IMMUNOHISTOCHEMISTRY IN GYNAECOLOGICAL PATHOLOGY- PART 1

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IMMUNOHISTOCHEMISTRY IN GYN PATHOLOGY

• very useful but sometimes overdone
• extensive sampling often more valuable (especially in ovarian neoplasms)
• use panel of markers
• panel should be FOCUSED depending on differential diagnosis
• beware unexpected positive and negative staining reactions
TOPICS TO DISCUSS

• typing of ovarian carcinoma
• uses of WT1
• primary versus secondary ovarian adenocarcinoma (adenocarcinoma of unknown origin, cytology specimens)
• markers of sex cord-stromal tumours
• typing of uterine carcinoma
• endometrial versus cervical adenocarcinoma
• cervical neuroendocrine carcinomas
• TTF1 in gynaecological neoplasms
TYPING OF OVARIAN CARCINOMA

• at present of limited therapeutic significance
• treatment more dependent on stage and grade
• NEW ERA OF TARGETTED THERAPY FOR OVARIAN CANCER
• ongoing trials regarding alternative therapeutic agents in clear cell and mucinous carcinoma (chemoresistant neoplasms)
REPRODUCIBILITY OF TYPING OF OVARIAN CANCER

- significant interobserver variability, especially for poorly differentiated tumours
- main problem is SEROUS/ENDOMETRIOID
- other problem is CLEAR CELL (true clear cell/clear cell change in serous or endometrioid)
- transitional - very subjective and poor agreement
USEFUL MARKERS

- WT1- most serous carcinomas positive
- p53- most high grade serous positive
- p16- most high grade serous positive
- hepatocyte nuclear factor 1 β- marker of clear cell carcinoma
WT1

- good marker of ovarian, tubal, peritoneal serous carcinomas
- endometrioid, clear cell, mucinous rarely diffusely positive
- useful in distinction between serous and endometrioid or serous and clear cell
- most ovarian undifferentiated and “transitional” carcinomas also positive
p16 and p53

• most ovarian high grade serous carcinomas positive

• most low grade serous, clear cell, mucinous and endometrioid negative or focally positive

• some high grade endometrioid p53 positive
CLEAR CELL VERSUS CLEAR CELL AREAS IN OTHER TUMOURS

- classic areas of serous or endometrioid
- true clear cell- solid, papillary, tubulocystic patterns, hobnail cells, eosinophilic stromal hyalinisation
- WT1, p16 (serous +ve; true clear cell -ve)
- ER (clear cell almost always -ve; serous often +ve, endometrioid usually +ve)
- hepatocyte nuclear factor 1 beta (useful marker of clear cell carcinoma)
POST -CHEMOTHERAPY

• increasing tendency to neoadjuvant chemotherapy followed by surgery
• ongoing trials (CHORUS in UK)
• post-chemotherapy serous carcinomas may have clear cytoplasm and mimic clear cell carcinoma
• maintain characteristic immunophenotype
HEPATOCYTE NUCLEAR FACTOR 1 BETA

- good marker of ovarian (and uterine) clear cell carcinoma (discovered from gene expression studies)
- endometriosis (associated and not associated with clear cell carcinoma) may be +ve
- occasionally other neoplasms +ve
- need more studies (and new monoclonal antibody)
WT1

- only consider nuclear staining
- normal fallopian tube epithelium positive
- useful marker of ovarian, tubal, peritoneal serous carcinoma
- other neoplasms positive- mesothelioma, OSCCHT, DSRCT, some ovarian sex cord-stromal tumours
- different antibodies (N-terminal, C-terminal)
USES

- confirmation of serous carcinoma (adenocarcinoma of unknown origin; typing of ovarian carcinoma; distinction from metastatic breast carcinoma)
- uterine versus extrauterine serous carcinoma
- OSCCHT versus mimics
COEXISTENT SEROUS CARCINOMAS

- USC may disseminate widely and involve ovaries, even within polyp (also EIC)
- OSC may involve uterus
- ? which is primary
- ? independent synchronous or metastatic disease
- management may differ
• WT1 may be of value
• much more likely to be positive in OSC than USC (however, some overlap with occasional USC diffusely positive)
• if WT1 negative, doubt whether OSC
OVARIAN SMALL CELL CARCINOMA OF HYPERCALCAEMIC TYPE

- usually young females (peak in 2nd and 3rd decades)
- may occur in older females
- hypercalcaemia in two-thirds
- WT1 almost always +ve
WT1

- antibodies against N and C terminals
- most commercially available are against N-terminal
- nuclear immunoreactivity diagnostically important
- N-terminal positive in serous carcinomas, mesothelioma
- C-terminal positive in DSRCT
- N-terminal positive in OSCCHT
PRIMARY VERSUS SECONDARY OVARIAN ADENOCARCINOMA

- also disseminated peritoneal adenocarcinoma and cytology specimens
- CK7/CK20
- CEA
- CDX2
- CA125
- CA19.9
- ER, PR
- WT1, TTF1
CK7 and CK20

- CK7+ve; CK20-ve profile not specific for GYN primary
- main use is in exclusion or confirmation of colorectal primary
METASTATIC COLORECTAL CARCINOMA

• histologically may mimic endometrioid (pseudoendometrioid), mucinous or rarely clear cell ovarian carcinoma
DISTINCTION FROM ENDOMETRIOID CARCINOMA

- triad of squamous elements, endometriosis, adenofibromatous areas are strong pointers in favour of ovarian endometrioid carcinoma
- IMMUNOHISTOCHEMISTRY
PANEL

• primary ovarian endometrioid carcinoma- CK7, ER, CA125+ve ; CK20, CEA , CDX2 negative

• colorectal adenocarcinoma- CK20, CEA, CDX2+ve; CK7, CA125, ER negative
• in distinction between primary ovarian mucinous adenocarcinoma and metastatic colorectal carcinoma, immunohistochemistry is of less value

• many primary ovarian mucinous carcinomas exhibit positivity with intestinal markers (CK20, CEA, CDX2, CA19.9), either focal or diffuse

• colorectal carcinomas with a mucinous appearance often exhibit focal CK7 positivity
In general:

• ovarian mucinous carcinomas are diffusely positive with CK7 and focally positive with CK20; may also be CEA, CA19.9, CDX2 positive

• mucinous colorectal carcinomas usually diffusely positive with CK20 and focally with CK7

EXCEPTIONS OCCUR
UPPER GI ADENOCARCINOMA

• stomach, pancreas, biliary tree
• very similar immunophenotypetype to primary ovarian mucinous neoplasms- NO RELIABLE MARKER TO DISTINGUISH (DPC4 may be of value but not in widespread use)
• pancreas often CA125 positive
ER/PR

- useful in adenocarcinoma in suggesting gynaecological or breast primary
- occasionally other carcinomas positive (pancreas may be PR +ve)
- pathologists often forget to use as part of panel
METASTATIC CERVICAL ADENOCARCINOMA

• in most cases diagnosis is obvious
• rarely ovarian mass is first manifestation
• small cervical adenocarcinomas may metastasise to the ovary
• value of p16 (cervix positive; ovarian endometrioid and mucinous carcinoma usually negative)

• also HPV studies (ISH or PCR)

• REMEMBER OVARIAN SEROUS CARCINOMA USUALLY POSITIVE
OVARY VERSUS BREAST

- association between breast and ovarian carcinoma (BRCA1/2)
- usually breast cancer develops first
- most patients with history of breast carcinoma who have pelvic mass will have new ovarian primary, almost always serous in type
- if poorly differentiated, morphological overlap between metastatic breast and serous
- can get micropapillary variant of breast carcinoma
- core biopsy often performed
USEFUL MARKERS

• ovarian serous- WT1, CA125, PAX 8 (useful new marker of non-mucinous ovarian carcinomas), PAX2
• breast- GCDFP15 (more specific/less sensitive), mammoglobin (more sensitive/less specific)

SOME OVERLAP AS ALWAYS BUT USEFUL PANEL
INHIBIN, CALRETININ , CD56

- useful markers of ovarian sex cord-stromal tumours
- inhibin most specific, CD56 most sensitive but less specific
- SOME CLASSIC GRANULOSA CELL TUMOURS INHIBIN NEGATIVE
- also often positive in UTROSCT
- ST1 (steroidogenic factor 1) useful marker of ovarian sex cord-stromal tumours
CD56

- extremely sensitive marker of ovarian sex cord-stromal tumour (84 of 85 cases)
- more sensitive than inhibin or calretinin
- lacks specificity (neuroendocrine tumours positive)
- membranous and weaker cytoplastic staining
ENDOMETRIOID CARCINOMA VERSUS SEX CORD-STROMAL TUMOUR

• sex cord-like areas in endometrioid carcinoma may mimic granulosa or Sertoli cell tumour
• pseudoendometrioid Sertoli-Leydig cell tumour
IMMUNOHISTOCHEMISTRY

- EMA and CK7- endometrioid adenocarcinoma
- inhibin, calretinin and CD56- sex cord tumour
- cytokeratins of limited value
- EMA almost invariably negative in sex cord-stromal tumour (except JGCT)
DISPLACED GRANULOSA CELLS

