11 Approach to a Patient with Peritoneal Metastases with Unknown Primary Site: Focus on Histopathological Evaluation

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11.1 Introduction

Most peritoneal metastases are secondary to other primary tumours whilst some rare tumours arise from the peritoneum itself. With the plethora of diagnostic investigations available, establishing the diagnosis and origin of peritoneal metastases is not a problem. Yet some situations can be challenging when an unsuspecting surgeon commits a diagnostic blunder or the primary tumour remains elusive despite a focused search. The curative treatment of PM is only for selected patients and comes with its own morbidity and cost [1]. Subjecting a patient to surgery where it is not indicated may lead to unnecessary morbidity and not uncommonly, early and symptomatic recurrence that can make the patient ineligible for systemic therapies [2]. Sugarbaker first reported the benefit of performing cytoreductive surgery and HIPEC in 15 patients with peritoneal metastases with undetermined primary site [3]. In this series, there were six patients with a poorly differentiated adenocarcinoma, four with adenocarcinoma and four with mucinous adenocarcinoma. Since the publication in 2001, progress has been made in molecular biology and diagnostic methods and newer and more effective systemic therapies have become available.

In many primary tumours, PM are a part of widespread metastatic disease. In a smaller percentage, they occur in isolation. Ovarian cancer is the exceptional tumour where peritoneal disease is not considered as distant metastases. The commonest tumours presenting to a peritoneal surface oncology unit are ovarian cancer, colorectal cancer, gastric cancer and rare peritoneal tumours like mucinous appendiceal tumours, peritoneal mesothelioma among others [4]. Commonest histological subtypes include adenocarcinomas, serous carcinomas, mucinous carcinomas and some rare tumours like round cell tumours and sarcomas. Either it is a common histology with an occult primary or an uncommon histology that needs to be accurately diagnosed. Peritoneal metastasis with an unknown primary site is a rare entity that has not been addressed separately. We look at the common histologies seen in peritoneal metastases, their commonest differential diagnosis and some peculiar situations in this chapter. The clinical aspects are touched in brief with greater stress on the

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pathological aspects of diagnosis. The uncommon histologies have been discussed in the preceding chapter.

11.2 Definition

Metastatic cancer of unknown primary (CUP) is defined as:

Histologically confirmed malignancy, for which no primary site is found despite an extensive diagnostic work-up [5].

Similarly, peritoneal metastases may occur in the absence of a known, identified primary malignancy.

11.3 Pathogenesis

There are two theories proposed to explain the development of CUP. The first hypothesis postulates that CUP does not undergo type 1 progression (from a premalignant lesion to malignant) but instead it follows a type 2 progression without forming a primary site. The second hypothesis supports that CUP follows the parallel progression model, where metastases can arise early in the development of a malignant process [6, 7].

There are some histologies that have a favourable outcome with treatment and are considered to be tumours with a good prognosis like papillary adenocarcinoma of the peritoneum and there are others that have a poor prognosis like adenocarcinoma having the marker profile of colonic origin [8].

11.4 Pathological Evaluation

Pathological evaluation of biopsy specimens or surgical specimens is the gold standard for establishing the diagnosis. Immunohistochemistry is a very useful adjunct to histopathological evaluation that is now considered an extension of routine pathological reporting. Molecular tests may be performed to confirm the diagnosis of rare tumours with known genetic alterations or to determine the diagnosis in cases where the histopathological evaluation is inconclusive and also to identify known and unknown therapeutic targets and are discussed in the following chapter.

It must be borne in mind that the pathological evaluation should not be performed in isolation, but keeping in mind the clinical history and other clinical findings. The other challenge is that in most instances, the diagnosis has to be made on a tissue sample that has been obtained by performing a transabdominal or laparoscopic biopsy and may be inadequate. Good coordination between the surgeons and pathologist is essential. Laparoscopic biopsy where possible is better as it allows better sampling. The morphology of the peritoneal deposits and disease extent can also be evaluated. Fluid samples alone may be submitted, but it is better if the evaluation is performed on biopsy specimens as often these are paucicellular and non-representative of the actual tumour.

In this chapter, we have broadly divided the tumours into five groups—adenocarcinomas, serous carcinomas, mucinous carcinomas, sarcomas and uncommon histologies. These groups are not mutually exclusive. The distinction between the subtypes of adenocarcinoma and serous carcinomas is not always clear and pathologists may put the same tumour in either group. The rare histologies are discussed elsewhere in this book.

11.4.1 Adenocarcinomas

Metastatic adenocarcinoma is perhaps the most common histological finding in peritoneal metastases. Though the exact incidence is not known, in majority of the cases, the underlying primary is from colorectum, stomach and ovaries. Other less common primaries presenting with isolated peritoneal metastases are endometrial adenocarcinoma, small bowel adenocarcinoma, appendiceal adenocarcinoma, cervical adenocarcinoma, pancreatobiliary adenocarcinomas and metastatic breast carcinoma. In majority of the cases, the primary site is evident on imaging.

11.4.1.1 Clinical Findings

In male patients, the commonest differentials would be colorectal, gastric and pancreaticobiliary

primaries, whereas in females it would be ovarian cancer, colorectal and gastric cancer. Though most of these tumours have an increased incidence in the older age groups, young age alone does not rule out any of the common primary tumours. There are no specific clinical findings in patients with PM that point towards the primary tumour site. The finding of PM may be incidental for investigations performed for non-specific symptoms or the presentation may be of advanced disease with ascites and its ensuing problems. A detailed clinical history of previous illnesses and treatments should be elicited. In rare situations, even when there is a history of pervious malignancy, PM may not be secondary but due to a primary tumour arising from the peritoneum. Upper and lower gastrointestinal endoscopy may not reveal a primary tumour and whole body imaging is negative for a primary. There is no pattern of peritoneal distribution that can point towards a particular diagnosis. The alteration in the tumour marker levels could give some clue about the primary site. There can be two clinical scenarios—bilateral ovarian metastases with peritoneal deposits with no other apparent primary site and PM alone with an occult primary. An appendiceal primary tumour should be suspected and searched by laparoscopic evaluation. It could be difficult, even by laparoscopic approach in case of extensive PM with massive involvement of ileocolic area.

The presence of bilateral ovarian tumours has a greater possibility of being metastatic than unilateral tumours though this is not binding. The features of an ovarian primary and metastases to the ovary may or may not been distinguished on imaging. The most common sites should be first ruled out like ovarian, colorectal and gastric and the less common sites considered thereafter.

11.4.1.2 Histopathological Findings

Majority of the colorectal and gastric tumours are adenocarcinomas and can be distinguished from other primaries based on histology alone. However, when the primary tumour is not evident, immunohistochemistry should be performed to confirm the origin. Figure 11.1 provides an algorithm for selecting the immunohistochemistry markers.

Epithelial ovarian cancer forms the commonest differential of metastatic adenocarcinoma to the peritoneum. There are five subtypes of which the endometrioid variety alone is discussed here. Serous, clear cell and mucinous adenocarcinomas are described in subsequent sections. Adenocarcinomas of the endometrioid variety constitute 10% of the ovarian epithelial tumours. Distinction between a primary ovarian endometrioid carcinoma and colorectal carcinoma is simple as primary ovarian endometrioid carcinomas are usually positive with CK7, estrogen

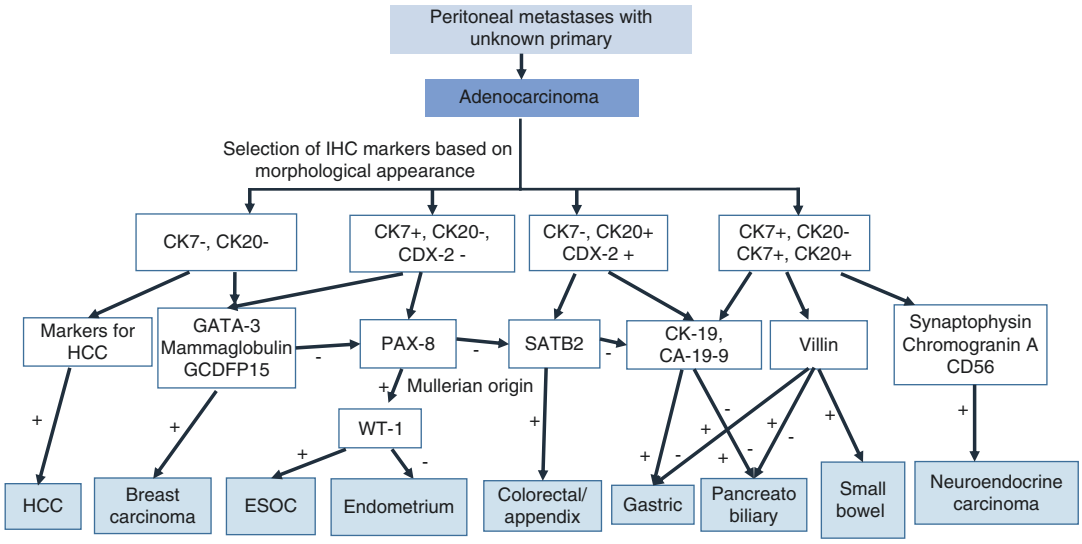

Fig. 11.1 Algorithm for determining the primary site in adenocarcinoma

receptor (ER), CA125 and PAX8 and negative with CK20, CEA and CDX2 whilst the converse immunophenotype is seen in metastatic colorectal adenocarcinomas [9–11]. There are two specific markers for ovarian cancer that should be considered to establish the diagnosis of an ovarian primary. This first is paired-box 8 (PAX8) that is a sensitive marker for tumours of the thyroid, kidney and thymus, and tumours derived from the Müllerian ducts [10–12].

It is also a specific marker for tumours of Mullerian origin and is expressed in nearly 95% of the ovarian epithelial tumours [13]. It represents the simplest way of confirming the ovarian origin of peritoneal metastases. Uncommonly, some high-grade tumours may not express PAX-8 and it may be difficult to differentiate at times, an endometrioid adenocarcinoma from other ovarian tumours. WTI is a useful marker for distinguishing endometrioid adenocarcinoma from the other more common serous subtype. WTI is a tumour suppressor gene that was first identified in the genitourinary system (kidney, ovary and testes) and is responsible for the coding of a transcription factor of 52–54 kDa important in cell growth and differentiation [14, 15]. It is responsible for the development of hereditary and sporadic types of Wilms tumours within the renal parenchyma. It is also involved in the structural and functional development of the gonads and is overexpressed in primordial and primary ovarian follicles [16]. In the normal mature ovary, WT1 is expressed in the ovarian surface epithelium and in stromal and granulosa cells [17]. In the tumour-bearing ovary, WT1 is characteristic of the serous subtype being rarely found in the others [17].

WT1 can thus be useful in the differential diagnosis of primary ovarian tumours with non-specific morphological features and also differentiating serous from the other subtypes. It also helps to exclude other primary tumours of uterine, breast, pancreatobiliary or gastrointestinal origin, exhibiting similar morphologic phenotype [17, 18]. Moreover, co-expression of WT1 and PAX8 has been recently demonstrated as a valuable association in confirming the ovarian origin of malignant effusions [19]. The endometrioid variety rarely expresses WTI and has a heterogeneous WT1 expression:

WT1 positivity implies that the tumour is either arising from the ovary or fallopian tube of peritoneum, whilst WT1 negativity indicates an ovarian tumour with origin in endometriosis foci [17].

The other common primary site is the lower gastrointestinal (GI) tract, specifically the colorectum. CDX2 is used to establish a colorectal/lower gastrointestinal origin.

Colorectal primaries need to be distinguished not just from ovarian primaries but also from gastric and pancreaticobiliary tumours and this may not always be clear on morphology alone. The typical immunohistochemistry profile of colorectal adenocarcinomas is expression of CK20 and CDX2 and lack of expression of CK7 [20]. However, CDX2 and CK20 have been shown to be positive in up to 21% each of gastric cancers and 14% and 21% of ovarian mucinous adenocarcinomas, respectively. Similarly, CK7 expression is seen in up to 50% of the gastric and ovarian carcinomas, more commonly, the mucinous ones. This combination of CK20 and CDX2, in one study, was more helpful in differentiating colorectal from pancreatic adenocarcinoma, which was only 2% CDX2 positive, 15% CK20 positive and predominantly CK7 positive (94%), and with only 3% of colorectal adenocarcinoma being CK7 positive [20].

