

March 2008



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NEWSPath

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ANATOMIC PATHOLOGY

Endometrial Hyperplasia and Carcinoma in Endometrial Polyps: Clinicopathologic and Follow-Up Findings

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The objectives of this study were: 1) to evaluate findings in follow-up hysterectomy specimens after a diagnosis of complex atypical hyperplasia or carcinoma in endometrial polyps (EMPs) for possible significance in management strategies; and 2) to identify features in these polyps, that are predictive of the presence of endometrial hyperplasia or carcinoma in subsequent hysterectomy. Records of all cases of EMPs with endometrial hyperplasia were retrieved from the files of New York University Medical Center from 1993 to 2005. Those cases with follow-up hysterectomy were selected for the study. Of the 29 patients with complex atypical hyperplasia within the polyp, 19 out of 29 (66%) patients had hyperplasia of the non-polyp endometrium, and adenocarcinoma was observed in 9 out of 29 (31%) patients on follow-up hysterectomy. The percentage of polyp area involved by the hyperplasia was predictive of finding endometrial disorder in subsequent hysterectomy ($P = 0.005$). Of the 8 patients with adenocarcinoma in situ (AIS) within the polyp 3 (38%) had myoinvasive adenocarcinoma. In contrast, in cases without AIS, 4 out of 21 (19%) had myoinvasive adenocarcinoma in follow-up hysterectomy. Eight of the nine cases with carcinoma in endometrial polyp had endometrial pathology on hysterectomy. Approximately two thirds of the patients with hyperplasia and 90% of patients with adenocarcinoma in endometrial polyps show endometrial pathology on subsequent hysterectomy. The above findings reinforce the need for hysterectomy especially in postmenopausal women with atypical complex hyperplasia or carcinoma in endometrial polyps even if these changes appear confined to the polyp in initial sampling.

International Journal Of Gynecological Pathology, Volume 27(1), January 2008, pp 45-48

Distinction of Hepatocellular Carcinoma From Benign Hepatic Mimickers Using Glypican-3 and CD34 Immunohistochemistry

**Coston, Wanda, Loera, Sofia, Lau, Sean, Ishizawa, Shin, Jiang, Zhong, Wu, Chin-Lee, Yen,
Yun, Weiss, Lawrence, Chu, Peiguo**

Distinguishing a well-differentiated hepatocellular carcinoma (HCC) from normal and cirrhotic liver tissue or benign liver nodules, such as hepatic adenoma (HA) and focal nodular hyperplasia (FNH), may be very difficult in some cases, particularly in small needle core biopsies. We studied the expression of Glypican-3 (GPC3) and CD34 in 107 cases of HCC, 19 cases of HA, and 16 cases of focal nodular hyperplasia (FNH). In addition, we studied GPC3 expression in 225 cases of nonhepatic human tumors with epithelial differentiation. Ninety-four of 107 cases (88%) of HCC showed focal or diffuse cytoplasmic GPC3 staining, whereas all HA and FNH cases were GPC3-negative, and only 7 of

225 cases (3%) of nonhepatic tumors with epithelial differentiation expressed GPC3. The sensitivity and specificity of GPC3 for HCC was 88% and 97%, respectively. There were three CD34 staining patterns observed in hepatic tissue: negative, incomplete positive, and complete positive. In negative staining pattern, only blood vessels in portal triads or rare sinusoidal spaces immediately adjacent to portal tracts were positive. The negative staining pattern was seen in normal or cirrhotic liver tissue only. The complete CD34 staining pattern showed virtually all sinusoidal spaces with CD34-positive staining throughout the lesion. The complete CD34 staining pattern was seen in virtually all cases of HCC and in only some cases of HA and FNH. The incomplete CD34 staining pattern was characterized by either CD34 positivity in virtually all sinusoidal spaces in some but not all nodules or CD34 positivity in the peripheral sinusoidal spaces adjacent to portal triads. The incomplete CD34 staining pattern was seen in rare cases of HCC and in most cases of HA and FNH. We conclude that GPC3 is a very specific marker not only for differentiating HCC from nonhepatic tumors with epithelial differentiation, but also for differentiating HCC from HA and FNH. GPC3 immunoreactivity, in combination with a complete CD34 immunostaining pattern, greatly facilitates the accuracy of distinguishing between malignant hepatic lesions and benign mimickers.

American Journal of Surgical Pathology. 32(3):433-444, March 2008.

Hepatocellular Carcinoma, Human Immunodeficiency Virus And Viral Hepatitis In The Haart Era

Douglas C Macdonald, Mark Nelson, Mark Bower, Thomas Powles

The incidence of hepatocellular carcinoma (HCC) in patients with human immunodeficiency virus (HIV) is rising. HCC in HIV almost invariably occurs in the context of hepatitis C virus (HCV) or hepatitis B virus (HBV) co-infection and, on account of shared modes of transmission, this occurs in more than 33% and 10% of patients with HIV worldwide respectively. It has yet to be clearly established whether HIV directly accelerates HCC pathogenesis or whether the rising incidence is an epiphenomenon of the highly active antiretroviral therapy (HAART) era, wherein the increased longevity of patients with HIV allows long-term complications of viral hepatitis and cirrhosis to develop. Answering this question will have implications for HCC surveillance and the timing of HCV/HBV therapy, which in HIV co-infection presents unique challenges. Once HCC develops, there is growing evidence that HIV co-infection should not preclude conventional therapeutic strategies, including liver transplantation.

