The Utility of Immunohistochemistry in the Differential Diagnosis of Gynecologic Disorders

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• ***Context.***—**Immunohistochemistry has assumed an increasing role in the identification and characterization of gynecologic disorders including lesions with deceptively bland morphology, uncommon and underdiagnosed neoplasms, and neoplasms with specific genetic alterations associated with overexpression or loss of expression of specific proteins. The diagnostic accuracy has been significantly improved owing to the discovery and increasing experience with the tumor-associated biomarkers, and the increasing demand for precise tumor classification to assess suitability for the expanding therapeutic modalities including clinical trials.**

Objective.—**To differentiate lesions of the gynecologic tract through the use of effective immunohistochemical panels.**

Data Sources.—**Literature review and authors' personal practice experience.**

Conclusions.—**The application of diagnostic and prognostic immunohistochemical panels has enabled pathologists to better guide therapeutic decisions and to better predict the clinical outcome. It is now well established that the use of ancillary testing, including immunohistochemistry, has a significant power in the identification, differentiation, and classification of reactive, premalignant, and malignant gynecologic disorders. This article discusses the utilities and pitfalls of the commonly used immunohistochemical markers in the context of overlapping morphologic features encountered in the uterus, ovaries, and fallopian tubes.**

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CERVIX

The Differential Diagnosis of Squamous Lesions of the Uterine Cervix

Immunohistochemistry in the cervix is helpful primarily in the differentiation of benign reactive epithelial changes from either squamous or columnar neoplasia.[1] In general, when managing a patient who is human papilloma virus (HPV) positive and has either atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion (LSIL) on cervical cytology, the pathologist does not have to distinguish minor reactive epithelial atypia from LSIL in the biopsy specimen in as much as either interpretation confers a similar outcome risk of high-grade squamous intraepithelial lesion (HSIL) (5%–10%) during the next 2 years of follow-up. The marker of proliferation (Ki-67), the expression of topoisomerase II-α and mini-chromosome maintenance protein-2 (Pro-Ex-C), and the HPV-associated cyclin-dependent kinase inhibitor 2A (p16) have been used to differentiate HSIL from several conditions with morphologic overlap, including reactive/reparative epithelial changes, immature squamous metaplasia, menopausal- and hormone-induced mucosal atrophy with atypia, and cytologic alterations induced by cautery artifact.[1] The expression of p16 is attributed to high-risk HPV infection.[2,3] A high index of Ki-67 nuclear staining and diffuse p16 nuclear and cytoplasmic expression involving most of the mucosal thickness are commonly associated with squamous intraepithelial lesions (SILs) arising from the squamocolumnar junction.[4] One caveat is that expression of Ki-67 can be seen well into the upper layers of immature inflamed squamous epithelium. Pro-Ex-C may be more useful in this setting owing to its improved specificity and lower background staining.[5] In either case, the p16 staining is the most decisive in distinguishing reactive changes from SIL.[6] Human papilloma virus in situ (IS HPV) hybridization may be helpful in distinguishing lesions from nonneoplastic epithelium but its value in routine practice is questionable given the availability of p16.[7] A second caveat is the distinction of LSIL from HSIL by p16 staining alone. On one hand, diffuse and full-thickness (block) staining has been associated with HSIL. On the other, we have found that this staining pattern in the cervix is strongly associated with any lesion arising at the squamocolumnar junction. Such lesions may be empirically at greater risk by their location in the squamocolumnar junction and associated high-risk HPV type. Nevertheless, many atypia that do not fulfill the criteria for HSIL occur at the squamocolumnar junction, are strongly p16 positive, and will regress on follow-up.[8] Thus, it is imperative that the pathologist be aware of the pitfall of using p16 *alone* as a "self-fulfilling prophet" for HSIL without considering the degree of atypia. This is particularly

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critical in young women for whom unnecessary loop electrosurgical excision procedure excision can increase the risk of premature rupture of membranes during pregnancy. One solution is to render a diagnosis of "SIL of uncertain grade (cervical intraepithelial neoplasia I–cervical intraepithelial neoplasia II)" when faced with this conundrum, emphasizing the option of a 6-month follow-up period during which at least half of such lesions will resolve.[9]

Trophoblastic neoplasms derived from placental extravillous trophoblast are uncommon tumors that involve the cervix and share histologic features with the squamous neoplasms. They are differentiated by their diffuse reactivity with the extravillous (intermediate) trophoblast markers human leukocyte antigen G (HLA-G), cluster of differentiation (CD)146 (Mel-CAM), and inhibin.[10] The use of glypican-3 (GP-3), another trophoblastic marker, is limited owing to its positivity in approximately 50% of squamous cell carcinomas.[11]

The Differential Diagnosis of Glandular Lesions of the Uterine Cervix

Similar to the squamous lesions, Ki-67 and p16 markers are commonly used to differentiate endocervical adenocarcinoma in situ (AIS) from endocervical glandular metaplasia, tubal metaplasia, microglandular hyperplasia, and endometriosis.[12] A prototype of endocervical AIS is a columnar lesion demonstrating increased Ki-67 proliferative index with strong and diffuse p16 nuclear and cytoplasmic reactivity, and frequently lacking the expression of estrogen receptor (ER) and vimentin. Caveats include early or superficial AIS in which Ki-67 staining may be less intense, and reactive epithelial changes in which Ki-67 staining may be strong.[13] Therefore, strong diffuse p16 staining is the most important distinguishing feature. However, diffuse strong p16 expression is also found in high-grade endometrial (including serous) adenocarcinomas involving the cervix and metastatic high-grade tubal or ovarian adenocarcinomas, requiring additional evaluation (outlined below), particularly in small biopsy specimens. In situ hybridization for HPV, if positive, will be helpful in accurately identifying the endocervical neoplasm. Moreover, strong (or complete absence of) nuclear p53 immunostaining will be seen in tumors from the endometrium or upper genital tract, while HPV-positive cervical neoplasms should show weak and heterogeneous staining. Ki-67 is also useful, albeit less so; although increased in AIS, it may be focally increased in endometriosis, tubal metaplasia, and microglandular hyperplasia and markedly elevated in reactive columnar epithelium. p16 will show focal reactivity only in these benign processes.[14,15] There are no markers yet available for clinical use to differentiate AIS and invasive adenocarcinoma, although improved histologic classification systems are being put forward to identify those problematic lesions with a very low risk of recurrence.

Endocervical Versus Endometrial Adenocarcinoma.—Estrogen receptor, progesterone receptor (PR), vimentin, carcinoembryonic antigen (CEA), p53, and IS HPV.

The distinction between endocervical and well-differentiated endometrial adenocarcinoma has significant therapeutic implications. Primary endocervical adenocarcinoma is managed differently from endometrial adenocarcinoma involving cervix. Endocervical adenocarcinoma is usually diffusely positive for p16 and CEA, negative or focally positive for ER and vimentin, and demonstrates positive nuclear staining on IS HPV. In contrast, low-grade endometrioid adenocarcinoma is diffusely positive for ER and vimentin, negative or focally positive for p16 and CEA, and is HPV negative.[16,17] The most useful marker is p16, provided careful attention is paid to the fact that endometrioid lesions can stain rather impressively albeit in a heterogeneous fashion.

While high-grade endocervical adenocarcinoma will invariably demonstrate strong diffuse p16 reactivity,[18] high-grade serous and some undifferentiated and high-grade endometrioid adenocarcinomas express strong, diffuse p16 reactivity similar to endocervical adenocarcinoma.[19–21] Tumor suppressor p53 (p53), and to a lesser degree, IS HPV testing, can be used to further characterize these tumors.[19,22,23] p53 is diffusely positive in some undifferentiated endometrial carcinomas, and in most high-grade serous and endometrioid carcinomas. In situ HPV test results are usually, but not always, positive in the primary endocervical adenocarcinoma and negative in the endometrial tumors. p63 has some value in outlining squamous carcinomas admixed with marked inflammatory infiltrates or distinguishing poorly differentiated carcinomas.[24]

UTERUS

The Differential Diagnosis of Preinvasive Endometrial Lesions

Atypical Hyperplasia Versus Benign/Reactive Endometrium.—Immunohistochemistry in endometrial hyperplasia has been used to identify lesions at a greater risk of progression to endometrioid adenocarcinoma.[25–29] The clonal loss of phosphatase and tensin homolog (PTEN) in 44% and paired box gene (PAX) 2 in 71% of endometrial intraepithelial neoplasia cases has been used as an investigative tool to outline the extent of the lesion in most cases.[29,30] The limitations to the universal use of both markers include the reduction or loss of PTEN expression in the secretory and atrophic glands of normal endometrium, and the spontaneous loss of both markers in normal endometria without identifiable endometrial intraepithelial neoplasia.[28–30] For these reasons, neither PTEN nor PAX2 are recommended as stand-alone tools in the diagnosis of neoplastic endometrioid precursor lesions of the endometrium.

