# Pathology of Peritoneal Metastases

## The Unchartered Fields

Olivier Glehen
Aditi Bhatt
*Editors*

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# Preface

This is a book on pathology of peritoneal metastases that has been edited and largely authored by surgeons which is unusual. Peritoneal surface oncology is a field that has always been at the cross roads—in the early years of evolution of surgical treatment, hyperthermia was being increasingly used to potentiate cancer therapy and thus was combined with the surgical treatment of peritoneal metastases, that is, cytoreductive surgery. The surgery itself had to prove its merit over systemic therapies and was burdened with proving the merit of another treatment that added to the morbidity. Similarly, disease biology was only partially understood and remains a major challenge for future progresses. While the prognostic factors were still being identified, and validated, oncology ushered into the era of genetics and molecular biology. And the gaps in understanding the pathophysiology of peritoneal metastases persisted. Pathological expertise has largely been directed at the diagnosis and classification of uncommon tumors.

During cytoreductive surgery that comprises of peritonectomy procedures and visceral resections, a large amount of tissue is submitted for histopathological evaluation. This remains a potential source of prognostic information regarding tumor biology. It provides a good opportunity to also study the patterns and pathways of peritoneal dissemination from various tumors.

In this book, we use these pathological findings to better explain the patterns and pathways of peritoneal cancer dissemination and their potential implications on clinical practice. We provide a rationale and recommendations for standardizing CRS procedures and evaluation of surgical specimens. In turn, we raise research question that can be addressed in future studies.

Some of the other aspects of pathological evaluation like pathological response to chemotherapy, diagnosis and classification of rare peritoneal tumors have also been covered in different chapters. Keeping in sync with the progress in molecular oncology, we look at the role of molecular oncology in the current and future management of peritoneal metastases.

We are grateful to all the contributors for lending their time and expertise to this book. We are also grateful to our pathology colleagues for their invaluable contribution to this work.

3 December 2019

Olivier Glehen
Aditi Bhatt

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# About the Editors

**Olivier Glehen** is a world renowned expert in peritoneal surface oncology and a member of the executive committee of the peritoneal surface oncology group international (PSOGI). He is the head of the General and Oncologic Surgery Department at Centre Hospitalier Lyon Sud (Hospices Civils de Lyon) and at the Lyon Sud Charles Mérieux Medical Faculty. His centre is one of centres that have pioneered the surgical treatment of peritoneal metastases in the world. He is director of the Peritoneal Carcinomatosis Research Group from the EMR 3738 (Claude Bernard Lyon 1 University).

He has published extensively about peritoneal metastases. He is at the head of RENAPE (French Network on rare peritoneal tumours) and BIG-RENAPE groups (National Clinic-Biological Database on Digestive Peritoneal Carcinomatosis). He is associate editor of European Journal of Surgical Oncology, Journal of Surgical Oncology and Journal of Peritoneum. Professor Glehen is one of the directors of the Inter-University Diploma on Peritoneal Carcinomatosis in France and his centre is a reference centre for the European Society of Peritoneal Surface Oncology (ESPSO) certified fellowship in peritoneal surface oncology. His centre performs more than 200 cytoreductive surgery and HIPEC (Hyperthermic Intraperitoneal Chemotherapy) procedures a year and is also one of the leading centers in the world that is developing PIPAC (Pressurized Intraperitoneal Aerosol Chemotherapy).

**Aditi Bhatt** is an Indian surgical oncologist specializing in the management of peritoneal surface malignancies with an experience of 10 years in the same. She is one of the founding members of the Society of peritoneal surface oncology, India, the Indian HIPEC registry and serves as the honorary secretary of the Asian Peritoneal Surface Malignancy Group.

She has published several scientific papers on the subject, edited two special issues of the Indian Journal of Surgical Oncology on the same and edited a book on peritoneal surface malignancies.

# 1 Mechanisms of Peritoneal Metastasis Formation

Yutaka Yonemura, Haruaki Ishibashi, Akiyoshi Mizumoto, Kazuo Nishihara, Yang Liu, Satoshi Wakama, Syouzou Sako, Nobuyuki Takao, Masumi Ichinose, Shun-ichi Motoi, Keizou Taniguchi, Sachio Fushida, Yoshio Endou, and Masahiro Miura

## 1.1 Introduction

It has long been considered that the establishment of peritoneal metastasis (PM) is a multi-step process, consisting of (1) detachment of cancer cells from the primary tumor, (2) adhesion of peritoneal free cancer cells (PFCCs) on the distant peritoneal surface, (3) invasion into the submesothelial tissue, and (4) proliferation accompanying with the angiogenesis and the induction of stromal tissue [1]. The process is called "trans-mesothelial metastasis" by Yonemura et al. [2] or "randomly proximal distribution" by Sugarbaker [3] (Fig. 1.1). Through the process, cancer cells with high malignant potential can metastasize on the peritoneum by concerted expression of metastasis-related genes [1–3]. Recently, new concepts of the formation of PM were proposed: i.e., (1) Trans-lymphatic metastasis and (2) superficial growing metastasis (Table 1.1) [3].

In this chapter, mechanisms of the formation of PM will be described in terms of the morphological, histological, and molecular biological aspects.

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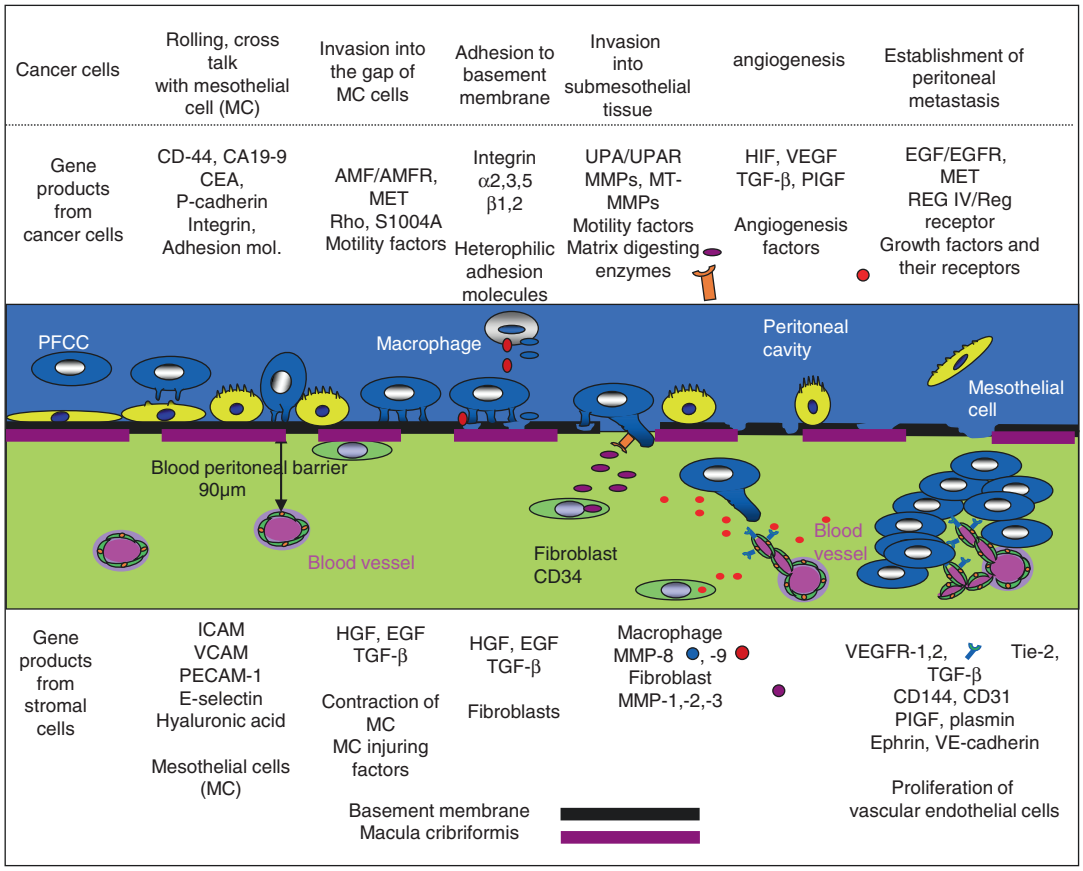


**Fig. 1.1** Trans-mesothelial metastasis as named by Yonemura et al. or randomly proximal distribution by Sugarbaker. Peritoneal free cancer cells (PFCCs) exfoliated from the serosal surface of primary tumors migrate into the peritoneal cavity, and adhere on the peritoneal surface (Process 1). During the rolling of PFCCs on the peritoneal surface, peritoneal mesothelial cells shrink by cytokines produced by PFCCs, resulting in the exposure of basement membrane and macula cribriformis (Process 2). PFCCs then migrate into the inter mesothelial space, and invade into the submesothelial tissue by degradation of extracellular matrix by matrix-digesting enzymes and by locomotive activity using motility factors (Process 3). Finally, cancer cells proliferate near the submesothelial blood vessels with introduction of tumor stromal tissues and neogenesis of tumor blood vessels (Process 4)

**Table 1.1** Three patterns of peritoneal metastasis according to the biological malignant potentials and the morphological feature of peritoneal free cancer cells

| Pattern of metastasis | Biologic malignant behavior | Cancer | Morphological features of PFCCs |
|---|---|---|---|
| Trans-mesothelial metastasis | High | Gastric, colorectal, pancreatic, biliary ovarian cancer | Single or small clusters |
| Trans-lymphatic metastasis<br>Omental milky spots (OMS)<br>Initial lymphatics outside OMS | High, moderate | Gastric, colorectal, ovarian cancer | Single or small clusters |
| Superficial growing metastasis | Moderate, low | AMN[a], GCS[b], mesothelioma, MCM[c], hepatoma | Large with or without mucinous material |

[a]*AMN* low-grade appendiceal mucinous neoplasm
[b]*GCS* granulosa cell tumor
[c]*MCM* multicystic mesothelioma

## 1.2 Mechanisms of Trans-mesothelial Metastasis

### 1.2.1 Mechanisms of Cancer Cell Spillage into the Peritoneal Cavity

The first step of PM is detachment of cancer cells from the serosal surface of the primary tumor in highly malignant tumors and the rupture of appendix vermiformis or ovary by the increased intrinsic pressure due to the proliferation on mucinous neoplasm. Additionally, during surgery, blood or lymphatic fluid contaminated with cancer cells may spill into the peritoneal cavity from damaged blood and lymphatic vessels. The exfoliated cancer cells in the peritoneal cavity are called peritoneal free cancer cells (PFCCs). PFCCs have high proliferative activities and can grow in the distant peritoneum.

In the process of detachment of cancer cells from the primary tumor, homophilic cell-cell adhesion molecules play important roles. Epithelial cells tightly interconnect with a tight junction on the apical membrane and an adherence junction on the basolateral membrane side (Fig. 1.2). Tight junction components are transmembrane proteins, claudin and occludin and the cytoplasmic scaffolding protein, ZO-1, -2, and -3, which bind the actin bundles of the cytoplasmic protein. Tight junction is mainly composed of claudin, which is observed as continuous bead-like particles expressed on the lateral membrane of cells [4]. The partition of the basilar membrane by the tight junction functions as a barrier to control the movement of materials and as a selective permeation channel [4]. However, dysfunction of the tight junction causes the loosening of cell-cell adhesion, resulting in edema. Disappearance of tight junction causes cells to disperse by the loss of polarity. Vascular endothelial growth factor (VEGF) and some cytokines reduce the function of tight junction, and cause edema [5].

Serial analyses of gene expression (SAGE) clarified the reduced expression of claudin in poorly differentiated adenocarcinoma of the stomach [6], in which reduced expression of claudin 4, 7 is found and the patients with gastric cancer showing low expression of claudin have a poor prognosis. In addition, ZO expression is

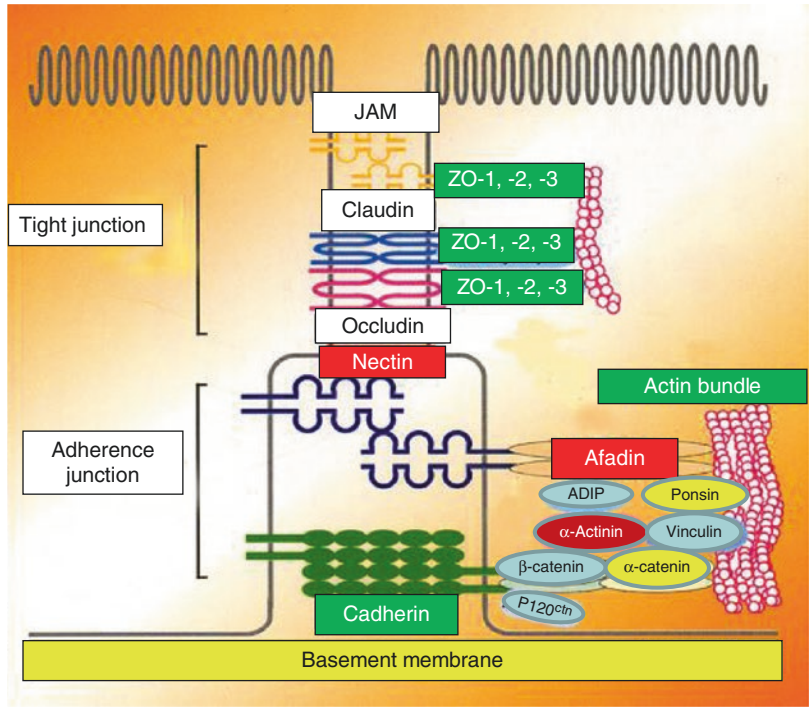


**Fig. 1.2** Molecules associated with homophilic cell-cell adhesion of epithelial cells. Epithelial cells interconnect with tight junction, located at apical membrane of cells, and with adherence junction on the basolateral membrane. Cancer cells showing the functional abnormalities of tight junction and adherence junction tend to disperse and detach from the serosal surface, spill from the primary tumor and migrate into the peritoneal cavity

also reduced in poorly differentiated adenocarcinoma which tends to establish PM [6, 7].

In the components of the adherence junction, E-cadherin expression is important. E-cadherin is a transmembrane glycoprotein, and cells tightly connect with the extracellular domain of the molecule on the basolateral membrane (homophilic adhesion). The intracellular domain of E-cadherin connects with α-, β-, γ-catenin and the complex controls the function of E-cadherin [8–10]. In gastric cancer, poorly differentiated adenocarcinoma especially macroscopic type-4 has a high potential of PM and the downregulation of E-cadherin expression is an important feature [11, 12]. The causes of reduced expression of E-cadherin are loss of the E-cadherin gene, point mutation, and the methylation of the promoter region [12]. In addition, the function of E-cadherin is reduced via the inhibition of the function of catenin molecules by the loss of the α-catenin gene, and phosphorylation of the tyrosine residue of β-catenin [13]. In colorectal cancer, downregulation of E-cadherin level is associated with poorly differentiated type, higher potential of metastasis, and progression [14]. The lower expression of E-cadherin in poorly differentiated colorectal cancer may explain the aggressive nature, and poorly differentiated adenocarcinoma is known as a subtype that frequently metastasizes on peritoneum [14, 15].

Accordingly, cancer cells with the functional abnormalities of tight junction and adherence junction tend to disperse and detach from the serosal surface, spill from the primary tumor and migrate into the peritoneal cavity.

### 1.2.2 Adhesion of PFCCs and Mesothelial Cells (Fig. 1.1, Process 1)

PFCCs migrate on the distant peritoneal surface and adhere to mesothelial cells during rolling on the mesothelial cell surface. PFCCs express several kinds of integrin molecules and adhere with their ligands expressed on mesothelial cells (Fig. 1.1, Process 1, 2). Peritoneal mesothelial cells express immunoglobulin-like intercellular adhesion molecules (ICAM-1, CD54) [16] and vascular cell adhesion molecules (VCAM-1, CD106) [17], and interact with integrin αLβ2 (LFA-1α, CD11a) expressed on PFCCs and inflammatory cells (heterotypic cell-cell adhesion).

Platelet-endothelial cell adhesion molecules (PECAM-1, CD31) play a role in the transmigration of white blood cells and PFCCs. After the process of slowing down the passage of leukocytes and PFCCs over activated vascular endothelium and peritoneal mesothelial cells ("rolling"), integrins are crucial for stopping the cells at the extravasation site and migration into submesothelial tissue [18].

Stopping is the result of the interaction of integrins on the leukocytes (β2 integrin, α4β1 (VLA-4), or α4β7) and immunoglobulin-like adhesion molecules (ICAM-1 and VCAM-1) on endothelial and mesothelial cells [19]. Next, for migration based on the interaction between β2 integrins on the leukocytes and ICAM-1, VCAM-1, and PECAM-1 on the endothelial cells, mesothelial cells adhere with inflammatory cells or PFCCs via homophilic adhesion of PECAM-1. The expression of VCAM-1, and E-selectin, is upregulated by IL-1β, TNF-1α, and IFN-γ, so the mesothelial cell adhesion can be facilitated by local inflammation [16, 20].

As shown in Fig. 1.3, several adhesion molecules are associated with the adhesion between mesothelial cells and PFCCs. The only classical cadherin expressed by mesothelial cells and PFCCs is P-cadherin, and this molecule could serve homophilic heterotypic adhesion (heterophilic adhesion by the same adhesion molecule) between PFCCs and mesothelial cells (Fig. 1.3) [21]. P-cadherin promotes intraperitoneal dissemination of ovarian cancer cells by facilitating tumor cell aggregation and tumor peritoneum interaction in addition to promoting tumor cell migration [22].

Hyaluronic acid (hyaluronate) is a mucoprotein with a molecular weight of 200,000–400,000, consisting of alternate binding of *N*-acetylglucosamine and glucuronic acid. This molecule is one of the components of extracellular matrices and acts as a cushion to support the

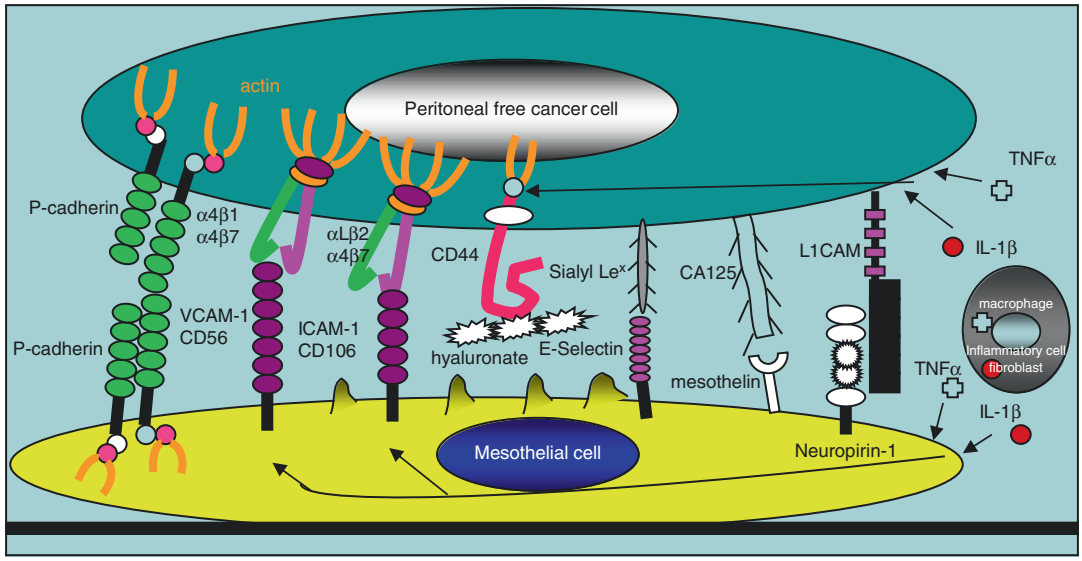


**Fig. 1.3** Heterotypic adhesion molecules associated with adhesion between cancer cells and mesothelial cells

cells by binding a considerable amount of water. Pericellular hyaluronate produced by mesothelial cells has a function as a lubricant (Fig. 1.3). CD44 is a transmembrane glycoprotein and acts as a receptor for hyaluronate. This molecule has four functioning domains, and hyaluronate binds with the extracellular domain of CD44. PFCCs expressing CD44 bind to the pericellular hyaluronate of the mesothelial cells. The CD44 gene has 20 exons and many translational products (v1–v10) are produced from alternative RNA splicing and post-translational modifications. Certain CD44 isoforms that regulate activation and migration of lymphocytes and macrophages may also enhance local growth and metastatic spread of tumor cells [23]. CD44v, v7 splice variants are expressed by some gastro-intestinal cancers, and considered as markers for their metastatic capability [24]. CD44v6 expressed on PFCCs accounts for the binding with mesothelial cells [24]. The TGF-β produced from the fibroblasts in the stroma of cancer tissue upregulates the CD44 expression from cancer cells [25]. Expression of CD44 by peritoneal mesothelial cells also seems to contribute to heterotypic cell adhesion by pancreatic cancer cells, and is upregulated by TNF-α/IL-1β from the peritoneal macrophages [26].

The sialyl Lewis-a (sLe$^a$) antigen is a carbohydrate structure present on cancer cells and has a structure of sialic form of Le$^a$ antigen with sialyl acid. The monoclonal antibody CA19-9 is a useful and popular tool to assess circulating sialyl Lewis-a epitopes in the blood of cancer patients. sLe$^a$ expressed on leukocytes binds to P-cadherin on the activated vascular endothelial cells, and the weak affinity interaction of sLe$^a$ and P-cadherin is considered as the initial force implicated in the "rolling" of extravasating leukocytes. E-selectin also can bind to sLe$^a$, and sLe$^a$ on cancer cells produces a similar interaction with E-selectin on the endothelial cells [27]. As shown in Fig. 1.3, E-selectin is also found on peritoneal mesothelial cells. The interaction of E-selectin and P-cadherin on mesothelial cells and sLe$^a$ on PFCCs has a role on the heterotypic adhesion in early pathophysiological event in PM.

CA125 is a cell surface mucin-like glycoprotein expressed in mesothelial cells, and is upregulated in malignant ovarian tumors [28, 29]. It is considered as a relatively specific circulating tumor marker in ovarian cancer patients. Mesothelin is expressed by the normal mesothelial cells, and soluble mesothelin is used to detect the overexpressed protein as a circulating tumor

marker for mesothelioma. Mesothelin binds with CA125 expressed on PFCCs and has a role as an adhesion molecule between ovarian cancer cells and mesothelial cells [30].

L1CAM (CD171), an adhesion molecule expresses on pancreas cancer and colorectal cancer cells and binds with neuropilin-1 (Fig. 1.3). It is upregulated by TGF-β1 [31]. Soluble L1CAM (sL1) binds to VEGF-A (165), and activates VEGFR-2, resulting in angiogenesis [32].

### 1.2.3 Morphological Changes of Mesothelial Cells (Fig. 1.1, Process 1), Submesothelial Invasion of PFCCs, Attachment of PFCCs to the Basement Membrane (Fig. 1.1, Process 2)

Mesothelial cells are flat and squamous-like cells and connect to each other with a tight junction. The diameter of mesothelial cell is approximately 25 μm (Fig. 1.4). Mesothelial cells provide a protective barrier against invading pathogens and PFCCs. The surface of activated mesothelial cells has a well-developed microvilli varying in length, shape, and density (Fig. 1.5). Cilia are also present on some resting mesothelial cells, but are more abundant on activated cells. They may be part of a sophisticated surveillance system that may respond to elicit discrete cellular responses [33].

ICAM-1 and VCAM-1 are expressed on the microvilli of mesothelial cells, and the materials correlate with the cross-talk of cells expressing integrins. Mesothelial cells produce IL-6, IL-1 (expressed by bacterial lipopolysaccharide), heat shock proteins, granulocyte-colony stimulating factor (G-CSF), IL-15, IL-1β, TNF-α, and epidermal growth factor (EGF) to have cross-talk, and these substances immediately react with the changes in the peritoneal environment [34, 35]. When PFCCs contact with mesothelial cells, many changes are found in the mesothelial cells (Figs. 1.4 and 1.5).

Akedo et al. reported three growth patterns of cancer cells are found when rat hepatoma cells were co-cultured with a rat mesothelial monolayer [36]. Tumor cells either formed "pile-up" nests upon the mesothelial monolayer, exhibited invasive growth between adjacent mesothelial cells (flattened tumor cell island), or failed to attach and grew in suspension.

When ascitic fluid was added into the medium of the mesothelial monolayer, the mesothelial cells took up a characteristic "round" morphology with separation of cell–cell contacts after

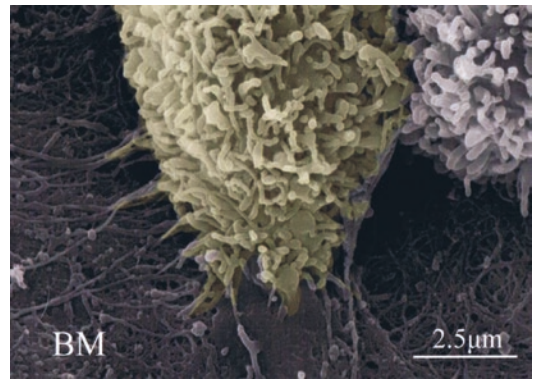


**Fig. 1.5** Activated human mesothelial cells. Microvilli expressed on cell surface of human mesothelial cells from resected specimens from pseudomyxoma peritonei

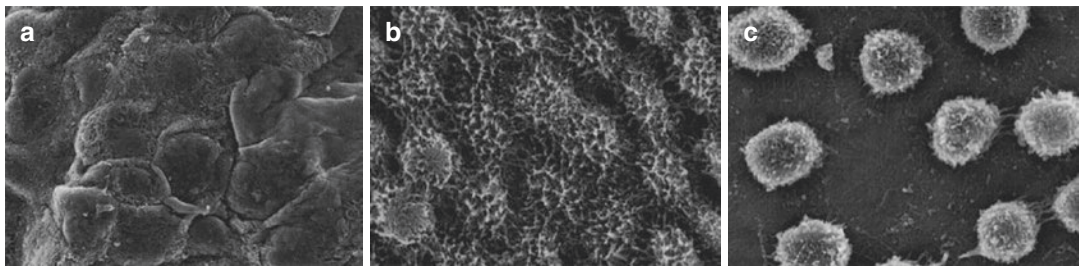


**Fig. 1.4** Morphological changes of mesothelial cells. (**a**) Normal, (**b**) shrinkage of cytoplasm, (**c**) separation and exposure of submesothelial basement membrane (human mesothelial cells)

20 h [37]. These results denote that the malignant ascitic fluid contains factors that induce the changes of mesothelial cell morphology (mesothelial cell injury factors). These factors are produced from cancer cells, peritoneal macrophages, and mesothelial cells [38].

### 1.2.4 Adhesion of PFCCS to the Submesothelial Basement Membrane (Fig. 1.1, Process 2 and Fig. 1.6)

After mesothelial cell contraction by cytokines, the submesothelial basement membrane is exposed (Fig. 1.4). The basement membrane consists of laminin, type IV collagen, heparin sulfate proteoglycan, entactin, and perlecan. Mesothelial cells and fibroblasts produce these elements. Current evidence suggests that adherence to the basement membrane of PFCCs is mediated via an integrin-ligand interaction.

The integrin molecule is a heterodimer consisting of an α and a β subunit and is expressed on the cell membrane. Integrins are the important molecules for cell-cell and cell-ECM adhesion. According to the combination of 17α subunits and 8β subunits, 24 kinds of integrins exist [39]. Many kinds of integrins are expressed from PFCCs, and the overexpression of integrins correlates with metastatic potential [40–42]. Integrin α2 and α3 expressions were significantly elevated in the peritoneal dissemination of gastric cancer [40, 41]. These α-integrins dimerize with β-subunits to form adhesion molecules for basement membrane proteins, including fibronectin, laminin, and collagen IV. Treatment with anti-β1 integrin antibody significantly inhibited the adherence of highly metastatic cell line on the peritoneum in an ex-vivo peritoneal model, suggesting a role for β1-mediated integrin adhesion to the submesothelial basement membrane [41]. In ovarian cancers, integrin α5β1 and α6β1 correlate with PM.

### 1.2.5 Invasion into the Submesothelial Tissue (Fig. 1.1, Process 3)

Factors associated with invasion into the submesothelial tissue are the autocrine motility factor (AMF)/AMF receptor, Rho/ROCK, S100A-4, and hepatocyte growth factor (HGF)/MET (receptor for HGF) [43–48].

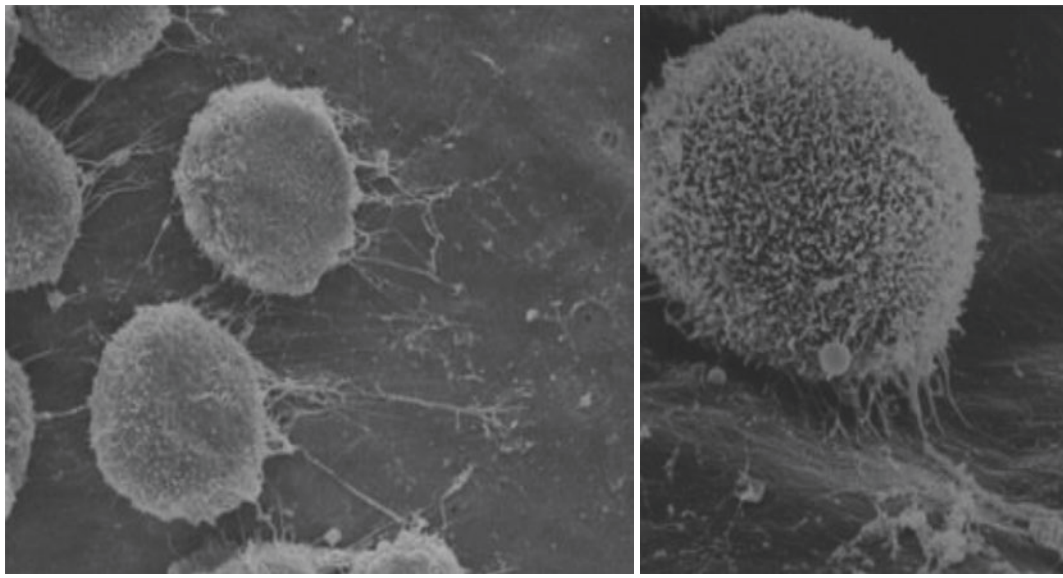

**Fig. 1.6** Highly metastatic cell line (MKN-45) from gastric cancer express filopodia, and attach to the basement membrane of human greater omentum

AMF is a 55 kDa protein, which stimulates chemotaxis and chemokinetics [49]. AMFR is a member of the tyrosine kinases, which are located on the cell membrane. Binding of AMF with AMFR stimulates changes in the cytoskeleton and formation of invadopodia, resulting in the induction of amoebic movement [43]. Type 4 gastric cancer is more significantly associated with PM than the other macroscopic types. In type 4 gastric cancer, expression of the AMFR protein was significantly higher than that in type 3 tumors [50]. Accordingly, poorly differentiated adenocarcinoma of the stomach has high motility by the activation of the AMF/AMFR cascade combined with downregulation of E-cadherin and claudin [7, 12, 50].

Rho is a G protein, which induces ruffling of the cell membrane in cooperation with effectors of its downstream, like mDia, Crk, Rac and ROCK, and FAK/paxillin [49]. Rho upregulates actin filaments by activation of mDia, and ROCK increases the contractile strength of myosin, which bridges actin filaments. Rho and Rac expressions were upregulated in poorly differentiated adenocarcinoma and advanced cancers in the late stage [43].

S100A4, a member of the S100 protein family, is known as a calcium-binding protein, and increases cell motility by activating myosin [44]. S100A4 activates myosin in lamellipodia expressed on the invasion front of cancer cells (Fig. 1.6) [45]. The actin filament that bridges myosin, combined with the vinculin connected with talin and the intracellular domain of integrin [45, 46]. In gastric cancer, S100A4 upregulation is significantly associated with poorly differentiated adenocarcinoma, lymph node metastasis, peritoneal dissemination, and a poor prognosis [46]. In addition, downregulation of E-cadherin and upregulation of S100A4 were found in type 4 gastric cancer [46]. Moriyama et al. reported that S100A4 gene was transfected into a non-invasive oral cancer cell line of OSC-19, and that the new cell line overexpressed S100 A4, showed significant invasive activity, and downregulated E-cadherin and β-catenin [47].

The scatter factor (SF) called hepatocyte growth factor (HGF) and its receptor of MET (a tyrosine kinase type receptor) are important molecules for cell motility and proliferation. When HGF binds with MET, MET is activated by the autophosphorylation of tyrosine residue on the intracellular domain and induces cell motility by activation of F-actin and microtubules. In the peritoneal cavity, HGF is produced from activated mesothelial cells, and fibroblasts, and induces mesothelial cell contraction and invasion of PFCCs through the intercellular space of mesothelial cells [48].

IL-1β, and TNF-α from peritoneal macrophages, fibroblasts, and inflammatory cells induce HGF production from mesothelial cells [51]. The HGF/MET paracrine cascade correlates with not only cancer cell motility but also proliferation and angiogenesis. Recently, molecular targeting therapy to control the cascade has been developed [52].

### 1.2.6 Destruction of Submesothelial Basement Membrane and Extracellular Matrix (ECM) and Invasion into Submesothelial Tissue (Fig. 1.1, Process 3)

The tissue between mesothelial cells and submesothelial arterial blood capillaries is named the peritoneal-blood barrier, and the average width is 90 μm (Figs. 1.1, 1.17 and 1.29) [53]. This barrier prohibits the diffusion of drugs administered by systemic chemotherapy. The diffusion length of oxygen from arterial blood capillaries is 100 μm, and PFCCs attached to the submesothelial basement membrane can survive by the oxygen nutritional supplement from blood vessels [54]. PFCCs with high invasive capacity destroy the ECM in peritoneal-blood barrier, invade near the arterial blood capillaries, and proliferate with angiogenesis (Fig. 1.1, Process 4).

The subperitoneal basement membrane between mesothelial cells and submesothelial stromal tissue is a thin membrane of 50–100 nm in width, and is composed of collagen type IV, laminin, entactin, heparin sulfate proteoglycan, and perlecan [55, 56]. Molecules associated

with the destruction of basement membrane are matrix metalloproteinases [MMPs: MMP-2, MMP-7, MMP-14 (MT1-MMP)] and plasmin. These molecules are produced from cancer cells, mesothelial cells, fibroblasts, inflammatory cells, and macrophages. Subperitoneal tissues are composed of dense network of ECM, which prohibits the movement of materials with molecular weight higher than 100,000. Cancer cells produce several kinds of matrix-digesting enzymes to destroy and invade into subperitoneal stromal tissue. An immunohistochemical study of gastric cancers revealed that urokinase-type plasminogen activator (UPA) is detected in the cytoplasm in 66% of gastric cancers [57]. UPA from gastric cancer and fibroblasts binds with its receptor (UPAR) on the cell membrane and is activated with plasmin and kallikrein. Activated UPA on the cell membrane activates plasminogen to plasmin [58]. Plasmin then degrades the ECM and further activates plasminogen and latent MMPs. UPA is specifically inactivated by plasminogen activator inhibitor-2 (PAI-2), and PAI-2 can inhibit the formation of experimental peritoneal carcinomatosis [59, 60]. UPAR expression in type 4 gastric cancers is significantly higher than that in other macroscopic types [57]. Fibroblasts accumulate in the stroma of the invasive front of type 4 gastric cancer. UPA secreted from fibroblasts is combined with UPAR on the cancer cells via the paracrine loop, leading to activation of plasmin in the cancer cells which help them to invade the stomach wall [60].

MMP family includes collagenases (MMP-1, MMP-8, and -13), gelatinases (MMP-2 and MMP-9), stromelysin-1, -2 (MMP-3, and MMP-10), transmembrane MMPs (MT-MMP families), and others: matrilysin, MMP-7; stromelysin-3, MMP-11; metalloesterase, MMP-12; and enamelysin, MMP-20. Activities of MMPs are controlled by the activation of proMMPs and inhibition by TIMPs (tissue inhibitor metalloproteinases), and MMPs are mutually activated by plasmin and the other MMPs. Four types of TIMPs have been reported and control the activity of MMPs, resulting in the degradation of collagen and the induction of fibrosis.

MMP genes are upregulated by IL-1, TNF-α, EGF, PDGF, and FGF. MMP-1, -2, -7, -13, and -14 (MT1-MMP) play roles in the stromal invasion of gastric cancer.

MMP-1 specifically cuts the helix structure of collagen types I, II, and III. In gastric cancer tissue, MMP-1 is secreted from the stromal fibroblasts. TGF-β produced from poorly differentiated adenocarcinomas of the stomach stimulates the proliferation of fibroblast in the invasive front [61]. Cancer cells invade the stroma utilizing MMP-1, and MMP-2 produced from the fibroblasts. TGF-β inhibits the proliferation of the epithelial cells. In contrast, TGF-β II receptor expression is downregulated in type 4 gastric cancer, which evades the inhibition of proliferation by TGF-β from fibroblasts [61].

MMP-2 (gelatinase A) degrades gelatin, collagen types IV, V, VII, X, and XI, fibronectin, elastin, and proteoglycan, which are components of the ECM [52]. TIMP-2 combines with activated MMPs and proMMP-2, and controls the activity and degradation of MMP-2. ProMMP-2 (72 kDa) when activated by MT1-MMP becomes active MMP-2 (62 kDa), which activates MMP-9 and MMP-13, resulting in the degradation of many kinds of ECM components.

MT-MMP is detected on the cell membrane (Fig. 1.7) and plays roles in cell migration,

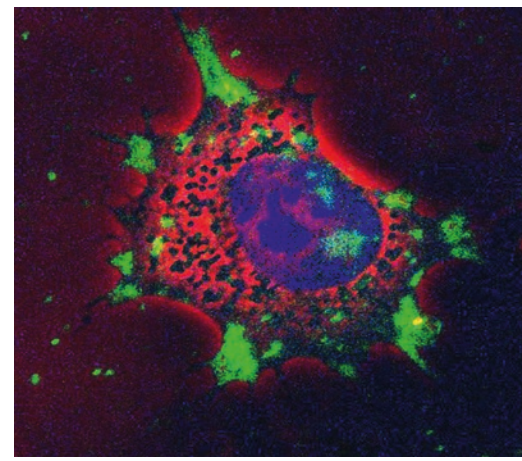

**Fig. 1.7** MT1-MMP expression on the invadopodia. Immunofluorescent staining with anti-MT1-MMP mAb for TMK-1 cells (gastric cancer cell line), transfected with MT1-MMP gene

differentiation, and morphological change by the degradation of the pericellular ECM. The MT-MMP family has 6 kinds of molecules (MT1-6-MMP).

MT1-MMP forms a homo-oligomer on the pseudopodia of cancer cells, and induces an efficient invasion by the degradation of their pericellular ECM [53]. MT1-MMP itself degrades collagen types I, II, and III, fibronectin, laminin, vitronectin, and aggrecan, and plays a role in the activation of proMMP-2 [54]. TIMP-2 combines with a catalytic domain of MT1-MMP. A complex of TIMP-2-MT1-MMP binds with proMMP-2 and forms a tertiary complex. ProMMP-2 becomes an intermediate active MMP-2 by the activation of neighboring MT1-MMP [55]. In poorly differentiated gastric cancers, the MMP-2 secreted from fibroblasts is activated by MT1-MMP. A paracrine loop of MMP-2 from fibroblasts and MT1-MMP on gastric cancer induces invasion and metastasis of gastric cancer [56].

MMP-7 (matrilysin) itself degrades collagen types I, II, III, and IV, aggrecan, laminin, and fibronectin, and can activate proMMP-1, -3, -8, and -9 secreted from cancer cells and fibroblasts. As a result, almost all ECM components can be degraded by MMP-7. A study of serial analyses of gene expression of gastric cancer revealed the overexpression of MMP-7 [57]. A highly metastatic cell line (MKN-45-P) on the peritoneal surface overexpressed MMP-7 [58]. Intraperitoneal administration of an antisense oligonucleotide against MMP-7 mRNA improved the survival of the mice bearing MKN-45-P [59]. The incidence of MMP-7 protein expression in the type 4 gastric cancer is significantly higher than that of the other macroscopic types [43].

MMP-13 is produced from cancer cells and chondrocytes and degrades collagen types I, II, and III. MMP-13 mRNA was expressed in 8 of 9 gastric cancer cell lines, and in these cell lines MMP-13 mRNA was coexpressed with MMP-2 and MT-1 MMP, which activate proMMP-13 [60]. MMP-13 mRNA expression was found in 61% of gastric cancer patients in stage IV disease [62], and the prognosis in patients with MMP-13 overexpressing tumor was significantly poorer than in those without MMP-13 expression. Patients with tumor expressing both MMP-13 and MT1-MMP showed the worst prognosis [60].

### 1.2.7 Proliferation in the Subperitoneal Tissue (Fig. 1.1, **Process 4: Angiogenesis and Proliferation**)

Tyrosine kinases play a major role in the proliferation of cancer cells. The interaction of the growth factors with the receptors activates signaling pathways and induces mitogenesis. Among these receptors, K-sam, EGFR, MET, vascular endothelial growth factor receptor (VEGFR), and ERBB are frequently involved in PM of various cancers. In gastric cancers, expressions of K-sam, EGFR, MET, and VEGFR are associated with proliferation and angiogenesis.

The *K-sam* gene encodes the receptors against fibroblast growth factor (FGF) and keratinocyte growth factor (KGF). When the *K-sam* gene product is activated, the ras-raf-MAP kinase cascade is activated and cell proliferation is induced [63]. In type 4 gastric cancer, *K-sam* gene amplification is a characteristic feature. In the poorly differentiated types of gastric cancer, expression of bFGF for the ligand of K-sam is significantly upregulated, and cancer cell proliferation is stimulated by the autocrine or paracrine loop [64]. In an immunohistochemical study of keratinocyte growth factor (KGF) and K-sam expression, the incidence of K-sam expression was significantly higher in type 4 gastric cancer than in the other types. In addition, patients with tumor coexpressing K-sam and KGF had significantly poorer prognosis [65]. Accordingly, the paracrine loop of K-sam/KGF/bFGF has an important role in the progression of gastric cancer, especially in poorly differentiated type and type 4 gastric cancer.

The epidermal growth factor receptor (EGFR) and its family of Her-2/ERBB-2, Her-3, and Her-4 are upregulated in 70% of all cancers [66, 67]. Signals of EGFR are transduced through the ras-raf-MAP kinase route, PI3K-Ak route, and Jak-STAT route, and they induce proliferation,

growth, and apoptosis. Type 4 gastric cancer co-expresses EGF and EGFR [68].

MET is associated with not only cell scattering, but also proliferation [69]. In gastric cancer, alteration of the domain induces a constant activation of the downstream components [70]. In addition, *c-met* gene amplification is detected in gastric cancer, and upregulates the signal transduction downstream of MET activated in a ligand-dependent or non-ligand-dependent manner [71]. Many molecular targeting strategies to decease MET function by controlling Try 1003 have been studied [72, 73].

When the diameter of a cancer nest is greater than 100 μm, oxygen and nutritional supplementation from preexisting blood vessels are not sufficient for survival of cancer cells. Accordingly, cancer cells that are located more than 100 μm apart from blood vessels will die off.

In such a situation, angiogenesis is induced by angiogenic factors secreted from cancer cells, and cancer tissue with newly formed vessels can be established. Proliferating cancer cells upregulate hypoxia inducible factor (HIF)-1α to induce angiogenesis [74], and the expression of vascular endothelial growth factor (VEGF) is stimulated [75, 76]. VEGF binds with VEGF receptor-1 and stimulates endothelial cell proliferation. Almost all cancer cells produce VEGF, which has a major role in the establishment of PM [77]. VEGF-C, which is a specific molecule for lymphangiogenesis, activates VEGFR-3 (flt-4) [64].

## 1.3 Trans-lymphatic Metastasis

In 2012, Yonemura reported a new concept of PM formation, called trans-lymphatic metastasis [78] (Fig. 1.8). Trans-lymphatic metastasis is the metastatic pathway by which the PFCCs migrate into the submesothelial initial lymphatic vessels through mesothelial stomata (Fig. 1.9), and holes of macula cribriformis (Fig. 1.10) [78]. Subperitoneal lymphatic vessels associated with trans-lymphatic metastasis are found in omental milky spots (OMS) and initial lymphatic vessels in parietal peritoneum, and small bowel mesentery.

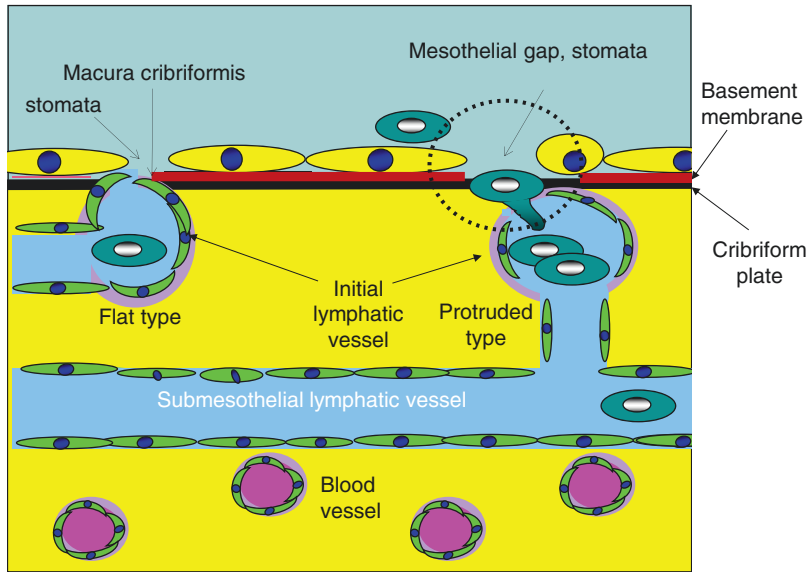


**Fig. 1.8** Initial lymphatic vessels, which directly connect with peritoneal cavity via mesothelial stomata and hole of macula cribriformis. Two types of initial lymphatic vessels on the parietal peritoneum. Flat type (Left) and protruded type (Right). The former is found on the Morrison's pouch, paracolic gutter, and small bowel mesentery. The latter is detected on the pelvic peritoneum. PFCCs migrated into initial lymphatic vessels through mesothelial stomata and holes of macula cribriformis

OMS are found on the omentum and are the small lymphatic organs for the migration of peritoneal inflammatory cells and the absorption of peritoneal fluid. Mean number of OMS in adult is 26/cm$^2$ and the diameters range from 15 to 800 μm [2, 79]. OSM is also linked to the dissemination of cancer cells [80]. Under scanning electron microscopy, OMS was found to have oval or round concave structure covered with cuboidal mesothelial cells (Fig. 1.11, upper right). After digestion of cuboidal mesothelial cells by 6 N KOH, concave pouch with holes of macula cribriformis is detected (Fig. 1.12). Under whole-mount preparation specimens stained with 5′-nucleotidase (5′-Nase) and alkali-phosphatase double enzyme staining, agglomerated blood capillaries are found under the macula cribriformis of OMS (Fig. 1.11, upper left). Additionally, 5′-Nase-enzyme-staining shows a lymphatic plexus under macula cribriformis of OMS (Fig. 1.13). Figure 1.14 is a vertical section of human OMS, stained with D2-40 monoclonal antibody (Mab). Lymphatic plexus stained brown with D2-40 Mab is found beneath the cuboidal mesothelial cells. Surface of OMS small gaps between cuboidal mesothelial cells similar to stomata on the diaphragm are found (Fig. 1.15), and macrophages are detected in the mesothelial gap (Fig. 1.16). Intraperitoneal inflammatory cells and PFCCs migrate into OMS lymphatic vessels through the mesothelial cell gap from peritoneal cavity to OSM lymphatic plexus [80, 81] (Figs. 1.11–1.16). These findings are similar to the mechanisms of the leukocyte extravasation into the inflammatory stroma [18]. These results indicate that lymphatic plexus of OMS can be considered a kind of initial lymphatic vessels.

