Application of Immunohistochemistry to Gynecologic Pathology

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Context.—A large variety of tumors and lesions arise in the female genital tract. Although the majority of these can be correctly recognized on routine hematoxylin-eosin–stained slides, occasional cases present a diagnostic challenge. Immunohistochemical stains are extremely useful in resolving many of these problematic cases. As the knowledge in this area is constantly expanding, it is useful to have this updated information in a review form for easy access.

Objective.—To present our current knowledge of immunohistochemistry of the lesions of the female genital tract in a readily accessible form.

Data Sources.—The review is based on previously published articles on this topic.

Conclusions.—Immunohistochemical stains help in reaching a conclusive diagnosis in a variety of problematic lesions seen in gynecologic pathology. As in any other system, immunohistochemical findings need to be interpreted in light of the clinical history and morphologic findings.

Immunohistochemical findings play a crucial role in the differential diagnosis of gynecologic lesions.1–3 Because of the limitations of space, this article is almost exclusively focused on the application of immunohistochemical findings in gynecologic pathology. Nevertheless, the significance of clinical and morphologic findings in arriving at a diagnosis or differential diagnosis in any branch of surgical pathology, including gynecologic pathology, cannot be overstated. Tumors often show aberrant expression of proteins, and none of the markers discussed in this study, with the possible exception of thyroglobulin, are specific for a particular tumor type. Therefore, the use of a panel of antibodies is generally recommended.

The article is organized by organ sites and further subcategorized by the differential diagnoses encountered in these locations. A panel of antibodies is suggested for each differential diagnosis based on our experience and published literature. These panels may be modified based on personal experience of the user and local availability of the antibodies.

OVARY

Primary Ovarian Adenocarcinoma Versus Adenocarcinomas Metastatic to the Ovary

This is the most common reason for immunohistochemical studies of ovarian lesions. Carcinomas that most commonly metastasize to the ovary include those from the endometrium, colon, and breast. Diffuse expression of cytokeratin (CK) 7 is seen in primary ovarian carcinoma, but such expression is not seen in colon carcinoma.4 WT1 and CA 125 are associated with ovarian carcinoma, but their expression varies with different histologic subtypes. WT15,6 and CA 1251,7,8 are expressed in most primary ovarian serous adenocarcinomas but are negative in a majority of ovarian clear cell and mucinous carcinomas.6 Endometrioid ovarian carcinoma is positive for CA 125 but usually negative for WT1.4 WT1 is usually negative in breast, gastrointestinal, and pancreatobiliary primaries. CA 125 is expressed in a minority of carcinomas other than those from ovary, including carcinomas from breast, endometrium, cervix, and lung. Approximately 40% of primary ovarian carcinomas are estrogen receptor (ER) and/or progesterone receptor (PR) positive.4,10 Carcinomas arising in breast and other sites in the female genital tract are also ER and PR positive, whereas carcinomas from other locations are ER and PR negative. CDX2 and β-catenin are useful markers for colon carcinoma, and gross cystic disease fluid protein 15 (GCDFP-15) is a useful marker for breast carcinoma. Immunohistochemical markers useful in differentiating primary ovarian carcinoma from metastatic carcinoma from a particular site are discussed later.

Suggested panel: CK7, CK20, WT1, ER, PR, GCDFP-15, CDX2, DPC4, p16, β-catenin.

Primary Endometrioid Carcinoma Versus Metastatic Colon Carcinoma

Because the expression of mucin may be low in colonic carcinoma, it is most often confused with an endometrioid carcinoma when metastatic to the ovary. Endometrioid carcinoma is positive for CK7, ER, PR, and CA 125 and negative for CK20,4,9–12 Colon carcinoma shows a reverse pattern (Figure 1, A and B). Other adenocarcinomas, in-
Figure 1. Colon adenocarcinoma, metastatic to the ovary, showing positive staining for cytokeratin (CK) 20 (A) and negative staining for CK7 (B) (original magnifications ×100).

Figure 2. Decidualized endometriosis in ovary. A. The arrow points to atrophic glandular epithelium that may be mistaken for endothelial lining (hematoxylin-eosin, original magnification ×100). B. Cytokeratin AE1/AE3 highlights the glandular structures (original magnification ×100).

Figure 3. Dysgerminoma and lymphoma may be distinguished from each other by staining for placental alkaline phosphatase (PLAP) and CD45. A. Dysgerminoma positive for PLAP (original magnification ×100). B. Lymphoma positive for CD45 (original magnification ×100).
cluding endometrial, endocervical, breast, and lung, are also positive for CK7 and negative for CK20; hence, these primary sites cannot be excluded based on this immunophenotype alone.

Suggested panel: CK7, CK20, ER, PR, CA 125.

**Primary Mucinous Carcinoma Versus Metastatic Colon Carcinoma**

Primary mucinous carcinomas are frequently positive for CK20,19,20, hence, CK20 cannot be used to distinguish these tumors from metastatic colon carcinoma. Cytokeratin 7 is more useful in this situation, as it is diffusely positive in ovarian carcinoma and negative or focally positive in colon carcinoma. Ovarian mucinous carcinoma is negative for β-catenin and positive for MUC5AC, whereas colon carcinoma shows a reverse pattern.4,9–15 CDX2 is a sensitive but nonspecific marker for colon carcinoma.13,16,17 A negative staining would favor ovarian primary. Mucinous tumors with intestinal type histology and immunostaining pattern may arise from ovarian teratomas.18

Suggested panel: CK7, CA 125, ER, PR, CDX2, MUC5AC.

**Primary Clear Cell Carcinoma Versus Metastatic Renal Clear Cell Carcinoma**

Renal cell carcinoma is negative for CK7 in contrast to ovarian clear cell carcinoma.15,16,17,19,26 Renal cell carcinoma is positive for CD10,19,21 whereas ovarian clear cell carcinoma is negative.

Suggested panel: CK7, CA 125, CD10, ER, PR.

**Primary Adenocarcinoma Versus Metastatic Gastric Carcinoma**

Occasional primary ovarian mucinous carcinomas may have signet ring cells. Usually this is a minor component of the tumor. Cytokeratin 7 and CK20 are not very helpful in this differential diagnosis as both these tumor types can be CK7 and CK20 positive.11,22,23 Nevertheless, positive staining for CK7, ER, and PR and lack of diffuse positive staining for CK20 would favor an ovarian origin.

Suggested panel: CK7, CK20, CA 125, ER, PR.

**Ovarian Adenocarcinoma Versus Metastatic Breast Carcinoma**

Breast carcinoma is usually positive for GCDFP-15 and negative for vimentin.4 Ovarian carcinomas are negative for GCDFP-15 and often positive for vimentin. If ER and PR data are available on a prior breast carcinoma, these data may be compared with the findings in the current tumor in the ovary.

Suggested panel: GCDFP-15, vimentin, ER, PR.

**Ovarian Adenocarcinoma Versus Metastatic Pancreatic and Bile Duct Carcinoma**

Pancreatic carcinomas often show loss of DPC4.24–27 Ovarian adenocarcinomas are usually negative for CA 19-9, which is expressed in both pancreatic and bile duct carcinomas.

Suggested panel: CA 19-9, DPC4, ER, PR.

**Ovarian Adenocarcinoma Versus Metastatic Cervical Adenocarcinoma**

Cervical adenocarcinoma is usually negative for ER and PR26–31 and positive for p16,12–35 whereas the reverse is true of primary ovarian carcinomas, except for serous ovarian carcinomas,38 which frequently show the same immunophenotype as cervical adenocarcinoma. However, primary papillary serous carcinomas are rare in the cervix.

Suggested panel: ER, PR, p16.

**Ovarian Adenocarcinoma Versus Epithelioid Mesothelioma**

Calretinin, thrombomodulin, and keratin 5/6 are the best positive markers for mesotheliomas.9–46 The best negative markers for mesothelioma are MOC-31, B72.3, Ber-EP4, CA 19-9, and Leu-M1.7,39,40,42,46–53


**Reactive Mesothelium Versus Implants or Serous Carcinoma**

Ber-EP4 and epithelial membrane antigen (EMA) are positive in implants and carcinoma but negative in reactive mesothelium. Calretinin shows a reverse pattern, with positive nuclear staining in mesothelium and usually lack of such staining in carcinomas.