Serous Carcinoma In Situ Versus Benign/Reactive Endometrium.—Serous intraepithelial carcinoma of the endometrium is considered an early form of malignancy with an approximately 5% risk of recurrence following surgical staging. It can also be associated with a concomitant pelvic or peritoneal serous carcinoma. The immunophenotype of intramucosal serous carcinoma is similar to the invasive carcinoma.[31] There is diffuse and strong expression of p53 exceeding 75% of the tumor cells with a similar increase in Ki-67 proliferative index for the serous tumor. This pattern is not found in the reactive endometrium or endocervix.[32] Nevertheless, the proliferative index can vary and there are lesions with a similar immunophenotype but lesser degrees of atypia that must be dealt with, including apparently benign clonal expansions with p53 mutations. For this reason, routine staining of benign-appearing endometrium for p53 is not encouraged at this time. Lesions with low proliferative indices, less pronounced atypia, and strong p53 staining are fortunately rare, but remain problematic and should be followed up.

The Use of Immunohistochemistry in the Classification of High-Grade Endometrial Carcinomas

High-grade carcinomas of the endometrium commonly include serous, clear cell, Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) grade 3 endometrioid and undifferentiated endometrial carcinomas, carcinosarcomas, and some mixed carcinomas and unclassifiable tumors. Diagnostic disagreement between pathologists in distinguishing between these tumors is not uncommon owing to their heterogeneity and overlapping hematoxylin-eosin (H&E) morphologic features.[33,34] Immunohistochemistry is helpful as an objective means for a better recognition and accurate classification, a better diagnostic reproducibility, and a reliable prediction of the clinical outcomes.[33,34] The ultimate question will be clinical utility, which will come with more effective targeted therapy. For now, the goal is to differentiate endometrioid from serous carcinoma and less commonly, clear cell carcinoma from either.

High-Grade Serous Versus Endometrioid Adenocarcinoma.—p53, β-catenin (nuclear), p16, PR, PTEN, AT-rich interactive domain–containing protein 1A (ARID1A), mismatch repair endonuclease PMS2 (PMS2), and mutator S homolog 6 (MSH6).

Serous carcinomas of the endometrium are treated more aggressively than endometrioid adenocarcinomas owing to their propensity for extrauterine spread, recurrence, and worse prognosis.[35,36] The distinction of serous from endometrioid carcinoma may be problematic on H&E, particularly in small biopsy samples and in tumors with ambiguous nonspecific features attributed to one or the other, such as slitlike spaces, glandular and solid architecture, and high nuclear grade.

Endometrioid histology has been associated with loss of PTEN expression, diffuse PR, and normal (heterogeneous/weak) p53 staining.[36–39] On the other hand, PTEN expression, focal or absent PR, and abnormal (diffusely strong or completely absent or null) p53 staining favor serous carcinoma.[23,33,34,40] Additional commonly used markers include p16, ARID1A, β-catenin, and mismatch repair proteins (MMRs). In approximately 90% of serous and one-third of clear cell and grade 3 endometrioid carcinomas, there is reactivity with p16.[19,37] The staining pattern of this marker is usually diffuse in the serous tumors and patchy or mosaic in FIGO 3 endometrioid adenocarcinoma. ARID1A is lost in approximately half of FIGO3 endometrioid adenocarcinomas but not in serous carcinomas.[41] Nuclear β-catenin is found in approximately 50% of endometrioid adenocarcinomas but not in serous carcinomas.[42–44] Aberrant expression of DNA MMRs is predominantly found in FIGO3 endometrioid adenocarcinomas, whereas serous carcinomas do not show this abnormality.[34] It has been shown that a combination of markers, including p53, p16, PTEN, and PR, may be superior to p53 alone in discriminating uterine serous versus endometrioid carcinoma.[40]

One approach is to perform p53 immunostaining on ambiguous carcinomas and classify them as endometrioid if the staining pattern is normal. If p53 staining is abnormal, PTEN, BRG1-associated factors 250 (BAF250), PMS2, and MSH6 stains may be useful in identifying concomitant endometrioid differentiation (R. Soslow, MD, written communication, 2013), alerting the clinician that the tumor may be a variant endometrioid tumor with loss of p53 function.

High-Grade Serous Versus Clear Cell Carcinoma.—p53, p16, PTEN, ARID1A (BAF250), and DNA MMR (PMS2, MSH6).

A useful diagnostic panel includes p53, PTEN, ARID1A, and DNA MMR. The typical serous carcinoma is strongly and diffusely positive for p53 and p16, whereas these 2 markers are less commonly positive in clear cell carcinoma. The limitations of the use of p53 include the reported cases of a variant of endometrial clear cell carcinoma that overexpresses p53.[45] Abnormal expression of PTEN, ARID1A, and DNA MMR is frequent in clear cell carcinoma and rare in serous carcinoma.[34] Hepatocyte nuclear factor–1β (HNF-1β) lacks the sensitivity and specificity for the distinction of these 2 tumors in the endometrium.[46]

Clear Cell Carcinoma Versus FIGO Grade 3 Endometrioid Adenocarcinoma.—HNF-1β, ER, and PR.

Although the role of immunohistochemistry is limited in the differential diagnosis of these 2 tumors, a panel consisting of PR and HNF-1β was suggested, with absent PR and diffuse HBF-1β expression favoring clear cell over endometrioid adenocarcinoma.[47–52] On the other hand, a useful panel of HNF-1β and ER was suggested in a recent study differentiating pure classic clear cell carcinoma of the endometrium from endometrioid and serous carcinomas.[52] Positive staining of more than 70% of tumor cells with HNF-1β and negative staining with ER characterized most clear cell carcinomas but not endometrioid or serous carcinomas.[52] It should be noted that HNF-1β is also expressed in endometriosis, Arias-Stella reaction, and occasional cases of endometrioid adenocarcinomas.[50,52] At this point, the value of HNF-1β in diagnosing clear cell carcinoma remains under study.

The Differential Diagnosis of Undifferentiated Carcinoma.—The reported prevalence of undifferentiated carcinoma (UC) of the endometrium in the recent literature is 9%.[53–55] The previously reported rare occurrence of this variant of aggressive endometrial carcinoma is attributed to underrecognition.[53–55] This tumor may occur in combination with low-grade endometrioid adenocarcinoma (dedifferentiated carcinoma), imparting a worse prognosis than expected.[57] On H&E, UC consists of a proliferation of medium to large-sized monotonous epithelial cells in solid sheets with complete absence of glandular formation (Figure 1, A) and with absence or minimal neuroendocrine differentiation (Figure 1, B).[54,55] These morphologic characteristics overlap with several endometrial tumors including FIGO 3 endometrioid adenocarcinoma, carcinosarcoma, endometrial stromal sarcoma, epithelioid sarcomas, serous carcinoma, lymphoma, plasmacytoma, small cell neuroendocrine carcinoma, embryonal rhabdomyosarcoma, and primitive neuroectodermal tumor (PNET).[34,53]

Immunohistochemistry is a valuable adjunct for a reproducible diagnosis of UC. Most UCs show focal expression of cytokeratin (CK)18, epithelial membrane antigen (EMA), and CK8/18 (CAM 5.2), and approximately 50% of the tumors show loss of at least 1 MMR protein (Figure 1, C).[34] They are predominantly negative or focally positive for ER, PR, and CK(AE1/AE3). Diffuse p53 expression is absent, but focal positivity may be encountered (Figure 1, D). The keratin stains usually show diffuse positivity in endometrioid and serous carcinomas. Diffuse ER, and PR, staining is also found in endometrioid adenocarcinoma and endometrial stromal sarcoma, but less frequently in UC. Undifferentiated carcinoma is negative for the sarcoma markers smooth muscle actin (SMA), desmin,