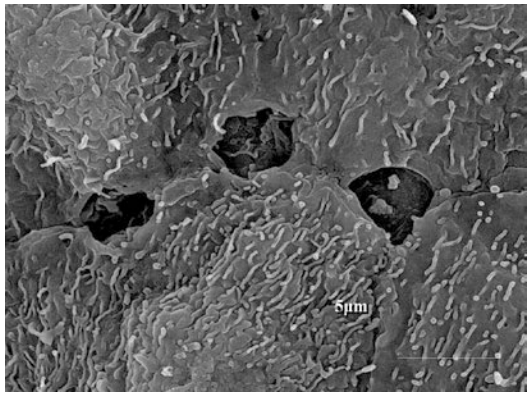


**Fig. 1.9** Stomata on the diaphragm. Gap between mesothelial cells of diaphragm, which connects with submesothelial lymphatic vessel through holes of macula cribriformis, located just below the mesothelial basement membrane

Structure of initial lymphatic vessels outside OMS is different. Figure 1.17 shows the locations of submesothelial lymphatic vessels and blood vessels of human parietal peritoneum. The part of the lymphatic vessels in the parietal peri-

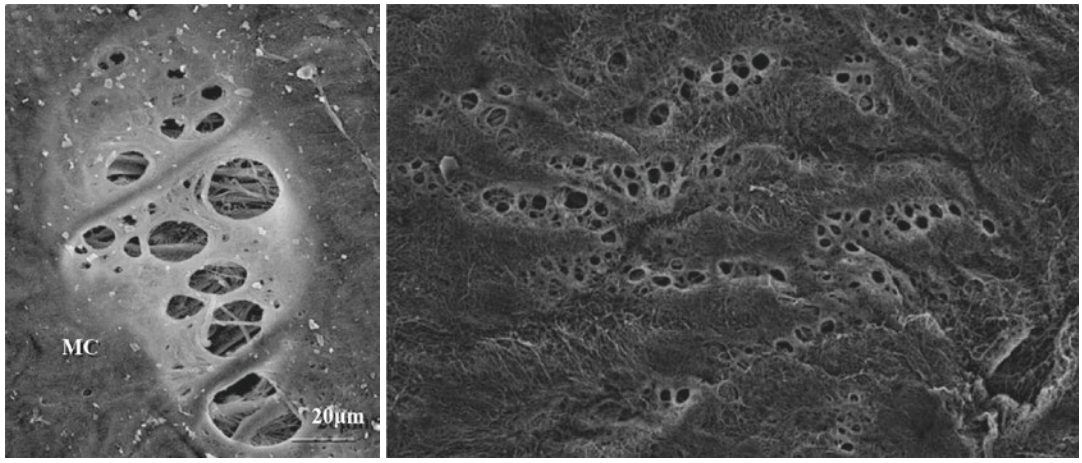


**Fig. 1.10** Holes of submesothelial basement membrane (Left). Macula cribriformis below basement membrane after 6N KOH cell maceration treatment. Diameters of the holes range from 5 to 30 μm (human peritoneum, Right)

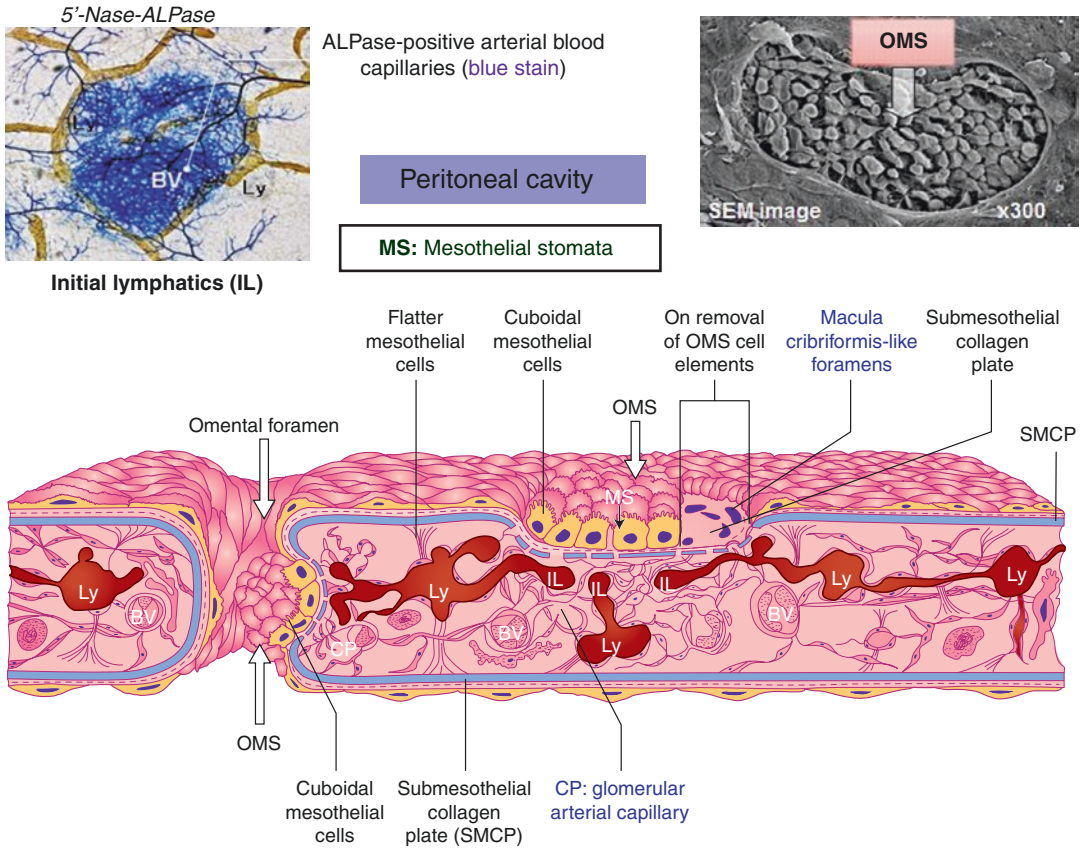


**Fig. 1.11** Omental milky spot stained with 5′-nucleotidase and alkali-phosphatase double enzyme staining (Upper left), electron microscopic finding (Upper right), and the schema of the structure. OMS is oval or round concave, and the cuboidal mesothelial cells cover the basement membrane of the bottom. Lymphatic plexus (red) is found below the macula cribriformis

toneum that are attached to macula cribriformis and mesothelial stomata are called initial lymphatic vessels (Fig. 1.8). There are two types of initial lymphatic vessels, i.e., flat type (Fig. 1.8, left, and Fig. 1.18) and protruded type (Fig. 1.8, right, and Fig. 1.19). The former type is found on the Morrison's pouch, paracolic gutter, and small bowel mesentery. After intraperitoneal injection of activated carbon CH40 [82], the tip of flat type of initial lymphatic vessel alone is stained with CH40. The blind-looped lymphatic vessels extending from the submesothelial lymphatic vessels are the protruded type, and their blind tips attach to the holes of macula cribriformis and stomata (Fig. 1.8, right, Fig. 1.19). Since there is no adhesion of CH40 except at the tip of initial lymphatic vessel, the tips of initial lymphatic vessels alone are considered to communicate with peritoneal cavity. The size of the holes of macula cribriformis ranges from 5 to 30 μm. PFCCs migrate into the initial lymphatic vessels through the stomata on the mesothelial surface without destruction of macula cribriformis (Fig. 1.20) and then proliferate in the lymphatic vessels (Fig. 1.21).

The triplet structure consisting of mesothelial stomata, hole of macula cribriformis, and initial lymphatic vessels is essential for the migration of PFCCs into the submesothelial lymphatic vessels (Fig. 1.22). When the stomata, hole of macula cribriformis, and the tip of initial lymphatic vessels are aligned in a row, direct communication between peritoneal cavity and submesothelial lymphatic vessels is established, resulting in the

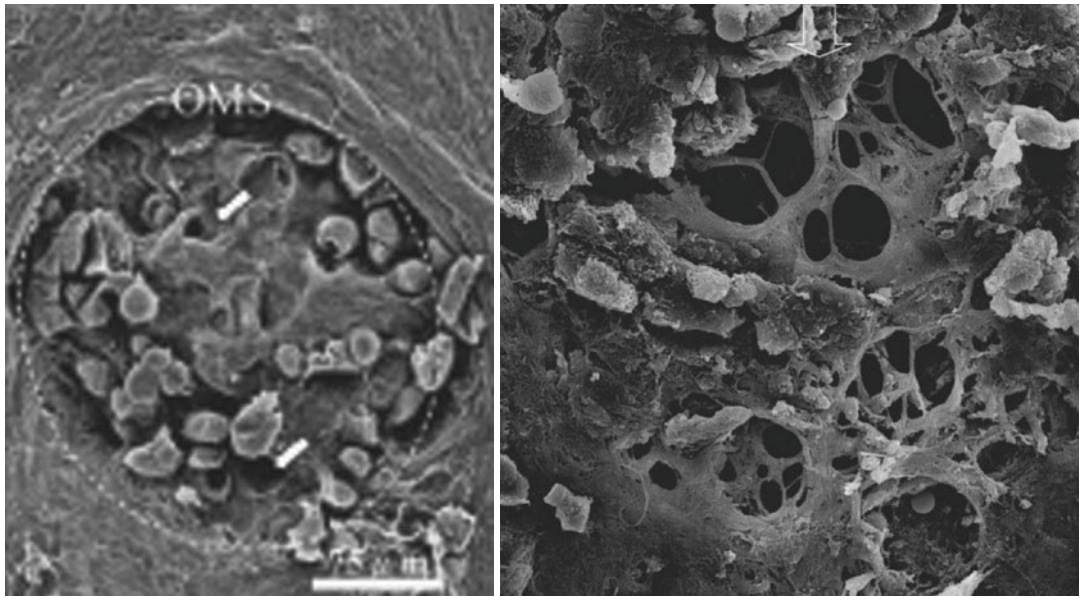


**Fig. 1.12** The bottom structure of OMS after digestion by 6 N KOH. Basement membrane is found beneath the cuboidal mesothelial cells. Holes of macula cribriformis cluster below the OMS basement membrane. Below the holes of macula cribriformis, lymphatic and vascular plexus are found (Fig. 1.11, upper left, Fig. 1.13)

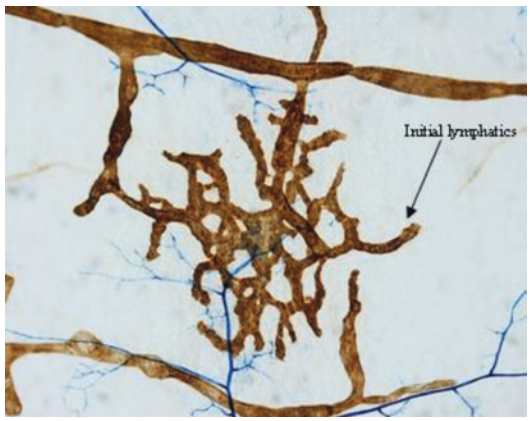


**Fig. 1.13** Lymphatic plexus locate under the macula cribriformis of OMS (5′-nucleotidase enzyme staining, omentum of Japanese monkey)

migration of PFCCs into the initial lymphatic vessels.

Lymphatic system of diaphragm is different from that of other parietal peritonea. Many lymphatic stomata (Fig. 1.9) and plenty of submesothelial lymphatic plexuses are detected by 5′-Nase enzyme staining (Fig. 1.23). Lymphatic fluid adsorbed from peritoneal cavity through diaphragmatic initial lymphatic vessels drains to the deep-seated lymphatic vessels of diaphragmatic muscle and then flows to the para-aortic lymph nodes via collecting lymphatic vessels in triangular ligaments or along subdiaphragmatic arteries, and to the lymphatic vessels along the internal mammary artery (Figs. 1.23 and 1.24). PFCCs are adsorbed on the stomata by negative pressure of inspiration and migrate into the diaphragmatic initial lymphatic vessels. Figure 1.25 shows the metastasis from colorectal cancer in the diaphragmatic lymphatic vessel.

Triplet structures (Fig. 1.22) are detected on the parietal peritoneum except on the anterior upper abdominal wall. The peritoneum of diaphragm, pelvis, paracolic gutter, Morrison's pouch, and perihepatic ligaments does not have any milky spots, but it does have the triplet structure. In the experimental study, intraperitoneal inoculation of cancer cells induces mesothelial cell contraction (Fig. 1.4), and cancer cells were detected in the submesothelial lymphatic vessels on day 3 after intraperitoneal inoculation [80].

On the small bowel mesentery 2 cm in from the attachment to small bowel, many milky spot-like

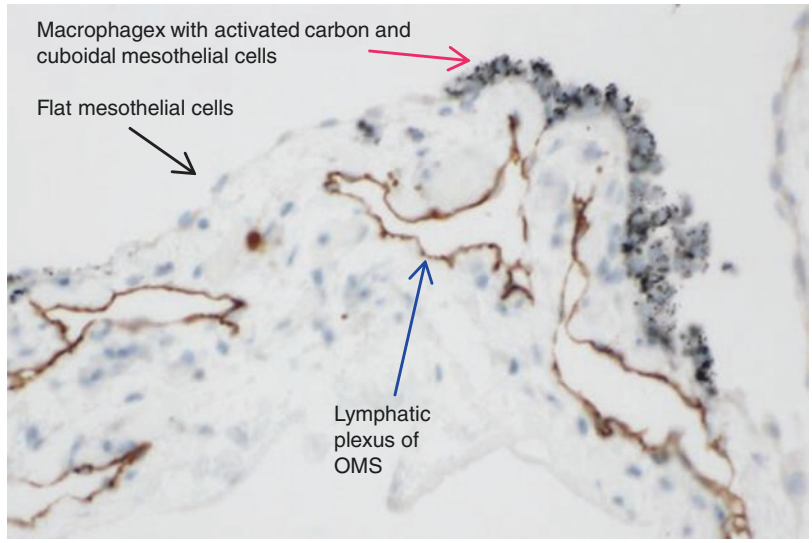


**Fig. 1.14** Human OMS stained with D2-40 monoclonal antibody. Lymphatic plexus (brown) are found beneath the cuboidal mesothelial cells

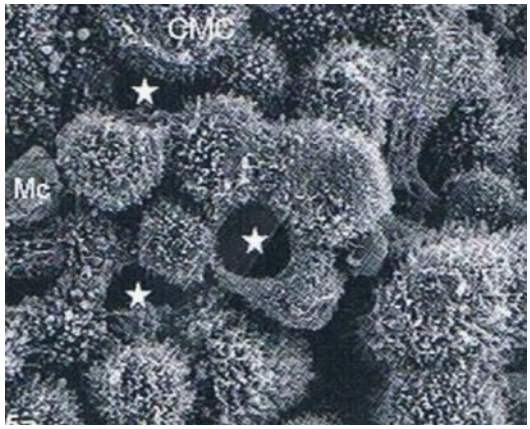


**Fig. 1.15** SEM findings of the surface of human OMS. Gaps (stars) between cuboidal mesothelial cells, and the gaps connect with macula cribriformis and initial lymphatic vessels

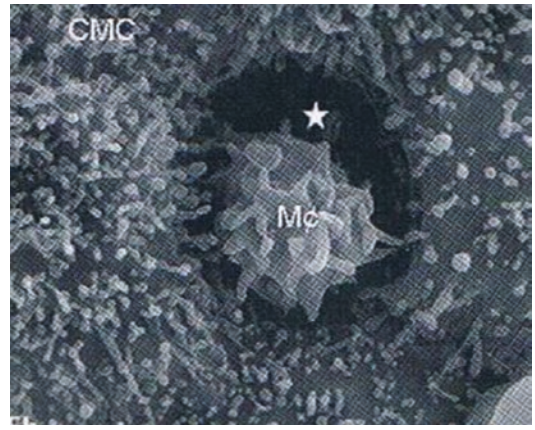


**Fig. 1.16** Macrophage is found in the gap (mesothelial stoma) between cuboidal mesothelial cells

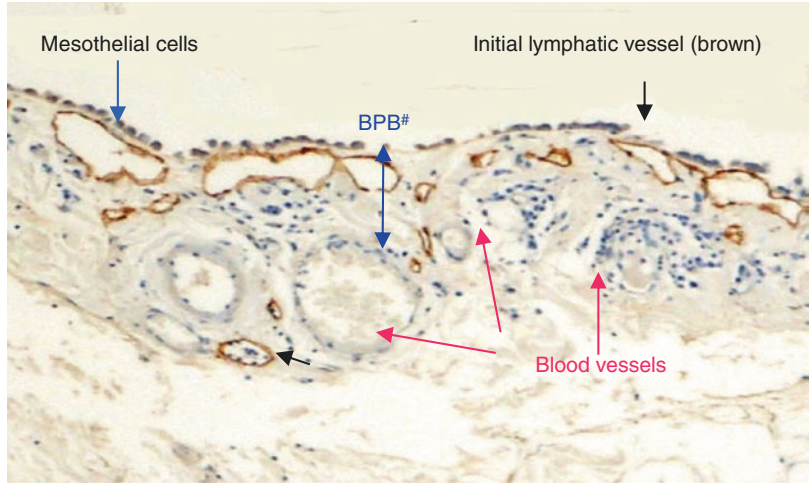


**Fig. 1.17** Normal structure of human Morrison's pouch stained by D2-40 monoclonal antibody. Initial lymphatic vessels attached to mesothelial cell gap. Blood vessels locate in the deeper subperitoneal tissue than lymphatic vessels. *BPB* blood peritoneal barrier

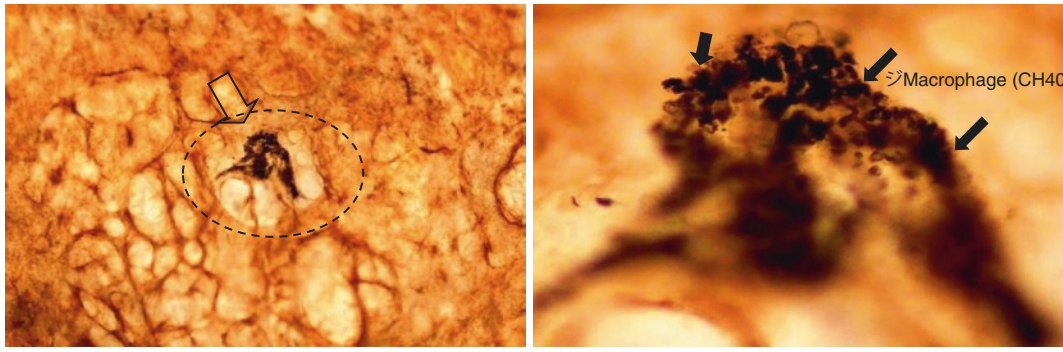


**Fig. 1.18** Flat type of initial lymphatic vessel of Morrison's pouch. Initial lymphatic vessel is stained black with CH40 (activated carbon), introduced intraperitoneally before sampling. There is no CH40 attachment on the subperitoneal lymphatic plexus except for flat type initial lymphatic vessel. Accordingly, the tip of lymphatic vessel stained with CH-40 is considered to contact with peritoneal cavity

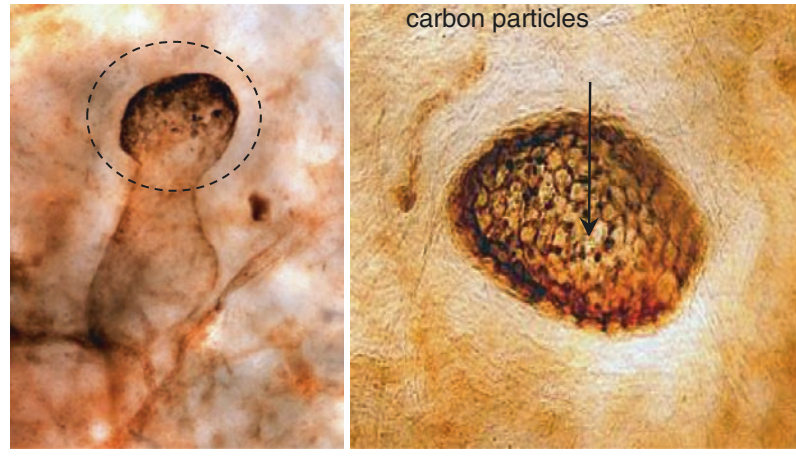


**Fig. 1.19** Protruded type of initial lymphatic vessel found in pelvic peritoneum. Tip of initial lymphatic vessel is stained with CH40, introduced intraperitoneally before sampling. There is no adsorption of CH40 except at the tip of initial lymphatic vessel (Left). CH40 particles adhere on the junction between lymphatic mesothelial cells

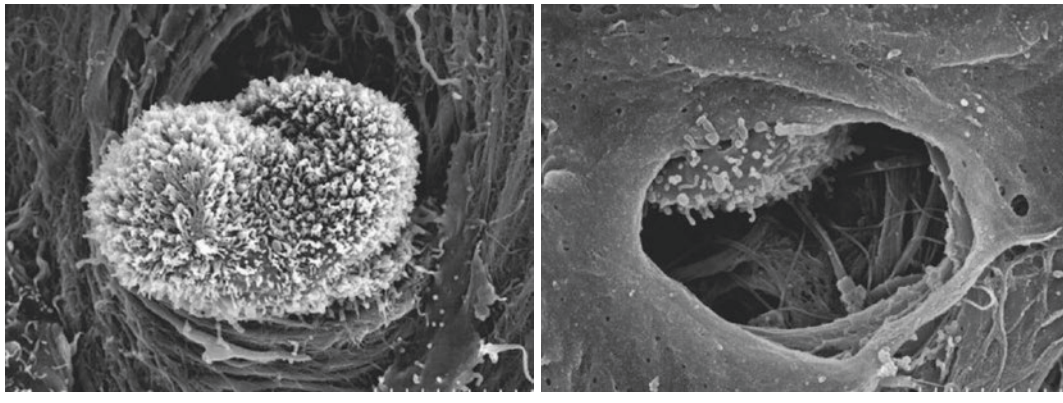

**Fig. 1.20** SEM findings of peritoneal free cancer cells from gastric cancer migrate into initial lymphatic vessels through the holes of macula cribriformis

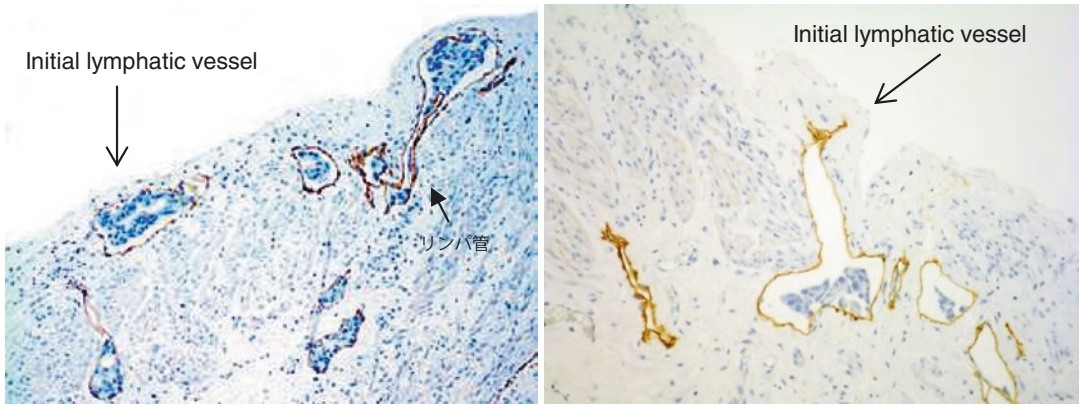


**Fig. 1.21** Findings of immunohistochemical staining using D2-40 monoclonal antibody for pelvic peritoneum from patients with gastric cancer. Gastric cancer cells proliferate in the flat type (Left) and protruded type of initial lymphatic vessels (Right)

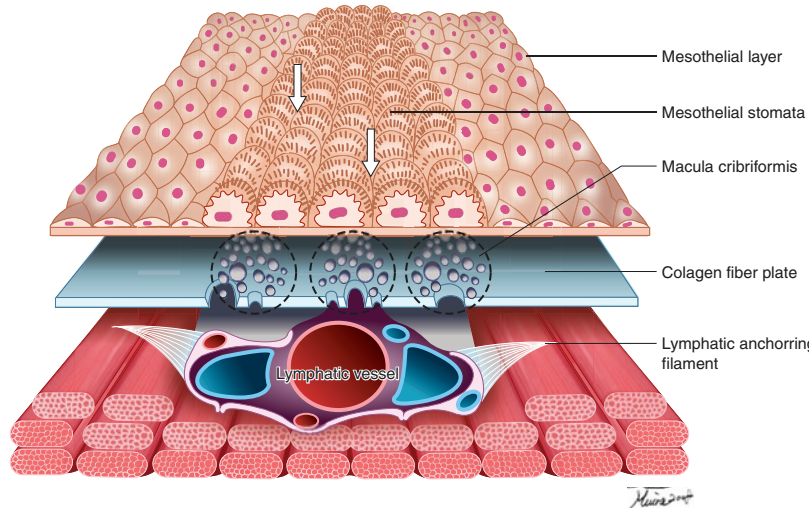


**Fig. 1.22** Schema of stomata, macula cribriformis, and initial lymphatic vessels. When the stomata, hole of macula cribriformis, and the tip of initial lymphatic vessels are aligned in a row, direct communication between peritoneal cavity and submesothelial lymphatic vessels is established, resulting in the migration of PFCCs into the initial lymphatic vessels

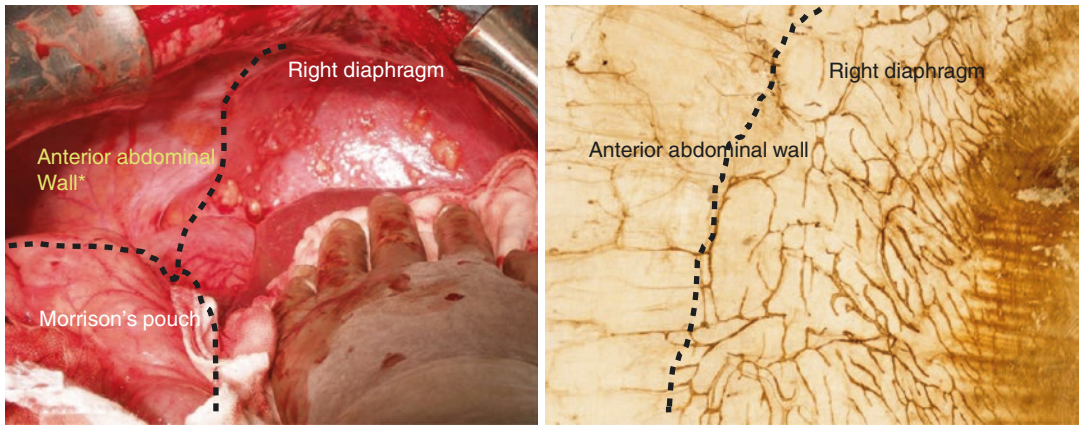


**Fig. 1.23** Metastatic nodules are found on the right diaphragm and Morrison's pouch, but are not detected on the anterior abdominal wall (Left, star). Diaphragmatic lymphatic network stained with 5′-Nase enzyme staining (Right). Lymphatic plexus is scarce on anterior abdominal wall, but plenty of lymphatic plexuses are detected on the subdiaphragmatic surface

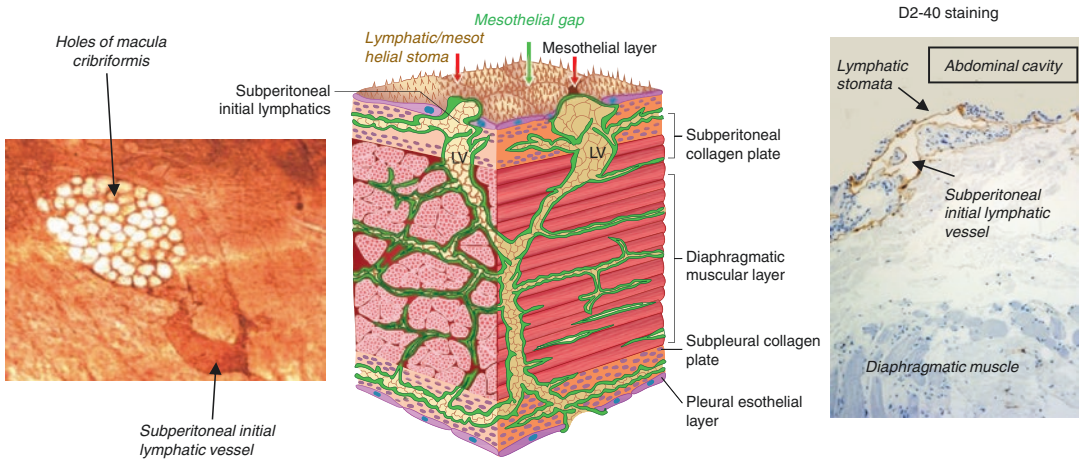


**Fig. 1.24** Diaphragmatic lymphatic system. Left: 5′-Nase enzyme staining shows the connection of holes of macula cribriformis and diaphragmatic initial lymphatic vessels. Middle: Schema of diaphragmatic lymphatic system. Mesothelial gaps that do not connect with initial lymphatic vessel are not called stomata, but those that communicate with initial lymphatic vessels are named mesothelial stomata. Right: Immunohistochemical staining using D2-40 monoclonal antibody shows the lymphatic stomata and diaphragmatic initial lymphatic vessels. PFCCs are adsorbed through the stomata by negative pressure of inspiration and migrate into the diaphragmatic initial lymphatics

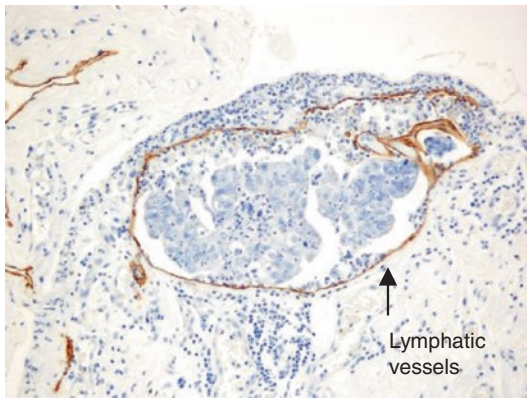


**Fig. 1.25** Lymphatic metastasis from colorectal cancer in diaphragmatic lymphatic vessel (immunohistochemical staining using D2-40 monoclonal antibody)

structures are found. On this special peritoneal area, round or oval shaped structures covered with cuboidal mesothelial cells are detected by SEM (Fig. 1.26). Below the cuboidal mesothelial cells, macula cribriformis is detected (Fig. 1.26, middle). Since the area is frequently involved in PM, and CH40 injected into peritoneal cavity adheres on the area, absorption of CH40 by initial lymphatic vessels is suggested. These results indicate that the metastasis in the area must be involved by trans-lymphatic metastasis (Fig. 1.26). Trans-lymphatic metastasis is found in gastric, colorectal, and pancreas cancer.

However, lymphatic system in peritoneum covering the rectus abdominis muscle between hypochondrium and semilunar arc is quite different from that of other parts of peritoneal surface. In this area, no initial lymphatic vessels or submesothelial lymphatic plexuses are detected. Lymphatic vessels locate in deep subperitoneal tissue 200 μm from the peritoneal surface (Fig. 1.27), and the blood vessels are also scarce. Accordingly, trans-lymphatic metastasis does not develop in the area. The peritoneal area must be involved at the late stage of PM and should be preserved when there is no macroscopic involvement on the sector.

## 1.4 Mechanisms of Superficial Growing Metastasis

PFCCs from appendiceal mucinous neoplasm (AMN) cannot metastasize through trans-mesenteric or trans-lymphatic metastasis, because

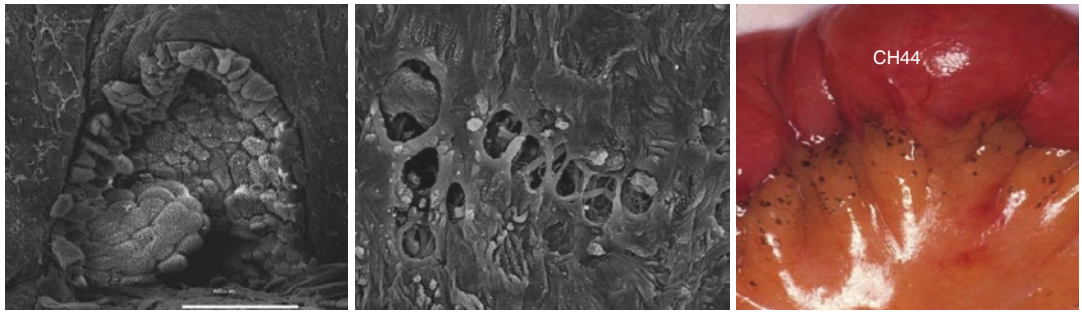


**Fig. 1.26** Milky spot-like structure detected on the small bowel mesentery in 2 cm from the attachment to small bowel (Left). Below the cuboidal mesothelial cells, holes of macula cribriformis are detected by SEM (Middle). CH40 injected into peritoneal cavity adheres on the peritoneal area, suggesting absorption of CH40 by initial lymphatic vessels (Right)

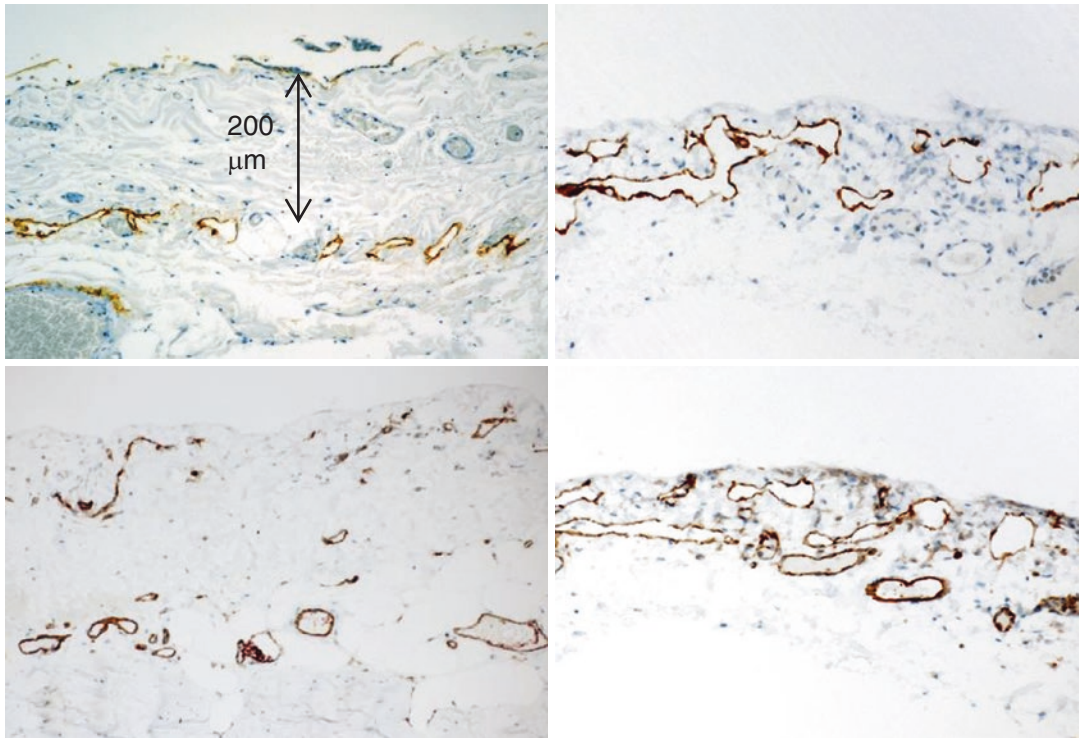


**Fig. 1.27** Lymphatic vessels of anterior abdominal wall between hypochondrium and semilunar arc. Lymphatic vessels are located 200 μm from the peritoneal surface (Left). Lymphatic vessels and blood vessels in falciform ligament located just below the mesothelial cells (Right). Upper left: Lymphatic vessels of anterior abdominal wall stained with D2-40 monoclonal antibody. Upper right: Lymphatic vessels of falciform ligament, stained with D2-40 monoclonal antibody. Lower left: Blood vessels of anterior abdominal wall, stained with CD31 monoclonal antibody. Lower right: Blood vessels of falciform ligament, stained with CD31 monoclonal antibody

PFCCs of AMN are large and covered with mucinous material (Fig. 1.28). They cannot migrate into the submesothelial tissue or initial lymphatics (Fig. 1.29).

However, AMN can establish PM in dependent areas such as on the pelvis, subdiaphragmatic surface, and greater omentum. They also grow in the pocket-like structure of omental

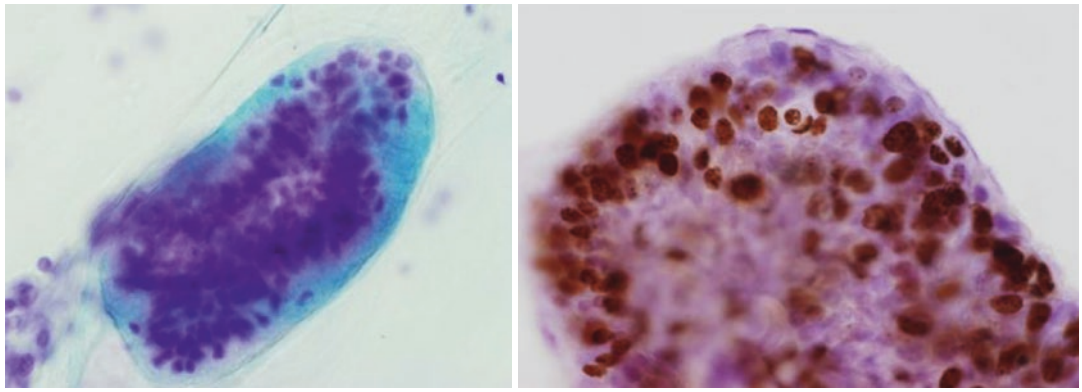

**Fig. 1.28** Peritoneal free cancer cells of appendiceal mucinous neoplasm. Neoplastic cells are covered with mucinous material and the diameter is several hundred micrometers (Left, Alcian blue staining). Neoplastic cells show high proliferative activity (Right, Immunohistochemical staining using MIB-1 monoclonal antibody)

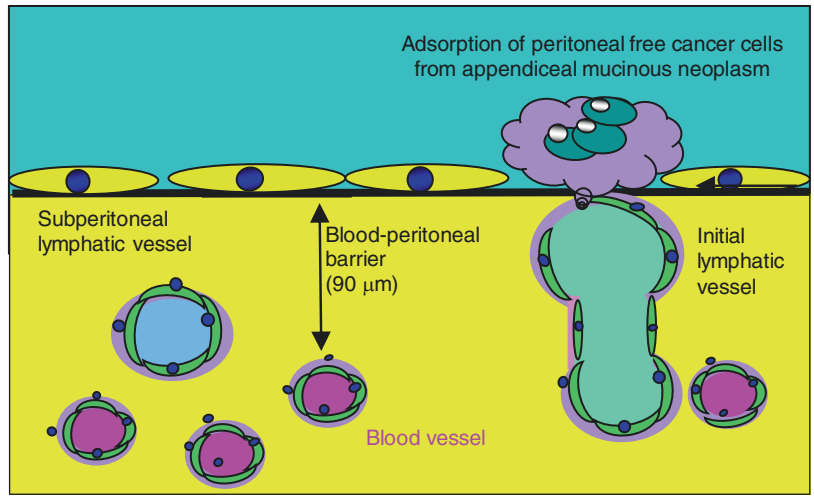


**Fig. 1.29** Mechanism of superficial growing metastasis. Peritoneal free cancer cells from appendiceal mucinous neoplasm attach on the pelvic peritoneum by the interaction of mucinous material and adhesion molecules (CD44) expressed on mesothelial cells and/or by gravity

bursa (inferior and superior recess of omental bursa), intersigmoid recesses, recesses in duodeno-jejunal folds, and ileocecal fossa.

A large volume of mucinous materials with tumor cells accumulates on the dependent peritoneal parts as a result of peritoneal fluid resorption by the negative pressure of initial lymphatic vessels and/or gravity [79, 83].

PFCCs of AMN attach on the pelvic peritoneal surface by gravity or by the interaction of adhesion molecules on the mesothelial cells and mucinous materials. As shown in Fig. 1.30, mucinous material attaches on the paravesical fossa, and immunohistochemical staining with CD31 shows newly formed vasculature in the mucinous material without epithelial cells. These results strongly suggest that angiogenesis factors released from mucinous materials induce angiogenesis in the mucinous stroma.

Figure 1.31 shows the metastasis on the surface of ovary by AMN. HE staining shows low-grade mucinous neoplasm growing on the surface of ovary (Fig. 1.31, upper left). Angiogenesis from the preexisting ovarian vasculature and epithelial cells in the proliferating phase (positive stain by MIB-1 antibody) are found.

On the omental surface, PFCCs with mucinous materials from AMN are adsorbed on OMS,

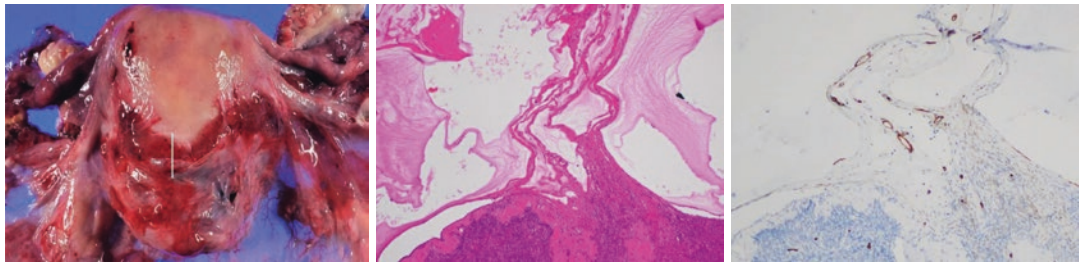

**Fig. 1.30** Mucinous material without epithelial cells accumulates on paravesical fossa (Left). HE staining of the vertical section of the bar in left photograph (Middle). Immunohistochemical staining using anti-CD31 monoclonal antibody shows newly formed vasculature (Right)

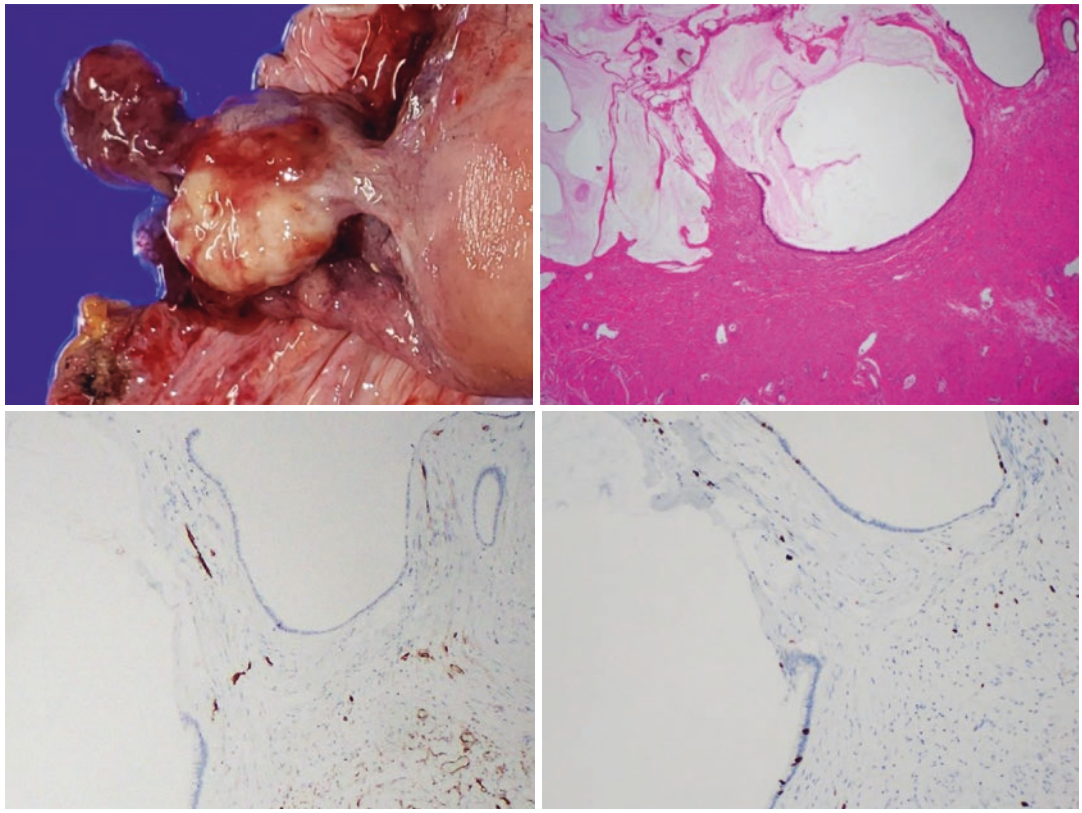

**Fig. 1.31** Superficial growing metastasis on ovarium from appendiceal mucinous neoplasm (AMN) (Upper left). AMN growing on the surface of ovary with production of mucinous material, and the neoplastic cells grow on the ovarian surface showing pushing invasion into the corpus of ovary (Upper right). Newly formed vasculatures from preexisting ovarian blood vessel are found (Immunohistological staining (HIS) using anti-CD31 Mab) (Left lower). Right lower photograph shows proliferative activities of tumor cells (IHS using MIB-1 mAb)

and many flat mucinous spots are found on the OMS (Fig. 1.32, left). HE staining shows three layers, consisting of a metastatic layer, inflammatory layer between metastatic layer and omentum, and normal omentum (Fig. 1.32, middle, and Fig. 1.33). Inflammatory layer shows CD34-positive interstitial tissues. CD34 is a glycoprotein expressed on interstitial stem cells and

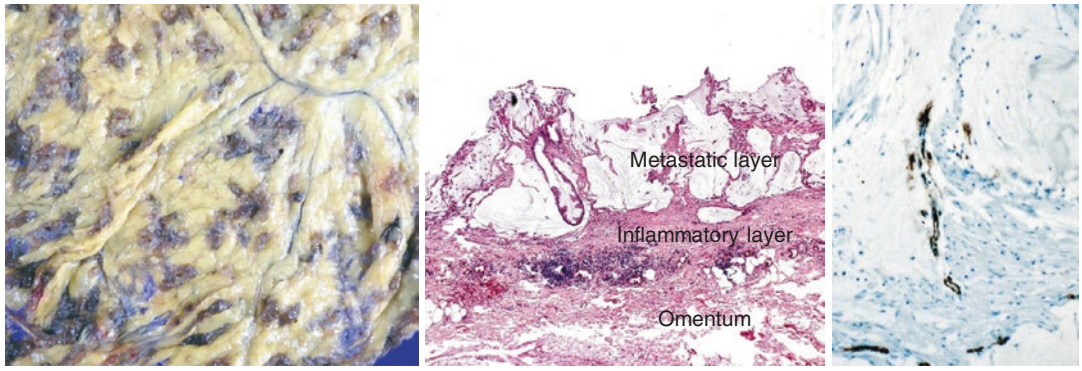


**Fig. 1.32** Superficial growing metastasis on greater omentum from appendiceal mucinous neoplasm (AMN). Flat mucinous spots adsorbed on the greater omentum (Left). AMN growing on the surface of omentum with production of mucinous material. Three layers, named metastatic layer, inflammatory layer, and omentum (Middle). Newly formed vasculatures from omental blood vessel are found (IHS using anti-CD31 MAb) (Right)

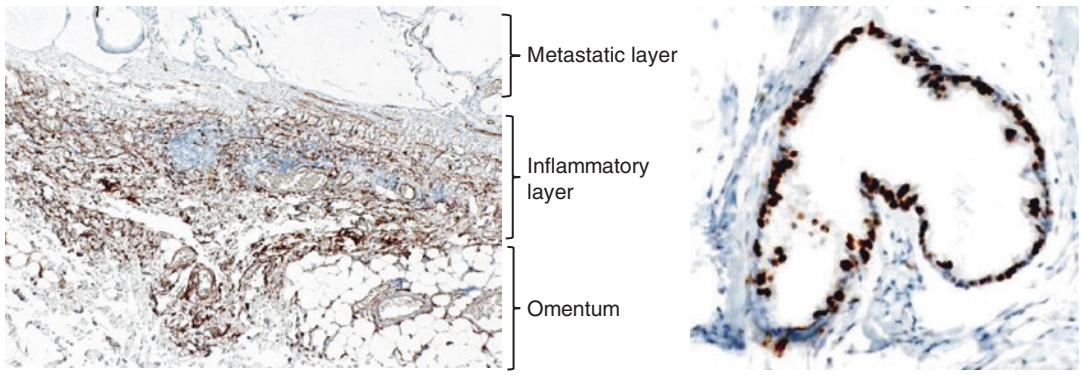


**Fig. 1.33** Three layers of superficial growing metastasis on the omentum. Inflammatory layer shows strong immunoreactivity against CD34 mAb (IHS using anti-CD34 mAb, Left). Immunohistochemical staining using MIB-1 Mab shows many positive staining on the nuclei of neoplastic cells from appendiceal mucinous neoplasm

immature vascular endothelial cells [83]. CD34 inhibits the maturation of the tissue, and disappears after the tissue maturation completes. Vascular neogenesis in the metastatic layer may be induced from the preexisting omental blood capillaries by the modulation of inflammatory layer. From these results, PFCCs proliferate on the peritoneal surface.