Suggested panel: calretinin, Ber-EP4, EMA.

**Ovarian Adenocarcinoma Versus Sex Cord Stromal Tumor**

Ber-EP4 and EMA are positive in carcinoma and negative in sex cord stromal tumors, with the exception of juvenile granulosa cell tumors, which may show positivity for EMA in up to 50% of the tumors.54 Epithelial membrane antigen is also negative in female adnexal tumors of Wolffian origin.

In contrast, sex cord stromal tumors are positive for inhibin,55,56 and calretinin, whereas ovarian carcinomas are usually negative. Cytokeratin AE1/AE3 staining is present in all carcinomas and in Sertoli cell tumors and may be present in granulosa cell tumors, often in a dotlike paranuclear pattern. A negative staining for AE1/AE3 supports a diagnosis of sex cord stromal tumor.


**Ovarian Adenocarcinoma Versus Adenocarcinoid**

Chromogranin, CD56, and synaptophysin are useful markers for carcinoids. Chromogranin is highly specific. CD56 and synaptophysin are less specific but more sensitive.12 The possibility of a metastasis from an appendiceal primary must be considered when diagnosing a case of ovarian adenocarcinoid.

Suggested panel: chromogranin, CD56.

**Ovarian Endometrioid Adenocarcinoma Versus Sertoli Cell Tumor**

Endometrioid adenocarcinoma and Sertoli cell tumors can look similar on morphology. Ovarian adenocarcinoma is positive for EMA and CK7, whereas Sertoli cell tumors are positive for α-inhibin and vice versa.60–63

Suggested panel: EMA, CK7, α-inhibin.

**Ovarian Adenocarcinomas Versus Embryonal Carcinoma**

Embryonal carcinomas are positive for human chorionic gonadotropin (hCG) and α-fetoprotein. OCT4 is positive in nuclei in embryonal carcinoma and dysgerminoma but is negative in most adenocarcinomas.65–66

**Immunohistochemistry in Gynecologic Pathology—Mittal et al**
 Origins of a Diffuse Peritoneal Adenocarcinoma in a Female

Expression of WT1 and to a lesser extent CA 125 favors an ovarian or müllerian primary peritoneal carcinoma. Most gastrointestinal, lung, and breast primaries are negative for WT1. Lung and thyroid adenocarcinomas are thyroid transcription factor 1 (TTF-1) positive as are neuroendocrine carcinomas in other locations.67–70 Malignant mesothelioma is also WT1 positive.

Suggested panel: CK7, CK20, WT1, CA 125, ER, PR, TTF-1, calretinin, DPC4, GCDFP-15, β-catenin.

Distinction of Endometriosis From Cystic Struma Ovarii

Struma ovari can be cystic and resemble an endometriotic cyst. Thyroglobulin is very useful in supporting a diagnosis of struma ovari.71

Ovarian Adenocarcinomas Versus Endodermal Sinus Tumor

α-Fetoprotein is positive in endodermal sinus tumor.

Ovarian Clear Cell Carcinoma Versus Dysgerminoma or Endodermal Sinus Tumor

Clear cell carcinoma is positive for AE1/AE3 and negative for placental alkaline phosphatase (PLAP). Dysgerminoma shows a reverse pattern, except that rarely focal AE1/AE3 staining may be seen.72 Endodermal sinus tumor is positive for α-fetoprotein and AE1/AE3.

Ovarian Lesions With Abundant Cytoplasm and Small Uniform Nuclei

These lesions include Leydig cell tumor, corpus luteum, decidual change, and decidualized endometriosis (Figure 2, A). A cytokeratin stain is helpful in identifying glandular components in decidualized endometriosis, as the glandular epithelium may be flattened and confused with endothelial lining (Figure 2, B). An immunostain for testosterone may confirm the diagnosis of Leydig cell tumor.

Ovarian Tumors With Small Blue Cells

A variety of tumors in the ovary may have a pattern of small blue cells.73 These include granulosa cell tumor (positive for inhibin), primary small cell carcinoma (variably positive for AE1/AE3, EMA, CD10, calretinin, WT1, and p53), metastatic small cell carcinoma (positive for TTF-1), dysgerminoma (positive for PLAP [Figure 3, A] and OCT474), lymphoma (positive for CD45; Figure 3, B), and immature teratoma (positive for neurofilament protein,75 synaptophysin, neuron-specific enolase).

Recommended panel: AE1/3, EMA, CD10, calretinin, WT1, TTF-1, CD45, inhibin, synaptophysin, neurofibrillary protein, PLAP.

Distinction Between Lymphangioma and Benign Cystic Mesothelioma

These lesions have similar morphology, but mesothelioma reacts with cytokeratin AE1/AE3,76 whereas lymphangioma would be positive for D2-40.77

FALLOPIAN TUBE

Adenocarcinoma In Situ Versus Normal Atypia

Normal tubal epithelium often appears atypical (Figure 4, A). This atypical epithelium may lead to a misdiagnosis of adenocarcinoma in situ (AIS) or the missed diagnosis of AIS in the fallopian tube. MB-1 and p53 are very useful in this differential diagnosis, as these are negative in normal epithelium (Figure 4, B) and show increased expression in the in situ carcinoma of the fallopian tube28 (Figure 4, C and D).

UTERINE CORPUS

Endocervical Adenocarcinoma Versus Uterine Endometrioid Carcinoma

Distinguishing between an endocervical adenocarcinoma of the usual type (ECA) and a uterine endometrioid carcinoma (UEC) can often be accomplished by routine histologic examination, but a significant degree of morphologic overlap between the entities prompts many pathologists to use immunohistochemistry for this differential diagnosis. Carcinoembryonic antigen (CEA) expression is more common in ECAs (up to 100%) than in UECs (up to 50% in some series), but this lack of discrimination should discourage its use as a single marker on an individual case basis.79–81 Carcinoembryonic antigen expression in UECs, which is usually weak and luminal, contrasts with that in ECAs, in which expression is cytoplasmic, luminal, and of strong intensity. Vimentin staining is a useful diagnostic adjunct because coexpression of vimentin and cytokeratin is seen in most UECs but not ECAs.82 The use of ER, PR, and p16 for the distinction of endocervical and endometrial adenocarcinomas has recently become popular.83–85 Using these markers as part of a panel makes use of frequent ER and PR expression in differentiated UECs and the association between high-risk human papillomavirus (HPV) infection, implicated in ECAs, and overexpression of p16. In summary, most International Federation of Gynecology and Obstetrics (FIGO) grades 1 and 2 UECs would express ER, PR, and vimentin, whereas most ECAs are ER and PR negative but express CEA and p16 diffusely (Table 1). Many UECs are negative with p16 immunostaining or show patchy positivity.

Making generalizations about the typical staining pattern of endocervical and endometrial carcinomas can be misleading. For example, there are reports suggesting that mesonephric, clear cell, and minimal-deviation endocervical adenocarcinomas may not be HPV-related,86 which calls into question the validity of the idea that “endocervical adenocarcinomas are p16 positive.” Data that are even better established suggest that endometrial adenocarcinomas, including types much more frequently encountered than these unusual cervical tumors, are heterogeneous and do not conform to the profiles discussed in the preceding paragraph.87 Specifically, serous carcinomas are consistently strongly and diffusely positive for p16, whereas FIGO grade 3 and clear cell carcinomas may show considerable staining as well.87 Serous carcinomas are well known to lack ER and PR expression; most clear cell carcinomas, in fact, are entirely negative for these markers. Therefore, the CEA, vimentin, ER, PR, and p16 panel should be used with a great deal of caution when the tumor in question could be of mesonephric, minimal deviation, serous, clear cell, FIGO grade 3 endometrioid or undifferentiated type.

We strongly advocate close clinical correlation when there is uncertainty about a tumor’s origin. Clinical and radiologic findings should outweigh immunohistochemi-
Figure 4. Fallopian tube epithelium can appear atypical (A; hematoxylin-eosin, original magnification ×100), but negative immunostains for p53 (B; original magnification ×100) and MIB-1 would exclude that diagnosis. In other cases, morphologic suspicion of intraepithelial serous carcinoma (C; MIB-1, original magnification ×100) would be confirmed by positive staining for p53 (D; original magnification ×100). The arrow in C points to the normal epithelium.

