Ancillary studies (Non-core)

Morphology remains the mainstay in ovarian carcinoma diagnosis. Diagnostic ancillary testing is currently based primarily on immunohistochemistry (IHC). Diagnostic immunohistochemical markers may assist in establishing a diagnosis of a primary ovarian carcinoma or aid in histotyping. It is beyond the scope of this dataset to present a detailed analysis (sensitivity, specificity, cut-off interpretation) but the most commonly used first-line immunohistochemical panels 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. Therefore, the results of immunohistochemical studies should always be interpreted in conjunction with the clinical, gross and microscopic features.[1,2]

The choice of ancillary tests for the distinction of a primary ovarian carcinoma from a metastatic malignancy (Table 6) depends on its morphological context and can be problematic particularly on small or cytological specimens.

Table 6: Ancillary tests to distinguish primary ovarian carcinoma from a metastasis.

Comparator #1	Comparator #2	Expressed/abnormal in comparator #1	Expressed/abnormal in comparator #2	References
Primary ovarian carcinoma	Benign mesothelial proliferation	Claudin 4, B72.3, Ber-EP4	Desmin	[3-9]
Primary ovarian carcinoma	Mesothelioma	Claudin 4, B72.3, Ber-EP4, Estrogen receptor (ER)[a]	Calretinin, BAP1	[4,10-12]
Ovarian endometrioid carcinoma	Lower gastrointestinal tract (colorectal and appendiceal)	CK7, PAX8[b], ER[a]	SATB2, CK20	[13]
Ovarian endometrioid carcinoma	Sex cord stromal tumour	EMA, CK7	Inhibin, Calretinin, SF1	[14]
Ovarian mucinous carcinoma	Lower gastrointestinal tract (colorectal and appendiceal)	CK7	SATB2, CK20	[13,15,16]
Ovarian mucinous carcinoma	Endocervical adenocarcinoma (human papilloma virus (HPV)-associated)		P16, HPV-PCR	[17,18]
Tubo-ovarian high grade serous carcinoma	Metastatic breast carcinoma	PAX8, WT1	GATA3	[19]
Tubo-ovarian high grade serous carcinoma	Endometrial serous carcinoma	WT1, p53	p53	[20,21]

[a] ER is absent in ovarian clear cell and mucinous carcinomas as well about 20% of endometrioid and high grade serous carcinomas.

[b] PAX8 is absent in 15% of ovarian endometrioid carcinomas.

In the distinction between a primary ovarian carcinoma and a benign mesothelial proliferation, a first line panel of claudin 4, B72.3 and desmin is slightly better than the traditional panel of MOC31 (or BerEP4), estrogen receptor (ER) and calretinin.[6] Claudin 4 can be superior to MOC31, BerEP4, or PAX8.[8] Expression of PAX8 in reactive mesothelial proliferations has been noted.[9,22-24] However, claudin 4 or BP72.3 may not be widely available. Desmin is an excellent second marker for differentiating primary ovarian carcinoma from reactive mesothelial proliferation,[3] which outperforms calretinin (positive, at least focally, in some serous carcinomas). WT1 is consistently positive in both serous and mesothelial proliferations but the combination of WT1 expression with abnormal p53 (p53abn) is characteristic of tubo-ovarian high grade serous carcinoma (HGSC), although some mesotheliomas can harbor a *TP53* mutation. If mesothelioma is in the differential diagnosis, BAP1 should be added. Bernardi et al (2020) showed that claudin 4 expression was completely sensitive and specific for metastatic carcinoma versus mesothelioma.[4]

Metastatic colorectal adenocarcinomas may mimic an endometrioid carcinoma or a mucinous neoplasm, either borderline or malignant. In the distinction between an ovarian endometrioid carcinoma and a metastatic colorectal adenocarcinoma, the following panel of markers may assist: CK7, CK20, PAX8, ER and SATB2.