Figure 1.34 shows the mesothelioma growing on the ovarian surface. There is no inflammatory layer, but newly formed blood vessels extending from preexisting ovarian vessels are found in the stroma. Angiogenesis factors from mesothelioma induce vascular neogenesis and proliferate on the peritoneal surface without invasion into the subperitoneal tissue.

This metastasis pattern is named superficial growing metastasis and PM from appendiceal and ovarian, mucinous neoplasms, mesothelioma, granulose cell tumor, and multicystic mesothelioma is established by superficial growing metastasis [15, 84] (Fig. 1.35).

Surgeons should understand the mechanisms of the peritoneal metastasis formation and perform peritonectomy in accordance with the biological malignancy and metastatic pattern of each tumor.

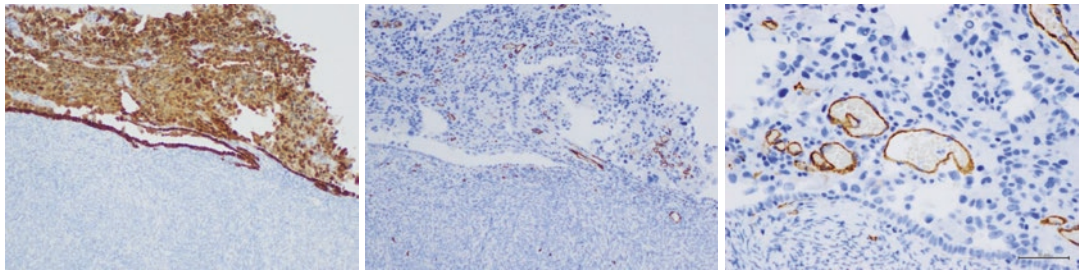

**Fig. 1.34** IHS using D2-40 mAb shows mesothelioma growing on the surface of ovary (Left). Newly formed vasculature extends from preexisting ovarian vessels (Middle). Many blood vessels are found in the superficial growing metastasis (Right)

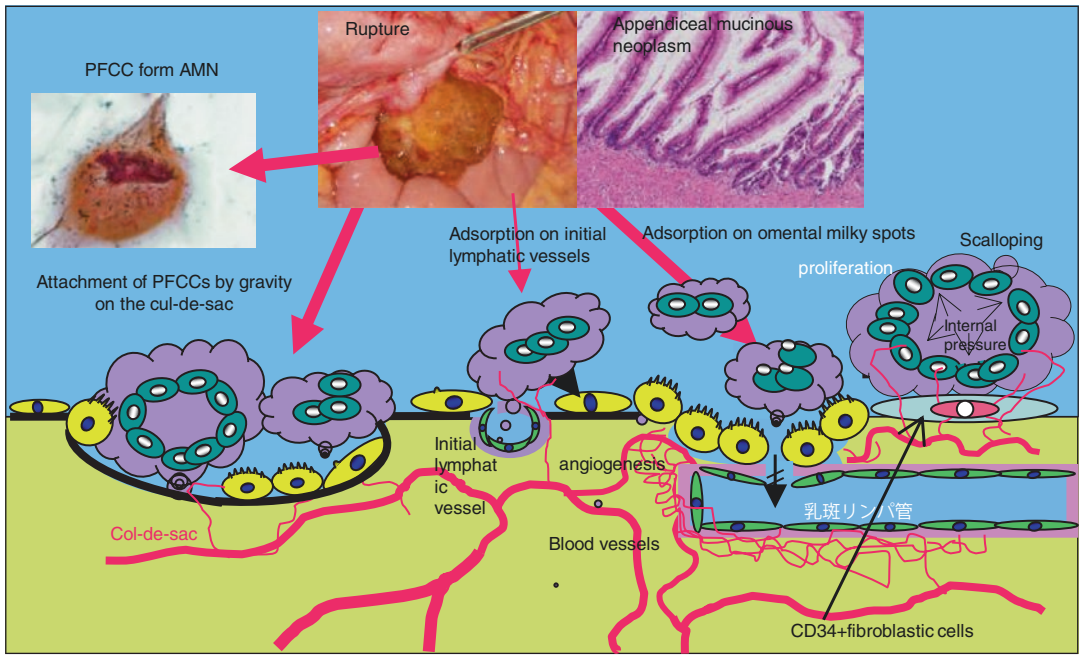


**Fig. 1.35** Schema of the mechanism of superficial growing metastasis [85]

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# 2 Extent of Peritoneal Resection for Peritoneal Metastases: Inferences from Pathophysiology

Aditi Bhatt and Olivier Glehen

## 2.1 Background and Introduction

It has been shown that surgical resection of the peritoneal metastases (PM) leads to an increased survival and cure in selected patients [1, 2]. One of the most important prognostic factors affecting the outcomes of this surgery is complete tumor removal or a complete cytoreduction [3]. The prognostic impact of complete resection has been established irrespective of the primary tumor site and disease extent [4, 5].

In most cancer surgeries, the goal is complete resection of the macroscopic disease [6]. For most primary tumors, the extent of surgical resection to be performed is predefined in terms of anatomical extent. A varying proportion of the surrounding normal tissue is excised to account for microscopic disease [7, 8]. For example, for a colonic primary, 5 cm of normal bowel is resected on both sides of the primary tumor [9]. Similarly, for metastatic disease to the liver, the goal of surgery is resection of the tumor(s) with 1 mm margins or free margins [10, 11].

Contrary to this, the goal of surgery for peritoneal metastases is complete removal of macroscopic disease. There is no consensus or guideline on the extent of the surrounding peritoneum that needs to be removed. This is partly because though considered locoregional disease, peritoneal metastases present as multiple nodules scattered over one or more areas of the peritoneum [12, 13].

The peritoneum is now considered an organ with its own blood supply, lymphatic drainage, and innervation [14]. Peritoneal metastases can give rise to secondary lymph node metastases. There is no consensus on the extent of lymphadenectomy to be performed for most PM except in case of peritoneal mesothelioma [15].

Broadly cytoreductive surgery (CRS) for peritoneal metastases is divided into peritonectomy procedures and visceral resections [16]. Peritonectomy procedures usually comprise of the five peritonectomies described by Paul Sugarbaker that comprise of resection of various portions of the parietal and visceral peritoneum (Table 2.1) [18]. These divisions and resections are largely anatomical and based on the disease distribution seen in pseudomyxoma peritonei (PMP) termed as the "redistribution phenomenon" [19]. Involvement of one region of the peritoneum merits performing a resection of that region of the peritoneum irrespective of the number and size of deposits [20]. Whereas the anatomical extent of each peritonectomy has

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**Table 2.1** The five peritonectomies described by Sugarbaker (from Ref. [17] with permission)

| Peritonectomy procedures | Structures included |
|---|---|
| Anterior parietal peritonectomy | Old abdominal incisions, umbilicus, epigastric fat pad |
| Right upper quadrant peritonectomy | Glisson's capsule |
| Left upper quadrant peritonectomy | Greater omentum and spleen |
| Pelvic peritonectomy | Uterus, ovaries, and rectosigmoid colon |
| Omental bursectomy | Gall bladder and lesser omentum |

been defined, it is not known whether resecting the peritoneum in this manner is needed for peritoneal metastases from other primary tumors in which the mechanisms and patterns of peritoneal dissemination are different. For some tumors, it is possible that only focal resection is sufficient.

To determine the extent of resection of surrounding tissue for any tumor, the mechanisms of tumor development and spread, the morphology of the tumor, the possibility of finding disease in the surrounding normal tissue, and the pattern of lymph node metastases should be known. This is followed by a study of the patterns of recurrence and the impact of varying extent of resection on survival.

In this chapter, we look at the above factors in relation to peritoneal metastases from some common primary tumors to determine the scientific basis of the extent of peritonectomy needed for these primary tumors and the extent of accompanying lymph node dissection that should be performed.

### 2.1.1 Evolution of Peritoneal Surface Oncology

The development of peritoneal surface oncology has occurred due to a better understanding of the disease biology and development of complex surgical techniques to remove peritoneal tumor deposits. The increase in understanding of the disease biology has occurred hand in hand with the evolution of these complex surgical procedures [21]. Over the years, clinicians have started treating peritoneal metastases as one disease entity. As pointed out by David Bartlett, this is because patients are symptomatic more often from peritoneal metastases and surgeons are called upon to manage these symptoms that severely bring down the quality of life [22].

The initial years were spent controlling the morbidity of surgery that was often accompanied by some form of intraperitoneal chemotherapy instillation and searching for prognostic factors to both quantify the benefit of the surgical procedure and select patients for surgery [23–25].

Most of the peritoneal tumors are secondary to other primaries with only a small proportion arising de novo from the peritoneum.

Peritoneal metastases arising from different primary tumors differ not only in biological behavior but also have different patterns of distribution in the peritoneal cavity and morphology of tumor nodules [12].

Thus, though the goal of CRS remains the same for all tumors, the extent of surrounding peritoneum that needs to be resected should vary.

## 2.2 Pathophysiology of Peritoneal Metastases and Its Clinical Implications

### 2.2.1 Peritoneal Metastatic Cascade

The development of peritoneal metastases has been described at length in existing scientific literature. Some of these reports are based on clinical observations, expression of different molecular markers, or from experimental studies. The evolution of PM is summarized in Fig. 2.1.

Peritoneal metastases arise in one of the following ways:

1. Dissemination from a primary tumor (gastric, colonic, and appendiceal tumors)
2. Primary tumor of the peritoneum (peritoneal mesothelioma, serous papillary adenocarcinoma)
3. Independent origins of the primary tumor and peritoneal implants (ovarian low malignant potential tumors, serous papillary peritoneal adenocarcinoma) [26]

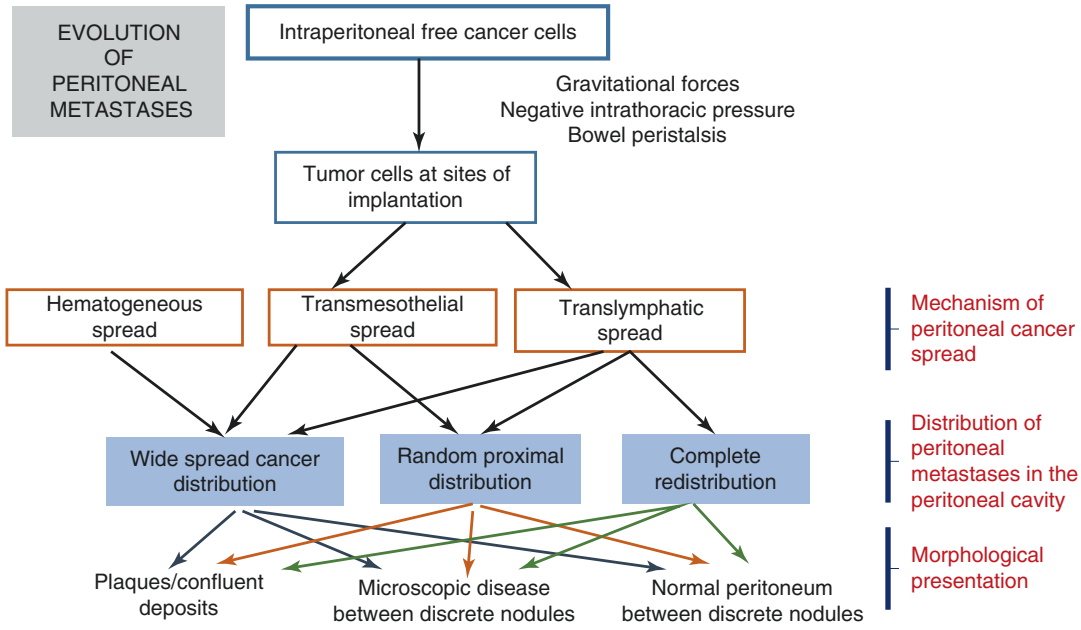


**Fig. 2.1** Evolution of peritoneal metastases—pathways of dissemination, intraperitoneal disease distribution, and morphological presentation

In 1931, Sampson first described the "life history of metastatic peritoneal implants" from an ovarian primary tumor which occurs in the following sequence: (1) escape of the cancer cells from the primary ovarian tumor into the peritoneal cavity, (2) migration of these cells to their site of implantation, (3) reaction of the peritoneal surface injured by the cancer cells so that fixation of the cancer in fibrin and organization of the fibrin occurs, and (4) progression of the cancerous implant at that site [27].

Cancer cells reach the site of implantation through three routes—transcoelomic or transmesothelial spread, translymphatic spread, and hematogenous spread. In the first two routes, single cells or clusters of cells are shed from the tumor spontaneously, due to surgical manipulation or spontaneous or iatrogenic rupture and thus gain access to the peritoneal cavity [28, 29].

In transmesothelial spread, cells then attach to the peritoneum, breach the mesothelial barrier and gain access to the submesothelial tissue and thereafter, proliferation is triggered (Fig. 2.2) [30].

The second route is the translymphatic route in which the tumor cells gain access to the subperitoneal tissue through the lymphatic stomata. Anatomical regions in the peritoneal cavity with a high concentration of lymphatic stomata are the greater omentum, appendices epiploicae of the colon, inferior surface of the diaphragm, falciform ligament, Douglas pouch, and small bowel mesentery [31, 32]. These regions are also rich in milky spots and therefore, common sites of peritoneal cancer spread [32]. The peritoneum covering the liver, serosa of the small bowel, and splenic capsule are devoid of these stomata and are therefore, involvement of these regions occurs in a very late stage of peritoneal cancer spread. Importantly, spread to distant regions of the peritoneal cavity is possible even in the absence of a carrier like ascitic fluid in translymphatic spread. It may be one route of rapid dissemination of peritoneal disease.

Some low-grade tumors like low-grade mucinous appendiceal tumors produce superficial deposits that do not infiltrate the mesothelial layer and are called "superficial growing metastases." One tumor may employ one or more of these pathways of peritoneal dissemination. In mucinous appendiceal tumors, the translymphatic pathway is employed while in gastric and

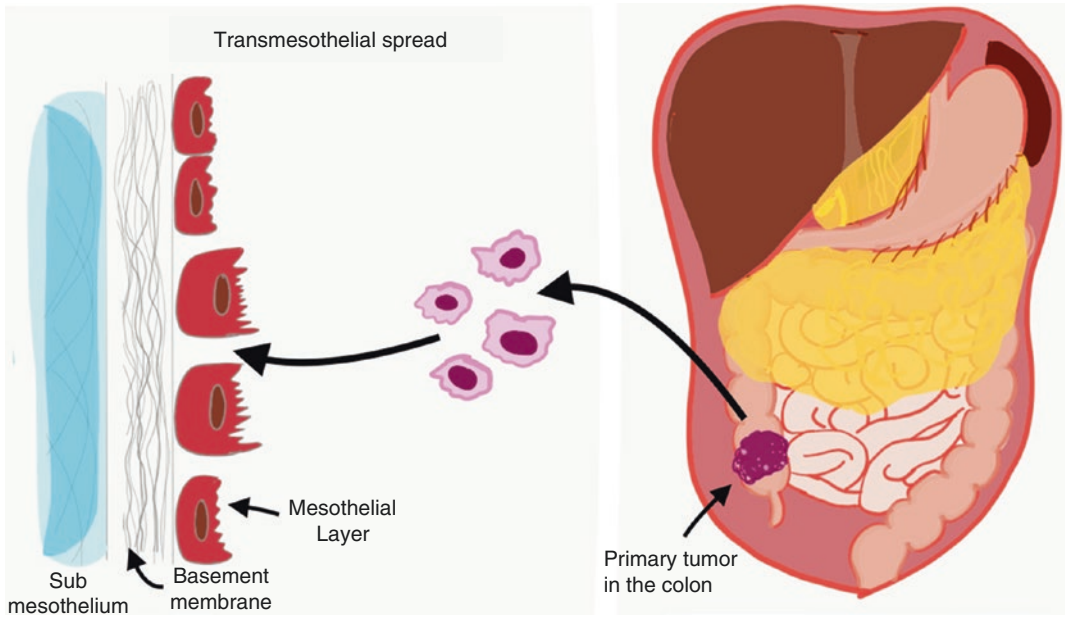


**Fig. 2.2** Diagramatic respresentation of transmesothelial spread in colorectal cancer. Tumor cells are shed from a primary in the colon and reach the subperitoneal space breaching the mesothelial barrier. (Adapted from Ref. [30] with permission)

colorectal cancer, both translymphatic and transmesothelial pathways are employed [33].

### 2.2.2 Distribution of Disease in the Peritoneal Cavity

The actual distribution of disease in the peritoneal cavity differs for tumors that employ the same pathways and this may be attributed to the inherent disease biology. For example, the translymphatic pathway is employed by appendiceal mucinous tumors giving rise to pseudomyxoma peritonei and also by the more aggressive cancers like gastric and colorectal cancer. In the former, the development of peritoneal metastases follows the flow of peritoneal fluid (the redistribution phenomenon) and the commonest disease sites are the pouch of Douglas, omentum, undersurfaces of the diaphragms, the falciform ligament, and the small bowel mesentery [34]. In contrast, such distribution is not seen in colorectal and gastric cancers in which proximal random distribution occurs and PM develop first in the vicinity of the primary tumor and then at other sites [17]. Three patterns of distribution of peritoneal metastases in the peritoneal cavity have been identified and described [17, 26].

1. Random proximal distribution (RPD)

   This pattern is typical of moderate-grade and high-grade cancers, such as adenocarcinomas and carcinoids of the appendix, nonmucinous colorectal cancer, and gastric cancer. In these tumors, there is early peritoneal implantation of tumor cells due to the presence of adhesion molecules on their surface and implantation occurs even in the presence of ascites. Peritoneal metastases typically develop in the vicinity of the primary tumor (Fig. 2.3).
2. Complete redistribution (CRD)

   This distribution is typical of pseudomyxoma peritonei (PMP) and diffuse malignant mesothelioma in which there is no adhesion to the peritoneal surface close to the primary tumor, due to the low biologic aggressiveness of tumor cells. The typical pattern of redistribution that is described above is seen (Fig. 2.4).
3. Widespread cancer distribution (WCD)

   This biological behavior is found in aggressive and undifferentiated tumors such as high-grade mucinous carcinoma peritonei arising from an appendiceal primary tumor, mucinous colorectal cancer, and mucinous

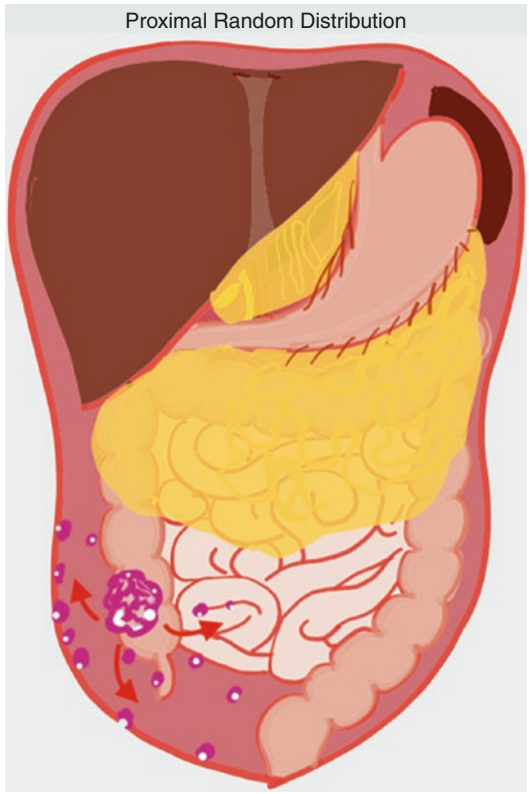


**Fig. 2.3** Proximal random distribution. Tumor cells shed from the primary tumor implant in the vicinity of the primary tumor

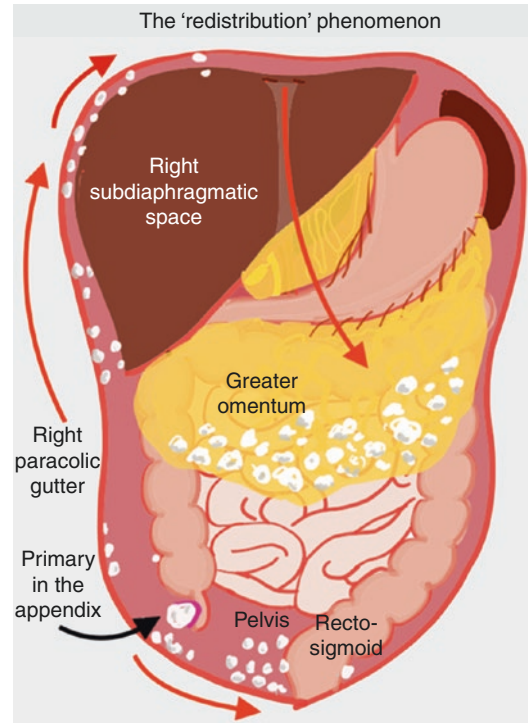


**Fig. 2.4** The "redistribution" phenomenon. Tumor dissemination from a perforated appendiceal tumor showing the pattern of redistribution. Tumor deposits are seen in the pelvis, right subphrenic region, omentum, and right paracolic gutter

ovarian cancer [35]. In these tumors, there is presence of adhesion molecules on the surface of cancer cells that produce a great amount of mucus, interfering with early cell adhesion. Deraco et al. have classified the pattern of distribution from various primary tumors as described in Table 2.2.

#### 2.2.2.1 Epithelial Ovarian Cancer

Though a lot has been published about development of peritoneal metastases in ovarian cancer, there is no clarity on the type of disease distribution. Epithelial ovarian cancer comprises of five biological different subtypes. Even in the serous subtype, that is the prototype for peritoneal dissemination, there are low-grade and high-grade tumors that are genetically and biologically different [36]. Transmesothelial spread is considered to be main pathway of peritoneal spread in ovarian cancer [27]. It has been proposed that high-grade serous ovarian cancer can spread by the hematogenous route to the peritoneum but conclusive evidence is lacking [37].

Though Deraco et al. have described the dissemination in serous ovarian cancer as random proximal, unlike colorectal and gastric cancer, the parietal peritoneum is involved first in serous cancer and visceral involvement comes after most regions of the parietal peritoneum have been involved. The disease distribution follows the flow of peritoneal fluid and certain sites are more commonly involved like the pelvis, hemidiaphragms, and omentum [26]. These regions are the dependent regions of the peritoneal cavity where absorption of peritoneal fluid occurs and also have a greater concentration of lymphatics.

Our preliminary study showed that small bowel involvement occurred after involvement of the diaphragmatic peritoneum [38]. This was in contrast to colorectal cancer where small bowel

**Table 2.2** Intraperitoneal distribution of peritoneal metastases arising from various primary tumors (adapted from Ref. [26] with permission)

| Histological subtype | Current classification | Random proximal distribution | Complete redistribution | Widespread cancer distribution |
|---|---|---|---|---|
| *Pseudomyxoma peritonei* | LGMCP | | + | |
| *Appendix cancer* | | | | |
| Cystadenocarcinoma G1 | LGMCP/HGMCP | | + | |
| | HGMCP/MAC | | | + |
| Adenocarcinoma | Non-mucinous adenocarcinoma | + | | |
| Carcinoid | Carcinoid | + | | |
| *Colorectal cancer* | | | | |
| Mucinous adenocarcinoma G1,2,3 | Mucinous adenocarcinoma G1,2,3 | | | + |
| Intestinal | Non-mucinous adenocarcinoma | + | | |
| *Gastric cancer* | | | | |
| Diffuse | Diffuse | + | | |
| Intestinal | Intestinal | + | | |
| *Ovarian cancer* | | | | |
| Serous | Serous | + | | |
| Mucinous | Mucinous | | | + |
| *Diffuse malignant mesothelioma* | Diffuse malignant mesothelioma | | + | |

Abbreviations: *LGMCP* low-grade mucinous carcinoma peritonei, *HGMCP* high-grade mucinous carcinoma peritonei, *MAC* mucinous adenocarcinoma, *G* grade

involvement often occurred without the involvement of the subphrenic peritoneum.

There are primary peritoneal tumors like primary peritoneal serous carcinoma that has a polyclonal origin and thus, disease is found on all peritoneal surfaces, but even in this tumor, the parietal peritoneum is the first site to develop disease.

### 2.2.3 Implications of Mechanism of Peritoneal Dissemination on Surgical Resection

Most surgeons do not have different surgical policies for different tumors.

Deraco et al., at the National Cancer Institute, Milan, perform a selective parietal peritonectomy that comprises of resection of macroscopically involved regions for limited peritoneal spread from tumors having a RPD pattern of dissemination like colorectal, gastric, and ovarian cancer [39]. Contrary to this, a complete parietal peritonectomy is performed for peritoneal carcinomatosis characterized by the CRD or WCD patterns. Patients with malignant peritoneal mesothelioma and PMP undergo a complete resection of the parietal peritoneum along with greater and lesser omenta even in the presence of localized disease. In a retrospective study of patients undergoing CRS for peritoneal mesothelioma at this center, the authors showed a benefit in overall survival in patients undergoing complete parietal peritonectomy compared to those undergoing selective parietal peritonectomy and complete parietal peritonectomy was an independent predictor of a longer overall survival [40].

Our recommendation for ovarian cancer is different as we believe that the distribution is not like colorectal and gastric cancer, but is widespread cancer distribution and hence we recommend more extensive peritoneal resection for these tumors. One retrospective study showed that there might be a role for complete parietal peritonectomy in serous epithelial ovarian cancer and primary peritoneal serous cancer but once again the evidence is preliminary [41]. Thus, there is likely to be a role for resection of normal, uninvolved regions for peritoneal metastases from certain primary tumors.

### 2.2.4 Morphology of Peritoneal Deposits and Morphological Evolution of Peritoneal Metastases

While the rationale for the extent of peritonectomy depends on the mode of dissemination and the disease distribution, the tumor morphology plays a role as well. There is variation in morphology of PM in different peritoneal regions and in PM arising from different primary sites.

Some tumors form isolated deposits and the surrounding peritoneum is usually free of disease. In other situation, microscopic disease is present in the surrounding peritoneum even when the peritoneum itself looks grossly normal. In patients with more extensive disease, the tumor deposits become confluent. One retrospective study found disease in normal peritoneum in 20.4% following neoadjuvant chemotherapy for ovarian cancer [42]. In our multi-centric study, it was seen in 27.2% with ovarian cancer, 12.2% with appendiceal tumors, and 26.6% with peritoneal mesothelioma [38]. Baratti et al. found microscopic disease in 50% of their patients with peritoneal mesothelioma [40].

Our prospective study showed that the PCI did not correlate with the pathological findings in more than 80% of the patients [38].

#### 2.2.4.1 Morphological Evolution of PM

Though the peritoneal metastatic cascade has been studied and described, the morphological evolution has not been studied. It may be presumed that in the early stages of development of peritoneal disease, the peritoneum appears normal. There is no accurate way except pathological evaluation of ruling out disease in the "normal looking" areas of the peritoneum. Surgeons often resect areas like the omentum, falciform ligament, and umbilical round ligament even in the absence of visible disease, expecting microscopic disease in those regions [43].

#### 2.2.4.2 Alteration in Morphology After Systemic Chemotherapy

PM present not just as discrete or confluent nodules but also as diffuse thickening and plaques [44]. Many patients receive systemic chemotherapy which alters the morphology of peritoneal deposits. Following a response to chemotherapy, areas harboring PM may become scarred or thickened, result in formation of adhesions or appear absolutely normal (Fig. 2.5) [45]. One study showed that following systemic chemotherapy for advanced ovarian cancer, nearly 15% of the patients had microscopic disease in areas that had a benign appearance [46]. For colorectal cancer, one retrospective study found a higher incidence of pathological complete response than what was predicted by intraoperative evaluation of disease [47]. Many a time, when the surgeon expects disease in a particular region and resects it, there is no tumor on histopathology and vice versa.

It is not possible to determine the presence or absence of disease with frozen section for each region. Innovative methods like 5-aminolevulinic acid guided fluorescence imaging have been used but are expensive and tedious and their value is still uncertain [46].

#### 2.2.4.3 Histological Subtype

Different histological subtypes of the same primary tumor have different morphology and

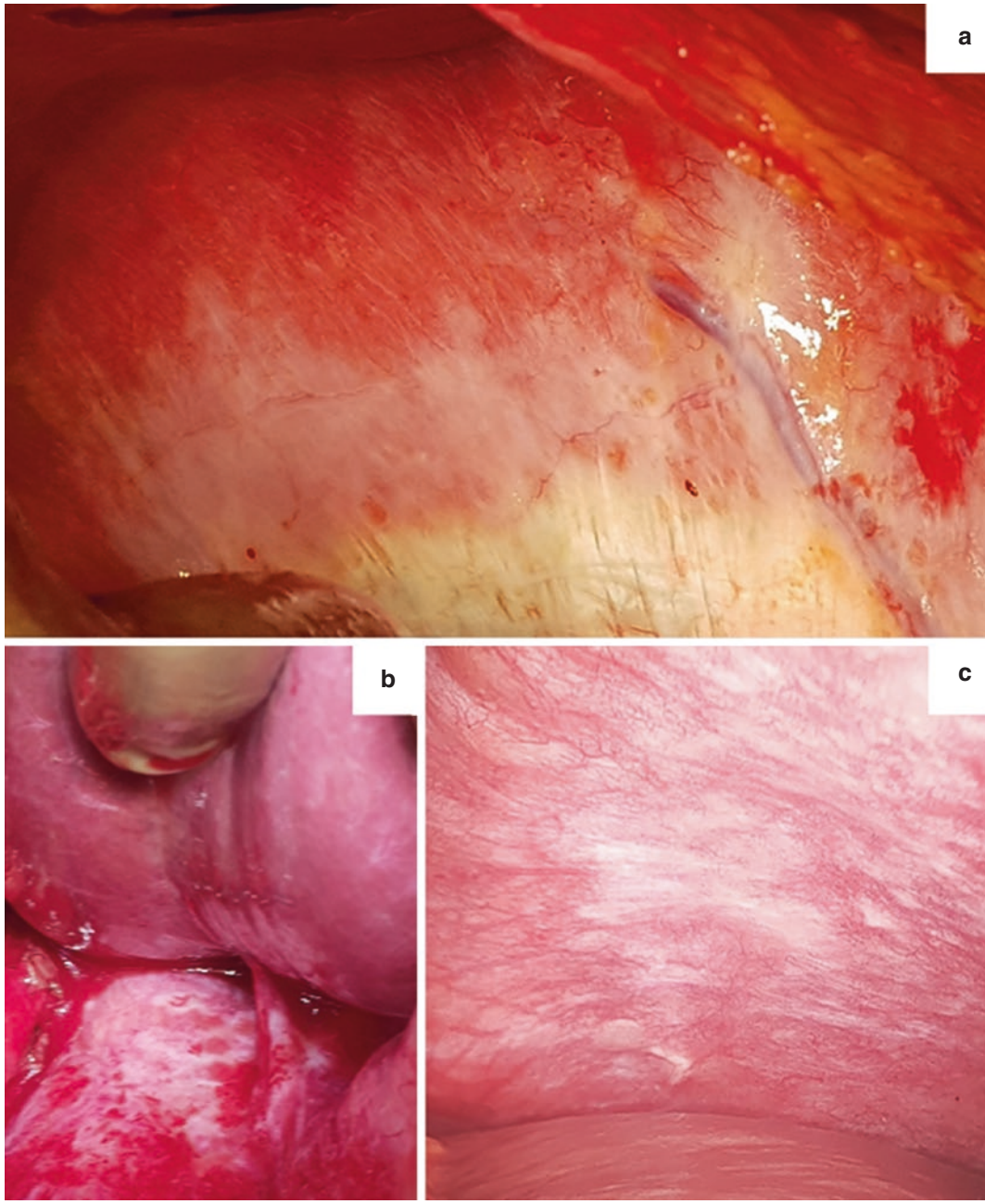


**Fig. 2.5** Morphological appearance of peritoneal deposits after systemic chemotherapy: (**a**) plaque like deposit in the right subphrenic peritoneum in high-grade serous ovarian cancer; (**b**) deposit in the peritoneum overlying the retrohepatic IVC in a patient with high-grade serous ovarian cancer; (**c**) residual plaque like deposits from signet ring cell carcinoma. On histopathological examination, residual disease was seen in areas shown in (**a**–**c**)

clinical behavior. Signet ring cell carcinoma presents and behaves differently from mucinous tumors with signet ring cells. The former has a diffuse plaque like appearance and seldom presents with localized disease. For mucinous carcinomas with signet ring cells, the visual appearance is not significantly different from tumors without signet ring cells (Fig. 2.6).

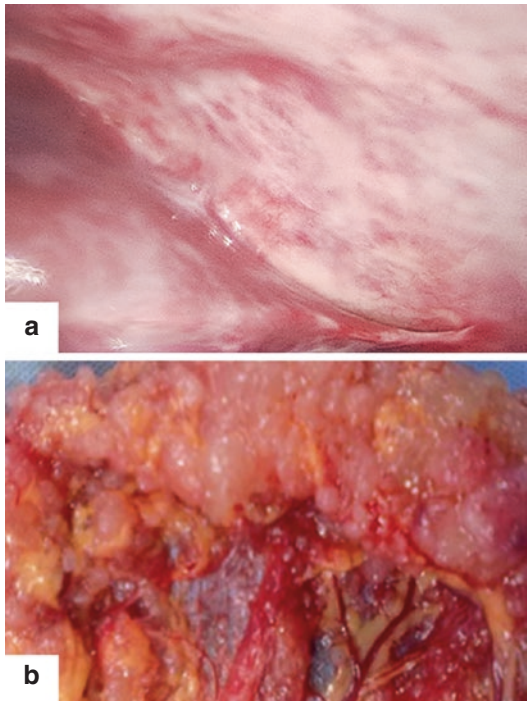


**Fig. 2.6** Morphological appearance of peritoneal deposits in mucinous and signet ring cell carcinoma. (**a**) Plaque like deposits in pure signet ring cell carcinoma. (**b**) Mucinous tumor with signet ring cells

### 2.2.5 Impact on the Extent of Surgical Resection

This has a bearing on the extent of peritoneal resection that is performed. For few small deposits in the rectouterine space, with no disease elsewhere in the pelvis, the extent of pelvic peritonectomy can vary from one surgeon to another—some may do a wide resection of the nodules or resection of the pouch of Douglas alone or go wider and remove the peritoneum over the bladder or resect the entire pelvic peritoneum including that in the iliac fossae (Fig. 2.7). All these would be considered a CC-0 resection. Whereas more extensive resection may not have much morbidity, resecting less peritoneum may be of consequence for some tumors like ovarian cancer where that normal peritoneum has a high probability of harboring microscopic disease. Contrary to this, for colorectal PM, less extensive resection may be sufficient as demonstrated by one retrospective study [48]. From the above, it may be concluded that the extent of peritonectomy should vary according to the primary tumor site.

Perhaps, the peritoneum is the only site where the extent of surgical resection is defined by the size of residual disease and not anatomically.

### 2.2.6 Resection of Uninvolved Regions

Apart from the extent of peritoneal resection, there are other issues that need to be addressed. Even when gross disease is present, some grey areas exist. For example, when there is presence of gross disease in the infracolic omentum, what is the extent of omentectomy that needs to be performed? Should the gastroepiploic arch be removed or not [49]? The basic information on pattern of disease distribution is not available in scientific literature. In ovarian cancer where the incidence of microscopic disease is high and there is a possibility of omental lymph node involvement, it may be prudent to resect the whole omentum with the arch. The same principle could be applied to mucinous appendiceal tumors with peritoneal dissemination and peritoneal mesothelioma. In others, we do not know. Currently it is not known which patients should have resection of areas like the falciform and round ligaments and omentum in the absence of gross disease. It has been recommended based on the results of one retrospective study that the omentum should be removed even if grossly normal in all patients undergoing CRS for colorectal PM [50]. Similarly, the gall bladder is resected along with the lesser omentum and hepatoduodenal ligament. The reasons for this being the possibility of microscopic disease on the serosal surface and prevention of future development of stones. There is an increased propensity for gall stone formation as a result of cholestasis due to damage to the vagal fibers during resection of the lesser omentum. Rather than trying to determine the extent of resection based on survival data which are influenced by many confounding factors which make it nearly impossible to determine the impact of extent of resection, the surgical principles should

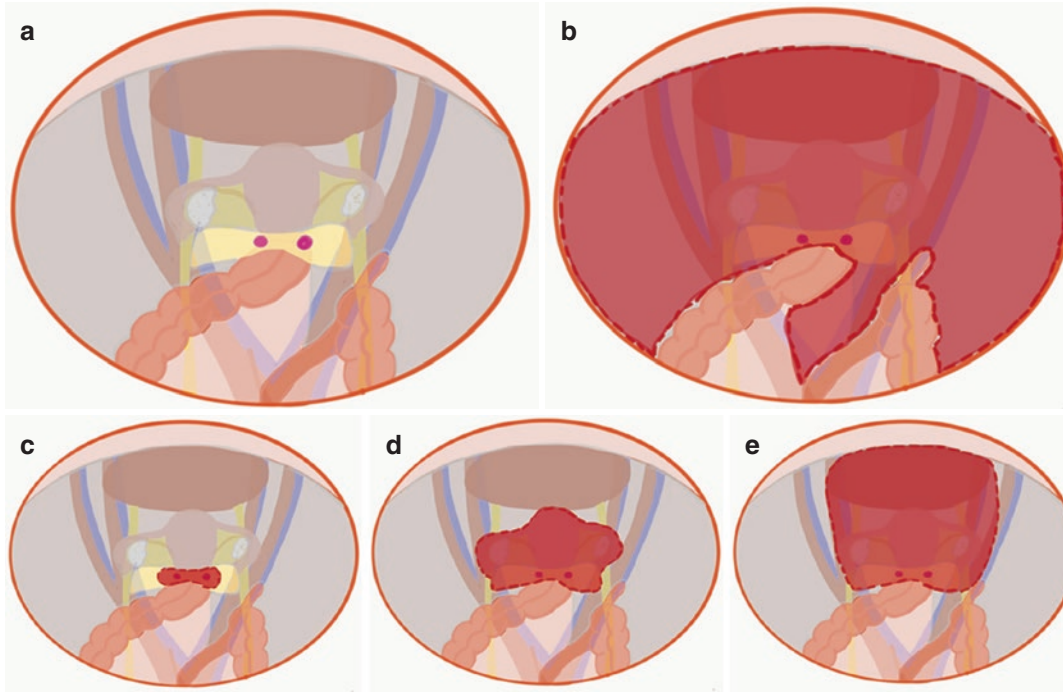


**Fig. 2.7** Variable extent of peritoneal resection that can achieve a complete cytoreduction: (**a**) deposits in the POD; (**b**) resection of the entire pelvic peritoneum as described by Sugarbaker, however, as shown in (**c**–**e**), resection of lesser amount of the pelvic peritoneum also comprises a complete cytoreduction

be based on the patterns of disease distribution and morphology as is the case for all other oncological resections. For the extremely low grade tumors like those arising from a mucinous appendiceal neoplasm, it may be acceptable to have residual disease especially when the morbidity is high but not for other tumors.

### 2.2.7 Primary Tumor Type

In addition to the primary tumors described above, sarcomas require specific mention. Though we have no evidence to support this recommendation, perhaps resection of normal regions like the omentum, falciform, and round ligament is not needed in sarcomas. Secondly, ovarian tumors should not be treated as one entity. Even among the epithelial tumors, the serous and non-serous subtypes are clinically and biologically different. The non-epithelial tumors should be treated differently from the epithelial tumors.

### 2.2.8 Lymphadenectomy in Addition to Cytoreductive Surgery

Lymph node metastases can arise secondary to peritoneal disease. The peritoneal fluid is regularly recycled by local lymphatics [51]. This leads to transfer of free floating cells to these lymphatics which then leads to lymph node metastases and subsequent distant metastases [52]. In addition, infiltration of the subperitoneal layer can lead to lymphatic and lymph node involvement.

Positive regional nodes are seen in 7–14% of the patients with peritoneal mesothelioma undergoing cytoreductive surgery [15, 52–55]. In one prospective study, lymph nodes were positive in 32.4% of which 15.7% were regional nodes (draining the primary tumor) and 13.6% were peritoneal nodes (in relation to peritoneal disease) [38]. Peritoneal nodes included those in relation to the resected bowel in 6.8%, in the subperitoneal

fat in 8 (4.1%), in relation to the omentum in 2 (1.0%) and paracardiac nodes in 3 (1.5%) [38].

Lymph node involvement secondary to peritoneal spread is not uncommon. But like non-metastatic disease, there is no guideline/recommendation for addressing lymph nodes. When enlarged nodes are found on imaging or intraoperatively, they are removed. For infrarenal retroperitoneal nodes, some surgeons perform a systemic lymphadenectomy in case of clinical suspicion. One of the pointers to occult metastases would be an increased peritoneal tumor burden in a particular region. The impact of increasing tumor burden on nodal positivity has been demonstrated in one retrospective study [56].

### 2.2.9 Prognostic Implications of Lymph Node Involvement

The involvement of lymph nodes has two important prognostic implications. When lymph node disease has been identified preoperatively, we may have to consider the curative potential of a surgical procedure in such scenarios. This is important if the lymph node stations cannot be addressed. One of the reasons for failure in the retroperitoneum could be the failure to address the occult nodal disease at the time of definitive surgery. Progression in regional nodes represents either growth of disease that has not been addressed surgically, or true disease progression.

The second would be planning adjuvant therapy. It must be borne in mind that in most instances, lymph node involvement is not picked up by pre-operative imaging. Disease-specific evaluation of pattern of lymph node involvement needs to be done.

## 2.3 Extent of Peritoneal Resection for PM Arising from Various Primary Tumors

Based on the above review, it can be concluded that there is more to cytoreductive surgery than achieving a complete removal of macroscopic disease. The issues that need to be specifically addressed are:

1. What is the amount of surrounding normal peritoneum that needs to be resected?
2. Is it necessary to remove the peritoneum in the entire region or is focal resection enough?
3. Should regions apart from the involved region be resected and which ones?
4. Which patients should have removal of the gastroepiploic arc?
5. Which patients should have resection of the normal looking omentum, falciform ligament, and umbilical round ligament?
6. In some peritonectomies, not all anatomical regions need to be resected in absence of gross disease—For example, should the Glisson's capsule be resected as part of right upper quadrant peritonectomy and the pancreatic capsule as part of omentectomy?
7. What is the extent of lymphadenectomy to be performed?

### 2.3.1 Studies Looking at the Extent of Peritoneal Resection

There are few studies that have looked at the extent of peritoneal resection irrespective of the primary site. Deraco et al. showed a benefit of complete removal of the parietal peritoneum over selective removal—that is resection of disease bearing areas alone [40]. However, this study was retrospective and no other study has addressed this issue for peritoneal mesothelioma. Similarly, there are two studies for ovarian cancer. Both are retrospective. One study showed a similar morbidity for the two procedures. The authors could not draw conclusion on the impact on survival due to heterogeneity of the data [41]. The other study showed a benefit of complete parietal peritonectomy but the quality of the study was poor as statistical difference between survival in the two surgical approaches was not mentioned and the methodology of patient selection was not described [57].

One study looked at the need to resect uninvolved "target regions" like the umbilical round ligament (URL), falciform ligament, greater and lesser omenta in the absence of visible disease. The incidence of involvement of the omentum

was more than 15% irrespective of primary tumor site. The falciform and round ligament were involved in >15 in the absence of visible disease in patients with mucinous appendiceal tumors, mesothelioma, and ovarian cancer and in <5% in patients with colorectal and gastric cancer [58]. The study was limited by relatively small numbers. However, the negative findings were compelling. Patients with gastric and colorectal cancer seldom had involvement of the falciform, umbilical round ligament, and lesser omentum in the absence of visible disease. Hence, involvement of these structures in every patient undergoing CRS may not be required. There are two other studies that looked at the involvement of the falciform and umbilical round ligament in the absence of visible disease. One did not look at disease-specific involvement [59, 60]. The other showed recurrence at the porta hepatis in 5% of the patients who did not have resection of the URL [53]. Clinical studies looking the extent of peritoneal resection, target region involvement, and lymph node involvement in patients with peritoneal metastases are summarized in Table 2.3.

It would be ideal to know the patterns of recurrence as well. Though irrespective of primary tumor site, most recurrences after CRS are intraperitoneal, the intraperitoneal disease distribution is not described. We recommend that the disease distribution in patients undergoing first and subsequent surgeries should be captured to study the patterns of recurrence.

### 2.3.2 Application in Clinical Practice and Question for Future Research

Published studies related to the extent of peritoneal resection are limited and most of them have many limitations. Similarly, there is limited information on the disease distribution in patients presenting with recurrent disease. To know the impact of extent of resection, ideally, the patterns of recurrence should also be known. In this situation, the surgical practice should be based on scientific rationale. There should be two main principles of resection—either the entire peritoneal region should be resected or a wide resection of the involved peritoneum should be performed with free margins. In the light of existing evidence, the extent of resection to be performed for some of the common tumors for which cytoreductive surgery is performed is described in Table 2.4. In addition, in patients receiving (neoadjuvant chemotherapy) NACT, it would be ideal to resect sites involved prior to NACT as there is no accurate way of determining the presence or absence of disease in those regions and the response to chemotherapy is often not sustained for long periods. At least the scarred or thickened areas should be resected. As regards the resection of the omentum, for most tumors the omentum should be resected even in absence of gross disease. In this situation, we recommend that the gastroepiploic arch should be preserved in all patients. When the omentum has gross disease, removal of the arch needs to be done though it is grossly free for certain tumor subtypes and preservation in all others if it is possible to get a complete cytoreduction without sacrificing it (Table 2.4). Similarly, not all tumors require removal of the falciform ligament and umbilical round ligament only for some tumors in the absence of visible disease. For the gall bladder, excision is performed when an omental bursectomy is performed in all cases. Removal of the Glisson's capsule and pancreatic capsule may not be done in any patient in absence of disease in those regions.