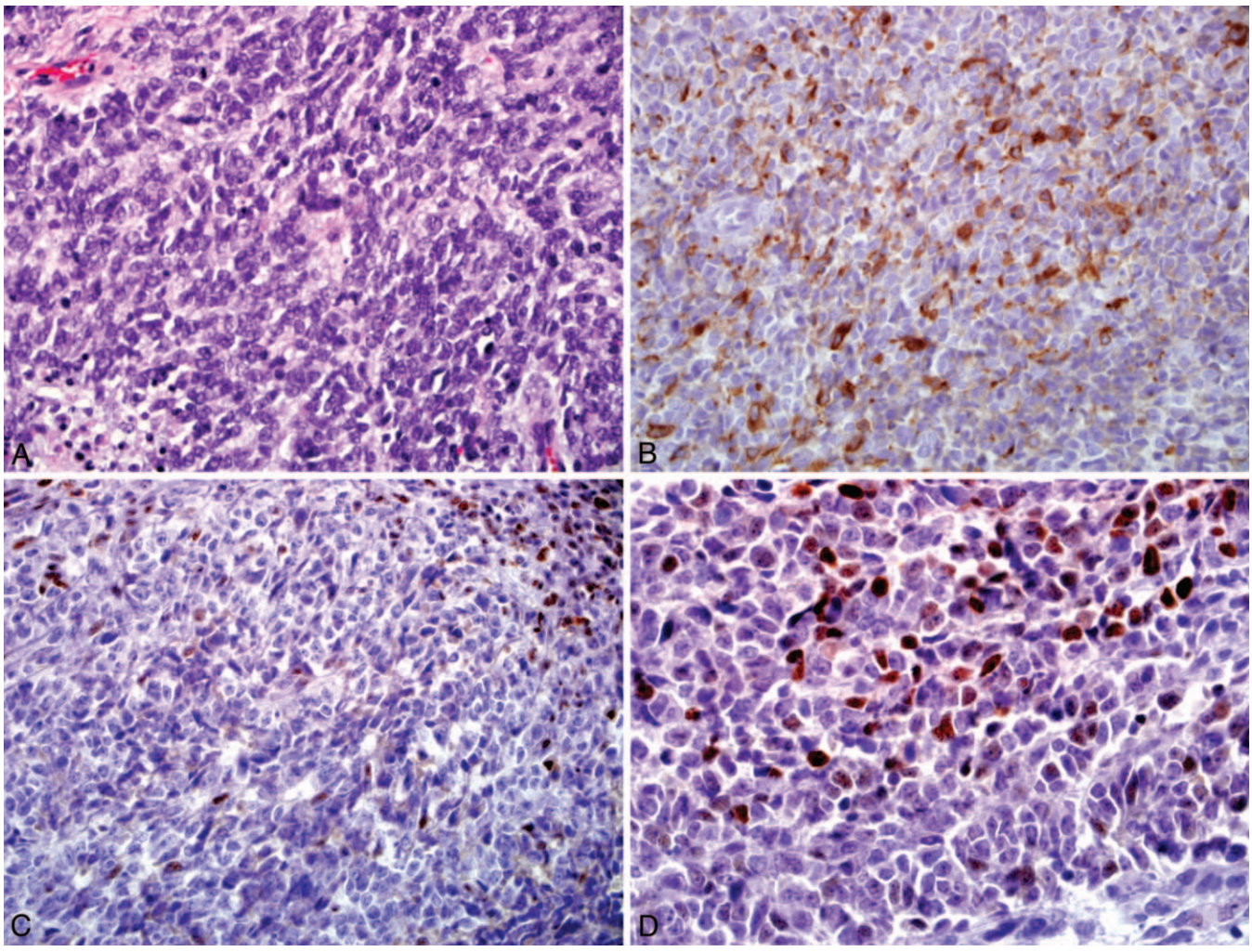

Figure 1. *A through D, A case of undifferentiated endometrial carcinoma on hematoxylin-eosin–stained section (A) with tumor cells focally positive for NSE (B) and negative for PMS2 (C), with wild-type expression of p53 (D) (hematoxylin-eosin, original magnification ×40 [A]; original magnification ×40 [B through D]). Abbreviation: NSE, neuron specific enolase.*

myogenin, and B-cell lymphoma 2 (bcl-2), negative or focally positive for the neuroendocrine markers chromogranin and synaptophysin, and negative for the lymphoma marker paired box protein Pax-5 (PAX5). Plasmacytoma is excluded by the absence of κ or λ light-chain restriction by in situ hybridization testing. It is worth noting that a wide variety of tumors express CK18, including breast, lung, and gestational trophoblastic neoplasms.

The Differential Diagnosis of Carcinosarcoma (Malignant Mixed Müllerian Tumor).—Malignant mixed müllerian tumor is a biphasic tumor with separate and distinct high-grade carcinomatous and sarcomatous components.[57] The differential diagnosis of carcinosarcoma includes biphasic carcinomas such as endometrioid adenocarcinoma with spindle cells and dedifferentiated carcinoma.

The most important exclusion when considering a carcinosarcoma is a metaplastic endometrioid carcinoma, which is facilitated by an index of suspicion for the histologic features of this entity. Immunohistochemistry can be used to identify the heterologous elements in carcinosarcoma although it may not influence management.[58] Rhabdomyoblastic differentiation is confirmed by positive staining with desmin, myogenin, and myo-D1. Chondroid and lipomatous heterologous elements are S100 positive, whereas carcinoma is rarely positive for this marker.[59,60] Epithelial membrane antigen, cytokeratins, and vimentin may stain both sarcoma and spindle cells in adenocarcinoma.[58] Carcinosarcomas frequently express p53 and Wilms tumor protein (WT1), and rarely ER and PR, in contrast to the spindle cell areas in endometrioid carcinoma with minimal to absent reactivity with p53 and WT1, and frequent expression of ER and PR.[37,61,62] The undifferentiated component of dedifferentiated carcinoma is negative for the sarcoma markers SMA, desmin, myogenin, and bcl-2, which are usually expressed in carcinosarcoma.[34]

The Differential Diagnosis of Uterine Mesenchymal Neoplasms

Uterine sarcomas are rare, accounting for fewer than 9% of all uterine malignancies, with leiomyosarcoma being the most frequent.[63] The smooth muscle neoplasms are reported to express several markers, including desmin, h-caldesmon, muscle-specific actin, SMA, vimentin, WT1, bcl-2, CD10, ER, PR, oxytocin receptors, and histone deacetylase 8.[58] Approximately one-third of all smooth tumors express cytokeratins, and the same proportion of myxoid and

epithelioid leiomyosarcomas are negative for the smooth muscle markers.[58]

Low-grade endometrial stromal sarcomas and endometrial stromal nodules are characteristically reactive with CD10, WT1, ER, and PR markers. Other positive markers include SMA, β-catenin, cytokeratin, and androgen receptors. Reactivity with CD34 and desmin is rare.[58] The metaplastic elements usually express the corresponding immunophenotype of endometrioid, sex cord, or smooth muscle differentiation.[64]

The criteria for diagnosis of undifferentiated sarcoma are not well defined. Undifferentiated sarcomas do not express the mesenchymal markers and panels that exclude carcinoma, melanoma, lymphoma, and leukemia may be required.[58]

Smooth Muscle Versus Endometrial Stromal Tumors.—Desmin, h-caldesmon, and SMA.

Uterine smooth muscle tumors are differentiated from endometrial stromal tumors by their reactivity with the smooth muscle markers desmin, h-caldesmon, and SMA.[65–67] Endometrial stromal tumors are usually negative for these markers unless mixed with a smooth muscle phenotype where focal reactivity may occur. Nuclear β-catenin was also used in the differentiation of low-grade endometrial stromal tumor from a cellular leiomyoma.[65,67,68]

Leiomyosarcoma Versus Leiomyoma.—Currently, there are no ideal immunohistochemical panels that can accurately distinguish between leiomyosarcoma (LMS) and leiomyoma (LM) in spite of the numerous tested antibodies. A commonly used panel consisting of p16, p53, and Ki-67, with diffuse p16 and p53 positivity and high Ki-67 proliferation index favoring LMS over LM,[69,70] is limited by the overlapping patterns described in the nonconventional variants of uterine smooth muscle tumors. The strong and diffuse p16 reactivity seen in LMS may also be encountered in the smooth muscle tumors of uncertain malignant potential (STUMPs) and LM with bizarre nuclei.[69,71–74] Similarly, diffuse p53 expression described in up to half of the cases of LMS may also be found to a lesser extent in LMs with bizarre nuclei and STUMP.[75,76] Moreover, mitotically active LMs and LMs with bizarre nuclei may have elevated Ki-67 proliferative index, simulating the pattern described in LMS.[69,71,74,76] Another potentially useful differentiating immunostain includes fascin described in most LMS, in approximately half of the STUMP cases, and rarely in leiomyoma.[77] Estrogen receptor positivity is used to support a gynecologic origin of LMS.[69]

Inflammatory Myofibroblastic Tumor Versus Malignant Mesenchymal Neoplasms.—Anaplastic lymphoma kinase.

Uterine inflammatory myofibroblastic tumor is a benign or locally recurrent spindle cell proliferation that has overlapping histologic features with benign and malignant endometrial stromal and smooth muscle tumors of the uterus. Anaplastic lymphoma kinase is a differentiating immunohistochemical marker with cytoplasmic reactivity in the inflammatory myofibroblastic tumor but not in the stromal or smooth muscle neoplasms.[78]

Perivascular Epithelioid Cell Tumor Versus LMS and Endometrial Stromal Sarcoma.—Human melanoma black 45 (HMB-45), melanoma antigen recognized by T-cells 1 (MART-1), S100, SMA, desmin, and CD10.

Perivascular epithelioid cell tumors (PEComas) are characterized by the proliferation of spindle and epithelioid cells with clear and granular cytoplasm and overlapping H&E features with smooth muscle and endometrial stromal tumors. Typical PEComas display immunoreactivity for SMA, desmin, and for the melanocytic markers HMB-45 and MART-1. When it shows negativity, S100 is helpful in excluding melanoma in HMB-45– and MART-1–positive cases. S100 shows positivity in approximately one-third of PEComas. Most of the smooth muscle and endometrial stromal tumors are predominantly negative for the melanocytic markers including S100.[79–82]

Gestational Trophoblastic Disorders

Trophoblastic populations emerging in early gestation include cytotrophoblast, syncytiotrophoblast, and extravillous implantation site trophoblast.[83] Several general markers, including CK(AE1/AE3), inhibin, CK18, CD10, and 3-β-hydroxysteroid dehydrogenase/Δ-5-4 isomerase (3βHSD), demonstrate diffuse and strong staining of these populations.[83–89] p63 and β-catenin are markers for the cytotrophoblast.[87] The implantation site trophoblast is derived from the cytotrophoblastic columns and is reactive with human placental lactogen (hPL) and to a lesser extent with human chorionic gonadotropin (hCG).[86] HLA-G and CD146, also known as Mel-CAM, are commonly used general markers for these extravillous early implantation trophoblasts.[10,58,90] In the villous, cytotrophoblasts abruptly differentiate to syncytiotrophoblasts, which are typically strongly reactive with hCG and inhibin and to a lesser extent with hPL and placental alkaline phosphatase (PLAP).[86,91,92]A third pathway of trophoblastic differentiation occurs in the development of the placental membranes and placental parenchyma. These are similar in type and characterized by the migration of cytotrophoblast from the villi entrapped in the chorionic membranes or in extravillous fibrin in the intervillous space or maternal surface. This cytotrophoblast undergoes an evolution to a mature extravillous trophoblast in the membranes or intervillous fibrin that is strongly inhibin positive. The transition from cytotrophoblast to mature extravillous trophoblast in these sites involves a "transitional" trophoblast cell type (so-called chorionic-type intermediate trophoblast) that retains p63 and also expresses PLAP. It loses p63 as it matures while acquiring more intense staining for inhibin.[93]