Pancreatic tumours also express CEA and CA-19-9. Bayrak et al. compared the use of the CK7 negative CK20 positive pattern, which had a sensitivity of 64% and specificity of 97%, with use of CDX2 positivity, which had a 78% sensitivity and 85% specificity in differentiating colorectal from gastric and pancreatic adenocarcinoma [21].

Another confirmatory marker for colorectal origin is SATB2.

SATB2 is part of the family of matrix attachment region-binding transcription factors and has developmental roles in craniofacial, neural and osteoblastic differentiation [22]. SATB2 is expressed in the epithelium of the lower gastrointestinal tract and is seen in only a few malignancies including colorectal/appendiceal adenocarcinomas, tumours of osteoblastic dif-

ferentiation and renal/urothelial carcinomas [23]. SATB2 is a specific marker of colorectal differentiation and is used to determine the origin of adenocarcinomas of unknown primary and distinguish primary ovarian mucinous adenocarcinomas from colorectal metastases. SATB2 as a solitary marker is reported to have a sensitivity of 93% and specificity of 77% but when combined with CK20 and CK7 expression, the sensitivity becomes 83% and specificity 100% as demonstrated in a large study [24, 25]. In comparison, the sensitivity and specificity of the CK7 negative, CK20 positive immunephenotype are 85% and 99%, respectively, and for the $CDX2^{+}$ immunophenotype these were 96% and 80%. Thus, when the primary is in situ, this marker does not add much to two-marker (CK7, CK20) combination or the three-marker combination (CK7, CK20, CDX2) [25–27]. The main application of SATB2 is to distinguish adenocarcinomas of colorectal origin from those of gastric and pancreatic origin [26, 27].

Most studies have shown a low expression in pancreaticobiliary and gastroesophageal tumours [27]. The only ones where the reported expression was high were the ones in which the threshold for positivity was low [28]. The expression of this marker is low even in lung and gynaecological adenocarcinomas which form the other differential diagnoses [28]. Pancreatic ductal carcinomas are also positive for CK8, CK17, CK18, CK19, CEA, CA19-9, Dupan-2, MUC1, MUC4 and MUC5AC [29–32].

Distinction from Breast Carcinomas

Breast carcinoma can be a rare differential diagnosis of adenocarcinoma of the peritoneum. It should be borne in mind when the morphology is not characteristic of GI or ovarian origin especially in female patients. PAX-8 and CA-125 are positive in endometrioid carcinomas and negative in breast cancer though CA-125 could be positive [33, 34]. Markers useful but not specific for breast cancer are GCDFP15, mammaglobin and GATA3 (usually negative in endometrioid carcinomas and positive in breast carcinomas) [35, 36]. A proportion of endometrioid adenocarcinomas may be mammaglobin positive [36].

Other Uncommon Primary Sites

When deemed necessary, TTF-1 and 2 can be used to rule out lung cancer. A high-grade neuroendocrine tumour can give the appearance of an adenocarcinoma and can be ruled out using chromogranin A, synaptophysin and the Ki-67 proliferation index [37]. A neuroendocrine tumour must be ruled out in poorly differentiated adenocarcinomas and poorly differentiated carcinomas. The other markers that are positive in all neuroendocrine tumours are PGP 9.5 and CD56 [38]. PM are usually part of widespread disease in these patients and are seen in over 15% of the patients [39]. Neuroendocrine tumours arising from the distal small bowel have a greater propensity for producing PM and lymph node metastases [40]. Some peculiar features of PM arising from these tumours are the small size of deposits (<5 mm) and mesenteric deposits along the blood vessels [41–44].

Carcinoids from the foregut and midgut are generally positive for chromogranin A and CD56, whilst those from the hindgut are usually negative [45–47]. Hindgut carcinoids on the other hand often express prostatic acid phosphatase [48]. A less helpful marker is CDX-2, which although positive for most colorectal carcinomas has an immunoreactivity of about 40% in well-differentiated carcinoids but has reported an 80% expression rate in poorly differentiated carcinoids [46, 49–51].

Hepatocellular carcinoma and small bowel adenocarcinoma are other rare differential diagnosis that should be considered. Small bowel tumours constitute 1–3% of all the gastrointestinal malignancies [52, 53]. Of the various tumours arising from the small bowel, adenocarcinomas are the commonest and constitute 30–45% of all the tumours [54, 55]. Small bowel adenocarcinoma is known to have a poor prognosis with a median overall survival ranging from 12 to 20 months [56, 57]. These tumours are CK7 positive in more than half of all cases, unlike normal small intestinal mucosa which is CK7 negative and colorectal adenocarcinomas which are CK7 negative and CK20 positive [58]. They are also positive for CK20, CDX-2 and villin [58].

The markers specific for hepatocellular carcinoma (HCC) are Glyican-3, CD34, AFP, CD10, CEA and HepPar-1 [59]. HCC includes its variant—fibrolamellar HCC and intrahepatic cholangiocarcinomas. These tumours express only a limited number of keratin markers, namely CK8 and CK18 and thus most metastatic carcinomas can be excluded as they generally express a larger variety of keratin markers such as CK5/6, CK7, CK14 or CK20 in comparison to HCC [60].

Many times the marker profiles overlap or do not give a clear pointer towards the primary. It is important to correlate the histology findings with the immunohistochemistry findings and not draw inferences from individual findings.

11.4.2 Serous Carcinomas

Serous carcinomas are the commonest variety of epithelial ovarian cancers that have a predilection for peritoneal spread. And hence, serous carcinoma is a common pathological diagnosis in patients with peritoneal metastases. Often the ovarian primaries are small in size and even inconspicuous. It has been shown that majority of the serous carcinomas arise from the fallopian tubes. The other less common sites of origin are the endometrium, cervix and the peritoneum itself [61]. The other differentials of a serous histology are peritoneal mesothelioma and breast cancer.

11.4.2.1 Clinical Presentation

Majority of the serous carcinomas are seen in women [61]. Primary peritoneal serous carcinoma is a rare entity in males. These cancers occur in older women, most of whom have attained menopause [62]. Most serous carcinomas are diagnosed in an advanced stage with disseminated peritoneal disease and ascites [63, 64]. A pelvic mass may or may not be present. Even when a pelvic mass is present, the site of origin may not be clear. The other peritoneal tumour that can mimic serous carcinoma is peritoneal mesothelioma.

11.4.2.2 Histopathological Findings

The histological features of high-grade serous carcinomas are diagnostic and consist of branching papillary fronds, slit-like fenestrations, glandular complexity, moderate to marked nuclear atypia with marked pleomorphism, prominent nucleoli, stratification, frequent mitoses and stromal invasion (irregular or destructive infiltration by small glands or sheets of cells) [65]. Psammoma bodies are common (Fig. 11.2). The stroma may be fibrous, oedematous, myxoid or desmoplastic. In comparison, low-grade tumours have extensive papillary features with many psammoma bodies, papillae, glands, cysts or irregular nests of cells with uniform round to oval nuclei and evenly distributed chromatin. The nuclear features are variable. The mitotic count is less than 10 per high power field [65]. The cells lie in a variable amount of fibrous stroma. Some of the ovarian tumours have clear cell features and are considered clear cell variants of serous carcinoma.

When the ovarian primary is not evident or the ovaries have been removed before, immunohistochemistry is required to establish the site of origin. Another presentation could be of a pelvic mass with peritoneal metastases and the ovarian origin is not clear (Fig. 11.3). As discussed above, PAX-8 is used to establish Mullerian origin and is negative in primary peritoneal serous

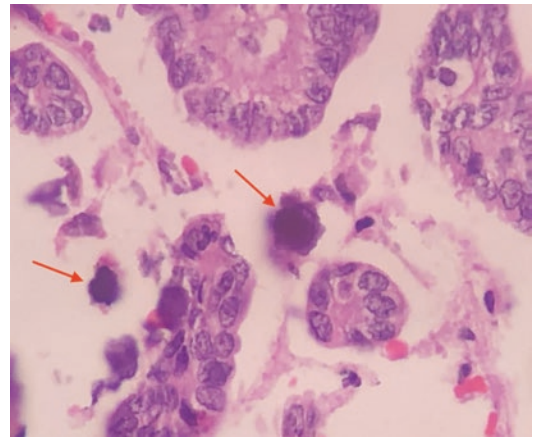

Fig. 11.2 Psammoma bodies which are characteristic of serous carcinomas

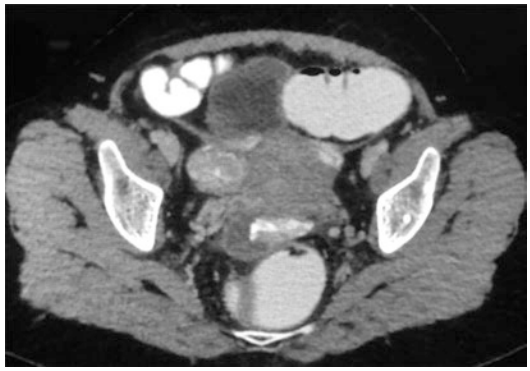

Fig. 11.3 Pelvic mass in a patient with serous carcinoma. The site of origin could be the ovary or the uterus. The prognosis is significantly worse in serous endometrial carcinomas

carcinomas. Moreover, some high-grade ovarian tumours may be PAX-8 negative. WTI is positive in present majority of the ovarian serous carcinomas.

WT1 is in contrast expressed in less than a third of the endometrial serous tumours [14]. However, in cases when both entities are WT1 positive, further investigations are needed to determine the primary site of origin [66]. The p53 expression can be similar in both the tumours. There may a situation in which both primaries co-exist. Making the distinction is important as endometrial serous carcinoma is a rare tumour and the outcomes with serous carcinoma of the endometrium are inferior to those obtained for serous ovarian carcinoma. It is believed that some of the primary peritoneal serous carcinomas originate from a latent endometrial serous carcinoma [67–69].

WT1 differentiates serous ovarian carcinomas exhibiting similar morphology to that of pure clear cell ovarian carcinoma, as WT1 is negative in the latter [70]. WT1 cannot distinguish an ovarian high-grade ovarian serous carcinoma from a primary peritoneal serous carcinoma or high-grade fallopian tube carcinoma. All these three entities express WT1 diffusely [17, 18].

Low-grade serous carcinomas usually present with large ovarian masses that infiltrate the surrounding peritoneal structures and are an uncommon cause of PM with unknown primary.

A common non-gynaecological malignancy that needs to be ruled out is peritoneal mesothelioma. Though it is a rare tumour, it is a peritoneal disease and thus may be seen more often in a peritoneal surface malignancy unit than other common cancers like breast cancer that present rarely with isolated peritoneal disease. Though histological features can point towards the diagnosis of peritoneal mesothelioma, immunohistochemistry is essential to establish the diagnosis and comprises of both positive and negative markers [71]. Peritoneal mesothelioma arises from a single cell line but has a spectrum of cytoarchitectural features that make it unique and often difficult to diagnose. The spectrum includes tumours that are entirely of epithelial or mesenchymal (sarcomatoid) type to a range of biphasic and intermediate forms [72]. The epithelial subtype is characterized by cuboidal or flattened epithelial-like malignant mesothelial cells with ample cytoplasm with distinct cellular membranes, and a relatively uniform, granular to vesicular nuclei. The subtypes of epithelial peritoneal mesothelioma are categorized by the patterns observed for the malignant epithelial component and include tubulopapillary, solid, deciduoid, storiform-like, fascicular-like, multicystic, papillary, microcystic and granular [73]. A positive calretinin, cytokeratins 5/6, WT-1, thrombomodulin and mesothelin stain, accompanied by a negative B72.3, CEA, CD15, Leu-M1 and BER-EP4 immunostain is highly suggestive of peritoneal mesothelioma [74].