- may mimic small cell or non-small cell carcinoma
- artefact of surgery or specimen dissection and may be associated crush artefact
- may involve ovarian tissue spaces or true vascular spaces
- rarely in tube or omentum
- awareness of phenomenon
- immunohistochemistry (inhibin and calretinin positive; CD56 negative)
Distinction Between Endometrial and Endocervical Adenocarcinoma

• morphological differences but can be similar
• looks for clues (squamous elements, foam cells)
• primary surgery may differ
• adjuvant therapy may differ
Panel

- ER
- vimentin
- monoclonal CEA
- p16
Endometrial adenocarcinoma of endometrioid type

- ER diffusely positive
- Vimentin diffusely positive
- CEA negative (squamoid areas may be positive) or rarely focally positive
- p16 negative, focal or diffuse (but patchy) positivity (squamous areas may be positive) (VERY FEW CASES TOTALLY NEGATIVE)
Endocervical Adenocarcinoma

• ER negative or weakly positive
• vimentin negative
• CEA usually positive
• p16 diffusely positive
p16 ASSOCIATION WITH HPV IN CERVICAL ADENOCARCINOMA

- usual type diffusely p16 positive in 42 of 43 cases (78% HPV positive)
- unusual morphological types diffusely p16 positive in 6 of 20 cases (others focally positive) (only 1 was HPV positive)
- DIFFUSE p16 POSITIVITY IN CERVICAL ADENOCARCINOMA NOT ALWAYS DUE TO PRESENCE OF HIGH RISK HPV
Pitfalls in Immunohistochemical Panel

- only useful for well differentiated tumours (endometrioid versus endocervical)
- small biopsies
- some endometrioid carcinomas are diffusely p16 positive (but still patchy)
- uterine serous carcinoma may be diffusely p16 positive (often also ER negative)
- mucinous carcinoma of endometrium (or mucinous areas in endometrioid) has inconsistent immunophenotype
UTERINE SEROUS VERSUS ENDOMETRIOID CARCINOMA

• papillary variants of endometrioid (including usual type, villoglandular and with small non-villous papillae)

• glandular variants of serous
SEROUS ADENOCARCINOMA

• NOT papillary serous adenocarcinoma
• many cases have predominant or exclusive glandular architecture
DISTINCTION BETWEEN SEROUS AND ENDOMETRIOID

- important
- surgical operation may differ
- adjuvant therapy may differ
- ultimate prognosis will differ
- DISTINCTION BASED ON MORPHOLOGY +/- IMMUNOHISTOCHEMISTRY
IMMUNOHISTOCHEMISTRY-
SEROUS VERSUS ENDOMETRIOID

• use a panel (ER, p63, p16)
• interpret along with morphology
• overlap in significant number of cases
CLASSIC IMMUNOPHENOTYPE

• endometrioid- ER+ve, p53-ve, p16-ve (or focal/patchy)
• serous- ER-ve, p53+ve, p16+ve

BUT SIGNIFICANT OVERLAP
Problems

- grade 3 endometrioid carcinomas
- mixed tumours
- immunohistochemical methods (especially p53)
- **overlapping immunophenotypes** (serous may be ER+ve and p53-ve) (endometrioid may be p16 positive)
CERVICAL NEUROENDOCRINE CARCINOMAS

- SCNEC/LCNEC
- important to make diagnosis since aggressive and require specific management
- SCNEC- may be overlap with small cell squamous
- LCNEC- may be overlap with poorly differentiated squamous or undifferentiated carcinoma
- primary versus secondary (especially from lung)
STUDY-SCNEC (n=13), LCNEC (n=8)

MARKERS

• AE1/3
• chromogranin, CD56, synaptophysin, PGP9.5
• TTF1
• p16
• p63,
• CK7/20
• neurofilament
• CD99
AE1/3

- 85% SCNEC and 75% LCNEC positive
NEUROENDOCRINE MARKERS

• 52% chromogranin positive
• 90% CD56 positive
• 90% synaptophysin positive
• 43% PGP9.5 positive
SCNEC

- 3 positive with all 4 neuroendocrine markers
- 6 positive with 3 markers
- 2 positive with 2 markers
- 2 positive with 1 marker
LCNEC

• 1 positive with all 4 markers
• 4 positive with 3 markers
• 3 positive with 2 markers
TTF1

- 71% positive
- 85% SCNEC
- 50% LCNEC
- immunoreactivity often diffuse
TTF1

- high percentage of cervical neuroendocrine carcinomas positive
- of no value in distinction from a pulmonary metastasis
- ? useful marker of neuroendocrine carcinoma
- different clones (SPT24 versus 8G7G3/1)
p16

- all except 1 case diffusely positive
- may be due to association with high risk HPV
- positivity may occur due to non-HPV related mechanisms (pulmonary small cell carcinomas often positive)
p63

- p53 homologue
- only nuclear immunoreactivity important
- in cervix, useful marker of squamous carcinoma
- most adenocarcinomas and neuroendocrine carcinomas negative
- useful in distinction between SCNEC and small cell squamous and between LCNEC and poorly differentiated squamous
p63

- 9 of 21 (7 SCNEC, 2 LCNEC) positive
- 5 with diffuse nuclear positivity
- occasional cases with cytoplasmic positivity
CK7/CK20/neurofilament

- CK7- 10 cases positive (6 SCNEC, 4 LCNEC)
- CK20- 4 cases positive (3 SCNEC, 1 LCNEC)
- neurofilament- 7 cases positive (3 SCNEC, 4 LCNEC)
Evidence of Merkel Cell Immunophenotype

- no case positive for both CK20 and NFT
- CK20 positivity described in small cell carcinomas in other organs (? of prognostic significance)
- Merkel cell polyomavirus negative (CM2B4)
- CK20 positivity in small cell neuroendocrine carcinoma of unknown origin not diagnostic of Merkel cell carcinoma
CD99

• marker of Ewing family of tumours
• 6 cases with membranous immunoreactivity (4 SCNEC, 2 LCNEC)
• 2 cases diffusely positive
• illustrates overlap with Ewing family of tumours
ASSOCIATION BETWEEN CERVICAL NEUROENDOCRINE CA AND AIS

• AIS found in several cases
• chromogranin positive cells in some cases
  (and some cases of pure AIS)
• ? neuroendocrine cells in AIS as origin of
  some neuroendocrine carcinomas
TTF1 IN GYNAECOLOGICAL NEOPLASMS

- unusual cases of struma ovarii (use with thyroglobulin)
- neuroendocrine neoplasms
- metastasis from lung/thyroid
- some gynaecological adenocarcinomas positive (some diffusely so)
UNUSUAL STRUMA OVARII

- clear cells
- oxyphil cells
- cystic struma ovarii
- unusual thyroid type adenocarcinomas
MALIGNANT STRUMA OVARII

• sometimes not typical papillary or follicular carcinoma
TTF1 IN GYN ADENOCARCINOMAS

- 7 of 19 ovarian serous carcinomas
- 1 of 28 cervical adenocarcinomas
- 6 of 31 uterine endometrioid adenocarcinomas
- 3 of 13 uterine serous carcinomas
CLINICAL HISTORY

- female 67
- found to have a right lung mass on routine radiological examination
- underwent right middle lobectomy
PAST MEDICAL HISTORY

- TAH and BSO four years previously for endometrial carcinoma
FURTHER IMMUNOHISTOCHEMISTRY

- endometrial carcinoma ER positive and focal strong TTF1 positivity
- lung carcinoma ER positive
TTF1 IN UTERINE ADENOCARCINOMAS

• ? more common in those which result in lung metastasis
• ? due to poor differentiation or result of a homing mechanism
IMMUNOHISTOCHEMISTRY IN GYNAECOLOGICAL PATHOLOGY

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Immunohistochemistry plays an important role in various diagnostic scenarios in gynaecological pathology. However, immunohistochemistry is sometimes overdone and it is always to be remembered that immunohistochemistry is an adjunct technique and an aid to careful morphological examination. Since no antibody is totally specific for any given tumour and since unexpected positive and negative staining reactions may occur, panels of markers should always be used and these panels should be carefully focused depending on the differential diagnosis under consideration. In many diagnostic scenarios, especially in the examination of an unusual ovarian neoplasm, judicious sampling is sometimes more useful than immunostains. However, when carefully used, immunohistochemistry can be extremely useful and is paramount in diagnosis in many cases. Several topics are discussed in this talk which aims to concentrate on new developments regarding immunohistochemistry in the female genital tract.

