Ancillary studies - Immunohistochemical markers (Recommended)

Reason/Evidentiary Support

Immunohistochemistry has many important applications in the field of ovarian neoplasia. There are a number of scenarios where immunohistochemical markers may assist in establishing a diagnosis of a primary ovarian epithelial malignancy or in tumour subtyping. It is beyond the scope of this dataset to present a detailed analysis of every scenario but major uses of immunohistochemistry are discussed. In general, panels of markers are better than reliance on individual markers and it should be remembered that no marker is totally specific or sensitive for any tumour type. Unexpected positive and negative staining reactions may occur and the results of immunohistochemical studies should always be interpreted in conjunction with the clinical, gross and microscopic features.

Markers of Use in Typing Ovarian Carcinomas

While most primary ovarian carcinomas are straightforward to type, on occasion it is difficult to distinguish between a high-grade serous carcinoma and a high-grade endometrioid carcinoma, or between a clear cell carcinoma and clear cell areas within a high-grade serous carcinoma or an endometrioid carcinoma. A panel of markers may help which should be tailored depending on the differential diagnosis. Approximately 80-90% of serous carcinomas (low-grade and high-grade) are positive with WT1, usually with diffuse immunoreactivity. In contrast, endometrioid and clear cell carcinomas are usually negative, although a small percentage of endometrioid carcinomas are positive. High-grade serous carcinomas exhibit aberrant “mutation-type” staining with p53 (see below) while low-grade serous carcinomas, clear cell carcinomas and most endometrioid carcinomas exhibit “wild-type” staining (focal and heterogenous); some high-grade endometrioid carcinomas exhibit aberrant p53 staining. p16 is diffusely positive (“block-type” staining) in most high-grade serous carcinomas while most low-grade serous carcinomas, clear cell carcinomas and endometrioid carcinomas exhibit patchy immunoreactivity. Clear cell carcinomas usually exhibit diffuse strong nuclear staining with hepatocyte nuclear factor 1-beta while other primary ovarian epithelial neoplasms are usually negative or focally positive. Napsin A is also a useful marker of clear cell carcinomas. ER is positive in most high-grade and low-grade serous carcinomas and endometrioid carcinomas while clear cell carcinomas are usually negative. Some of these markers have helped establish that most neoplasms which were previously classified as mixed high-grade serous and endometrioid and mixed high-grade serous and clear cell represent high-grade serous carcinomas with pseudoendometrioid areas and areas of cytoplasmic clearing.

On occasion, especially in a biopsy specimen, it may be problematic to differentiate between a low-grade and a high-grade serous small carcinoma. The most useful marker in this scenario is p53 (“mutation-type” staining in high-grade serous carcinoma; “wild-type” staining in low-grade serous carcinoma).

Distinction Between Primary and Secondary Ovarian Adenocarcinoma

The distinction between a primary ovarian adenocarcinoma and metastatic adenocarcinoma from various sites may be problematic. Metastatic colorectal adenocarcinomas may mimic an endometrioid carcinoma or a mucinous neoplasm of intestinal type, either borderline or malignant. In the distinction between an ovarian endometrioid adenocarcinoma and a metastatic colorectal adenocarcinoma with a pseudoendometrioid pattern, a panel of markers may assist. While there may be immunophenotypic overlap of individual markers, primary ovarian endometrioid carcinomas are usually positive with CK7, ER, CA125 and PAX8 and negative with CK20, CEA and CDX2 while the converse immunophenotype is the rule in metastatic colorectal adenocarcinomas. In distinguishing between a primary ovarian mucinous tumour and a metastatic colorectal adenocarcinoma, immunohistochemistry is less helpful. This is because many primary ovarian mucinous neoplasms exhibit CK20 positivity, usually focal but sometimes widespread. They are also commonly positive, sometimes diffusely so, with CEA, CDX2 and CA19.9. The expression of these enteric markers is a reflection of intestinal differentiation in primary ovarian mucinous neoplasms. However, the pattern of coordinate expression of CK7/CK20 may assist in distinguishing between a primary ovarian mucinous tumour and a metastatic colorectal adenocarcinoma with a mucinous appearance. Although either marker can be positive in both tumours, primary ovarian mucinous neoplasms are often diffusely positive with CK7 while CK20 is variable; conversely metastatic colonic adenocarcinoma is usually diffusely positive with CK20 and focally positive with CK7 when this marker is expressed. Thus, CK7 immunopositivity is typically of greater extent than CK20 immunopositivity in primary ovarian mucinous tumours and CK20 staining is more extensive than CK7 in metastatic colonic adenocarcinoma.

Metastatic pancreatic or biliary adenocarcinoma may mimic a primary ovarian mucinous neoplasm of intestinal type, either borderline or malignant and immunohistochemistry is of limited value. Most commonly, these tumour types are diffusely positive with CK7 while CK20 is variable, being negative, focally or diffusely positive. CEA, CA19.9 and CDX2 may be positive. An absence of staining with DPC4 (DPC = deleted in pancreatic cancer) may be a useful pointer towards a pancreatic adenocarcinoma since this nuclear transcription factor is inactivated in about 50% of pancreatic
Metastatic breast carcinomas of ductal type may mimic a high grade serous carcinoma or an endometrioid carcinoma. It is a not uncommon scenario that a patient with a history of breast carcinoma is found to have a pelvic mass or a disseminated peritoneal malignancy. In most cases, this will represent a new tubo-ovarian high grade serous carcinoma; such patients may or may not have underlying BRCA1/2 mutation. In distinguishing between a metastatic breast carcinoma and a tubo-ovarian high grade serous carcinoma, markers which may be useful are PAX8, CA125 and WT1 (usually positive in high grade serous carcinomas and negative in breast carcinomas, although occasionally the latter are CA125 or WT1 positive) and GCDFP15, mammoglobin and GATA3 (usually negative in high grade serous carcinomas and positive in breast carcinomas).18-20 A similar panel of markers is useful in the distinction between an endometrioid carcinoma and a metastatic breast carcinoma, although WT1 is negative in endometrioid carcinomas and a proportion of these may be mammoglobin positive.21

Rarely, a metastatic cervical adenocarcinoma of usual type (HPV related) in the ovary may mimic a primary ovarian mucinous or endometrioid neoplasm.22 Diffuse p16 immunoreactivity in such cases may be useful in suggesting a metastatic cervical adenocarcinoma.