Calretinin, WT1, CK5/6, D2-40 and mesothelin are generally immunoreactive in peritoneal mesothelioma but can also be positive in gynecologic and non-gynecologic adenocarcinoma [75].

There are some extremely well-differentiated papillary mesotheliomas that need to be distinguished from benign mesothelial proliferation.

Cytological examination of ascitic fluid removed by paracentesis rarely results in a positive finding. If cells are recovered, they frequently resemble hyperplasic mesothelial cells with insufficient atypia present for a confident diagnosis.

Calretinin is one of the first markers that was found to be useful in the diagnosis of mesothelioma. Calretinin is currently regarded as being the most sensitive and one of the most specific of the positive mesothelioma markers. Because of this, it has been recommended as one of the primary markers in the various panels that are currently used in the diagnosis of mesothelioma [76]. Calretinin is often expressed in all histologic types of mesothelioma, in contrast to other commonly used mesothelioma markers, such as keratin 5/6, Wilms' tumour 1 (WT1) protein and podoplanin, which are often expressed in epithelioid mesotheliomas, but are usually absent in sarcomatoid mesotheliomas [77].

Although the reaction reported for this marker in mesotheliomas is usually strong and diffuse and that seen in adenocarcinomas is most frequently restricted to small focal areas of the tumour, diffuse strong positivity can occasionally occur in adenocarcinomas [78]. In addition, it should be emphasized that there are differences in calretinin expression among the different types of carcinomas. The reported percentages of calretinin expression in recent investigations ranged from 6% to 10% in lung adenocarcinomas, 31% to 38% in serous carcinomas and 0% to 10% in renal cell carcinomas [79–85].

D2-40 is a monoclonal antibody directed against M2A antigen, a surface sialoglycoprotein originally detected in association with germ cell neoplasia and foetal testicular gonocytes [86]. D2-40 has demonstrated a selective immunoreactivity for lymphatic endothelium and thus, has been used to demonstrate lymphatic invasion by primary tumours and as a marker of certain vascular lesions [87–90].

It is also a novel marker of cells with a mesothelial phenotype and is useful for making a distinction between peritoneal mesothelioma and adenocarcinoma. The sensitivity and specificity of this antibody is comparable or superior to other mesothelioma markers and it can be used to confirm the diagnosis of peritoneal mesothelioma when the conventional marker profile is inconclusive [71].

Mesothelin is highly sensitive for malignant mesothelioma, but its specificity is relatively low since other tumours including ovarian cancer may exhibit mesothelin positivity. Nevertheless, diffuse and strong membranous mesothelin expression serves as a strong indicator of epithelioid mesothelioma as opposed to ovarian carcinoma [74, 91]. Mesotheliomas have a high proportion of CK7 positivity and usually do not express CK20 akin to ovarian primary tumours [92].

Peritoneal mesotheliomas are also characterized by strong and diffuse membranous EMA positivity (expression on the luminal aspects of the tumour cells) though this staining pattern does not distinguish them from adenocarcinomas. ER positivity in malignant mesothelioma is a rare phenomenon, and indicates the likelihood of a serous carcinoma rather than a mesothelioma [93]. ER-α is rarely expressed in mesothelioma (highest rate of expression—10%), with most studies showing expression to be absent in both pleural and peritoneal disease. Similarly, PR is generally reported as negative in peritoneal mesothelioma. One study showed PR positivity in 7% of 71 patients [93–98].

Although WT-1 protein is highly sensitive for epithelioid mesotheliomas, it has no benefit in discriminating from serous carcinomas [99].

Immunohistochemistry panels should be chosen keeping in mind the histological features and should include both positive and negative markers (Table 11.1). Not just positivity but the type of staining should also be considered. Peritoneal mesothelioma can be a second primary in a patient with a known malignancy and the possibility of this diagnosis should be kept in mind (Fig. 11.4).

Another differential diagnosis is breast carcinoma. Metastatic breast carcinomas of ductal type can mimic a papillary serous or endometrioid ovarian cancer. The finding of a pelvic mass and/or disseminated peritoneal disease is not uncommon in a patient with a history of breast cancer and usually represents a new malignancy of ovarian origin. Yet, the rare possibility of metastatic breast disease needs to be considered and ruled out. As mentioned above, PAX-8, CA-125 and WT-1 are positive in serous carcinomas and nega-

tive in breast cancer though WT-1 and CA-125 could be positive [34, 35]. Markers useful but not specific for breast cancer are GCDFP15, mammaglobin and GATA3 (usually negative in serous carcinomas and positive in breast carcinomas) [36, 37]. An algorithm for determining the primary site in peritoneal metastases with serous histology is provided in Fig. 11.5.

Table 11.1 Common IHC markers for establishing the diagnosis of peritoneal mesothelioma

Immunohistochemistry markers for peritoneal mesothelioma
Positive markers
Calretinin
Cytokeratins 5/6
WT1
Podoplanin
Thrombomodulin
D240
Mesothelin
Negative markers
Claudin-4
TTF-1
PAX-8
CEA
BER-EP4
Prognostic markers
Nuclear grade
Mitotic count
Ki-67

11.4.3 Mucinous Carcinomas

Mucinous peritoneal metastases commonly arise from appendiceal tumours, colorectal tumours and ovarian tumours. Other primary sites include the pancreas, urachus and cervix. The term pseudomyxoma peritonei is reserved for patients with mucinous ascites and the characteristic pattern of redistribution. In rare situations, high-grade mucinous carcinoma peritonei may be present without any apparent primary [100]. Either the primary has been removed during a prior surgical procedure and the diagnosis missed or it is a true case of peritoneal carcinomatosis with unknown primary. It is not known if mucinous tumours can arise de novo from the peritoneum.

11.4.3.1 Clinical Presentation

A large proportion of the mucinous PM are from appendiceal origin. The diagnosis may be an incidental finding on imaging performed for other reasons. The appendiceal primary itself may be small and not evident on imaging. Ovarian metastases can be present even in low-grade mucinous carcinomas. When ovarian mucinous tumours are found, an appendiceal primary should always be ruled out. Tumour markers are helpful but seldom diagnostic. A colonoscopy is performed for all patients to rule out a colorectal

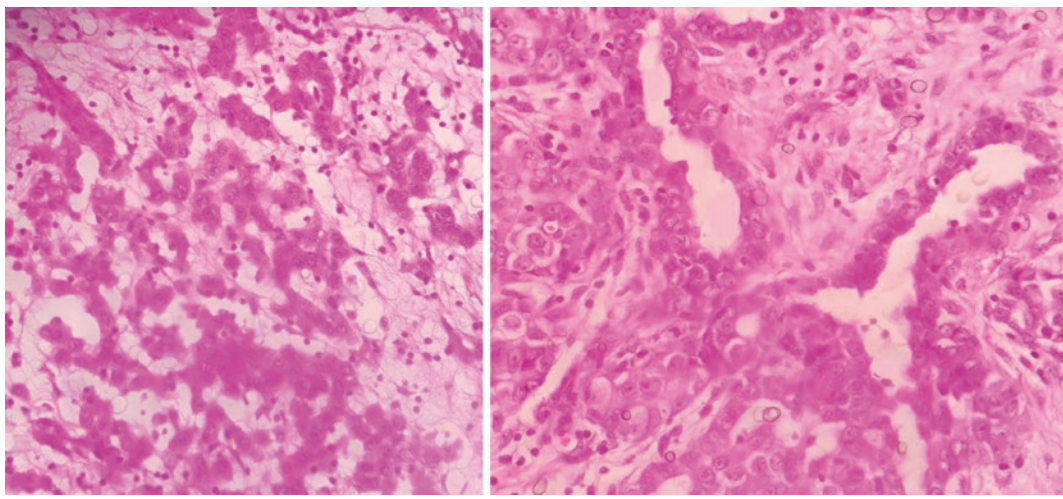

Fig. 11.4 Histological findings in the peritoneal biopsy suggestive of peritoneal mesothelioma several years after the initial diagnosis of breast cancer. The immunohistochemistry profile was in favour of a peritoneal mesothelioma

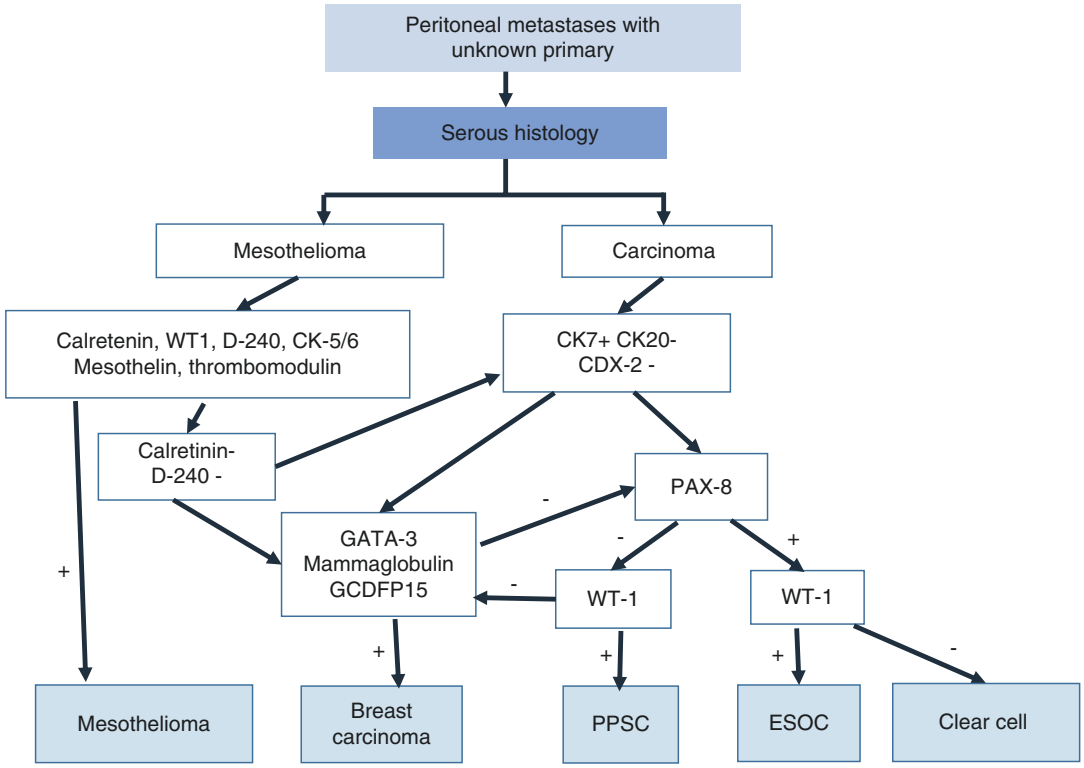

Fig. 11.5 Algorithm for determining the primary site in peritoneal metastases with serous histology. *PPSC* primary peritoneal serous carcinoma, *ESOC* epithelial serous ovarian carcinoma

primary. Other primaries like the urachus, mucinous pancreatic tumour may or may not be apparent on imaging. The symptom of passing mucous in urine is typical of an urachal tumour.