HLA-G is useful in the differential diagnosis of a placental site trophoblastic tumor (PSTT). Inhibin is also a reasonable marker to distinguish PSTT from a squamous carcinoma. HLA-G is not expressed either in cervical squamous cell carcinoma or endometrial adenocarcinoma.[58] However, 10% of endocervical adenocarcinomas,[94] 40% of breast adenocarcinoma,[95] and 66% of esophageal adenocarcinomas are HLA-G positive.[96] Similarly, inhibin-α is expressed in 20% of melanomas,[94,97] 20% of endocervical adenocarcinomas,[94] 22% of large cell lung carcinomas,[98] and 10% of high-grade ovarian serous carcinomas,[99] in addition to most ovarian gonadal stromal tumors. Inhibin-α is expressed in only 40% of exaggerated placental site reaction and intermediate trophoblastic tumors.[85]

Complete Hydatidiform Mole Versus Partial Mole and Nonmolar Villous Hydropic Degeneration.—Complete moles are precursor lesions of choriocarcinoma, necessitating their accurate differentiation from partial moles and nonmolar villous hydropic degeneration that display overlapping histologic features. The loss of p57 expression in the villous cytotrophoblast and stromal cells is a characteristic feature of complete moles.[100–102] This marker is expressed in the partial mole and nonmolar aborted

placental tissue, and also in the intermediate trophoblast of the complete moles.[100–102] The use of p57 is particularly helpful in the early first trimester product of conception tissue, since the H&E morphologic features of complete molar gestation are not well developed. With rare exceptions (there always are) p57 staining will make this distinction (rarely, p57 can be expressed from a retained maternal segment of chromosome 10). One important caveat is staining interpretation, and the pathologist must pay attention to antibody concentration and weak false positives.

Placental Implantation Site Versus Decidua.—Under most circumstances, decidua can be distinguished from extravillous trophoblast in the implantation site. However, when implantation site must be discriminated in a small sample, immunohistochemistry is useful in detecting residual mononuclear and binuclear intermediate trophoblastic cells with cytologic similarities to the decidual cells in the curettage tissue devoid of chorionic villi and typical syncytiotrophoblasts. The trophoblastic markers hPL, PLAP, and keratin CK(AE1/AE3) were used for this purpose.[103] When used simultaneously, hPL and CK(AE1/A3) demonstrated the highest sensitivity and specificity in detecting trophoblastic tissue that is not recognized on H&E sections, and therefore excluding ectopic gestation.[103]

Placental Site Nodule Versus Epithelioid Trophoblastic Tumor.—Both lesions share the same transitional (chorionic) extravillous trophoblastic markers including strong staining with CK18, HLA-G, and p63 and negative or weakly and focally positive staining with hPL. However, Ki-67 proliferative index is less than 10% in the placental site nodule and more than 10% in the epithelioid trophoblastic tumor.[87,94,104]

Exaggerated Placental Site Nodule and Placental Site Trophoblastic Tumor.—Both lesions share the same general and implantation site–associated extravillous trophoblast (Figure 2, A) and trophoblastic markers including strong staining with CK18, HLA-G, hPL (Figure 2, B) and lack of staining with p63 (Figure 2, C). The differentiating marker Ki-67 stains positively in 15% ± 7% of the tumor cells, and in fewer than 10% to 15% of cells in the exaggerated placental site (Figure 2, D).[87,94,105]

The Differential Diagnosis of Epithelioid Trophoblastic Tumor, PSTT, Choriocarcinoma, and Poorly Differentiated Carcinomas.—Squamous cell carcinoma and extravillous trophoblastic tumors display histologic similarities including the epithelioid appearance of the tumor cells with cytoplasmic eosinophilia. In addition, both squamous cell carcinoma and epithelioid trophoblastic tumor (ETT) express p63 and may share the same anatomic location in the cervix with a pushing infiltration pattern.[106]

Both ETT and PSTT are positive for HLA-G, CD10, and hCG, and negative for CK5/6, unlike squamous cell carcinoma, which shows an opposite pattern.[94] Lack of p16 expression is reported in ETT, in contradistinction to squamous cell carcinoma.[95]

Choriocarcinoma may not be distinguished from the extravillous trophoblastic tumors owing to the predominance of the extravillous trophoblast in both tumors.[94,95] However, a tumor with predominant hCG-positive cells favors choriocarcinoma over ETT and PSTT.[105] Moreover, Ki-67 tumor labeling index in choriocarcinoma exceeds 70% as opposed to 15% to 20% in both extravillous trophoblastic tumors.[105]

OVARY

The Differential Diagnosis of Serous Carcinoma of the Ovary

The 2-grade classification system of ovarian serous carcinomas as high and low grade is currently widely accepted because of its reproducibility, ease of application, and correlation with the underlying genetic alterations paralleling their biologic behavior.[107–111] Serous carcinomas are usually positive for CK7, carcinoma antigen 125 (CA 125), and WT1 and negative for CK20.[112–114] Serous borderline tumors are negative for p53, whereas strong expression of this marker is observed in up to 50% of serous carcinomas.[51,115] High-grade serous carcinomas usually show diffuse and strong p53 expression with a high Ki-67 proliferative index, in contrast to the low-grade serous tumors.[116] WT1 is also expressed in borderline serous ovarian and peritoneal tumors but not in metastatic endometrial serous carcinomas.[115]

Ovarian Serous Versus Clear Cell Carcinoma.—HNF-1β, WT1, ER, PR, p53, and ARID1A.

Not uncommonly, high-grade serous carcinoma (HGSC) displays foci of cytoplasmic clearing mimicking ovarian clear cell carcinoma. The distinction of HGSC from clear cell carcinoma is critical since the latter may respond poorly to conventional chemotherapy and clinical trials may be contemplated.[117] Immunohistochemistry has provided a valuable tool in the evaluation of epithelial tumors with abundant clear cells departing from the classical H&E appearance of clear cell or HGSC. HNF-1β is upregulated in clear cell carcinoma and its overexpression is detected by immunohistochemical staining.[48,49] p53 is mutated in most cases of HGSC and in approximately 25% of clear cell carcinomas. A prototype of clear cell carcinoma is HNF-1β (+), WT1 (–), ER (–), PR (–), and p53 (– or +).[51,118] On the other hand a prototype of HGSC is HNF-1β (–), WT1 (+), ER (+), PR (+), and p53 (+). Additional findings include deficient ARID1A expression in up to 57% of ovarian clear cell carcinoma but not in HGSC.[119,120]

Clear cell carcinoma with prominent papillary architecture may resemble serous tumors of low malignant potential. WT1 and ER are useful differentiating biomarkers since clear cell carcinoma is negative for both markers, in contradistinction to serous tumors of low malignant potential.[121]

Ovarian High-Grade Serous Versus Well-Differentiated or Moderately Differentiated Endometrioid Adenocarcinoma.—WT1, p53, and ARID1A.

Glandular and cribriform patterns are common in serous carcinoma, imparting a significant morphologic overlap with endometrioid adenocarcinoma. It has been argued that the distinction between these 2 entities may not be necessary since stage-stratified treatment and prognosis is not different. Many experts advocate for the classification of high-grade nonpapillary, nonmucinous, and nonclear cell carcinomas as high-grade serous unless the classical pattern of endometrioid adenocarcinoma of the endometrium is easily discernible.[122,123] However, the identification of WT1 expression as a feature of high-grade ovarian serous carcinoma, not found in endometrioid carcinoma, paved the way for a more reproducible differentiation.[124–127] Approximately 20% of HGSCs are negative for WT1. On the other hand, the more recent finding of deficient ARID1A expression in endometrioid but not in serous carcinoma provided an additional marker in the differentiation of these tumors.[129] The loss of ARID1A expression is limited to 30%