Table 1. Endocervical Adenocarcinoma* Versus Endometrioid Adenocarcinoma (FIGO Grades 1 and 2)†

<table>
<thead>
<tr>
<th></th>
<th>CEA</th>
<th>Vimentin</th>
<th>ER/PR</th>
<th>p16</th>
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<tbody>
<tr>
<td>Endocervical</td>
<td>+</td>
<td>N</td>
<td>N</td>
<td>+</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>N</td>
<td>+</td>
<td>N</td>
<td>–/+</td>
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* Human papillomavirus associated.
† CEA indicates carcinoembryonic antigen; ER/PR, estrogen receptor/progesterone receptor; +, positive; N, negative; and –/+ , mostly negative. CEA staining is cytoplasmic.

Nuclear vacuoles favor microglandular hyperplasia. Unlike endocervical adenocarcinoma, microglandular hyperplasia is usually CEA negative and shows variable expression of ER and PR, which means that these markers are not useful when mucinous endometrial adenocarcinoma is under consideration. A more useful immunohistochemical panel would include vimentin and MIB-1. Microglandular hyperplasia lacks anti-vimentin immunoreactivity and usually shows only occasional nuclei (less than 1%) positive with MIB-1. In contrast, most endometrioid adenocarcinomas and mucinous endometrial adenocarcinomas express vimentin and demonstrate a proliferation index of at least approximately 10%. Suggested panel: vimentin, MIB-1.

Microglandular Hyperplasia Versus Mucinous Endometrial Adenocarcinoma

These can appear strikingly similar. Older age, luminal squamous metaplasia, at least moderate cytologic atypia, easily found mitotic activity, and stromal foam cells favor mucinous endometrial adenocarcinoma, whereas younger age, recent history of hormone use, and sub-nuclear vacuoles favor microglandular hyperplasia. Unlike endocervical adenocarcinoma, microglandular hyperplasia is usually CEA negative and shows variable expression of ER and PR, which means that these markers are not useful when mucinous endometrial adenocarcinoma is under consideration. A more useful immunohistochemical panel would include vimentin and MIB-1. Microglandular hyperplasia lacks anti-vimentin immunoreactivity and usually shows only occasional nuclei (less than 1%) positive with MIB-1. In contrast, most endometrioid adenocarcinomas and mucinous endometrial adenocarcinomas express vimentin and demonstrate a proliferation index of at least approximately 10%. Suggested panel: vimentin, MIB-1.

Arias-Stella Reaction Versus Clear Cell Carcinoma

The Arias-Stella reaction is a well known mimic of clear cell carcinoma. A recent study reported only 3 of 27 Arias-Stella cases showing greater than 5% nuclear labeling with MIB-1; this contrasted with clear cell carcinomas, in which 9 of 11 cases showed at least 5% labeling. Therefore, although clear cell carcinomas are generally much more proliferative than Arias-Stella reactions, there is some overlap. p53 immunohistochemical results were similar.
Most Arias-Stella reactions were negative, and only occasional cases showed p53 expression greater than 25%. Clear cell carcinomas tended to demonstrate larger percentages of p53-positive nuclei, but only 7 of 11 contained greater than 25% positive nuclei.

Suggested panel: none.

Invasive Endometrial Cancer Versus Noninvasive Endometrial Cancer Involving Adenomyosis

CD10 expression is characteristic of endometrial stroma, whereas it is significantly less frequently observed in myometrial smooth muscle. Several authors have used this observation to study whether CD10 is useful for distinguishing invasive endometrial cancer from adenocarcinomas involving adenomyosis.91,92 They hypothesized that CD10 expression surrounding adenocarcinoma in the myometrium would support a diagnosis of adenomyosis over invasive carcinoma.91,92 Instead, their articles report that a rim of CD10-positive tissue frequently envelops many myoinvasive adenocarcinomas, leading to the mistaken impression of a carcinoma that colonizes adenomyosis.91,92 There are also occasional problems involving endometrial stroma that fails to express CD10. In these cases, the endometrial stroma surrounding an endometrial cancer in- stromal cases showed p53 expression greater than 25%.

Suggested panel: MIB-1, p53.

Uterine Serous Carcinoma Versus UEC

Immunohistochemistry, useful diagnostically, has also been used to highlight these tumors' divergent pathoge- netic pathways. Strong p53 immunoreactivity (overexpression) is seen in more than 80% of uterine serous carci- nomas (USCs) (Figure 5, A through F; Figure 6, A); in contrast, p53 is overexpressed in less than 20% of UECs93,94 (Figure 5, G; Table 2). p53 expression in UECs is negative or weak in grade 1 to grade 2 tumors, whereas it is not seen in complex atypi- cal hyperplasia. These results are supported by the pres- ence of p53 gene mutations in 90% of USCs and in only 10% to 20% of UECs.95-96 Nearly all UECs demonstrating p53 gene mutations are FIGO grade 3 (Figure 5, H). p53 immunohistochemistry is therefore not recommended when the differential diagnosis includes FIGO grade 3 UEC. p53 immunostaining is most likely to be of help when the differential diagnosis includes architecturally well-differentiated endometrioid adenocarcinomas, including those with papillary features,97 and glandular tumors with high nuclear grade. p53 immunostaining, along with MIB-1, p16, and ER and PR (see following), are also particularly useful for the distinction of intraepithelial serous carcinoma (endometrial intraepithelial carcinoma) and cytologically nonneoplastic proliferations, including metaplasias98 and radiation atypia.

Immunohistochemical evaluation for p53 antibodies is generally straightforward, but there are several complexities to keep in mind. Patchy and/or focal and/or weak p53 labeling does not constitute overexpression and does not correlate with either p53 gene mutation or serous differen- tiation. The primary consideration should be whether there is p53 overexpression, which many pathologists de- fine as expression in greater than 75% of tumor cell nuclei. Next, as many as 10% of USCs do not overexpress p53,93 they still differ from most FIGO grade 1 and 2 UECs in this respect because USCs, when p53 negative, are often entirely devoid of staining. UECs, in contrast, frequently show patchy, weak staining.

Additional markers of interest include MIB-1, p16, ER, and PR (Figure 6, B). MIB-1 is especially useful when a serous diagnosis is considered and none of the tumor cells expresses p53. Diffuse, strong (>75% of cells) MIB-1 stain- ing in this context would still support USC, especially if the expression profile for p16 and ER and PR were consis- tent with this diagnosis.95 p53 overexpression along with diffuse MIB-1 staining is much more commonly en- countered in USC.95 Although p16 is well known as a sur-rogate marker for HPV in the lower genital tract, it is also characteristically diffusely and strongly expressed in USC,94,95 in which HPV infection is not considered to play an important role in pathogenesis. The pathways leading to p16 expression in USC have not been elucidated. More than 90% of USCs are strongly p16 positive, as compared with less than 10% of UECs and 50% of clear cell carci- nomas.96 In contrast to that typically seen in USCs, p16 expression in UECs and clear cell carcinomas is almost always patchy and weak. Considerably greater degrees of p16 staining are seen in FIGO grade 3 UECs as compared with well-differentiated tumors. Estrogen receptor and PR expression is prevalent in FIGO grades 1 and 2 UECs, but it is generally weaker in serous and FIGO grade 3 UECs and negative in clear cell carcinomas.96-103 Presence of β-catenin and loss of expression of phosphatase and tensin homolog (PTEN) are also features of endometrioid carci- noma (Figure 6, C and D).

Suggested panel: p53, MIB-1, p16, ER, PR.