TYPING OF OVARIAN CARCINOMAS

Ovarian carcinomas comprise a heterogeneous group of neoplasms. Each tumour type has a different underlying pathogenesis and natural behaviour. Although currently, management of ovarian carcinoma is largely dependent on factors such as tumour grade and stage, and not usually on cell type, it is important to accurately type ovarian carcinomas to ascertain whether the various neoplasms have a different behaviour independent of stage and other clinicopathological parameters. Typing is also important
since some tumours, such as mucinous and clear cell carcinoma, seem to exhibit a poor response to platinum-based chemotherapy. In fact, tumour type may be a more reliable predictor of response to chemotherapy than tumour grade and there are ongoing trials regarding different therapeutic regimes in ovarian clear cell and mucinous carcinomas. Morphology remains the mainstay in typing of ovarian carcinomas but immunohistochemistry may be of value as a supplement in problematic cases. From personal experience, typing is reproducible for well differentiated tumours of the four most common types, namely serous, endometrioid, clear cell and mucinous (there may be problems in mucinous carcinomas in distinguishing between a primary and secondary neoplasm and there is significant variability amongst pathologists as to what constitutes invasion in ovarian mucinous neoplasms but typing a tumour as mucinous is generally not problematic). However, there are significant problems with regard to typing poorly differentiated carcinomas. Many of the difficulties relate to the categories of high grade serous, high grade endometrioid and undifferentiated carcinoma in which there is morphological overlap. Another source of disagreement is the categorization of clear cell areas within ovarian carcinomas, specifically whether these represent a clear cell carcinoma or component of clear cell carcinoma or clear cell areas within a serous, endometrioid or undifferentiated carcinoma.

Some pathologists tend to diagnose poorly differentiated ovarian carcinomas as serous in type while others classify them as endometrioid or mixed serous and endometrioid. My personal opinion is that the majority of these are serous carcinomas. In this distinction, WT1 immunohistochemical staining may be of value. Most primary ovarian (as well as primary peritoneal and tubal) serous carcinomas exhibit diffuse nuclear positivity with WT1 while most endometrioid adenocarcinomas are negative or focally positive. p53 may also be of value in that most serous carcinomas are diffusely positive while most endometrioid adenocarcinomas are negative, although some high grade endometrioid neoplasms are positive. p16 (p16INK4A), a cyclin-dependent kinase IV inhibitor which is integral to pRb mediated control of the G1-S phase transition of the cell cycle, is more likely to be diffusely and strongly positive in serous than in endometrioid adenocarcinomas (the majority of high grade serous carcinomas are diffusely positive...
with p16 while most low grade serous, endometrioid, mucinous and clear cell carcinomas are negative or focally positive). Vimentin and nuclear β-catenin positivity favours an endometrioid adenocarcinoma, although not all cases are positive. In problematic cases, I would recommend WT1 immunohistochemical staining as an adjunct to help distinguish between a high grade serous and a high grade endometrioid adenocarcinoma.

Characteristically in ovarian clear cell carcinoma, an admixture of growth patterns is present, including solid, glandular, tubulocystic and papillary. Hobnail cells and eosinophilic hyalinised stroma are common features. Most clear cell carcinomas are diagnosed without difficulty but there is a tendency to overdiagnose clear cell carcinoma or a clear cell carcinoma component within a mixed neoplasm due to the presence of clear cell areas within other types of ovarian carcinoma, especially serous and to a lesser extent endometrioid. The presence of more typical areas of serous or endometrioid adenocarcinoma are useful pointers in diagnosis (sometimes a combination of clear cell and endometrioid adenocarcinoma occurs) and it is stressed that the mere presence of clear cells does not constitute a clear cell carcinoma. WT1 is usually negative in ovarian clear cell carcinoma, as is p53 and p16. ER may also be of value in that most clear cell carcinomas are negative while many serous and endometrioid adenocarcinomas are positive. Recently, hepatocyte nuclear factor 1 beta has emerged as a useful immunohistochemical marker of ovarian (and uterine) clear cell carcinoma. Most of the other morphological subtypes are negative, although there has been only limited investigation of the expression of this marker in morphological types of ovarian carcinoma other than clear cell.

Transitional carcinoma is a rare variant of ovarian epithelial malignancy which is subject to considerable interobserver variability in diagnosis. Some, including myself, make the diagnosis rarely and feel that most cases which are so-diagnosed are serous or endometrioid carcinomas with a transitional-like growth pattern while others diagnose transitional carcinoma not uncommonly. Ovarian transitional carcinomas are negative with markers which are commonly expressed in urothelial transitional carcinoma, such as
CK20, uroplakin III, thrombomodulin and p63. Rather, they exhibit a CK7 positive/CK20 negative immunophenotype, similar to other ovarian carcinomas, and exhibit Mullerian rather than urothelial differentiation. They often exhibit nuclear positivity with WT1, suggesting that many represent variants of high grade serous carcinoma. In contrast, Brenner tumours exhibit true urothelial differentiation and express markers which are commonly positive in normal urothelium and urothelial neoplasms, such as uroplakin III, thrombomodulin and p63. Many undifferentiated ovarian carcinomas are WT1 positive, suggesting that these merely represent the extreme end of the spectrum of poor differentiation in serous carcinomas.

Rare hepatoid carcinomas arise within the ovary; these may or may not be associated with a component of more usual epithelial tumour. In the absence of the latter, these neoplasms are morphologically indistinguishable from a metastatic hepatocellular carcinoma or a metastatic hepatoid carcinoma from other organs, such as the stomach. All these tumours, in addition to hepatoid yolk-sac tumour, exhibit a similar immunophenotype with positive staining with α fetoprotein (αFP) and hepPAR 1.

There is an increasing tendency to administer up-front chemotherapy to patients with advanced ovarian carcinoma. The morphological features of post-chemotherapy ovarian carcinoma often differ markedly from that of native untreated tumour. In some cases, it may be difficult to identify residual tumour cells while in other cases a misdiagnosis of clear cell carcinoma may be made because of the presence of abundant clear cytoplasm, a direct consequence of chemotherapy treatment. It has recently been demonstrated that ovarian carcinomas treated by pre-operative chemotherapy retain their chemonaive immunophenotype even if the morphological response is marked. Most advanced stage ovarian carcinomas are serous in type and following chemotherapy treatment are immunoreactive with antibodies such as CK7, CA125, WT1, ER, p16 and p53, markers which are characteristically positive in serous carcinomas. These markers may be useful in identifying residual tumour cells and in typing the neoplasm when a pre-chemotherapy biopsy has not been obtained.
DISTINCTION BETWEEN PRIMARY AND SECONDARY OVARIAN ADENOCARCINOMA

Most ovarian adenocarcinomas are readily classified as primary or metastatic. However, significant problems still arise in distinguishing between a primary ovarian adenocarcinoma of endometrioid or mucinous type and a metastatic adenocarcinoma. Although these difficulties have been highlighted in recent years, problems still arise. With a history of a carcinoma elsewhere and with bilateral ovarian adenocarcinomas, the possibility of a secondary is likely to be immediately considered by the pathologist. However, patients with no known history of a primary extraovarian neoplasm may present with a secondary adenocarcinoma in the ovary, which may be unilateral or bilateral, and this is an area where immunohistochemistry may be of value, although there are significant diagnostic pitfalls. At this point, I will note that it is my experience that immunohistochemistry is often resorted to with undue haste and without careful consideration of the gross and microscopic pathological features. Almost always there are features which should result in a definitive diagnosis of or strong consideration of a metastasis; of course, in these cases immunohistochemistry may be extremely useful to support the gross and microscopic suspicion of a secondary. I will discuss in the following sections the immunophenotype of adenocarcinomas of diverse organs, especially concentrating on those primary sites which are likely to metastasize to the ovary or present with disseminated peritoneal malignancy as ovarian cancer commonly does. The value of immunohistochemistry in defining the relationship between the two neoplasms in the not uncommon scenario of simultaneously occurring adenocarcinomas of the uterine corpus and ovary is discussed in a separate section below.

Metastatic Colorectal Adenocarcinoma

Differential cytokeratin (CK7 and CK20) staining has received much attention in the literature. The main value of these markers is in the distinction between a primary ovarian endometrioid or mucinous adenocarcinoma and a metastatic colorectal
carcinoma, especially between an endometrioid adenocarcinoma and a colorectal carcinoma with a pseudo-endometrioid appearance. The former are usually diffusely CK7 positive and CK20 negative while the latter exhibit the converse immunophenotype, although there may be focal CK7 immunoreactivity in a metastatic colorectal adenocarcinoma and focal CK20 staining of an ovarian endometrioid adenocarcinoma. In this distinction, I usually combine CK7 and CK20 with CA125 and oestrogen receptor (ER) (typically positive in an ovarian endometrioid adenocarcinoma) and carcinoembryonic antigen (CEA) and CDX2 (typically positive in a colorectal adenocarcinoma). CDX2 is an intestinal transcription factor which is diffusely positive with nuclear immunoreactivity in most colorectal carcinomas. However, focal positivity may be seen in ovarian endometrioid adenocarcinomas and occasional cases are diffusely positive (personal observations). Squamous morules in endometrioid carcinomas are also typically diffusely CDX2 positive. The histological features useful in the distinction between an ovarian endometrioid adenocarcinoma and a metastatic colorectal adenocarcinoma have been extensively described and will not be repeated here, except to mention that one or more of the triad of endometriosis, adenofibromatous areas and squamous differentiation may be useful pointers to an endometrioid adenocarcinoma.

