction that may include stromal loosening or oedema, a desmoplastic reaction and an inflammatory infiltrate, particularly an eosinophilic infiltrate, at the point of invasion. Giant cells may be present adjacent to small invasive foci.

Squamous and glandular cervical intraepithelial neoplasia (CIN & CGIN) and early invasive disease

Cervical carcinomas usually arise from dysplastic epithelium. Squamous cell carcinoma, the most common type, evolves from cervical intraepithelial neoplasia (CIN) while adenocarcinoma develops from cervical glandular intraepithelial neoplasia (CGIN). The diagnosis of frankly invasive disease does not create a diagnostic problem. In contrast, confident diagnosis of the earliest stage of invasive disease arising from a dysplastic precursor lesion is problematic. The diagnostic process is further complicated by a lack of concordance concerning the nature (and behaviour) of certain diseases associated with the cervix e.g. CIN3-like squamous carcinoma. In this session, an attempt has been made to clarify the features that are suggestive of definitive stromal invasion and to highlight the features of those unusual carcinomas that are often misinterpreted as non-invasive disease.

CIN3 with features of impending invasion

CIN3 with features suggesting early, or impending, stromal invasion has been described. These features include: extensive CIN3 involving both the surface epithelium and deep endocervical crypts; expansion of the involved crypts often with evidence of central comedo-type necrosis, and focal squamous (or eosinophilic) maturation. Deeper levels should always be performed on such lesions to rule out the presence of foci of early microinvasion.

CIN3-like invasive squamous cell carcinoma

Squamous carcinoma of the uterine cervix with a CIN3-like growth pattern is a recently described variant of squamous cell carcinoma of the cervix. It is characterized by a squamous neoplastic lesion similar to CIN3 occupying deep endocervical crypts with some irregularity and a back-to-back arrangement. These lesions show very prominent comedo-necrosis, quite obvious intra-lesional squamous maturation, and frequent peripheral bulging of what appear to be deep endocervical crypts. This pattern gives a false impression of CIN3 involving complex crypts that may have been cut tangentially. However, these nests are often seen in association with small tongues of invasion surrounded by stromal loosening and various degrees of inflammation. Similar appearances may also be seen deep within the endocervical stroma of the hysterectomy specimen. At present, due to a lack of awareness of these histological features, the majority of such lesions in a small biopsy or a loop excision are being reported as equivocal, or CIN3 with areas suspicious of invasion. This would certainly necessitate further biopsies until a definitive diagnosis of invasion is

made. Conversely, such a pattern may lead to a false assurance of very early stage disease. On receiving such equivocal reports, some gynaecologists might perform a further LLETZ, while others may opt for close follow-up of the patient. Due to the small number of reported cases of this type of squamous carcinoma, the long-term prognosis has yet to be characterized

Cervical glandular intraepithelial neoplasia (CGIN)

CGIN is often discovered in loop excisions for CIN. The distribution of CGIN is most commonly unifocal, although multifocal and circumferential distributions may also be seen, usually affecting both the surface epithelium and the underlying crypts. Midline disease, involving either CGIN or CIN (or both) is very common and the examination of midline blocks from hysterectomy specimens will result in the identification of CGIN lesions in over 90% of patients. CGIN is usually found adjacent to the squamo-columnar junction, or at the edge of CIN, invasive squamous carcinoma or invasive adenocarcinoma.

The dysplastic or neoplastic endocervical epithelium usually expresses carcinoembryonic antigen (CEA). The proliferative marker MIB1, shows numerous positive nuclei in the abnormal epithelium; in contrast, in normal epithelium, MIB1 expression, when present, is only detected in isolated nuclei. P53 expression is frequent in endocervical adenocarcinoma and suggests that mutation of the P53 gene may be important in the evolution of some cases of endocervical adenocarcinoma. However, the significance of scattered P53-positive cells, which may be seen in endocervical adenocarcinoma in situ and in non-neoplastic glandular lesions of the endocervix, is uncertain.