There are some other aspects which need to be evaluated in clinical studies before they are widely adopted in clinical practice. We recommend bowel resections performed for bowel surface deposits should include resection of the draining lymph node stations—that is they should be performed according to the same principles that are used to resect a primary tumor. This is particularly for rectal resections and isolated small bowel or colonic deposits when the surgery is potentially curative. When multiple small bowel sites are involved, the curative potential of the surgery is questionable and such resections may not be performed. Similarly, for mucinous appendiceal tumors, lymph node involvement is not

**Table 2.3** Clinical studies on extent of peritoneal resection and lymph node dissection

| First author/year | Prospective/ retrospective | No. of patients | Primary site | Goals of the study | Major findings | Comments |
|---|---|---|---|---|---|---|
| *Extent of peritoneal resection* | | | | | | |
| Baratti et al. 2012 [40] | Retrospective | 60 | Mesothelioma | TPP versus SPP | TPP was an independent predictor of a longer overall survival | Small sample size, retrospective study |
| Sinukumar et al. 2019 [41] | Retrospective | 69 | Ovary, serous epithelial, stages IIIC and IVA | TPP versus SPP | Morbidity similar in the two groups. Survival better in TPP group | Small sample size, heterogeneous population |
| Somashekhar et al. 2019 [57] | Retrospective | 163 | Various | TPP versus SPP | Longer survival with TPP | p-value for survival difference in the two arms not provided; patients selection criteria not described |
| *Target region resection* | | | | | | |
| Bhatt et al. 2019 [58] | Prospective | 191 | Various | Involvement of falciform, URL, omenta | Low incidence of involvement of URL and falciform in colorectal and gastric cancer | Small sample size considering multiple primary sites |
| Jayakrishnan et al. 2014 [59] | Retrospective | 71 | Various | Involvement of URL and falciform | 5.3% had involvement in the absence of visible disease | All the discrepancies were seen in patients with appendiceal and ovarian cancer |
| Sugarbaker et al. 2008 [60] | Retrospective | 710 | Mucinous appendiceal tumors | Recurrence at porta hepatis | In 5%, recurrence was at the porta hepatis | The authors recommend resection of the umbilical round ligament in all patients |
| Passot et al. 2019 [50] | Retrospective | 337 | Colorectal | Involvement of greater omentum | 17.7% greater omentum involvement in the absence of visible disease | Full study not published, only abstract available |
| *Peritoneal disease distribution* | | | | | | |
| Bhatt et al. 2019 [42] | Retrospective | 69 | Ovary, serous epithelial, stages IIIC and IVA | Patterns of response of chemotherapy | Parietal peritoneum responds last | Small sample size, retrospective study |
| *Lymph node involvement*[a] | | | | | | |
| Bhatt et al. 2019 [42] | Retrospective | 69 | Ovary, serous epithelial, stages IIIC and IVA | Patterns of response to chemotherapy and lymph node involvement | >30% had positive regional nodes | Small sample size, retrospective study |

(continued)

**Table 2.3** (continued)

| First author/year | Prospective/ retrospective | No. of patients | Primary site | Goals of the study | Major findings | Comments |
|---|---|---|---|---|---|---|
| Bhatt et al. 2019 [38] | Prospective | 191 | Various | Patterns of peritoneal and lymph node involvement in patients undergoing CRS | Lymph nodes were positive in 32.4% (15.7% were regional nodes and 13.6% peritoneal nodes) | Small sample size considering multiple primary sites |
| Kammar et al. 2019 [56] | Retrospective | 91 | Ovarian, epithelial, advanced, and recurrent | Correlation between depth of rectal wall infiltration and mesorectal lymph node involvement | 42.5% with rectal wall infiltration confirmed on pathology had mesorectal lymph node involvement | Retrospective study, patients undergoing primary CRS and CRS for recurrence were included |
| Deraco et al. 2012 [15] | Retrospective | 83 | Mesothelioma | Lymph node involvement and impact on survival | Lymph node involvement in nearly 30% of the evaluated patients | Non-evaluation of regional nodes and positive regional nodes both had a negative impact on survival |

Abbreviations: *TPP* total parietal peritonectomy, *SPP* selective parietal peritonectomy, *URL* umbilical round ligament
[a]Only studies involving cytoreductive surgery were included

**Table 2.4** Principles of resection of various peritoneal regions according to the primary tumor site

| Primary site | Principle of peritoneal resection | Resection of "target regions" in the absence of visible disease[a] | Omental resection in the absence of visible disease | Resection of gastroepiploic arch in omentum with visible disease[b] | Resection of the lesser omentum in the absence of visible disease | Resection of uninvolved regions of parietal peritoneum[c] |
|---|---|---|---|---|---|---|
| Colorectal cancer (non-mucinous) | Wide excision with free margins | No | Yes | No | No | No |
| Colorectal mucinous tumors | Resection of the involved peritoneal region | Yes | Yes | Yes | Yes | Yes |
| Appendiceal mucinous tumors | Resection of the involved peritoneal region | Yes | Yes | Yes | Yes | Yes |
| Appendiceal adenocarcinoma (non-mucinous) | Wide excision with free margins | No | Yes | No | No | No |
| Gastric cancer | Resection of the involved peritoneal region | Yes | Yes | Yes | Yes | No |
| Serous epithelial ovarian cancer | Resection of the involved peritoneal region | Yes | Yes | Yes | Yes | Yes |
| Epithelial ovarian cancer (Non serous) | Resection of the involved peritoneal region | Yes | Yes | Yes | No | No |
| Epithelial ovarian cancer (mucinous) | Resection of the involved peritoneal region | Yes | Yes | Yes | No | Yes |
| Non-epithelial ovarian cancer | Wide excision with free margins | No | No | No | No | No |
| Peritoneal mesothelioma | Resection of the involved peritoneal region | Yes | Yes | Yes | Yes | Yes |
| Sarcomas | Wide resection with free margins | No | No | No | No | No |

[a]Falciform ligament and umbilical round ligament
[b]When the arch itself is not involved by the tumor deposits
[c]Requires further evaluation in clinical studies

**Table 2.5** Recommendations for resection of regional nodes and involved bowel segments

| Primary site | Lymph node dissection[a] | Resection of other regional nodes/non-regional nodes | Radical resection of involved segment of the bowel[b] |
|---|---|---|---|
| Colorectal cancer (non-mucinous) | No | No | Yes |
| Colorectal mucinous tumors | No | No | Yes |
| Appendiceal mucinous tumors | No | No | Yes[c] |
| Appendiceal adenocarcinoma (non-mucinous) | No | No | Yes |
| Gastric cancer | No | No | Yes |
| Serous epithelial ovarian cancer | Yes | Yes | Yes |
| Epithelial ovarian cancer (Non serous) | Yes | Yes | Yes |
| Epithelial ovarian cancer (mucinous) | No | No | Yes |
| Non-epithelial ovarian cancer | No | No | Yes |
| Peritoneal mesothelioma | Yes | Yes | Yes |
| Sarcomas | No | No | Yes |

[a]Nodal stations not directly draining the bowel like iliac, obturator, periportal, and supradiaphragmatic node
[b]Needs further clinical evaluation
[c]Only for high-grade tumors

common and this recommendation does not hold (Table 2.5). Other nodal stations like pelvic and para-aortic nodes, periportal and supradiaphragmatic paracardiac nodes may be resected when suspicious on imaging or found enlarged during surgery when the surgery is performed with a curative intent. For advanced epithelial ovarian cancer and peritoneal mesothelioma, removal of iliac, obturator, and lower para-aortic nodes should be performed for all patients. The periportal and paracardiac nodes should be removed as far as possible for patients with peritoneal mesothelioma and if suspicious for epithelial ovarian cancer. In high-grade malignancies, nodal involvement would preclude a curative resection and the role of surgery itself may be questionable in these situations. For certain tumors, resection of normal parietal peritoneal regions could be performed.

We admit that most of these recommendations are based on level 5 evidence and our own experience and propose that future studies should be carried out to determine the patterns of peritoneal dissemination and disease distribution in greater detail. However, till such evidence is generated, one has to continue to treat patients and this outline could be used to guide the extent of surgery that is performed for each patient. We believe that such a stratification could help standardize the existing practices. Though resecting certain regions may not have a significant impact on the post-operative outcomes, it is important that surgical procedures are standardized.

## 2.4 Conclusions

There is a strong rationale but limited evidence to vary the extent of peritoneal resection according to the primary tumor type. In light of existing evidence, some tumors like serous epithelial ovarian cancer, peritoneal mesothelioma, and mucinous appendiceal tumors with peritoneal dissemination merit more extensive resection and the entire involved region of the peritoneum should be resected in these patients according to the five peritonectomies described by Sugarbaker. For other tumors, the peritonectomy procedure should be tailored to widely resect the involved region. Bowel resections that are performed for peritoneal implants should include a regional lymphadenectomy as is performed for a primary tumor at that site for certain tumors when the surgery is potentially curative. Lymphadenectomy

should be a part of cytoreductive surgery for certain tumors like serous epithelial ovarian cancer and peritoneal mesothelioma. A systematic method of synoptic reporting of pathological specimens of cytoreductive surgery should be developed and adopted by all peritoneal surface malignancy centers to capture important information regarding the disease distribution within the peritoneal cavity and morphology of peritoneal metastases from different tumors. This can in future be used to establish standard guidelines for such resections.

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# 3 Therapeutic Rationale and Data Set for Reporting Cytoreductive Surgery Specimens

Aditi Bhatt, Nazim Benzerdjeb, Suniti Mishra, and Olivier Glehen

## 3.1 Introduction

Cytoreductive surgery (CRS) has emerged as the cornerstone of potentially curative treatment for selected patients with peritoneal metastases (PM) arising from various primary sites [1]. It essentially comprises of removal of disease bearing areas of the parietal and visceral peritoneum and the adjacent viscera. Depending on the extent of disease, the extent of resection and thus tissues that are submitted for histopathological analysis vary. Even when the disease is limited, certain normal looking areas like the omentum, falciform ligament and umbilical round ligament may be resected due to the high probability of the presence of microscopic disease in those regions.

Unlike other metastatic sites like the liver and the lung where cancer spread occurs through the haematogenous route, spread to the peritoneum occurs through the transmesothelial, lymphatic or haematogenous routes or a combination of these [2]. The peritoneum itself is an organ with its own blood supply and lymphatic drainage and is in close contact with all the viscera and other structures in the abdominal cavity [3]. Peritoneal metastases on the visceral peritoneum often infiltrate the underlying visceral wall and thus produce secondary lymph node metastases for e.g. involvement of mesorectal nodes secondary to infiltration of the rectal wall. Similarly, infiltration of the parietal peritoneum can lead to tumour spread to the subperitoneal nodes, retroperitoneal and mediastinal nodes. Involvement of supradiaphragmatic cardiophrenic angle nodes appears to be one example of regional node involvement in relation to peritoneal disease [4]. However, for PM, pathological findings usually comprise of looking for the presence or absence of tumour, its histological subtype and the depth of organ infiltration. There are many other pathological findings that (may) have prognostic value (Table 3.1).

There are no existing guidelines for pathological evaluation and synoptic reporting of peritonectomy or cytoreductive surgery specimens [5]. As in non-metastatic disease, the surgical stage is determined by the histopathological analysis of the resected tumour specimen as is used to guide further therapy, the histopathological analysis of CRS specimens can also yield crucial prognostic information which, as further evidence is generated, can affect the way these patients are

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**Table 3.1** Pathological findings that are/may be of prognostic value

| Pathological finding |
|---|
| Grading/histological classification |
| Evaluation of the disease extent—pathological PCI |
| Pathological response to chemotherapy |
| Evaluate regional lymph nodes—in relation to the primary tumour and peritoneal disease |
| Distribution of disease in the peritoneal cavity |
| Morphological presentation of peritoneal metastases |

treated. This chapter provides an outline of histopathological evaluation of CRS specimens and its therapeutic rationale.

## 3.2 Anatomical Considerations

The peritoneum is broadly divided into the parietal and visceral peritoneum [6]. The visceral peritoneum includes peritoneum covering the viscera including the bowel and its mesenteries, the lesser and greater omenta. The parietal peritoneum lines the anterior and posterior abdominal walls. The peritoneum is densely adherent to the underlying structures in some regions which makes it imperative to resect the underlying structure to completely remove the peritoneum. It is loosely attached in others with a generous amount of intervening connective tissue which makes resection relatively easier.

### 3.2.1 Peritoneal Regions

From a surgical perspective, there are five peritonectomies described by Sugarbaker (Table 3.2) [7]. However, each of these represents a large area of the peritoneal cavity and includes one or more peritoneal spaces and recesses bound by peritoneal ligaments. These spaces and fossae play an important role in the circulation of the intraperitoneal fluid and therefore tumour dissemination (Fig. 3.1) [8]. Peritoneal ligaments are double layers of the peritoneum that support an intraperitoneal structure. The omentum and bowel mesenteries are examples of peritoneal ligaments [6]. Other ligaments include the falciform and triangular ligaments that bind the bare area of the liver, the hepatoduodenal ligament

**Table 3.2** The five peritonectomies described by Sugarbaker (from Ref. [7] with permission)

| Peritonectomy procedures | Structures included |
|---|---|
| Anterior parietal peritonectomy | Old abdominal incisions, umbilicus, epigastric fat pad |
| Left upper quadrant peritonectomy | Greater omentum and spleen |
| Right upper quadrant peritonectomy | Glisson's capsule |
| Pelvic peritonectomy | Uterus, ovaries and rectosigmoid colon |
| Omental bursectomy | Gall bladder and lesser omentum |

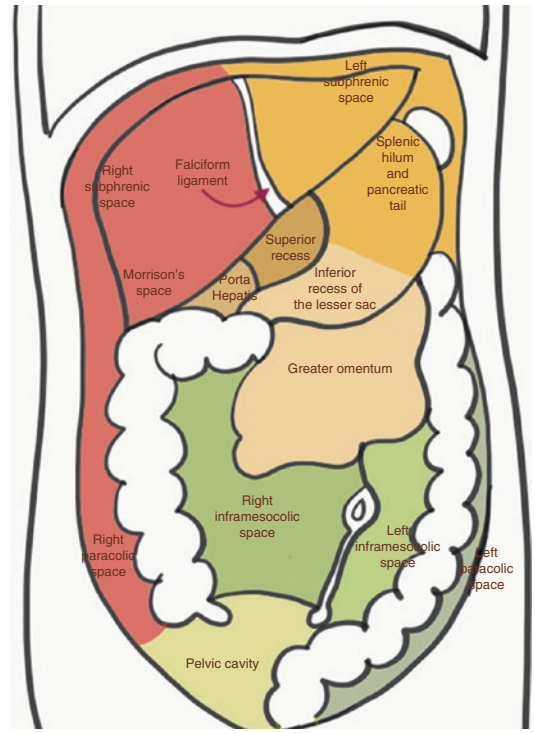


**Fig. 3.1** The peritoneal spaces. (Adapted from Ref. [3] with permission)

that contains the portal structures, the gastrohepatic ligament that contains the left gastric vessels, the gastrosplenic ligament which connects the greater curve to the spleen and contains the short gastric vessels, and the splenorenal ligament which contains the pancreatic tail [8, 9]. The transverse colon and mesocolon divide the peritoneal cavity into the supramesocolic and inframesocolic compartments (Fig. 3.1) [10]. The supramesocolic compartment is divided into the left and right subphrenic spaces by the falciform ligament. The right space

includes the subphrenic space and the subhepatic space (Morrison's pouch). One the left side is the lesser sac that has multiple recesses that communicate with the left subphrenic space cranially and the greater omentum caudally. The lesser sac is divided by fold of peritoneum containing the left gastric artery into a smaller superior recess that is in close proximity to the caudate lobe and a larger inferior recess that lies between the stomach and the pancreatic body. Sometimes, the inferior recess communicates with a potential space between the leaves of the greater omentum [11].

Similarly, the inframesocolic compartment is subdivided by the root of the small intestine mesentery into the right and the left inframesocolic space and the pelvis [7]. The left space is larger than the right and communicates with the pelvis. The right space is cordoned off from the pelvic cavity by the root of the mesentery [12].

The paracolic spaces (gutters) are located lateral to the peritoneal reflections of the left and right sides of the colon. The right paracolic gutter is larger than the left and communicates freely with the right subphrenic space. The connection between the left paracolic gutter and the left subphrenic space is partially limited by the phrenicocolic ligament. Both the right and left paracolic gutters communicate with the pelvic spaces [12].

In men, the most gravity-dependent site is the rectovesical space of the pelvic cavity. In women, it is the retrouterine space (the pouch of Douglas). The pelvic space is divided into right and left halves by the medial umbilical folds containing the obliterated umbilical arteries and further into the medial and lateral inguinal fossae by the inferior epigastric artery on each side [12].

### 3.2.2 Lymphatic Drainage

Experimental studies have shown that the lymphatic drainage of the peritoneum occurs largely through the visceral peritoneum and goes first to the retroperitoneal nodes (superior mesenteric, periportal and celiac groups) and then via the thoracic duct to the mediastinal nodes (Fig. 3.2) [13, 14]. Lymph from the parietal peritoneum goes to the mediastinum via the transdiaphragmatic lymphatics [15]. Inguinal lymph nodes are considered regional lymph nodes [16]. The peritoneal fluid is regularly recycled by local lymphatics [17]. This leads to transfer of free floating cells to these lymphatics which then leads to lymph node metastases and subsequent distant metastases [17]. In addition, infiltration of the subperitoneal layer can lead to lymphatic and lymph node involvement.

Knowledge of lymph node drainage patterns could also aid in cytoreductive surgery. Survival in most cancers is directly proportional to the completeness of cytoreduction [18]. If the lymph node disease is not adequately addressed, the survival may be adversely affected despite complete resection of the peritoneal disease. Moreover, involvement of lymph nodes may be a poor prognostic marker. The impact on survival has not been conclusively demonstrated so far perhaps due to heterogeneous patient populations in most studies comprising of patients with differing sensitivity to systemic chemotherapy.

### 3.2.3 Subperitoneal Nodes

The peritoneum comprises of a layer of mesothelial cells resting on a basement membrane underlying which is the subperitoneal space, rich in lymphatics and containing various amounts of adipose tissue [19]. Peritoneal metastases can lead to secondary involvement of subperitoneal lymph nodes. In one prospective study, involvement of such subperitoneal nodes was found in 4.1% of 191 patients undergoing CRS for PM from various primary sites [20].

### 3.2.4 Omental Nodes

The omentum is rich in lymphatics. Omental nodes are usually located along the greater curve in proximity to the arc of Barkov [20]. Similarly, lesser omental nodes can be found around the blood vessels of the lesser curve [9]. Omental lymph nodes were involved in nearly 10% of the patients in our prospective study [19].

### 3.2.5 Retroperitoneal Nodes

The involvement of retroperitoneal nodes secondary to peritoneal metastases has been demonstrated in experimental studies. Multiple pathways

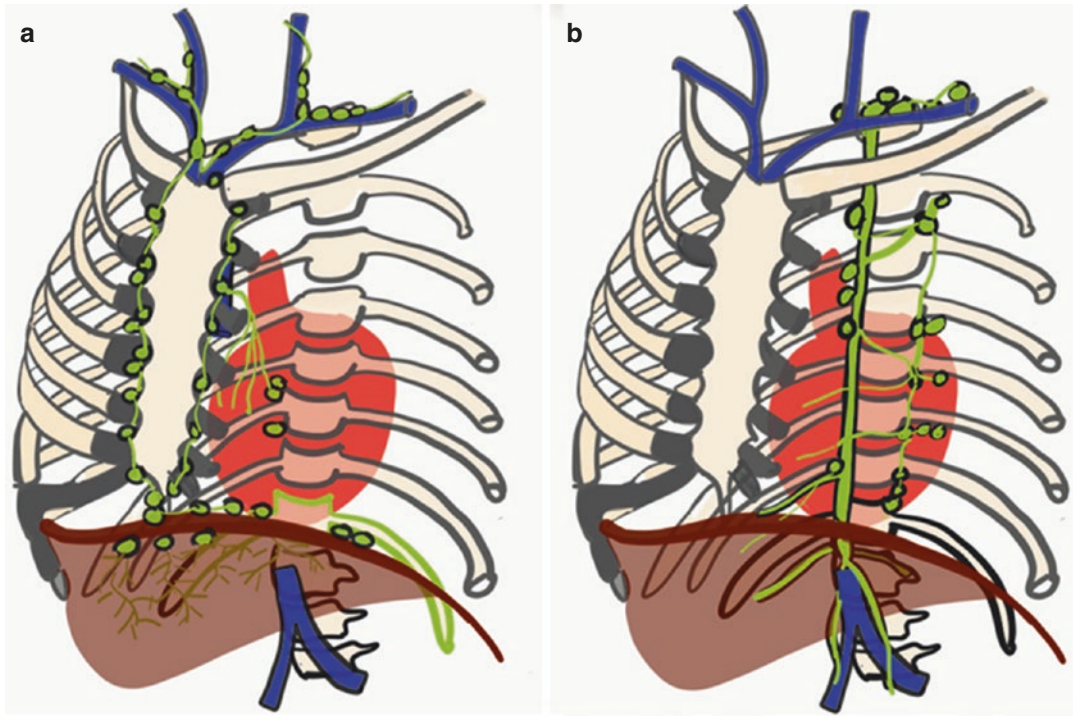


**Fig. 3.2** Lymphatic drainage from the peritoneal cavity to the supra-diaphragmatic nodes: (**a**) Trans-diaphragmatic route—Lymphatics of the diaphragm drain the peritoneal lymphatics and then drain into the supra-diaphragmatic nodes. The supra-diaphragmatic nodes are divided into two groups—retrosternal and paracardiac. (**b**) Posterior route—the posterior route drains all the lymphatics from the deep lymphatic vessels inferior to the diaphragm (iliac, para-aortic and mesenteric) and the superficial lymphatic vessels inferior to the level of umbilicus. The vessels unite to form the cisterna chyli and the thoracic duct, which travels in the posterior mediastinum and opens into the junction of the left subclavian and internal jugular vein. (Adapted from Ref. [28] with permission)

exist for drainage of the peritoneal cavity including lymph nodes in the celiac, superior mesenteric and periportal groups [21–23]. Similarly, the pelvic lymph nodes comprising of the iliac and obturator nodes and nodes around the infra-renal aorta and inferior vena cava can be involved secondary to peritoneal disease [24]. In ovarian cancer, involvement of these nodes could be secondary to the primary tumour itself or to the peritoneal disease.

In one study on ovarian cancer, the extent of nodal involvement correlated with the extent of peritoneal disease [25]. The para-aortic nodes were more commonly involved than pelvic nodes and the authors found that pelvic node involvement was more often secondary to the peritoneal disease rather than the primary tumour [25]. Another study found a relation between the lesion score in the pelvic regions and involvement of pelvic and para-aortic nodes [26]. The involvement of other nodes has not been specifically studied in relation to peritoneal disease in humans. But in the authors' experience, involvement of periportal nodes is often seen in patients with peritoneal metastases.

### 3.2.6 Paracardiac and Mediastinal Nodes

There are two major groups of supra-diaphragmatic paracardiac nodes as described by Rouviáere [27]. Group 1 is the anterior prepericardiac group that comprises of one single median group placed in the retroxiphoid region and a lateral group on either side placed behind the seventh anterior costal-cartilage junctions. Group 2 comprises of 2 aggregates located around the phrenic nerve, one on either side, with the left one often being absent [27]. Their afferent lymphatics drain portions of the diaphragm,

liver, pleura and anterior abdominal wall. Group 1 further drains into the internal mammary nodes and group 2 into the anterior mediastinal nodes. There are two routes of lymphatic drainage of the peritoneal cavity as described above. One is the anterior route in which the Rouviáere's nodes are involved [28]. The other is the posterior route in which mediastinal nodes are involved directly through the thoracic duct which drains the deep retroperitoneal nodes. Thus, mediastinal node involvement can occur secondary to parietal peritoneal metastases without involvement of paracardiac nodes. It is believed that the anterior root is the more common route [28]. The involvement of paracardiac nodes has been shown in ovarian and colorectal cancer and peritoneal mesothelioma [16, 29, 30]. Though the prognostic significance has not been consistently demonstrated, involvement of these nodes has been associated with an increased disease burden [31].

This knowledge of the nodal drainage of the peritoneal cavity is important both for the surgeon and the pathologist.

## 3.3 Pathological Evaluation of Cytoreductive Surgery Specimens

### 3.3.1 The Surgeon's Role

#### 3.3.1.1 Labelling of Surgical Specimens by the Surgeon

The five peritoneal resections are divided into five regions that is the pelvic, anteroparietal, right and left upper quadrant (including the omentum) and omental bursa (lesser omentum and lesser sac) [7]. Each of these can be further divided into anatomical sub-regions (Fig. 3.3). Each region includes a large area of the peritoneum. Not the entire peri-

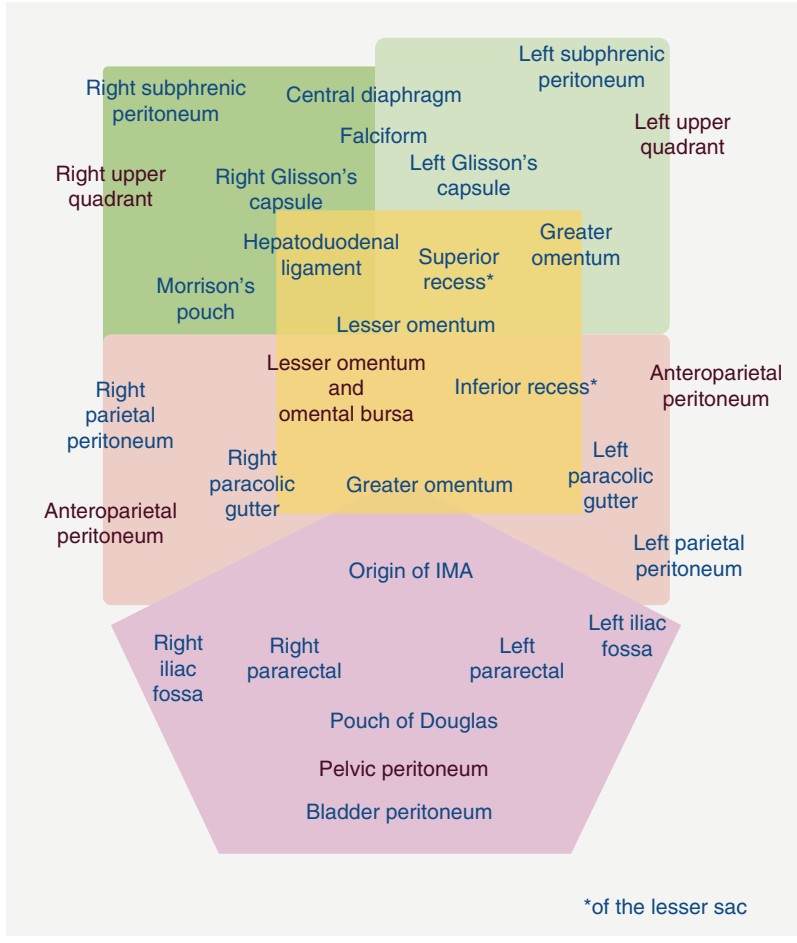


**Fig. 3.3** Peritoneal regions included in the five peritonectomies described by Sugarbaker

toneum in a region is involved in every patient and one or more sub-regions are involved. The prognostic implications of involvement of sub-regions remain unknown. We recommend defining each sub-region by either sending it as a separate specimen or marking it out for the pathologist in an en-bloc resection specimen. An example of identifying various regions on an en-bloc peritonectomy specimen is shown in Fig. 3.4.

The Glisson's capsule is identified separately from the right upper quadrant peritonectomy specimen. Other peritoneal regions like the pancreatic capsule, omental bursa, hepatoduodenal ligament and areas of mesenteric peritoneum are identified separately. The corresponding PCI region can be determined using the 'PROMISE' internet application [32].

### 3.3.1.2 Morphological Description

Peritoneal deposits are not morphologically the same in every patient. Some tumours present as few discrete nodules with normal intervening peritoneum. In others, the deposits are more numerous and may be confluent at some places (Fig. 3.5). The morphology varies not just according to the primary tumour site but also the time point at which surgery is performed. Morphological alterations and changes occur following administration of systemic therapies [33]. Following a response to chemotherapy, areas harbouring PM may become scarred or thickened, result in formation of adhesions or appear absolutely normal. It is not always possible to accurately determine the presence or absence of disease on visual inspection alone. Some morphological changes like thickening are not uniformly associated with disease. The surgeon must provide the morphological description to the pathologist in the pathological requisition form. This will ensure that areas showing subtle changes are not missed by the pathologist. Very small nodules may not be

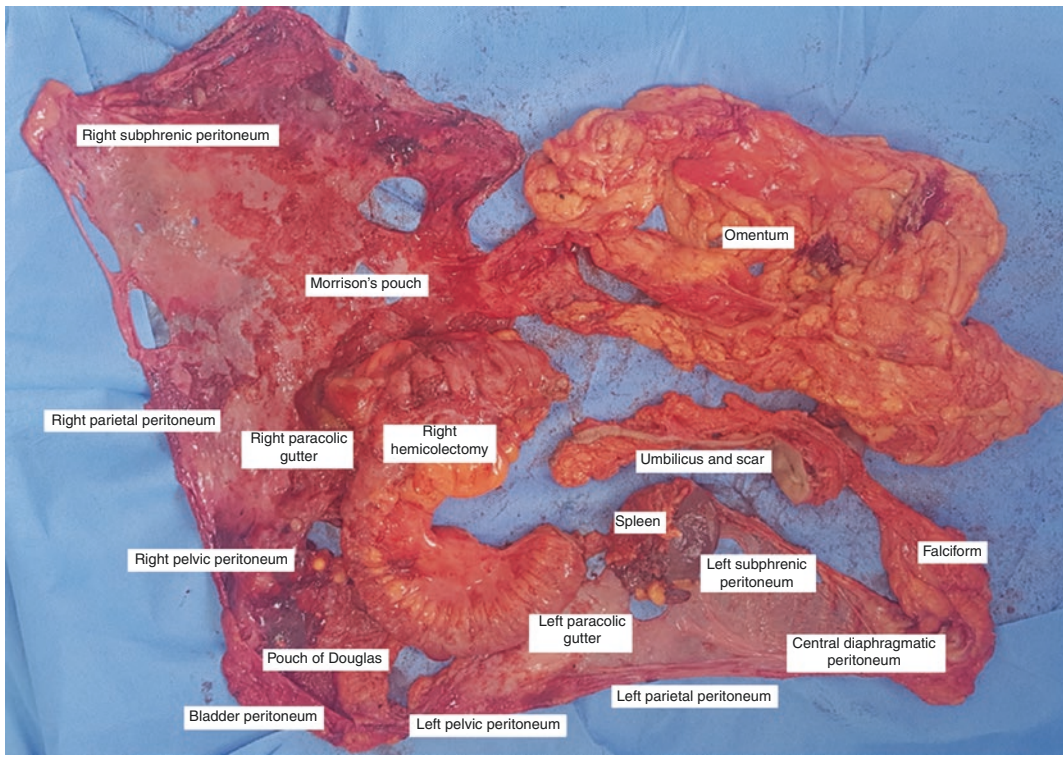


**Fig. 3.4** Identification of various peritonectomy regions on the resected en-bloc specimen

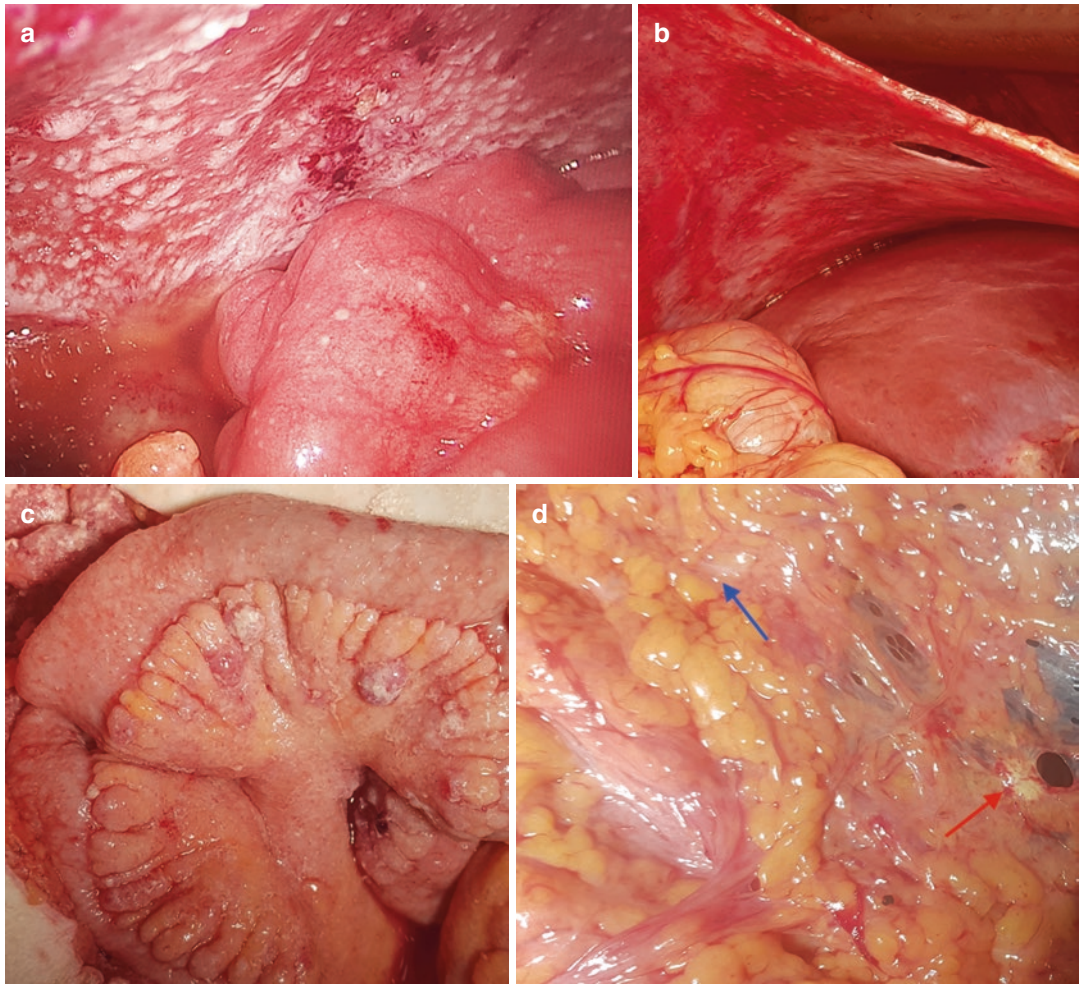


**Fig. 3.5** Variations in morphological appearance of peritoneal deposits: (**a**) nodular peritoneal deposits in gastric cancer, the intervening peritoneum is also thickened; (**b**) thickened peritoneum post chemotherapy in ovarian cancer with few macular deposits; (**c**) peritoneal mesothelioma with thickened mesenteric peritoneum representing diffuse involvement; (**d**) small nodule (red arrow) and areas of scarring (blue arrow) in serous ovarian cancer following chemotherapy

appreciable once the specimen has been fixed in formalin. The morphological presentation of the peritoneal metastases can be described as one or more of the following:

- Discrete tumour nodules
- Confluent nodules
- Plaques
- Omental cake
- Adhesions
- Scarring
- Thickened peritoneum
- Normal peritoneum

The size of the largest nodule in each region must be mentioned as well. Chemotherapy-related changes may not be appreciable after fixation and such changes if present should be documented and communicated with the pathologist.

### 3.3.2 The Pathologist's Role

#### 3.3.2.1 Handling of Specimens by the Pathologist

Ideally, the gross findings should be recorded on fresh specimens but this is usually not possible.

Fixation may help in identifying tumour nodules better (Fig. 3.6). Fixation is performed as early as possible, ideally within few hours to prevent any degradation of proteins and nucleic acids that might occur during cold ischaemia especially when biomarker evaluation is contemplated [34, 35]. Fixation in 10% neutral buffered formalin (4% formaldehyde solution) is performed for 6–48 h [36]. Longer or shorter fixation times may adversely affect biomarker testing, whereas under-fixation can result in poor tissue morphology [37]. Adequate fixation also allows thinner sections to be taken from the tumour and proper sampling of lymph nodes. The bowel regions may be opened up to facilitate permeation of the fixative [38]. In this situation, it may be required to pin the specimen on a cork board and then immerse it in formalin for some time.

Acidic fixatives (e.g. Bouin) can cause rapid degradation of nucleic acids and accelerated fixation with heated formalin can lead to alteration in tissue morphology and should not be performed [39, 40].

#### 3.3.2.2 Gross Description and Sectioning

The pathologist describes the gross findings in each region in detail comprising of the following:

**Peritonectomy Specimen(s)**

- The three-dimensional measurement (in millimetres) of the specimen and its integrity
- The presence or absence of tumour
- A description of the tumour deposit—discrete nodules, confluent nodules, plaques or any other
- The maximum diameter of the largest tumour nodule (in millimetres)
- The presence or absence of other nodules

One or more sections are taken from the largest nodule depending on the size of the nodule. This is extrapolated from guidelines for evaluation of primary tumours where sections are taken at 3–4 mm intervals (though most of the times sections are taken at 1 cm intervals) [41]. Thus, depending on the size of the nodule, on an aver-

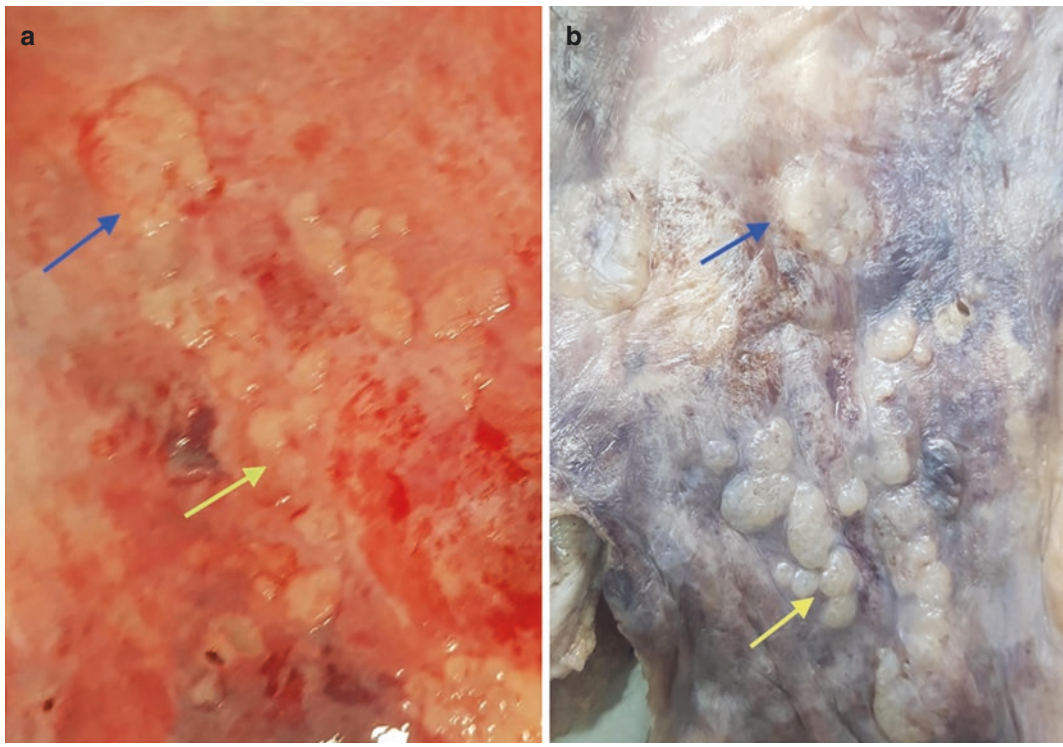


**Fig. 3.6** (**a**) Tumour nodules as seen in freshly resected peritoneum (blue and yellow arrows). (**b**) The same nodules after fixing the specimen in formalin

age 1–3 sections will be needed. For very large deposits, most pathologists take 4–5 sections representing different regions. Additional sections should be taken from one of the adjacent nodules and from the normal peritoneal surface. Currently, the therapeutic implications of such information are not known. For plaques and confluent nodules, a minimum of 2 sections are taken from the whole plaque/region. We recommend 2 sections as is commonly done for primary tumours. There is no evidence/guidelines or similar recommendations by other authors to support this recommendation. In absence of gross tumour, the clinical history is considered. If chemotherapy has been administered before, a minimum of 5 sections is taken from the region to designate a complete response. This recommendation is extrapolated from the guidelines for documenting a complete response in rectal cancer [42]. If no prior therapy has been given and normal peritoneal has been resected, 1–2 sections from the region should be taken. After sectioning, the specimens are embedded in paraffin [43].

#### Adjacent Lymph Nodes

The adjacent fat that is removed along with the peritonectomy specimen is evaluated for the presence of lymph nodes. The lymph nodes are counted and a gross description provided similar to the reporting of other regional nodes.

Standard criteria for sectioning of nodes is followed. All lymph nodes identified are sampled as follows: whole node if <4 mm; central block through longest axis for larger nodes [42]. Any other dissected regional nodes are examined for the presence of disease.

#### Adjacent Viscera

Adjacent viscera are examined for the presence or absence of tumour deposits.

The size of the largest nodule, presence of other nodules and distribution of the nodules are provided. The presence or absence of tumour at the margins of resected ends of bowel is mentioned. Sections from the area of deepest infiltration of the organ and two adjacent areas are taken. The evaluation of lymph nodes in the attached mesentery is performed as in case of a primary tumour in the bowel even if the same is absent.

#### Evaluation of the Omentum

The international collaboration on cancer reporting (ICCR) has laid down guidelines for handling of the omentum in ovarian cancer [44]. Our recommendations are based on these guidelines. Three dimensions of the omentum should be provided. The size of the specimen can be helpful to determine the extent of sampling that is needed. In the setting of a grossly involved omentum, submitting one block for histological examination is considered sufficient [45, 46]. However, most pathologists will take more than one section and we also recommend the same. In patients who have received neoadjuvant chemotherapy, where histological assessment of tumour response to therapy is needed, examination of 4–6 blocks/sections of omentum is recommended [44].

#### Evaluation of the Normal Peritoneum

As described above, in some tumours there is microscopic disease in the intervening normal peritoneum between tumour nodules [47]. Our own prospective study has shown the presence of disease between tumour nodules in over 50% of the patients with ovarian cancer, mucinous appendiceal tumours and peritoneal mesothelioma [19]. Even in non-metastatic disease, there is a recommendation to take sections from normal tissue adjacent to the primary tumour [38]. Hence, we recommend taking at least one section from the normal peritoneum adjacent to the tumour in each region.

### 3.3.2.3 Microscopic Findings

The microscopic findings should include the following:

#### The Histological Tumour Type

This histological diagnosis is made for each tumour following the guidelines specific for each tumour. For certain tumours, further classification or grade is important. This is dealt with in different chapters in this book. Briefly, for colorectal cancer, certain subtypes require special mention.

Mucinous tumours—these are defined by the presence of >50% extracellular mucin [48].

Signet ring cell carcinomas—these are carcinomas having >50% of signet ring cells [48]. Those with fewer than 50% are classified as adenocarcinoma with signet ring cells. The exact percentage of signet ring cells should be specified. For appendiceal tumours with peritoneal dissemination, we recommend the use of the PSOGI consensus classification or the AJCC-8 classification [49, 50]. The grade of the primary tumour and peritoneal disease should be provided separately. The other details like cellularity and type of invasion should be mentioned in addition to the histological subtype. For epithelial ovarian cancer, mention of the histological subtype should be made [51]. Similarly, for peritoneal mesothelioma, the list of pathological subtypes is provided in Table 3.3 [52].

### Presence or Absence of Organ Infiltration and Its Depth

For any resected viscera, the presence of tumour deposit is confirmed on microscopy. The type of involvement is specified—for solid organs like the liver—whether the capsule alone or the parenchyma is involved. For hollow viscera—the deepest layer of the wall that is involved—serosa, muscularis or mucosa should be mentioned. For appendiceal mucinous tumours, the type of invasion—infiltrative or pushing—should be mentioned [53]. And in this regard, it is important to mention whether the infiltration is by mucin or epithelial cells as the prognosis is worse when epithelial cells are present in addition to mucin. In peritoneal mesothelioma, the presence or absence of infiltration of the subperitoneal fat should be mentioned. The margins of the resected bowel and mesenteric lymph nodes are examined for the presence of disease.

**Table 3.3** Histological subtypes of malignant mesothelioma

| Histological subtype |
|---|
| **Localized** |
| *Benign* |
| • Adenomatoid tumour |
| • Localized fibrous tumour |
| **Diffuse** |
| *Borderline* |
| • Multi-cystic mesothelioma |
| • Papillary well-differentiated mesothelioma |
| *Malignant mesothelioma* |
| • Epitheloid mesothelioma |
| • Biphasic (mixed) mesothelioma |
| • Sarcomatoid mesothelioma |

### Chemotherapy Response Grade (Following Neoadjuvant Systemic/Regional Chemotherapy)

Pathological response to chemotherapy has an impact on survival for some tumours. For different tumours, there are different scoring systems. Currently, such scores are in use for ovarian cancer, colorectal cancer and gastric cancer. Some of them have been externally validated like the Bohm score for high-grade serous epithelial ovarian cancer (Table 3.4) [54]. The French scoring system developed by Passot et al. is the

**Table 3.4** Chemotherapy response grade for high-grade serous epithelial ovarian cancer (from Ref. [54] with permission)

| Criteria for chemotherapy response grade | |
|---|---|
| CRG 1 | No or minimal tumour response. Mainly viable tumour with no or minimal regression-associated fibroinflammatory changes, limited to a few foci; cases in which it is difficult to decide between regression and tumour-associated desmoplasia or inflammatory cell infiltration |
| CRG 2 | Appreciable tumour response amid viable tumour that is readily identifiable. Tumour is regularly distributed, ranging from multifocal or diffuse regression-associated fibroinflammatory changes with viable tumour in sheets, streaks, or nodules to extensive regression-associated fibroinflammatory changes with multifocal residual tumour, which is easily identifiable |
| CRG 3 | Complete or near-complete response with no residual tumour OR minimal irregularly scattered tumour foci seen as individual cells, cell groups or nodules, up to 2 mm maximum size. Mainly regression-associated fibroinflammatory changes, or in rare cases no or very little residual tumour in the complete absence of any inflammatory response. It is advisable to record whether there is no residual tumour or whether there is microscopic residual tumour present. |

**Table 3.5** The BIG-RENAPE group's scoring of pathological regression in peritoneal metastases from colorectal cancer

| Histological response | Type of regression |
|---|---|
| No residual tumour cell | Fibrosis |
| <50% residual tumour cells | Infarct-like necrosis |
| >50% residual tumour cells | Colloid response |

**Table 3.6** The peritoneal regression grading score (PRGS) (from Ref. [58] with permission)

| | Peritoneal regression grading score | |
|---|---|---|
| Grade | Tumour cells | Regression features |
| PRGS 1—complete response | No tumour cells | Abundant fibrosis and/or acellular mucin pools and/or infarct-like necrosis |
| PRGS 2—major response | Regressive changes predominant over tumour cells | Fibrosis and/or acellular mucin pools and/or infarct-like necrosis predominant over tumour cells |
| PRGS 3—minor response | Predominance of tumour cells | Tumour cells predominant over fibrosis and/or acellular mucin pools and/or infarct-like necrosis |
| PRGS 4—no response | Solid growth of tumour cells (seen at the lowest magnification) | No regressive changes |

only existing classification specific for colorectal cancer [55]. Briefly, the response is classified into three groups: no residual cancer cells in all specimens (complete response), 1–49% residual cancer cells (major response) and 50% or more residual cancer cells (minor or no response). For patients with multiple specimens, a mean of values is used to define the pathological response. This classification is based on the classifications used for liver metastases [56]. Tumour regression results in partial or complete disappearance of malignant cells and replacement of the tumour by fibrous or fibroinflammatory granulation tissue and/or mucinous acellular pools and/or infarct-like necrosis [56]. Hence, the type of response is further classified as a fibrotic, necrotic or colloid. The BIG-RENAPE group has devised a classification for pathological response based on the above which is described in Table 3.5.

The Japanese system of classification is common for all tumours and is extrapolated from the classification for gastric cancer [57]. Ef-0 reflects no pathologic response or response in less than one-third of the tumour tissue, Ef-1 means that the cancer is detected in the tumour tissue ranging from one-third to less than two-thirds of the tumour tissue, Ef-2 reflects the degeneration of cancer tissue in more than two-thirds of the tumour tissue, while Ef-3 responds to complete disappearance of the cancer cells.