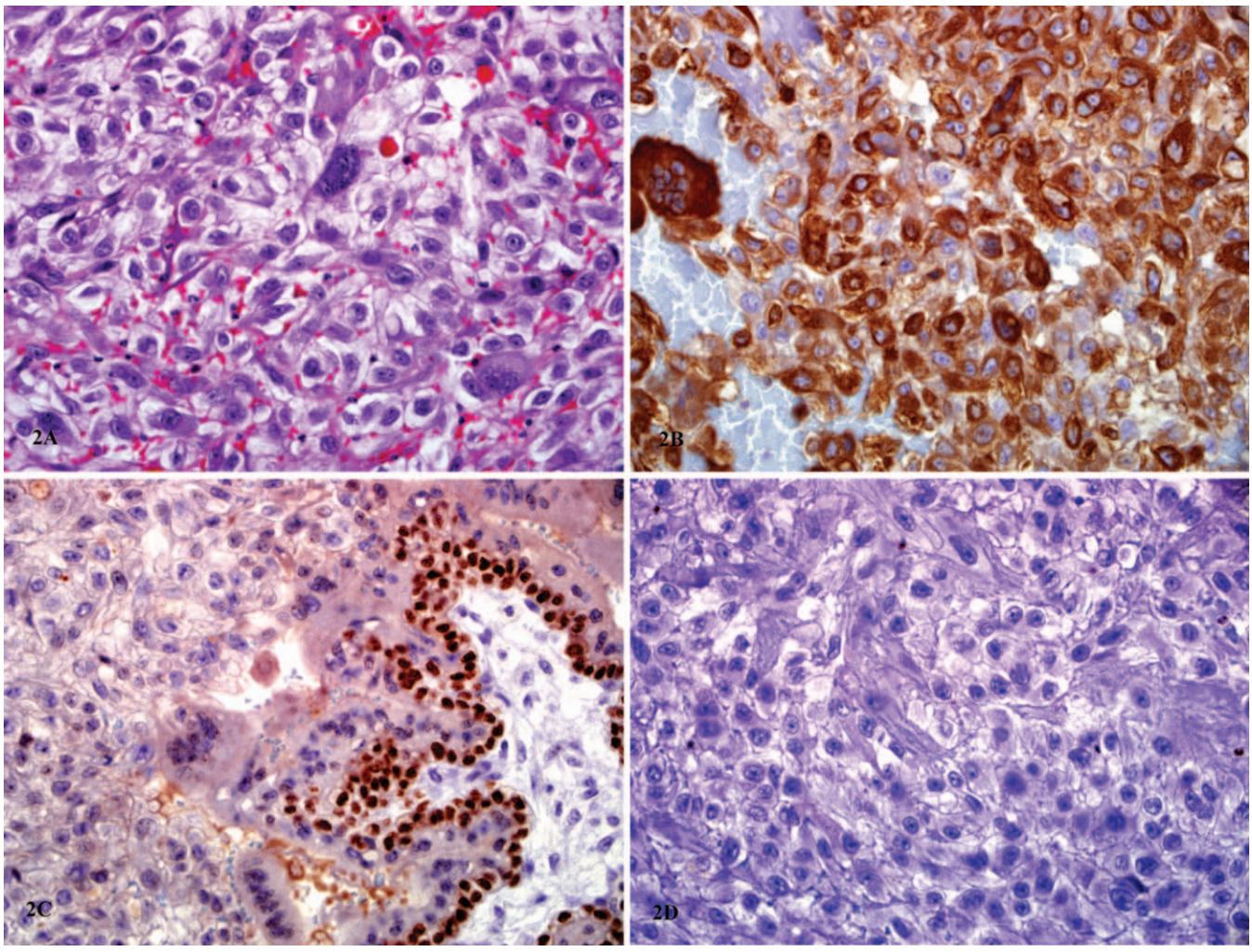

Figure 2. *A through D, Exaggerated placental site on hematoxylin-eosin–stained section (A) with intermediate trophoblastic cells positive for hPL (B), negative for p63 in the extravillous trophoblast (as compared to p63-positive villous trophoblast) (C), and negative for Ki-67 (in this field) (D) (hematoxylin-eosin, original magnification ×40 [A]; original magnification ×40 [B through D]). Abbreviation: hPL, human placental lactogen.*

of ovarian endometrioid adenocarcinomas.[120,124,128] A prototypic HGSC is WT1 (+), ARID1A (+), and p53 (+, strong, diffuse). On the other hand, WT1 (–), ARID1A (– or +), and p53 (– or weak and focal) favors endometrioid adenocarcinoma.[129]

Based on the literature, the distinction of a high-grade serous from a high-grade endometrioid or transitional carcinoma cannot be made reliably by immunohistochemistry alone, in as much as both are typically strongly p53, p16, and WT1 positive and PAX2 negative. Evidence is emerging that the serous and endometrioid "variants" of these high-grade carcinomas may signify disparate cellular origins, an issue that is germane to the concept of tubal versus ovarian carcinogenesis. This invites speculation regarding the role of salpingectomy as a cancer preventive and focuses attention to the tubal-peritoneal-ovarian region for further clues to cancer development.[130] The endometrioid variants, also called "SET" tumors (solid, pseudoendometrioid, transitional), may be more common in women with germline breast cancer *BRCA1* or *BRCA2* mutations, emphasizing the potential importance of their recognition by pathologists.[131]

Ovarian High-Grade Serous Carcinoma Versus Mesothelioma.—PAX8, claudin-4, epithelial-specific antigen/Ep-CAM (Ber-EP4, MOC-31), calretinin, and podoplanin (D2-40).

Several immunohistochemical markers were used in the differentiation of epithelioid mesothelioma and serous carcinoma, including the mesothelial markers calretinin, podoplanin, keratin 5/6, and thrombomodulin, and the carcinoma markers MOC-31, Ber-EP4, blood group 8 (BG-8 Lewis^{y}), tumor-associated glycoprotein 72 (TAG-72, B72.3), CD15 (leu-M1), and carbohydrate antigen 19-9 or cancer antigen 19-9 (CA19-9).[132–134] Recently, a limited panel consisting of PAX8 and claudin-4 was demonstrated equally efficient in differentiating the 2 neoplasms. Nearly all carcinomas were positive for PAX8 and membranous claudin-4, whereas none of the mesotheliomas exhibited reactivity with these markers.[135,136] Moreover, vascular endothelial growth factor receptor 2 (VEGFR2) was identified as a supplemental marker to identify malignant mesothelioma with promising results. Strong expression of VEGFR was demonstrated in malignant epithelioid mesothelioma but rarely in other epithelial tumors.[137]

Table 1. Summary of Useful Markers in Mucinous Tumors Involving the Ovary

Marker	OV	P	AP	LGI	UGI	L	UR	B
CK7	+	+	− or +	− or +[a]	+	+	− or +[a]	+
CK20	+ or −[a]	+ or −	+	+	− or +	− or +	+	−
CK17	−	+	ND	−	− (S)	ND	ND	ND
PAX8	− or +	−	−	−	−	−	ND	−
CDX-2	− or +[a]	+ or −	+	+	+	− or +	+	−
TTF-1 (N)	−	ND	ND	−	−	− or +	ND	−
DPC4	+	+ or −	ND	+	+	−	ND	ND
β-Catenin (N)	−	+ or −	+	+ or −	− or +	ND	+	ND
MUC2	− or +	+ or −	+ or −	+	+	+ or −	ND	− or +
MUC5AC	+	+	− or +	+	− or +	+	ND	− or +
MUC6	− or +	+ or −	−	− or +	− or +	ND	ND	− or +
Villin	+ or −	ND	ND	+	+	−	+	− or +
CEA-P	− or +	+	ND	+	+	+	ND	ND
HNF-1β	− or +	+	ND	ND	ND	ND	ND	ND
ER	− or +	−	−	−	−	−	ND	−

Abbreviations: AP, appendiceal; B, breast lobular carcinoma with signet rings; CEA-P, carcinoembryonic antigen, polyclonal; CK, cytokeratin; DPC4, deleted in pancreatic cancer 4; ER, estrogen receptor; HNF-1β, hepatocyte nuclear factor-1β; L, lung mucinous carcinoma; LGI, lower gastrointestinal; N, nuclear; ND, no data; OV, ovarian; P, pancreatic; S, stomach; TTF-1, thyroid transcription factor–1; UGI, upper gastrointestinal; UR, urachal carcinoma; +, usually greater than 75% of cases are positive; –, fewer than 5% of cases are positive; + or –, usually more than 50% of cases are positive; – or +, fewer than 50% of cases are positive.

[a] Focal or patchy.

Rare examples of hybrid tumors have been described, in which tumor cells stained positively for both calretinin and PAX8. While such tumors are unlikely to confound the pathologist in routine practice, it is important to be aware of such variants.

The Differential Diagnosis of Mucinous Ovarian Tumors

Metastatic mucinous tumors involving the ovary commonly display characteristic gross and microscopic features. However, individual patterns suggestive of the primary site, such as segmental epithelial necrosis and garland epithelium in metastatic colorectal carcinoma, are not specific.[138,139] Similarly, the mucinous epithelium of metastatic pancreatic, colorectal, endocervical, urachal, and lung adenocarcinomas may appear benign or as borderline ovarian primary tumor, suggesting an origin of the neoplasm in the ovary. The use of selected immunohistochemical markers in conjunction with the microscopic characteristics of individual mucinous tumors is valuable in accurately differentiating these tumors. Table 1 depicts the commonly used immunohistochemical markers used to differentiate the mucinous tumors involving the ovaries.