Immunohistochemistry for DNA Mismatch Repair Proteins in Endometrial Carcinoma (Markers of Microsatellite Instability)

Up to one quarter of UECs demonstrate high levels of microsatellite instability (MSI-H), which has been implicated in the pathogenesis of UECs but not USCs.104-106 We are now able to detect the expression status of 4 DNA mismatch repair proteins, the loss of which gives rise to MSI-H.107-111 The most common cause of abnormal DNA mismatch repair protein expression (involving as many as 80% of all MSI-H endometrial carcinomas) is hypermethylation of the hMLH1 promoter, which results in loss of MLH1 and PMS expression. Tumors that arise from this pathway occur sporadically. Most are well- or moderately differentiated UECs.110,112-115 Rare undifferentiated endometrioid carcinomas have also been described.115 There may be a good clinical reason to identify MSI-H tumors—most affected patients (presumably those with hMLH1 promot- er hypermethylation) have a superior prognosis when compared with patients with endometrial carcinomas that are not MSI-H.110 This situation stands in contrast to the remaining 10% of MSI-H endometrial cancers. These re- sult from a mutation, usually of hMSH2, that occurs in the setting of hereditary nonpolyposis colorectal cancer. Mutation of hMSH2 usually gives rise to loss of MLH1 and PMS2 expression, whereas mutation in hMSH2 usually leads to loss of MSH2 and MSH6 expression. Identifying abnormal expression of these proteins may therefore help clinicians and geneticists determine whether a
Figure 5. Uterine serous carcinoma (USC) versus uterine endometrioid carcinoma (UEC). USC shows diffuse and intense nuclear immunoreactivity for p53 (hematoxylin-eosin, original magnification ×100 [A]; p53, original magnification ×100 [B]), as does intraepithelial serous carcinoma (endometrial intraepithelial carcinoma) (hematoxylin-eosin, original magnifications ×100 and ×400 [inset] [C]; p53, original magnifications ×100 and ×400 [inset] [D]). USC can demonstrate a glandular architectural pattern (E; hematoxylin-eosin, original magnification ×100). Diffuse and intense p53 immunoreactivity in glandular USC (F; original magnification ×100) can provide support for this entity when atypical endometrial hyperplasia and endometrioid adenocarcinoma are considerations. Although low-grade endometrioid adenocarcinomas rarely overexpress p53, high-grade examples (G; hematoxylin-eosin, original magnification ×100) can show p53 overexpression (H; original magnification ×100).
Figure 6. Markers of emerging importance in distinguishing uterine endometrioid carcinoma (UEC) and uterine serous carcinoma (USC). Although USC characteristically overexpress p53 (**A; original magnification ×100**), endometrioid adenocarcinomas, especially when gland-forming, frequently express estrogen receptors (**B; original magnification ×100**) and commonly show at least focal nuclear and cytoplasmic staining with anti-β-catenin (**C; original magnification ×100**) and loss of expression of phosphatase and tensin homolog (**D; original magnification ×100**). **A** illustrates a mixed serous and endometrioid carcinoma in which the serous component overexpresses p53. **D** is reprinted with permission from the American Journal of Surgical Pathology.281

Table 2. Uterine Serous Versus Uterine Endometrioid Carcinoma*

<table>
<thead>
<tr>
<th></th>
<th>p53†</th>
<th>ER/PR</th>
<th>MIB-1</th>
<th>p16</th>
<th>β-Catenin</th>
</tr>
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<tbody>
<tr>
<td>Serous</td>
<td>+/−</td>
<td>−/+</td>
<td>High</td>
<td>+</td>
<td>Normal</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>−/+</td>
<td>+/−</td>
<td>Low</td>
<td>−‡</td>
<td>Normal or abnormal§</td>
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* ER/PR indicates estrogen receptor/progesterone receptor; +/−, mostly positive; and −/+ , mostly negative.
† Only staining greater than 75% of cells is scored as positive.
‡ Patchy and weak staining is common.
§ Only nuclear staining is considered abnormal.

Interpreting immunostains111 for expression of MLH1, MSH2, MSH6, and PMS2 requires some practice because the goal is detection of expression loss. To score a protein as abnormally expressed, one should find strong and diffusely positive internal controls, such as the nuclei of endometrial stroma and nonneoplastic endometrium and verify complete absence of expression in tumor cell nuclei. A common pitfall is scoring protein expression loss when the internal controls are not optimal. Another common pitfall is scoring protein expression as retained when expression is encountered in hyperplasia but not in carcinoma. If no expression is found in a carcinoma, but DNA mismatch repair proteins appear retained in the accompanying hyperplasia, the case should be interpreted as DNA mismatch repair protein loss. Loss of MLH1 almost always accompanies loss of PMS2, and loss of MSH2 almost always accompanies loss of MSH6, with only occasional exceptions. The appearance of loss of MLH1 with MSH2 or loss of MLH1 with MSH6 should be questioned and the case reassessed. Occasional cases show loss of MSH6 alone or only PMS2.

Suggested panel: MLH1, MSH2, MSH6, PMS2.

Endometrial Stromal Versus Smooth Muscle Neoplasms

CD10 and h-caldesmon are useful markers in this differential diagnosis (Figure 7, A through C; Figure 8, A and B). CD10 is expressed in endometrial stroma and endometrial stromal neoplasms, including low-grade endometrial stromal sarcoma (ESS) and endometrial stromal nodule.117-119 (Table 3). Diffuse and strong membranous expression is found in only approximately 50% of classical-appearing ESSs. The remainder show patchy staining, with a cytoplasmic and membranous pattern. CD10 is also occasionally expressed in uterine smooth muscle neoplasms and UECs. Smooth muscle actin (SMA) can be expressed in both stromal and...
smooth muscle cells, but a lack of desmin expression is consistent with endometrial stromal differentiation in the right context. h-Caldesmon, an actin and tropomyosin-binding protein, has also been reported to support smooth muscle differentiation.\textsuperscript{120,121} h-Caldesmon, more specific for smooth muscle differentiation when compared with desmin, distinguishes uterine smooth muscle tumors from conventional endometrial stromal tumors, which uniformly lack h-caldesmon reactivity (Figure 7, A and B). Endometrial stromal tumors almost always express ER and

Figure 7. Endometrial stromal neoplasms. An endometrial stromal sarcoma (ESS) (A; hematoxylin-eosin, original magnification ×100) is negative for h-caldesmon (B; original magnification ×100). Endometrial stromal sarcomas nearly always express CD10 (C; original magnification ×100) and estrogen receptor (D; original magnification ×100) and progesterone receptors. Several histologic mimics of metastatic ESS, including solitary fibrous tumor, can express progesterone receptors (E; original magnification ×400) and CD10 as well. Although many ESSs express CD10 strongly and diffusely, some express CD10 in a patchy pattern, which overlaps with that seen in solitary fibrous tumor and hemangiopericytoma, both tumors with a staghorn vascular pattern. A and B reprinted with permission from Churchill Livingstone.\textsuperscript{280} C through E reprinted with permission from Modern Pathology.\textsuperscript{131}

Figure 8. Endometrial stromal neoplasms with smooth muscle differentiation. A, Areas resembling endometrial stroma (left) contrast with areas resembling smooth muscle (right) (hematoxylin-eosin, original magnification ×100). B, CD10 marks endometrial stromal cells, whereas muscle markers, such as h-caldesmon, mark zones showing smooth muscle differentiation (CD10, original magnification ×100). Endometrial stromal neoplasms are in general diagnosed when conventional-appearing endometrial stroma is present; the diagnosis should not be abandoned solely because of divergent differentiation, such as smooth muscle differentiation, illustrated here. Reprinted with permission from Churchill Livingstone.\textsuperscript{280}
PR, as can uterine smooth muscle tumors. WT1 expression has been described in endometrial stromal and smooth muscle tumors as well.\textsuperscript{122}

Several endometrial stromal tumor variants exist: smooth muscle variant (also referred to as mixed endometrial stromal and smooth muscle tumor and stromal myoma),\textsuperscript{123,124} fibromyxoid variant,\textsuperscript{123,125} sex cord variant, including the “uterine tumor resembling ovarian sex cord tumor,”\textsuperscript{125,126} and variants including endometrioid glands or epithelioid cells.\textsuperscript{127} Understanding the immunophenotype of these tumors is based on the acknowledgment that the variant elements often lose the phenotype of endometrial stroma and acquire the phenotype of the corresponding variant or metaplastic element.\textsuperscript{122} For example, the smooth muscle in a smooth muscle variant of endometrial stromal nodule expresses muscle markers and may be negative for CD10.\textsuperscript{118,124} The endometrioid glands in an ESS, epithelioid variant, would express epithelial markers, such as EMA but not necessarily CD10. Similarly, the sex cord component of an endometrial stromal nodule, sex cord variant, might express inhibin and lose CD10 expression.\textsuperscript{128} Hybrid forms also exist, further complicating interpretation; some stromal neoplasms with sex cord features contain elements that coexpress muscle markers and cytokeratins.\textsuperscript{118}

Suggested panel: CD10, desmin, h-caldesmon.