World J Gastroenterol 2008 March 21;14(11): 1657-1663

Epstein-Barr Virus-Associated Epithelial and Mesenchymal Neoplasms

Deyrup AT.

Epstein-Barr virus (EBV) is a ubiquitous human pathogen that usually maintains a harmonious relationship with its host. Rarely, this host-virus balance is perturbed, causing a diverse group of malignancies in both immunocompetent and immunosuppressed patients. In addition to its role in hematologic malignancies (Burkitt lymphoma, subsets of Hodgkin and T-cell lymphomas, posttransplant lymphomas), EBV has been implicated in both epithelial (undifferentiated nasopharyngeal carcinoma, a subset of gastric adenocarcinomas) and mesenchymal (EBV-associated smooth muscle tumor, inflammatory pseudotumor-like follicular dendritic cell tumor) neoplasms. This review will focus on EBV-associated epithelial and mesenchymal neoplasms.

Hum Pathol. 2008 Apr;39(4):473-83.

MOLECULAR PATHOLOGY

Molecular Testing in Solid Tumors An Overview

Jennifer L. Hunt

Molecular testing in anatomic pathology is going to become more and more important during the next decade as we develop assays that can aid in diagnosis, prognosis, and predicting response to therapy. The anatomic pathologist needs to be familiar with the different assays available but also needs to be able to discern which are going to become standard of care and which will not. Three different types of tumors are reviewed: thyroid cancer, oligodendroglioma, and lung carcinoma. Molecular assays that are currently in use or on the near horizon, including translocation analyses for *RET-PTC* and *PPAR_γ-PAX8*, point mutation analysis for *BRAF* and epidermal growth factor receptor, and genetic loss for 1p and 19q, are discussed.

Traditionally, pathologists have relied on the hematoxylin-eosin–stained slide to make diagnoses. Prognostic indicators were limited to those that could be seen at the light microscopic level and included such variables as surgical margin status, lymph node metastases, and perineural and angiolymphatic invasion. These tools remain at the core of pathology practice for clinical care of patients. In the past 2 decades, the analysis of protein expression by immunohistochemical staining has become an integral tool for assessment of pathology specimens. More recently, molecular testing is being integrated into very specific areas in diagnostic pathology. Molecular testing in anatomic pathology serves various functions. The testing can be used diagnostically to assist in classifying or subclassifying tumors or other lesions. Clinical molecular testing can also be used for prognostic purposes. Finally, testing for molecular mutational events can be performed to analyze potential to respond to therapeutic agents.

The technologies that are used in assays for mutational events in anatomic pathology are often based on standard molecular biology techniques and assays. Many of the clinical assays rely on amplification of nucleic acids (polymerase chain reaction [PCR]) and hybridization techniques (fluorescent in situ hybridization [FISH]). Three types of tumors are discussed in this brief review, with particular emphasis on some of the specific molecular assays that are currently in use or in development for these organ systems. Several specific techniques are discussed in reference to clinical applicability in samples from the anatomic pathology laboratory.

THYROID CARCINOMA: *RET-PTC*, *PPAR__α-PAX8*, AND *BRAF*

Thyroid tumors have several well-identified molecular mutations. These have given us a better understanding of pathogenesis and now are making their way into use for clinical diagnostic testing. In fact, several studies have now reported utility of molecular testing in thyroid specimens preoperatively in fine-needle aspiration samples in final pathology diagnosis. The 2 most common types of mutations in thyroid cancers are translocations and point mutations. Translocations, or intrachromosomal rearrangements, between the *RET* proto-oncogene and various partner genes are found in up to 40% of papillary carcinomas. The most common partner genes are *H4 (PTC1)* and *ELE1 (PTC3)* (Figure 1).

These translocations, and particularly *RET-PTC3*, are more common in radiation-induced tumors. The other translocation that is notable in thyroid cancers occurs in follicular carcinomas. Up to 40% of follicular carcinomas harbor a translocation between the *PAX8* gene and the *PPAR_α* gene (*PAX8-PPAR_α*). Oncocytic carcinomas do not usually have this *PAX8-PPAR_α* translocation. However, recent evidence has suggested that some cases of follicular variant of papillary carcinoma may harbor this translocation. Translocations can be difficult to detect in paraffin samples. Many translocation breakpoints occur in large introns and may not be highly clustered, making PCR from genomic DNA very difficult. The most common strategy for testing is to use the messenger RNA (mRNA), in which the introns are spliced out, resulting in consistent fusion of exons from the 2 different partner genes in the mRNA. Unfortunately, mRNA is not of high quality when obtained from paraffin samples, and so many of the translocation assays are designed for fresh or frozen tissue. Another testing option for translocations that has proven to be highly successful in paraffin-embedded tissues is FISH. Dual color probes, or chromosome-specific paints, will localize each chromosome involved in the translocation. After hybridization in the assay, the abnormal fusions or breaks can be identified.