A panel including CK7, CK20, deleted in pancreatic cancer 4 (DPC4)/SMAD4, and PAX8 is efficient for an initial categorization of these tumors. Primary ovarian mucinous tumors are diffusely positive for CK7 and may show variable and patchy CK20 staining. This pattern is shared with metastatic tumors arising in the upper gastrointestinal (GI) tract and pancreatobiliary system. On the other hand, mucinous tumors arising in mature cystic teratoma may diffusely express CK20 and homeobox protein CDX-2 (CDX2), and completely lack CK7 positivity, mimicking metastatic tumors arising in the lower GI tract. Moreover, there is no loss of DPC4/SMAD4 in primary ovarian mucinous carcinoma, making it a helpful marker in the differentiation from pancreatobiliary and lower GI tumors. The loss of expression of this marker excludes ovarian primary mucinous carcinoma. However, caution should be taken, since the loss of expression of DPC4/SMAD4 has been reported in up to 11% of müllerian tumors with or without mucinous differentiation.[140] Interestingly, none of these tumors were ovarian mucinous carcinomas or borderline mucinous tumors. However, DPC4 expression is retained in approximately half the cases of pancreatobiliary and 90% of colorectal carcinomas.[58,141]

Mucinous Ovarian Versus Mucinous Pancreatobiliary Adenocarcinoma.—Typically, an ovarian primary tumor is SAMD4/DPC4 (+), β-catenin nuclear (–), CK17 (–), HNF-1β (– or +), CEA-P (– or +), PAX8 (– or +) mucin (MUC) 2 (– or +), and MUC6 (– or +). On the other hand, metastatic pancreatobiliary carcinoma is SAMD4/DPC4 (+ or –), β-catenin nuclear (+ or –), CK17 (+), HNF-1β (+), CEA-P (+), PAX8 (–) MUC2 (–) (unless colloid carcinoma of the pancreas, which is MUC2 [+]), and MUC6 (+ or –). SAMD4/DPC4-negative tumors are usually metastatic and additional differentiating markers may be used accordingly.[142–146]

Mucinous Ovarian Versus Appendiceal Adenocarcinoma.—Ovarian mucinous tumors are β-catenin (–), CDX2 (– or +, patchy), and MUC5AC (+), whereas metastatic appendiceal carcinoma is β-catenin (+), CDX-2 (+), and MUC5AC (– or +).[142–150]

Mucinous Ovarian Versus Mucinous Lower GI Tract Adenocarcinoma.—Most ovarian mucinous tumors are MUC2 (– or +), villin (– or +), CDX2 (– or +), and CK7 (+), whereas mucinous lower GI tract adenocarcinomas are MUC2 (+/–), villin (+), CDX-2 (+), and CK7 (– or + patchy).[143,150,151]

Mucinous Ovarian Versus Mucinous Upper GI Tract Adenocarcinoma.—Ovarian mucinous tumors display the following pattern: MUC2 (– or +), CDX-2 (– or +), MUC5AC (+), ER (– or +), PAX8 (– or +), and β-catenin-nuclear (–). On the other hand, metastatic mucinous upper GI carcinomas are MUC2 (+), CDX-2 (+/–), MUC5AC (– or +), ER (–), PAX8 (–), and β-catenin nuclear (– or +).[143,152,153]

Mucinous Ovarian Versus Mucinous Lung Adenocarcinoma.—Mucinous ovarian carcinoma demonstrates the following differentiating pattern: ER (– or +), PAX8 (– or +), CEA-P (+), MUC2 (– or +), thyroid transcription factor-1 (TTF-1) (–), and MUC5AC (+). On the other hand, metastatic mucinous lung adenocarcinoma is ER (–), PAX8 (–), CEA-P (+), MUC2 (+ or –), TTF-1 (– or +), and MUC5AC (– or +).[154,155]

Table 2. Ovarian Germ Cell Tumors Versus Ovarian Epithelial Tumors and Melanoma								
Marker	DYSG	YST	EMBR	CHORIO	SEROUS	CLCC	OST	MEL
SALL4	+	+	+	+ or −[a]	−	− or +	−	ND
SOX2	+ or −	−	+	−	+	ND	ND	− or +
NANOG	+	−	+	−	+	ND	−	ND
D2-40	+	− or f+	−	−	−	ND	−	−
PLAP	+	− or +	+	− or +	+ or −	− or f	−	−
GP-3	− or +	+	−	+	−	− or +	−	−
OCT4	+	−	+	−	−	− or +[b]	− or +	−
CD117	+	+ or −	− or +	−	− or +	−	− or +	− or +[c]
AFP	−	+	− or +	−	−	− or +	− or +	−
hCG	− or f+	−	− or +	+	−	−	−	ND
CD30	−	−	+	−	−	ND	ND	−
PAX8	−	−	−	?	+	+	− or +	−
HNF1-β	−	+	−	ND	−	+	ND	ND
EMA	−	− or f+	−	+ or −	+	+[d]	−	−
Inhibin-α	−	−	−	+	− or +	−	+	− or +
FOXL2	−	−	ND	ND	−	−	+	ND

Abbreviations: AFP, α-fetoprotein; CHORIO, choriocarcinoma; CLCC, clear cell carcinoma; DYSG, dysgerminoma; D2-40, podoplanin; EMA, epithelial membrane antigen; EMBR, embryonal carcinoma; f, focal; FOXL2, forkhead box protein L2; GP-3, glypican-3; hCG, human chorionic gonadotropin; HNF1-β, hepatocyte nuclear factor-1β; MEL, malignant melanoma; ND, no data; OCT4, octamer-binding transcription factor 4; OST, ovarian gonadal stromal tumors; SALL4, Sal-like protein 4; SEROUS, high-grade serous carcinoma; YST, yolk sac tumor; +, usually greater than 75% of cases are positive; −, fewer than 5% of cases are positive; + or −, usually more than 50% of cases are positive; − or +, fewer than 50% of cases are positive.

[a] Reactivity in mononuclear cells.
[b] Reactivity is reported in fewer than 10% of tumor cells.[169]
[c] Cytoplasmic reactivity, not membranous.
[d] Rare cases may show reactivity in fewer than 30% of tumor cells.

Mucinous Ovarian Versus Mucinous Urachal Adenocarcinoma.—A differentiating panel includes β-catenin (–), CDX-2 (– or +), CK 7 (+), and CK20 (+ or –, patchy) for mucinous ovarian carcinomas, whereas metastatic urachal carcinoma displays the following pattern: β-catenin (+), CDX-2 (+), CK7 (– or +, patchy), and CK20 (+).[156]

Mucinous Ovarian Versus Endocervical Adenocarcinoma.—Endocervical adenocarcinoma may metastasize to the ovary, with a few cases reported without evidence of invasion in the primary endocervical tumor. Characteristically, the metastatic tumor may mimic benign, borderline, and malignant mucinous adenocarcinomas of the ovary. Diffuse positive nuclear and cytoplasmic p16 staining and positive inclusions by in situ hybridization for HPV are useful differentiating features from ovarian mucinous primary tumors. However, a panel consisting of p16 and in situ hybridization for HPV are not useful for the detection of the rare metastatic non-HPV–related adenocarcinomas of cervix including minimal deviation adenocarcinoma.[157]

Mucinous Ovarian Versus Metastatic Lobular Carcinoma With Signet Ring Cell Differentiation.—Mucinous ovarian neoplasms are usually negative for gross cystic disease fluid protein 15 (GCDFP-15) and transcription factor GATA-binding protein 3 (GATA-3), whereas the reverse is true for metastatic carcinoma. Other useful markers are mouse double minute 2 homolog (MDM2) (+), MUC5AC (+), CA19-9 (+), PAX8 (– or +), CK20 (+ or –, patchy), and PR (–) for ovarian primary tumor, and MDM2 (– or +), MUC5AC (– or +), CA19-9 (– or +), PAX8 (–), CK20 (–), and PR (– or +) for breast carcinoma.[143,158,159]

The Differential Diagnosis of Ovarian Clear Cell Carcinoma

Clear cell carcinoma is commonly identified by its papillary pattern with minimal stratification of epithelial lining and hyalinized stroma. However, there are several reported architectural and cytologic variations that may pose differential diagnostic problems with other epithelial, gonadal stromal, and germ cell neoplasms. A summary of the commonly used markers for the differentiation from other neoplasms with overlapping histologic similarities is presented in Table 2.