CGIN is classified into low- and high-grade categories. High-grade lesions are almost certainly precursors of invasive adenocarcinoma, while the premalignant nature of the low-grade lesions is questionable; in practice, the diagnosis of a low-grade glandular lesion is rarely made in isolation.

High-grade CGIN usually exhibits pronounced architectural and cytological abnormalities. Architecturally, the abnormal crypts tend to aggregate together in small clusters or lobules and there may be papillary infolding and outpouching. Cytologically, there is nuclear enlargement and hyperchromasia, depletion of cytoplasmic mucin, stratification (or pseudostratification) of cells, loss of nuclear polarity; easily identifiable mitotic figures are seen in the luminal aspect of the cells and apoptotic bodies are seen towards the basement membrane. The presence of mitotic figures, in the absence of any inflammation, almost certainly indicates CGIN and a cribriform pattern raises the suspicion of early invasion.

SMILE

The term 'stratified mucin-producing intraepithelial lesions' or 'SMILEs' was recently introduced to describe unusual cervical intraepithelial lesions characterized by the presence of a stratified epithelium similar in architecture to a high-grade CIN but with the conspicuous spacing of nuclei in the lower-to-middle epithelial layers. This change is usually associated with the presence of diffuse cytoplasmic mucin, or occasionally, with more discrete cytoplasmic vacuoles. Another important feature of

this lesion is the rounded contour of the epithelial–stromal interface, similar to that seen in glandular neoplasia. SMILE often coexists with either a squamous (CIN) or glandular (CGIN) precursor lesion and when invasive carcinoma is present, the majority contain either glandular or adenosquamous differentiation (or both). It has been suggested that this subset of precursor lesions is of columnar cell origin rather than an adenosquamous origin. As SMILE originates in concert with a wide spectrum of lesion phenotypes in the cervical transformation zone, it is considered as a marker of phenotypic instability. It is, therefore, important to identify SMILEs and ensure a complete examination of specimens containing this unusual precursor lesion. SMILEs are distinguished from benign metaplasia by nuclear hyperchromasia and a high MIB-1 index. SMILEs do not express keratin-14 and stain variably for p63. When present, P63 expression is confined to the basal areas of SMILEs and is absent in areas of columnar differentiation. The distribution and immunophenotype of SMILEs is consistent with a neoplasm arising from reserve cells in the transformation zone.

Microinvasive adenocarcinoma

This represents the earliest stage of invasive adenocarcinoma and is classified in the same way as its squamous counterpart. It has recently been shown that early invasive cervical adenocarcinoma, with a depth of invasion of 3 mm (or less) and a horizontal spread of 7 mm (or less) has little potential for nodal metastasis or recurrence. It seems possible that the FIGO definition (1994) of early cervical cancer may also be applicable in its present form to early cervical adenocarcinoma. However, recurrence of FIGO stage IA1 cervical adenocarcinoma has rarely been reported.

Features suggestive of early invasion are the presence of a back-to-back arrangement of the involved glands, a cribriform pattern, or the identification of solid nests with irregular borders. Other features that are found to be very useful in identifying early stromal invasion include: the presence of focal squamoid cytoplasmic change of the abnormal epithelium [similar to the early buds of microinvasive squamous carcinoma and thinning and attenuation (or stretching) of part of the glandular wall in an ‘elastic band-like’ manner]. These changes are often accompanied by a loosening of the periglandular connective tissue and a florid inflammatory response). Another important feature is the proximity of the abnormal glands to thickened blood vessels. The microscopic measurement of microinvasion is usually assessed from the surface glandular mucosa to the deepest invasive gland. However, there is usually a problem deciding whether CGIN with foci of early stromal invasion that involves the entire thickness of the LETZ is considered a microinvasive or frankly invasive carcinoma. In the author's department, in such a situation, the practice is to measure the distance between the deepest invasive nest and the surface, and to comment that frankly invasive carcinoma cannot be excluded in the biopsy. In these circumstances, a deeper LETZ might be necessary to enable a better assessment before making a decision regarding the final treatment

Histopathological challenges in the diagnosis of endometrial hyperplasia and carcinoma

The classification of endometrial hyperplasias and neoplasms often causes diagnostic problems. This session aims to provide a broad overview of the terminology and microscopic features of endometrial hyperplasias, emphasizes the morphological

features that are useful in categorizing these and in discriminating them from endometrial carcinoma

Endometrial hyperplasia

The term endometrial hyperplasia refers to a group of proliferative abnormalities of the endometrium, which are characterized by an increase in the amount of endometrium, alterations in glandular architecture and changes in the gland:stroma ratio. These lesions are a frequent cause of diagnostic problems in routine histopathological practice.