11.4.3.2 Histopathological Findings

When mucinous ovarian tumours and peritoneal implants are present, a lower gastrointestinal primary is always ruled out. However, mucinous tumours of the intestinal type can arise de novo from the ovary. Most of these tumours arise from a mature cystic teratoma and may show massive mucin secretion, goblet cells, carcinoid-like patterns, pseudomyxoma ovarii and peritonei, and signet ring cells characteristic of a gastrointestinal phenotype. Mucinous ovarian tumours can be borderline or malignant. These tumours may not always be CK-7 positive and CK-20 negative like the other ovarian epithelial tumours. Primary mucinous ovarian tumours can exhibit CK20 positivity, which is usually focal but can be diffuse. Focal and at times diffuse positivity is seen for CEA, CDX2 and CA19.9 as well [101]. This may make distinction from a colorectal tumour difficult. However, the pattern of coordinate expression of CK7/CK20 may be useful [102]. Although either marker can be positive in both tumours, primary ovarian mucinous neoplasms are usually diffusely positive with CK7 whilst CK20 is variable; conversely, metastatic colonic adenocarcinoma is usually diffusely positive with CK20 and shows focal positivity for CK7 [102]. As mentioned above, CDX2 will be expressed by appendiceal and colorectal primaries and not by ovarian primary tumour, but can vary. SATB2 is the confirmatory test for colorectal origin. Mucinous tumours arising from teratomas can express colorectal markers and need to be distinguished from metastases which is done by demonstration of teratomatous foci. However, when the mucinous component is huge, it may not be possible to find these foci.

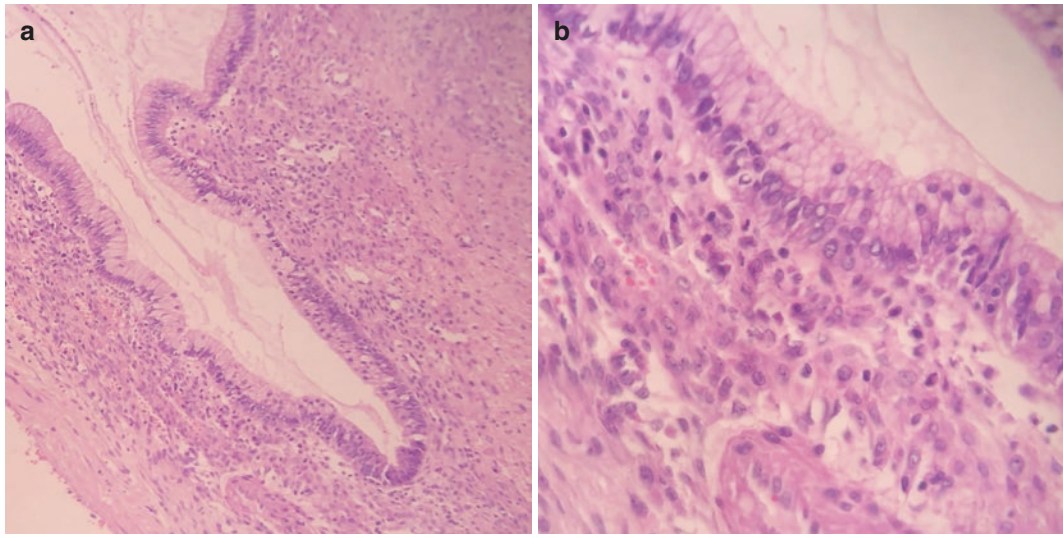

Fig. 11.6 (**a**, **b**) Peritoneal deposits from low-grade mucinous neoplasm of the ovary

The other markers expressed by these tumours are HepPar-1 and villin [103]. Figure 11.6 shows the histological features of a low-grade mucinous tumour arising from the ovary. As shown in Fig. 11.7, this tumour expressed CK-7, CA-125 and PAX-8 and was negative for CDX2, CK20 and WT1. Urachal primary tumours have similar expression to the colorectal primaries. They are diffusely positive for CK-20, CDX-2, MUC-2 and MUC-5 AC, and CK-7 expression is variable [104].

When the ovaries have been submitted for pathological examination, there are some histological features that can help in differentiating an ovarian from appendiceal primary. Involvement of both ovaries and surface implants are more likely in metastatic disease [105]. Large size and smooth external surfaces are not always associated with metastatic disease, especially in mucinous tumours. Histologically, features favouring metastasis to the ovary include retraction artefact separating tumour epithelium from underlying stroma, a scalloped pattern, infiltrative invasion, vascular invasion, hilar involvement, dissecting mucin (pseudomyxoma ovarii) and signet ring cells [106]. In contrast, back-to-back neoplastic glands with no intervening stroma, periglandular cuffing by cellular ovarian-type stroma, histiocyte aggregates, background endometriosis or associated primary teratomatous elements favour a primary ovarian neoplasm [105–107]. Conventionally, lower gastrointestinal mucinous tumours are diffusely positive for CK-20, CDX-2, MUC-2 and MUC-5 AC and were variably positive for CK-7. Mucinous ovarian tumours can arise from an immature teratoma too.

Tumour Grade

Mucinous PM arising from the appendix and ovary can be high grade or low grade. With the other primary sites, the tumours usually have a high grade.

Rare Differentials

Rarely, a metastatic cervical adenocarcinoma of usual type (HPV related) in the ovary may mimic a primary ovarian mucinous or endometrioid neoplasm [108]. Diffuse p16 immunoreactivity in such cases may be useful in suggesting a metastatic cervical adenocarcinoma. These tumours can present with mucinous peritoneal metastases.

Some rare situations that mimic mucinous peritoneal carcinomatosis have been enlisted by Carr et al. Malignant mesotheliomas in rare situations can have intracellular mucinous material rich in hyaluronic acid giving the appearance of signet ring cells [109]. These cells stain

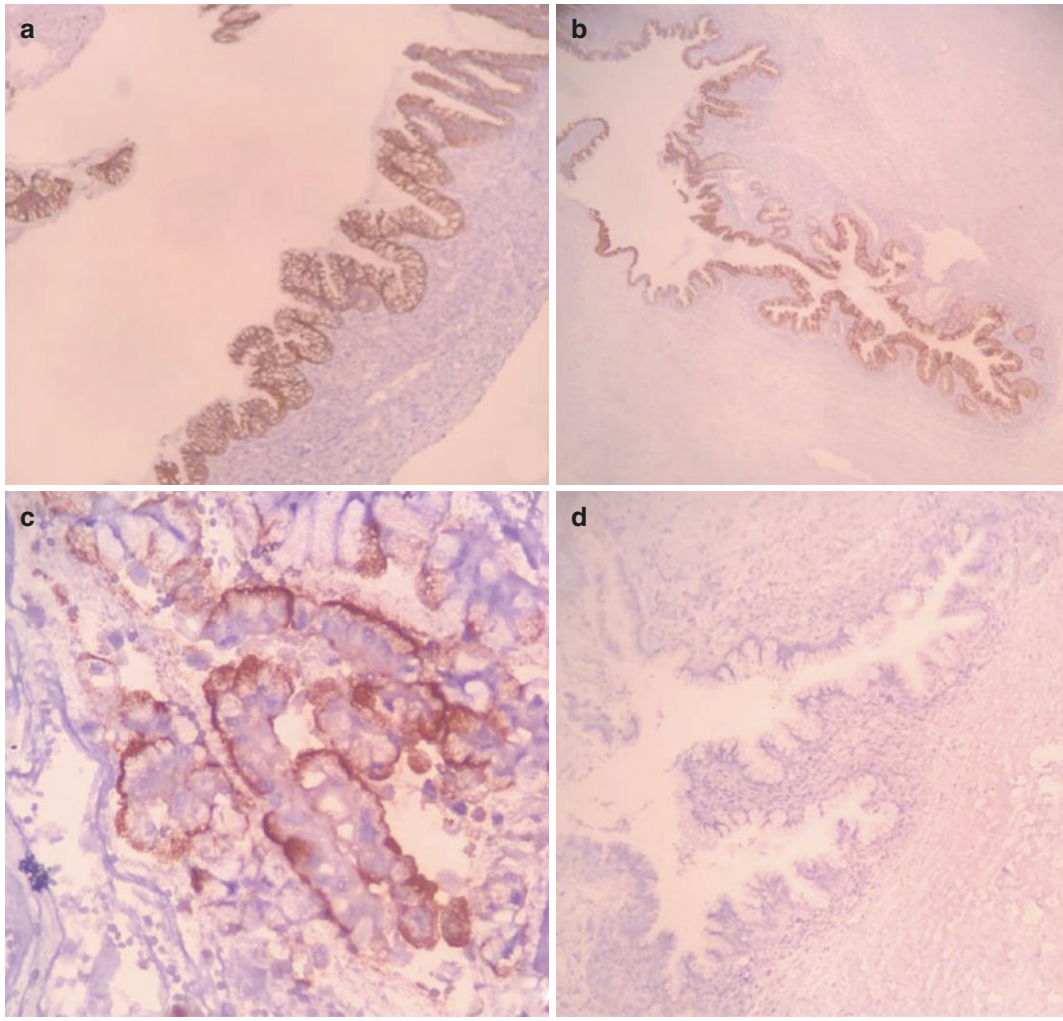

Fig. 11.7 Tumour cells express CK-7 (**a**), CA-125 (**b**), PAX-8 (**c**) and are negative for CDX-2 (**d**), CK-20 (not shown) and WT1 (not shown)

positive with mucin stains but can be distinguished as mesotheliomas when appropriate markers are used. Claudin-4 expression is seen in carcinomas and not mesotheliomas and can be used to make the distinction. The histological features should alert the pathologist of an alternative diagnosis [110, 111]. Myxoid change occurring in endometriosis and papillary mesothelioma can mimic mucinous peritoneal carcinomatosis [112, 113]. An algorithm for determining the primary site in mucinous peritoneal metastases is provided in Fig. 11.8.

11.4.4 Peritoneal Sarcomas

After the lungs and bones, the peritoneum is a common site of spread from soft tissue sarcomas. Nearly 30% of the sarcomas present with intra-abdominal disease. The commonest sarcomas metastasizing to the peritoneum are retroperitoneal liposarcomas, uterine leiomyosarcomas and low-grade and high-grade endometrial stromal sarcomas [114]. Low-grade endometrial stromal sarcoma can arise from the ovaries and the peritoneum itself [115].

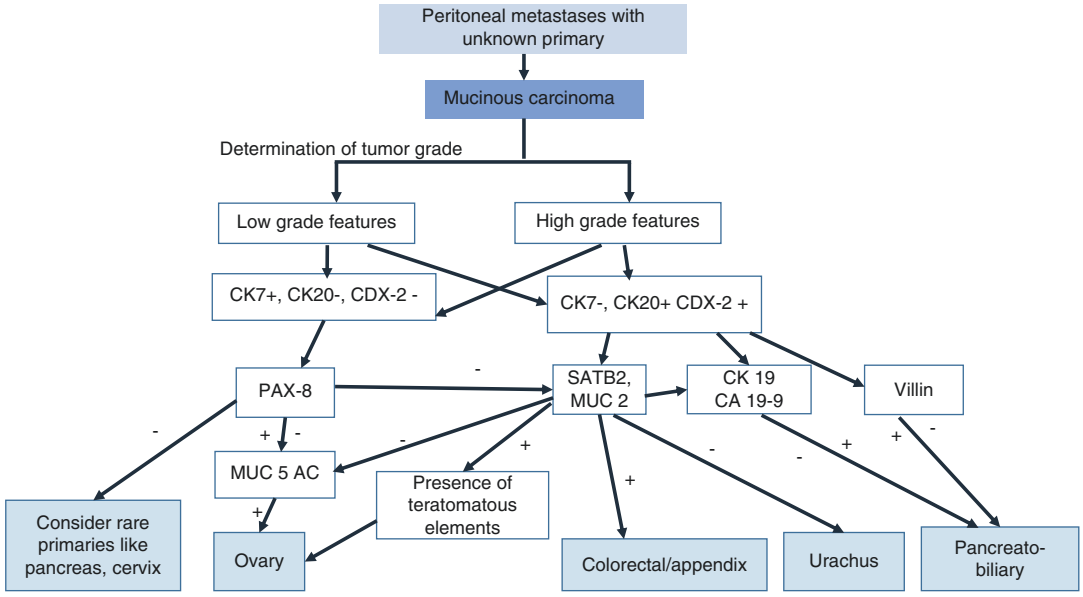

Fig. 11.8 Approach to a patient with mucinous peritoneal carcinomatosis

PM from sarcomas can be present at the time of diagnosis but usually occur in the recurrent setting and are largely due to tumour spillage during surgery. Some rare tumours like epithelioid leiomyosarcomas and gastrointestinal stromal tumours can arise from the omentum or peritoneum itself. In most cases, the primary site is apparent or there is a history of treatment of the primary tumour. The peritoneal sarcomas still require a search for a primary site before attributing the origin to the peritoneum. Peritoneal sarcomatosis with unknown primary has not been described.