Immunohistochemistry is less useful in distinguishing between a primary ovarian mucinous carcinoma or borderline mucinous tumour and a metastatic colorectal adenocarcinoma with a mucinous appearance, although it may still be helpful. Problems arise because colorectal carcinomas with a mucinous appearance may exhibit CK7 positivity. Moreover, most primary ovarian mucinous carcinomas and borderline tumours are of intestinal type and, as a consequence, often express enteric markers such as CK20, CA19.9, CEA and CDX2 and are negative with Mullerian markers such as ER and CA125. CK20 staining is usually focal while immunoreactivity with the other enteric markers may be focal or widespread. Helpfully, primary ovarian mucinous tumours usually retain their diffuse CK7 positivity, although this is not always the case. There is also a subset of primary ovarian mucinous tumours which arise in a teratoma which may be totally overgrown by the mucinous neoplasm. These mucinous tumours often exhibit overt intestinal differentiation and closely mimic a colorectal
adenocarcinoma. Not unexpectedly, they may exhibit an identical immunophenotype to a primary colorectal adenocarcinoma and are often diffusely positive with enteric markers such as CK20, CEA and CDX2 and CK7 negative. Other markers which have been reported to be of value in distinguishing between a primary ovarian endometrioid or mucinous adenocarcinoma and a metastatic colorectal adenocarcinoma include villin, β catenin, MUC2 and P504S (racemase), these typically being positive in colorectal carcinomas. However, in general these may be of limited value in an individual case since many primary ovarian mucinous carcinomas exhibit intestinal differentiation and may be positive, at least focally, with one or more of these markers. It is stressed that β catenin staining should be nuclear in order to be of diagnostic value since this pattern of immunoreactivity is associated with β catenin or adenomatous polyposis coli (APC) gene mutation which is characteristic of most colorectal adenocarcinomas. Some ovarian endometrioid adenocarcinomas also exhibit nuclear positivity, often confined to the squamous elements, since they are associated with β catenin mutation.

Occasional metastatic colorectal adenocarcinomas in the ovary have a clear cell appearance and potentially might be misdiagnosed as a primary ovarian clear cell carcinoma, although the morphological features of the two tumours are almost always significantly different. An identical panel of markers used to distinguish a metastatic colorectal adenocarcinoma from a primary ovarian endometrioid adenocarcinoma is useful.

**Metastatic Pancreatic and Biliary Adenocarcinoma**

When these neoplasms metastasise to the ovary they may closely resemble a primary ovarian mucinous tumour, either malignant, borderline or rarely even benign. Although these are often bilateral ovarian neoplasms with associated omental and peritoneal disease, this is not always the case and occasionally metastatic pancreatic or biliary adenocarcinoma presents as a large unilateral solid or cystic mass, apparently confined to the ovary. Immunohistochemistry plays a limited role in the distinction between metastatic pancreatic or biliary adenocarcinoma and a primary ovarian mucinous tumour
since they exhibit a similar immunophenotype with regard to differential cytokeratin staining, ie they are most commonly diffusely positive with CK7 and focally with CK20. However, any pattern of CK7 and CK20 staining potentially occurs in mucinous carcinomas of the ovary, pancreas and biliary tree. These neoplasms also exhibit a similar immunophenotype with regard to CEA, CA19.9 and CDX2, potentially being negative, focally or diffusely positive with each of these markers, such that in an individual case immunohistochemistry is of little value. These tumours are usually ER negative. DPC4 staining may be of value since in approximately 50% of pancreatic adenocarcinomas this tumour suppressor gene is inactivated by allelic loss with resultant absence of immunoreactivity. Negative staining with DPC4 is therefore suggestive of a pancreatic primary while positive staining is of no value. Pancreatic adenocarcinomas are often positive with CA125 and uncommonly exhibit nuclear immunoreactivity with PR. Rare acinar cell carcinomas of the pancreas metastasise to the ovary and cause problems in diagnosis. These neoplasms exhibit positivity with trypsin and, or, chymotrypsin.

**Metastatic Gastric Adenocarcinoma**

Immunohistochemistry is also of limited value in distinguishing between a metatastic gastric adenocarcinoma and a primary ovarian mucinous tumour. With regard to the immunophenotype of gastric adenocarcinoma, similar comments pertain to those already discussed for pancreatic and biliary adenocarcinoma. However, most metastatic gastric carcinomas in the ovary have the typical appearance of a Krukenberg tumour with signet ring cells which are very rare in a primary ovarian mucinous adenocarcinoma. Occasional metastatic gastric adenocarcinomas in the ovary have an intestinal pattern and mimic a primary ovarian endometrioid or mucinous carcinoma. Again, immunohistochemistry is of little value.

**Metastatic Appendiceal Adenocarcinoma**
Appendiceal adenocarcinoma may spread to involve one or both ovaries, more commonly the right in the case of unilateral involvement. The ovarian involvement may occur in the setting of pseudomyxoma peritonei (PMP) or outside of this setting. It is beyond the scope of this review to detail the relationship between the coexistent appendiceal, ovarian and peritoneal disease in PMP, except to say that there is abundant evidence that, in the majority of cases, the appendix is the primary source of disease. Some of this evidence comes from immunohistochemical studies demonstrating that the epithelial elements at all sites express enteric markers, such as CK20, CEA, MUC2 and CDX2 and are CK7 negative, in keeping with intestinal differentiation. However, in rare cases of PMP no appendiceal lesion is found in spite of examining the appendix in its entirety, the ovary being the source of the disease. In these cases, the ovarian mucinous tumour typically exhibits overt intestinal differentiation with diffuse CK20, CEA and CDX2 immunoreactivity and is thought to represent an intestinal mucinous neoplasm arising within a teratoma; other teratomatous elements may or may not be identified. It seems that only overt intestinal type mucinous epithelium has the capacity to give rise to PMP. Metastatic appendiceal carcinomas in the ovary outside the setting of PMP may comprise a mucin secreting adenocarcinoma resembling a colorectal primary and, on other occasions, a signet ring adenocarcinoma, morphologically resembling a gastric primary. In my experience, the appendix is often not considered as a possible site of primary with a metastatic ovarian mucinous or signet ring carcinoma. In some cases, the appendiceal neoplasm has a goblet cell carcinoid-like appearance with focal immunoreactivity for neuroendocrine markers. Immunohistochemistry may be of value in distinguishing between an appendiceal and a gastric primary for a signet ring carcinoma. Most metastatic gastric signet ring carcinomas are positive with CK7 while CK20 is variable. Conversely, most appendiceal signet ring carcinomas exhibit an overt enteric immunophenotype and are diffusely CK20, CEA and CDX2 positive while CK7 is negative or focally positive.

**Metastatic Breast Carcinoma**
Breast carcinoma metastatic to the ovary may mimic a primary ovarian endometrioid, serous or undifferentiated carcinoma. It is a not uncommon scenario that a patient with a history of breast carcinoma presents with a pelvic mass and the differential diagnosis lies between metastatic spread and a separate ovarian primary. Some of these patients have a germline BRCA1 or BRCA2 mutation, although this is a not uncommon scenario in patients without these mutations. In most cases, the pelvic tumour represents a new ovarian primary, usually of serous type. Immunohistochemically, metastatic breast carcinoma is usually positive with CK7 and negative with CK20. ER and PR are often positive, as is HER2. Hormone receptor positivity may be useful both in diagnosis and in determining the likely response to adjuvant therapy. Hormone receptor positivity is, of course, not specific for a metastatic breast cancer since many primary ovarian and other gynaecological malignancies are positive. Gross cystic disease fluid protein 15 (GCDFP15) and mammaglobin are commonly positive in breast carcinomas. The former is a relatively specific marker of breast cancer but its sensitivity is low. Mammaglobin is a more sensitive marker of breast carcinoma than GCDFP15 but is less specific since some gynaecological adenocarcinomas are positive, although expression of this marker has not been extensively studied in primary ovarian adenocarcinomas. In the distinction between a metastatic breast carcinoma and an ovarian high grade serous carcinoma, WT1 and CA125 may be of value since these are commonly positive in serous carcinomas but usually negative in metastatic breast carcinoma, although a small percentage of the latter may be positive with both markers. It has been shown recently that PAX8 is of value in the distinction between a metastatic breast carcinoma (PAX8 negative) and an ovarian serous or endometrioid adenocarcinoma (usually PAX8 positive). In this distinction, PAX8 has been found to be more sensitive and more specific than WT1. Another recent study suggested PAX2 to be of value in the distinction between an ovarian serous carcinoma (PAX2 positive) and a breast carcinoma (PAX2 negative).

Metastatic Cervical Adenocarcinoma
Cervical adenocarcinoma rarely metastasises to the ovary, usually in a patient with a known history of a cervical neoplasm or the metastasis is discovered at radical hysterectomy for the cervical tumour. However, occasionally the metastatic lesion is discovered in a patient who is not known to have a cervical neoplasm. The metastatic tumour may closely mimic a primary ovarian endometrioid or mucinous carcinoma and a high index of suspicion by the pathologist is needed to make the diagnosis. In the distinction between a primary ovarian endometrioid carcinoma and a metastatic cervical adenocarcinoma with an endometrioid appearance, ER and p16 staining may be of value. ER is usually diffusely positive in an ovarian endometrioid carcinoma and negative or focally positive in a cervical adenocarcinoma. Conversely, diffuse p16 staining (this is a surrogate of the presence of high risk human papilloma virus (HPV) in the cervix) is the rule in most cervical adenocarcinomas while the majority of ovarian endometrioid carcinomas are negative. These markers may be combined with molecular studies to demonstrate the presence of HPV. Similarly, p16 staining and HPV studies may be useful in distinguishing a primary ovarian mucinous carcinoma or borderline tumour from a metastatic cervical adenocarcinoma. ER is of little value in this regard as both tumour types are typically negative. As stated earlier, many high grade ovarian serous carcinomas are diffusely positive with p16 due to non-HPV related mechanisms; morphologically there is usually little resemblance between these and a metastatic cervical adenocarcinoma.