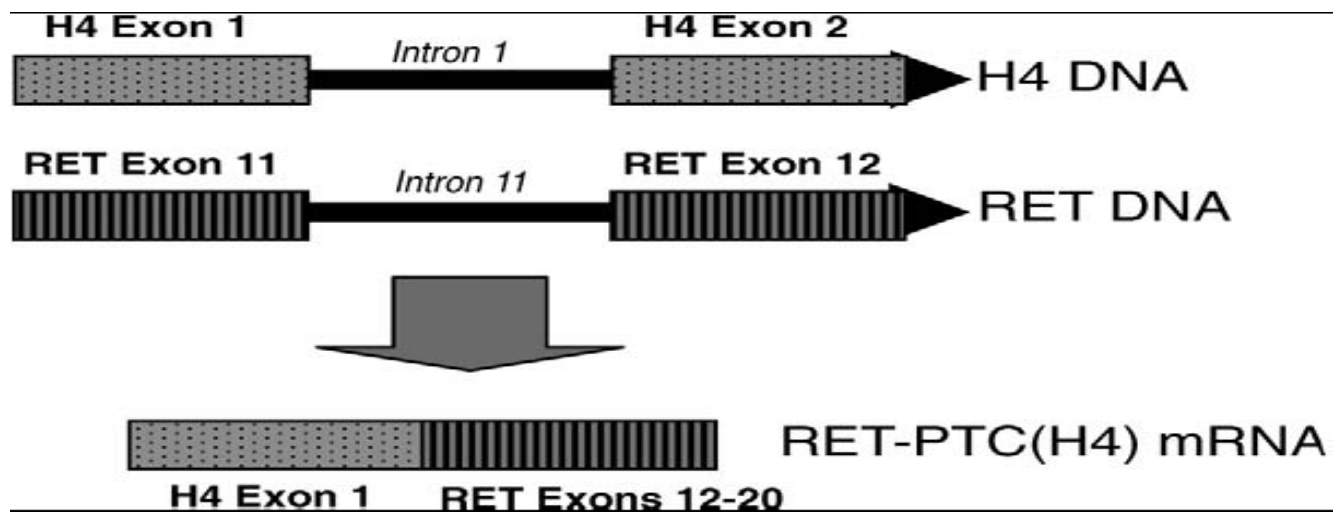


Figure 1. This diagram demonstrates the *RET-PTC1* rearrangement, in which exon 1 of the H4 gene is fused to exons 12 to 20 of the RET gene.

Finally, the overexpression of a fusion protein driven by the translocation can be detected by immunohistochemistry in certain instances. Antibodies for RET and PPAR_α have been used in this way. This approach is usually the least specific because protein expression can be increased through a variety of mechanisms. The final notable mutation in papillary carcinoma is found in the *BRAF* gene, which encodes for a serine threonine kinase. This point mutation is a T-to-A transition at codon 600 (nucleotide 1799). This mutation is thought to be the most common mutation in papillary carcinomas and is estimated to be found in 40% to 60% of cases. The mutation is more frequently found in conventional and tall-cell variant tumors and is uncommon in follicular variant of papillary carcinoma. Point mutations can be detected by a variety of assays, most of which are based in PCR techniques. These assays include standard direct gene sequencing, quantitative PCR, and newer platforms, such as pyrosequencing. All of the assays are fairly reliable for identifying cases with the characteristic *BRAF* gene mutation in papillary thyroid carcinoma.

OLIGODENDROGLIOMA AND LOSS OF 1p AND 19q

In brain tumors, concurrent loss of 1p and 19q is associated with oligodendroglioma differentiation. This mutational profile has also been shown to correlate with responses to chemotherapy. By contrast, loss of heterozygosity (LOH) mutations in *p53* and *p16* may be associated with a poor survival or with tumor progression. Because of the strong prognostic and diagnostic value of 1p and 19q loss analysis, some institutions are testing oligodendrogliomas and other primary glial neoplasms for these genetic abnormalities. The areas of 1p and 19q that are lost are fairly large and have been called the *minimal deletion regions*. Usually, multiple markers are used to cover the region (Figure 2). Loss of chromosomal arms can be assessed by FISH or by PCR, using an LOH analysis. FISH for loss of genetic material on fixed tissue sections can be difficult to interpret because of incomplete nuclei from drop-out in tissue sectioning. However, with appropriate validation, this is an excellent diagnostic test. Loss of heterozygosity analysis relies on our ability to discriminate between the 2 inherited copies of a particular gene. Polymerase chain reaction–based technologies have evolved to do this type of assessment using the polymorphisms in the human genome. These polymorphisms occur frequently

with an estimated periodicity of at least 1 polymorphism per 1000 base pairs. Loss of heterozygosity analysis usually uses short tandem repeat polymorphisms. These are areas in the genome at which there is a short sequence of DNA that is repeated. The sequence of the repeat unit is the same from person to person, but it is repeated for a variable number of times; these polymorphisms are inherited. For PCR assays to assess for LOH, primers are designed to flank a location-specific short tandem repeat polymorphism. In a person who is heterozygous for that gene locus, the PCR amplicons will have different lengths because there are 2 copies of the gene with different numbers of repeat units. Polymerase chain reaction products of different sizes will migrate at different speeds in both gel and capillary electrophoresis analyses. In tumor cells that have lost 1 copy of a normally heterozygous short tandem repeat polymorphism (LOH), there will be only 1 PCR product. Both LOH and FISH assays are commonly used to detect loss of 1p and 19q in oligodendrogliomas. These assays provide valuable clinical prognostic and therapeutic information.