Endometrioid carcinoma 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.[25] Markers which are useful to distinguish between them include inhibin, calretinin and SF-1 versus EMA, PAX8, BerEP4 and CK7.[25-30]

Simultaneous involvement of the endometrium and ovaries by an endometrioid carcinoma is not uncommon.[31,32] IHC and molecular testing are of little value in ascertaining the relationship between the tumours as synchronous dual primaries versus metastasis since it has been shown that in almost all such the tumours are clonally related.[33-35] However, an indolent behaviour can be anticipated if both tumours are low grade; the endometrial tumour shows less than 50% myometrial invasion; substantial lymphovascular invasion is absent; and only the endometrium and one ovary and no other site is involved.[36] These tumours can be designated as synchronous.

In the distinction between an ovarian mucinous carcinoma and a metastatic colorectal adenocarcinoma or appendiceal neoplasm, as well as the macroscopic and microscopic findings, with large size and unilaterality being more in keeping with primary ovarian mucinous carcinoma, a panel of CK7, CK20, CDX2 and SATB2 may assist.[13,15,16] The use of IHC to distinguish primary ovarian mucinous carcinoma from metastatic adenocarcinoma of upper gastrointestinal origin (pancreatic, hepatobiliary, gastric) is limited. An absence of staining with SMAD4 (DPC4) may suggest a pancreatic adenocarcinoma since staining of this nuclear transcription factor is lost in about 50% of pancreatic adenocarcinomas.[37] Conversely, DPC4 is expressed in virtually all primary ovarian mucinous neoplasms. Rarely, a metastatic human papillomavirus (HPV)-associated endocervical adenocarcinoma may mimic a primary ovarian mucinous or endometrioid neoplasm.[38] Diffuse p16 immunoreactivity in such cases may be useful in suggesting a metastatic cervical adenocarcinoma, but performing HPV testing is more specific.[17,18,39]

Metastatic triple negative ductal breast carcinomas may mimic a tubo-ovarian HGSC. In a patient with a history of breast carcinoma and germline *BRCA1*/2 mutation who is found to have a pelvic mass or a disseminated peritoneal malignancy, most often this will represent a new tubo-ovarian HGSC. A panel of PAX8, WT1 and GATA3 is helpful.[19,40-42] However, in the setting of triple negative breast carcinomas, GATA3 expression is often limited or completely negative.

With a serous carcinoma involving the endometrium and one or both tubes/ovaries, correct site assignment becomes important because only tubo-ovarian HGSC are eligible for poly ADP ribose polymerase inhibitors (PARPi) at this time, but this could change. WT1 and p53 staining may be of some value in distinguishing between an endometrial serous carcinoma with metastasis to the tube/ovary, a 'drop metastasis' in the endometrium from a tubo-ovarian HGSC or independent synchronous neoplasms. Differences in staining between the sites, especially with both markers, suggest the latter. Absence of WT1 staining is a relatively specific indicator of endometrial primary site because almost all tubo-ovarian HGSC show diffuse WT1 staining (approximately 2% show partial or complete absence).[43,44] On the contrary, while WT1 expression is consistent with a tubo-ovarian HGSC, approximately one third of endometrial serous carcinoma exhibit WT1 staining (often focal).[20,21,43-49]

While most primary ovarian carcinomas are straightforward to histotype on well sampled specimens, on occasion it is difficult to distinguish between a HGSC and a high grade endometrioid carcinoma (Table 7). The recommended panel is a combination of WT1 and p53.[50] Diffuse strong WT1 expression in combination with abnormal mutation-type p53 staining is highly sensitive and specific for HGSC. If it is not possible to distinguish between high grade serous and endometrioid carcinoma, these cases could be submitted for cancer susceptibility screening and predictive testing for both histotypes (*BRCA1/2* mutation testing and mismatch repair (MMR) protein expression). HGSC with clear cell areas and clear cell carcinoma can be distinguished by a combination of WT1, napsin A/HNF1B and ER.[2] HGSC can be distinguished from low grade serous carcinomas (LGSC) by p53 and from mucinous carcinoma by WT1.[51] Endometrioid carcinoma can be distinguished from clear cell carcinoma by napsin A, HNF1B and progesterone receptor (PR).[1] Endometrioid and mucinous carcinomas can be distinguished by PR and vimentin.[51-53]

Table 7: Ancillary tests to distinguish serous and endometrioid carcinomas.