**Uterine Smooth Muscle Neoplasms**

**Leiomyoma and Leiomyosarcoma.**—One of the most difficult differential diagnoses in gynecologic pathology concerns the distinction of atypical leiomyomas from leiomyosarcomas. Morphologic criteria are useful and remain the standard, but the relative deficiencies of the approach are well known. Expression of ER, PR, Bcl-2, and MIB-1 in uterine smooth muscle tumors has been studied.\textsuperscript{130–136} Leiomyosarcomas express more p53 and MIB-1 than leiomyomas but less Bcl-2 and ER and PR. In the presence of nuclear atypia, MIB-1 and/or p53 expression of more than 15% favors a diagnosis of leiomyosarcoma over smooth muscle tumors of uncertain malignant potential or cellular leiomyoma.\textsuperscript{137} MIB-1 expression may be useful in confirming the impression of mitotic activity, especially in poorly preserved specimens in which the distinction of mitotic figures from apoptotic bodies is not straightforward. Expression of ER, PR, p53, and MIB-1 have prognostic significance in leiomyosarcomas,\textsuperscript{136} but these lose significance in multivariate models in which stage remains the most important prognostic factor.

**Epithelioid Smooth Muscle Tumors.**—Epithelioid smooth muscle tumors possess some characteristics that separate them from the more common smooth muscle tumors composed of spindle-shaped cells. They can simulate a number of neoplasms with epithelioid features, including primary and metastatic carcinomas, melanomas, trophoblastic tumors, endometrial stromal tumors, and tumors with sex cord differentiation. Although using immunohistochemistry can narrow the differential diagnosis, its use is complicated in this group of tumors. The limited immunohistochemistry data for uterine epithelioid smooth muscle tumors indicate that they can demonstrate cytokeratin expression, at least focally.\textsuperscript{118,138} Carcinomas, on the other hand, often demonstrate stronger and more diffuse anticytokeratin immunoreactivity. Using desmin alone without a panel of markers is also subject to problems because many uterine epithelioid smooth muscle tumors lose diffuse desmin and h-caldesmon expression.\textsuperscript{118} A reasonable panel for this differential diagnosis would include CAM 5.2 cytokeratin, AE1/AE3 cy-

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>SMA</th>
<th>Desmin</th>
<th>h-Caldesmon</th>
<th>CD10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>+/-</td>
<td>R</td>
<td>N</td>
<td>+/-</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

* SMA indicates smooth muscle actin; +/-, mostly positive; R, rare; N, negative; +, positive; and -/+ , mostly negative.

**Table 3. Endometrial Stromal Versus Smooth Muscle Tumor**

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>CD10</th>
<th>ER</th>
<th>PR</th>
<th>CD14</th>
<th>AE1/AE3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>-/+</td>
</tr>
<tr>
<td>Solitary fibrous tumor/hemangiopericytoma</td>
<td>+/-</td>
<td>N</td>
<td>-/+</td>
<td>+</td>
<td>N</td>
</tr>
<tr>
<td>Monophasic synovial sarcoma</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>+/-</td>
</tr>
</tbody>
</table>

* ER indicates estrogen receptor; PR, progesterone receptor; +, positive; N, negative; -/+ , mostly negative; and +/+, mostly positive.

**Table 4. Endometrial Stromal Sarcoma Versus Pericytomatous Tumor**

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tokeratin, desmin, h-caldesmon, and SMA. S100 and CD10 could be added if melanoma and ESS were considered.

Perivascular Epithelioid Cell Tumor.—The morphologic criteria for distinguishing complete hydatidiform moles from mimickers are robust, particularly when the tissue is derived from a second trimester conceptus. Detection of abnormal gestations in the first trimester has led to diagnostic difficulties, however, because increased villous size and trophoblastic proliferation are less pronounced than in tissue derived from more advanced gestations. Immunohistochemical evaluation with antibodies against the p57 protein, a maternally transcribed gene product, has emerged as a useful adjunct to morphologic, flow cytometric, and cytogenetic study of molar tissue. Whereas p57 protein is expressed in intermediate trophoblasts of complete moles, partial moles, and nonmolar abortuses, only complete hydatidiform moles lack p57 expression in cytotrophoblasts and villous stromal cells.

Undifferentiated Endometrial Carcinoma Versus Undifferentiated Uterine Sarcoma Versus Carcinosarcoma

If clearly defined and separable carcinomatous and sarcomatous components are not seen on examination of routinely stained slides, it is often difficult or impossible to substantiate a diagnosis of carcinosarcoma (malignant mixed müllerian tumor). In some monophasic tumors showing pleomorphism, using an antibody panel that includes cytokeratins and a variety of mesenchymal-associated markers often reveals a confusing immunophenotype—coexpression of epithelial and mesenchymal markers or complementary expression of both. CD10 antibodies are not recommended in this setting because CD10 immunoreactivity is not indicative of endometrial stromal differentiation when the tumor in question is cytologically high grade. If one encounters distinct, geographic zones that express cytokeratins exclusively and separate, distinct zones that express mesenchymal markers exclusively, it is reasonable to consider a diagnosis of carcinosarcoma. The very rare undifferentiated endometrial carcinoma expresses cytokeratins and/or EMA only focally and lacks ER and PR expression; they have also been reported to lose expression of MLH. Otherwise, diffuse, strong keratin expression supports carcinoma and diffuse muscle marker expression without keratin immunoreactivity supports a sarcoma with a myogenous phenotype. Other patterns of immunoreactivity are probably not informative.

Emerging data support the previously discredited idea that heterologous differentiation in carcinosarcoma is a poor prognostic indicator. In this regard, immunohistochemistry is a powerful method of confirming an impression of rhabdomyoblastic differentiation. Myogenin or myoD1, sensitive and specific markers for skeletal muscle differentiation, can be used for this purpose.

Considerations about a tumor whose morphology is undifferentiated include undifferentiated primary or metastatic carcinomas, melanoma, lymphoma/leukemia, and undifferentiated uterine sarcoma. If there is concern about these tumors, it is reasonable to evaluate with antibodies against cytokeratin, S100, leukocyte common antigen, CD43, and a muscle marker.

Suggested panel: cytokeratin, desmin, S100; consider myogenin or myoD1 to confirm rhabdomyoblastic differentiation; consider leukocyte common antigen and CD43 to exclude lymphoma and leukemia when appropriate.

Early Complete Hydatidiform Mole Versus Partial Mole and Hydropic Abortus

The morphologic criteria for distinguishing complete hydatidiform moles from mimickers are robust, particularly when the tissue is derived from a second trimester conceptus. Detection of abnormal gestations in the first trimester has led to diagnostic difficulties, however, because increased villous size and trophoblastic proliferation are less pronounced than in tissue derived from more advanced gestations. Immunohistochemical evaluation with antibodies against the p57 protein, a maternally transcribed gene product, has emerged as a useful adjunct to morphologic, flow cytometric, and cytogenetic study of molar tissue. Whereas p57 protein is expressed in intermediate trophoblasts of complete moles, partial moles, and nonmolar abortuses, only complete hydatidiform moles lack p57 expression in cytotrophoblasts and villous stromal cells.

Suggested antibody: p57.

Placental Site Nodule Versus Squamous Cell Carcinoma

Placental site nodules are composed of intermediate trophoblast cells within a nodular, hyalinized stroma. In curettage specimens, these lesions can be confused with keratinizing squamous carcinoma. An immunohistochemical panel with inhibin, Ki-67, CK18, and p16 is suggested. CK18 and inhibin, although expressed in trophoblastic lesions, are generally not expressed diffusely in squamous neoplasms. p16 is reportedly negative in trophoblastic lesions.

Suggested panel: inhibin, CK18, p16, MIB-1.

Placental Site Trophoblastic Tumor Versus Placental Site Nodule

Both placental site trophoblastic tumor cells and intermediate cells in a placental site nodule stain with cytokeratin and frequently with human placental lactogen (hPL) (Figure 9). The Ki-67 index (percentage of positively staining cells) is close to zero in placental site nodules; placental site trophoblastic tumors have an index of 14% (±7%)(±7%). Exaggerated implantation sites also have a low Ki-67 index. A new strategy for distinguishing placental site nodule and placental site trophoblastic tumor is to use p63 immunohistochemistry; p63 is expressed in the intermediate trophoblasts of placental site nodule but not in placental site trophoblastic tumor. One should be aware that the epithelioid trophoblastic tumor, a cousin to placental site trophoblastic tumor, typically expresses p63.
the lesion in question expresses p63 and has a low proliferative rate, a diagnosis of placental site nodule should be considered, but if the proliferative rate exceeds approximately 10%, a diagnosis of epithelioid trophoblastic tumor should be entertained.