11.4.4.1 Clinical Presentation

The endometrial stromal sarcomas are seen only in women. There are no specific clinical features and a detailed history should be elicited. Peritoneal recurrence can occur after several years in both low- and high-grade uterine sarcomas and a history of hysterectomy for a mass is usually present. Ascites is usually absent. The sarcomatosis may be an incidental finding or present with vague abdominal symptoms. In more aggressive tumours like epithelioid leiomyosarcomas, there is ascites with debilitation. The general condition is well preserved in most other cases even in presence of extensive disease. Whole body imaging should be performed to rule out metastases at other sites.

11.4.4.2 Histopathological Features

Each of the sarcomas has distinct histological features and immunohistochemistry and molecular marker profile that is well defined. The problem arises when the diagnosis has to be made on a small sample usually obtained through a trucut biopsy or when the tumours have poor differentiation. We discuss the histopathological features and immunohistochemistry profile of commonest peritoneal sarcomas—endometrial stromal sarcomas, uterine leiomyosarcomas and liposarcomas.

Endometrial Stromal Sarcomas

Endometrial stromal sarcoma (ESS) has been divided into low and high grades in the world health organization (WHO) 2014 classification. High-grade sarcomas are defined by the presence a recurrent chromosomal translocation—t(10; 17) (q22; p13) resulting in *YWHAE-NUTM2A or YWHAE-NUTM2B* genetic fusions (collectively referred to as *YWHAE-NUTM2*) [116].

These rearrangements are mutually exclusive with the *JAZF1/SUZ12/EPC1/PHF1* genetic rearrangements seen in low-grade endometrial stromal sarcomas.

ESS in its commonest form is composed of a proliferation of small, round monomorphic cells with scanty cytoplasm and round to oval nuclei with smooth nuclear contours, which resembles endometrial stroma in the proliferative phase [117–120]. Tumour cells are concentrically arranged around the vascular channels. In the low-grade ESS, mitotic activity is usually low (usually <5/10 HPF). Hyalinization is present and is usually mild though extensive hyalinization may been seen at times. Ischaemic necrosis may be observed. These features are typical of low-grade ESS. These tumours show positive staining for CD10, estrogen receptor (ER) and progesterone receptor (PR) irrespective of the genotypes, and the staining pattern is generally diffuse in adequately fixed tumour samples though it may be patchy and focal in some instances [121–123]. There may be focal patchy staining for smooth muscle actin, caldesmon and/or desmin, with smooth muscle marker staining being more extensive in *JAZF1 LGESS* showing smooth muscle differentiation. The ki-67 proliferation index (<5%) is low and nuclear cyclin D1 expression is typically weak and focal (<5%). KIT expression may be present and tends to be weak and very focal [124–127]. DOG1 expression is consistently absent in low-grade ESS [128]. High-grade ESS on the other hand has characteristic diffusely positive staining for cyclin D1 and is negative for CD10, ER and PR receptors. There is strong cytoplasmic c-KIT staining. Areas of low-grade ESS are seen in *YWHAE-NUTM2 ESS*. The term undifferentiated uterine sarcoma (UUS) is now used for tumours which were previously classified as endometrial undifferentiated sarcomas and they can arise from smooth muscles as well.

UUS is a high-grade sarcoma and exhibits a combination of severe nuclear atypia and high mitotic rate. UUS is a diagnosis of exclusion and often has tumour necrosis. It should be distinguished from other sarcomas (i.e. leiomyosarcoma, rhabdomyosarcoma, high-grade ESS), mixed epithelial-mesenchymal uterine tumours (sarcoma-predominant carcinosarcoma or sarcomatous overgrowth of adenosarcoma), uterine carcinomas (undifferentiated or dedifferentiated endometrial carcinoma) and secondary involvement of the uterus by extra-uterine soft tissue sarcomas [115].

On immunohistochemistry, it can be positive for CD10 and hormone receptors, hence it is important to not regard CD10 as evidence of endometrial stromal differentiation [115]. It may show very focal positive staining for smooth muscle actin, but the presence of positive staining for more than one smooth muscle markers should raise the suspicion for leiomyosarcoma or malignant PEComa [115]. Focal keratin and EMA staining, when encountered in a suspected UUS that demonstrates nuclear uniformity, should prompt a careful investigation into the possibility of undifferentiated or dedifferentiated endometrial carcinoma [115].

Leiomyosarcomas

Leiomyosarcomas have a combination of diffuse moderate-to-severe nuclear atypia, greater than 10 mitotic figures per 10 high power fields (HPF) and presence of (coagulative) tumour-cell necrosis. The presence of any two of these features is essential for the diagnosis of a uterine leiomyosarcoma [129].

In addition to the spindle cell variety, there is an epithelioid variant that is characterized by the presence of rounded or polygonal cells that have a microscopic appearance of ‘epithelial cells’ in at least 50% of the tumour [130]. Immunohistochemical study is sometimes necessary to confirm the smooth muscle nature.

Unfortunately, the tumour cells in about 20% of epithelioid smooth muscle tumours express cytokeratins (as in carcinomas) and less often myogenic markers such as desmin [131, 132].

Though the diagnosis of LMS is usually made on light microscopy, in cases of uncertainty due to poor differentiation, a combination of these markers can be used to determine the smooth muscle origin.

The overexpression of p16 has been identified in 86.7%, 86% and 51% of uterine LMS in

three studies [133–135]. The frequency of overexpression of p53 protein in uterine LMS has been variable and has ranged from 13% to 56.5% [136–139].

Oestrogen, progesterone and androgen receptors are expressed in about 30–40% of LMS. Immunoreactivity with these markers may provide a target for treatment. Even though some LMS show immunoreactivity for CD117 (C-KIT) but there is no underlying KIT oncogenic mutation or KIT phosphorylation, targeted treatment with imatinib is ineffective [140–142].

Liposarcomas

The commonest sarcoma causing intraperitoneal dissemination is a liposarcoma. Liposarcomas are the commonest retroperitoneal tumours and are prone to develop recurrence when they arise in this location as compared to others.

Liposarcomas account for 20% of all soft tissue sarcomas in adults and is the most common retroperitoneal sarcoma [143, 144]. Five histological subtypes of liposarcoma in order of increasing malignant behaviour are well differentiated, dedifferentiated, myxoid, round cell and pleomorphic. Most retroperitoneal liposarcomas are of the well-differentiated and dedifferentiated subtypes [145]. Liposarcomas can also arise intraperitoneally from the omentum and mesentery and present as large intraperitoneal masses [146]. Retroperitoneal liposarcoma is known to recur frequently with multiple intra-abdominal masses after resection [147].

Local recurrence is more common when well-differentiated liposarcoma (WDLPS) arises in the retroperitoneum, mediastinum or paratesticular region and is a cause of morbidity and mortality, as is the emergence of dedifferentiated disease [148]. Dedifferentiated liposarcoma (DDLPS) is a high-grade and aggressive disease, arising most commonly within the retroperitoneum, and is associated with high rates of local and metastatic recurrence and a disease-specific mortality that is six times that of WDLPS [149].

Histologically, WDLPS appears as a proliferation of mature and variably pleomorphic adipocytes intersected by fibrous septa and containing single, enlarged, hyperchromatic nuclei [150]. DDLPS is characterized by more highly cellular areas of high-grade undifferentiated sarcoma typically transitioning abruptly within a background of WDLPS. In most liposarcomas, the histological features alone are enough to make a diagnosis. Immunohistochemistry is a useful adjunct to establish the diagnosis and aid differentiation from non-malignant conditions. The combination of CDK4, MDM2 and p16 is useful in the histologic diagnosis of WDLPS and DDLPS [151]. The *MDM2* gene and its neighbouring gene *CDK4* are amplified, which can be detected by molecular methods such as reverse transcription–polymerase chain reaction (RT-PCR) and FISH [152]. The resultant *MDM2* and *CDK4* protein overexpression can be detected by IHC [152].

p16 is the most sensitive and specific marker for detecting WDLPS/DDLPS, and the combination of *CDK4* and *p16* is of more discriminatory value than the combination of either with *MDM2*, the least sensitive and specific of the three markers [151].

These markers are used to distinguish atypical lipomatous tumour from lipoma as well as dedifferentiated liposarcoma from undifferentiated sarcoma, especially when both markers show positivity. It should be remembered that pleomorphic liposarcoma (PLPS) and myxoid liposarcoma (MLPS) are negative for *MDM2* and *CDK4* [103].

Myxoid liposarcoma (MLPS) accounts for approximately 30% of LPSs and is clinically and pathologically distinct from WD/DDLPS [153]. Over 90% of MLPSs contain a pathognomonic t(12; 16) (q13; p11) translocation that results in expression of the *FUS-DDIT3* fusion protein, whereas a smaller proportion carries *EWSR1-DDIT3* gene fusions [154]. Microscopically, MLPS has small, round-to-oval, non-adipocytic mesenchymal tumour cells alongside a variable number of immature lipoblasts on a background of prominent myxoid stroma. Round cell LPS is now recognized as a high-grade, more cellular variant of MLPS that is associated with worse outcomes [153, 155].

PLPS is a rare and clinically aggressive LPS subtype. Typically arising in the limbs or, less commonly, the trunk or retroperitoneum, PLPS

histologically appears as a high-grade undifferentiated sarcoma without recognizable lineage and contains a variable number of pleomorphic lipoblasts.

Characteristically, PLPSs have complex karyotypes consisting of multiple chromosomal losses and gains, indicating pathogenesis driven by complex and variable molecular events [156].

11.5 Future Directives

During the last few decades, molecular biology has been added to armamentarium of diagnostic pathology. Molecular biology techniques are used to diagnose and subclassify tumours, predict response to therapies and identify therapeutic targets [157].

The development of molecular tumour subclassifications and targeted therapies was facilitated by an improved knowledge of genetic aberrations. Oncogenes and tumour suppressor genes were identified, and their association with metastatic pathways discovered. Next-generation sequencing techniques have helped speed up this process [158]. At present single gene analysis with mutation-specific PCR and Sanger- or pyrosequencing is most commonly used in diagnostic molecular pathology [159]. Molecular tests alone are seldom used for diagnostic purposes currently. They are used to subclassify tumours and identify mutations that can be treated with specific drugs.

Another development in molecular pathology is the analysis of DNA released by dying normal or tumour cells, also termed as cell-free DNA (cfDNA) which can be used as an alternative to tissue biopsy in certain instances. The term 'liquid biopsy' is used for such an analysis [157]. This test requires drawing of a sample of 5–10 mL of peripheral blood as opposed to the more invasive process of deriving a tissue sample. Circulating tumour DNA (ctDNA) is not formalin fixed and thus, any alteration caused by it is avoided. Though there are several indications now for performing a 'liquid biopsy', most of these are still undergoing clinical validation [157, 160].

Tumours have intra-tumoural and inter-metastatic genetic heterogeneity [161]. A tissue biopsy often does not capture the whole spectrum of genetic changes in a tumour. Circulating tumour DNA (ctDNA) that is also detectable in blood may better represent the genetic composition of different tumour compartments. A further advantage is that DNA modifications caused by formalin fixation of tissue and the resulting artefacts in DNA sequencing are not present in ctDNA [162]. However, currently, for the initial tumour diagnosis a tissue biopsy is essential. The biggest challenge in the analysis of cell-free tumour DNA (ctDNA) is the often low frequency of mutated alleles in cfDNA. The amount of ctDNA is variable and ranges from 0.01% to more than 50% of the whole cfDNA [131].