Another scoring system has been developed by Solass et al. and is used to evaluate response following pressurized intraperitoneal aerosolized chemotherapy (PIPAC) [58]. The classification is provided in Table 3.6 and is termed as peritoneal regression grading score (PRGS). Four quadrant peritoneal biopsies are performed and a grade is assigned to each region. The recommended minimum size of the biopsy sample is 3–5 mm. The mean score and highest grade are both recorded. This system is used for other peritoneal tumours as well and showed good reproducibility for each biopsy and for the mean and maximum PRGS per biopsy set [59]. It is also recommended to take peritoneal liquid for cytological analysis for each PIPAC procedure, but its clinical value is unclear in setting of PIPAC.

We recommend following the French classification until more definitive evidence is available favouring a particular classification since it has been devised specifically for colorectal cancer. Similarly, for high-grade serous ovarian cancer, the Bohm score should be preferred.

The important unanswered question is how many regions to evaluate for the pathological response. It is possible that the grade of response varies in different regions. Hence, the response in all regions should be evaluated and the one with the least favourable response should be considered.

### Evaluation of Lymph Nodes

All lymph nodes sampled should be evaluated for the presence or absence of disease. The number

of sampled nodes and positive nodes should be reported for each site separately. Extracapsular extension present should be reported. Presence of tumour deposits other than lymph nodes should be distinguished. A tumour deposit in the anatomical region of a lymph node is considered a lymph node provided some normal nodal tissue is identified [16].

#### Calculation of the Pathological PCI

The peritoneal cancer index (PCI) that quantifies the disease in 13 abdominal regions is one of the most important prognostic factors in peritoneal surface oncology [60]. It is calculated during surgery based on the subjective evaluation of the disease in each abdominal region. Our preliminary evidence shows that the pathological evaluation of the peritoneal cancer extent differs from the surgical evaluation in nearly 80% of the patients [19]. Hence, we recommend computing of the pathological PCI similar to the surgical PCI for all patients. For the lesion size, the gross tumour size computed by the pathologist is considered. Microscopic examination should further confirm the presence of disease. If the presence of disease is confirmed on microscopy, then the gross tumour size is considered for computing the lesion score in that particular region. If there is no tumour on microscopy, then that particular region is given a score of zero. Immunohistochemistry tests may be performed to determine the presence or absence of disease in a particular region. Figure 3.7 shows an algorithm for calculating the pathological PCI. The structures in each region of Sugarbaker's PCI can be defined using Fig. 3.8.

### 3.3.3 Peritoneal Fluid Cytology

The sampling of peritoneal fluid should be performed soon after opening the abdominal cavity. The fluid is obtained by peritoneal lavage or sampling of the ascetic fluid if present. To perform a peritoneal lavage, 200 mL of isotonic saline solution (NaCl 0.9%) is instilled into the abdominal cavity (50 mL for each quadrant: right upper, right lower, left upper and left lower) [61]. After 2 min, 50 mL of fluid are taken out from the pelvis and is centrifuged at 2000 rpm for 10 min at room temperature. The cell pellet is aspirated, smeared onto a glass slide and fixed with methanol. Smears are stained with a 5% Giemsa solution. Positive samples are defined as the presence of at least three tridimensional clusters of malignant cells (epithelial cells with elevated nuclear-cytoplasmic ratio and vacuolated cytoplasm).

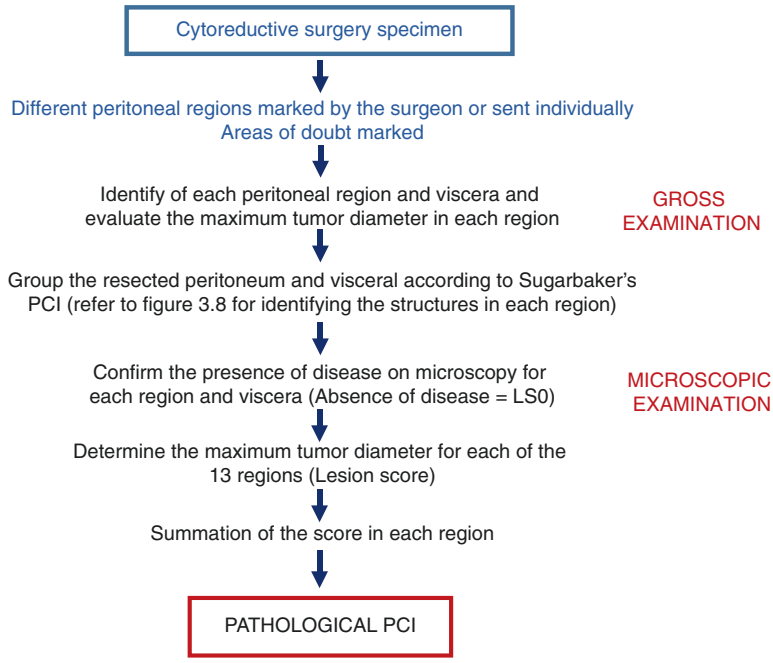


**Fig. 3.7** Algorithm for calculating the pathological PCI

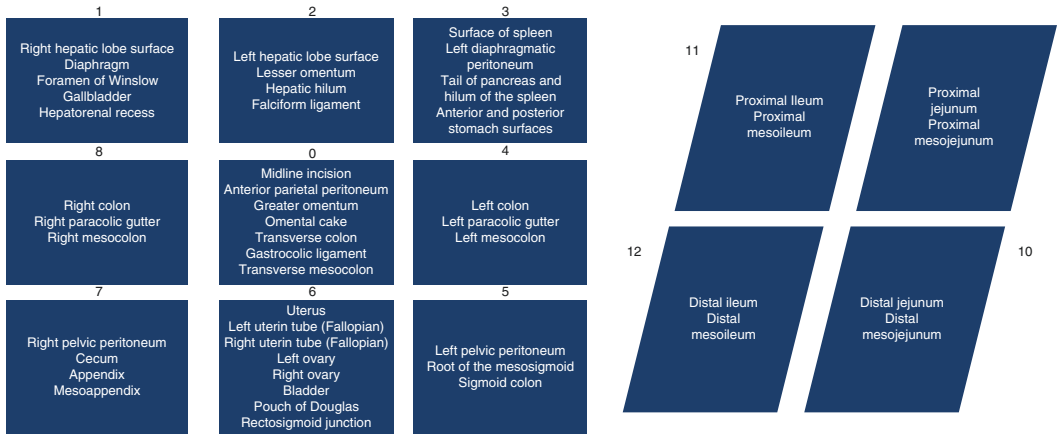


**Fig. 3.8** Structures in each of the 13 regions of Sugarbaker's PCI

| Pathological finding | Increasing score → | | | |
|---|---|---|---|---|
| PCI | 0–9 | 10–19 | 20–29 | 30–39 |
| Lymph nodes | Regional nodes negative, peritoneal nodes negative | Regional nodes positive, Peritoneal nodes negative | | Regional nodes positive, Peritoneal nodes positive |
| Pathological response to chemotherapy | Complete response | Near complete response | Moderate response | Poor response |
| Regional distribution | Around the primary | Lower regions | Middle regions | Upper regions |
| Morphological presentation | No tumor in normal peritoneum adjacent to tumor nodules | | Presence of tumor in the normal peritoneum adjacent to tumor nodules | |

**Fig. 3.9** Pathological variables that may have prognostic value and could be used to compute a scoring system to stage peritoneal metastases from different primary tumours

Negative samples are defined by the absence of malignant cells and dubious cases by the presence of one or two clusters of abnormal epithelial cells (large size, abnormal nuclear-cytoplasmic ratio or enlarged nucleus) [62]. Cell blocks are prepared and immunohistochemistry may be used to confirm the presence of tumour in doubtful situations.

## 3.4 Summary and Recommendations

We have brought out some of the pathological factors that may have prognostic value in this chapter. The prognostic value of the histological subtype/grade has been demonstrated for many tumours. The other factors like pathological PCI, pathological response to chemotherapy, presence of microscopic disease adjacent to tumour nodules and regional lymph node value may have prognostic value which needs to be determined. Hence, we recommend a systematic capturing of all this information. The above parameters could in future used to compute a staging system for PM (Fig. 3.9). As with TNM staging, common parameters like the tumour stage and lymph node involvement correlate with prognosis across different tumour types, we hypothesize that the factors listed in Fig. 3.7 will have a bearing on the prognosis irrespective of the primary tumour site. This of course needs further prospective evaluation, development of a scoring system and its validation.

A data set of reporting these specimens is provided here (Figs. 3.10, 3.11 and 3.12). It is difficult to have one format for reporting all tumours. This form covers all major aspects pertaining to the most common tumours presenting to a pathologist. As the surgical procedure is complex and time consuming for the surgeon, so is the pathological evaluation for the pathologist. Our prospective study of 191 patients showed that the average number of blocks prepared by the pathologist was 45 per patient [19].

**Data set for reporting of cytoreductive surgery specimens**

**Demographic information**

Given name/First name _____ Family name/Surname _____

Date of Birth _____ Sex Male ____ Female _____

Hospital _____ Hospital Number _____

Data of surgery _____ Date of receipt _____

Date of reporting _____ ..... Laboratory number _____

Surgeon _____.... Pathologist _____

**Relevant History**

Primary tumor site _____ Peritoneal metastases- Synchronous ___ Metachronous _____

Prior histological diagnosis - _____

Slides of previous diagnosis - reviewed _____ Not reviewed _____

Prior surgical score _____

Prior chemotherapy Yes___ / No_____ For primary _____ For PM _____

Neoadjuvant chemotherapy Yes _____ No _____

Previous intraperitoneal chemotherapy.. Yes____ .. No _____

Description of gross findings with list of specimens*

| No | Description | Size of the specimen (3 dimensions in millimeters) | Tumor (present /absent) | Maximum tumor diameter (in mm) | Presence of other tumor nodules (Yes/No) | No of sections taken | Section from normal peritoneum (Yes/No) |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

* Please list all the specimens as they are removed
Detailed pathological PCI (microscopic findings)

**Fig. 3.10** Data set for synoptic reporting of cytoreductive surgery specimens-1

Detailed pathological PCI (microscopic findings)

| Region | Structure | Size of largest deposit | Other deposits present/absent | Presence of disease in normal peritoneum | Pathological regression grade | PCI* Score (for the entire region) |
|---|---|---|---|---|---|---|
| 0 | Midline incision | | | | | |
| | Anteroparietal peritoneum (Right) | | | | | |
| | Anteroparietal peritoneum (Left) | | | | | |
| | Greater omentum | | | | | |
| | Transverse colon | | | | | |
| | Transverse mesocolon | | | | | |
| 1 | Right hepatic surface (Glisson's capsule) | | | | | |
| | Right diaphragmatic peritoneum | | | | | |
| | Foramen of Winslow | | | | | |
| | Gall bladder | | | | | |
| | Morrison's pouch | | | | | |
| 2 | Left hepatic surface (Glisson's) | | | | | |
| | Lesser omentum | | | | | |
| | Hepatic hilum | | | | | |
| | Falciform ligament | | | | | |
| | Caudate lobe surface | | | | | |
| | Lesser sac superior recess | | | | | |
| | Lesser sac inferior recess | | | | | |
| | Central diaphragm | | | | | |
| 3 | Splenic surface | | | | | |
| | Tail of pancrease/splenic hilum | | | | | |
| | Left diaphragmatic peritoneum | | | | | |
| | Anterior surface of the stomach | | | | | |
| | Posterior surface of the stomach | | | | | |
| 4 | Left colon | | | | | |
| | Left paracolic peritoneum | | | | | |
| | Left mesocolon | | | | | |
| | Splenic flexure of colon | | | | | |
| 5 | Left pelvic peritonuem | | | | | |
| | Sigmoid colon | | | | | |
| | Sigmoid mesocolon | | | | | |
| | Appendices epiploicae | | | | | |
| 6 | Uterus | | | | | |
| | Left fallopian tube | | | | | |
| | Left ovary | | | | | |
| | Right fallopian tube | | | | | |
| | Right ovary | | | | | |
| | Bladder | | | | | |
| | Pouch of Douglas | | | | | |
| | Rectosigmoid junction | | | | | |
| 7 | Right pelvic peritoneum | | | | | |
| | Caecum | | | | | |
| | Appendix | | | | | |
| | Mesoappendix | | | | | |
| 8 | Right colon | | | | | |
| | Right paracolic peritoneum | | | | | |
| | Right mesocolon | | | | | |
| | Appendices epliplicae | | | | | |
| 9 | Proximal jejural surface | | | | | |
| | Proximal jejural surface (mesenteric) | | | | | |
| | Proximal meso jejunum | | | | | |
| 10 | Distal jejural sunface | | | | | |
| | Distal jejural sunface (mesenteric) | | | | | |
| | Distal meso jejunum | | | | | |
| 11 | Proximal ileal surface | | | | | |
| | Proximal ileal surface (mesenteric) | | | | | |
| | Proximal meso ileum | | | | | |
| 12 | Distal ileal surface | | | | | |
| | Distal ileal surface (mesenteric) | | | | | |
| | Distal meso ileum | | | | | |
| | Total | | | | | |

**Fig. 3.11** Data set for synoptic reporting of cytoreductive surgery specimens-2

Details of visceral involvement

| Organ | Primary tumour / metastatic disease | Margins (involved/ uninvolved) | Depth of tumour infiltration (in mm) | Mucosa (involved/ uninvolved) | No of involved nodes/no of sampled nodes |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**Other details**

**Description of microscopic findings**

_____

_____

_____

_____

_____

Histological tumor type _____

Grade _____ Differentiation _____
Classification used _____

Primary tumour (if resected)
Site _____..... Size _____ Grade _____.....Differentiation_____
Margins _____.....Pathological stage _____
Classification used_____

Total no of nodes sampled _____
Total number of positive nodes _____

**Regional nodes**

| Site | Total nodes sampled | No of positive nodes |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

**Peritoneal nodes**

| Site | Total nodes sampled | No of positive nodes |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

**Fig. 3.12** Data set for synoptic reporting of cytoreductive surgery specimens-3

**Ascitic fluid**- Positive _____. Negative _____. Not submitted _____

**Chemotherapy response**
Score used _____
Overall score _____
Pathological complete response Yes___/ No_____

Final diagnosis

_____
_____
_____
_____
_____
_____

Signature _____ Date _____

**Fig. 3.12** (continued)

## 3.5 Conclusions

Pathological evaluation of cytoreductive surgery specimens can yield many prognostic factors that can in future guide the treatment of these patients. It is complex and requires considerable effort on the part of the pathologist as well as a coordination between the surgeon and the pathologist. Standardized methods of synoptic reporting of CRS specimens should be developed by consensus and include calculation of the pathological PCI, description of the morphology, distribution of peritoneal disease, nodal involvement and pathological response to chemotherapy in addition to determining the histological subtype/grade.

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# 4 Colorectal Peritoneal Metastases: Correlating Histopathological Findings and Disease Biology

Aditi Bhatt and Olivier Glehen

## 4.1 Introduction

Roughly 10% of the patients with colorectal peritoneal metastases (PM) undergo cytoreductive surgery (CRS) and HIPEC with a curative intent [1]. Patient selection and surgical decision making play a key role in achieving optimal outcomes much more as compared to other cancers. And the two most important prognostic factors are the PCI and a complete cytoreduction [2]. Despite complete cytoreduction, nearly 70% of the patients develop a peritoneal recurrence [3]. Even in patients who have optimal disease burden and optimal surgical and systemic chemotherapies, peritoneal recurrence remains a problem. There are several prognostic scores that have been developed to determine the prognosis and benefit of surgery, but they can be computed only during or after surgery [4, 5].

The search for newer prognostic markers continues for this heterogeneous disease [6]. Colorectal cancer is an anatomically, pathologically, and genetically heterogeneous disease. Recent data has shown that left sided and right sided colonic tumors have different outcomes with CRS and HIPEC and similarly, colonic and rectal tumors fare differently [7, 8].

One aspect that has received little attention is the distribution of disease in the peritoneal cavity. Involvement of certain regions like the diaphragmatic peritoneum and small bowel has been associated with poorer outcomes [9, 10]. The different pathways of peritoneal disease spread in colorectal cancer can result in PM that differ in clinical behavior and distribution in the peritoneal cavity. So far all this data about the pathways of spread comes from experimental studies that cannot duplicate the environment in vivo. Pathological finding of disease in a region is the most accurate way of studying the patterns of peritoneal dissemination. Neoadjuvant chemotherapy is administered to many patients with both resectable and unresectable disease. The pathological response to chemotherapy has prognostic value but has not been utilized for therapeutic decision making [11]. As research shifts its focus to molecular subtyping, routine histopathological findings could be further exploited to evaluate prognosis and treat patients. This chapter looks at potential prognostic information that can be derived from histopathological evaluation of cytoreductive surgery specimens of patients with colorectal PM.

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## 4.2 The Histological Subtype of Colorectal Cancer

Conventional colorectal adenocarcinomas arise from adenomas and the adenoma carcinoma sequence is followed [12]. Colorectal cancer has been treated as one entity baring the need for local radiotherapy for rectal cancers. In recent times, it has been shown that colonic primaries are different biologically from their rectal counterparts with the former having a better survival compared to the rectal tumors [13, 14]. The differing mutation profile has been now identified and described [14].

There are two histological types that require special mention as the incidence of PM in these patients is higher—mucinous and signet ring cell tumors. Mucinous tumors tend to be right sided, whereas no such difference is seen in the signet ring cell carcinomas. Approximately, 10–15% of all colorectal cancers are of the mucinous variety [15]. The proportion of these tumors in patients with PM undergoing surgery is higher than patients undergoing treatment of the primary tumor. The histological features do not vary between a mucinous colonic and a mucinous rectal primary.

### 4.2.1 Mucinous Adenocarcinomas

Mucinous adenocarcinomas are a variant of adenocarcinomas with >50% of the tumor mass composed of extracellular mucin [16]. Mucinous adenocarcinomas also have a high incidence of microsatellite instability (MSI-H) and mutations of genes in the *RAS-RAF-MEK-ERK* pathway (*RAS/MAPK* pathway) [17, 18]. However, the factors involved in the development of the mucinous colorectal adenocarcinoma and their prognostic implications are not yet well understood. There are no low-grade mucinous neoplasms in the colon like in the appendix. In the appendix, there are likely to be two different origins of mucinous tumors. One is the mucinous tumors like low-grade mucinous neoplasms and high-grade mucinous neoplasms that give rise to pseudomyxoma peritonei (PMP) and may over a period of time evolve into an infiltrative mucinous adenocarcinoma. The other type is the mucinous adenocarcinomas that arise de novo and also produce mucinous peritoneal implants. In the colorectum, only the second type of tumors arise. There are no low-grade mucinous neoplasms showing "pushing invasion" that give rise to PMP. Colorectal mucinous adenocarcinomas are always high grade and can be well, moderately, or poorly differentiated. They can give rise to PMP.

### 4.2.2 Signet Ring Cell Tumors

Signet ring cell carcinomas are a variant of adenocarcinoma with >50% signet ring cells [19]. These tumors constitute 0–1% of all colorectal adenocarcinomas [20]. Any percentage of signet ring cells should be reported and the exact percentage documented. Signet ring cell tumors do not have the same molecular profile as the mucinous tumors. In this setting, two types of signet ring cell tumors must be distinguished—there are mucinous adenocarcinomas with a varying proportion of signet ring cells and there are signet ring cells tumors that do not arise in the setting of a mucinous adenocarcinoma. In the former, the peritoneal deposits are similar to other mucinous adenocarcinomas while in the latter, there are diffuse plaque like deposits without the mucinous appearance (Fig. 4.1a). The second variety usually presents with extensive disease involving all peritoneal surfaces (Fig. 4.1b). A mucinous tumor with signet ring cells may still be offered cytoreductive surgery but pure signet ring cell carcinomas usually present with extensive disease that precludes such treatment.

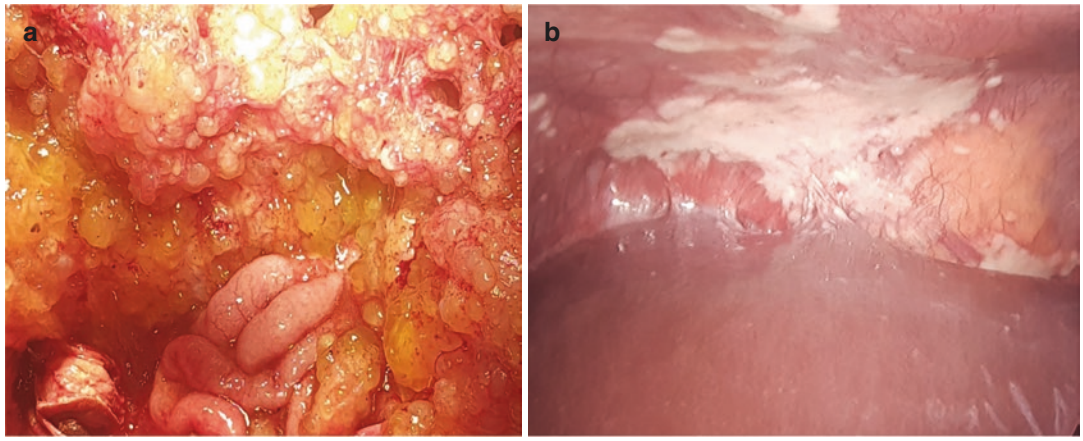


**Fig. 4.1** Differing morphological appearance of mucinous adenocarcinoma with signet ring cells (**a**) and pure signet ring cell carcinoma (**b**)

## 4.3 Pathogenesis of Colorectal Peritoneal Metastases

The pathogenesis of colorectal peritoneal metastases (PM) includes mechanisms of peritoneal spread, disease distribution in the peritoneal cavity, and morphological evolution of the peritoneal deposits. Colorectal cancer cells can reach the peritoneal cavity after being shed from the primary tumor either due to serosal involvement, tumor perforation, tumor manipulation during surgery or from transected blood vessels and lymphatics during surgery [21–23]. Following this, there are two pathways for development of peritoneal metastases—the transmesothelial pathway and the translymphatic pathway. An alternative pathway of peritoneal metastases is the hematogenous route.

### 4.3.1 Routes of Peritoneal Dissemination

#### 4.3.1.1 Transmesothelial Spread

In the transmesothelial pathway, once a viable, free cancer cell is present in the peritoneal cavity, adhesion to the peritoneal surface is required in order to ultimately invade the peritoneum, proliferate, and produce peritoneal deposits [24]. In the postoperative period, production of reactive oxygen species and inflammatory cytokines leads to upregulation of specific cell surface adhesion molecules leading to increased adhesiveness of cancer cells [25]. Surgical trauma caused to the peritoneum is also known to increase the adhesiveness and metastatic potential of free intraperitoneal cells. This creates a milieu that favors the development of PM [26]. The "transmesothelial peritoneal metastatic cascade" and its associated molecular events have been described. Lemoine et al. described 6 major steps in this cascade [27]. First is detachment from the primary tumor and gaining access to the peritoneal cavity. The second step involves transport of the tumor cells along predefined routes in the peritoneal cavity. The third step involves the attachment to the distant peritoneum and the fourth, invasion of the subperitoneal space. The underlying connective tissue provides the necessary scaffold for tumor proliferation. The final step involves angiogenesis, which sustains tumor proliferation and facilitates further metastatic growth [25]. These steps do not occur in isolation but are a continuous and interdependent process [27]. The

molecular events associated with each of these steps are listed in Table 4.1.

### 4.3.1.2 Translymphatic Spread

In the translymphatic pathway, the tumor cells gain access to subperitoneal lymphatics. This is through the lymphatic stomata that are present in abundance on the greater omentum: appendices epiploicae of the colon, the inferior surface of diaphragm, the falciform ligament, Douglas' pouch, and the small bowel mesentery [28]. They then reach the subperitoneal lymphatic sinuses and subsequently, the peritoneal surface. Proliferation takes place in the subperitoneal lymphatic space [29]. These processes are further facilitated by milky spots that are abundant in the omentum [30]. In contrast, there are no lymphatic stomata and milky spots on the liver capsule, the peritoneum covering the abdominal wall, the serosal surface of the small bowel or the splenic capsule. These peritoneal parts are not affected until the late stages of peritoneal dissemination. The exact molecular pathways underlying translymphatic spread are not known [27]. It has been proposed that some molecular events are common in both transmesothelial and translymphatic spread [27]. It is not known to what extent this pathway is employed in colorectal cancer and what its clinical implications are. The lack of involvement of regions like the falciform ligament and umbilical round ligament in patients with limited and advanced disease points towards this pathway not being the main route of dissemination.

**Table 4.1** Molecular mechanisms underlying transmesothelial peritoneal spread of colorectal cancer (from Ref. [27] with permission)

| Metastatic event | Molecules/molecular pathways involved |
|---|---|
| Detachment from the primary tumor | *Spontaneous tumor cell shedding*<br>E-cadherin ↓<br>N-cadherin ↑<br>*EMT*<br>PC1 and PC2 ↑<br>Interstitial fluid pressure ↑<br>intra-operative seeding of tumor cells during surgery |
| Peritoneal transport | Mucinous ascites<br>Actin microfilament system<br>Lamellipodia, Filipodia |
| Attachment to distant peritoneum | *Transmesothelial dissemination*<br>ICAM-1 ↑, PECAM-1, VCAM-1 ↑<br>TNF-α, IL-1β, IL-6, INF-γ<br>β1 integrin subunit<br>CD43, CD44<br>Hyaluron<br>*Translymphatic dissemination*<br>Lymphatic stomata<br>Milky spots |
| Invasion into the subperitoneal space | *Rounding of mesothelial cells*<br>HGF/SF ↑<br>c-MET ↑<br>Destruction of the mesothelial monolayer<br>Tumor-induced apoptosis<br>Fas ligand, Fas<br>*Adherence to the basement membrane*<br>Integrins<br>*Invasion of the peritoneal-blood barrier*<br>MMP-1, MMP-2, MMP-7, MMP-9, MMP-13, MMP-14 ↑<br>TIMP-1, TIMP-2, TIMP-3, TIMP-4<br>μPA/μPAR<br>Plasminogen activator inhibitor-1 and -2 |
| Proliferation and angiogenesis | *Proliferation*<br>EGFR, EGF, TGFα<br>IGF, IGF-binding protein 3<br>Angiogenesis<br>HIF-1α, HIF-1β<br>VEGF, VEGFR |

Abbreviations: *E-cadherin* epithelial-cadherin, *N-cadherin* neural-cadherin, *EMT* epithelial to mesenchymal transition, *PC* polycystin, *ICAM* intercellular adhesion molecule, *PECAM* platelet-endothelial cell adhesion molecule, *VCAM-1* vascular cell adhesion molecule-1, *TNF-α* tumor necrosis factor-α, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *INF-γ* interferon-γ, *CD43* sialophorin, *HGF* hepatocyte growth factor, *SF* scatter factor, *MMP* matrix metalloproteinases, *TIMP* tissue inhibitor metalloproteinases, *μPA* urokinase plasminogen activator, *μPAR* urokinase plasminogen activator receptor, *EGFR* epidermal growth factor receptor, *EGF* epidermal growth factor, *TGFα* tumor growth factor-α, *IGF-1* insulin-like growth factor 1, *HIF* hypoxia inducible factor, *VEGF* vascular endothelial growth factor, *VEGFR* vascular endothelial growth factor receptor

#### 4.3.1.3 Hematogenous Spread

It was for long believed that occurrence of colorectal PM represents end stage disease and can be treated only with a palliative intent. Subsequently, it was shown that at least in some patients the disease remains confined to the peritoneal cavity and may behave as locoregional disease and based on this understanding of the disease biology, cytoreductive surgery with or without HIPEC has been used as a locoregional treatment. But not more than 15% of the patients are eligible for this treatment and a large proportion of the patients present with extensive peritoneal disease that cannot be treated with CRS and HIPEC or with involvement of multiple extraperitoneal sites which precludes this treatment too. Some investigators argue that if cancer cells can reach other site through the blood stream, the same should hold true for the peritoneum [31, 32]. The seed-soil hypothesis also supports this proposition as different tumors have a propensity to metastasize to different sites. Moreover, as compared to other cancers like ovarian cancer, the incidence of positive peritoneal fluid cytology is low even in patients with established peritoneal metastases [32–34]. Though peritoneal metastases are more common in patients with serosal involvement, patients without serosal involvement develop metastases as well which could be explained by this route.

All these findings support that dissemination of colorectal cancer to the peritoneum can occur through the hematogenous route as well. Based on our observations, patients with colorectal PM could be classified into three groups based on the mode of dissemination and could be associated with the disease extent (Fig. 4.2).

### 4.3.2 Distribution of Peritoneal Metastases in the Peritoneal Cavity

The disease distribution in the peritoneal cavity in colorectal peritoneal metastases has been described as "**random proximal distribution**" which means that tumor cells implant in the vicinity of the primary tumor [35]. This is due to the increased aggressiveness and expression of adhesion molecules on the surface of tumor cells which facilitate cell adhesion [34, 35]. Even when ascites is present, this pattern is seen. Such distribution can be seen in patients who have undergone surgery and develop metachronous disease or those presenting with synchronous metastases. The sequence of involvement

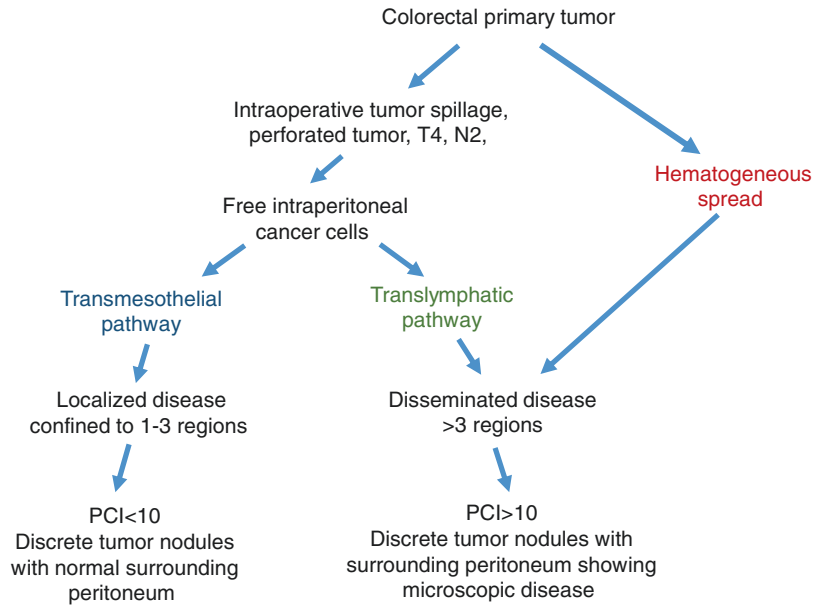


**Fig. 4.2** Different pathways for genesis of colorectal peritoneal metastases and correlation with disease extent. *Regions refer to the peritoneal regions described in the Dutch simplified PCI (described later)

of different regions is not known. In our prospective multi-centric study of patients undergoing cytoreductive surgery, it was observed that the disease involves lower and middle regions first and the upper region is involved at a later stage [36]. Roughly, only 10–15% of the patients undergoing cytoreductive surgery have involvement of the diaphragmatic peritoneum. One study showed that diaphragm involvement was associated with a high PCI and poor prognosis [37].

Thus, disease spread from the primary tumor to the adjacent regions and then the more distant regions. This knowledge is however limited. For example, in case of tumor involving the splenic flexure of the colon, the adjacent peritoneum and that in the left paracolic gutter are involved first. But it is not known what the sequence of involvement of other structures is. Does the tumor spread centrifugally in the abdomen or will it go to the pelvis first or the left upper quadrant first and subsequently involve other regions? Many investigators believe that it is the failure to recognize occult disease that leads to increased peritoneal recurrence and methods of intraoperative tumor detection are being developed to overcome this [37–39]. These techniques are expensive and tedious and time consuming. If the order of involvement of different regions is known, surgical resection can target sites likely to be involved. An old Dutch study comparing various tools for quantifying the extent of peritoneal disease showed that, in 102 patients, the commonest site of disease was the pelvis, followed closely by the omentum and transverse colon and then the right lower quadrant (Table 4.2). The small bowel was involved more often compared to the right upper quadrant, subhepatic space, and left upper quadrant. However, there was no correlation with the primary tumor site [41].

Many patients present with ascites, omental caking, and extensive disease on all peritoneal surfaces. This pattern of spread is akin to **widespread cancer distribution** that is seen high-grade malignancies like appendiceal mucinous adenocarcinoma in which there is early formation of ascites [40]. It could represent progressive dissemination from a disease that was initially limited or it could represent hematogenous spread that was widespread to start with. When the disease spreads in a progressive manner, there is usually a discrepancy in the appearance of tumor deposits in different regions. In some patients with extensive disease, there is a great deal of homogeneity in the deposits in different regions which indicates that all sites were involved at the same time. This is of interest in sequencing locoregional and systemic therapies in these patients. If the spread is hematogenous, systemic therapy becomes mandatory. The role of locoregional therapy in this situation may be questioned irrespective of the response to chemotherapy. If the spread is however transmesothelial in responders to systemic chemotherapy that have resectable disease, CRS may be considered. Currently, there is no accurate way of telling one from the other. Though most peritoneal surface oncology teams would include systemic chemotherapy in the treatment of limited colorectal PM, there is no concrete evidence demonstrating its benefit [42].

**Table 4.2** Proportion of 102 patients having involvement of different peritoneal regions according to the Dutch simplified PCI (from Ref. [40] with permission)

| Peritoneal region | No of patients affected $N = 102$ (%) |
|---|---|
| Pelvis | 90 (88.2) |
| Omentum and transverse colon | 87 (85.2) |
| Small bowel and mesentery | 78 (76.4) |
| Right lower abdomen | 70 (68.6) |
| Subhepatic space and stomach | 37 (36.2) |
| Right subphrenic space | 37 (36.2) |
| Left subphrenic space | 25 (24.5) |

One finding that can point towards hematogenous dissemination is the peritoneal deposits at the ends of the vasa recta.

Circulating tumors cells (CTCs) are known to be a risk factor for developing metastatic disease and the presence of CTCs has been associated with a poor prognosis following CRS and HIPEC [43, 44]. The presence or absence of CTCs could point towards the pathway of peritoneal dissemination and this can be a point for future research.

#### 4.3.2.1 Transition from Localized to More Extensive Disease

The pathways of peritoneal spread have largely been studied in experimental studies. The clinical evolution of PM is not known. It is not known if the same tumor can employ more than one pathway or different tumors exclusively employ different pathways. It seems more plausible that the former is true. Based on this assumption, in tumors that employ only the transmesothelial pathway, disease spreads to regions in proximity of the primary tumor. There is limited circulation of free cells due to less frequent formation of ascites in early disease. Progression to more extensive disease can occur if the disease is left untreated. The subperitoneal lymphatics connect different regions of the peritoneal cavity and thus may lead to rapid spread of the disease in the peritoneal cavity [45]. Progression from less to more extensive can be a slower process when only the transmesothelial pathway is employed and may become a more rapid process when the translymphatic pathway is employed (Fig. 4.3). In patients who have had prior surgery, it may be presumed that tumor cells trapped in fibrous tissue that is laid down postoperatively have a limited capacity to spread to other regions and the disease may remain limited for some time [36, 46]. In some others, disease can spread rapidly to the other regions. The other cancers that employ the translymphatic pathway are ovarian cancer and mucinous appendiceal tumors. In both of these, a large proportion of the patients present with disseminated disease.

Much less is known about hematological spread but it can be presumed that is present with extensive disease. Sugarbaker has hypothesized that each peritoneal deposit serves as a source of free cancer cells and thus, not only does the peritoneal deposit itself grow in size but produces other deposits as well [47]. Our hypothesis is that an alternative pathway is employed for rapid dissemination in some patients, whereas in others the transition is slower. And this would explain why some patients with disease progression on chemotherapy with resectable disease still experience a survival benefit after complete cytoreductive surgery [48].

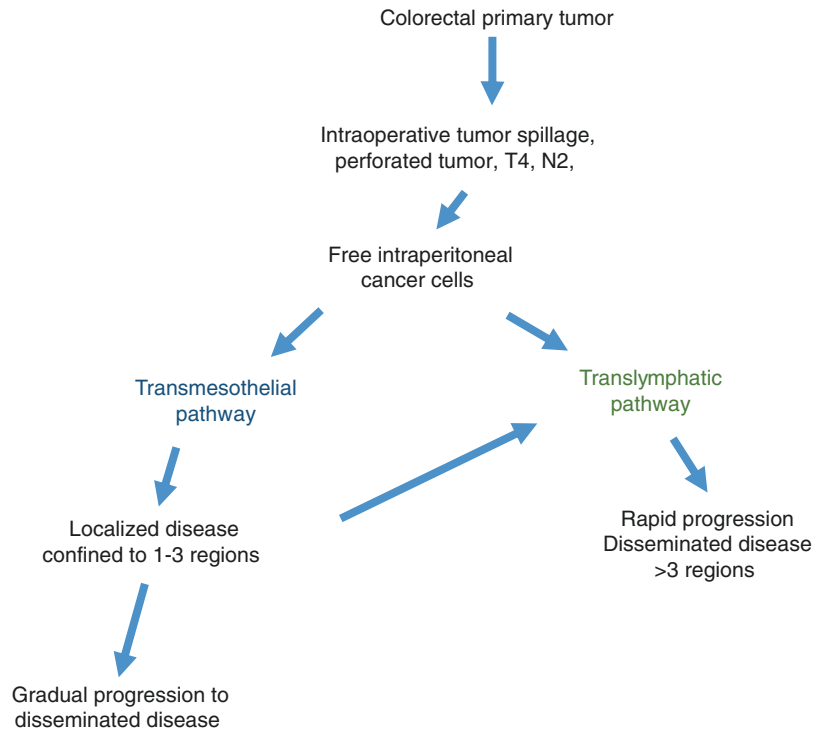


**Fig. 4.3** Probable routes of transition from limited to extensive peritoneal metastases

This understanding of the disease biology is very important for both therapeutic and preventive strategies. For example, one of the reasons for failure of prophylactic and second look surgical trials could be the mode of dissemination [49, 50]. It may not be possible to prevent hematogenous spread and perhaps even translymphatic spread with locoregional therapies. Secondly, the early detection of PM may not alter the survival as it represents a lead time bias as seen with screening strategies for primary tumors. It also provides proof for the hypothesis that PM can remain limited in extent for a certain period of time before becoming disseminated. Second look surgery is performed to detect peritoneal spread in an early stage but not all cases of limited spread are found on second look. Some are detected during routine imaging as well. And the other reason for failure of these trials may be the surveillance itself. These clinical trials are performed at expert centers where expert radiologists can identify peritoneal disease early. An average radiologist may miss such findings. Imaging has evolved significantly since the trial protocols were made [51, 52].

Even in high-grade serous epithelial cancers, rapid progression to stage 3 disease occurs and screening strategies have largely been ineffective [53]. Colorectal peritoneal metastases may not be an ideal comparison. But even though the risk factors for peritoneal dissemination are known, the incidence of PM in such patients is only 30% for some of the common risk factors and in most patients PM occur without any known high-risk features [54].

#### 4.3.2.2 Findings of Our Prospective Study

In our prospective study (unpublished results) of 100 patients, there were 34 patients with tumors of the right colon and 61 with left sided tumors including 10 rectal tumors. Involvement of the upper regions was seen in 32%. However, only 19 of these 32 patients had involvement of the diaphragmatic peritoneum. Involvement of the upper region was associated with a higher surgical and pathological PCI. It was never seen in absence of involvement of either the lower or middle regions. Contrary to this, the middle and lower regions were involved in isolation. Similarly, small bowel involvement was less commonly seen as compared to lower and middle region involvement and was rarely seen without involvement of either of these regions. These findings correlate well with the findings of the study by Verwaal et al. of 102 patients in which the pelvis and omentum and transverse colon were the most commonly involved sites [40]. Moreover, these are the findings in patients who have been selected to undergo CRS and have had a complete CRS. It can be inferred that patients with limited disease have transmesothelial spread that is seen in the vicinity of the primary tumor. This is explained by the increased incidence of involvement of the lower and middle regions in which the colon and rectum lie. We believe that the other two pathways seldom produce localized disease. In our study, none of the patients had involvement of the falciform and umbilical round ligament [55]. This is contrary to other tumors like ovarian cancer and appendiceal mucinous tumors in which involvement of these structures is common. If colorectal PM with limited spread employed these pathways, then these regions should be involved more commonly as they represent sites having high concentration of peritoneal lymphatics.

Though these results are from a prospective study with a modest number of patients, they are important. Not just the distribution but the evolution of peritoneal disease is not known. The clinical setting provides the best scenario for studying this evolution. Experimental studies cannot account for the situation in vivo and the various confounding factors [56–58]. The main drawback is that it excludes a lot of patients with PM that did not undergo cytoreductive surgery and plausibly had more extensive disease.

Looking at patients who undergo iterative procedures, it is usually those who had a low-moderate PCI at the first surgery. In a multicentric collaborative study of 231 patients undergoing iterative procedures for colorectal PM, 56% had a PCI of <10 and 31% had a PCI of 11–20 during the first surgery [59]. Most of the patients undergoing such procedures could

be the ones that had transmesothelial spread alone. Disease recurrence may be due to intraoperative spillage which has not been dealt with despite maximal locoregional therapy or occult disease that could not be identified or progression of disease that has employed other pathways of spread.

### 4.3.3 Morphology of Peritoneal Deposits

Colorectal peritoneal metastases can have a varied morphology pointing towards varied underlying disease biology. There are discrete tumor deposits with normal surrounding peritoneum in some patients, whereas in others, the tumor nodules themselves are more numerous and the surrounding peritoneum has microscopic disease. It may be hypothesized that in transmesothelial spread, the peritoneal disease comprises of solitary deposits with little abnormality in the surrounding peritoneum (Fig. 4.4). In ovarian cancer and peritoneal mesothelioma, in nearly half the patients, peritoneum surrounding the tumor nodules has microscopic disease. This is seen less often in colorectal cancer and it is likely to be related to translymphatic or hematogenous spread (Fig. 4.5). Translymphatic spread is common in mucinous tumors and thus a high PCI. In mucinous appendiceal tumors, widespread cancer distribution is seen which is the result of early formation of mucinous ascites resulting from the rupture of a mucinous tumor. In a study looking at the prognostic impact of PCI in mucinous and non-mucinous peritoneal carcinomatosis, the median PCI for mucinous tumors was 20.5 compared to 8 for non-mucinous tumors [60]. Though this may reflect a selection bias as most surgeons do not use a PCI cut-off for mucinous tumors, it also shows that in general mucinous tumors present with more advanced disease. As stated earlier, mucinous colorectal tumors are different and the widespread cancer distribution that is seen in these tumors is likely to be due to translymphatic spread rather than the early formation of ascites.

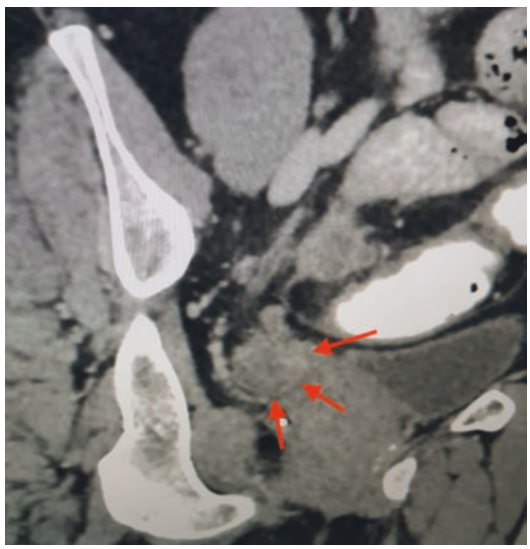

**Fig. 4.4** CT scan showing solitary metachronous peritoneal deposit 12 months after surgical treatment of the primary tumor

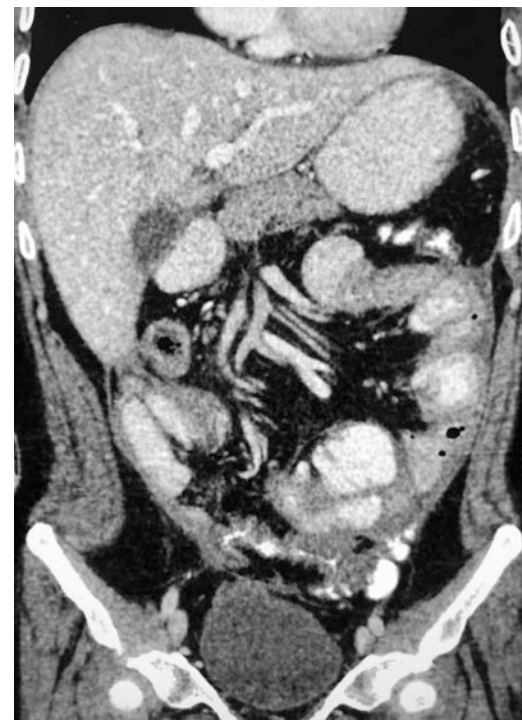

**Fig. 4.5** CT scan showing extensive disease in another patient 12 months after surgical treatment of the primary tumor. Possibly the routes of peritoneal dissemination are different in the two patients (in Fig. 4.5 and Fig. 4.6)

### 4.3.4 Clinical Implications

For transmesothelial spread, the surgical resection can comprise of limited resection of the disease bearing peritoneum. For patients in whom the spread is translymphatic or hematogenous, more extensive resection comprising of the entire peritoneal region (according to the five peritonectomies described by Sugarbaker) should be performed [61]. Whereas locoregional therapy comprising of cytoreductive surgery and HIPEC may be sufficient for patients with transmesothelial spread, systemic chemotherapy and locoregional therapies are required for tumors employing the other two pathways of spread. The former are the patients who can be cured with surgery; however, the benefit of surgery is not just providing a cure or a long disease free interval but also preventing the symptoms of peritoneal metastases [62]. The results of a randomized trial bear proof for this fact [63]. Though the median progression free survival was 13 months, the overall survival was 43 months and surgery had the additional benefit of preventing the metastases from becoming symptomatic even when the disease progressed. Trials looking at the benefit of systemic chemotherapy should look at these three broad subgroups based on the pathway of peritoneal dissemination. In addition, what should be borne in mind is the use of neoadjuvant chemotherapy and biological therapy that alters the morphology of peritoneal metastases and reduces the accuracy of visual inspection in estimating the PCI. It is the disease extent before NACT that should be considered. The use of NACT does not produce a dramatic response as it does in ovarian cancer and the PCI does not reduce by more than 2–3 points [64]. When PM develop from transmesothelial spread, surgery perhaps has a greater role and systemic therapies are an adjunct to surgery, whereas in patients with translymphatic and hematogenous spread, systemic therapy would be the mainstay and surgery would be an adjunct to these therapies. As stated above, the long overall survival despite early disease progression seen in various prospective and retrospective studies and clinical trials demonstrates the importance of systemic therapies in the management of colorectal PM [65].

Further proof of this hypothesis comes from the findings of a recent study that showed that patients who developed metachronous disease early had poor outcomes with CRS and HIPEC [66]. It shows that it is not transmesothelial spread but an alternative mechanism that is work and such patients need better systemic therapies. Adjuvant locoregional therapies also failed to demonstrate benefit in preventing peritoneal spread [67]. Whereas one may argue that it represents a non-optimal therapeutic regimen and there is no flaw in the strategy itself, it is more likely that an alternative pathway is responsible for the peritoneal spread and cannot be dealt with locoregional therapy.

At many centers, it is a policy to give neoadjuvant chemotherapy to all patients of colorectal PM irrespective to the disease extent [2]. For patients with a low PCI, surgery can be performed even when there is disease progression provided a complete cytoreduction is possible [49]. More caution should be exercised while applying this strategy to patients with a higher PCI as surgery may fail to control such disease. Passot et al. in their study of 93 patients showed that even if response to systemic chemotherapy improve survival, interesting long-term survival was obtained for progressive patients undergoing complete cytoreduction [49]. Information about the disease extent in patients undergoing surgery with progressive disease is not available but would be interesting to know.