Suggested antibodies: MIB-1; consider p63.

Choriocarcinoma Versus Placental Site Trophoblastic Tumor Versus Poorly Differentiated Neoplasm

Choriocarcinoma is a biphasic tumor consisting of syncytiotrophoblast cells and mononuclear cells including both intermediate trophoblasts and cytotrophoblasts. A placental site trophoblastic tumor may enter the differential diagnosis when multinucleated giant cells are present and serum hCG levels are elevated. Choriocarcinomas contain numerous syncytiotrophoblasts, intimately associated with mononuclear trophoblasts. In contrast, some placental site trophoblastic tumors contain randomly distributed syncytiotrophoblasts amongst sheets of intermediate trophoblasts. Because the extent and distribution of hCG immunoreactivity is a rather good indication of the number and location of syncytiotrophoblasts, it can be used to distinguish choriocarcinoma and placental site trophoblastic tumor.

An immunohistochemical panel of hCG, CK18, hPL, and inhibin is recommended for distinguishing a trophoblastic neoplasm from a poorly differentiated carcinoma (Figure 9). Trophoblastic tumors express CK18 diffusely, but squamous lesions do not. Inhibin is a generally good marker to distinguish choriocarcinoma and placental site trophoblastic tumor. If p63 is negative and human placental lactogen (hPL) is diffusely positive, the lesion is either a placental site nodule (PSN) or an epithelioid trophoblastic tumor (ETT). If p63 is diffusely positive and only focally positive for hPL, the lesion is either a placental site nodule (PSN) or an epithelioid trophoblastic tumor (ETT). These lesions can be distinguished based on the Ki-67 labeling index. If p63 is diffusely positive and only focally positive for hPL, the lesion is either a placental site nodule (PSN) or an epithelioid trophoblastic tumor (ETT). These lesions can be distinguished based on the Ki-67 labeling index. +++, diffusely positive; +, patchy staining; +, focally positive; and −, negative. Courtesy of Dr Ie-Ming Shih.

VULVA AND VAGINA

Vulvovaginal Mesenchymal Lesions

A wide variety of mesenchymal lesions can arise in the vulvovaginal region. Lesions specific to, or characteristic of this site include aggressive angiomyxoma, angiomyoﬁbroblastoma, cellular angioﬁbroma, superﬁcial myoﬁbroblastoma of the lower female genital tract, ﬁbroepithelial stromal polyyp, and smooth muscle tumors. In addition, any soft tissue tumor can potentially arise in the vulvovaginal region. Most of the mesenchymal lesions that are encountered here are morphologically bland, and distinction between the various entities may be problematic. Immunohistochemistry plays a limited role, but there are occasional cases when markers may be useful. Almost all of the aforementioned lesions are positive with ER, PR, and vimentin. Most exhibit desmin positivity and α-smooth muscle actin (α-SMA), and CD34 may be positive or negative. Therefore, with regards to the immunophenotype, there is considerable overlap between the various entities. Cellular angioﬁbroma differs from the others in that, although vimentin, ER, and PR positive, it is typically desmin and α-SMA negative because this lesion exhibits ﬁbroblastic rather than myoﬁbroblastic differentiation. As a result, negative staining with desmin may be a useful diagnostic pointer, although a minority of cases of the other lesions mentioned are also negative. Positivity of aggressive angiomyxoma with ER and PR has resulted in the use of gonadotropin-releasing hormone agonists in the management of lesions that are not amenable to surgical resection. Administration of these agents may result in marked shrinkage of aggressive angiomyxomas.
Immunohistochemical staining with HMGA2 may be of value in the diagnosis of aggressive angiomyxoma. Aggressive angiomyxoma usually exhibits nuclear positivity, whereas most of the other vulvovaginal mesenchymal lesions are negative. Staining with HMGA2 may also be of value in the assessment of margins in resection specimens and in the determination of the presence or absence of residual disease in wider excisions. It is stressed that any mesenchymal lesion, benign or malignant, may potentially occur in the vulvovaginal region. The immunophenotype of these does not differ from when they occur at more usual sites. Recently, extragastrointestinal stromal tumors have been described arising in the vulvovaginal region. The immunophenotype is usually p16 negative. It has been claimed that p53 positivity of the intraepithelial cells of primary vulvar Paget disease may result in a false impression of MIB-1–positive cells in the upper aspects of the epithelium or partial denudation of the surface epithelium. MIB-1 is of no value in the distinction between koilocytosis and cervical intraepithelial neoplasia (CIN) 1 and in the separation of adjacent lesions in the CIN spectrum. Its main value is in the distinction between high-grade CIN (CIN 2 and 3) and mimics such as atrophic squamous epithelium, transitional metaplasia, and immature squamous metaplasia. In normal cervical squamous epithelium, nuclear MIB-1 staining is confined to the parabasal cells, whereas in CIN 3 it is full thickness (Figure 11, A; Figure 12, A). There is also a high proliferation index in CIN 2. Atrophic squamous epithelium exhibits minimal proliferative activity, with only focal staining of the basal and parabasal cells (Figure 11, B), and the proliferation index is low in transitional metaplasia and immature squamous metaplasia. MIB-1 may be of value in the interpretation of cauterized squamous epithelium at resection margins because the normal patterns of immunoreactivity are typically retained, even with marked cautery artefact. Tangential sectioning of normal squamous epithelium or partial denudation of the surface epithelium may result in a false impression of MIB-1–positive cells in the upper aspects of the epithelium.

p16 may be of value in the distinction between HPV-related invasive vulvar squamous carcinomas (diffuse p16 positivity) and the more common non–HPV-related squamous carcinomas (p16 negative or focally positive, especially at the advancing edge of the tumor).

Suggested panel: MIB-1, p16, p53.

Vulvar Paget Disease

Immunohistochemistry may be useful both in the diagnosis of vulvar Paget disease and in the distinction of primary from secondary Paget disease. The neoplastic cells of Paget disease are positive with CAM 5.2 (Figure 10, A) and CEA, helping to exclude mimics such as pagetoid Bowen disease, melanocytic lesions, and mycoses fungoides. The cells of primary vulvar Paget disease are typically positive for CK7 (Figure 10, B) and GCDFP-15 and commonly also react with HER-2/neu (Figure 10, C) and androgen receptor. They are typically ER and PR negative. As well as being of use in diagnosis, these markers may assist in the assessment of margins and in the identification of small foci of dermal invasion. Most cases of pagetoid Bowen disease are CK7 negative, although CK7 positivity has been described in a small number of cases.

Cervicovaginal stromal tumors are typically c-Kit and CD34 positive but negative with desmin and hormone receptors. They contain activating c-kit mutations.

Suggested panel: ER, PR, desmin, α-SMA, HMGA2.

Vulvar Intraepithelial Neoplasia

The value of markers in the distinction between dysplastic squamous lesions and nondysplastic mimics is covered more fully in the section on the cervix. In the vulva, the morphologic features of koilocytosis, the hallmark of HPV infection, may not be as obvious as in the cervix. Consequently it may be difficult to diagnose a viral-induced lesion. Immunohistochemical staining with the proliferation marker MIB-1 may be of value. In true HPV infection of the vulva, MIB-1 positive clusters are present in the middle and upper thirds of the epithelium. The cells of high-grade vulvar intraepithelial neoplasia (VIN 2 to 3) express MIB-1 throughout much of the full epithelial thickness. This may be of value in the distinction between high-grade VIN and atrophic squamous epithelium. p16 may also be of use. The most common type of VIN (undifferentiated or Bowenoid VIN) is associated, in most cases, with high-risk HPV. As discussed in the “Cervix” section, p16 may be regarded as a surrogate marker of the presence of high-risk HPV. Therefore, most cases of undifferentiated VIN are p16 positive. In contrast, differentiated VIN is not typically associated with HPV and is usually p16 negative. It has been claimed that p53 may assist in the diagnosis of differentiated VIN. This may be a difficult diagnosis morphologically because the features are subtle. Intense positive nuclear staining for p53 in the basal and suprabasal cells, usually secondary to p53 mutation, is characteristic of differentiated VIN. However, it is clear that p53 positivity may also occur in the basal cell layers of nonneoplastic lesions such as lichen sclerosis and inflammatory conditions because of mechanisms other than p53 mutation.

p16 may be of value in the distinction between HPV-related invasive vulvar squamous carcinomas (diffuse p16 positivity) and the more common non–HPV-related squamous carcinomas (p16 negative or focally positive, especially at the advancing edge of the tumor).