LUNG ADENOCARCINOMA AND EPIDERMAL GROWTH FACTOR RECEPTOR

Lung carcinogenesis is complex and has predominantly been associated with smoking-related genetic damage. One of the common mutations found in lung cancer is a point mutation in the *RAS* oncogene. These mutations are often clustered in 2 different hot spots and can be fairly easily detected by direct sequencing. Many other mutations in other genes, including tumor suppressor genes, have also been described in lung carcinogenesis.

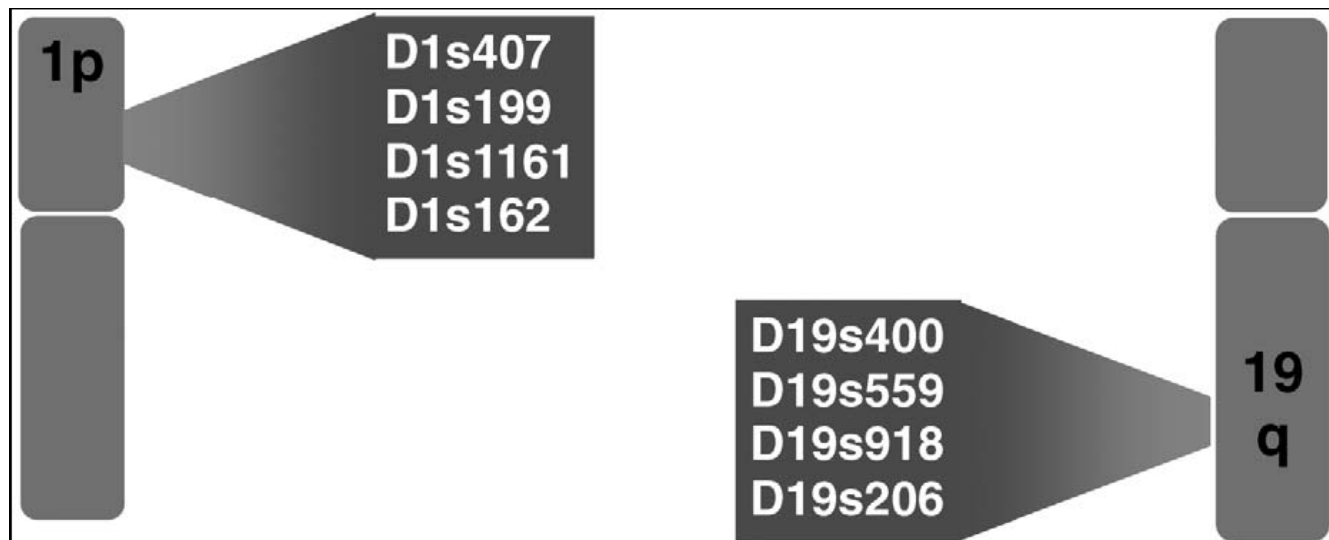


Figure 2. This diagram demonstrates the approach used to select short tandem repeats covering a minimal deletion region for 1p and 19q. These short tandem repeats fall within the minimal deletion region.

Trade Name	Generic Name	Company	FDA Approval
Iressa	Gefinitib	AstraZeneca (Wilmington, Del)	Limited for lung
Tarceva	Erlotinib	OSI (Melville, NY); Genentech (South San Francisco, Calif)	Lung, pancreatic
Erbix	Cetuximab	ImClone (New York, NY)	Colon

Clinical Feature	EGFR Mutant, %
Never-smokers	63
Smokers	22
Female	55
Male	23
Adenocarcinoma	47
Other tumor type	1.7

* These data demonstrate the percentage of cases from each clinical group of patients that have EGFR mutations in studies originating in Asia.

The recent discovery of alterations in epidermal growth factor receptor (*EGFR*) in lung carcinomas has been intriguing, especially because there are drugs that are directed against *EGFR* that have become potential targeted therapies for lung carcinoma (Table 1). Epidermal growth factor receptor is also known as *c-Erb-B1* and *HER1*. Epidermal growth factor receptor and the closely related family members *HER2/neu* (*c-Erb-B2*) and *Erb-B3* and *Erb-B4* are all cell membrane receptors that have intrinsic tyrosine kinase activities. Epidermal growth factor receptor is a 170-kd protein, 1186 amino acid transmembrane protein that has an extracellular ligand-binding domain, a transmembrane domain, and an intracellular tyrosine kinase domain. As with other oncogenes, *EGFR* can be assessed at several different levels: At the genomic level, we can identify mutations in the DNA sequence or copy number changes. At the mRNA level and the protein expression level, the amount of gene expression or the protein expression can be analyzed. In lung cancers, studies have detected alterations in *EGFR* at each level. In lung carcinomas, overexpression of EGFR by immunohistochemistry is often seen. Variable results have been reported regarding the prognostic significance of EGFR expression. Gene amplification by FISH has also been described in lung carcinomas, and recent reports do suggest that amplification has prognostic and therapeutic implications. One of the most interesting *EGFR* alterations has been recently described. Mutations have been found clustered in the tyrosine kinase domain of the *EGFR* gene, occurring almost exclusively in lung carcinomas. They are thought to be highly specific to certain populations of patients with specific tumors: young women, never-smokers, and adenocarcinomas (Table 2). Despite much work on other types of tumors, there have been very few reports of *EGFR* mutations outside of lung carcinomas. The reported rates of *EGFR* mutation in lung cancers are variable and are obviously highly dependent on the population being tested. The *EGFR* mutations are clustered in the region of exons 18 to 21, which is the area that encodes the ATP-binding pocket of the tyrosine kinase domain of *EGFR*. The most common mutations