### 4.3.5 Future Directives

Some molecular markers have been identified as predictors of poorer outcome following cytoreductive surgery and HIPEC. A multi-centric retrospective study showed that patients with mutations in *RAS/RAF* oncogenes were predictive of a poorer survival [68]. Another study showed that none of the patients with *BRAF* mutation survived for more than 24 months following surgery [69]. The negative impact of *VEGF* expression was brought out by another bi-institutional study [70]. Another study showed a favorable outcome in patients with *MSI-H* (microsatellite

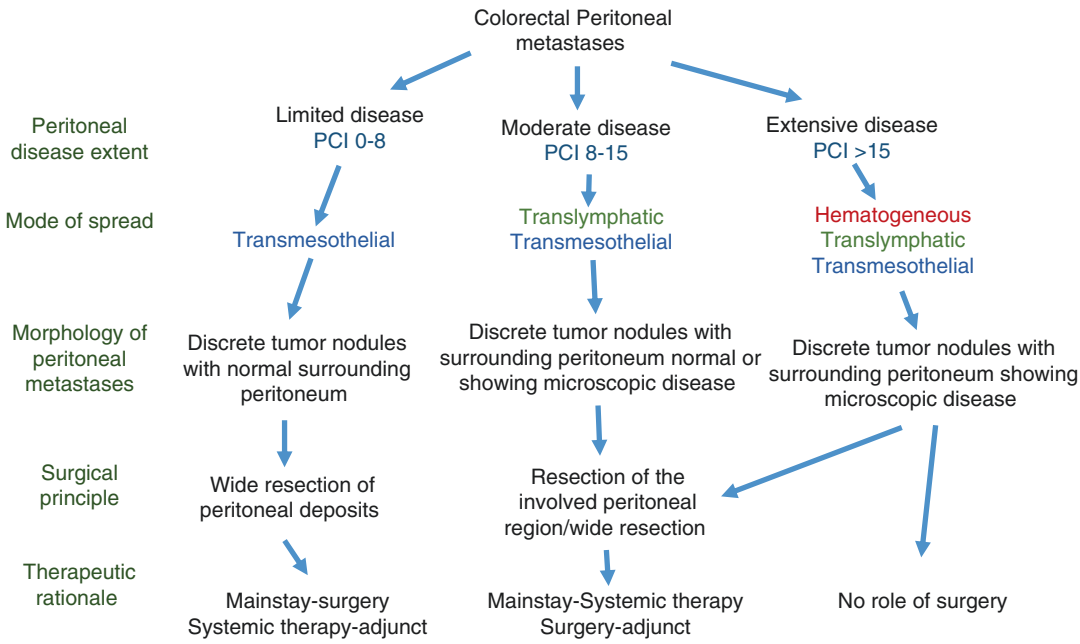


**Fig. 4.6** Correlation between pathway of peritoneal spread, disease extent, and morphological presentation of peritoneal metastases in patients with colorectal cancer

instability-high) tumors and a less favorable outcome in patients with mucinous and signet ring cell tumors [71]. Whereas these studies point towards biomarkers predictive of a poor prognosis, it is difficult to extrapolate it to clinical practice. For example, should surgery be refused to a patient carrying *BRAF* mutation? No data exists correlating the PCI with *BRAF* mutations. Do such tumors present with localized disease? We propose that all patients should be sub-classified into three groups according to the disease extent (Fig. 4.6). This is an arbitrary division based on the fact that not all limited peritoneal disease is an accidental finding. Many times it is picked up on routine imaging. As shown in the recent COLOPEC trial, nearly 70% of patients in both the control and experimental group had peritoneal metastases detected on surveillance and invasive diagnostic methods. However, 30% of the patients in both groups were not eligible for CRS at the time of detection of PM. Thus, invasive and non-invasive surveillance methods can detect PM in some patients at an early stage. In others, progression occurs which cannot be prevented or detected early, thus suggesting that alternative methods of peritoneal dissemination work that cannot be prevented by locoregional strategies.

The simplified peritoneal cancer index (SPCI) developed by the Dutch group was similar to Sugarbaker's PCI [72, 73]. In the score, the peritoneal cavity is divided into 7 regions instead of 13 and scored like Sugarbaker's PCI (Fig. 4.7). What is of interest is that the number of regions involved had an impact on survival in addition to the absolute score [40]. Involvement of 2–3 regions was associated with the most favorable outcomes. If we extrapolate this to Sugarbaker's PCI, it would come to involvement of 4–5 regions and an average PCI score of around 8–10 (Fig. 4.8). Involvement of more than 5 regions is a contra-indication for the procedure [72]. Extrapolating to Sugarbaker's PCI, this would come to an average PCI of 15–20. Thus, group 1 would have patients with a PCI of 0–8, group 2, 9–15 and group 3, >15. Our hypothesis is that different mechanisms of peritoneal dissemination play a predominant role in patients in these three groups. We hypothesize that when the transmesothelial route is the sole route of

| Region | Tumor size | None | <1 cm | 1–5 cm | >5 cm |
|---|---|---|---|---|---|
| | Score | 0 | 1 | 2 | 3 |
| 1 | Pelvis | | | | |
| 2 | Right lower region | | | | |
| 3 | Greater omentum, transverse colon and spleen | | | | |
| 4 | Right subphrenic space | | | | |
| 5 | Left subphrenic space | | | | |
| 6 | Subhepatic space and lesser omentum | | | | |
| 7 | Small bowel and small bowel mesentery | | | | |
| | Total (0–21) | | | | |

**Fig. 4.7** The Dutch simplified peritoneal cancer index (SPCI)

**Fig. 4.8** Comparison between Sugarbaker's PCI and the SPCI

| Comparison of PCI and SPCI | | |
|---|---|---|
| Lesion size score | PCI | SPCI |
| 0 | None | None |
| 1 | <0.5 cm | <2.0 cm |
| 2 | 0.5–5.0 cm | 2.0–5.0 cm |
| 3 | >5.0 cm | >5.0 cm |
| Total maximum score | 39 | 21 |

**Table 4.3** Information that should be captured in pathological assessment of cytoreductive surgery specimens of colorectal PM

| Surgical PCI | Pathological PCI | No. of regions involved | List of involved regions (Sugarbaker's PCI) | List of involved regions (Dutch simplified PCI) | Disease in regions with PCI-0 | Disease in normal peritoneum adjacent to tumor nodules |
|---|---|---|---|---|---|---|

dissemination, the PCI is seldom more than 10. When the disease is more extensive, other pathways play a predominant role. In addition to recording the absolute value of the PCI, the number of involved regions and sites of involvement should be recorded [74]. We could either divide the peritoneal cavity into 7 regions like the Dutch or a more simplified way would be into upper (regions 1, 2, and 3), middle (regions 0, 4, and 8), lower (region 5, 6, and 7), and small bowel regions (regions 9, 10, 11, and 12 of Sugarbaker's PCI). It would be interesting to see the impact of the regions involved on survival. To further study the morphology of peritoneal deposits, additional sections should be taken from the surrounding normal peritoneum and the pathological PCI should be calculated for every patient [36]. When information is captured prospectively in this manner, it would be possible to test our hypothesis, understand the disease biology better and correlate peritoneal spread with biomarker abnormality.

We also propose that the surgical PCI and pathological PCI should both be computed (Table 4.3). The surgical PCI is important for making treatment decisions during surgery. Our prospective study showed that the surgical and pathological PCI correlate only in 20% of the patients with colorectal PM. Often, the PCI was zero on pathological examination [36].

## 4.4 Mechanisms of Formation of Ovarian Metastases and Relation to Peritoneal Metastases

In women, a frequent site of the progression of peritoneal metastases is the ovaries, especially in the premenopausal women. In one retrospective study, when both the ovaries were macroscopically normal, the incidence of involvement was 17% and when one ovary had macroscopic disease and the other looked normal, the incidence of occult disease was 45% [75]. Evers et al. reported ovarian metastases in 52% of the patients undergoing CRS and HIPEC [76]. A third of these patients had only microscopic disease in the ovaries.

The term Krukenberg's tumor is frequently used for all ovarian metastases [77]. The ovarian metastases originally described by Krukenberg are defined histologically and have a significant component (arbitrarily defined as >10% of the tumor) of mucin-filled signet ring cells [78, 79]. Ovarian metastases can precede the onset of PM, occur synchronously with PM or a patient with OM may never develop PM. In a small percentage, PM may precede the development of OM. The reported incidence of peritoneal metastases in patients with ovarian metastases ranges from 30% to 72% [80, 81].

Honore et al. classified ovarian metastases as a high-risk factor for developing PM with an incidence of more than 30% [54].

The exact mechanism of tumor spread is still unknown but it is believed to occur through one of the three routes—lymphatic, hematogenous, or transcoelomic [82–84]. There is rationale to support all three mechanisms of development and it is possible that it is not just one particular pathway that is employed but different routes for different patients.

The lymphatic route is believed to be the most likely route of cancer spread with several supporting evidence.

1. Microscopically, the hilum and cortex have demonstrated lymphatic permeation.
2. Many cases of ovarian metastases have been reported in which the primary tumor is confined to mucosa and submucosa. In this situation, given the rich lymphatic network in the gastrointestinal mucosa and submucosa, the only logical explanation is that the tumor spread has occurred through the translymphatic route.
3. The risk of metastasis is higher with increased number of positive metastatic lymph nodes.
4. Lack of involvement of the peritoneum without any evidence of seedings, adhesion, or tumor infiltrates favors other pathways as opposed to the transcoelomic route [85].

It has also been proposed that ovarian metastases develop due to transcoelomic spread and are a part of the spectrum of peritoneal disease [86]. Many patients present with synchronous ovarian and peritoneal metastases and in those with ovarian metastases alone, PM develop subsequently in a large majority. This is further supported by the increased incidence of ovarian metastases in premenopausal women. The raw area created on the surface of the ovary during ovulation provides a site for attachment and subsequent proliferation of intraperitoneal free cancer cells. Contrary to this, there are some findings that support an alternative pathway of spread. There are no signs of peritoneal dissemination in most cases of ovarian metastases and the capsular surface of affected ovaries is usually smooth, without tumor deposits [87]. At times it is more than one route that is employed [85]. Ovarian metastases have been associated with an increased incidence of BRAF mutations [88].

In patients undergoing CRS and HIPEC, the presence of ovarian metastases did not lead to an inferior overall survival in one retrospective study [89]. Another study in which only oophorectomy was performed showed a median survival of around 25 months [88]. Perhaps, this study included patients with more advanced disease that may not have been amenable to CRS or it was not performed when possible.

None of these studies reports what proportion of patients have true Krukenberg's tumors as opposed to metastases without features of Krukenberg's tumor. From a pathological perspective, it may be different and may have clinical implications which are not known.

It remains uncertain if ovarian metastases truly are a spectrum of peritoneal metastases. This assumption has come from the high incidence of PM in patients with OM and supporting molecular data is lacking. Even in patients with ovarian metastases, prophylactic procedures have not been useful in preventing peritoneal recurrence and prolonging survival [50]. Though initial studies showed that a systematic second look with HIPEC was of benefit in preventing peritoneal recurrence, a randomized trial failed to demonstrate the benefit of such a strategy [50, 80, 90].

## 4.5 Pathological Response to Systemic Chemotherapy

Neoadjuvant systemic therapy is often used in patients with colorectal PM. It has the benefits of preventing/controlling metastatic disease at other sites, reducing the disease extent and identifying patients that do not benefit from cytoreductive surgery [2]. The pathological response to neoadjuvant chemotherapy (NACT) has prognostic value in patients undergoing surgery for colorectal liver metastases [91]. Similarly, one study demonstrated its prognostic value in patients with colorectal PM. In this study of 115 patients, a pathological complete response at all sites was seen in nearly 10% of the patients [11].

Tumor regression results in partial or complete disappearance of malignant cells and replacement of the tumor by fibrous or fibroinflammatory granulation tissue and/or mucinous acellular pools and/or infarct-like necrosis [92]. Residual tumor cells are hyperchromic and usually show nuclear atypia (karyorrhexis, pyknosis, or enlargement of nuclei) and presence of giant cells and apoptotic figures. Tumor cells get replaced by fibrotic scar tissue comprising of fibroblasts and bundles of collagen. The tumor itself can produce a fibroinflammatory response and this must be distinguished from a pathological response to systemic therapy. Chemotherapy-induced fibrosis has the presence of foamy macrophages which distinguish it from a fibroinflammatory response [93, 94]. Fibroinflammatory changes may be seen around the tumor in the absence of any treatment [95]. Chemotherapy can also produce infarct-like necrosis which should be distinguished from tumor necrosis. Tumor necrosis which is also called "dirty necrosis" is seen at the center of the tumor and is due to loss of blood supply at the center of the tumor. There is patchy distribution of nuclear debris. The necrotic area is mixed with viable cells and the periphery of the necrotic area is also surrounded by these. There is a rapid transition from viable cells to dying cells with pyknotic nuclei and to anucleate cells. In contrast, infarct-like necrosis comprises of large confluent areas of eosinophilic cytoplasmic remnants located centrally within a lesion with no or minimal nuclear debris [96]. The necrotic tissue is often surrounded by a layer of hyaline-like fibrosis with foamy macrophages. Cholesterol clefts, microcalcifications, and hemosiderin are sometimes present in between the necrotic cells. The third type of response is a "colloid response" which is characterized by the presence of mucin pools with or devoid of viable cells that replace the tumor cells (Figs. 4.9 and 4.10). This is similar to the acellular mucin pools seen in rectal cancer following neoadjuvant chemoradiotherapy [11, 97, 98]. When there are no viable tumor cells, it is a pathological complete response. Post-treatment mucin pools are less basophilic than untreated colloid carcinomas and may have small areas of fibrosis within them. These must be distinguished from acellular mucin seen in patients with PMP of appendiceal origin. Such mucin is characterized by the ingrowth of blood vessels (organizing mucin) and the presence of acellular mucin indicates the presence of disease.

There is only one scoring system developed by Passot et al. from Lyon-Sud Hospital specific for pathological response to NACT in colorectal cancer. The pathological response is based on the determination of the percentage of viable tumor cells with respect to the area of each nodule [99]. The response is classified into three groups; no residual cancer cells in all specimens (complete

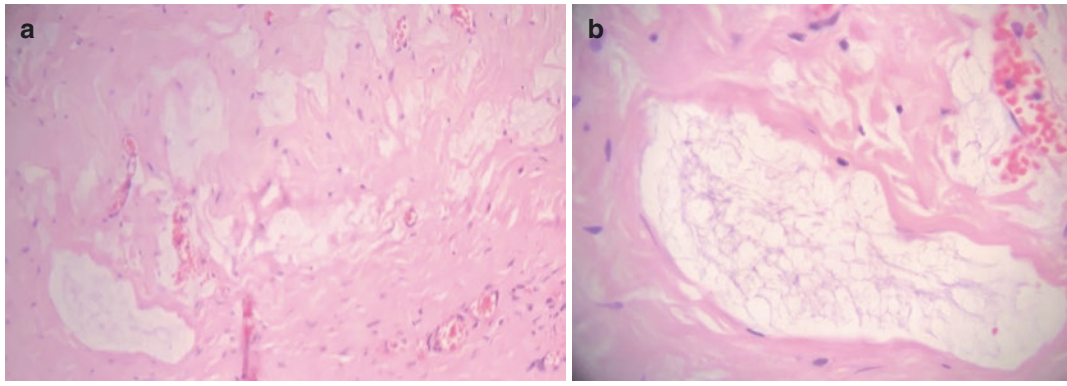


**Fig. 4.9** Acellular mucin pools seen after a complete pathological response to neoadjuvant chemotherapy in a patient with signet ring cell carcinoma (**a**, **b**)

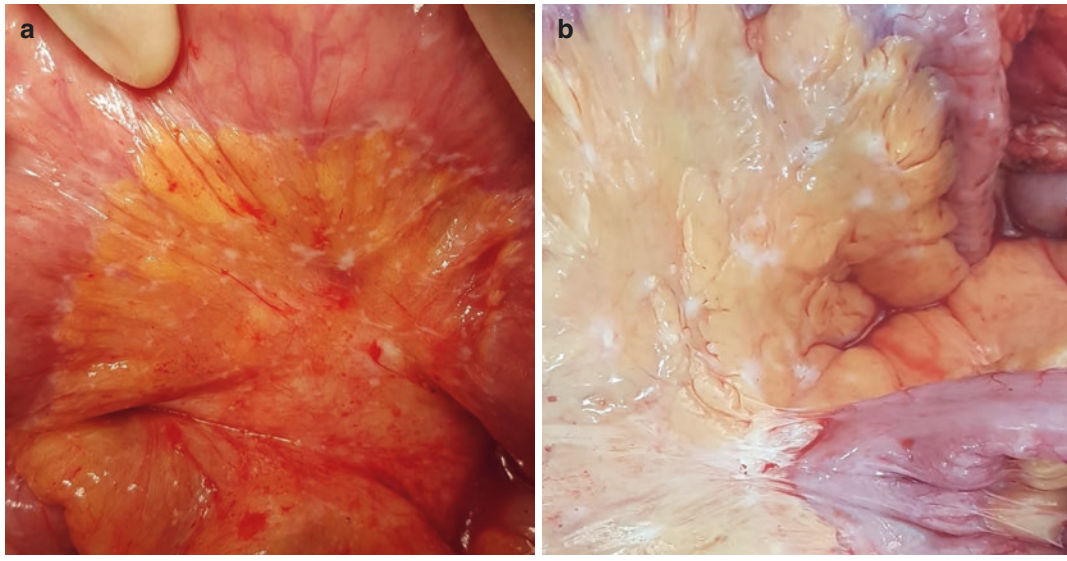


**Fig. 4.10** Intraoperative appearance of peritoneal deposits on small bowel and mesentery in the same patient as in Fig. 4.9 (**a**, **b**). It is not possible to predict the grade of response on visual appearance alone

response), 1–49% residual cancer cells (major response), and 50% or more residual cancer cells (minor or no response). For patients with multiple specimens, a mean of values is used to define the pathological response. The classification is based on the classifications used for liver metastases [100]. This scoring system has not been validated. The BIG-RENAPE group further looks at the type of necrosis extrapolating from the prognostic significance of "infarct-like necrosis" in colorectal liver metastases [94]. The classification for pathological response to NACT that is currently used by this group is as follows.

**Histological response**
- No residual tumor cell
- < 50% residual tumor cells
- 50% residual tumor cells

**Type of regression**
- Fibrosis
- Infarct-like necrosis
- Colloid response

There are two other classifications. The first one is from Japan. This classification has been extrapolated from the response in gastric cancer

and divides the response into four categories [10]. Ef-0 reflects no pathologic response or response in less than one-third of the tumor tissue, Ef-1 means that the cancer is detected in the tumor tissue ranging from one-third to less than two-thirds of the tumor tissue, Ef-2 reflects the degeneration of cancer tissue in more than two-thirds of the tumor tissue, while Ef-3 responds to complete disappearance of the cancer cells.

Another scoring system has been developed by Solass et al. and is used to evaluate response following pressurized intraperitoneal aerosolized chemotherapy (PIPAC) [92]. The classification is provided in Table 4.4 and is termed as peritoneal regression grading score (PRGS). Four quadrant peritoneal biopsies are performed and a grade is assigned to each region. The recommended minimum size of the biopsy sample is 3–5 mm. The mean score and highest grade are both recorded. This system is used for other peritoneal tumors as well.

Passot et al. showed that patients with a complete response had a survival benefit and so did patients with a major response [11]. However, they could not identify any significant predictive factor for the response. Pathological response to NACT only has prognostic value and has not treatment implications so far. As pointed out by Sugarbaker, patients who have a complete/near complete response to NACT may derive little benefit from the addition of HIPEC [101]. CRS in such patients is useful for staging and establishing a complete response [101]. The main drawback is that the response is known only after surgery and cannot be predicted preoperatively. Such scores are only for patients who have received a single line of chemotherapy for peritoneal metastases. The value of such scores in patients who have received more than one line of chemotherapy is not known.

**Table 4.4** The peritoneal regression grading score (PRGS)

| | Peritoneal regression grading score | |
|---|---|---|
| Grade | Tumor cells | Regression features |
| PRGS 1—complete response | No tumor cells | Abundant fibrosis and/or acellular mucin pools and/or infarct-like necrosis |
| PRGS 2—major response | Regressive changes predominant over tumor cells | Fibrosis and/or acellular mucin pools and/or infarct-like necrosis predominant over tumor cells |
| PRGS 3—minor response | Predominance of tumor cells | Tumor cells predominant over fibrosis and/or acellular mucin pools and/or infarct-like necrosis |
| PRGS 4—no response | Solid growth of tumor cells (seen at the lowest magnification) | No regressive changes |

## 4.6 Conclusions

Routine histopathological findings represent an untapped source of information about the disease biology of colorectal PM that is still only partially understood. The pathological disease extent as determined by the pathological PCI, the distribution of disease in the peritoneal cavity, the morphology of peritoneal deposits, and the response of NACT should be recorded systematically for all patients undergoing cytoreductive surgery. This information can be correlated with biomarker abnormalities to understand the varied clinical behavior of colorectal PM and devise different preventive and therapeutic strategies for different patients.

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# 5 Epithelial Serous Ovarian Cancer: Patterns of Peritoneal Dissemination and their Clinical Implications

Aditi Bhatt, Loma Parikh, Suniti Mishra, and Olivier Glehen

## 5.1 Introduction

Peritoneal involvement in epithelial ovarian cancer is considered loco-regional disease and classified as stage III. Over 75% of the patients are diagnosed with peritoneal involvement and it represents the commonest site of recurrence which occurs in over 80% of the patients after first-line therapy [1, 2].

The peritoneal metastatic cascade in epithelial ovarian cancer has been described by many investigators. Epithelial ovarian cancer employs the transmesothelial and translymphatic pathways for peritoneal dissemination [3]. The disease distribution in the peritoneal cavity differs from that in other cancers that employ the same routes and has not been studied in detail. It is known the pelvic spread occurs before extra-pelvic spread. But what is the sequence of involvement of upper abdominal regions? Is the greater omentum always the first extra-pelvic site to be involved? Does diaphragm involvement occur before or after omental involvement and in what percentage of patients with omental involvement is diaphragm involvement seen? Most surgeons can tell from experience that omental involvement comes first but it has not been studied systematically. Similarly, it is known that small bowel involvement comes at a later stage and is perhaps the first site to respond to chemotherapy. But there is only preliminary data on the patterns of response to neoadjuvant chemotherapy [4]. The surgical goal in ovarian cancer is complete removal of all visible disease [5]. However, this is largely dependent on the extent of exploration performed by the surgeon. Disease in the right subphrenic peritoneum could be missed if the liver is not completely mobilized as it is often present only on the tendinous portion of the diaphragm and the retro-hepatic region. If the sequence and incidence of involvement of various regions are known, resection can be based on the probability of involvement of that region and not just visual findings which are often inaccurate in determining the presence or absence of disease. This chapter reviews the pathways and patterns of peritoneal dissemination in epithelial serous ovarian cancer (ESOC), disease distribution in the peritoneal cavity and patterns of response to systemic chemotherapy and their implication on clinical practice. The impact of the histological subtype is also discussed.

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## 5.2 Histological Subtype

### 5.2.1 High- and Low-Grade Serous Carcinomas

Serous carcinoma is the commonest subtype of epithelial ovarian cancer. ESOCs constitute nearly 75% of the epithelial ovarian tumours [6]. They are further classified as low grade and high grade. The low- and high-grade tumours are genetically and biologically distinct entities. Low-grade serous carcinomas arise from definite precursor lesions like benign serous cystadenomas, adenofibromas and serous borderline tumours, a transition that takes place in a stepwise manner. Serous cystadenomas and adenofibromas are considered to be surface epithelial inclusion cysts. Their transition to low-grade serous carcinoma is promoted by *KRAS* and *BRAF* mutation [7–9].

On the other hand, most high-grade serous tumours are now known to arise from precursor lesions in the fallopian tube known as serous tubal intraepithelial carcinomas (STICs) that progress to high-grade ESOC with subsequent involvement of the ovarian surface and rapid peritoneal dissemination [10]. There is early loss of *TP53* function followed by loss of *BRCA* function leading to deficiency in homologous recombination repair of double-strand DNA breaks [11, 12].

Clinically, high-grade ESOC has been characterized by psammomatous calcification on CT, peritoneal carcinomatosis, relatively small ovaries and a highly elevated serum CA-125 level [13]. Figure 5.1 shows the type of TP53 immunostaining characteristic of high-grade ESOC.

Low-grade ESOC is considered to arise from serous borderline tumours and may have features of serous papillary borderline tumours like a papillary architecture and internal branching pattern, like that of a sea anemone (Fig. 5.2) [14]. Therefore low-grade ESOC may resemble these tumours that tend to present as large ovarian masses [15]. Figure 5.3 shows the CT images of low-grade serous carcinoma presenting with a large pelvic mass. These large masses can infiltrate the pelvic peritoneum or adhere to it or they may be adherent to the peritoneal deposits. They are usually always resectable as the retroperitoneum is not involved. ESOCs are tumours that typically colonize the mesothelial cell layer but invade no further, leaving the deeper lamina largely intact [16].

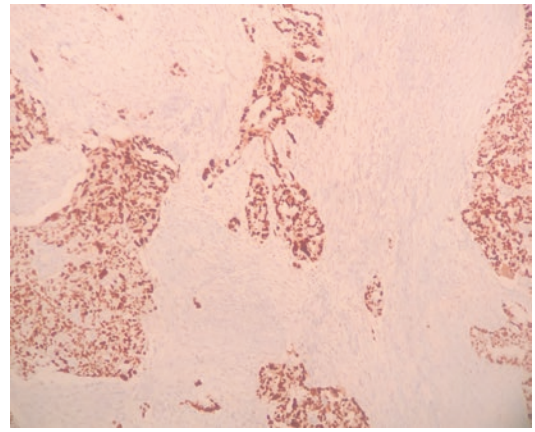

**Fig. 5.1** TP53 immunostaining characteristic of high-grade epithelial serous ovarian cancer

Even in high-grade ESOC, cancer spreads readily within the peritoneal cavity but its metastatic growths only invade the surface of affected organs [16]. An extra-peritoneal approach is preferred in these situations to completely resect the primary tumour and peritoneal deposits.

Retroperitoneal involvement is not seen unless a prior surgery in the region has led to retroperitoneal tumour implantation.

### 5.2.2 Distinguishing Serous Tumours from Other Histological Subtypes

The histopathological, morphological, and molecular features of the high-grade ESOC (Fig. 5.4) and other subtypes are different and have been clearly described elsewhere [17]. However, sometimes there are mixed tumours or those with overlapping features that make it dif-

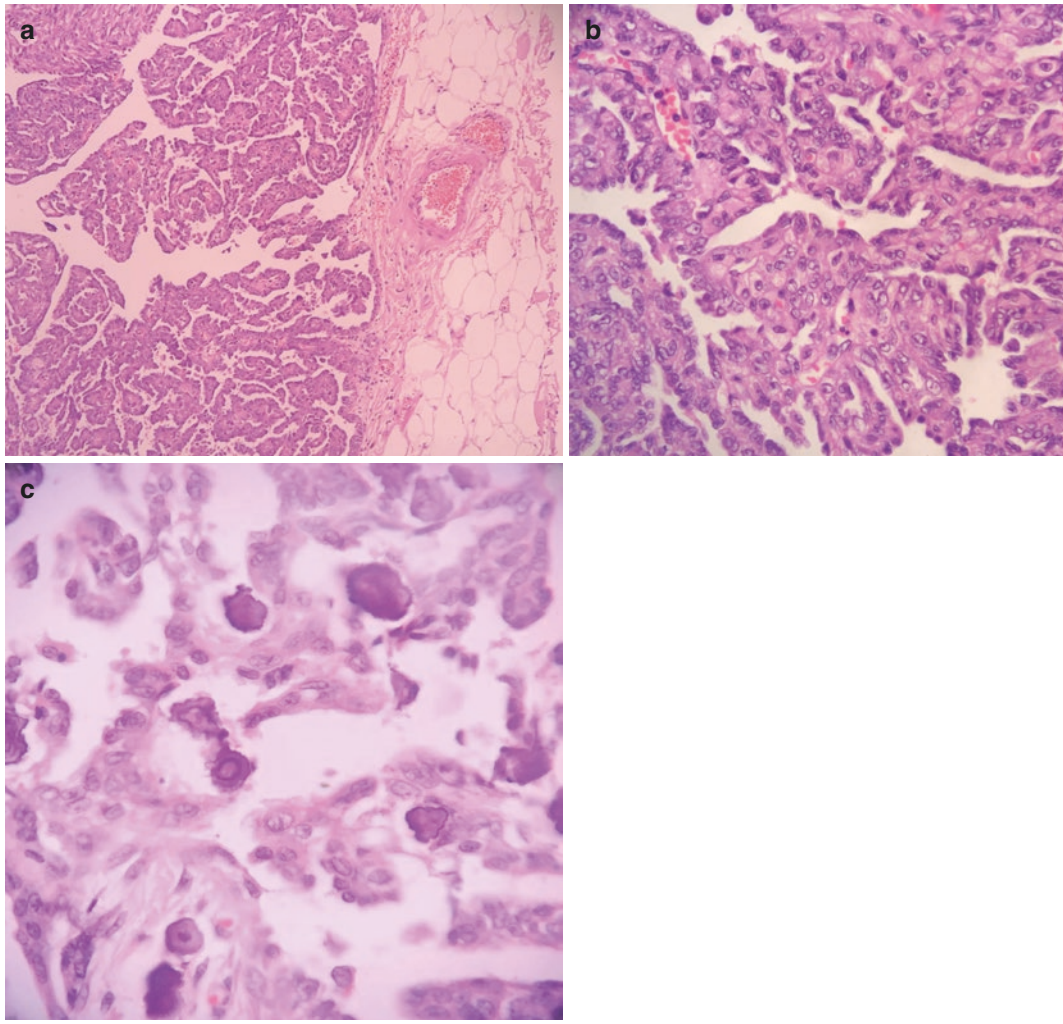


**Fig. 5.2** Typical histological features of a low-grade epithelial serous ovarian cancer: (**a**) at 10× magnification, (**b**) at 40× magnification, and (**c**) psammomatous calcifications

ficult to assign the correct subtype. Histological features of the serous subtype can be confused with clear cell features and immunohistochemistry can be used to make the distinction. In 1973, the World Health Organization (WHO) defined ovarian clear cell carcinomas as tumours with clear cells growing in solid, tubular or glandular patterns with hobnail cells lining cysts and tubules and this was further updated in 2003 (Fig. 5.5) [17, 18].

Where the histological features are confusing, immunohistochemistry can be used to distinguish them from serous tumours using markers like hepatocyte nuclear factor 1-beta (HNF1B), napsin A, Wilms tumour 1 (WT1), oestrogen receptor (ER), progesterone receptor (PR) and tumour protein 53 (p53). Clear cell carcinomas stain positive for HNF1B and napsin A and negative for WT1, ER, PR and p53, whereas the reverse is seen in high-grade ESOC (Fig. 5.6) [19–21].

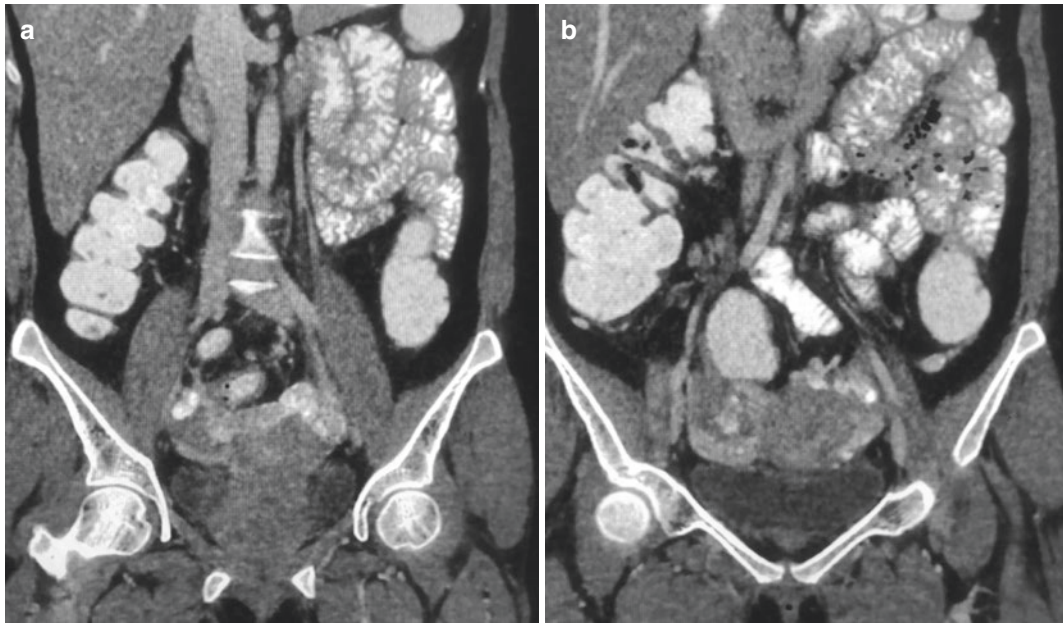


**Fig. 5.3** (**a**, **b**) CT scan images of a patient with low-grade serous carcinoma presenting with a large pelvic mass. Such masses can be resected completely using the extraperitoneal approach

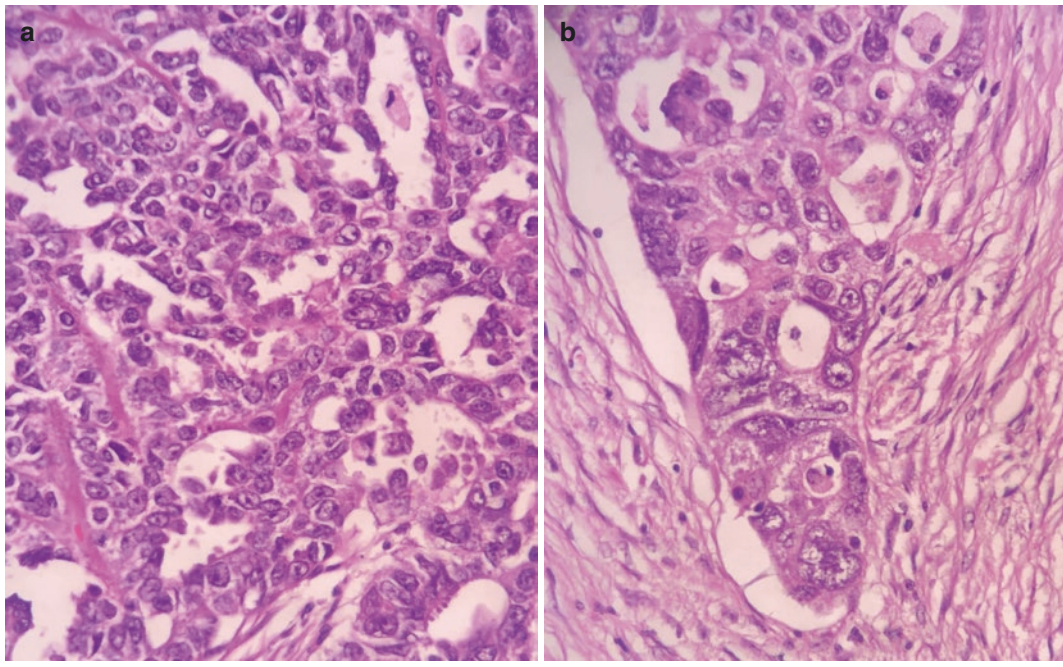


**Fig. 5.4** Histological features of high-grade serous carcinoma of the ovary: (**a**) at 10× magnification and (**b**) at 40× magnification

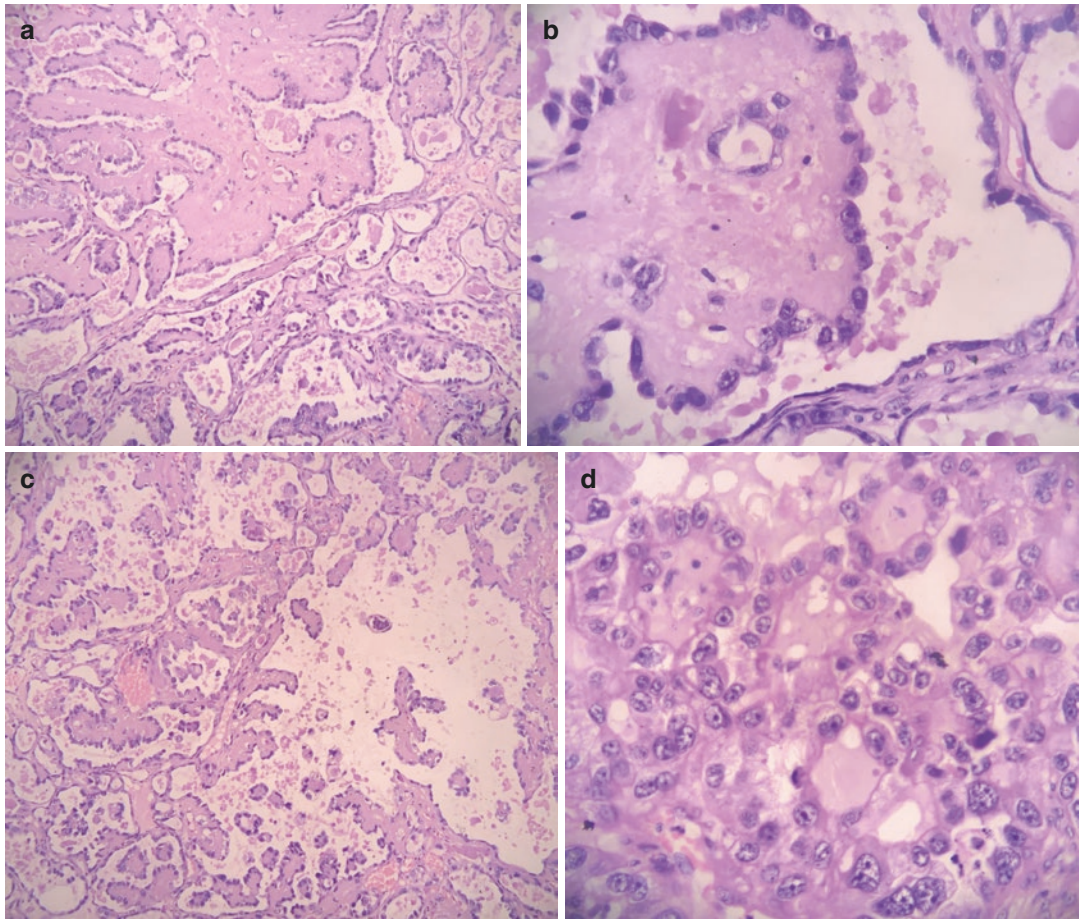


**Fig. 5.5** Histological features of clear cell carcinoma of the ovary. Hyalinized stroma is seen in (**a**) and (**b**) and hobnail pattern in (**c**) and (**d**): (**a**, **c**) at 10× magnification and (**b**, **d**) at 40× magnification

However, it is not always possible to distinguish between the two in this manner.

Han et al. reported on a series of tumours of mixed serous and clear cell histology, with the stage, immune-phenotypes and mitotic activity similar to that of pure serous carcinomas, and concluded that they most probably represent pure serous epithelial ovarian cancer with clear cell changes [22]. In a study by Gilks et al., comprising of 575 patients with early stage clear cell carcinomas, 23% of the tumours were misdiagnosed as ESOC at the time of initial diagnosis [23]. Unlike ESOC, the reported accuracy of frozen section has been less than 50% for clear cell carcinomas. In one study, frozen section diagnosis was accurate for clear cell carcinomas only 41% of the time [24].

Such tumours are now classified as serous carcinomas with clear cell features and represent a variant of high-grade serous epithelial ovarian cancer. Clear cell carcinomas also have a higher frequency of AT-rich interactive domain 1A [SWI-like] gene (*ARID1A*) and phosphatidylinositol 3-kinase (*PI3K*) pathway mutations [25].

Endometrioid and clear cell carcinomas both arise in the setting of endometriosis and it is not uncommon for them to present as a mixed ovarian carcinoma [26–28].

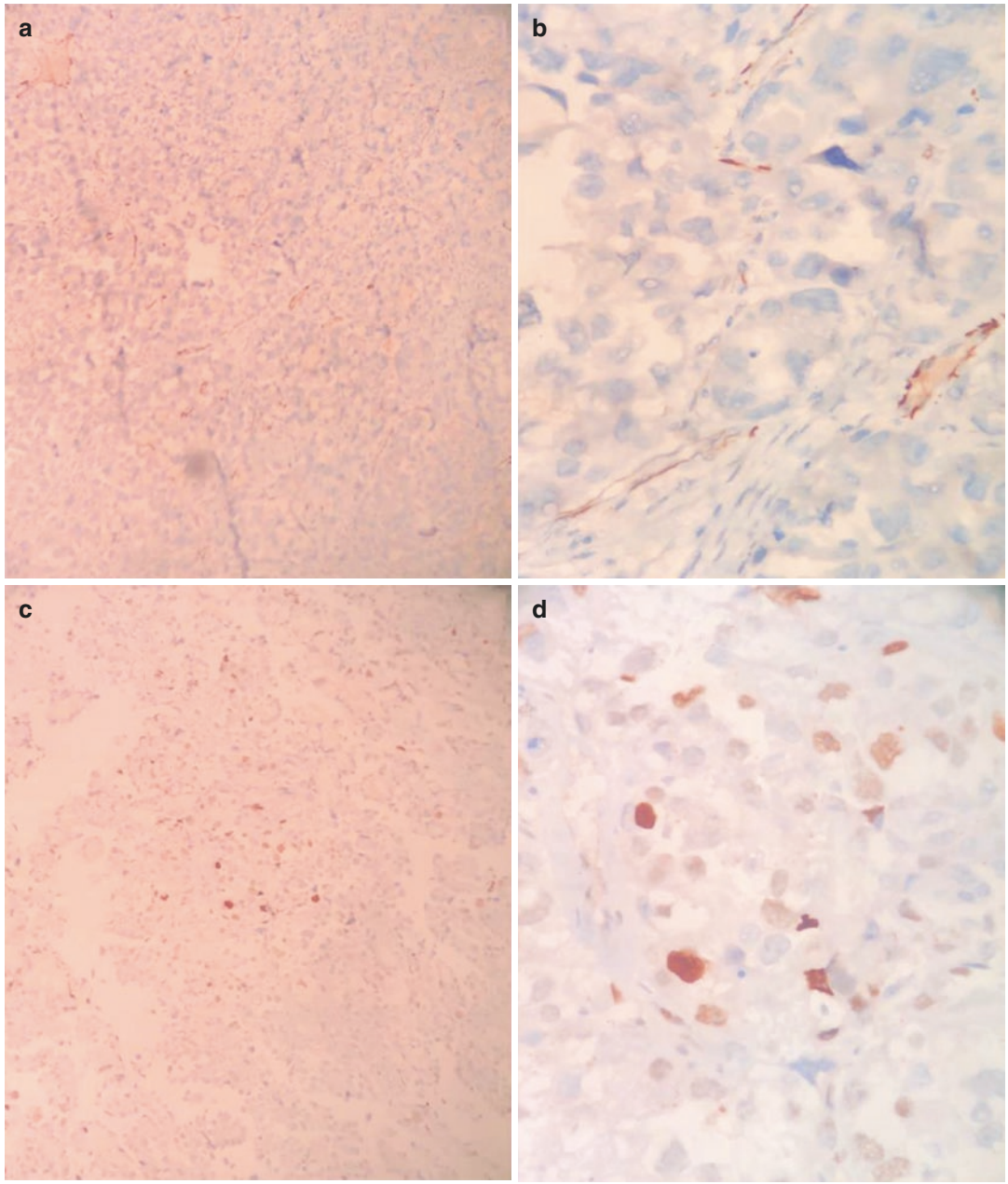


**Fig. 5.6** Immunostaining in clear cell carcinoma of the ovary of the tumour in Fig. 5.5: (**a**) Lack of p53 expression; (**b**) lack of WT1 expression; (**c**, **d**) focal expression of napsin. The diagnosis was made histological features

Mixed endometrioid-clear cell carcinomas account for approximately 1.3% of epithelial ovarian cancers and represent the most common mixed ovarian carcinoma [29].

Radiologically, clear cell carcinomas present as a unilocular or multilocular cystic mass with eccentric mural nodules [30, 31]. Coexistent endometriosis is another key finding in these tumours [32]. Clinically, these tumours present as more infiltrative deposits as compared to serous cancer. Locally advanced disease confined to the pelvis is seen more often compared

to ESOC. Though at this point, surgical treatment decisions do not vary according to the subtype except for the mucinous variety, it is important to correctly classify the tumour as this may in future be used to develop targeted therapies. At the same time, it is still unclear if the surgical approach and extent of peritoneal resection should be the same for all subtypes as discussed later in this chapter.

## 5.3 Lessons from the FIGO Staging of Ovarian Cancer

The Federation International of Gynecology and Obstetrics introduced a clinical staging system for ovarian cancer in 1988 that was revised in 2013 (Table 5.1) [33]. In light of evidence supporting the origin of high-grade ESOC from the fallopian

**Table 5.1** Comparison of the old and new FIGO classifications

| FIGO 2013 | | FIGO 1988 | |
|---|---|---|---|
| I | Tumour confined to the ovaries or fallopian tube(s) | I | Tumour confined to the ovaries |
| IA | Tumour limited to one ovary (capsule intact) or fallopian tube | IA | Tumour limited to one ovary (capsule intact) |
| IB | Tumour limited to both ovaries of fallopian tubes | IB | Tumour limited to both ovaries |
| IC | Tumour limited to one or both ovaries or fallopian tubes with any of the following | IC | Tumour limited to one or both ovaries with any of the following: capsule ruptured; tumour on ovarian surface; malignant cells in ascites |
| IC1 | Surgical spill | IC1/2 | Malignant cells in ascites/peritoneal washings |
| IC2 | Capsule ruptured before surgery or tumour on the surface of the ovary or fallopian tube | ICa/b | Capsule ruptured before surgery/surgical spill |
| IC3 | Malignant cells in ascitic fluid | | |
| II | Tumour involves one or both ovaries or fallopian tubes with pelvic extension or primary peritoneal tumour | II | Tumour involves one or both ovaries or fallopian tubes with pelvic extension |
| IIA | Extensions and/or implants on ovaries and/or uterus and/or fallopian tubes | IIA | Extensions and/or implants on ovaries and/or uterus and/or fallopian tubes |
| IIB | Extension to other pelvic intraperitoneal tissues | IIB | Extension to other pelvic intraperitoneal tissues |
| | | IIC | Pelvic extension with malignant cells in ascites |
| III | Tumour spread to peritoneum outside the pelvis and/or spread to retroperitoneal lymph nodes | III | Tumour spread to peritoneum outside the pelvis and/or spread to retroperitoneal lymph nodes |
| IIIA1 | Positive retroperitoneal lymph nodes only | IIIA | Microscopic peritoneal metastases outside the pelvis |
| IIIA1(i) | Metastases ≤1 mm | | |
| IIIA1(ii) | Metastases >1 mm | | |
| IIIA2 | Microscopic extra-pelvic involvement | | |
| IIIB | Macroscopic extra-pelvic peritoneal metastases <2 cm | IIIB | Macroscopic extra-pelvic peritoneal metastases <2 cm |
| IIIC | Macroscopic extra-pelvic peritoneal metastases >2 cm | IIIC | Macroscopic extra-pelvic peritoneal metastases >2 cm and/or regional lymph node involvement |
| IV | Distant metastases excluding peritoneal metastases | IV | Distant metastases excluding peritoneal metastases |
| IVA | Pleural fluid with positive cytology | | |
| IVB | Parenchymal metastases and metastases to extra-abdominal organs | | |

tubes, the primary tumour in this classification can be either in the ovary or the fallopian tube. There is no stage I for primary peritoneal serous cancer (PPSC) for which staging begins from Stage II. Figure shows a systematic approach to determining the primary tumour site in these group of tumours.