Suggested panel: MIB-1, p16, p53.

CERVIX

Preinvasive Cervical Squamous Lesions

The value of proliferation markers, such as MIB-1 (which reacts against the Ki-67 antigen), in the assessment of preinvasive cervical squamous lesions has been widely investigated. MIB-1 is of no value in the distinction between koilocytosis and cervical intraepithelial neoplasia (CIN) 1 and in the separation of adjacent lesions in the CIN spectrum. Its main value is in the distinction between high-grade CIN (CIN 2 and 3) and mimics such as atrophic squamous epithelium, transitional metaplasia, and immature squamous metaplasia. In normal cervical squamous epithelium, nuclear MIB-1 staining is confined to the parabasal cells, whereas in CIN 3 it is full thickness (Figure 11, A; Figure 12, A). There is also a high proliferation index in CIN 2. Atrophic squamous epithelium exhibits minimal proliferative activity, with only focal staining of the basal and parabasal cells (Figure 11, B), and the proliferation index is low in transitional metaplasia and immature squamous metaplasia. MIB-1 may be of value in the interpretation of cauterized squamous epithelium at resection margins because the normal patterns of immunoreactivity are typically retained, even with marked cautery artefact.

brake on the cell cycle by inactivating the cyclin dependent kinases that phosphorylate Rb protein. In cervical lesions associated with high-risk HPV infection, there is functional inactivation of Rb by HPV E7 protein with a resultant accumulation of p16 protein, because normally Rb inhibits transcription of p16. In most cases, diffuse p16 positivity in the cervix can be regarded as a surrogate marker of the presence of high-risk HPV (Figure 12, B). However, it is clear that focal or even diffuse p16 expression in the cervix and other tissues may occur as a result of non-HPV-related mechanisms. The role of p16 as a diagnostic aid in gynecologic pathology has recently been reviewed. There is diffuse p16 staining, nuclear, cytoplasmic, or a combination, involving the full thickness of the epithelium in most cases of CIN 2 and 3 because of the presence of high-risk HPV. However, although diffuse p16 staining involving the full thickness of the epithelium is a relatively specific indicator of high-grade CIN, a negative reaction does not exclude this because some cases of high-grade CIN are negative. It has been suggested that p16 may assist in identifying low-grade CIN lesions that are associated with high-risk HPV and that have an increased risk of progression. In one study, almost all cases of CIN 1 associated with high-risk HPV were p16 positive, whereas a large majority of cases not associated with high-risk HPV were negative. In most CIN 1 cases that are p16 positive (the percentage of positive cases has varied widely between studies), immunoreactivity has been confined to the lower third to half of the epithelium. There is minimal or negative p16 staining in normal, atrophic, and inflamed cervical squamous epithelium. It can be appreciated that, in the evaluation of cervical squamous lesions, MIB-1 and p16 have broadly similar uses and in practice the 2 markers are often best used together because they are complementary to each other.

**Suggested panel:** MIB-1, p16.

### Distinction of Cervical AIS From Benign Mimics

In the World Health Organization classification, the terms adenocarcinoma in situ and glandular dysplasia are used to encompass the spectrum of premalignant endocervical glandular lesions. In the United Kingdom, cervical glandular intraepithelial neoplasia is the most commonly used term; high-grade cervical glandular intraepithelial neoplasia equates to AIS and low-grade cervical glandular intraepithelial neoplasia to glandular dysplasia. The distinction between AIS and benign mimics, especially tuboendometrial metaplasia (TEM) and endometriosis, although usually straightforward, may on occasions be problematic. The combination of MIB-1, Bcl-2, and p16 may be of value. The MIB-1 proliferation index is typically less than 10% in TEM and endometriosis, whereas in AIS it is generally greater than 30%; in the majority of cases it is much greater with most nuclei exhibiting positivity (Figure 12). It is usually not necessary to undertake a formal count as there are typically only scattered positive nuclei in TEM and endometriosis, whereas in AIS most nuclei are positive. However, in some instances there is overlap with occasional cases of AIS having a low proliferation index and some benign lesions, especially en-
Figure 11. Cervical intraepithelial neoplasia 3 exhibits full-thickness immunoreactivity with MIB-1 (A), whereas atrophic squamous epithelium is negative (B) (original magnifications ×100).

Figure 12. Cervical adenocarcinoma in situ exhibiting a high MIB-1 proliferation index (A) and diffuse p16 positivity (B) (original magnifications ×100).

Figure 13. Prostatic metaplasia in cervix exhibiting positivity with prostatic acid phosphatase (A; original magnification ×100). Staining with 34BE12 highlights the basal layer of the glandular epithelium (B; original magnification ×100).
dometriosis, exhibiting a proliferation index in excess of 30%, meaning that, in an individual case, MIB-1 may not be of value. Bcl-2 may be useful as TEM and endometriosis typically exhibit diffuse cytoplasmic positivity, whereas AIS is generally negative.230,231 Adenocarcinoma in situ usually, although not always, exhibits p16 positivity because of the presence of high-risk HPV.258,230–233 Some cases of TEM and endometriosis are p16 positive but usually focally so, in contrast to the diffuse staining seen in AIS. Vimentin may be of value as TEM and endometriosis typically exhibit cytoplasmic positivity, whereas AIS is negative.235 Similarly TEM and endometriosis usually exhibit nuclear ER positivity, whereas AIS is generally negative or focally positive. Mononuclear CEA may be of value in that cytoplasmic positivity is characteristic of a premalignant or malignant glandular lesion.235,236 However, some cases of AIS are CEA negative, and conversely there may be luminal positivity in benign glandular lesions and even normal endocervical glands. The markers discussed may be useful in distinguishing between cauterized normal endocervical glands or cauterized TEM and the cauterized glands of AIS at loop resection margins. Glandular dysplasia (which is rarely diagnosed in pure form without coexistent AIS) has not been extensively investigated using the aforementioned markers and would be expected to exhibit a relatively low MIB-1 proliferation index. However, p16 may be of use in that most cases of glandular dysplasia studied have been positive because of their association with high-risk HPV.230

A variety of other markers have been investigated in an attempt to distinguish between AIS and benign mimics, including antibodies against p53, CD44, mucus-related antigens, and blood group antigens.238–240 However, these markers have not gained a foothold in routine pathology practice.

Suggested panel: MIB-1, Bcl-2, p16, monoclonal CEA.

Markers of Cervical Mesonephric Lesions

CD10 has recently been described as a marker of mesonephric glands within the cervix and elsewhere in the female genital tract.242–244 Cervical mesonephric remnants, in most cases, exhibit luminal CD10 positivity. It is relatively rare for other benign cervical glandular lesions to be CD10 positive. However, cervical and endometrial adenocarcinomas may be positive, albeit not typically with a luminal pattern. Therefore, CD10 immunoreactivity in a cervical adenocarcinoma cannot be taken as proof of a mesonephric origin. CD10 is a widely used marker of normal and neoplastic endometrial stroma.245 However, the cervical stroma immediately surrounding endocervical glands is CD10 positive, illustrating that in the cervix CD10 is of limited value in helping to confirm a diagnosis of endometriosis.242

Other markers that are positive in benign mesonephric remnants and mesonephric adenocarcinomas, in a variable percentage of cases, include androgen receptor, vimentin, calretinin, and inhibit.246 Estrogen receptor, PR, and monoclonal CEA are typically negative. Diffuse p16 positivity has been described in cervical mesonephric glands, presumably because of non–HPV-related mechanisms.

Suggested panel: CD10, vimentin, calretinin, ER.