**Metastatic Pulmonary Adenocarcinoma**

Pulmonary adenocarcinomas rarely metastasise to the ovary usually, but not always, in a patient with a known primary lung tumour. These may mimic an ovarian serous, endometrioid or mucinous adenocarcinoma. Primary pulmonary and ovarian adenocarcinomas are usually diffusely positive with CK7 and negative with CK20. Thyroid transcription factor 1 (TTF1) is a sensitive marker of a primary pulmonary adenocarcinoma. However, we have recently shown that some gynaecological adenocarcinomas of ovarian, endometrial and cervical origin are positive, usually with focal staining but occasionally with diffuse immunoreactivity. Positive ovarian tumours
have included both those of serous and endometrioid type. ER may be of value in that many ovarian serous and endometrioid carcinomas are positive while pulmonary adenocarcinomas are negative. Pulmonary small cell carcinomas may also metastasise to the ovary and are TTF1 positive but small cell neuroendocrine carcinomas arising in other organs may also be immunoreactive.

**Metastatic Renal Carcinoma**

Rarely a renal clear cell carcinoma may metastasise to the ovary and potentially be mistaken for a primary ovarian clear cell carcinoma. Usually the morphological appearances differ with a characteristic admixture of architectural patterns in the primary ovarian neoplasm which commonly arises in endometriosis. A panel of markers may be of use. Renal clear cell carcinoma is usually negative with both CK7 and CK20 and positive with CD10 and renal cell carcinoma (RCC) marker. Conversely, primary ovarian clear cell carcinomas are almost invariably diffusely CK7 positive and negative with RCC marker; some may be CD10 positive. ER immunoreactivity is uncommon in primary ovarian clear cell carcinomas but occasional tumours are positive. Therefore, positive ER staining is suggestive of a primary ovarian clear cell carcinoma but negative staining is of no value. As stated earlier, ovarian transitional carcinomas express Mullerian and not urothelial markers. Positive staining with urothelial markers, such as uroplakin III, thrombomodulin and p63, may assist in confirming a metastatic urothelial transitional carcinoma in the ovary.

**ADENOCARCINOMA OF UNKNOWN PRIMARY IN PERITONEAL OR OMENTAL BIOPSY**

I have already detailed those markers of value in distinguishing between a primary ovarian adenocarcinoma and a metastatic adenocarcinoma from various sites. These may also be useful in the not uncommon scenario of a metastatic adenocarcinoma of unknown
origin in a peritoneal or omental biopsy or in a peritoneal fluid specimen. In a peritoneal fluid specimen, I would also carry out Ber EP4 immunocytochemical staining in order to exclude a mesothelioma, diffuse positivity with this marker being supportive of an adenocarcinoma. There are a few other general comments with regard to the pathological interpretation of an adenocarcinoma in an omental or peritoneal biopsy which are worth emphasizing. The first is that the likely primary site should be formulated on the histological appearances before immunohistochemistry is undertaken. A large majority (80-90%) of advanced stage (stage 3 and 4) ovarian carcinomas are serous in type and, as discussed, diffuse nuclear WT1 immunoreactivity is extremely useful in confirming a serous carcinoma which could have arisen either in the ovary, fallopian tube or peritoneum. Primary ovarian, tubal and peritoneal serous carcinomas are all usually diffusely WT1 positive, in contrast to uterine serous carcinoma. With a mucinous adenocarcinoma in a peritoneal or omental biopsy in a female the ovary is commonly suggested as a likely primary site. However, primary ovarian mucinous carcinomas are relatively uncommon and advanced stage ovarian mucinous carcinomas are rare; therefore with a mucinous adenocarcinoma in a peritoneal or omental biopsy, an ovarian primary is unlikely, the colorectum, pancreas, biliary tree, appendix or stomach being more likely sites of primary. In my experience, if the ovary is mentioned as a possible primary site in the pathology report the patient is often referred to a gynaecological oncologist because surgical debulking is typically indicated for an advanced ovarian cancer in contrast to the situation pertaining to other primary sites. Some pathologists erroneously equate a CK7 positive/CK20 negative immunophenotype in an adenocarcinoma of unknown origin with an ovarian primary; it is stressed that adenocarcinomas of many organs exhibit this pattern of differential cytokeratin staining and that the main value of differential cytokeratin staining is in helping to confirm or exclude a primary colorectal neoplasm. CA125 positivity is also often erroneously equated with an ovarian adenocarcinoma but this is a relatively unspecific marker, with adenocarcinomas of many sites potentially being positive.
ENDOMETRIAL CARCINOMAS INVOLVING OVARY AND SYNCHRONOUS ENDOMETRIAL AND OVARIAN CARCINOMAS

A not uncommon scenario is simultaneous involvement of the uterine corpus and one or both ovaries by an adenocarcinoma. Most commonly, these adenocarcinomas are endometrioid in type but sometimes they are serous. With different morphological tumour types, for example an endometrioid adenocarcinoma in the uterus and a serous adenocarcinoma in the ovary, it is clear that these represent independent primary neoplasms, although mixed endometrioid and serous carcinomas not uncommonly occur within the uterus and the serous element may preferentially metastasise even when this represents a minor component of the primary neoplasm. The most common scenario is the presence of an endometrioid adenocarcinoma within the uterus and one or both ovaries. This association is found in approximately 5% of uterine endometrioid adenocarcinomas and 10-15% of ovarian endometrioid adenocarcinomas. In such cases, the neoplasms may represent synchronous independent primaries or metastasis from the uterus to the ovary or vice versa. Careful pathological examination (discussed below) is the basis for deciding the relationship between the tumours. The question of whether these represent synchronous independent or metastatic carcinomas is of importance since adjuvant therapies may differ, as will the prognosis. For example, with an early stage, low grade endometrioid adenocarcinoma of the uterine corpus and a separate independent stage IA or IB well differentiated endometrioid adenocarcinoma of the ovary, it is likely that no adjuvant therapy will be given. Conversely, adjuvant therapy is indicated with an ovarian tumour which has spread to the uterus (stage II) or with an endometrial carcinoma metastatic to the ovary (stage IIIA).

It is currently considered that early stage, low grade endometrioid adenocarcinomas involving the uterus and one or both ovaries most likely represent synchronous independent primary neoplasms. The prognosis in such cases is usually good. Careful pathological examination using “common sense” criteria is the basis for deciding the relationship between the uterine and ovarian neoplasms. Adjacent endometrial hyperplasia in the case of the uterine tumour and endometriosis (which may be subtle) or
a component of benign or borderline adenofibroma in the case of the ovarian neoplasm are pointers towards an origin in these organs. With a deeply myoinvasive endometrial tumour exhibiting prominent lymphovascular invasion and tumour deposits in and on the surface of both ovaries, a uterine primary with ovarian metastasis is likely. The pattern of ovarian involvement may be a clue to a secondary neoplasm; for example, a nodular pattern of tumour growth, surface implants and prominent lymphovascular invasion within and surrounding an ovary which shows significant preservation of its parenchyma are clues to metastatic involvement. With endometrioid adenocarcinomas involving the uterus and one or both ovaries, immunohistochemistry is of no value in ascertaining the relationship between the tumours as the immunophenotype of a primary ovarian and uterine endometrioid adenocarcinoma is essentially identical.

With a serous carcinoma involving the uterus and one or both ovaries, the situation is different. Uterine serous carcinoma (USC) has a marked propensity for extrauterine spread which may occur even with a small primary tumour apparently confined to the endometrium. Similarly, the presumed precursor lesion of USC, serous endometrial intraepithelial carcinoma (serous EIC), may be associated with extrauterine involvement without demonstrable endometrial stromal or myometrial invasion. USC and serous EIC may arise within endometrial polyps and in such cases may be associated with extrauterine involvement. It is theoretically possible that, analogous to the situation with endometrioid carcinomas, the uterine and extrauterine (usually ovarian, peritoneal or omental) disease could represent independent primary neoplasms, indicative of a “field-change” effect. However, currently with serous neoplasia it is considered much more likely that multifocal disease represents spread from one organ to another. The pattern of ovarian involvement described above may be a clue that one is dealing with a metastatic neoplasm in that organ. In problematic cases, WT1 staining may assist in distinguishing between a primary USC with metastasis to the ovary and independent synchronous neoplasms or metastasis from the ovary to the endometrium. Most ovarian serous carcinomas (and primary tubal and peritoneal serous carcinomas) exhibit diffuse nuclear positivity with WT1. In contrast, USC is usually negative. However, the percentage of USCs exhibiting WT1 immunoreactivity has varied between studies and some cases are
positive, occasionally with diffuse immunoreactivity. It can be summarized that, although the published studies are somewhat contradictory and there is overlap, diffuse WT1 positivity in a serous neoplasm favours an ovarian, tubal or peritoneal origin. In contrast, negative staining is a pointer towards a primary uterine neoplasm.