Two important changes from the point of view of peritoneal involvement were:

### 5.3.1 Down-staging of regional lymph node involvement to stage IIIA from IIIC

Less than 10% of the patients with ovarian cancer have retroperitoneal lymph node involvement beyond the pelvis without peritoneal involvement [34–37]. These are now classified as IIIA1 and not IIIC as they have a better prognosis than patients with peritoneal involvement [38, 39].

### 5.3.2 Sub-classification of stage 1C

A ruptured ovarian tumour or those with surface involvement of the ovary with peritoneal deposits were classified as IC. In the old classification, this included patients with iatrogenic rupture, spontaneous rupture before surgery as well as those with malignant pelvic ascites proven on cytology. It is now subdivided into stage IC1, IC2 and IC3 (IC1 is a surgical spill; IC2 includes capsule ruptured before surgery or tumour on ovarian or fallopian tube surface; IC3 includes malignant cells in ascites or peritoneal washings). In a retrospective study of 870 patients of which 56.8% had serous tumours, 119 patients had stage IC disease and 53 of these has IC3. The survival in IC3 was significantly inferior to that obtained in the other two groups (Fig. 5.7) [40].

The point to be kept in mind here is that pelvic ascites occurs in patients with ovarian cancer without extra-pelvic metastases. This early formation of ascites or presence of positive peritoneal washings may have a role to play in peritoneal dissemination and disease distribution in the peritoneal cavity.

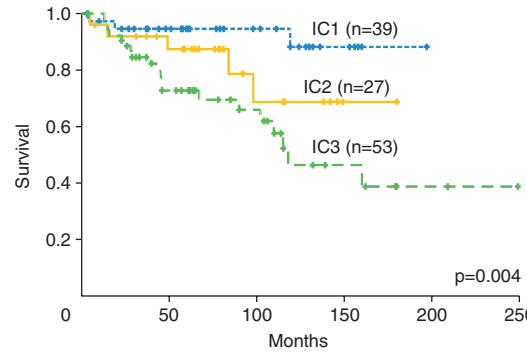


**Fig. 5.7** Overall survival in patients with stage IC ovarian cancer according to the new FIGO classification. (From Ref. [40] with permission)

### 5.3.3 Diagnosis of Serous Cancers in Stage II Disease

A very small percentage of serous cancers are picked up in stage II. As opposed to other histological subtypes, stage II in serous cancer is involvement of the pelvic peritoneum. Infiltration of adjacent structures is not the case in serous ovarian cancer. It may be seen in the low-grade tumours and other histological subtypes. The pelvic disease alone is seldom voluminous and widespread peritoneal involvement is more common. In a series of 220 patients treated by Seidman et al., only 2 out of 118 patients with serous carcinoma presented with stage II disease 5 with stage one. The clear cell and endometrioid subtypes had over 50% diagnosed in stages I and II [41].

PPSC can be diagnosed in Stage II, which means that the origin of this tumour lies in the pelvic peritoneum. Previously, there was no stage II for PPSC.

## 5.4 Pathways of Peritoneal Dissemination

Ovarian cancer has been the prototype for peritoneal disease. The peritoneal metastatic cascade was first described by Sampson in 1931 as the 'life history of metastatic peritoneal implants from an ovarian primary tumour'. The following steps occur in ovarian carcinogenesis: (1) escape

of cancer cells from the primary ovarian tumour into the peritoneal cavity, (2) migration of these cells to their site of implantation, (3) reaction of the peritoneal surface injured by the cancer cells leading to the fixation of the cancer cells in fibrin and organization of the fibrin, and (4) progression of the cancerous implant at that site [42].

Cancer cells reach the site of implantation through three routes—transcoelomic or transmesothelial spread, translymphatic spread and haematogenous spread. In the first two routes, single cells or clusters of cells are shed from the tumour spontaneously, due to surgical manipulation or spontaneous or iatrogenic rupture and thus gain access to the peritoneal cavity [43, 44]. The distribution of cancer cells is influenced by three factors—gravitational force, negative upward pressure exerted by diaphragmatic movements and peristaltic activity of the gastrointestinal tract [45].

In transmesothelial spread, cells then attach to the peritoneum, breach the mesothelial barrier and gain access to the submesothelial tissue and thereafter, proliferation of the cells is triggered (Fig. 5.8) [46].

The second route is the translymphatic route in which the tumour cells gain access to the subperitoneal tissue through the lymphatic stomata. Anatomical regions in the peritoneal cavity with a high concentration of lymphatic stomata are the greater omentum, appendices epiploicae of the colon, inferior surface of the diaphragm, falciform ligament, Douglas pouch and small bowel mesentery [47, 48]. Some of these regions are also rich in milky spots and therefore, common sites of peritoneal cancer spread [48]. The peritoneum covering the liver, serosa of the small bowel and splenic capsule are devoid of these stomata and therefore, involvement of these regions occurs in a very late stage of peritoneal cancer spread.

Both transmesothelial and translymphatic spread occur in ESOC.

It has been proposed that high-grade ESOC spreads by the haematogenous route to the peritoneum but conclusive evidence is lacking [49].

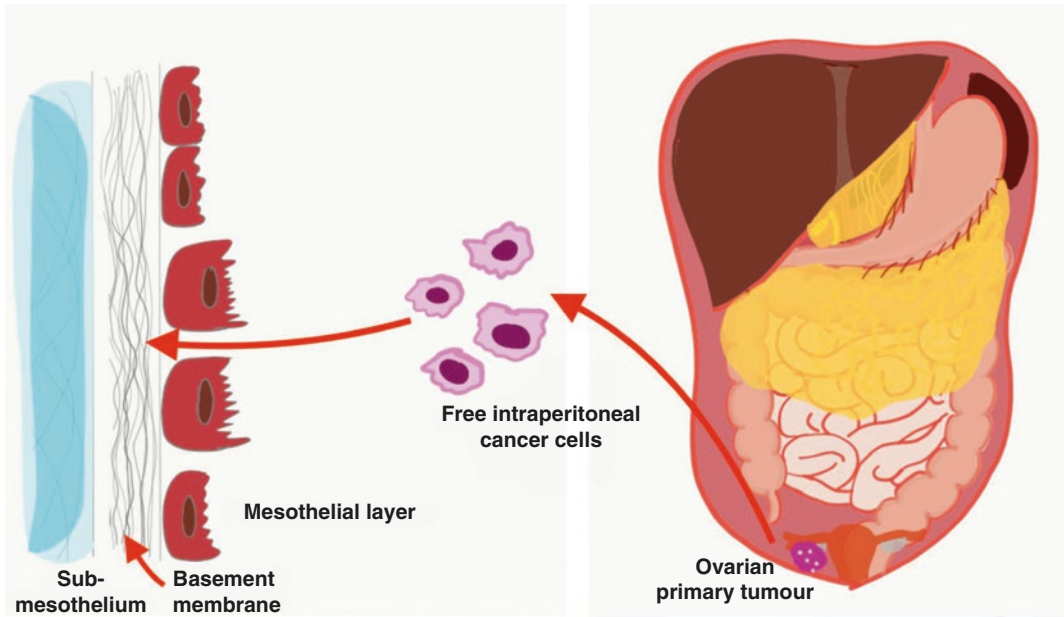


**Fig. 5.8** Transmesothelial spread in ovarian cancer—Cancer cells are shed from the primary tumour leading to the accumulation of free intraperitoneal cancer cells that attach to the mesothelial layer, invade the submesothelial tissue and produce metastatic deposits

### 5.4.1 Molecular Events in Peritoneal Dissemination

The propensity for epithelial ovarian cancer to spread to the peritoneum and/or other organs is governed by the mutational status of the *p53* gene [50]. Ninety-six percent of all cases belonging to high-grade ESOC carry mutations in the *tp53* gene, which could occur at multiple locations within the gene sequence. The molecular events in the stepwise development of ovarian peritoneal metastases have been described. A membrane type-1 matrix metalloproteinase plays a pivotal role in the spontaneous release of cell–cell adherent sheets, which later form spheroids [51, 52]. The next step is to facilitate mesothelial cell invasion.

ESOC cells can prime mesothelial cells to facilitate peritoneal adhesion. It has been shown that ESOC cells can secrete exosomes enriched for CD44 molecule (CD44). These exosomes could be internalized by mesothelial cells, resulting in the secretion of matrix metallopeptidase 9 (MMP-9), which aids in the invasion of mesothelial cells [53]. Mesothelial cells also secrete lysophosphatidic acid (LPA), which aids in mesothelial adhesion of ESOC cells expressing receptors for LPA [53, 54].

After breaching the mesothelial monolayer, the cancer cells quickly adhere to the submesothelial matrix, which is predominantly composed of collagens type I and III, using both α2β1- and α3β1-integrins [55, 56]. MT1-MMP is a major interstitial collagenase enabling invasion and anchorage of metastatic ovarian cancer cells in the submesothelial matrix [57]. Three-dimensional collagen I is instrumental in upregulating the transmembrane collagenase membrane type 1 matrix metalloproteinase (MT1-MMP) via several mechanisms [58, 59]. Epidermal growth factor receptor (EGFR)-dependent modulation of MT1-MMP surface dynamics was also found to contribute to transition to a more invasive phenotype of ovarian cancer cells [60].

Thus, several molecular interactions between cancer and mesothelial cells establish successful cell–cell adhesion during mesothelial adhesion. Disseminating cancer cells take advantage of secreted molecules produced by mesothelial cells and can reprogram their gene expression to aid in peritoneal adhesion. Likewise, ageing can amplify the process of peritoneal carcinomatosis by providing more permissive conditions for cancer cell adhesion. Importantly, ESOC cells themselves express proteins that enable their attachment and tissue invasion.

This knowledge of the molecular pathways is particularly important for developing new systemic treatments for ovarian cancer.

## 5.5 Disease Distribution in Ovarian Cancer

A knowledge of disease distribution is important in ovarian cancer as the peritoneum represents a large area and the surgeon should know where to look for disease in each situation. Moreover, the morphological appearance of peritoneal disease is variable and as discussed below, the presence of disease is difficult to determine on visual inspection in many situations. Some tumours employ the same pathways for peritoneal spread but the actual distribution of disease in the peritoneal cavity differs and this may be attributed to the inherent disease biology.

The distribution of disease in the peritoneal cavity can be described as one of the following [61, 62]:

1. Random proximal distribution (RPD), in which early peritoneal implantation is due to the presence of adhesion molecules on the surface of tumour cells and occurs even in the presence of ascites. Tumour implants form in the vicinity of the primary tumour first and other regions are involved at a later stage. This is typical of moderate-grade and high-grade cancers, such as adenocarcinomas and carcinoids of the appendix, non-mucinous colorectal cancer, and gastric cancer.

2. Complete redistribution (CRD), in which there is no adhesion to the peritoneal surface close to the primary tumour, due to the low biologic aggressiveness of tumour cells. The tumour cells follow the flow of peritoneal fluid and get implanted in the dependent regions like the pelvis, retro-hepatic and right sub-hepatic regions. In addition, sites rich in lymphatics and milky spots like the subphrenic regions and omentum have a greater concentration of tumour deposits.
3. Widespread cancer distribution (WCD), in which there is presence of adhesion molecules on the surface of cancer cells that produce a great amount of mucus, interfering with early cell adhesion. This biological behaviour is found in aggressive and undifferentiated tumours such as high-grade mucinous carcinoma peritonei arising from an appendiceal primary tumour, mucinous colorectal cancer and mucinous ovarian cancer [63].

Based on the above, the pattern of distribution in high-grade ESOC cancer should be 'random proximal distribution'. But it has been observed that the parietal peritoneum is involved first and disease follows the pattern of redistribution [64]. The early formation of ascites may be responsible for this. Certain sites are involved early in the course of peritoneal spread like the omentum, pelvic peritoneum, right paracolic gutter and right subphrenic peritoneum. At the molecular level, an experimental study showed that cells invade the mesothelium independently or form clusters known as spheroids. Aggregated ovarian tumour cells in vitro have shown increased complement resistance because of insufficient penetration of antibodies and complement into the spheroids [65]. The interaction between α5β1 integrin and fibronectin has been implicated in the process of spheroid formation in ovarian cancer cells in vitro [66]. After attachment, ovarian cancer spheroids have been shown to disaggregate on and invade live human mesothelial cell monolayers [67].

It has been shown that patients with adherent cells have a longer survival than those that have free cells which are responsible for peritoneal dissemination. Tan et al. performed a review of literature to determine if the transmesothelial spread was an active or passive process [68]. They concluded that though certain sites were favoured because of the flow of peritoneal fluid, it was also the metastatic potential of the cells shed in the peritoneal cavity and the milieu of each site that contributed to its involvement by tumour. The authors hypothesized that the ovarian primary and peritoneal disease were likely to be monoclonal in origin—i.e. arising from the same primary site. This was subsequently proven by molecular analysis [69]. In low-grade serous cancer, it was shown that the primary tumour and peritoneal deposits had independent origins.

In a retrospective study of 214 patients in which 67.7% has ESOC and nearly 90% has stage III and IV disease, the pelvic peritoneum was the commonest site to be involved, followed by the colon and then the diaphragm [70]. The authors, however, did not report the incidence of involvement of the greater omentum. Moreover, it was not specified which part of the colon was involved by direct disease extension of the primary tumour or by peritoneal deposits. In a prospective multi-centric study, it was shown that serous cancer involved the lower regions (regions 5, 6 and 7 according to the description in Sugarbaker's peritoneal cancer index first) followed by the middle regions (0, 4, 8—mainly the omentum) and then the upper regions (1, 2, 3) [64]. None of the patients had involvement of the upper regions without involvement of the lower regions. The small bowel was the last site to be involved. Disease on the small bowel surface and mesentery was not seen in the absence of disease in all three regions of the peritoneal cavity (Fig. 5.9). Ninety five percent of the patients in this study had serous epithelial ovarian cancer.

Based on available evidence, it can be inferred that in ovarian cancer, the disease distribution is similar to that seen in mucinous appendiceal tumours and can be termed as 'widespread

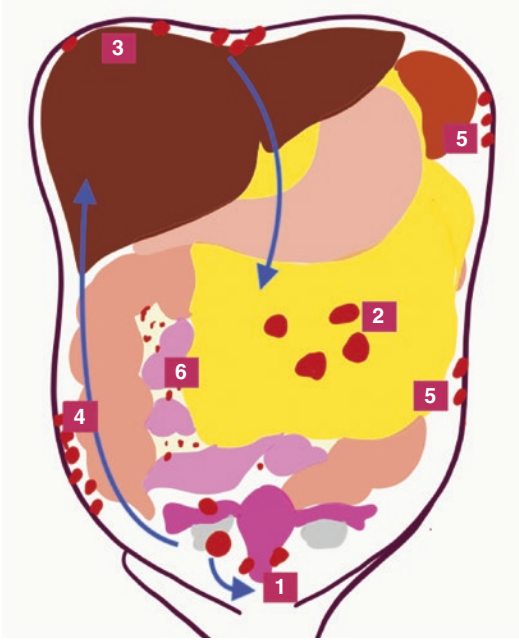


**Fig. 5.9** Sequence of involvement of various peritoneal regions in ovarian cancer—the pelvic peritoneum is the first site to be involved, followed by the omentum, right subphrenic region, right paracolic gutter, left subphrenic and paracolic regions and lastly, the small bowel and its mesentery

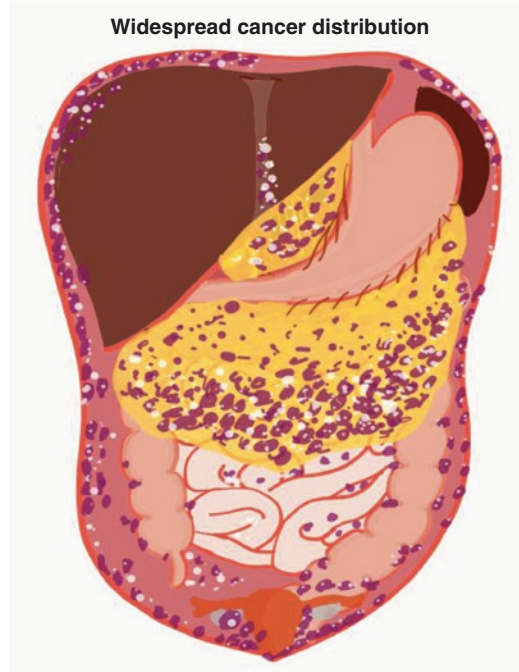


**Fig. 5.10** Widespread cancer distribution in ovarian cancer—regions that are involved first like the pelvic peritoneum, right subphrenic region and omentum have more extensive disease compared to regions that are involved later

cancer distribution' (Fig. 5.10). Parietal peritoneal sites are involved first, specifically sites that have a greater concentration of lymphatics and are sites of absorption of peritoneal fluid. The visceral peritoneum (except for the greater omentum) is involved at a later stage. The visceral peritoneum has a lower concentration of lymphatics as compared to the parietal peritoneum which may be in part responsible for this difference. Secondly, the peristaltic activity of the small bowel may be responsible for its involvement at a later stage.

It must be noted that the visceral peritoneum that covers the mesentery of the bowels is different from the one overlying the bowel serosa. The serosa of the small bowel is devoid of lymphatics [71]. Involvement of the omenta, visceral peritoneum over the small and large bowel mesentery is seen before the involvement of the peritoneum overlying the bowel serosa.

Sugarbaker described increased concentration of peritoneal deposits at three sites where the motility of the GI tract is restricted—the rectosigmoid junction, the ileocaecal junction and the pyloric region in mucinous appendiceal tumours [72]. This is also seen in ESOC.

## 5.6 Pathological Response to Systemic Chemotherapy

Patients with advanced ESOC not amenable to upfront cytoreduction undergo neoadjuvant chemotherapy followed by surgery. This gives an opportunity to make an objective assessment of the pathological response to NACT. There are two important aspects to be considered in this situation—the grade of pathological response to chemotherapy and the pattern of response to chemotherapy.

### 5.6.1 Grade of Response

It was hypothesized that the degree of response to systemic chemotherapy in ovarian cancer

should correlate with survival as shown in other cancers like those arising from the breast and the rectum. Böhm et al. developed a 3-tiered classification which was based on the scores used in rectal cancer [73]. From the initial 6 grades, the authors finally devised a 3-tiered score that correlated with survival. This score has been validated by other investigators [74, 75]. This score looks at both the residual tumour as well as the architecture and tumour microenvironment in high-grade epithelial serous ovarian cancer alone (Table 5.2). The authors studied a test cohort of 60 patients and validation cohort of 71 and used a modification of the Dworak system and demonstrated good inter-observer reproducibility and significant association with clinical outcome. In the original score, the term is chemotherapy response score (CRS score). We have used the term 'chemotherapy response grade—CRG' to avoid confusion with cytoreductive surgery (CRS). This score has been used and validated only for high-grade ESOC.

**Table 5.2** Criteria for the chemotherapy response grade (chemotherapy response score) (from Ref. [73] with permission)

| Chemotherapy response grade | Histopathological findings |
|---|---|
| CRG 1 | No or minimal tumour response. Mainly viable tumour with no or minimal regression-associated fibro-inflammatory changes, limited to a few foci; cases in which it is difficult to decide between regression and tumour-associated desmoplasia or inflammatory cell infiltration. |
| CRG 2 | Appreciable tumour response amid viable tumour that is readily identifiable. Tumour is regularly distributed, ranging from multifocal or diffuse regression-associated fibro-inflammatory changes with viable tumour in sheets, streaks, or nodules to extensive regression-associated fibro-inflammatory changes with multifocal residual tumour, which is easily identifiable. |
| CRG 3 | Complete or near-complete response with no residual tumour OR minimal irregularly scattered tumour foci seen as individual cells, cell groups, or nodules up to 2 mm maximum size. Mainly regression-associated fibro-inflammatory changes or, in rare cases no or very little residual tumour in the complete absence of any inflammatory response. It is advisable to record whether there is no residual tumour or whether there is microscopic residual tumour present. |

*Note*: Regression-associated fibro-inflammatory changes consist of fibrosis associated with macrophages, including foam cells, mixed inflammatory cells and psammoma bodies, as distinguished from tumour-related inflammation or desmoplasia

CRG 1 denotes no or minimal tumour response (mainly viable tumour with minimal regression-associated fibro-inflammatory changes limited to a few foci) (Fig. 5.11). CRG 2 denotes partial response (multifocal or diffuse regression-associated fibro-inflammatory changes, with viable tumour ranging from diffuse sheets, streaks or nodules, to extensive regression with multifocal but easily identifiable residual tumour) (Fig. 5.12). CRG 3 denotes complete or near-complete response (mainly regression, with few irregularly scattered individual tumour cells or cell groups (all measuring less than 2 mm), or no residual tumour identified) (Fig. 5.13).

Regression-associated fibro-inflammatory changes consist of fibrosis associated with macrophages, including foam cells, mixed inflammatory cells and psammoma bodies, as distinguished from tumour-related inflammation or desmoplasia. The presence of fibrosis, residual tumour and inflammatory changes should all be taken into account while scoring the response [76]. When fibrotic changes are seen in the absence of residual tumour, the inference is tumour regression. As opposed to this, fibrosis seen in the vicinity of residual tumour is considered to be desmoplasia and not regression. In some cases, fibrotic changes are accompanied by an inflammatory response termed as a fibro-inflammatory response and this is considered to be a sign of regression. Psammoma bodies may be seen at the site of previous tumour and are also a sign of regression of tumour. Due to the disappearance of tumour, they may at times appear more numerous (Fig. 5.14). Sometimes, the fibro-inflammatory changes are not seen and the cells appear bizarre (Fig. 5.15) in response to chemotherapy. This is considered to be chemotherapy response grade 1.

The Böhm or CRS score is recommended by the international council for cancer reporting (ICCR)

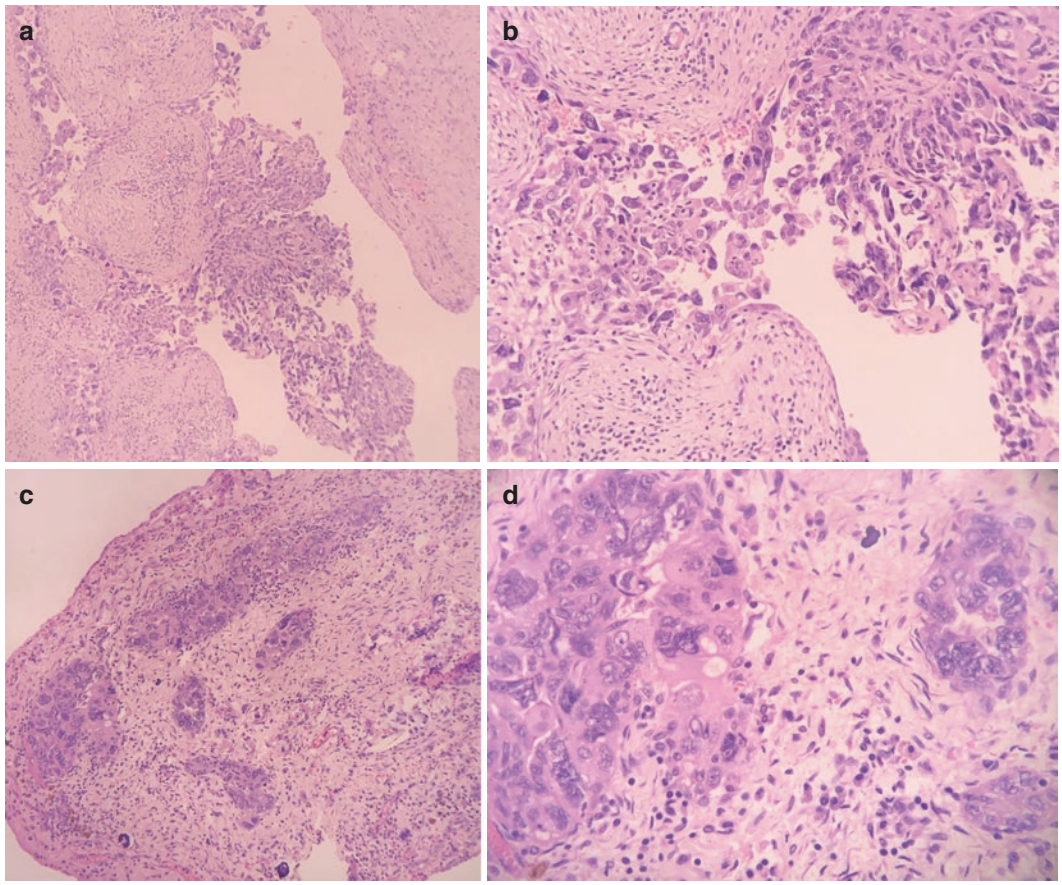


**Fig. 5.11** Histological features of chemotherapy response grade 1: (**a**, **b**) Show tumour deposits with minimal inflammatory response. (**c**, **d**) Show deposit on the fallopian tube with no inflammatory changes: (**a**, **c**) at 10× magnification and (**b**, **d**) at 40× magnification

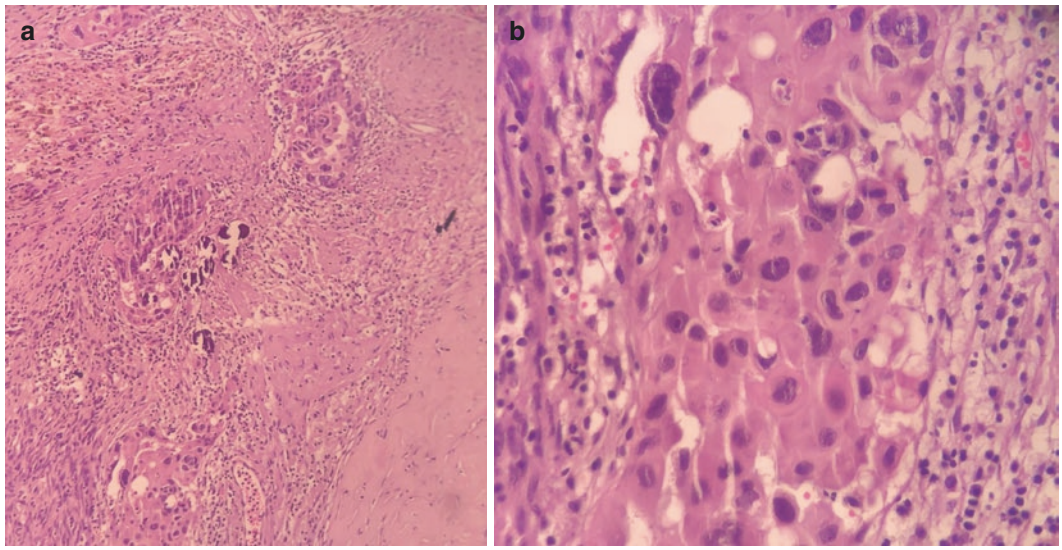


**Fig. 5.12** Histological features of chemotherapy response grade 2: Viable tumour cell surrounded by inflammatory cells and areas of hyalinization: (**a**) at 10× magnification and (**b**) at 40× magnification

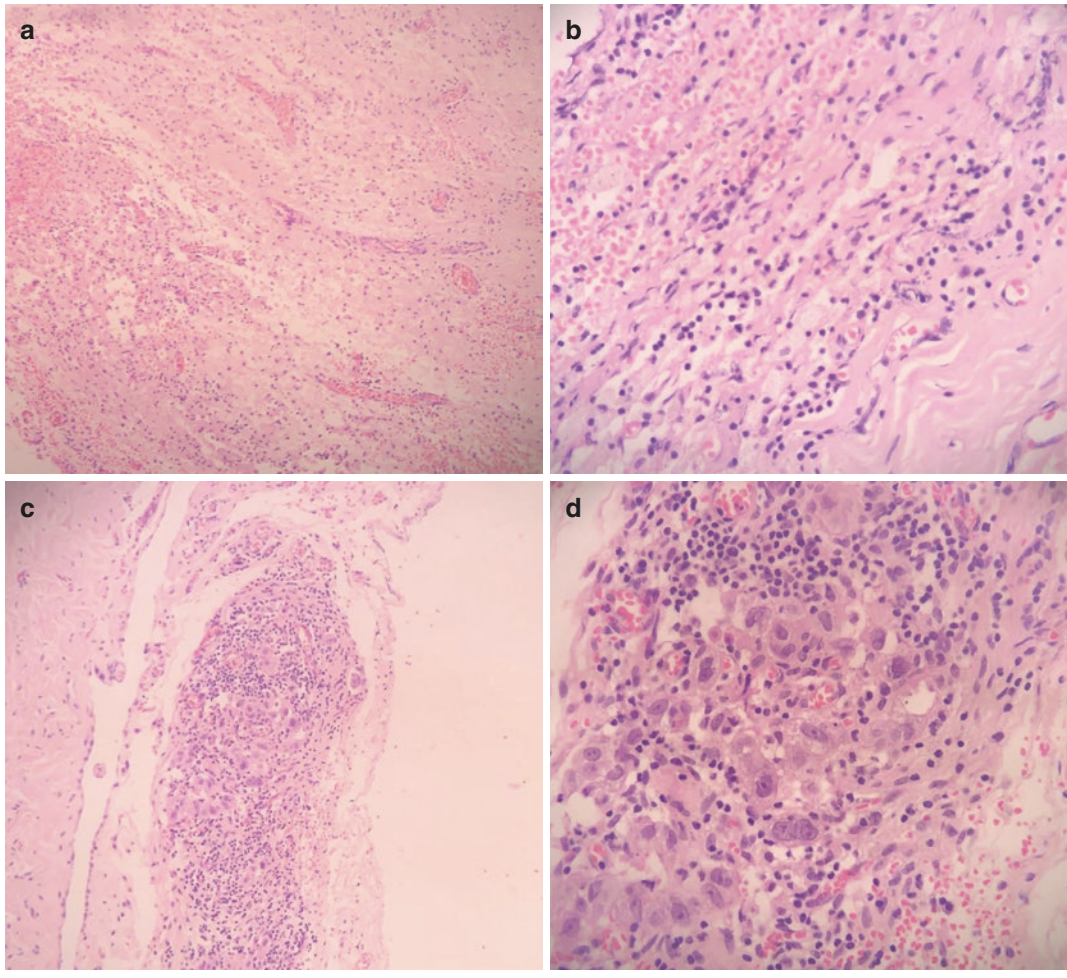


**Fig. 5.13** Histological features of chemotherapy response grade 3: (**a**, **b**) Show no residual disease with fibro-inflammatory changes. (**c**, **d**) Another region with tumour less than 2 mm and the surrounding fibro-inflammatory changes: (**a**, **c**) at 10× magnification and (**b**, **d**) at 40× magnification

[77]. Scoring is done on a single H&E stained section from the omentum. As per the guidelines, 6–7 sections are studied from the omentum and the one with the least regression or score is considered [77]. The amount of viable tumour should be assessed; this may or may not show degenerative changes in the form of nuclear atypia, smudging of the nuclear chromatin and cytoplasmic clearing. As a guide, >95% of tumour should be viable for a score of 1, and <5% for a score of 3.

In the score by Böhm et al., the response is evaluated only at two sites—in the primary ovarian tumour and in the omentum. In their evaluation of survival, the authors just looked at the response in the omentum. They state that the ovaries should not be considered since chemotherapy seldom eradicates the tumour completely and the response in the peritoneal sites is more important. In the original study, only one section from the omentum was evaluated. Böhm et al. do not consider evaluating other peritoneal sites and it may be considered logical since the omentum is perhaps one of the first regions to be involved and one of the last to respond. However, there could be a varying response in different regions as is seen in other tumours like breast and rectum and in colorectal PM as well.

In one retrospective study, in which this score was prospectively evaluated, the heterogeneity was usually between the ovaries and the peritoneal disease [4]. The ovaries seldom showed a pathological complete response and had a lower score as compared to the peritoneal regions. The response in each resected region was evaluated and in some patients, there was a discrepancy in the CRS score in different regions (Fig. 5.16) [64]. It is not known whether the chemotherapy response should be assessed in all peritoneal sites. Apart from the initial validation by Böhm et al., other investigators have evaluated the score only in the omentum and shown its impact on survival [74, 75]. There is a small percentage of patients in whom there is a complete response in the omentum but residual disease in the pelvis. Even when such a patient has CRG 3 in the omentum, the CRG would usually remain the same. A recent study showed that the involvement of other peritoneal sites was associated with a shorter survival independent of other prognostic factors including the Böhm score (Fig. 5.17) [78]. In this study, the prognostic value of the ovarian chemotherapy response score was also demonstrated (Fig. 5.18).

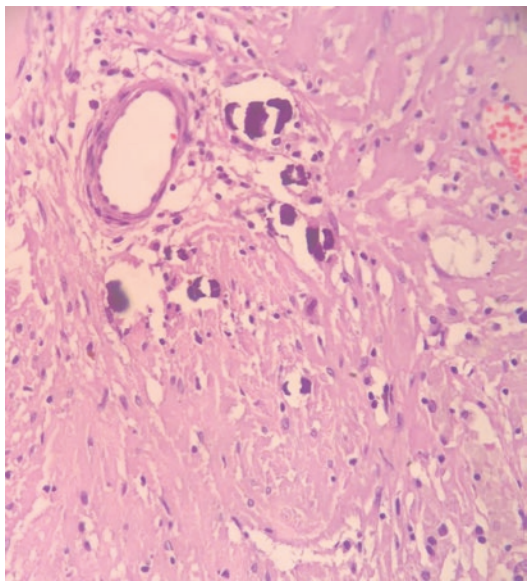

**Fig. 5.14** Psammoma bodies with no residual tumour cells representing a good response to chemotherapy

There are no therapeutic implications of this score at present—whether a different line of chemotherapy should be used in poor responders has not been addressed. When the impact on survival is evaluated, CRG 1 and 2 are usually clubbed together and compared to CRG 3 [73]. The ICCR still recommends reporting CRG 1 and 2 independently as a difference may be observed in larger series.

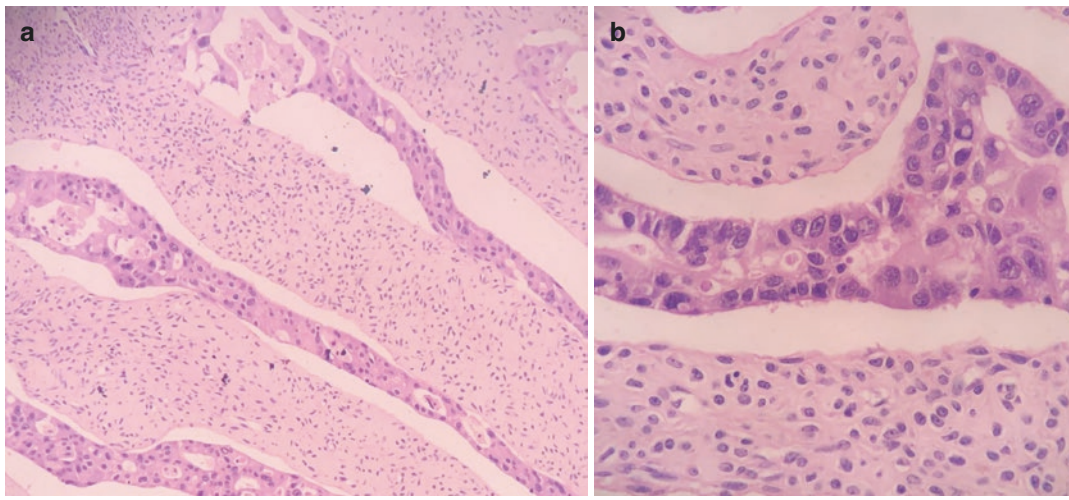


**Fig. 5.15** Change in the architectural pattern and morphology of tumour cells following chemotherapy. No fibro-inflammatory changes are seen and hence this is considered chemotherapy response grade 3: (**a**) at 10× magnification and (**b**) at 40× magnification

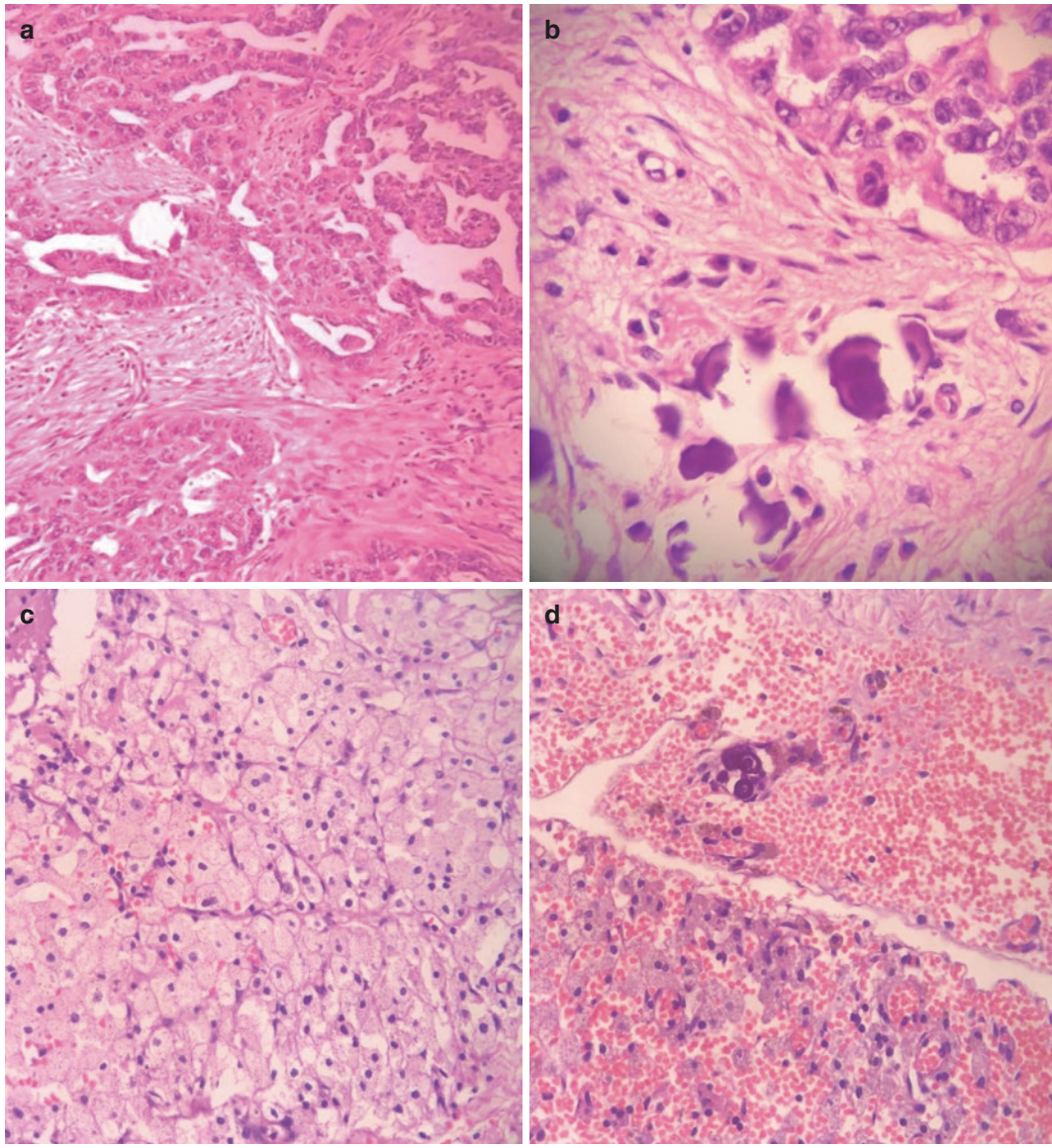


**Fig. 5.16** Different peritoneal regions showing different chemotherapy response grade in the same patient: (**a**, **b**) Show tumour in the omentum with no chemotherapy-related changes. (**c**, **d**) Show a peritoneal region with no residual disease and fibro-inflammatory changes representing chemotherapy response grade 3: (**a**, **c**) at 10× magnification and (**b**, **d**) at 40× magnification

Secondly, there is no way to predict the pathological response to chemotherapy. In the study by Böhm et al., it was shown that the reduction in CA-125 could not be correlated to the response to chemotherapy. Good and poor responders both experienced a significant reduction in CA-125 following chemotherapy. Thus, though the chemotherapy response score correlates with survival, it is only of prognostic value. We presume that the radiological response could be predictive of the CRG and patients who do not show any residual disease on imaging except in the ovaries could have CRG 3 and contrary to this those with any amount of extra-ovarian residual disease on imaging are likely to have CRG 1 or 2. However, this is just a hypothesis and needs to be evaluated.

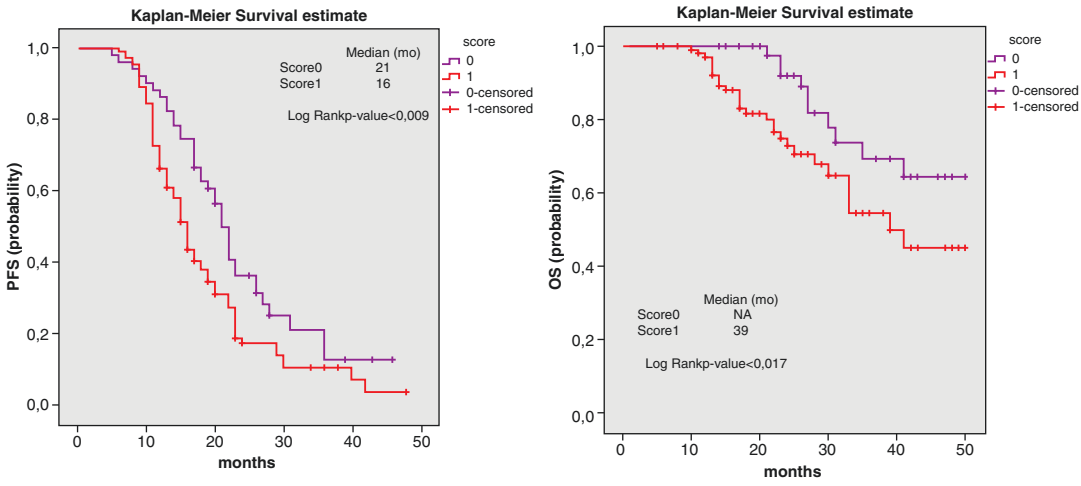


**Fig. 5.17** Impact of chemotherapy response grade in peritoneal regions on progression free and overall survival using a 2-tier classification system. (From Ref. [78] with permission)

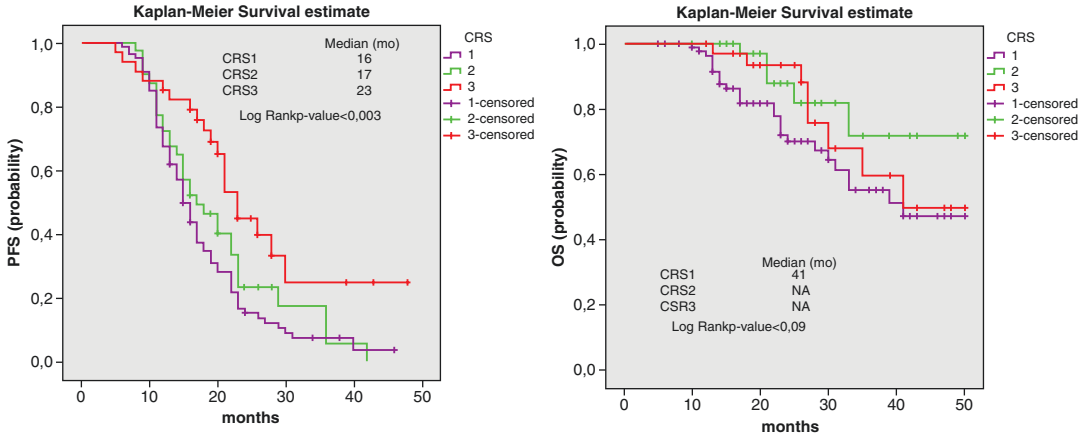


**Fig. 5.18** Impact of chemotherapy response grade in the ovaries on progression free and overall survival using a 3-tier classification system. (From Ref. [78] with permission)

Even in patients with CRG 1 and 2, the disease is usually completely resectable. Thus, this score must be distinguished from platinum sensitivity. The median progression free survival in patients with scores of 1 and 2 was still 11.2 months and 43% of the patients with CRG 2 and 41% with CRG 1 went on to develop platinum resistant disease [73].

Other systems of classification of response have been developed but have not been validated.

### 5.6.2 Implications in Patients Undergoing HIPEC

Recently, a randomized phase III trial showed a survival benefit of HIPEC in patients undergoing interval cytoreductive surgery [79]. It has been hypothesized that residual tumour following NACT contains chemotherapy resistant cells [80]. This does not imply that the patient has platinum resistant disease but the cells themselves are more resistant and the benefit may be due to

the ability of cisplatin combined with hyperthermia to overcome chemotherapy resistance. When there is minimum residual disease following chemotherapy, i.e. CRG 3, it may be presumed that the benefit of HIPEC is less. This aspect should be further evaluated as those patients likely to have a marginal benefit could be spared the morbidity of the procedure. However, the CRG score is only available postoperatively.

### 5.6.3 Other Plausible Clinical Applications

Patients with CRG 1 and 2 are prone to early recurrence. As stated above, a poor pathological response to systemic chemotherapy does not always lead to platinum resistant disease, and therefore, switching over to second-line therapy may not be considered despite CRG 1 and 2. But these patients may benefit from maintenance therapy. Currently, there is no study looking at the correlation between CRG and BRCA mutation status. It is likely that BRCA mutated patients have a greater incidence of CRG 3 and a complete pathological response. A correlation between the two could be used in future to guide treatment decisions.

### 5.6.4 Association with Lymph Node Involvement

The response in lymph nodes was not included in the score by Böhm et al. One retrospective study found that none of the patients with positive regional lymph nodes had CRG 3 or a complete response [4]. Positive nodes were seen in nearly 35% of the patients with CRG 1 and 2. The correlation between CRG and nodal positivity should be studied further.

### 5.6.5 Pattern of Response

It is not known if all sites of disease respond the chemotherapy in the same manner and to the same extent. It is not known which regions respond first and which later.

A retrospective study of 79 patients undergoing interval cytoreductive surgery showed that the response to NACT follows a specific pattern [4]. The peritoneal cavity was divided into the upper, middle and lower regions comprising of regions 1, 2 and 3, 0, 4 and 8 and 5, 6 and 7, respectively, of Sugarbaker's peritoneal cancer index. Regions that were first involved by tumour were the last to have a complete response. A complete response at the primary in the ovary was seen in only two patients which concurs with other reports. In four others, there was residual disease in the ovaries with a complete response at all other sites. The next most common site of residual disease was the lower region with 89.8% patients having residual disease in this region, followed by the omentum (residual disease in 77.2%), the upper region in 56.9% and the middle region in 29.1%. Thus, the last site to respond were the ovaries, preceded by lower region, the omentum, upper region, then middle region and then the small bowel (Fig. 5.19). This sequential response was seen in each patient. An explanation for this may be the high concentration of disease in these regions with multiple clones of tumour cells and a higher probability of harbouring drug resistant clones [61]. Disease on the small bowel was not seen in the absence of disease in both the lower and upper regions and disease in the upper region was not seen in absence of disease in the lower region. This study was retrospective with a small number of patients and the authors recommended prospective evaluation in a large series. It is important to know the pattern of response for intraoperative decision making. Surgeons generally resect sites of residual disease and sometimes there are very small remnant tumour deposits that are difficult to identify without a thorough exploration. It has been shown that normal and benign looking areas after NACT harbour microscopic disease in around a fifth of the patients [81, 82]. Knowledge about the pattern of response would be useful in guiding surgical decisions which are otherwise based only on visual inspection.

Factors affecting response to chemotherapy have not been studied. Disease in the visceral peritoneum is usually the first to respond. This may be because the visceral peritoneum has a lower concentration of lymphatics. Sub-peritoneal lymphatics have a greater concentration of chemotherapy resistant cells and may be responsible for the persistence of disease in regions where they are more numerous.