The 1994 World Health Organization (WHO) classification is the most commonly used classification system for endometrial hyperplasias. This is based on the observation that the presence of cytological atypia in endometrial hyperplasias is closely linked to progression to carcinoma. Accordingly, the WHO classification divides endometrial hyperplasias into two categories depending on the presence or absence of cytological atypia. The first category, termed endometrial hyperplasia, is defined by a lack of cytological atypia. The second category is termed atypical endometrial hyperplasia and includes those lesions that show cytological atypia. Each of these main categories is further subdivided into simple and complex subtypes, the former being defined by a lack, and the latter by the presence, of glandular architectural abnormalities. The term 'simple endometrial hyperplasia' refers to non-atypical hyperplasia without glandular architectural abnormalities, whereas 'complex endometrial hyperplasia' denotes non-atypical hyperplasia with glandular architectural abnormalities. The term 'simple atypical hyperplasia' is rarely used in practice, as there is considerable controversy about the validity of this category. Thus, in routine histopathological practice the term 'atypical endometrial hyperplasia' refers to complex atypical hyperplasia.

The validity of this terminology has been challenged on the grounds of observer variability and preliminary findings on the biology of endometrial hyperplasias. The authors of these studies have proposed modifications to the existing terminology. However, the proposed new schemes have not been adequately evaluated, and it is advised that, for the present, histopathologists continue to adhere to the WHO classification.

Simple endometrial hyperplasia

Simple endometrial hyperplasia usually involves the entire endometrium with resulting diffuse endometrial thickening. On microscopic examination it is characterized by glands showing marked variation in size and shape. Some of the glands may be normal in size, but many are cystically dilated. The glands are separated by abundant stroma. Glandular crowding should be either absent or minimal and focal. The glandular outline may be round or slightly irregular, but budding and branching should be either absent or minimal. The epithelial lining closely resembles that seen in proliferative endometrium. The epithelial cells are columnar, pseudostratified and arranged with the long axis of their nuclei at right angles to the basement membrane. The nuclei are round or oval with evenly dispersed chromatin and inconspicuous nucleoli. Mitotic activity is present but may be variable. By definition, there is no cytological atypia or epithelial disorganization.

In simple hyperplasia, the stroma is abundant and the stromal cells resemble those seen in the normal proliferative phase. They have small oval nuclei with very scanty cytoplasm and poorly demarcated cytoplasmic borders. Variable mitotic activity is also seen within the stroma.

Complex endometrial hyperplasia

In complex hyperplasia, there is disorderly glandular proliferation at the expense of the stroma. The endometrial glands are crowded with a complex branching architecture. The glands are separated by relatively scanty stroma. Complex endometrial hyperplasia is often focal and frequently coexists with simple endometrial hyperplasia.

The glands show marked variation in size and shape with branching and papillary infoldings. They are lined by columnar epithelial cells that lack cytological atypia. The cytonuclear features are essentially similar to those seen in simple hyperplasia, but there may be increased pseudostratification as a result of increased epithelial proliferation. Mitotic activity is variable. The stroma is diminished and compressed between the glands.

As the endometrial hyperplasias represent a morphological continuum there is no clear dividing boundary between simple and complex hyperplasia. Minor degrees of glandular budding may still be considered as within the spectrum of simple

hyperplasia. However, glandular crowding is caused by a reduction in the amount of stroma and is therefore best regarded as a feature of complex hyperplasia.