MARKERS OF OVARIAN SEX CORD-STROMAL TUMOURS

Ovarian sex cord-stromal tumours comprise a heterogeneous group of neoplasms which may mimic a range of other lesions and as such immunohistochemistry is often resorted to as an aid to diagnosis. In recent years, several antibodies have come to the fore as useful markers of this group of neoplasms. Probably the best known are inhibin and calretinin. Although inhibin is positive in a high percentage of sex cord-stromal tumours, some cases are negative, including occasional examples of typical adult granulosa cell tumour. This may shake the confidence of the pathologist and it is stressed that a diagnosis of adult granulosa cell tumour and other sex cord-stromal tumours can be confidently made in the absence of inhibin immunoreactivity. Inhibin immunoreactivity is usually maintained in recurrent and metastatic granulosa cell tumours. Many purely fibromatous neoplasms are inhibin negative as often are poorly differentiated Sertoli-Leydig cell tumours; most of the other morphological types of sex cord-stromal tumour are positive in the majority of cases. In most, but not all, studies which directly compare the two markers, calretininin has been shown to be a slightly more sensitive, but less specific, marker than inhibin of the sex cord-stromal group of tumours as a whole. Other markers which may be positive in this group of neoplasms are CD99, melan A, CD10, ER, PR, relaxin-like factor, Mullerian inhibiting substance, S100 and WT1, although all of these have limitations with regard to specificity and, or, sensitivity and are of limited value in diagnosis. In fact, they may result in diagnostic confusion; for example, CD10 positivity in a sex cord-stromal tumour may lead to consideration of an endometrial stromal sarcoma if it is not realised that the former group of neoplasms may be positive. It has recently been demonstrated that WT1 is positive in most ovarian Sertoli cell
tumours and is useful in distinguishing these from endometrioid neoplasms and carcinoid tumours. WT1 may be positive in other sex cord-stromal tumours but the range of immunoreactivity in these neoplasms has not been extensively investigated. CD10 is a well known marker of endometrial stromal neoplasms which when occurring in the ovary, either as a primary neoplasm or metastatic from the uterus, may mimic a sex cord-stromal tumour. CD10 is positive in many sex cord-stromal tumours, albeit usually with low intensity, in contrast to the diffuse strong immunoreactivity which is the rule in most endometrial stromal neoplasms. Recently, it has been shown that CD56 is an extremely sensitive marker of sex cord-stromal tumours with positivity, usually both membranous and cytoplasmic, in almost all cases. However, although an extremely sensitive marker, the value of CD56 is somewhat limited by its poor specificity as many other neoplasms which may be in the differential diagnosis of a sex cord-stromal tumour, such as carcinoid tumour, are positive. In the sometimes problematic distinction between a sex cord-stromal tumour and an endometrioid adenocarcinoma with a sex cord-like pattern or between an endometrioid adenocarcinoma and a pseudoendometrioid Sertoli-Leydig cell tumour a panel of markers is recommended, including inhibin, calretinin, CD56, epithelial membrane antigen (EMA) and CK7. EMA and CK7 are positive in endometrioid carcinomas but are usually negative in ovarian sex cord-stromal tumours, although some juvenile granulosa cell tumours have been shown to be positive with EMA. Many sex cord-stromal tumours are positive with cytokeratin markers, often with dot-like cytoplasmic immunoreactivity, and these are no value in the distinction from an epithelial neoplasm. Inhibin and other sex cord markers stain non-neoplastic luteinised ovarian stromal cells which may be numerous in many ovarian neoplasms. This may result in problems with interpretation and close attention should be paid to which cells are positive.

**OVARIAN NEOPLASMS COMPOSED OF SMALL ROUND CELLS**

The differential diagnosis of an ovarian neoplasm composed of small round cells is wide. The prototypical ovarian neoplasm composed of small round cells is ovarian small cell
carcinoma of hypercalcaemic type (OSCCHT). Although termed OSCCHT, only about two-thirds of these neoplasms are associated with paraneoplastic hypercalcaemia and so the presence of a normal serum calcium does not preclude this diagnosis; moreover, occasionally other ovarian neoplasms, such as dysgerminoma and clear cell carcinoma, may be associated with hypercalcaemia. A variety of other tumours may enter into the differential diagnosis of an ovarian neoplasm composed of small round cells, including ovarian small cell carcinoma of pulmonary (neuroendocrine) type, metastatic small cell neuroendocrine carcinoma, the various small round cell tumours of childhood, malignant lymphomas and leukaemias, sex cord-stromal tumours (including adult and juvenile granulosa cell tumour and poorly differentiated Sertoli-Leydig cell tumour), undifferentiated carcinoma, malignant melanoma, desmoplastic small round cell tumour (DSRCT) and peripheral and central primitive neuroectodermal tumour (PNET). The immunophenotype of many of these neoplasms is well known and identical to when they occur at more usual sites. Only a few points are emphasised here. It is stressed that a panel of markers should always be used and careful morphological examination is paramount in diagnosis because of immunophenotypic overlap between the various neoplasms. Extensive sampling may also assist; for example some primary ovarian small cell carcinomas of pulmonary type are associated with a component of more usual surface epithelial neoplasm which may be focal.

WT1 (antibody against N-terminal) is a useful marker of OSCCHT; a large majority of cases exhibiting diffuse nuclear positivity. OSCCHT is variably positive with cytokeratins, EMA, Ber EP4, calretinin, CD10 and p53 but WT1 is currently the most useful immunohistochemical marker. Germ cell markers, such as OCT4 and c-kit, are negative, arguing against a germ cell origin for this enigmatic neoplasm. The immunophenotype of the large cell variant of OSCCHT does not differ significantly from the more usual type. Some other neoplasms in the differential diagnosis such as undifferentiated carcinoma, endometrial stromal sarcoma and sex cord tumours may also be WT1 positive. WT1 is negative in primary ovarian small cell carcinoma of pulmonary type. DSRCT, which in females may mimic an ovarian malignancy because of bilateral ovarian involvement, exhibits a polyphenotypic immunophenotype with coexpression of
epithelial, mesenchymal and neural markers, including anti-cytokeratins, EMA, vimentin, neurone specific enolase, CD57, CD15 and Ber EP4. Cytoplasmic dot-like desmin immunoreactivity is especially common. DSRCT also exhibits nuclear positivity with WT1 but with an antibody against the C-terminal; with antibodies against the N-terminal there is characteristically cytoplasmic staining. TTF1 is positive in metastatic pulmonary small cell carcinoma but it is stressed that neuroendocrine carcinomas primary in other organs may also be positive. Both central and peripheral PNETs may occur in the ovary, the former usually as a component of a teratoma. The latter is positive with CD99 and FLI-1, although neither of these are specific markers; for example CD99 may be positive in sex cord-stromal tumours, rhabdomyosarcoma and some malignant lymphomas, all of which may enter into the differential diagnosis of an ovarian small round cell neoplasm.

MARKERS OF VALUE IN TYPING ENDOMETRIAL CARCINOMA

A dual model of endometrial carcinogenesis is well established. Type 1 carcinoma, the prototype of which is endometrioid adenocarcinoma, is usually a low grade, oestrogen-dependent neoplasm which arises on a background of endometrial hyperplasia in the perimenopausal or early postmenopausal age group. In contrast, type 2 carcinoma, the prototype of which is uterine serous carcinoma (USC) is a high grade neoplasm which is not oestrogen-dependent and which usually arises in elderly postmenopausal women from an atrophic endometrium. It is important to distinguish between type 1 and a type 2 neoplasm since treatments may differ, as does the prognosis. Usually the morphological distinction between an endometrioid adenocarcinoma and USC is straightforward but, on occasions, it may be difficult for a number of reasons and it should be remembered that combined endometrioid and serous neoplasms are not uncommon. Endometrioid adenocarcinoma may have a focal or diffuse papillary growth pattern, including the villoglandular variant and the variant referred to as endometrioid carcinoma with small non-villous papillae, and this may result in overdiagnosis of USC. Conversely a glandular variant of USC exists with little or no papillary formation which may result in diagnosis of an endometrioid adenocarcinoma. This is an argument for the nomenclature USC rather than uterine papillary serous carcinoma. The nuclear features are different.
between endometrioid adenocarcinoma and USC but, in problematic cases, immunostaining with p53, p16 and ER may be of value. Endometrioid adenocarcinoma, especially of low grade, is usually positive with ER and is largely negative with p53. However, some endometrioid adenocarcinomas, particularly but not exclusively those of high grade, may be positive with p53 and negative with ER. Some of these p53 positive cases are not associated with p53 mutation and this may represent accumulation of wild-type p53. Conversely, USC characteristically exhibits diffuse nuclear positivity with p53 and is negative with ER. The combination of p53 and ER may be useful in diagnosing USC, especially the glandular variant. Diffuse positivity with ER and negative staining with p53 in a papillary endometrial adenocarcinoma is against a USC. As already stated, the glandular variant of USC may be overlooked and misdiagnosed as an endometrioid adenocarcinoma. Generally, in endometrioid adenocarcinomas with good glandular formation, the nuclear features are low grade and, although exceptions occur, the nuclear and architectural grades are usually broadly similar. In a tumour with good glandular differentiation throughout, but with marked cytologic atypia, a diagnosis of the glandular variant of USC should be considered. p53 and ER may be useful in this scenario, as already described. However, some cases exhibit unexpected staining patterns; for example, some serous carcinomas are ER positive and/or p53 negative. p16 may also be useful in the distinction between a uterine endometrioid and a serous carcinoma. The latter are typically diffusely p16 positive while the former may be negative, focally positive or occasionally diffusely immunoreactive (see section below on distinction between endometrial and cervical adenocarcinoma). Even in those cases of endometrioid adenocarcinoma which exhibit diffuse p16 immunoreactivity, positive staining is usually patchy with areas of negative staining.