11.6 Conclusions

Peritoneal metastases can present with an occult primary. Careful evaluation of the clinical details, histopathological and immunohistochemistry evaluation can lead to a diagnosis in most cases. Awareness about the common and uncommon tumours giving rise to PM can facilitate the diagnostic process. Molecular tests can be useful adjuncts to conventional histopathological evaluation.

References

1. Rajan F, Bhatt A. Evolving role of CRS and HIPEC: current indications. In: Bhatt A, editor. Management of peritoneal metastases-cytoreductive surgery, HIPEC and beyond. Singapore: Springer; 2018.
2. Kusamura S, Moran BJ, Sugarbaker PH, Levine EA, Elias D, Baratti D, Morris DL, Sardi A, Glehen O, Deraco M, Peritoneal Surface Oncology Group International (PSOGI). Multicentre study of the learning curve and surgical performance of cytoreductive surgery with intraperitoneal chemotherapy for pseudomyxoma peritonei. Br J Surg. 2014;101(13):1758–65. https://doi.org/10.1002/bjs.9674. Epub 20 Oct 2014.

3. Sebbag G, Shmookler BM, Chang D, Sugarbaker PH. Peritoneal carcinomatosis from an unknown primary site. Management of 15 patients. Tumori. 2001;87(2):67–73.
4. Passot G, Vaudoyer D, Villeneuve L, et al. What made hyperthermic intraperitoneal chemotherapy an effective curative treatment for peritoneal surface malignancy: a 25-year experience with 1,125 procedures. J Surg Oncol. 2016;113:796–803.
5. Pavlidis N, Fizazi K. Cancer of unknown primary (CUP). Crit Rev Oncol Hematol. 2005;54:243–50.
6. Frost P. Unknown primary tumours: an example of accelerated (type 2) tumor progression. Basic Life Sci. 1991;57:233–7.
7. Klein CA. Parallel progression of primary tumours and metastases. Nat Rev Cancer. 2009;9(4):302–12.
8. Pavlidis N, Khaled H, Gaafar R. A mini review on cancer of unknown primary site: a clinical puzzle for the oncologists. J Adv Res. 2015;6(3):375–82. https://doi.org/10.1016/j.jare.2014.11.007. Epub 21 Nov 2014.
9. McCluggage WG. Recent advances in immunohistochemistry in the diagnosis of ovarian neoplasms. J Clin Pathol. 2000;53:558–60.
10. McCluggage WG. Recent advances in immunohistochemistry in gynaecological pathology. Histopathology. 2002;46:309–26.
11. McCluggage WG, Young RH. Immunohistochemistry as a diagnostic aid in the evaluation of ovarian tumors. Semin Diagn Pathol. 2005;22:3–32.
12. Ozcan A, Steven SS, Hamilton C, Anjana K, Coffey D, Krishnan B, Truong LD. PAX 8 expression in non-neoplastic tissues, primary tumors, and metastatic tumors: a cornprehensive immunohistochemical study. Mod Pathol. 2011;24:751–64.
13. Chai H, Ren Q, Fan Q, Ye L, Du G, Du H, et al. PAX8 is a potential marker for the diagnosis of primary epithelial ovarian cancer. Oncol Lett. 2017;14:5871–5. https://doi.org/10.3892/ol.2017.6949.
14. Al-Hussaini M, Stockman A, Foster H, McCluggage WG. WT-1 assists in distinguishing ovarian from uterine serous carcinoma and in distinguishing between serous and endometrioid ovarian carcinoma. Histopathology. 2004;44(2):109–15.
15. Oka Y, Tsuboi A, Oji Y, Kawase I, Sugiyama H. WT1 peptide vaccine for the treatment of cancer. Curr Opin Immunol. 2008;20(2):211–20.
16. Makrigiannakis A, Amin K, Coukos G, Tilly JL, Coutifaris C. Regulated expression and potential roles of p53 and Wilms' tumor suppressor gene (WT1) during follicular development in the human ovary. J Clin Endocrinol Metab. 2000;85(1):449–59.
17. Bárcena C, Oliva E. WT1 expression in the female genital tract. Adv Anat Pathol. 2011;18(6):454–65.
18. Liliac L, Carcangiu ML, Canevari S, Căruntu ID, Ciobanu Apostol DG, Danciu M, Onofriescu M, Amălinei C. The value of PAX8 and WT1 molecules in ovarian cancer diagnosis. Rom J Morphol Embryol. 2013;54(1):17–27.
19. Zhao L, Guo M, Sneige N, Gong Y. Value of PAX8 and WT1 immunostaining in confirming the ovarian origin of metastatic carcinoma in serous effusion specimens. Am J Clin Pathol. 2012;137(2):304–9.
20. Dennis JL, Hvidsten TR, Wit EC, et al. Markers of adenocarcinoma characteristic of the site of origin: development of a diagnostic algorithm. Clin Cancer Res. 2005;11(10):3766–72. https://doi.org/10.1158/1078-0432.CCR-04-2236.
21. Bayrak R, Haltas H, Yenidunya S. The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7−/20+ phenotype is more specific than CDX2 antibody. Diagn Pathol. 2012;7(1):1–11. https://doi.org/10.1186/1746-1596-7-9.
22. FitzPatrick DR, Carr IM, McLaren L, et al. Identification of SATB2 as the cleft palate gene on 2q32–q33. Hum Mol Genet. 2003;12(19):2491–501. https://doi.org/10.1093/hmg/ddg248.
23. Conner JR, Hornick JL. Metastatic carcinoma of unknown primary: diagnostic approach using immunohistochemistry. Adv Anat Pathol. 2015;22(3):149–67. https://doi.org/10.1097/pap.0000000000000069.
24. Magnusson K, de Wit M, Brennan DJ, et al. SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. Am J Surg Pathol. 2011;35(7):937–48. https://doi.org/10.1097/PAS.0b013e31821c3dae.
25. Dragomir A, de Wit M, Johansson C, Uhlen M, Ponten F. The role of SATB2 as a diagnostic marker for tumors of colorectal origin: results of a pathology-based clinical prospective study. Am J Clin Pathol. 2014;141(5):630–8.
26. Lin F, Shi J, Zhu S, et al. Cadherin-17 and SATB2 are sensitive and specific immunomarkers for medullary carcinoma of the large intestine. Arch Pathol Lab Med. 2014;138(8):1015–26. https://doi.org/10.5858/arpa.2013-0452-oa.
27. Brandler TC, Jelloul F, Soto D, Das K, Rosen L, Bhuiya TA. Young investigator challenge: cadherin-17 and SATB2 in cytology specimens: do these new immunostains help in differentiating metastatic colorectal adenocarcinoma from adenocarcinomas of other origins? Cancer Cytopathol. 2015;123(12):706–13.
28. Berg KB, Schaeffer DF. SATB2 as an immunohistochemical marker for colorectal adenocarcinoma: a concise review of benefits and pitfalls. Arch Pathol Lab Med. 2017;141(10):1428–33. https://doi.org/10.5858/arpa.2016-0243-RS.
29. Goldstein NS, Bassi D. Cytokeratins 7, 17, and 20 reactivity in pancreatic and ampulla of vater adenocarcinomas. Percentage of positivity and distribution is affected by the cut-point threshold. Am J Clin Pathol. 2001;115:695–702.
30. Park SY, Kim HS, Hong EK, et al. Expression of cytokeratins 7 and 20 in primary carcinomas of the

stomach and colorectum and their value in the differential diagnosis of metastatic carcinomas to the ovary. Hum Pathol. 2002;33:1078–85.
31. Ji H, Isacson C, Seidman JD, et al. Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. Int J Gynecol Pathol. 2002;21:391–400.
32. Chhieng DC, Benson E, Eltoum I, et al. MUC1 and MUC2 expression in pancreatic ductal carcinoma obtained by fine-needle aspiration. Cancer. 2003;99:365–71.
33. Nonaka D, Chiriboga L, Soslow RA. Expression of pax8 as a useful marker in distinguishing ovarian carcinomas from mammary carcinomas. Am J Surg Pathol. 2008;32:1566–71.
34. Tornos C, Soslow R, Chen S, et al. Expression of WT1, CA125, and GCDFP-15 as useful markers in the differential diagnosis of primary ovarian carcinomas versus metastatic breast cancer to the ovary. Am J Surg Pathol. 2005;29:1482–9.
35. Liu H, Shi J, Wilkerson ML, et al. Immunohistochemical evaluation of GATA3 expression in tumors and normal tissues: a useful immunomarker for breast and urothelial carcinomas. Am J Clin Pathol. 2012;138:57–64.
36. Bhargava R, Beriwal S, Dabbs DJ. Mammaglobin vs GCDFP-15: an immunohistologic validation survey for sensitivity and specificity. Am J Clin Pathol. 2007;127:103–13.
37. Ruiz-Tovar J, Alonso HN, Morales CV, Lobo ME, Sanjuanbenito DA, Martinez ME. Peritoneal carcinomatosis secondary to carcinoid tumour. Clin Transl Oncol. 2007;9:804–5.
38. Mertz H, Vyberg M, Paulsen SM, et al. Immunohistochemical detection of neuroendocrine markers in tumors of the lungs and gastrointestinal tract. Appl Immunohistochem. 1998;6:175–80.
39. Vasseur B, Cadiot G, Zins M, et al. Peritoneal carcinomatosis in patients with digestive endocrine tumors. Cancer. 1996;78:1686–92.
40. Vinik AI, Thompson N, Eckhauser F, Moattari R. Clinical features of carcinoid syndrome and the use of somatostatin analogue in its management. Acta Oncol. 1989;28:389–402.
41. Gonzalez RS, Liu EH, Alvarez JR, Ayers GD, Washington MK, Shi C. Should mesenteric tumor deposits be included in staging of well differentiated small intestine neuroendocrine tumors? Mod Pathol. 2014;27:1288–95.
42. Chambers AJ, Pasieka JL, Dixon E, Rorstad O. Role of imaging in the preoperative staging of small bowel neuroendocrine tumors. J Am Coll Surg. 2010;211:620–7.
43. Søreide O, Berstad T, Bakka A, Schrumpf E, Hanssen LE, Engh V, Bergan A, Flatmark A. Surgical treatment as a principle in patients with advanced abdominal carcinoid tumors. Surgery. 1992;111:48–54.
44. Elias D, Lefevre JH, Duvillard P, Goéré D, Dromain C, Dumont F, Baudin E. Hepatic metastases from neuroendocrine tumors with a thin slice CT scan and pathological examination: they are many more than you think. Ann Surg. 2010;251:307–10.
45. Kimura N, Pilichowska M, Okamoto H, et al. Immunohistochemical expression of chromogranins A and B, prohormone convertases 2 and 3, and amidating enzyme in carcinoid tumors and pancreatic endocrine tumors. Mod Pathol. 2000;13:140–6.
46. Al-Khafaji B, Noffsinger AE, Miller MA, et al. Immunohistologic analysis of gastrointestinal and pulmonary carcinoid tumors. Hum Pathol. 1998;29:992–9.
47. Fahrenkamp AG, Wibbeke C, Winde G, et al. Immunohistochemical distribution of chromogranins A and B and secretogranin II in neuroendocrine tumours of the gastrointestinal tract. Virchows Arch. 1995;426:361–7.
48. Sobin LH, Hjermstad BM, Sesterhenn IA, et al. Prostatic acid phosphatase activity in carcinoid tumors. Cancer. 1986;58:136–8.
49. Barbareschi M, Roldo C, Zamboni G, et al. CDX-2 homeobox gene product expression in neuroendocrine tumors: its role as a marker of intestinal neuroendocrine tumors. Am J Surg Pathol. 2004;28:1169–76.
50. La Rosa S, Rigoli E, Uccella S, et al. CDX2 as a marker of intestinal EC-cells and related well-differentiated endocrine tumors. Virchows Arch. 2004;445:248–54.
51. Jaffee IM, Rahmani M, Singhal MG, et al. Expression of the intestinal transcription factor CDX2 in carcinoid tumors is a marker of midgut origin. Arch Pathol Lab Med. 2006;130:1522–6.
52. Dabaja BS, Suki D. Pro B, et al. Adenocarcinoma of the small bowel: presentation, prognostic factors, and outcome of 217 patients. Cancer. 2004;101:518–26.
53. Locher C, Malka D, Boige V, et al. Combination chemotherapy in advanced small bowel adenocarcinoma. Oncology. 2005;69:290–4.
54. Overman MJ, Kopetz S, Wen S, et al. Chemotherapy with 5-fluorouracil and a platinum compound improves outcomes in metastatic small bowel adenocarcinoma. Cancer. 2008;113:2038–45.
55. Talamonti MS, Goetz LH, Rao S, et al. Primary cancers of the small bowel: analysis of prognostic factors and results of surgical management. Arch Surg. 2002;137:564–70.
56. North JH, Pack MS. Malignant tumors of the small intestine: a review of 144 cases. Am Surg. 2000;66:46–51.
57. Frost DB, Mercado PD, Tyrell JS. Small bowel cancer: a 30-year review. Ann Surg Oncol. 1994;1:290–5.
58. Chen ZM, Ritter JH, Wang HL. Differential expression of alpha-methylacyl coenzyme a racemase in adenocarcinomas of the small and large intestines. Am J Surg Pathol. 2005;29:890–6.