Atypical endometrial hyperplasia

The current terminology subdivides this category into simple and complex atypical hyperplasia. Although a theoretical possibility, cytological atypia in the context of architecturally simple hyperplasia is in practice extremely rare and its existence is disputed. Unless otherwise qualified, the term 'atypical endometrial hyperplasia' refers to architecturally complex hyperplasia with cytological atypia. By definition, atypical endometrial hyperplasia is characterized by a complex glandular architecture, glandular crowding, epithelial cells showing the cytological hallmarks of malignancy and lack of endometrial stromal invasion.

On low power, the glands have a complex architecture. The epithelial cells show increased nuclear:cytoplasmic ratio and cytonuclear pleomorphism. There is loss of epithelial polarity so that the long axis of epithelial cell nuclei is no longer at right angles to the basement membrane or in parallel to each other. The nuclei are hyperchromatic with coarse chromatin, large nucleoli and irregular nuclear membrane. There is variable mitotic activity and occasional atypical mitoses are seen. The atypical glands may be admixed with glands lined by non-atypical epithelial cells. The degree of atypia present is not graded in routine practice as this is poorly reproducible and has not been shown to have prognostic implications.

Atypical endometrial hyperplasia is by definition a non-invasive lesion. The assessment of endometrial stromal invasion can be extremely difficult because of the architectural complexity of the endometrial epithelial–stromal interface and by the absence in the endometrium of fixed anatomical structures, such as a muscularis mucosa, that can be used as a reference point. Because of the difficulty of establishing stromal invasion in endometrial biopsies and the natural reluctance of histopathologists to diagnose adenocarcinoma without unequivocal features of invasion, 'atypical endometrial hyperplasia' may be overdiagnosed in endometrial curettings. These factors may explain the finding of adenocarcinoma in 17–43% of uteri resected following a diagnosis of atypical endometrial carcinoma.

It appears that the extent of atypia is the single most important feature that helps discriminate atypical hyperplasia from carcinoma. Focal atypia and the presence of non-atypical glands admixed with the atypical glands strongly support a diagnosis of atypical hyperplasia. Conversely, the presence of generalized atypia supports a

diagnosis of adenocarcinoma. Other features that favour a diagnosis of adenocarcinoma are: labyrinthine, and confluent glandular patterns, cribriform appearance, macroglands having multiple bridges, microacini, an excessive papillary pattern, stromal desmoplasia and neutrophilic inflammation.

There may be some instances where, despite applying these criteria, a confident distinction cannot be made between atypical hyperplasia and well-differentiated endometrial adenocarcinoma. In these borderline cases, the report should clearly state the element of uncertainty. Depending on the clinical picture, these patients may need additional investigation with hysteroscopy and imaging techniques and a rebiopsy. In my view it is these cases which may benefit from the terminology of endometrial intraepithelial neoplasia EIN.

Terminological problems

There has been recent attempt to improve the diagnosis reproducibility of endometrial hyperplasia, first by combining simple and complex hyperplasia in a single category (endometrial hyperplasia) and second by introducing a new terminology "endometrial intraepithelial neoplasia (EIN)", which is considered as a precancerous lesions, characterized by a monoclonal growth of crowded glands, with epithelial cytological changes. It has to be more than 1mm in diameter.

The term 'endometrial adenocarcinoma in situ' has been used in a very ambiguous and confusing way. Some authors have used this term to indicate atypical endometrial hyperplasia, while others have equated it to endometrial adenocarcinoma confined to the endometrium (FIGO stage IA). The use of this term is best avoided because of the lack of clarity associated with it in the context of the endometrium and because it is not included in the WHO classification of endometrial hyperplasias.

More recently, 'endometrial intraepithelial carcinoma' have been used to denote a lesion some have considered to be the precursor to uterine serous carcinoma. This lesion represents non-invasive high grade serous carcinoma. Histologically, this lesion is often seen within the confine of an endometrial polyp or in an atrophic endometrium with or without associated uterine serous papillary carcinoma elsewhere in the uterus.. The abnormal cells which are indistinguishable from serous papillary carcinoma cells, are usually seen replacing normal surface epithelium or colonising normal glands. Endometrial intraepithelial carcinoma should not be used for a clear

cut endometrial carcinoma that is limited to the endometrium with no myometrial invasion (FIGO IA).

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