Previous reports have drawn attention to the propensity for USC and its presumed precursor lesion endometrial intraepithelial carcinoma (EIC) to involve and be largely confined to otherwise benign endometrial. EIC and USC may focally involve polypoid or non-polypoid endometrium and be easily overlooked. With EIC and small foci of USC arising in benign endometrial polyps or non-polypoid endometrium, there is usually an abrupt transition from glands lined by atrophic or weakly proliferative endometrial
epithelium to glands lined by cells with nuclear features characteristic of EIC or USC. Pre-existing glands in EIC are lined by cells with markedly pleomorphic nuclei, a high nuclear to cytoplasmic ratio, nuclear hyperchromatism, prominent nucleoli and a high mitotic rate, often with abnormal mitoses. p53 and MIB1 staining may be useful in highlighting the areas of EIC which, if they are focal, may cause problems in diagnosis. The cells of EIC (and USC) are characteristically strongly positive with p53, exhibit a high proliferation index with MIB1 and are ER negative. This contrasts with the surrounding endometrial epithelial cells which are ER positive, p53 negative and exhibit a low proliferation index with MIB1. An occasional problem in endometrial polyps and non-polypoid endometrium is that metaplastic changes may occur which can be associated with a degree of nuclear atypia. In such instances, staining with the aforementioned antibodies may be useful in distinguishing metaplastic epithelia (p53 negative or focally positive, low proliferation index with MIB1, ER positive) from EIC. Many epithelial metaplasias within the endometrium are not entirely negative but exhibit a pattern of staining which has been referred to as weak and heterogenous.

MARKERS USEFUL IN THE DISTINCTION BETWEEN ENDOMETRIAL AND ENDOCERVICAL ADENOCARCINOMA

On occasions, adenocarcinoma is present in preoperative endometrial and cervical biopsies and ascertaining the site of origin may be difficult. This is of importance in that a simple hysterectomy is usually performed for an endometrial cancer whereas radical hysterectomy is generally undertaken for a cervical carcinoma. Preoperative imaging may or may not assist in determining the tumour origin. Problems may also occur in a hysterectomy specimen where tumour involves both the uterine corpus and the cervix and the choice of adjuvant therapy may depend on the site of origin. Typically the morphology differs between a usual cervical adenocarcinoma and an endometrial adenocarcinoma of endometrioid type, with foam cells and squamous elements being more common in the latter neoplasms, but there may be considerable histological overlap.
A panel of markers comprising ER, vimentin, p16 and monoclonal CEA may be of value. Endometrial adenocarcinomas of endometrioid type typically exhibit diffuse nuclear ER and cytoplasmic vimentin positivity. CEA is usually negative or focally positive, although the squamous elements which are common in these neoplasms may be immunoreactive. In contrast, cervical adenocarcinomas are usually, but not always, CEA positive. Vimentin is usually negative and ER is typically negative or there is focal weak positivity. p16 may also be of use in distinguishing between an endometrial adenocarcinoma of endometrioid type and a cervical adenocarcinoma. Cervical adenocarcinomas usually exhibit diffuse p16 positivity due to the presence of high risk HPV while endometrial adenocarcinomas of endometrioid type are typically negative or focally positive. Some case are diffusely positive but even then positive areas are usually admixed with areas of negative staining. The squamous elements of endometrioid carcinomas may be positive. Uterine serous carcinoma (similar to ovarian serous carcinoma) is often diffusely positive with p16, due to non-HPV related mechanisms, and it is stressed the panel of markers discussed is only of value in distinguishing a usual cervical adenocarcinoma from an endometrial adenocarcinoma of endometrioid type. Molecular studies for HPV may also be of value since cervical adenocarcinomas are typically positive whereas endometrial adenocarcinomas are almost always negative. The panel of markers discussed also assists in cases of subtle cervical stromal invasion by endometrioid adenocarcinoma of the uterine corpus. In such cases, the tumour within the cervix may be morphologically bland without a stromal reaction and may mimic cervical adenocarcinoma in situ, a primary cervical adenocarcinoma or even cervical mesonephric remnants. Rarely, an endometrioid adenocarcinoma of the uterine corpus and a premalignant or malignant endocervical glandular lesion coexist and the aforementioned panel of markers helps to clarify the relationship between the two neoplasms.

The question also arises as to the immunophenotype of a mucinous adenocarcinoma of the endometrium and an endometrioid adenocarcinoma of the cervix; is the immunophenotype more dependent on the site of origin or the pattern of differentiation? One study which addressed this issue found that if a tumour exhibited diffuse positivity
with ER and vimentin then it was almost certainly of endometrial origin. I have observed that some mucinous adenocarcinomas of the endometrium and endometrioid carcinomas with mucinous differentiation may be vimentin negative and CEA positive, this immunophenotype overlapping with that of a cervical adenocarcinoma. However, these endometrial neoplasms typically retain their nuclear ER positivity and are p16 negative or focally positive. I also stress that in an individual tumour, unexpected staining reactions may occur with one or more of the markers discussed. This can result in potential diagnostic problems and illustrates that the immunohistochemistry is always to be interpreted in light of the clinical, radiological, gross pathological and microscopic findings.

**IMMUNOHISTOCHEMISTRY OF CERVICAL NEUROENDOCRINE CARCINOMAS**

In attempting to confirm neuroendocrine differentiation, the four most commonly used markers are chromogranin, CD56, synaptophysin and PGP9.5. In our series of 21 cases of cervical neuroendocrine carcinoma (13 small cell neuroendocrine carcinoma (SCNECs), 8 large cell neuroendocrine carcinoma (LCNECs)), chromogranin, CD56, synaptophysin and PGP9.5 were positive in 57, 90, 90 and 43% of cases respectively. All neoplasms were positive with at least one of the four markers. Chromogranin is widely regarded as the most specific neuroendocrine marker available but, as illustrated by our study, the sensitivity is lower than that of other markers with the exception of PGP9.5. Although some neoplasms were diffusely positive, there were several cases where chromogranin appeared negative on initial inspection but high power examination revealed small foci of cytoplasmic positivity with a punctate pattern. Thus, when evaluating chromogranin immunoreactivity, careful inspection of the entire slide at high power is necessary. Of the four most commonly used neuroendocrine markers, PGP9.5 seems the least sensitive while CD56 and synaptophysin are the most sensitive. This is broadly in agreement with the results of a previous study where 22 of 25 cervical SCNECs stained with CD56 compared to 16 of 25 with synaptophysin and 8 of 25 with chromogranin. However, CD56 lacks specificity and cervical carcinomas of non-
neuroendocrine type can be positive, including some squamous carcinomas and adenocarcinomas (personal observations). In attempting to confirm neuroendocrine differentiation, it is best to utilise a panel of markers, including chromogranin, synaptophysin and CD56, since sometimes only one of these is positive. Only 4 of our cases were positive with all four neuroendocrine markers. Although all SCNECs were positive with at least one neuroendocrine marker, I make the point that this diagnosis can be made on the basis of the typical morphological features, even if all the neuroendocrine markers are negative. p63 (discussed later) may be of value in this regard in the distinction from a small cell squamous carcinoma. While at present, it is considered that a diagnosis of cervical LCNEC requires neuroendocrine marker positivity in the context of an appropriate morphology, it is possible that as more experience with these neoplasms is gathered, a diagnosis will be possible on morphology alone in the absence of neuroendocrine marker positivity, as has been suggested for the corresponding pulmonary neoplasms.