Comparator #1	Comparator #2	Expressed/abnormal in comparator #1	Expressed/abnormal in comparator #2	References
High grade serous carcinoma	Endometrioid carcinoma (grade 3)	WT1, p53		50
High grade serous carcinoma	Clear cell carcinoma	WT1, Estrogen receptor	Napsin A, HNF1B	2,54
High grade serous carcinoma	Low grade serous carcinoma	p53		51
High grade serous carcinoma	Mucinous carcinoma	WT1		2
Endometrioid carcinoma	Clear cell carcinoma	Progesterone receptor	Napsin A, HNF1B	55
Endometrioid carcinoma	Mucinous carcinoma	Progesterone receptor, Vimentin		52
Low grade serous carcinoma	Endometrioid, clear cell, mucinous	WT1		2

Biomarkers are not necessary if the features are unequivocally those of serous tubal intraepithelial carcinoma (STIC), however if there is diagnostic uncertainty, both p53 and Ki-67 staining should be performed.[56] The cells must exhibit abnormal (mutation-type) p53 staining.[57,58] The Ki-67 proliferation 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 Ki-67 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 IHC is undertaken (morphological features and aberrant p53 staining are also needed).[56]

While many prognostic biomarker studies have been published for HGSC, none provide sufficient stratification to influence management.

This is different for endometrioid carcinoma where three recent studies validated that the same molecular subtype assignment of their uterine counterparts showed prognostic stratification.[59-61] The four molecular subtypes are *Polymerase epsilon* (*POLE)* mutated with the longest survival, mismatch repair deficient (MMRd) and no specific molecular profile (NSMP) cases with intermediate survival and p53abn cases with the shortest survival. In particular, assessing the latter may supplant grading. Assessing the MMR status also serves genetic Lynch syndrome (LS) screening and might provide predictive information. The NSMP group is the largest in ovarian endometrioid carcinoma, as it is in endometrial endometrioid carcinoma. Further stratification of this group might require other biomarkers. For example, PR expression status and/or *CTNNB1* mutation status both have been shown to be associated with survival across all ovarian endometrioid carcinomas, but have not been studied within the NSMP group.[62-66]

There are no validated prognostic biomarkers for ovarian clear cell or mucinous carcinoma. However, p53 status might inform about the course of mucinous borderline tumours. A recent study showed that p53abn mucinous borderline tumours were associated with a higher risk of death.[67] While there are no current therapeutic options for these patients, the converse information that p53 normal mucinous borderline tumours are at very low risk of disease progression can be useful in some clinical circumstances.[68]

Tubo-ovarian HGSCs with proven *BRCA1/2* mutations (germline or somatic) are likely to respond to PARPi. If modern IHC supported histotyping is performed, *BRCA1/2* mutations are confined to HGSC so *BRCA1/2* testing can be restricted to this histotype.[69] Difficult cases (e.g., differential diagnosis with grade 3 endometrioid) can also be tested at the discretion of the pathologist. Several clinical trials showed effects of PARPi in the *BRCA1/2* wild-type but homologous repair deficient group.[70] It can be anticipated that eligibility for PARPi will be expanded. Several competing proprietary homologous repair deficiency (HRD) tests (mutational signatures, genomic scars etc.) are being marketed, with an alternative approach to testing being an expanded gene panel that includes proven HRD genes such as *RAD51C, RAD51D, BRIP1, PALB2* among others.[71]

The United States Food and Drug Administration (FDA) has approved immunotherapy for MMRd tumours irrespective of site. Universal MMRd testing is recommended for ovarian endometrioid carcinoma to screen for hereditary LS.[72] While MMRd is rarely observed in prototypical clear cell carcinomas, some cases with ambiguous morphology between endometrioid and clear cell carcinoma are MMRd and even with the use of diagnostic IHC panels these cases might be diagnosed as clear cell carcinoma. While MMRd in clear cell carcinoma is uncommon, all cases reported in the literature were proven or probable LS.[73-76] Hence, if funding is not restricted, clear cell carcinoma might also be tested for LS. Alternatively, a features-based screening for clear cell carcinoma is possible (ambiguous/mixed morphology between endometrioid/clear cell carcinoma, microcystic architecture and intratumoural stromal lymphocytic infiltrate, presence of synchronous endometrial and ovarian carcinoma).[73] Age cut-offs have limited value.