involve exons 19 and 21, which account for more than 95% of mutations. Exon 19 mutations are deletion mutations, usually involving between 10 and 20 base pairs of the exon 19 sequence, beginning around codon 746; occasional exon 19 point mutations are also seen (codon 719). Exon 21 mutations are almost always point mutations, involving codon 858 with a T-to-G transversion in most cases. The relationship between somatic *EGFR* sequence mutations and response to anti-EGFR therapies has been controversial. Initial reports were promising, with demonstrated tumor shrinkage and benefits in surrogate endpoints. It appeared that mutations in the *EGFR* gene were more frequent in patients who responded to anti-EGFR therapies. However, importantly, all of these reports also acknowledge that some patients without identifiable mutations responded to anti-EGFR therapy. More recent studies have failed to demonstrate long-term benefit in survival advantages with treatment with anti-EGFR therapies. Therefore, testing for the point mutations and deletion mutations in the *EGFR* gene has not become standard practice at this time.

CONCLUSION

This overview provides several examples of molecular tests in different stages of clinical applicability. The life cycle of new diagnostic assays is complex. Some assays quickly and appropriately become the standard of care. Others are brought into clinical practice slowly, as needs evolve and develop. Still others show initial promising results but are abandoned when they fail to provide value in practice or when clinical management changes over time. The assays discussed provide examples that fit into each of these categories. Molecular testing in anatomic pathology will almost certainly become critical for providing optimal patient care during the next 5 to 10 years. As more assays are developed that provide valuable diagnostic, prognostic, and therapeutic information for patient management, anatomic pathologists will need to be familiar with the literature to order appropriate ancillary studies. Advanced molecular assays are best interpreted in an integrated fashion, with basic hematoxylin-eosin morphology and immunohistochemical findings remaining an essential component of the overall diagnosis.

Arch Pathol Lab Med—Vol 132, February 2008

BOTTOM LINE

ACP BEST PRACTICE

Dissection and Reporting of the Organs of the Female Genital Tract

M K Heatley

The aim of this article is to provide as comprehensive a review as possible of the techniques in use in dissecting and sampling the major specimens encountered in gynaecological practice, whether these have originated from gynaecological oncologists or from gynaecologists who specialise in non-malignant conditions. A brief description of relevant histology is provided where considered necessary for completeness.

Where possible I have listed material in boxes rather than providing it as free text in order to save space and in the hope that these lists will double as checklists when dissecting and describing these specimens or finalising the report for the clinician. Obviously no list can be exhaustive and it goes without saying that any temptation to pigeonhole features of a given specimen into the necessarily limited series of options included should be resisted. Common tumour types (e.g., adenocarcinoma, transitional cell carcinoma and squamous cell carcinomas), and metastases, melanoma, lymphomas and leukaemias, may occur at any site and have been omitted from these checklists to save space. Finally, I have tried to avoid duplicating material provided in other classification systems unless they have interesting associated pathological feature, an obvious example being the association between clear cell carcinoma of the vagina and diethylstilboestrol (DES) exposure in utero.

LYMPH NODES

It may seem odd that a paper dealing with the female genital organs should begin with an account of how lymph nodes should be handled, but it is worth describing it at this point as these specimens may be obtained either as therapeutic lymphadenectomy specimens or as part of a sampling procedure for cancers at any of the sites described below and the comments are therefore applicable to all these situations.

The TNM system specifies that ordinarily six lymph nodes are recovered from an inguinal and 10 from a pelvic lymphadenectomy, but intriguingly the failure to achieve the number does not alter the nodal staging. It is preferable that the surgeon submits the nodes from each group he wishes to have assessed separately, as it is often impossible to do this reliably once the anatomical landmarks have been lost when the tissue is removed from the body. Large lymph nodes may need to be sectioned to fit in a cassette, and more than one cassette may need to be used, although several small nodes may be processed intact together in a single cassette. A record should be made of which node goes in which cassette (e.g., first node in cassette A, second and third nodes in cassette B, and so on). Every lymph node is examined in its entirety unless obviously replaced by tumour when only one section need be taken providing one is confident that any pericapsular spread has been included in the section.