**Distinction Between Ovarian Endometrioid Carcinoma and Sex Cord-Stromal Tumour**

Some primary ovarian carcinomas, especially of endometrioid type, may closely mimic an ovarian sex cord-stromal tumour, either a granulosa cell tumour or a Sertoli cell tumour. Conversely, some Sertoli-Leydig cell tumours have a pseudoendometrioid appearance and can mimic an endometrioid neoplasm.23 Markers which are useful to distinguish between an endometrioid neoplasm and a sex cord-stromal tumour include inhibin, calretinin and steroidogenic factor-1 (SF-1; positive in sex cord-stromal tumours) and epithelial membrane antigen and CK7 (positive in epithelial neoplasms).1-3,23-25

**Diagnosis of Serous Tubal Intraepithelial Carcinoma (STIC)**

Biomarkers are not necessary if the features are unequivocally those of STIC but if there is diagnostic uncertainty, both p53 and MIB1 staining should be performed.26 The cells must exhibit aberrant p53 staining (see definition below). The MIB1 proliferative index is increased, typically in the region of 40% to nearly 100% with most cases showing focal areas exceeding 70%. However, some cases of STIC exhibit a lower MIB1 proliferation index and it has been suggested that at least 10% of the nuclei should be positive for a diagnosis of STIC in cases where immunohistochemistry is undertaken (morphological features and aberrant p53 staining are also needed).26

**Two Patterns of Aberrant p53 Staining**

There is significant variability amongst pathologists in the interpretation of p53 staining. Pathologists are often unaware that many normal tissues and tumours unassociated with TP53 abnormalities express p53 protein. Such staining is usually focal and weak and somewhat variable from area to area (referred to as “wild-type” p53 staining), although on occasions many of the nuclei are positive, albeit with variable intensity. The degree of positive staining can be affected by varying the antibody concentration used.27 This pattern of staining is found in many normal tissues (non-neoplastic epithelia, stromal and lymphoid cells which can act as an internal positive control) and neoplasms not related to TP53 mutation. Rather than this “wild-type” staining, it is the diffuse intense pattern of nuclear immunoreactivity which should be interpreted as “positive” and which is correlated with TP53 missense mutations. Typically in excess of 75% and sometimes almost all of the nuclei are intensely positive. It should also be appreciated that totally absent p53 staining (as stated, there is usually an inbuilt positive control with “wild-type” staining of non-neoplastic tissues) is also indicative of aberrant p53 immunoreactivity.28,29 This pattern of immunoreactivity is in keeping with a null (including non-sense, frame shift or splice site) TP53 mutation resulting in complete absence of detectable protein. To summarise, it is not simply negative or positive staining but rather patterns of p53 immunoreactivity which are of importance. Diffuse intense nuclear immunoreactivity and totally absent staining (“all or nothing”) are aberrant patterns (“mutation-type” staining) and in keeping with an underlying TP53 mutation while “wild-type” staining is not.

**Distinction Between Ovarian and Uterine Carcinoma**

A not uncommon scenario is simultaneous involvement of the uterine corpus and one or both ovaries by an adenocarcinoma. Most commonly, the adenocarcinomas are endometrioid in type but sometimes they are serous.30,31 With endometrioid adenocarcinomas involving the uterus and one or both ovaries, immunohistochemistry is of little or no value in ascertaining the relationship between the tumours as the immunophenotype of a primary ovarian and uterine endometrioid adenocarcinoma is essentially identical.

With a serous carcinoma involving the uterus and one or both ovaries, WT1 staining may be of some value in distinguishing between a uterine serous carcinoma with metastasis to the ovary, metastasis from the ovary/tube to
the endometrium (“drop metastasis”) and independent synchronous neoplasms, the latter being unlikely.\textsuperscript{3,4,9,32} Most tubo-ovarian serous carcinomas exhibit diffuse nuclear positivity with WT1 while most uterine serous carcinomas are negative. However, there is some overlap in that a proportion of uterine serous carcinomas are WT1 positive (the percentage has varied between studies) and a small percentage of tubo-ovarian high-grade serous carcinomas are WT1 negative.\textsuperscript{4-9} It can be summarized that, although there is some overlap, diffuse WT1 positivity in a serous neoplasm favours a tubo-ovarian origin. In contrast, negative staining is a pointer towards a primary uterine neoplasm.

**Distinction Between Serous and Mesothelial Proliferation**

On occasion it may be difficult to distinguish between a serous proliferation (borderline or malignant) and a mesothelial proliferation (reactive or neoplastic). Florid reactive mesothelial proliferation may occur in association with endometriosis and mimic an endometrioid carcinoma.\textsuperscript{31} A suggested panel of markers in this situation would include BerEP4, ER and PAX8 (usually positive in serous proliferations and endometrioid carcinomas) and calretinin and CK5/6 (usually positive in mesothelial proliferations). WT1 is usually positive in both serous and mesothelial proliferations.

**References:**