59. Wong H, Chu P. Immunohistochemical features of the gastrointestinal tract tumors. J Gastrointest Oncol. 2012;3(3):262–84.
60. Johnson DE, Herndier BG, Medeiros LJ, et al. The diagnostic utility of the keratin profiles of hepatocellular carcinoma and cholangiocarcinoma. Am J Surg Pathol. 1988;12:187–97.
61. Hatano Y, Hatano K, Tamada M, Morishige KI, Tomita H, Yanai H, Hara A. A comprehensive review of ovarian serous carcinoma. Adv Anat Pathol. 2019;26(5):329–39. https://doi.org/10.1097/PAP.0000000000000243.
62. Ramalingam P. Morphologic, immunophenotypic, and molecular features of epithelial ovarian cancer. Oncology (Williston Park). 2016;30(2):166–76.
63. Micco M, Sala E, Lakhman Y, Hricak H, Vargas HA. Role of imaging in the pretreatment evaluation of common gynecological cancers. Womens Health (Lond). 2014;10(3):299–321.
64. Saida T, Tanaka YO, Matsumoto K, Satoh T, Yoshikawa H, Minami M. Revised FIGO staging system for cancer of the ovary, fallopian tube, and peritoneum: important implications for radiologists. Jpn J Radiol. 2016;34(2):117–24.
65. Ehdaivand S. Serous carcinoma. PathologyOutlines.com website. https://www.pathologyoutlines.com/topic/ovarytumorserouscarcinoma.html. Accessed 1 Nov 2019.
66. Sumathi VP, Al-Hussaini M, Connolly LE, Fullerton L, McCluggage WG. Endometrial stromal neoplasms are immunoreactive with WT-1 antibody. Int J Gynecol Pathol. 2004;23(3):241–7.
67. Shimizu M, Toki T, Takagi Y, et al. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. Int J Gynecol Pathol. 2000;19:158–63.
68. McCluggage WG. WT1 is of value in ascertaining the site of origin of serous carcinomas within the female genital tract. Int J Gynecol Pathol. 2004;23:97–9.
69. Chen W, Husain A, Nelson GS, et al. Immunohistochemical profiling of endometrial serous carcinoma. Int J Gynecol Pathol. 2017;36:128–39.
70. McCluggage WG. Immunohistochemical markers as a diagnostic aid in ovarian pathology. Diagn Histopathol. 2008;14(8):335–51.
71. Davidson B. New diagnostic and molecular characteristics of malignant mesothelioma. Ultrastruct Pathol. 2008;32:227–40.
72. Battifora H, McCaughey WTE. Tumors of the serosal membranes. Washington, DC: Armed Forces Institute of Pathology; 1994.
73. Munkholm-Larsen S, Cao CQ, Yan TD. Malignant peritoneal mesothelioma. World J Gastrointest Surg. 2009;1(1):38–48. https://doi.org/10.4240/wjgs.v1.i1.38.
74. Ordóñez NG. What are the current best immunohistochemical markers for the diagnosis of epithelioid mesothelioma? A review and update. Hum Pathol. 2007;38:1–16.
75. Husain AN, Colby T, Ordóñez N, et al., International Mesothelioma Interest Group. Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. Arch Pathol Lab Med. 2013;137(5):647–67.
76. Comin CE, Novelli L, Boddi V, et al. Calretinin, thrombomodulin, CEA, and CD15: a useful combination of immunohistochemical markers for differentiating pleural epithelial mesothelioma from peripheral pulmonary adenocarcinoma. Hum Pathol. 2001;32:529–36.
77. Ordóñez NG. The diagnostic utility of immunohistochemistry and electron microscopy in distinguishing between peritoneal mesotheliomas and serous carcinomas: a comparative study. Mod Pathol. 2006;19:34–48.
78. Davidson B, Nielsen S, Christensen J, et al. The role of desmin and N-cadherin in effusion cytology: a comparative study using established markers of mesothelial and epithelial cells. Am J Surg Pathol. 2001;25:1405–12.
79. Carella R, Deleonardi G, D'Errico A, et al. Immunohistochemical panels for differentiating epithelial malignant mesothelioma from lung adenocarcinoma: a study with logistic regression analysis. Am J Surg Pathol. 2001;25:43–50.
80. Abutaily AS, Addis BJ, Roche WR. Immunohistochemistry in the distinction between malignant mesothelioma and pulmonary adenocarcinoma: a critical evaluation of new antibodies. J Clin Pathol. 2002;55:662–8.
81. Ordóñez NG. Immunohistochemical diagnosis of epithelioid mesothelioma: a critical review of old markers, new markers. Hum Pathol. 2002;33:953–67.
82. Cathro HP, Stoler MH. The utility of calretinin, inhibin, and WT1 immunohistochemical staining in the differential diagnosis of ovarian tumors. Hum Pathol. 2005;36:195–201.
83. Martignoni G, Pea M, Chilosi M, et al. Parvalbumin is constantly expressed in chromophobe renal carcinoma. Mod Pathol. 2001;14:760–7.
84. Osborn M, Pelling N, Walker MM, Fisher C, Nicholson AG. The value of mesothelium-associated T antibodies in distinguishing between metastatic renal cell carcinoma and mesotheliomas. Histopathology. 2002;41:301–7.
85. Lugli A, Forster Y, Haas P, et al. Calretinin expression in human normal and neoplastic tissues: a tissue microarray analysis on 5233 tissue samples. Hum Pathol. 2003;34:994–1000.
86. Marks A, Sutherland DR, Bailey D, et al. Characterization and distribution of an oncofetal antigen (M2A antigen) expressed on testicular germ cell tumors. Br J Cancer. 1999;80:569–78.
87. Kahn HJ, Bailey D, Marks A. Monoclonal antibody D2-40, a new marker of lymphatic endothelium,

reacts with Kaposi's and a subset of angiosarcomas. Mod Pathol. 2002;15:434–40.
88. Fogt F, Pascha TL, Zhang PJ, et al. Proliferation of D2-40-expressing intestinal lymphatic vessels in the lamina propria in inflammatory bowel disease. Int J Mol Med. 2004;13:211–4.
89. Fogt F, Zimmerman RL, Ross HM, et al. Identification of lymphatic vessels in malignant, adenomatous and normal colonic mucosa using the novel immunostain D2-40. Oncol Rep. 2004;11:47–50.
90. Kahn HJ, Marks A. A new monoclonal antibody, D2-40, for detection of lymphatic invasion in primary tumors. Lab Investig. 2002;82:1255–7.
91. Ordóñez NG. Value of mesothelin immunostaining in the diagnosis of mesothelioma. Mod Pathol. 2003;16:192–7.
92. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. Mod Pathol. 2000;13:962–72.
93. Ordóñez NG. Value of estrogen and progesterone receptor immunostaining in distinguishing between peritoneal mesotheliomas and serous carcinomas. Hum Pathol. 2005;36:1163–7.
94. Tandon RT, Jimenez-Cortez Y, Taub R, Borczuk AC. Immunohistochemistry in peritoneal mesothelioma: a single-center experience of 244 cases. Arch Pathol Lab Med. 2018;142(2):236–42. https://doi.org/10.5858/arpa.2017-0092-OA. Epub 19 Oct 2017.
95. Barnetson RJ, Burnett RA, Downie I, Harper CM, Roberts F. Immunohistochemical analysis of peritoneal mesothelioma and primary and secondary serous carcinoma of the peritoneum. Am J Clin Pathol. 2006;125(1):67–76. https://doi.org/10.1309/8fch-q3vp-bwm7-b5x9.
96. Comin CE, Saieva C, Messerini LH. Caldesmon, calretinin, estrogen receptor, and Ber-EP4: a useful combination of Immunohistochemical markers for differentiating epithelioid peritoneal mesothelioma from serous papillary carcinoma of the ovary. Am J Surg Pathol. 2007;31(8):1139–48. https://doi.org/10.1097/pas.0b013e318033e7a8.
97. Tong GX, Chiriboga L, Hamele-Bena D, Borczuk AC. Expression of PAX2 in papillary serous carcinoma of the ovary: immunohistochemical evidence of fallopian tube or secondary müllerian system origin? Mod Pathol. 2007;20(8):856–63. https://doi.org/10.1038/modpathol.3800827.
98. Trupiano JK, Geisinger KR, Willingham MC, et al. Diffuse malignant mesothelioma of the peritoneum and pleura, analysis of markers. Mod Pathol. 2004;17(4):476–81. https://doi.org/10.1038/modpathol.3800067.
99. Hashi A, Yuminamochi T, Murate S-I, Iwamoto H, Honda T, Hoshi K. Wilms tumor gene immunoreactivity in primary serous carcinomas of the fallopian tube, ovary, endometrium, and peritoneum. Int J Gynecol Pathol. 2003;22:374–7.
100. Delhorme JB, Severac F, Averous G, Glehen O, Passot G, Bakrin N, Marchal F, Pocard M, Lo Dico R, Eveno C, Carrere S, Sgarbura O, Quenet F, Ferron G, Goéré D, Brigand C, French National Network of Peritoneal Surface Malignancies (RENAPE). Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for pseudomyxoma peritonei of appendicular and extra-appendicular origin. Br J Surg. 2018;105(6):668–76. https://doi.org/10.1002/bjs.10716. Epub 7 Feb 2018.
101. Bhatt A, Mishra S, Parikh L, et al. Essentials for pathological evaluation of peritoneal surface malignancies and synoptic reporting of cytoreductive surgery specimens—a review and evidence-based guide. Indian J Surg Oncol. 2019;332 https://doi.org/10.1007/s13193-019-00897-7.
102. Vang R, Gown AM, Barry TS, et al. Cytokeratins 7 and 20 in primary and secondary mucinous tumors of the ovary: analysis of coordinate immunohistochemical expression profiles and staining distribution in 179 cases. Am J Surg Pathol. 2006;30:1130–9.
103. Binh MB, Sastre-Garau X, Guillou L, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data. Am J Surg Pathol. 2005;29(10):1340–7.
104. Liu Y, Ishibashi H, Hirano M, Takeshita K, Mizumoto A, Ichinose M, Nishino E, Kashu I, Yamamoto Y, Sugarbaker PH, Yonemura Y. Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy for pseudomyxoma peritonei arising from urachus. Ann Surg Oncol. 2015;22(8):2799–805.
105. Leen SL, Singh N. Pathology of primary and metastatic mucinous ovarian neoplasms. J Clin Pathol. 2012;65:591–5.
106. Carr NJ, Bibeau F, Bradley RF, Dartigues P. The histopathological classification, diagnosis and differential diagnosis of mucinous appendiceal neoplasms, appendiceal adenocarcinomas and pseudomyxoma peritonei. Histopathology. 2017;71(6):847–58. https://doi.org/10.1111/his.13324.
107. Stewart CJ, Ardakani NM, Doherty DA, Young RH. An evaluation of the morphologic features of low-grade mucinous neoplasms of the appendix metastatic in the ovary and a comparison with primary ovarian mucinous tumors. Int J Gynecol Pathol. 2014;33:1–10.
108. Ronnett BM, Yemelyanova AV, Vang R, et al. Endocervical adenocarcinomas with ovarian metastases: analysis of 29 cases with emphasis on minimally invasive cervical tumours and the ability of the metastases to simulate primary ovarian neoplasms. Am J Surg Pathol. 2008;32:1835–53.
109. Cook DS, Attanoos RL, Jalloh SS, Gibbs AR. 'Mucin-positive' epithelial mesothelioma of the peritoneum: an unusual diagnostic pitfall. Histopathology. 2000;37:33–6.
110. Facchetti F, Lonardi S, Gentili F, et al. Claudin 4 identifies a wide spectrum of epithelial neoplasms and represents a very useful marker for carcinoma