In the report, the number of lymph nodes recovered at each site, the number involved and a record of whether there are extranodal deposits or evidence of extracapsular spread is included.

It has been my practice for many years to submit all tissue including that which appears to be macroscopically fat for histology. Whilst this undoubtedly increases the lymph node count as fat replaced nodes may be macroscopically indistinguishable from fat, I have never located tumour in these sections.

CERVIX

Cervical polyps

Unless very large, when a longitudinal slice through the specimen including the base of the stalk is representative, all the tissue is embedded. Although it is said that endocervical polyps may give rise to changes that are confused with borderline nuclear change or dyskaryosis on cervical cytology, they

may be associated with a genuine CIN lesion in the adjacent cervix (in 2.7% (95% confidence interval (CI) 0.5% to 13.8%) of cases in a personal series).

In the report, the precise origin of the tissue (endocervical, endometrial, mixed (i.e., of probable lower segment origin)) and the diagnosis are given. The presence or absence of invasive malignancy or any other abnormalities should also be described.

Manchester repair

These are now rare, but I would handle them as the cervix from a hysterectomy for benign disease as described below.

Cervical punch biopsy

Box 1: Histological assessment of cervical punch biopsies

- Presence/absence of ectocervical squamous epithelium, endocervical glandular epithelium and deeper tissue (ie, endocervical crypts and stroma)
- Presence/absence of dysplasia: squamous CIN III, II or I; epithelial abnormality of uncertain significance; glandular dysplasia – cervical intraepithelial glandular neoplasia/adenocarcinoma in situ
- Evidence of wart virus infection
- Presence/absence of invasive malignancies

The macroscopic description, which is usually limited to a dimension, can be left to the histotechnology staff, though large biopsies such as a wedge specimen may require sectioning to fit into a cassette. Eosin-stained formalin does not assist in orientating cervical biopsies in my experience, and technologists have complained that it hampers distinguishing stroma and epithelium when orientating the specimen. They have found it helpful to receive the biopsy placed stromal side down on filter paper, even when the specimen detached from the paper, because it retains a flat base.

We routinely examine six histological levels from each block. The greatest yield is obtained in the first three sections, with examination of the further three levels resulting in an increase in the grade of CIN in about 10% of cases. Arguably, therefore, fewer levels need be cut if local arrangements allow the pathologist to be confident that discrepancies between the biopsy and highest grade of dyskaryosis on the previous smear can be reliably identified.

The content of the report is determined by the National Health Services Cervical Screening Program and is summarised in box 1.

All grades of CIN, including ungradable CIN and epithelial abnormality of uncertain significance, should be described, starting with the highest. The presence of viral features such as koilocytosis, warty features or a flat condyloma is mentioned after the CIN.

An invasive malignancy may be encountered, and estimates of type and grade are possible, though it must be stressed to the clinician verbally or in writing that the biopsy may be non-representative and that the adequacy of excision cannot usually be predicted on this type of specimen. These specimens are not usually sufficiently well orientated to allow a reliable assessment of the depth of invasion and further, ulceration of the surface epithelium reduces this measurement's reliability; however, an overall approximate estimation of the dimension may be useful, providing it is made clear verbally or in the report that this measurement is for guidance and may not be reliable.

Distinctions should be made between those specimens that fail to explain the cytological and colposcopic findings because they are technically inadequate, and those that are adequate but fail to account for the referral findings.

Loop excision specimen/cone

Box 2: Information to be included in the report of a cervical loop excision/cone biopsy specimen

Cervical intraepithelial neoplasia (CIN):

- The specimen is measured and the number of blocks taken recorded
- All grades present are noted, the highest should be recorded first
- Are both lips involved?
- Is CIN confined to the endocervical canal?
- Number of blocks containing CIN
- Involvement of endocervical crypts if present
- Presence of CIN at specimen edges: ecto, endocervical and deep lateral edges
- Presence of endocervical epithelium at the end of the canal
- Presence/absence of invasion
- (If three or more blocks are involved the tumour may be more than 7 mm across)
- Evidence of wart virus infection

Glandular dysplasia/intraepithelial neoplasia:

- Grade high/low
- Extent
- Completeness of excision
- The UK National guidelines comment that a note should be included in the report that glandular lesions of the cervix have a high risk of multifocality and residual disease in the form of skip lesions may persist up the canal; similarly, a note should be included that the presence of CIN at a specimen limit prohibits a diagnosis of microinvasive carcinoma

These are usually performed when confirming a cytological or colposcopic diagnosis of squamous CIN, though they are increasingly performed for cervical intraepithelial glandular neoplasia (CIGN), to

excise an ectropion, and rarely to diagnose and quantify a known clinical or colposcopic invasive cancer.