Overall, clear cell carcinoma is positive for cytokeratin, CK7, PAX8, EMA, CD15, and high-molecular-weight cytokeratins. They are generally negative for CK20, WT1, GP-3, and show minimal staining with ER and PR.[58] Some tumors may also express diffuse p53.[160] The accumulating experience with the recently described HNF-1β shows promising results. This marker displays diffuse, intense nuclear staining in clear cell carcinoma and yolk sac tumor, but not in the other tumors with histologic similarities (Table 2).[50,51]

The differential diagnosis of ovarian clear cell carcinoma includes epithelial ovarian neoplasms (discussed above), dysgerminoma, yolk sac tumor, granulosa cell tumor, and steroid cell tumor (discussed below). It has been reported that both kidney injury molecule-1 (KIM-1) and von Hippel–Lindau tumor suppressor (pVHL) were frequently expressed in ovarian clear cell carcinomas and showed negativity in ovarian serous carcinomas.[161,162] Metastatic clear cell renal cell carcinoma (RCC) is usually negative for CK7, ER, and PR and more likely to express CD10 and RCC marker.[58] Both tumors are positive for HNF-1β, PAX8, pVHL, and KIM-1.[58,161] Additional differentiating markers include PAX2, expressed in most RCCs and rarely in clear cell carcinoma. When RCC is excluded, pVHL and KIM-1 are useful markers for the differentiation of ovarian and uterine clear cell carcinoma from other metastatic carcinomas such as lung, colon, and pancreas.[162]

Germ Cell Tumors of the Ovary

Immunohistochemistry is a valuable tool in reliably classifying and differentiating the malignant germ cell neoplasms from other gynecologic and nongynecologic tumors, impacting their clinical management.[163,164] The commonly used nuclear markers include Sal-like protein 4

(SALL4), octamer-binding transcription factor 4 (OCT4), NANOG protein, and SRY (sex-determining region Y)–box 2 (SOX2). SOX2 is also a cytoplasmic marker. The commonly used membranous markers include CD117, D2-40, CD30, PLAP, and GP-3. PLAP and GP-3 are also cytoplasmic markers.[58] When used in combination with the non–germ cell tumor markers, PLAP and SALL4 reliably separate germ cell tumors from epithelial and gonadal stromal neoplasms in most cases (Table 2).[58,165,166] SALL4 is specific for germ cell tumors with only focal weak staining reported in ovarian clear cell and metastatic gastrointestinal adenocarcinomas, and no reactivity with granulosa, theca, or ovarian stromal cell tumors.[167,168]

The Differential Diagnosis of Dysgerminoma.—CD117, PLAP, OCT4, NANOG, D2-40, hCG, and CK(AE1/AE3).

Dysgerminoma displays a characteristic membranous staining with CD117 not found in embryonal carcinoma or yolk sac tumor.[58] Both dysgerminoma and embryonal carcinoma usually display strong nuclear staining with OCT4 and NANOG.[166,168,169] When embryonal carcinoma is excluded, OCT4 is a sensitive and specific marker for dysgerminoma, since it shows positivity in nearly all dysgerminoma cases but not yolk sac tumor or choriocarcinoma, and only rare cases of clear cell carcinoma show reactivity, with fewer than 10% of the tumor cells.[169] D2-40 stains positively in dysgerminoma and its use is helpful in excluding embryonal carcinoma.[170] Dysgerminoma is negative for EMA and shows only focal cytoplasmic dot or rimlike staining with CK(AE1/AE3), in contradistinction to the epithelial tumors that usually demonstrate diffuse reactivity with these markers.[171] The tumor cells with syncytiotrophoblastic differentiation in dysgerminoma show positive cytoplasmic staining with the hCG marker, but overall the tumor cells are negative for this marker.[58] Dysgerminoma is negative for the melanoma marker S100, the yolk sac tumor marker AFP, the lymphoma markers, and the neuroendocrine markers.[58] Since dysgerminoma is a common component of mixed germ cell tumors of the ovary, it is essential to carefully choose the tissue block(s) with the atypical patterns to exclude other germ cell elements.

Dysgerminoma Versus Yolk Sac Tumor.—NANOG, OCT4, SOX2, CD117, D2-40, GP-3, and AFP.

Most of the growth patterns of yolk cell tumor are unlikely to cause confusion with dysgerminoma. However, foci of solid growth pattern and cytoplasmic clearing within a yolk sac tumor may resemble dysgerminoma. Yolk sac tumors do not stain for NANOG, CD117, D2-40, or OCT4.[170,172,173] However, they are positive for α-fetoprotein (AFP) and GP-3 and show diffuse strong cytoplasmic staining for cytokeratin, as compared to the negative staining with these markers in dysgerminoma.[174] SOX2 is another useful differentiating marker owing to its specificity when embryonal carcinoma and other epithelial ovarian tumors are excluded. None of the yolk sac tumors stained positively for this marker, whereas two-thirds of dysgerminomas stained positively for SOX2.[173,175] Dysgerminoma and yolk sac tumor are positive for SALL4, and approximately half of yolk sac tumors show some staining with PLAP and CD117, as compared to the diffuse and strong staining in most dysgerminoma cases.[172]

Dysgerminoma Versus Embryonal Carcinoma.—SALL4, OCT4, NANOG, CD117, D2-40, CD30, and CK(AE1/AE3).

Dysgerminomas with marked nuclear atypia and increased mitosis or with poor fixation may mimic embryonal carcinoma.[174] Diffuse keratin staining is against the diagnosis of dysgerminoma. Strong positive staining with CD30 and CK(AE1/AE3), and negative staining with CD117 and D2-40 in a SALL4- and OCT4-positive tumor, is diagnostic of embryonal carcinoma. OCT4, NANOG, and SALL4 stain positively in both embryonal carcinoma and dysgerminoma.[166,176–178]

Dysgerminoma Versus Lymphoma.—CD117, D2-40, OCT3/4, SALL4, CK(AE1, AE3), and CD45.

Usually there are light microscopic differentiating features such as vesicular nuclei in dysgerminoma as opposed to the coarsely granular chromatin in lymphoma. However, the monotonous proliferation of tumor cells in lymphoma may resemble dysgerminoma. The dysgerminoma markers show negativity in lymphoma, while the B-cell markers show positivity in lymphoma and negativity in dysgerminoma. Most of the reported cases of lymphoma in the ovary are of the B-cell type.[174,179]

Dysgerminoma Versus Clear Cell Carcinoma.—OCT4, SALL4, CD117, CK7, EMA, HNF-1β, and PAX8.

Overlapping H&E similarities include solid growth pattern, lymphocytic infiltrate, clear cytoplasm, and prominent nucleoli. Staining with CK7 and EMA may be focally positive in dysgerminoma, but clear cell carcinoma is diffusely and strongly positive for these markers. Similarly, clear cell carcinoma may focally express the commonly used dysgerminoma markers such as SALL4, OCT3/4, PLAP, and D2-40. However, PAX8- and HNF-1β–positive tumor cells reliably exclude dysgerminoma.[169,170,172,180–183]

Dysgerminoma Versus Melanoma.—MART-1, S100, SOX10, HMB-45, SALL4, OCT3/4, and D2-40.

The melanoma tumor cell markers MART-1, S100, SOX10, and HMB-45 show positivity in melanoma, and negativity in dysgerminoma. Similarly, the dysgerminoma tumor cell markers, SALL4, OCT3/4, and D2-40 show negativity in melanoma. Cytoplasmic CD117 reactivity may be encountered in melanoma and should not be confused with the membranous reactivity commonly found in dysgerminoma.[174]

The Differential Diagnosis of Yolk Sac Tumor

Yolk sac tumors are characterized by multiple growth patterns frequently encountered in the same tumor. Moreover, yolk sac tumor occurs as a component of mixed germ cell tumor of the ovary or mixed with endometrioid adenocarcinoma, requiring evaluation with immunohistochemical panels individually tailored to identify each component. The endodermal and microcystic patterns are the most common and do not cause diagnostic challenges. They are usually confirmed by diffuse reactivity with SALL4, AFP, and GP-3 and negative staining with CK7 and EMA. Traditionally, AFP is used owing to its high specificity in excluding dysgerminoma and embryonal carcinoma. AFP is limited, however, by its low sensitivity and absent, weak, or focal staining of the solid areas of the tumor.[167,184,185] GP-3 positive staining in yolk sac tumor and negative staining in embryonal carcinoma is used as an additional marker in differentiating these 2 tumors.[186–188] It should be noted that GP-3 stains positively in choriocarcinoma and metastatic hepatocellular carcinoma and may be expressed in immature teratoma, metastatic acinar carcinoma of the pancreas, and squamous cell carcinoma of the cervix.[174]

Yolk Sac Tumor Versus Ovarian Clear Cell Carcinoma.—SALL4, AFP, GP-3, CK7, and EMA.

The main differential diagnosis of yolk sac tumor is clear cell carcinoma with its tubulocystic, papillary, and solid growth, and stromal hyaline material resembling features encountered in yolk cell tumor. The use of the common immunohistochemical panels in differentiating these 2 tumors may be challenging owing to the recently reported overlapping immunohistochemical features. Approximately one-third of clear cell carcinomas stain positively with the yolk sac tumor markers AFP and GP-3.[184,185,189] In addition, the broad germ cell tumor marker SALL4 may also stain rare cases (approximately 7%) of clear cell carcinomas. EMA and CK7 are reported to stain fewer than 30% of clear cell carcinoma tumor cells. The use of the clear cell marker HNF-1β is limited owing to its reactivity with both tumors.

Yolk Sac Tumor Versus Embryonal Carcinoma.—OCT3/4, NANOG, SOX2, GP-3, and CD30.

CD30, OCT3/4, NANOG, and SOX2 stain positively in most cases of embryonal carcinoma and negatively in yolk sac tumors, making them robust differentiating markers between the 2 neoplasms. GP-3 stains positively in yolk sac tumor and negatively in embryonal carcinoma.