versus mesothelioma diagnosis in pleural and peritoneal biopsies and effusions. Virchows Arch. 2007;451:669–80.
111. Facchetti F, Gentili F, Lonardi S, Bercich L, Santin A. Claudin-4 in mesothelioma diagnosis. Histopathology. 2007;51:261–3.
112. McCluggage WG, Kirk SJ. Pregnancy associated endometriosis with pronounced stromal myxoid change. J Clin Pathol. 2000;53:241–2.
113. Diaz L, Okonkwo A, Solans EP, Bedrossian C, Rao MS. Extensive myxoid change in well differentiated papillary mesothelioma of the pelvic peritoneum. Ann Diagn Pathol. 2002;6:164–7.
114. Bhatt A, Ramakrishnan AS. Rare indications for cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. In: Bhatt A, editor. Management of peritoneal metastases—cytoreductive surgery, HIPEC and beyond. Singapore: Springer; 2018.
115. Lee CH, Nucci MR. Endometrial stromal sarcoma—the new genetic paradigm. Histopathology. 2015;67(1):1–19. https://doi.org/10.1111/his.12594. Epub 22 Jan 2015.
116. Lee CH, Ou WB, Marino-Enriquez A, et al. 14-3-3 fusion oncogenes in high-grade endometrial stromal sarcoma. Proc Natl Acad Sci U S A. 2012;109:929–34.
117. Chang KL, Crabtree GS, Lim-Tan SK, et al. Primary uterine endometrial stromal neoplasms. A clinicopathologic study of 117 cases. Am J Surg Pathol. 1990;14:415–38.
118. Evans HL. Endometrial stromal sarcoma and poorly differentiated endometrial sarcoma. Cancer. 1982;50:2170–82.
119. Hendrickson MR, Tavassoli FA, Kempson RL. Mesenchymal tumours and related lesions. In: World Health Organization classification of tumours pathology and genetics of tumours of the breast and female genital organ. Lyon: IARC Press; 2003.
120. Norris HJ, Taylor HB. Mesenchymal tumors of the uterus. I. A clinical and pathological study of 53 endometrial stromal tumors. Cancer. 1966;19:755–66.
121. Chu PG, Arber DA, Weiss LM, et al. Utility of CD10 in distinguishing between endometrial stromal sarcoma and uterine smooth muscle tumors: an immunohistochemical comparison of 34 cases. Mod Pathol. 2001;14:465–71.
122. McCluggage WG, Sumathi VP, Maxwell P. CD10 is a sensitive and diagnostically useful immunohistochemical marker of normal endometrial stroma and of endometrial stromal neoplasms. Histopathology. 2001;39:273–8.
123. Lee CH, Marino-Enriquez A, Ou W, et al. The clinicopathologic features of YWHAE-FAM22 endometrial stromal sarcomas: a histologically high-grade and clinically aggressive tumor. Am J Surg Pathol. 2012;36:641–53.
124. Lee CH, Ali RH, Rouzbahman M, et al. Cyclin D1 as a diagnostic immunomarker for endometrial stromal sarcoma with YWHAE-FAM22 rearrangement. Am J Surg Pathol. 2012;36:1562–70.
125. Klein WM, Kurman RJ. Lack of expression of c-kit protein (CD117) in mesenchymal tumors of the uterus and ovary. Int J Gynecol Pathol. 2003;22:181–4.
126. Nakayama M, Mitsuhashi T, Shimizu Y, et al. Immunohistochemical evaluation of KIT expression in sarcomas of the gynecologic region. Int J Gynecol Pathol. 2006;25:70–6.
127. Caudell JJ, Deavers MT, Slomovitz BM, et al. Imatinib mesylate (gleevec)-targeted kinases are expressed in uterine sarcomas. Appl Immunohistochem Mol Morphol. 2005;13:167–70.
128. Lee CH, Liang CW, Espinosa I. The utility of discovered on gastrointestinal stromal tumor 1 (DOG1) antibody in surgical pathology-the GIST of it. Adv Anat Pathol. 2010;17:222–32.
129. Hendrickson MR, Tavassoli FA, Kempson RL, et al. Mesenchymal tumours and related lesions. In: Tavassoli FA, Devilee P, editors. World Health Organization classification of tumours: pathology and genetics of tumours of the breast and female genital organs. Lyon: IARC Press; 2003. p. 236–43.
130. Bell SW, Kempson RL, Hendrickson MR. Problematic uterine smooth muscle neoplasms. A clinicopathologic study of 213 cases. Am J Surg Pathol. 1994;18:535–58.
131. Oliva E, Young RH, Amin MB, et al. An immunohistochemical analysis of endometrial stromal and smooth muscle tumors of the uterus: a study of 54 cases emphasizing the importance of using a panel because of overlap in immunoreactivity for individual antibodies. Am J Surg Pathol. 2002;26:403–12.
132. Rizeq MN, van de Rijn M, Hendrickson MR, et al. A comparative immunohistochemical study of uterine smooth muscle neoplasms with emphasis on the epithelioid variant. Hum Pathol. 1994;25:671–7.
133. Bodner-Adler B, Bodner K, Czerwenka K, et al. Expression of p16 protein in patients with uterine smooth muscle tumors: an immunohistochemical analysis. Gynecol Oncol. 2005;96:62–6.
134. Atkins KA, Arronte N, Darus CJ, et al. The use of p16 in enhancing the histologic classification of uterine smooth muscle tumors. Am J Surg Pathol. 2008;32:98–102.
135. O'Neill CJ, McBride HA, Connolly LE, et al. Uterine leiomyosarcomas are characterized by high p16, p53 and MIB1 expression in comparison with usual leiomyomas, leiomyoma variants and smooth muscle tumours of uncertain malignant potential. Histopathology. 2007;50:851–8.
136. de Vos S, Wilczynski SP, Fleischhacker M, et al. p53 alterations in uterine leiomyosarcomas versus leiomyomas. Gynecol Oncol. 1994;54:205–8.
137. Blom R, Guerrieri C, Stal O, et al. Leiomyosarcoma of the uterus: a clinicopathologic, DNA flow cytometric, p53, and mdm-2 analysis of 49 cases. Gynecol Oncol. 1998;68:54–61.
138. Jeffers MD, Farquharson MA, Richmond JA, et al. p53 immunoreactivity and mutation of the p53 gene

in smooth muscle tumours of the uterine corpus. J Pathol. 1995;177:65–70.
139. Hall KL, Teneriello MG, Taylor RR, et al. Analysis of Ki-ras, p53, and MDM2 genes in uterine leiomyomas and leiomyosarcomas. Gynecol Oncol. 1997;65:330–5.
140. Lasota J, Jasinski M, Sarlomo-Rikala M, et al. Mutations in exon 11 of c-Kit occur preferentially in malignant versus benign gastrointestinal stromal tumors and do not occur in leiomyomas or leiomyosarcomas. Am J Pathol. 1999;154:53–60.
141. Rushing RS, Shajahan S, Chendil D, et al. Uterine sarcomas express KIT protein but lack mutation(s) in exon 11 or 17 of cKIT. Gynecol Oncol. 2003;91:9–14.
142. Serrano C, Mackintosh C, Herrero D, et al. Imatinib is not a potential alternative treatment for uterine leiomyosarcoma. Clin Cancer Res. 2005;11:4977–9, author reply 9–80.
143. Dalal KM, Antonescu CR, Singer S. Diagnosis and management of lipomatous tumors. J Surg Oncol. 2008;97:298–313.
144. Kim EY, Kim SJ, Choi D, et al. Recurrence of retroperitoneal liposarcoma: imaging findings and growth rates at follow-up CT. AJR. 2008;191:1841–6.
145. Milic DJ, Rajkovic MM, Pejcic VD. Primary omental liposarcoma presenting as an incarcerated inguinal hernia. Hernia. 2005;9(1):88–9.
146. Fotiadis C, Zografos GN, Karatzas G, Papachristodoulou A, Sechas MN. Recurrent liposarcomas of the abdomen and retroperitoneum: three case reports. Anticancer Res. 2000;20(1B):579–83.
147. Jaques DP, Coit DG, Hajdu SI, Brennan MF. Management of primary and recurrent soft-tissue sarcoma of the retroperitoneum. Ann Surg. 1990;212:51–9.
148. Singer S, Corson JM, Demetri GD, et al. Prognostic factors predictive of survival for truncal and retroperitoneal soft-tissue sarcoma. Ann Surg. 1995;221:185–95.
149. Henricks WH, Chu YC, Goldblum JR, et al. Dedifferentiated liposarcoma: a clinicopathological analysis of 155 cases with a proposal for an expanded definition of dedifferentiation. Am J Surg Pathol. 1997;21:271–81.
150. Lee A, Thway K, Huang PH, Jones RL. Clinical and molecular spectrum of liposarcoma. J Clin Oncol. 2018;36(2):151–9.
151. Thway K, Flora R, Shah C, Olmos D, Fisher C. Diagnostic utility of p16, CDK4, and MDM2 as an immunohistochemical panel in distinguishing well-differentiated and dedifferentiated liposarcomas from other adipocytic tumors. Am J Surg Pathol. 2012;36(3):462–9.
152. Wei S, Henderson-Jackson E, Qian X, Bui MM. Soft tissue tumor immunohistochemistry update: illustrative examples of diagnostic pearls to avoid pitfalls. Arch Pathol Lab Med. 2017;141(8):1072–91.
153. Fletcher CDM, Hogendoorn PCW, Mertens F, et al. WHO classification of tumours of soft tissue and bone. Washington, DC: IARC Press; 2013.
154. Crozat A, Åman P, Mandahl N, et al. Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature. 1993;363:640–4.
155. Fiore M, Grosso F, Lo Vullo S, et al. Myxoid/round cell and pleomorphic liposarcomas: prognostic factors and survival in a series of patients treated at a single institution. Cancer. 2007;109:2522–31.
156. Conyers R, Young S, Thomas DM. Liposarcoma: molecular genetics and therapeutics. Sarcoma. 2011;2011:483154.
157. Müllauer L. Milestones in pathology-from histology to molecular biology. Memo. 2017;10(1):42–5. https://doi.org/10.1007/s12254-016-0307-z. Epub 9 Jan 2017.
158. Buermans HP, den Dunnen JT. Next generation sequencing technology: advances and applications. Biochim Biophys Acta. 2014;1842(10):1932–41. https://doi.org/10.1016/j.bbadis.2014.06.015.
159. Harrington CT, Lin EI, Olson MT, Eshleman JR. Fundamentals of pyrosequencing. Arch Pathol Lab Med. 2013;137(9):1296–303. https://doi.org/10.5858/arpa.2012-0463-RA.
160. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579–86. https://doi.org/10.1200/JCO.2012.45.2011.
161. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer. 2012;12(5):323–34. https://doi.org/10.1038/nrc3261.
162. Do H, Dobrovic A. Sequence artifacts in DNA from formalin-fixed tissues: causes and strategies for minimization. Clin Chem. 2015;61(1):64–71. https://doi.org/10.1373/clinchem.2014.223040.