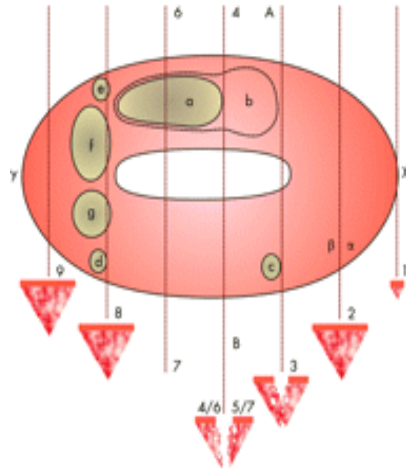
Cone and large loop excision specimens of cervix

These two specimen types are handled essentially in the same way. The report should indicate whether the specimen was received in more than one piece, and which blocks originated from which piece of tissue. The tissue may be painted with Indian ink and rinsed with acetic acid to ensure the ink stays on the tissue to denote the specimen edges, although diathermy artifact, if severe, may fulfill this role in some large loop specimens. Some pathologists use different colours to mark anterior and posterior surfaces, but I find the ink often runs, causing confusion. I do not encourage clinicians to pin the pieces making up a fragmented specimen on corkboard, as I find this damages the epithelial surface.

A permanent photographic record of the specimen should be made using the digital or Polaroid camera. Loop excisions should be sectioned transversely at regular intervals. Use of a pre-calibrated cutting board facilitates this, providing the tissue is well fixed and a sharp knife such as a skin graft blade is used. The cutting board I use is prefixed at 3 mm intervals. Each slice is turned to the right and embedded in individual cassettes, and as a result the tissue is examined at equal intervals throughout.

It is useful to standardise the sequence with which blocks are processed. If the clinician has orientated the specimen, I label the blocks from the extreme left of the specimen (i.e., surface X) moving toward the right, and number those from the anterior lip before the posterior lip (Fig 1). I mark the surface of the block opposite to the one I wish to have embedded downward, and thus sectioned first, with red ink just in case the tissue should turn in the processing cassette before or during opening. I prefer to put each piece into a separate cassette and mark where they have come from on a photograph of the specimen, and only divide slices if they are too big to fit into the cassette, as introducing a metal instrument into the canal damages the epithelium, especially the columnar epithelium. If it is necessary to do this, I squeeze the transverse aspects of the tissue slice and make the canal pout so I can insert the knife without it making contact with and damaging the epithelium. Over the years, I have found that it is a good policy to minimise any manipulation of these specimens, as the epithelium, particularly if there is extensive CIN III, may be very friable and excessive handling may result in its being denuded. The system described above is illustrated in the diagram (Fig 1) where the initial slices (1 and 2) show ectocervix, but as the slices are progressively taken there is a gradual emergence of the columnar epithelium in the crypts around the canal (3) and then the canal itself including the squamocolumnar junction (4–7). The transverse (Fig 1, A and B) and first lateral edges (Fig 1, X) of the specimen are examined. Some pathologists turn the first block through 180 deg before embedding it, arguing that by doing so they get a greater profile of tissue but I would suggest that the resulting section (alpha) is merely a mirror image of the section (beta) that is cut from the block that follows and that unless this first block is routinely turned through 180 deg re-embedded and a further section (representing the edge denoted by X in Fig 1) is cut, the first 3 mm of tissue are left unrepresented histologically.

Figure 1 Diagram showing blocking system and its rationale for loop and cone biopsy specimens.



If the cone is more than 25 mm long apical blocks are taken and embedded in the first cassette(s).

All the tissue should be submitted for histology.

I resist routinely examining multiple levels, as I believe that it is uneconomic in technical and medical time and as experience has shown that should it be necessary to invert the block to examine the obverse side, it can cause technical difficulties if excessive levels were taken initially. Should there be a discrepancy between the previous cytological or the colposcopic diagnoses and the histological features, or if the section, particularly the squamocolumnar junction, is incomplete, I examine a single deeper level because in 6% (95% CI 3.5% to 10.2%) of cases this has resulted in a significant increase in the grade of CIN identified and in 2.5% (95% CI 1.1% to 5.7%) of cases it has allowed its identification for the first time.

Inverting the last block (Fig 1, section 9) may be necessary to demonstrate involvement of lateral edge Y (some pathologists argue it is unnecessary to do this as the presence of CIN 3 mm from any edge (e.g., in section 9) has the same prognostic significance as if definite margin involvement is encountered). If CIN I is present at such a margin, recent work suggests there is no increased risk of recurrent disease over patients with clear margins.

If stromal invasion is noted in two consecutive blocks (e.g., Fig 1, sections 4 and 6) I cut further levels from these blocks, and I turn the block preceding the first of these since the largest tumour profile may be in the preceding slice. Thus inverting block 3 and sectioning into it may provide the best assessment of the cross-sectional tumour size in tumour b (Fig 1).

Report

The presence of CIN should be noted and all grades present recorded. I usually make a note of which blocks are involved in brackets after the grade of CIN, as it is useful if I need to demonstrate this in a hurry at a multidisciplinary meeting, and also because it gives a semiquantitative indication of the extent of the disease. I also record whether both lips are involved, if disease is confined to the

endocervical canal, as this may not be visible colposcopically, or if it involves the endocervical crypts. The prognostic importance of these criteria may simply be that they provide an indication of the overall size of the area of abnormality. The condition of the specimen edges and the presence of endocervical or squamous epithelium at the end of the canal is recorded systematically. The presence of glandular dysplasia/intraepithelial neoplasia, which in the UK is graded as low or high grade, is recorded along similar lines (see box 2).