Sex Cord–Stromal Tumors

Sex cord–stromal tumors constitute 5% to 12% of all ovarian neoplasms. Granulosa cell tumor is the most common malignant sex cord tumor, accounting for 1% to 2% of all malignant ovarian tumors. Most of the diagnostic challenges encountered in the differentiation of tumors in this category are resolved with the use of immunohistochemistry. The commonly used markers inhibin, calretinin, forkhead box protein L2 (FOXL2), steroidogenic factor 1 (SF-1), WT1, and EMA are supplemented by additional immunohistochemistry depending on the differential diagnosis. Other positively staining markers, such as CD99, CD56, vimentin, and estrogen and progesterone receptors, are also used. For instance, CD56 shows positivity in sex cord–stromal tumors, but its use is to exclude endometrioid adenocarcinoma, which is predominantly CD56 negative. Diagnostic mimics such as serous carcinoma, carcinoid tumor, endometrial stromal sarcoma, and extraovarian neuroendocrine carcinoma also express CD56.[190–192]

The Differential Diagnosis of Granulosa Cell Tumor.—EMA, cytokeratin, inhibin, calretinin, FOXL2, and SF-1.

Granulosa cell tumors are usually positive for inhibin, calretinin, FOXL2, and SF-1. They are negative for the epithelial markers CK7 and EMA, and the germ cell tumor marker SALL4.[193–198] The absence of staining with these 3 markers has a robust diagnostic value in excluding germ cell tumors and metastatic or primary carcinomas that demonstrate overlapping histologic features.[193,196] Inhibin-α (cytoplasmic) is a sensitive and specific marker, and calretinin (nuclear and cytoplasmic) is a less specific but more sensitive marker.[196,197,199–203] Both adult and juvenile granulosa cell tumors express FOXL2 nuclear staining, with a sensitivity ranging between 75%[203] and 100%.[204,205] FOXL2 is also reported in female adnexal tumor of wolffian origin (FATWO) and 50% of Sertoli-Leydig cell tumors.[204] SF-1 is another promising nuclear stain with reported positivity in all adult and juvenile granulosa cell tumors[194,202] and most fibromas, thecomas, Sertoli cell tumors, and steroid cell tumors[202,206] and reported negativity in yolk sac tumors,[194] carcinoid tumors, and primary and metastatic ovarian carcinomas.[206,207] WT1 stains positively in most cases of granulosa cell tumor and sex cord–stromal tumors.[196,202,208] CD56 stains positively in granulosa cell tumor but its use is limited owing to its expression in a large variety of mimics including small cell carcinoma, large cell neuroendocrine carcinoma, fibrothecoma, endometrioid and serous carcinomas, and endometrial stromal sarcomas. CD56 is useful in distinguishing between granulosa cell tumor and normal ovarian follicles.[192]

Granulosa Cell Tumor Versus Lymphoma.—The small darkly stained cells of lymphoma infiltrating around ovarian follicles may resemble granulosa cell tumor, uncommonly causing a diagnostic challenge. Most lymphomas involving the ovary are B-cell lymphomas identified by staining with CD20 and CD45 (leukocyte common antigen) markers, and lack of staining with the granulosa cell markers inhibin and calretinin. Granulosa cell tumor is negative for the lymphoma stains CD20 and CD45.[209]

Granulosa Cell Tumor Versus Carcinoid.—Inhibin, calretinin, chromogranin, SF-1, and WT1.

Carcinoid tumors are sometimes associated with teratoma and may display insular and trabecular patterns resembling granulosa cell tumor. Although the differentiation of both tumors may be reliably achieved from the characteristic nuclear and cytoplasmic H&E features, immunohistochemistry is helpful in the cases with diagnostic difficulties. Carcinoid tumors are positive for the neuroendocrine marker chromogranin and negative for inhibin, calretinin, SF-1, and WT1. The reverse pattern is found in granulosa cell tumor. It should be noted that WT1 stains positively in 70% of granulosa cell tumors.[192,196,202–210]

Granulosa Cell Tumor Versus Poorly Differentiated Adenocarcinoma.—Inhibin, calretinin, FOXL2, EMA, and CK7.

Primary and/or metastatic carcinomas of the ovary may display overlapping features with granulosa cell tumor. EMA is used initially to exclude adenocarcinoma since granulosa cell tumor in negative for this marker.[180] Adenocarcinomas are usually diffusely and strongly positive for EMA and CK7, and negative for inhibin, calretinin, and FOXL2. Granulosa cell tumor is usually negative for EMA and CK7, and positive for inhibin, calretinin, and FOXL2.[172] Juvenile granulosa cell tumor may focally express EMA. Other markers, such as nuclear E-cadherin, demonstrated positivity in 90% of granulosa cell tumors and in none of the cases with carcinoid or adenocarcinoma.[99]

Granulosa Cell Tumor Versus Low-Grade Endometrial Stromal Sarcoma.—CD10, inhibin, CD99, and calretinin.

Low-grade endometrial stromal tumors usually demonstrate a characteristic vascular network of small arterioles and tonguelike growth pattern. Inhibin, calretinin, and CD99 are expressed in adult granulosa cell tumor but not in the endometrial stromal sarcoma. On the other hand, CD10 staining is strongly positive in endometrial stromal sarcoma but usually weak or negative in granulosa cell tumor.[67,202,211]

Granulosa Cell Tumor Versus Small Cell Carcinoma of Hypercalcemic Type.—EMA and inhibin.

Small cell carcinoma of hypercalcemic type (SCCHT) is a very aggressive rare form of ovarian carcinoma morphologically similar to small cell carcinomas from other sites. Its immunophenotypic characterization is limited owing to the rarity of the tumor.[212] Commonly, SCCHT is positive for p53, WT1, CD10, EMA, CK(AE1/AE3), and calretinin, and negative for CK5/6, inhibin, S100, CD99, TTF-1, and chromogranin.[213,214] Inhibin-positive and EMA-negative

staining of a densely cellular tumor with scant cytoplasm distinguishes granulosa cell tumor from SCCHT. WT1 and calretinin cannot be used as differentiating markers owing to their positivity in both tumors.

Juvenile Granulosa Cell Tumor and Steroid Cell Tumors Versus Clear Cell Carcinoma.—FOXL2, inhibin, calretinin, EMA, and keratin.

Both juvenile granulosa cell tumor and steroid cell tumor usually express inhibin, calretinin, and FOXL2. Juvenile granulosa cell tumor may express EMA (focally) and keratin. Clear cell carcinoma is diffusely positive for EMA and keratin, and negative for the other markers. Steroid cell tumors are positive for keratin, inhibin, calretinin, FOXL2, SF-1, and MART-1 and are negative for WT1.[58,172] It should be noted that PAX8, which usually stains positively in clear cell carcinoma, is not a differentiating marker since it may also be expressed in the gonadal stromal tumors.[58]

The Differential Diagnosis of Sertoli Cell and Sertoli-Leydig Cell Tumors

The sertoliform variant of endometrioid adenocarcinoma may show overlapping features with Sertoli cell and Sertoli-Leydig cell tumors. Sertoli–stromal cell tumors are positive for calretinin, inhibin, SF-1, and WT1.[190,202,203,215,216] The use of FOXL2 is limited, since it is not expressed in approximately half of the cases of Sertoli-stromal tumors, particularly the retiform variant.[204] CK7, CAM 5.2, CK8/18, CK(AE1/AE3), and cytokeratin may show variable degrees of reactivity in up to half of the cases of Sertoli stromal tumors.[190,195,215] Their use is limited in excluding adenocarcinoma with certainty. However, EMA is negative in all cases of Sertoli-stromal tumors.[190,215] It should be noted that epithelial markers, neuroendocrine markers, muscle markers, and hepatocyte markers may show reactivity with heterologous elements found in Sertoli-Leydig cell tumors.[217,218]

MART-1 shows positivity in steroid cell tumors and in the Leydig cells of Sertoli-Leydig cell tumor, but not in the Sertoli cell component.[202] A negative MART-1 and positive FOXL2 tumor is unlikely to be a steroid cell tumor.

In the Sertoli cell tumors expressing neuroendocrine markers and showing morphologic overlap with carcinoid tumor, ER and PR positivity favors Sertoli-stromal cell tumor.[117,215,219]

The Differential Diagnosis of Fallopian Tube Lesions

Primary epithelial tumors of the fallopian tube and paratubal region demonstrate similar morphologic and immunohistochemical features as those of the ovary and peritoneum. Differentiating markers characteristic of the anatomic location of these tumors do not exist.

Immunohistochemistry helps in the differential diagnosis of adenomatoid tumors with an infiltrative pattern or cytoplasmic vacuoles resembling signet cells and raising the possibility of adenocarcinoma. Adenomatoid tumor is positive for the mesothelial markers CK5/6, calretinin, and WT1,[220,221] and negative for the epithelial markers BerEP4 and CEA.[222]

Similarly, immunohistochemistry helps in differentiating FATWO, a rare tumor arising in the broad ligament, mesosalpinx, and ovary,[58] from the low-grade endometrioid adenocarcinoma variant of the fallopian tube. Unlike endometrioid adenocarcinoma, FATWO is positive for inhibin and calretinin.[223,224] It is also positive for cytoplasmic CD10 and negative for EMA.[224–226]

The immunophenotype of serous carcinoma of the fallopian tube is similar to that of the ovary and peritoneum. Most tumors are positive for WT1, p53, CK7, and CA125. Serous tubal in situ carcinoma is differentiated from reactive epithelium by its strong and diffuse reactivity with p53 and increased Ki-67 labeling index, unlike the reactive epithelium.[227–231] The p53 signature lesion, a serous cancer precursor in the fimbria, is identified by its reactivity with p53 and lack of Ki-67 proliferative activity and cytologic atypia of intraepithelial carcinoma.[229,230,232,233]

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