No other molecular targeted therapies are approved. Hormone receptor expression assessment might be requested by oncologists before commencing hormonal therapy for endometrioid or LGSC.[65] No predictive cut-offs have been established and the expression of ER and PR should be reported descriptively. About 5% of LGSCs harbor a *BRAF* V600E mutation and case reports suggest promising results with BRAF inhibitors.[77] *HER2* amplifications occur in 18% of ovarian mucinous[78] and 7-14% of ovarian clear cell carcinoma.[79]

Ovarian carcinomas represent a heterogeneous group of tumours. In recent years, molecular pathology has been instrumental in demonstrating that ovarian carcinomas are not a single entity, but a group of tumours with diverse morphology, natural history, and pathogenesis.[80] While molecular investigations at present do not have a significant role in diagnosis, prediction of prognosis or determination of treatment in ovarian, tubal and peritoneal carcinomas, this may change in the future, especially with the introduction of PARPi therapy for HGSC.

High grade serous carcinomas (HGSCs) are chromosomally unstable tumours, in which *TP53* mutations are ubiquitous. Germline or sporadic, genetic or epigenetic, alterations in *BRCA1* and *BRCA2* also occur. A pathogenetic model has been proposed, starting with early *TP53* alteration, followed by *BRCA1* loss, leading to deficiency in homologous recombination repair of double strand breaks, triggering chromosomal instability with gene copy number variation. The Cancer Genome Atlas (TCGA) performed an integrated genomic analysis of 489 high grade ovarian serous carcinomas.[81] Mutations in *TP53* were seen in 96% of the cases. There was a low prevalence, but there were statistically recurrent somatic mutations in nine further genes, including *NF1, BRCA1, BRCA2, RB1* and *CDK12*. Copy number alterations and promoter hypermethylation events were detected in 168 genes. The most common amplifications were detected in *CCNE1, MYC* and *MECOM*. Deletions were identified in *RB1, NF1* and *PTEN*. Hierarchical clustering analysis identified four transcriptional subtypes, three microRNA subtypes, four promoter methylation subtypes, and a transcriptional signature associated with survival. In 33% of the tumours, alterations in *BRCA* genes, either somatic or germline mutations or promoter hypermethylation were present. Defects in DNA repair by homologous recombination, secondary to mutations in *BRCA1*, *BRCA2* or related genes, or by mechanisms not yet elucidated, are seen in approximately 50% of HGSCs, and HRD is a predictive marker for response to PARPi therapy.[82,83] At present there is no single agreed upon predictive assay for HRD/prediction of response to PARPi.

Low grade serous carcinomas (LGSCs) are closely related to serous borderline tumours, and show frequent mutations in the MAPK pathway (*KRAS*, *BRAF*, *NRAS*), prognostically unfavourable alterations in *CDK2A* and mutations in *USP9X* [64,84] PR is an unfavourable prognostic marker.[65]

The molecular events in endometrioid carcinoma are similar to the uterine counterpart. The main molecular alterations are: *CTNNB1* mutation (50%), microsatellite instability (13%), and mutations in the *PTEN* (20%), *KRAS*, *PIK3CA, TP53, and POLE* genes. The molecular subtypes from the uterine counterpart are equally prognostic in ovarian endometrioid carcinomas, as discussed earlier.[59,85]

Clear cell carcinoma shows frequent *ARID1A* and *PIK3CA* mutations. Alterations in *KRAS* and *TP53* are unusual. *HER2* amplifications are uncommon.

Mucinous carcinomas frequently harbour genomic loss of *CDKN2A*, *KRAS* and *TP53* mutations often co-occurring and *HER2* amplifications.[86] In mucinous tumours with areas of carcinoma admixed with foci of benign or borderline mucinous tumour, *KRAS* mutations have been demonstrated in all components, suggesting that this represents an early event during tumourigenesis. *TP53* mutations are implicated in the progression from mucinous borderline tumour to carcinoma and, as discussed earlier, a recent study demonstrated a higher risk of death for patient with mucinous borderline tumour harbouring a *TP53* mutation.[67]

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