Tumours that are visible to the naked eye are staged as 1b, but, if microscopic, the tumour should be measured using an eyepiece graticule or the vernier stage on the side of the microscope, to establish if it exceeds the criteria for stage 1a1 tumours (7 mm across by 3 mm deep, below originating epithelium in the crypt or intact surface), as it may warrant aggressive surgical treatment. The dimensions should be measured on the section showing the greatest profile. This provides two of the three dimensions cited (ie, one transverse dimension across the section and the depth). Even if the tumours are <7 mm across x 3 mm deep, the third dimension may exceed 7 mm, and Burghardt, who developed the system of examining parallel sections, advocated multiplying the greatest dimension by 1.5 to arrive at this "third dimension". Traditionally British pathologists have sought to establish this third dimension by multiplying the number of involved slices by their thickness. Thus in tumour b (Fig 1), where three 3 mm slices are involved, this dimension is up to 9 mm, whereas tumour with two slices involved is no more than 6 mm thick. To ensure the third dimension of the tumour is not underestimated, I turn the block preceding the block from which the section first showing tumour was cut, through 180 deg to exclude invasive tumour in it. In tumour a, this is slice 3 (Fig 1) and it is not involved, confirming that it involves only two slices, and, as each of them is 3 mm thick, it is less than 6 mm (ie, stage 1a1).

In contrast, with respect to tumour b in Fig 1, the initial sections would show tumour in slices 4 and 6, which are in continuity but not in section 3. Turning section 3 reveals an invasive component suggesting invasion over three slices (up to 9 mm, and indeed the largest cross-sectional dimensions would be located as a result of this process). This method probably overestimates the third dimensions in some cases, but is justified on the basis that tumour is better over treated than under treated.

The consequence of this method of arriving at this third dimension is that the only person who can assess what it is, is the person who cut the case, as only he/she can be confident as to whether the blocks were cut at 2, 3 or 4 mm intervals and if they were of equal thickness. Thus the person cutting the case should usually be deferred to when invasive tumour involving more than one contiguous slice is encountered; this issue may cause problems especially when cancer centre pathologists are reviewing the work of others. (Note: microscopic tumour invasion and early stromal invasion are descriptive terms that are no longer used to describe tumours that are less than 1 mm deep). Another area of controversy is when several small foci of microinvasion, none of which is more than 7x7x3 mm deep but which are separated by more than 7 mm, are encountered, as in foci c and d in Fig 1. We will assume that foci d and e in the same slice are also separated by more than 7 mm of non-invasive tissue. The FIGO and TNM classifications give no advice as to how best to proceed in this scenario. In my view it is illogical to stage widely separated foci that may be confined to one or two cells as being of stage 1b and presumably therefore warranting radical surgery, whereas two foci each 3.4 mm across (Fig 1, f and g) separated by less than 0.1 mm of tissue and thus occupying a lateral dimension of less than 7 mm are stage 1a1 and do not warrant such treatment despite having a greater overall volume. Burghardt, in his paper, describes adding the volume of such tumours together, and indeed his

prognostic data are based on this strategy. In my experience, most clinicians appreciate this problem and judge each case on its merits after discussion with the patient (box 2).

Deep resection edges (the so-called top hat)

Large specimens may warrant being treated as above but on occasion it may be best to place an inked orientation mark on the specimens to ensure the distal resection margin is sectioned first and process them intact. In this situation, consideration should be given to ordering levels at cut up. If this option is taken, a photograph must be available that should be marked to indicate how the specimen was orientated.

Invasive tumours are typed using the World Health Organization (WHO) system, which may be supplemented by consulting the International Society of Gynaecological Pathologists' modification.

Other useful information is the presence or absence of lymphatic/vascular invasion; although this does not alter the stage of the lesion some surgeons will opt for more radical surgery if it is extensive or if the primary tumour shows adenocarcinomatous differentiation. Some pathologists also comment as to whether the border of the tumour is confluent or infiltrative.

The description for the dissection of hysterectomy specimens in patients with CIN or invasive cervical tumours is included in the section dealing with hysterectomies below.

Tissue trauma

Opening cone biopsy and loop excision specimens may damage the epithelium lining the endocervical canal and may also result in underestimating the dimensions of a peripherally placed invasive tumour; this practice and attempts at "clock facing" an intact specimen should be discouraged.

Endocervical curettings

Box 3: Histological assessment of endometrial samples

- Phase of cycle (proliferative, secretory, menstrual), inactive, atrophic, postmenopausal)
- Inflammation/stromal reaction if present
- Hyperplasia (disordered proliferative endometrium, simple or complex architectural hyperplasia with/without cytological atypia), intraepithelial neoplasia
- Malignancy (endometrial adenocarcinoma should be typed and graded)
- Vascular lymphatic and myometrial invasion if present

I have not seen a specimen of this type for many years. They should be discouraged, since if malignant endocervical pathology is present it may render attempts to assess the presence and depth of stromal invasion impossible. Their value therefore seems to be confined to saying whether or not abnormal

epithelium is present and even then it may not be possible to grade it; this information can be extracted by a cytopathologist from a properly handled cytology specimen.

Journal of Clinical Pathology 2008; 61:241-257