Cervical Minimal Deviation Adenocarcinoma (Adenoma Malignum)

Minimal deviation adenocarcinoma is an extremely well-differentiated adenocarcinoma that may be confused with normal endocervical glands or a variety of benign cervical glandular lesions, especially in small biopsy specimens. The most common morphologic subtype is the mucinous variant, known as adenoma malignum. Gastric mucins are present in many cases of adenoma malignum. HIK1083, a monoclonal antibody against gastric gland mucins, is positive in a proportion of cases and, as a consequence, may be useful in diagnosis.248,249 Normal endocervical glands are almost always negative, although focal areas of positivity may be present in usual cervical adenocarcinomas.250 Cytoplasmic monoclonal CEA positivity may be of value in distinguishing adenoma malignum from normal or hyperplastic endocervical glands, which are usually CEA negative.44,251 However, some cases of adenoma malignum are negative,251,252 and conversely there may be luminal positivity in hyperplastic or normal endocervical glands. In contrast to most usual cervical adenocarcinomas, adenoma malignum is often p16 negative because many cases are not associated with high-risk HPV.253 The benign endocervical glandular lesion “lobular endocervical glandular hyperplasia, not otherwise specified” also, in some cases, exhibits a pyloric gland immunophenotype and is positive with HIK1083.254 It has been suggested that this may represent a precursor of adenoma malignum, although this is unproven.255,256 p16 positivity, unassociated with the presence of HPV, has been demonstrated in cases of lobular endocervical glandular hyperplasia, not otherwise specified.256

It has been suggested that a combination of ER and α-SMA immunohistochemistry may be useful in the distinction between adenoma malignum and normal or hyperplastic endocervical glands.257 With normal and hyperplastic glands, the stroma is ER positive and largely α-SMA negative. Because of the desmoplastic stromal response in adenoma malignum, the stromal cells are largely ER negative and there are increased numbers of α-SMA-positive myofibroblasts.

Suggested panel: HIK1083, monoclonal CEA, ER, α-SMA.

Prostatic Tissue in the Cervix

Ectopic prostatic tissue has been described in the cervix in the last few years.258,259 This is possibly more common than is realized because cases may go unrecognized if the diagnosis is not considered and appropriate immunohistochemistry performed. The prostatic tissue is usually located deep within the cervical stroma, either at the transformation zone or on the ectocervix, and there is almost always a combination of glandular and squamous elements. Typically, a double cell layer of glandular epithelium is present, similar to the eutopic prostate. Confirmation is easy, once the diagnosis is considered, using antibodies against prostate-specific antigen (PSA) and prostatic acid phosphatase (PrAP; Figure 13, A). However, PSA may be negative and PrAP staining may be focal. 34βE12 highlights the basal cell layer of glandular epithelium (Figure 13, B), and ER is positive in the squamous elements.259 The basal cell layer of the glandular epithelium is usually CD10 positive and the glandular elements are
androgen receptor positive.\textsuperscript{259} α-Methylacyl-CoA racemase has been positive in most cases tested.\textsuperscript{259}
Suggested panel: PSA, PrAP, 34βE12.

**Distinction Between Endometrial and Cervical Adenocarcinoma**

Adenocarcinoma may be present in preoperative endometrial and cervical biopsies and ascertaining the site of origin may be difficult. Problems may also occur in a hysterectomy specimen when tumor involves both the uterine corpus and the cervix. In general, the morphology differs between a usual cervical adenocarcinoma and an endometrial adenocarcinoma of endometrioid type, but there may be considerable overlap. A panel of markers comprising ER, vimentin, p16, and monoclonal CEA is of value.\textsuperscript{260–263} Endometrial adenocarcinomas of endometrioid type typically exhibit diffuse nuclear ER and cytoplasmic vimentin positivity. Carcinoembryonic antigen and p16 are usually negative or focally positive, although the squamous elements that are common in these neoplasms may stain. In contrast, cervical adenocarcinomas are usually, but not always, diffusely positive with CEA and p16, the latter because of the association with high-risk HPV. Vimentin is usually negative, and ER is typically negative or there is focal weak positivity. In occasional endometrioid adenocarcinomas of the uterine corpus, there is diffuse p16 positivity, and uterine serous carcinoma (similar to ovarian serous carcinoma) is often diffusely positive.\textsuperscript{264–266} Presumably because of non–HPV-related mechanisms. It is stressed that the panel of markers discussed is only of value in distinguishing a usual cervical adenocarcinoma from an endometrial adenocarcinoma of endometrioid type. The panel of markers also assists in the recently highlighted cases of subtle cervical stromal invasion by endometrioid adenocarcinoma of the uterine corpus.\textsuperscript{267} In such cases, the tumor within the cervix may be morphologically bland without a stromal reaction and can mimic cervical AIS, a primary cervical adenocarcinoma, or even cervical mesonephric remnants. In those rare cases in which an endometrioid adenocarcinoma of the uterine corpus and a premalignant or malignant endocervical glandular lesion coexist, the panel of markers discussed helps to clarify the relationship between the 2 neoplasms.

The question also arises as to the immunophenotype of a mucinous adenocarcinoma of the endometrium and an endometrioid adenocarcinoma of the cervix. One study that addressed this issue found that if a tumor exhibited diffuse ER and vimentin positivity, then it was almost certainly of endometrial origin.\textsuperscript{270} We stress that in any individual tumor, unexpected staining reactions may occur with one or more of the markers discussed. This can result in potential diagnostic problems and illustrates that the immunophenotype is always to be interpreted in light of the clinical, radiologic, gross pathologic, and microscopic findings.

Suggested panel: ER, vimentin, monoclonal CEA, p16.

**Cervical Neuroendocrine Neoplasms**

Establishing a diagnosis of a small cell or large cell neuroendocrine carcinoma is important because these are highly aggressive neoplasms that may be managed differently from the more common types of cervical cancer. It may be difficult to distinguish between a small cell neuroendocrine carcinoma and a small cell nonkeratinizing squamous carcinoma, especially on a small biopsy specimen. Similarly, the distinction between a large cell neuroendocrine carcinoma and an undifferentiated carcinoma or a poorly differentiated squamous or adenocarcinoma may be problematic.\textsuperscript{271} Neuroendocrine markers, such as chromogranin (a specific but relatively insensitive neuroendocrine marker), synaptophysin, PGP9.5, and CD56 assist.\textsuperscript{272} CD56 is rather nonspecific, with carcinomas of non-neuroendocrine type not uncommonly being positive.\textsuperscript{272} Cervical small cell neuroendocrine carcinomas, like their pulmonary counterparts, are commonly only focally positive with neuroendocrine markers and some cases are negative, especially on small biopsies.\textsuperscript{272} A diagnosis of small cell neuroendocrine carcinoma may be made when neuroendocrine markers are negative, if the morphology is typical. p63 may be of value in the distinction between a small cell neuroendocrine and a small cell squamous carcinoma in that most squamous carcinomas exhibit nuclear positivity, whereas neuroendocrine carcinomas are typically negative.\textsuperscript{273} Cervical adenocarcinomas are usually p63 negative, and this marker may be of use in confirming a poorly differentiated cervical carcinoma to be squamous in type (p63 positive).\textsuperscript{275} Positive staining with neuroendocrine markers is probably a prerequisite to diagnosing a cervical large cell neuroendocrine carcinoma. A high index of suspicion is required to diagnose these neoplasms because some do not have an overt neuroendocrine growth pattern and, as a result, are often misdiagnosed as an undifferentiated carcinoma or a poorly differentiated squamous or glandular carcinoma.

Neuroendocrine carcinomas of the cervix are usually p16 positive because of the presence of high-risk HPV.\textsuperscript{274–276} Recently, TTF-1 nuclear positivity has been described in cases of primary cervical large cell neuroendocrine carcinoma,\textsuperscript{277} and cervical small cell neuroendocrine carcinomas may also be positive.\textsuperscript{278} TTF-1 positivity is, in fact, not uncommon in extrapulmonary neuroendocrine tumours.\textsuperscript{279} Typical and atypical carcinoids, although extremely rare, are also part of the spectrum of neuroendocrine neoplasms of the cervix and are positive with neuroendocrine markers.

Suggested panel: chromogranin, PGP9.5, synaptophysin, CD56, p63.

**Distinction Between Cervical Microglandular Hyperplasia and Mucinous Adenocarcinoma of the Endometrium**

This is discussed in the “Uterine Corpus” section.

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