Our study illustrates that TTF1 nuclear immunoreactivity is very common in cervical neuroendocrine carcinomas with positive staining in 11 (85%) and 4 (50%) cases of SCNEC and LCNEC respectively. Altogether 71% of cases were positive. Staining may be focal but is commonly diffuse. TTF1 is a widely used and well known useful immunohistochemical marker of thyroid and pulmonary neoplasms, including primary pulmonary adenocarcinomas and small cell carcinomas. However, TTF1 may be positive in primary neuroendocrine carcinomas of other organs. For example, TTF1 nuclear immunoreactivity was found in 39% of small cell carcinomas of the urinary bladder in one study and in another 80% of extrapulmonary small cell carcinomas were positive, although in most studies the percentage of extrapulmonary small cell carcinomas positive with TTF1 is much less than this. There has been only limited investigation of TTF1 immunoreactivity in cervical neuroendocrine carcinomas. We previously reported TTF1 nuclear immunoreactivity in three cervical LCNECs and other studies have found positivity in a small number of cervical SCNECs. As stated, our study illustrates that cervical neuroendocrine carcinomas of small cell and large cell type are commonly positive with TTF1 and this should not be misconstrued as evidence of a metastasis from
a primary pulmonary neoplasm. The proportion of cervical SCNECs exhibiting TTF1 positivity is greater in our series than in other studies. The reason for this is not clear but may be due to the use of different antibodies and/or methods of staining. One study which examined TTF1 immunoreactivity in colonic adenocarcinomas found that with the SPT24 anti-TTF1 clone, there was staining of a small number of neoplasms which were all were negative with the 8G7G3/1 clone. The authors concluded that the diagnostic value of TTF1 depends on the antibody clone used. The SPT24 clone seems to have a stronger affinity for TTF1 protein but may result in a few positive colorectal adenocarcinomas. We used an antibody against the SPT24 clone while most other studies used antibodies against the 8G7G3/1 clone. Given the high percentage of cervical neuroendocrine carcinomas that are TTF1 positive, we feel that immunoreactivity with this marker may be a useful pointer that a poorly differentiated neoplasm represents a neuroendocrine carcinoma. However, it should be borne in mind that some gynaecological adenocarcinomas, including primary cervical adenocarcinomas, exhibit TTF1 nuclear immunoreactivity with occasional cases being diffusely positive. In one study, more gynaecological adenocarcinomas were TTF1 positive using antibodies against the SPT24 clone than against other clones. Whether those cases which stain with antibodies against the SPT24 clone but not with antibodies against the 8G7G3/1 clone represent false positive staining or is simply a result of increased affinity for TTF1 protein (and by implication false negative staining of some cases with antibodies against the 8G7G3/1 clone) is not clear and should be determined by further study.

All the neuroendocrine carcinomas in our study, except one LCNEC, were diffusely positive with p16 which, in the cervix, is regarded as a surrogate marker of the presence of high risk HPV. While in most cases, diffuse p16 immunoreactivity may be secondary to the presence of oncogenic HPV, diffuse p16 expression may also occur in various neoplasms, such as ovarian and uterine serous carcinomas and leiomyosarcomas, due to non-HPV related mechanisms and pulmonary small cell carcinomas are commonly positive with this marker.
p63 is regarded as a useful immunohistochemical marker of squamous differentiation within a cervical carcinoma, as most squamous carcinomas exhibit diffuse nuclear positivity while most adenocarcinomas and neuroendocrine carcinomas are negative or focally immunoreactive. In one study, 75% of cervical squamous carcinomas were diffusely positive compared to no adenocarcinomas or neuroendocrine carcinomas. Neuroendocrine carcinomas of small cell and large cell type may be mistaken for a squamous carcinoma and, in conjunction with neuroendocrine markers and TTF1, p63 staining is useful in this distinction. However, we found a significant number of cervical neuroendocrine carcinomas to exhibit nuclear immunoreactivity with p63, including 5 with diffuse positivity. These cases were also positive with three or four neuroendocrine markers. Thus, p63 nuclear immunoreactivity in a cervical neoplasm cannot be taken as unequivocal evidence of a squamous carcinoma. There were three cases which exhibited cytoplasmic p63 immunoreactivity. This is considered to represent non-specific staining.

Several of our cases exhibited positive staining with CK20, including one with diffuse immunoreactivity and one with cytoplasmic dot-like staining. Merkel cell carcinomas (primary cutaneous neuroendocrine carcinomas) are commonly CK20 positive with cytoplasmic dot-like immunoreactivity and positivity of some of our cases suggests a Merkel cell immunophenotype. This has been described in primary small cell carcinomas at extracutaneous sites, for example the salivary gland, the oesophagus and the vagina. In fact, small cell carcinomas of the major salivary glands have been divided into pulmonary type and Merkel cell type on the basis of CK20 immunoreactivity and this has been shown to be of prognostic significance with CK20 positive neoplasms having a better prognosis. It would be interesting to investigate whether CK20 immunoreactivity in a cervical neuroendocrine carcinoma is of prognostic significance, as it is in the salivary gland. In order to further investigate a possible Merkel cell immunophenotype, we stained our cases with neurofilament, a sensitive marker of Merkel cell carcinoma. In 7 cases, there was positive staining of scattered individual or small groups of cells. No case was positive with both CK20 and neurofilament. While CK20 and neurofilament immunoreactivity can be taken as evidence of a Merkel cell immunophenotype, it is also
possible that positivity with these antibodies is merely indicative of the fact that highly aggressive neoplasms may abberantly express various markers. CK20 immunoreactivity in primary cervical neuroendocrine carcinomas also illustrates that positivity with this marker cannot be taken as unequivocal evidence of a Merkel cell carcinoma when faced with a neuroendocrine carcinoma of unknown origin. CK20 immunoreactivity has been reported previously in a small number of cervical SCNECs but only a limited number of cases have been stained with this marker. In one study, 1 of 11 cervical SCNECs expressed CK20. Recently, a polyomavirus (referred to as Merkel cell polyomavirus) has been discovered which is present in most cutaneous Merkel cell carcinomas but not in SCNECs in other organs. We stained our CK20 positive cases with the CM2B4 monoclonal antibody generated against a predicted antigenic epitope on the Merkel cell virus T antigen. In a recent tissue microarray study, 75% of Merkel cell carcinomas were immunoreactive with CM2B4 but all skin tumours with a combined squamous and neuroendocrine phenotype were negative, as were all pulmonary neuroendocrine carcinomas. In our study, the 4 CK20 positive cases were negative with CM2B4, arguing against a true Merkel cell carcinoma. Several of our neoplasms expressed CK7 which is typically negative in neuroendocrine carcinomas arising in various organs. Similar to the expression of CK20, this may indicate aberrant staining in an aggressive neoplasm.

CD99 staining was undertaken to investigate whether there is immunohistochemical overlap with neoplasms in the Ewing family of tumours (EFT) or primitive neuroectodermal tumour, occasional examples of which have been described in the cervix and elsewhere in the lower female genital tract. Several of our cases were positive, including two with diffuse membranous immunoreactivity. Neoplasms in the EFT and cervical neuroendocrine carcinomas both typically occur in young females and these tumours may be difficult to distinguish, especially if cytokeratins and some or all neuroendocrine markers are negative in the latter. To complicate matters, some neoplasms in the EFT exhibit immunoreactivity with cytokeratins and neuroendocrine markers. Given these observations, we undertook molecular studies for HPV in 4 cases which exhibited CD99 immunoreactivity. One case each contained HPV16 and 18, arguing against a neoplasm in the EFT since these are not known to be HPV associated
and in the others there was insufficient tumour DNA for HPV analysis. Most neuroendocrine carcinomas of the cervix of small cell and large cell type are associated with oncogenic HPVs, with HPV 18 being the most common infecting viral type.

If a diagnosis of a neuroendocrine carcinoma of small cell or large cell type is made on a punch biopsy of an obvious cervical neoplasm, then chemotherapy is generally given and surgical resection not undertaken, even with a tumour confined to the cervix. This is because of the high risk of systemic spread. Given this fact, adjacent tissues are usually not available for histological examination. However, there is thought to be an association between cervical neuroendocrine carcinomas and premalignant or malignant endocervical glandular lesions. In two of our neoplasms, adjacent endocervical glands exhibited AIS and interestingly in both of these cases, scattered individual chromogranin positive neuroendocrine cells were present in the AIS. This has been noted previously in a single cervical SCNEC and raises the possibility that neuroendocrine cells in AIS are the origin of at least some cervical neuroendocrine carcinomas. We stained 13 cases of cervical AIS with chromogranin and individual positive cells were present in 4 of these. Argyrophilic cells were noted previously in 7 of 25 cases of cervical AIS.

**TTF1 IMMUNOREACTIVITY IN GYNAECOLOGICAL ADENOCARCINOMAS**

TTF1 is widely regarded as a useful immunohistochemical marker of primary thyroid and pulmonary neoplasms. Most primary pulmonary adenocarcinomas and neuroendocrine carcinomas are positive. As discussed, TTF1 may also be positive in neuroendocrine carcinomas arising primary in other organs. In the ovary, TTF1 may be of value in diagnosing unusual morphological variants of struma ovarii, including those with clear cells or oxyphilic cells and cystic variants, and thyroid type adenocarcinomas. In two recent studies, we have shown TTF1 nuclear immunoreactivity in a significant percentage of primary adenocarcinomas of the cervix, uterine corpus and ovary. In most cases, positive staining was focal but occasionally it was diffuse. TTF1 was positive in 7 of 19 ovarian serous carcinomas, 1 of 28 cervical adenocarcinomas, 6 of 31 uterine endometrioid adenocarcinomas and 3 of 13 uterine serous carcinomas. This illustrates
that TTF1 nuclear immunoreactivity in an adenocarcinoma is not diagnostic of a pulmonary primary.