The only exception to the visceral peritoneal regions is the greater omentum. The omentum is perhaps the first extra-pelvic region to be involved and the last to respond. Little is known about the lesser omentum.


**Fig. 5.19** Probable sequence of response of various peritoneal sites to neoadjuvant chemotherapy in serous epithelial ovarian cancer. The small bowel is the first site to respond, followed by the left subphrenic and paracolic regions, the right paracolic and subphrenic regions, the omentum, the pelvic peritoneum and lastly, the ovaries

## 5.7 Morphology of Peritoneal Deposits

Ovarian cancer peritoneal deposits can have a varied morphology. Serous carcinomas tend to form surface deposits that coalesce to form plaques. In patients with extensive disease, these can infiltrate the underlying structure like the bowel and diaphragm muscle. In patients undergoing surgery upfront, a surgeon may not have much difficulty in distinguishing involved and uninvolved regions of the peritoneum. However, the morphological evolution of peritoneal deposits is not described. Even in patients who have not received chemotherapy, normal looking peritoneum can have microscopic disease [64]. High-grade ESOC has rapid progression to stage III disease and early detection is not possible even when asymptomatic patients are screened. Epithelial ovarian cancer is the only one in which ascites with malignant cells is seen in stage I disease. It may be assumed that in the early stages of development of PM, the peritoneum looks absolutely normal. The different morphological presentations of peritoneal metastases with and without neoadjuvant chemotherapy are listed in Table 5.3.

**Table 5.3** Morphological presentations of ovarian peritoneal deposits

| | Probability of harbouring disease | | |
|---|---|---|---|
| Lesion type | Primary CRS | Interval CRS | Secondary/salvage CRS |
| Tumour nodule | Certain | Certain | Certain |
| Confluent nodules | Certain | Certain | Certain |
| Plaques | Certain | Certain | Certain |
| Scarring | Possible[a] | Possible | Possible |
| Thickening | Possible | Possible | Possible |
| Tumour adhesions | Certain | Certain | Certain |
| Other adhesions | Possible[a] | Possible | Possible |
| Normal peritoneum | Possible | Possible | Possible |

[a]Scarring and adhesions due to prior surgery or other causes are less likely to harbour disease in patients undergoing CRS upfront

Sugarbaker has described the evolution of peritoneal deposits in colorectal cancer [83]. Each peritoneal deposit serves as a source for cancer cells. Even small tumour nodules can shed cancerous cells that form new implants. This exfoliation process causes a far more rapid disease progression, and all quadrants of the abdominal cavity are involved in the disease process within a few months [84].

This is unlike liver metastases in which tumour masses grow and remain confined to the liver till there is necrosis of the mass leading to disruption of capillaries within it and a consequent release of cells into the systemic circulation [85]. This results in metastases at other sites particularly the lung. This process of metastases in the liver resulting in metastases in the lungs and other systemic sites may take many months and even years. It may not occur at all with a response to chemotherapy or if a liver resection is successful [86].

In ovarian cancer, the FIGO staging makes a distinction according to the size of largest extrapelvic deposit between stages IIIB and IIIC and thus, this mode of dissemination may be occurring in ESOC as well.

It is not known which regions of the peritoneum get involved at what stage. For example, if there is disease in the pelvic peritoneum, omentum and right upper quadrant regions, what is the probability of finding disease in the left upper quadrant peritoneum.

Ovarian cancer is perhaps the only solid tumour in which surgery following neoadjuvant chemotherapy for locally advanced disease produces inferior survival compared to surgery performed upfront. For other tumours like those of the breast and rectum, survival following neoadjuvant therapy is non-inferior [87]. One of the reasons for this is difficulty in assessing response both before and during surgery [88]. Unlike other tumours, advanced ovarian cancer comprises of multiple tumour nodules scattered over part or whole of the peritoneum. The morphological response may range from shrinkage in the tumour nodules to thickening, scarring and completely normal appearance of previous tumour bearing areas (Fig. 5.20). Moreover, benign looking areas following NACT are known to harbour microscopic disease. Thus, surgery after NACT is difficult as it is impossible for the surgeon to accurately predict the presence or absence of disease in each region and perhaps for this reason, surgery should be performed with the intention of resecting previous disease sites. Pathological findings in resected cytoreductive surgery specimens provide the rationale for this [4]. There is very limited evidence supporting the use of such an approach, and future studies could look at this aspect of treatment which could significantly improve the survival of these patients. As regards the visceral resections in this setting, it is often possible to remove the thickened visceral peritoneum following a good response to chemotherapy and some of the organs involved prior to chemotherapy could thus be spared. This requires a careful inspection of involved regions and meticulous surgical dissection. When there is extensive scarring or multiple small nodules, it would be more prudent to resect the organ or underlying segment of the bowel. Surgery following NACT should not be less radical.

Two retrospective studies looked at the role of resecting previous disease sites in ovarian cancer. One study compared 34 patients undergoing CRS upfront to 110 undergoing interval CRS and found a similar survival in two groups [89]. Another study of 54 patients showed that when previous disease sites were resected as opposed to sites of residual disease alone, the incidence of peritoneal recurrence was significantly lower [90]. These studies provide evidence for evaluating this approach further. New ways of intraoperative intraperitoneal exploration such as fluorescence should be more evaluated and may help 'surgeon's eyes' to better detect areas that should be removed.

## 5.8 Extent of Peritoneal Resection for Ovarian Cancer

Parietal peritoneum lines the anterior and posterior abdominal wall and includes the pelvic, bilateral anteroparietal and right and left upper quadrant peritoneum. The omenta and the small bowel mesentery are part of the visceral peritoneum.

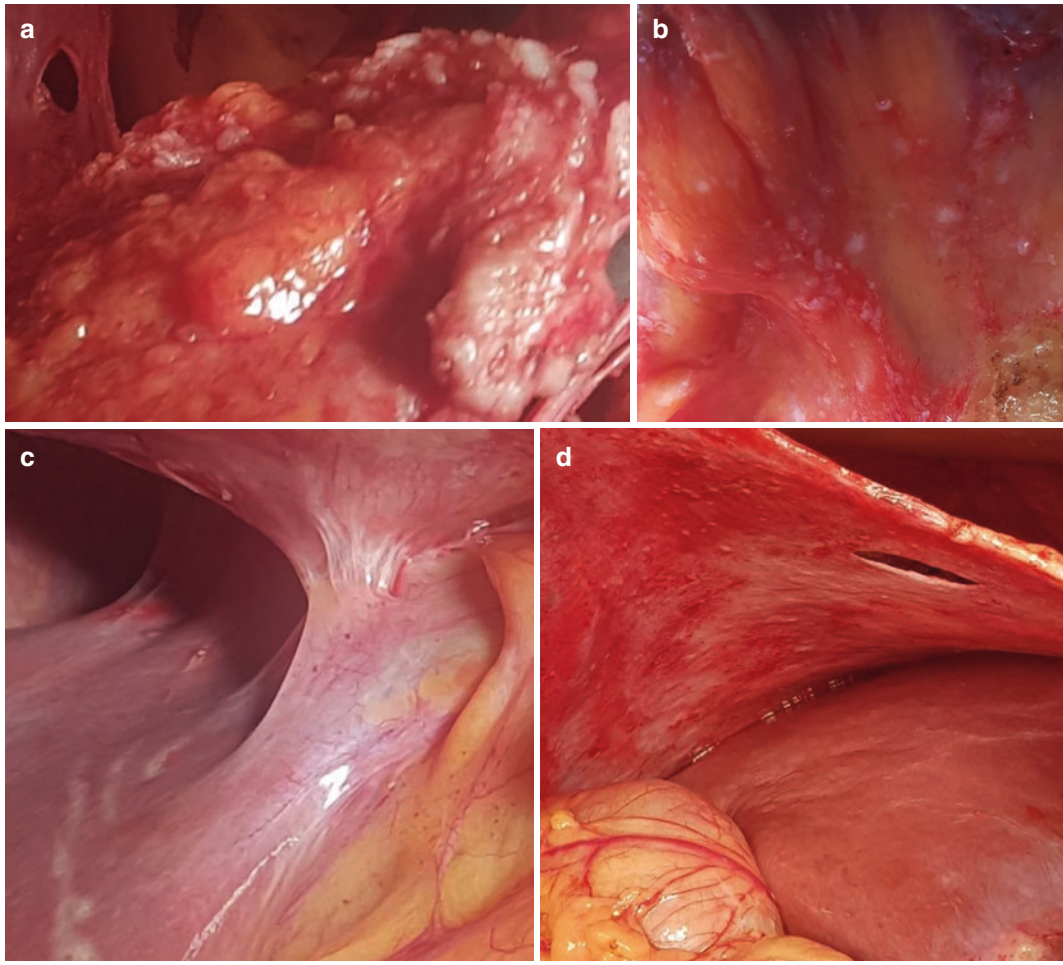


**Fig. 5.20** Varied morphological appearance of peritoneal deposits following neoadjuvant chemotherapy: (**a**) gross residual disease in the omentum; (**b**) nodular deposits on the small bowel mesentery; (**c**) scarring over the falciform ligament; (**d**) thickening with small plaques on the subphrenic peritoneum. On microscopy, residual tumour was seen in all these regions

Peritonectomy procedures were first formally described by Sugarbaker [91]. The parietal peritoneum is divided into five regions, each spreading over a large area of the peritoneal cavity. In presence of any amount of disease in a particular region, surgeons either resect the surrounding peritoneum or the entire peritoneum in that region. In ovarian cancer, it has been shown that microscopic disease is present in normal areas both in proximity to and at a distance from the tumour deposits. And hence it would be ideal to resect the whole peritoneal region. The peritoneal regions described by Sugarbaker are based not just on anatomical location but also represent different regions according to the structural and physiological properties of the peritoneum.

The goal of surgery in ovarian cancer is resection of all macroscopic diseases. Guidelines do not recommend how much of normal peritoneum to resect and whether to resect uninvolved regions of the peritoneum that are at a risk of harbouring occult disease except the greater omentum. When there is disease in the pelvis, omentum and right upper quadrant peritoneum, the probability of microscopic disease in the uninvolved left upper quadrant may be increased. Recurrences in ovarian cancer are

peritoneal in more than half the patients and occur in regions of the peritoneum that were not resected during surgery.

The principles of surgery in ovarian cancer are different from other sites of non-metastatic disease in which areas prone to harbour microscopic disease are resected. One retrospective study looked at the role of resecting the entire parietal peritoneum in patients who had received NACT and showed that it could be performed with a low morbidity [82]. However, due to heterogeneous patient population, no further conclusions could be drawn from it.

Our prospective study comprising of 110 patients with ovarian cancer showed a high incidence of involvement of the umbilical round ligament, falciform ligament and lesser omentum in patients with advanced ovarian cancer. The involvement increased with increasing PCI (unpublished results).

As described elsewhere in this book, we recommend that the entire peritoneal region should be resected in ovarian cancer (Fig. 5.21). Target regions like the falciform, lesser omentum and umbilical round ligament can be resected in the absence of visible disease especially in patients who have disease beyond the pelvis (based on unpublished results). The role of complete parietal peritonectomy should be evaluated further and is supported by pathological findings.

## 5.9 Recurrent Disease in the Peritoneum

Secondary/salvage cytoreductive surgery has a role in ovarian cancer [92]. The recurrence usually occurs in peritoneal sites that were not previously resected. This can either be disease that was overlooked by the surgeon in the first surgery or occult disease that was not resected or may represent recurrence in regions that were previously uninvolved [93]. Once again the same question arises regarding the extent of resection that is needed in these patients. Little is understood about the pattern of peritoneal involvement in cases of true recurrence. Most studies describe recurrence as 'intra-abdominal' that would include both peritoneal and nodal recurrence or 'peritoneal' that refers solely to peritoneal disease [94]. It is difficult to make any recommendations for such patients. For those who have a peritoneal recurrence following a suspected incomplete previous cytoreduction, the same principles that apply to first-line therapy could be applied. However, it is difficult to obtain similar results as disease that persists after one or more lines of chemotherapy has chemotherapy resistant cells and is almost impossible to cure with surgery alone. Surgery could be of benefit in prolonging the time to recurrence which is inevitable. The role of surgery will always be weighed

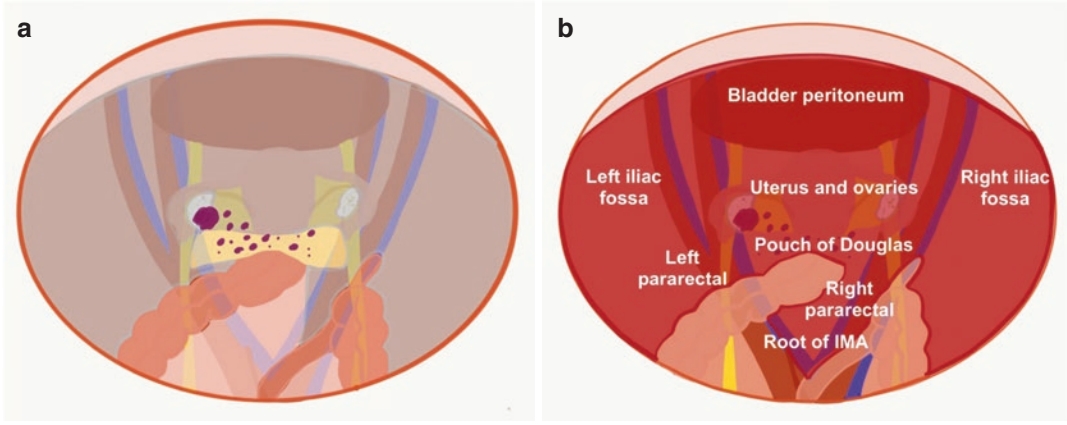


**Fig. 5.21** Extent of peritoneal resection to be performed for ovarian cancer. (**a**) Ovarian primary tumour with deposits in the pouch of Douglas. (**b**) The entire pelvic peritoneum should be resected in such a situation. The different regions comprising the pelvic peritoneum are shown here

against systemic therapies and even high-grade ESOC becomes a heterogeneous group with some subsets of patients in whom the margin of benefit from surgery is even smaller.

## 5.10 Lymph Node Involvement

Regional lymphadenectomy has been recommended for all patients with advanced ovarian cancer. One randomized trial has shown a benefit of performing systematic lymphadenectomy in all patients who had a complete cytoreduction in progression free but not overall survival [36]. Another recent trial showed no benefit in systematic lymphadenectomy in patients who did not have clinically suspicious nodes though around 50% of these nodes were positive [95]. Retroperitoneal lymphadenopathy can be secondary to the primary tumour itself or due to peritoneal deposits [96]. One retrospective study showed a correlation between the lesion score in pelvic peritoneal regions and lymph node involvement. Increased incidence of lymph node involvement was seen in patients with lesion score of 3 [97]. In addition, other nodes like mesorectal nodes, nodes in the mesentery of resected segments of bowel, periportal nodes and omental nodes may be involved secondary to peritoneal disease. The correlation between depth of rectal wall involvement and lymph node involvement has been similarly demonstrated [98]. One study showed that around 10% of the patients had positive peritoneal nodes secondary to peritoneal disease. This study included not just patients undergoing surgery upfront but also those with recurrent disease or having interval CRS. Nodal disease portends a poorer prognosis. The clinical implications of involvement of regions apart from infra-renal retro peritoneal nodes are not known but can be presumed to have a worse outcome.

## 5.11 Conclusions

In epithelial serous ovarian cancer, the pattern of involvement of different peritoneal regions and response to chemotherapy needs to be studied in greater detail. From the existing evidence, parietal peritoneal regions are involved first and harbour disease for a longer period as compared to the visceral regions (excluding the greater omentum). The entire region of the peritoneum harbouring disease should be resected. The role of more extensive resection in the primary and interval settings should be evaluated further. Chemotherapy response grade should be determined for all patients receiving NACT and should include evaluation of the parietal peritoneal regions as well. Future studies can look at the potential use of the pathological response to chemotherapy as a factor to guide treatment decisions. Clinical factors predictive of a near-complete/complete pathological response need to be identified in order to be able to predict it before surgery.

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# 6 Peritoneal Mesothelioma: Disease Biology and Patterns of Peritoneal Dissemination

Marcello Deraco, Nadia Zaffaroni, Federica Perrone, Antonello Cabras, Shigeki Kusamura, Marcello Guaglio, Matteo Montenovo, and Dario Baratti

Mesothelioma is a rare neoplasm arising from the mesothelial cells lining the pleura, peritoneum, pericardium, and tunica vaginalis layer of testis [1]. Diffuse malignant peritoneal mesothelioma (DMPM) represents about one-fifth to one-third of all forms of mesothelioma.

Age-adjusted incidence rates of DMPM in the Surveillance, Epidemiology, and End Results (SEER) database were 1.2 per 1,000,000 person-year in men and 0.8 per 1,000,000 person-year in women during the years 1973–2003. In Europe, crude incidence rate during the years 1995–2002 was 1.3 per 1,000,000 person-year for both genders [2]. An increase of 5–10% in the annual mortality rate will be observed worldwide at least until 2020. The disease has likely already reached its incidence peak in the USA, but the peak is expected during the present decade in Europe and Australia [3].

The role of asbestos exposure in DMPM has not been clearly established as in the pleural forms. It is estimated that 58% of men and only 20% of women with DMPM had past asbestos exposure [4]. No asbestos exposure is documented in about 20–40% of DMPM, thus suggesting that other factors may be the culprit. Simian Virus 40 (SV40) is a possible co-factor in mesothelioma oncogenesis, and the hypothesis of a genetic susceptibility with an autosomal dominant pattern is based on observations gathered in Cappadocia [5, 6].

DMPM has been traditionally regarded to as an end-stage disease and treated with options that were merely palliative and minimally effective, such as surgical debulking and/or palliative systemic chemotherapy (sCT). The interest in this disease on part of biological and clinical researchers was poor. Only in recent years, an increasing number of patients with DMPM have been treated with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), resulting in remarkable survival improvements and increased interest in this disease. This chapter reviews several relevant issues regarding DMPM, with a special focus on basic science and translational researches carried out in our institution to investigate the molecular and cellular mechanisms underlying the proliferative potential and resistance to therapy of this disease.

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## 6.1 Pathology of Peritoneal Mesothelioma

Tumors arising from the mesothelial cells lining the abdominal cavity encompass a wide spectrum of biological aggressiveness [7]. Adenomatoid tumor and solitary fibrous tumor are truly benign lesions that very unlikely recur after simple excision. The former is a solitary asymptomatic lesion which most often involves genital region peritoneum in reproductive-aged women. Solitary fibrous tumor affects primarily men in their sixth decade [8]. The multicystic variant of PM (MCPM) and well-differentiated papillary variant of PM (WDPPM) are exceedingly uncommon entities with borderline malignant potential. At the other extreme, DMPM is a rapidly lethal malignancy, with a median survival of only 1 year when treated with standard therapies. Classification of PM according to clinical presentation, biological behavior, and pathological features is shown in Table 6.1.

DMPM is macroscopically characterized by multiple variably sized grey-white nodules throughout the abdominal cavity. As the disease progresses, the nodules become confluent to form plaques, masses, bowel encasement, or uniformly cover the peritoneal surfaces. Abundant effusion is often present.

Similar to its more frequent pleural counterpart, DMPM is classified as epithelial, sarcomatoid, or biphasic (mixed) [9]. However, the incidence of biphasic tumors is lower than in pleural disease, and pure sarcomatoid DMPM is rare. Epithelial DMPM is composed of polygonal, oval, or cuboidal cells exhibiting cytonuclear features and architectural formations ranging from well-differentiated to anaplastic/pleomorphic appearance. Sarcomatoid tumors and the sarcomatoid component of biphasic DMPM consist of spindle cells arranged in fascicle or storiform pattern [10].

Epithelial DMPM can be further categorized according to the patterns of the epithelial component. The tubulopapillary pattern is one of the most common patterns. It consists of a mixture of small tubules and papillary structures with fibrovascular cores lined by bland flat, cuboidal, or polygonal cells. The solid pattern consists of nests, cords, or sheets of round, oval,

**Table 6.1** Classification of peritoneal mesothelioma

| Clinical presentation | Biological behavior | Histological subtype | Histological pattern | Prevalence % |
|---|---|---|---|---|
| Localized | Benign | Adenomatoid tumor | | Uncommon |
| | | Solitary fibrous tumor | | Uncommon |
| | Malignant | Epithelial/ Sarcomatoid/ Biphasic (mixed) | Tubulopapillary, solid, signet-ring cells | Uncommon |
| Diffuse | Borderline | Multicystic | | Uncommon |
| | | Papillary well-differentiated | | Uncommon |
| | Malignant | Epithelial | Tubulopapillary | 75–80% |
| | | | Solid | |
| | | | Small cells | |
| | | | Adenomatoid | |
| | | | Acinar | |
| | | | Clear cells | |
| | | | Signet-ring cells | |
| | | | Deciduoid | |
| | | | Rhabdoid | |
| | | Biphasic (mixed) | | 10–15% |
| | | Sarcomatoid | Desmoplastic | 4–6% |
| | | | Lympho-histiocytoid | |
| | | | Anaplastic | |
| | | | Giant cell | |

or polygonal cells with abundant eosinophilic cytoplasm and round, vesicular nuclei with prominent nucleoli. The adenomatoid (microglandular), acinar, clear-cell, deciduoid, signet-ring cell, small-cell, and rhabdoid patterns are rare. Sarcomatoid DMPM may demonstrate anaplastic, giant-cell, and desmoplastic features, or osteosarcomatous/chondrosarcomatous areas [8–10]. A very rare form of localized malignant peritoneal mesotheliomas (LMPM) has been reported and characterized by uncommon sharply circumscribed tumors of the serosal membrane with the microscopic appearance of diffuse malignant mesothelioma but without any evidence of diffuse spread [11].

Lymph-node metastases within and outside the abdominal cavity can occur even at the initial manifestation of DMPM. Node involvement has been reported in 7–14% of patients undergoing extensive cytoreductive surgery. By contrast, metastatic disease outside the abdominal cavity is uncommon, except for direct invasion of pleural spaces through the diaphragm [12].

Multicystic and well-differentiated papillary peritoneal mesothelioma are rare variants that generally affect reproductive-aged women with no history of asbestos exposure and show indolent clinical behaviors. MCPM forms multiple variably sized thin-walled cysts involving primarily the pelvis, but often spreading throughout the abdominal cavity. Microscopically, these cysts are separated by fibrous/adipose septa and lined by single layers of flattened to cuboidal cells with no or little atypia. WDPPM is characterized by well-developed papillary structures with fibrovascular core. MCPM is often associated with previous abdominal surgery, inflammation, or endometriosis. However, early recurrences requiring multiple surgical interventions, transformation into truly malignant disease, lymph-node involvement, and even death have been described. This, along with the reported clear evidence of diffuse disease distribution throughout the peritoneum and invasion into peritoneal surfaces, suggests that MCPM and WDPPM should be considered as borderline or low-malignant potential conditions, rather than truly benign tumors [13, 14].

## 6.2 Diagnosis of Peritoneal Mesothelioma

According to initial symptoms, DMPMs were categorized into three groups: "wet type," presenting with symptoms of malignant ascites causing an increase in abdominal girth, a "dry-painful type" presenting with a focal mass seen at computed tomography (CT) scan usually causing pain, and a "combined type" characterized by both pain and ascites [4]. In a more recent series of 81 DMPM Italian patients, ascites, abdominal pain, and asthenia were the most frequent symptoms, followed by weight loss, anorexia, abdominal mass, fever, diarrhea, and vomiting; 13% of patients presented with abdominal hernia. Systemic symptoms such as thrombocytosis and anemia were present in 73% of cases. About 25% of female patients came to medical attention due to non-specific gynecological symptoms [15].

Contrast-enhanced CT scan is currently the preferred diagnostic radiological tools for DMPM. CT features of PM have been defined as "dry" and "wet," which correspond to wet or dry-painful type clinical types. The radiological "dry" appearance consists of peritoneal-based lesions and the "wet" appearance consists of ascites, irregular, or nodular peritoneum thickening and omental mass [16, 17]. CT scan is also useful in patient selection for a comprehensive surgical approach. The presence of a tumor mass >5 cm in the epigastric region and loss of normal architecture of the small bowel and its mesentery correlate with a low likelihood to perform an adequate surgical cytoreduction (residual lesions $\leq$2.5 cm), that is a predominant prognostic variable [18].

Circulating tumor markers could be used as an adjunct to clinical and radiological assessment. In 2006, our group reported CA125 above normal limits in 53.3% and CA15.3 in 48.5% of 60 patients undergoing CRS/HIPEC. On the contrary, CEA and CA19.9 were mostly normal. Also, serial CA125 measurements paralleled with tumor growth or regression after CRS/HIPEC [19]. More recently, we have assessed the diagnostic and prognostic role of mesothelin and osteopontin, which are markers currently used

in pleural mesothelioma [20]. Using the optimal diagnostic cut-offs selected by ROC methodology, mesothelin attained 100% specificity and 100% positive predictive value in the differential diagnosis between DMPM and peritoneal dissemination of unknown origin. Additionally, osteopontin correlated with survival at multivariate analysis (hazard rate 6.46; 95% CI 1.81–23.05; $p = 0.004$), and it might be a prognostic marker to select DMPM patients for aggressive treatment approaches.

According to the consensus of expert pathologists from the International Mesothelioma Interest Group (Chicago, IL, October 2006), the diagnosis of DMPM must always be based on an adequate biopsy in the context of appropriate clinical, radiological, and surgical findings [18]. Cytology still plays a limited role in the primary diagnosis. Laparoscopy is a tool to perform biopsies, especially when there is no tumor deposit amenable to imaging-guided percutaneous biopsy, due to the unfavorable anatomic sites or small volume disseminated disease. Laparoscopy can also provide an opportunity to evaluate the peritoneal disease burden and to assess the feasibility of optimal cytoreductive surgery [21].

The first step for the diagnosis is hematoxylin–eosin staining. Demonstration of stromal invasion into visceral or parietal peritoneum (or beyond) is the key feature in the differential diagnosis with reactive mesothelial proliferations [22, 23]. Any gastrointestinal carcinoma and, in women, ovarian, primary peritoneal, and, more rarely, lobular breast carcinoma should be considered for the differential diagnosis of epithelial DMPM. The differential diagnosis for sarcomatoid DMPM includes sarcoma and other spindle cells neoplasms, such as sarcomatoid renal carcinoma and, particularly for biphasic DMPM, synovial sarcoma [8]. Since no immunohistochemical marker is entirely specific and sensitive for mesothelioma, the standard is to use panels of positive and negative markers. Mesothelioma is characterized by positive staining for EMA, calretinin, Wilms tumor-1 antigen, cytokeratin 5/6, HBME-1, podoplanin, and mesothelin. Depending on the tumor being considered in the differential diagnosis, CEA, Leu-M1, Ber-Ep4, claudine, B72.3, Bg8, and MOC-31 can be used as negative marker [8, 9, 22–24].

## 6.3 Comprehensive Treatment of Peritoneal Mesothelioma

DMPM has been traditionally treated by palliative or debulking surgery. Systemic/intraperitoneal chemotherapy and abdominal irradiation have been used in malignant variants. The results of these treatments were quite disappointing, accounting for median survival of about 12 months [25–32]. However, DMPM tends to remain within the peritoneal surfaces of the abdominal cavity all over its clinical course. Lymph-node and extra-abdominal metastases develop rarely and mostly in the late disease progression. In the last two decades, these notions have evolved into the rationale base of a comprehensive local-regional approach to treat DMPM with a curative intent by extensive CRS and hyperthermic intraperitoneal chemotherapy (HIPEC) to eradicate the microscopic residual disease [33].

In 1996, Sugarbaker described five peritonectomy procedures to surgically remove all of the peritoneal linings of the abdominopelvic cavity: (1) right upper quadrant peritonectomy; (2) left upper quadrant peritonectomy with greater omentectomy and splenectomy; (3) lesser omentectomy with stripping of the omental bursa; (4) right colectomy with stripping of the right paracolic gutter; (5) pelvic peritonectomy with sigmoidectomy and (in women) hysterectomy and bilateral adnexectomy [33].

In recent years, a few modifications have been undertaken to adapt the original technique to DMPM clinical and pathological features. The most relevant technical contributions from our center during a 20-year experience with this disease are the innovative concept that a systematic complete parietal peritonectomy (including both macroscopically involved and normal surfaces) regardless of disease distribution is associated with better survival because of DMPM biological characteristics and dissemination pattern with frequent microscopic (not visible) peritoneal disease

[34], the importance of nodal sampling and the impact of node metastases on prognosis [12], and the technique of mesenteric peritonectomy, with partial or complete stripping of the serosal layer from both sides of the mesentery [35].

An additional important concept is that that CRS must be aimed at removing all visible tumors. Numerous studies have stratified survival on the basis of the completeness of cytoreduction and this surgical endpoint is the major prognostic factor not only in DMPM, but also in all peritoneal surface malignancies [36]. This is generally explained by the limited penetration of locally delivered drugs in tumor tissue: only 2–3 mm. On the contrary, the pharmacological advantages of intraperitoneal administration consist in higher local-regional drug concentration with minimal systemic toxicity. Also, the intra-operative time setting allows optimal distribution of chemotherapeutic agents before the development of postoperative adhesions and tumor cell entrapment in scar tissue, which can contribute to disease recurrence. Finally, mild hyperthermia (41–43 °C) has a direct cytotoxic effect, increases the efficacy of antiblastic agents, such as mitomycin-C and platinum compounds, as well as their penetration into tumor tissue [33, 35].

The most relevant literature series of CRS/HIPEC in DMPM are reported in Table 6.2. Median survival ranged from 30 to 92 months, and improved with growing experience, as it was 4–5 years in the most recent updates [37–51]. One French, one American, and one international multi-institutional series have collected 249, 211, and 405 patients, respectively [46–48]. The international study was sponsored by the Peritoneal Surface Oncology Group International (PSOGI) and included patients treated in eight centers from 1989 to 2009 with major operative morbidity of 46%, mortality of 2%, median survival of 53 months, and 5-year survival of 47% [46].

We reported operative long-term outcomes for 108 patients treated with complete CRS/HIPEC (post-cytoreduction residual disease ≤2.5 mm). Treatment-related morbidity and mortality were 38.9% and 1.9%, respectively. Median survival was 63.2 months. Interestingly, there were 19 (43.6%) actual survivors of the 39 patients with potential follow-up >7 years, suggesting that patients surviving >7 years may be cured. On multivariate analysis, epithelioid histology and negative lymph node correlated with both overall survival and progression-free survival [45].

**Table 6.2** Selected literature series of CRS/HIPEC for peritoneal mesothelioma

| Center [Ref.] | Pts n. | HIPEC | Follow-up (months) | Median OS (months) | 5-year OS |
|---|---|---|---|---|---|
| Winston-Salem, NC [37] | 34 | CDDP or MMC | 72 | 41 | 17% |
| Bethesda, MD [38] | 49 | CDDP | 28 | 92 | 59% |
| Turin, It [39] | 42 | CDDP + DX | 72 | 65 | 44% |
| New York, NY [40] | 54 | CDDP + MMC | 48 | 55 | 50% |
| Washington, DC [41] | 62 | CDDP + DX | 37 | 79 | 50% |
| Villejuif, Fr [42] | 26 | OX ± IRI | 54 | NS | 68% |
| Sydney, Au [43] | 20 | CDDP + DX | 18 | 30 | NS |
| Basingstoke, UK [44] | 76a | CDDP + DX | NS | 98 | NS |
| Milan, It [45] | 108 | CDDP + DX | 49 | 63 | 52% |
| International [46] | 401 | Various | 33 | 53 | 47% |
| Bethesda, Pittsburgh, Baltimore [47] | 211 | CDDP or MMC | NS | 38 | 26% |
| Lyon, FR [48] | 28 | CDDP + MMC | 34 | 37 | NS |
| Pittsburgh, PA [49] | 65 | CDDP + MMC | 37 | 46 | 39% |
| Washington, DC [50] | 205 | CDDP + DX | 31 | 77 | 52% |
| RENAPE [51] | 249 | Various | 24 | NR | 80% |

*CDDP* cisplatin, *DX* doxorubicin, *MMC* mitomycin-C, *OX* oxaliplatin, *IRI* irinotecan, *NS* not stated, *NR* not reached, *5FU* 5 fluorouracil, *OS* overall survival, *HIPEC* hyperthermic intraperitoneal chemotherapy, *EPIC* early postoperative intraperitoneal chemotherapy

As patients not amenable to CRS/HIPEC, due to advanced or not resectable disease, are concerned, scarce data on the role of systemic chemotherapy are available. This may be, at least in part, explained by the rarity and inherent difficulties of radiologic assessment of DMPM. A variety of systemic agents have been extrapolated from pleural mesothelioma treatment. More recent studies have demonstrated improved outcomes with pemetrexed in combination with cisplatin/carboplatin. Pemetrexed is a multi-targeted antifolate that inhibits thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase. Activity of combinations of pemetrexed-based combinations was observed in two expanded access programs, with response rates of 15–30% and median survival 13–15 months in the palliative setting [52, 53]. Pemetrexed has been tested also in combination with gemcitabine [54].

Limited data are also available on systemic chemotherapy (sCT) in combination with CRS/HIPEC in the adjuvant or neoadjuvant setting. We have retrospectively analyzed 116 DMPM patients treated with CRS/HIPEC from 1995 to 2011. Sixty of them had preoperative sCT, 30 had postoperative sCT, and 26 no sCT. Platinum and pemetrexed were given to 55 cases. Preoperative sCT was not associated with complete cytoreduction or severe morbidity, but also with no survival differences among preoperative, postoperative, and no sCT groups [55]. In a recent multi-institutional French study, preoperative sCT was associated with worse survival at multivariate analysis (HR = 2.30; 95% CI = 1.07–4.94; $p = 0.033$) [56].

## 6.4 Clinical and Pathological Prognostic Factors

Several predictive factors for overall survival in patients with DMPM have been identified. Beside the completeness of cytoreduction, disease stage, which is generally quantified by peritoneal cancer index (PCI), was identified as a prognostic factor by Yan [57]. Schaub created a nomogram to predict survival that was partly based on PCI [58]. Male sex and older age have been also associated with poorer prognosis [47, 49, 59]. The histological type is one of the most consistent prognostic factors, as worse outcomes have been repeatedly reported for sarcomatoid and biphasic DMPM [45, 46, 58]. Magge showed that there may be no benefit from CRS-HIPEC in sarcomatoid and biphasic groups, with a median survival of 10.5 as compared with 51.5 months in epithelioid DMPM [49]. On the contrary, a recent PSOGI registry study reported better results in patients with biphasic histology undergoing CCR-0 cytoreduction, with a median survival of 7.8 years, thus suggesting that biphasic DMPM should no longer be considered as an absolute contraindication [60].

The prognostic impact of lymph-node metastases has been reported in both single center and multi-institutional series [45, 46]. Individual studies have also identified mitotic rate [40, 45, 61], GLUT-1 expression [48], preoperative CA-125 [19, 58], telomere maintenance mechanisms [62], estrogen receptors [63], BCL2 [64], MUC-1 [65], BAP1, NF2, CDKN2A [66], mitotic index and pattern of growth [67], PD-L1 [61], and preoperative thrombocytosis [68] as predictors of poorer survival.

We recently developed an algorithm by means of conditional inference tree model [69]. This model relies on pre-cytoreduction PCI and tumor proliferative index measured by Ki-67 using immunohistochemistry. Three prognostic subsets were defined: (I) Ki-67 ≤9%; (II) Ki-67 >9% and PCI 17; and (III) Ki-67 >9% and PCI >17. The median OS for subsets I, II, and III were 86.6, 63.2, and 10.3 months, respectively. The model had an acceptable discriminant capacity with a bootstrap-corrected Harrell c-index of 0.74.

## 6.5 Prognostic Biomarkers and Therapeutic Targets (Fig. 6.1)

The discovery of new targeted therapies could be the key for improving the prognosis of patients affected by diffuse malignant peritoneal mesothelioma (DMPM) which is known to be relatively resistant to traditional chemotherapy. Thus

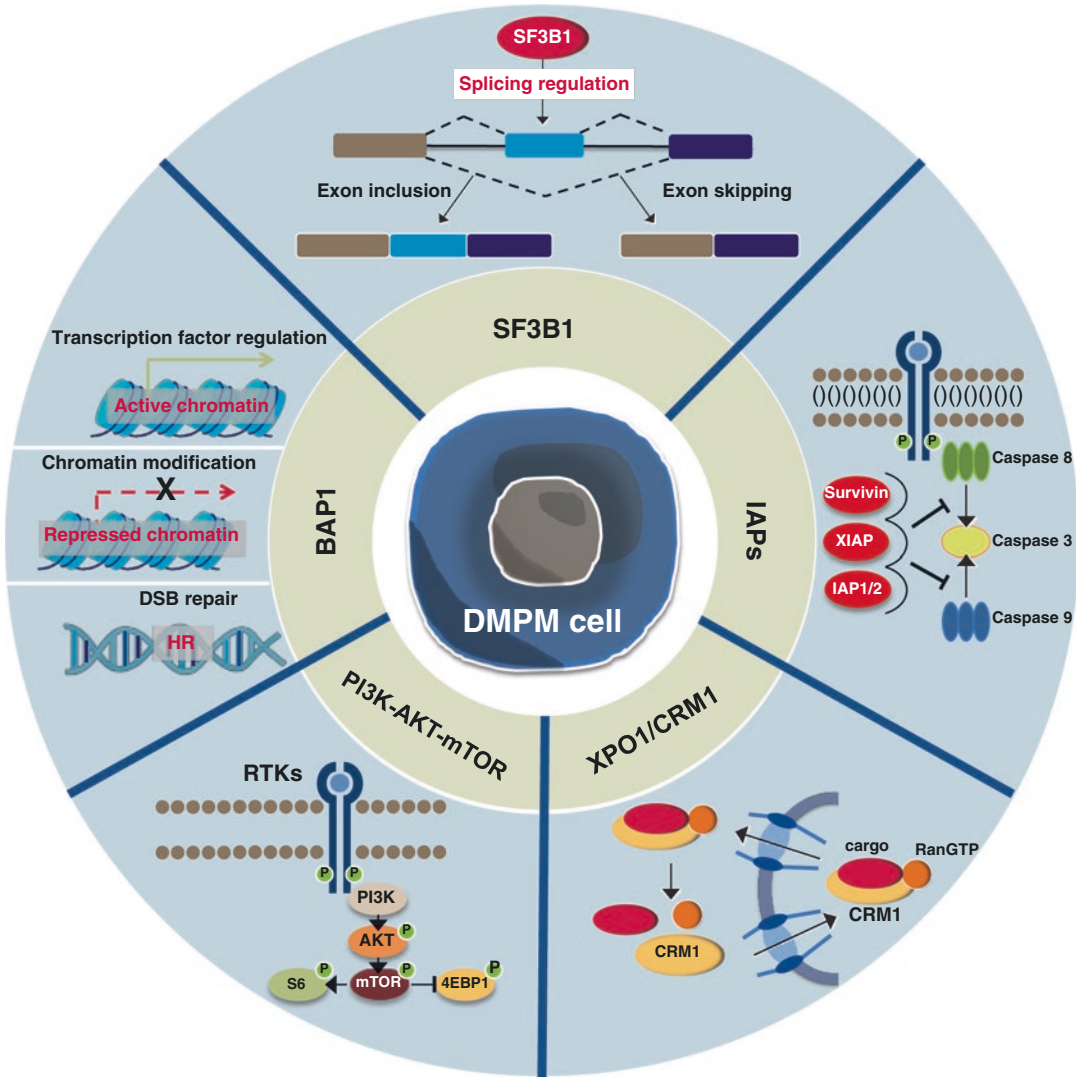


**Fig. 6.1** Genes/pathways altered in DMPM with potential as biomarkers and/or therapeutic targets

far, a limited number of studies have focused on the identification of deregulated pathways in DMPM that can be specifically targeted to obtain a direct therapeutic effect or to increase the tumor sensitivity to conventional anticancer agents.

It was initially demonstrated that the dysregulation of apoptotic pathways may play a role in the relative chemoresistance of DMPM and that survivin and other members of the inhibitors of apoptosis protein family (i.e., IAP-1, IAP-2, and X-IAP), which are overexpressed in most DMPMs, could represent new therapeutic targets. Indeed, it was found that RNAi-mediated survivin knockdown in DMPM cells enhanced both spontaneous and drug-induced apoptosis [70], thus supporting the notion that survivin inhibitors may provide new approaches to the treatment of the disease. In this context, it was reported that nortopsentin analogues (1H-pyrrolo[2,3-*b*]pyridine derivatives) reduced proliferation and induced a caspase-dependent apoptotic response in DMPM cell lines, which were paralleled by a significant decline of the expression of the active Thr(34)-phosphorylated form of the anti-apoptotic protein survivin, as a consequence of CDK1 inhibition [71]. Survivin

exclusively relies on exportin 1 (XPO1/CRM1) to be shuttled into the cytoplasm and performs its anti-apoptotic function. It was demonstrated that selinexor, a clinical stage XPO1/CRM1 inhibitor, induced dose-dependent inhibition of DMPM cell growth, cell cycle arrest at G1-phase, and caspase-dependent apoptosis, which were paralleled by a time-dependent reduction of cytoplasmic survivin levels. Most importantly, orally administered selinexor caused a significant antitumor effect in subcutaneous and orthotopic DMPM xenografts without appreciable toxicity [72]. Collectively, these findings highlight the interference with survivin expression and function as a novel therapeutic option for DMPM.

Additional interesting targets that may have clinical utility in DMPM are represented by PI3K-AKT-mTOR pathways. Indeed, expression and activation of PI3K, AKT, mTOR, S6, and 4EBP1 have been documented by biochemical analyses in a series of DMPM clinical samples and activity of mTOR inhibitors has been demonstrated in vitro in a human DMPM cell line [73]. Consistently, a gene expression profile study revealed the upregulation of genes related to PI3K and mTOR signaling pathways, which was significantly correlated with shortened survival of DMPM patients [74]. Activation of these pathway is likely sustained by NF2 deletion and a ligand-dependent activation and co-activation of multiple receptors tyrosine kinase, such as EGFR, PDGFRB, and MET, described in DMPM [73, 75]. Such finding may explain the low efficacy of single-agent anti-EGFR therapy reported in DMPM patients, despite a predominant EGFR overexpression/activation, thus supporting the use of combined treatments [76, 77]. Coherently, a combined inhibition of PI3K and mTOR signaling was effective in two young women with papillary indolent DMPM enabling long-term survival despite disease recurrence [78].

In the last years, results from studies aimed at dissecting the genomic landscape of DMPM improved the knowledge of the molecular biology of this rare tumor and identified additional potential therapeutic targets. Specifically, it was revealed that over 70% of DMPMs harbor *BRCA1* associated protein 1 (*BAP1*) inactivating mutation or copy number loss and/or loss of protein expression, making *BAP1* the most commonly altered gene in this malignancy [79–82]. BAP1 is a tumor suppressor and deubiquitinase, localized to the nucleus where it regulates chromatin remodeling and maintains genome integrity. Thus, a reduced BAP1 activity results in the accumulation of DNA-damaged cells and in an increased susceptibility to the development of malignancy. Results from several studies support the specificity of BAP1 protein loss assessed by immunohistochemistry as a helpful diagnostic marker for the pathologic identification of mesothelioma [83, 84]. By contrast, the prognostic role of loss of BAP1 in DMPM is still controversial. Indeed, a study showed that loss of BAP1 immunostaining did not correlate with DMPM patients' outcome [61], whereas better overall survival for patients with BAP1 mutations, protein expression loss, or at least one of these alterations, independently of tumor histological subtype, age, and sex, was reported in another study [82].

Inactivating mutations and focal deletion of neurofibromin 2 (*NF2*), which encodes the cytoskeletal scaffolding protein Merlin, and mutations of the two epigenetic regulatory genes DDX3X and SETD2 are also relatively common in DMPM, indicating that transcriptional deregulation is a key oncogenic mechanism in mesothelial tumorigenesis [80, 81]. This notion is also supported by the finding that a significant fraction of DMPMs show loss of 3p21 locus, in which are located other chromatin modifiers and epigenetic regulatory genes, such as SMARCC1 and PBRM1 [85]. Interestingly, DMPMs harboring 3p21 locus or presenting BAP1 loss (BAP1 haploinsufficiency) also show a differential expression of a set of genes involved in both chromatin remodeling and DNA damage repair mechanisms [85]. DMPMs carrying inactivating alterations affecting BAP1 and other transcriptional regulators may represent a molecular subgroup with altered transcriptional programs that may benefit from inhibitors of epigenetic modifiers, including histone deacetylases and the histone methyltransferase EZH2, that seem to be promising in preclinical setting [86, 87].

BAP1 haploinsufficiency also seems to predict a distinct immunogenic class of DMPMs. Indeed, this subgroup is characterized by both the presence of an inflammatory tumor microenvironment and PD-1/PD-L1 expression [85]. If confirmed, these interesting findings could open an additional therapeutic opportunity for this subset of DMPM patients since BAP1 haploinsufficiency may confer sensitivity to immune checkpoint inhibitors. In this context, the combination of anti-CTLA4 and anti-PD-L1 monoclonal antibodies was active and safe in mesothelioma patients recently enrolled into the phase 2 trial NIBIT-MESO-1 [88]. PD-L1 expression had already been reported in half of DMPMs, with a frequency similar or even higher compared to pleural mesothelioma [89, 90]. Although, in the trial NIBIT-MESO, PD-L1 expression did not seem to correlate with clinical response or overall survival, the correlation between BAP1 loss and PD-L1 expression deserves further investigations.

ALK rearrangements have been described in a small subset (3%) of younger women (>40 years) affected by DMPM without genetic alterations in BAP1, SETD2 or NF2. This was an exciting finding suggesting that a restricted subset of selected patients may benefit from treatments with ALK inhibitors [91].

Results from an extensive exome sequencing of a large collection of pleural mesothelioma specimens showed the presence of mutations affecting the splicing factor 3b subunit 1 (SF3B1), which encodes an essential component of the spliceosome, as well as the histone methyltransferase SETD2 and the DEAD-box RNA helicases DDX51 and DDX3X, which are also involved in RNA processing and splicing [92]. In addition, this study unraveled several mesothelioma-specific splice alterations, most of which were independent of splice site mutations. Recently, we found that spliceosomal genes are differentially upregulated in DMPM cells compared to normal tissues. In addition, the expression of SF3B1, as assessed by immunohistochemistry in tissue microarrays of 64 DMPM specimens, was found to correlate with poor patients' clinical outcome in univariate and multivariate analysis [93]. SF3b modulators (Pladienolide-B, E7107, Meayamycin-B) showed potent in vitro cytotoxic activity in the low nanomolar range. Differential splicing analysis of Pladienolide-B-treated cells revealed abundant alterations of transcripts involved in cell cycle, apoptosis, and other oncogenic pathways. E7107 demonstrated remarkable in vivo antitumor efficacy, with significant improvement of survival rates compared to vehicle-treated controls [93]. Collectively, such data indicate SF3B1 as a novel potential prognostic factor and designate splicing as a promising therapeutic target in DMPM.

MicroRNAs (miRNAs) are endogenous small non-coding RNA molecules that negatively regulate gene expression in a variety of biological processes by translation inhibition, cleavage, or degradation of target mRNAs. The value of miRNAs as novel biomarkers and targets for cancer therapy is now widely recognized. In this context, several preclinical studies utilized miRNA targeting approaches for improving the therapy of pleural mesothelioma [94]. However, no information is currently available on the expression/functional role of miRNAs in DMPM with the only exception of miR-34a [95]. The expression and biological effects of miR-34a, which is one of the most widely deregulated miRNAs in cancer, have been evaluated in a cohort of 45 DMPM and 7 normal peritoneum specimens as well as in 5 DMPM cell lines. The miRNA was found to be significantly downregulated in DMPM clinical specimens and cell lines. In addition, miR-34a reconstitution in DMPM cells significantly inhibited proliferation and tumorigenicity, induced an apoptotic response, and declined invasion ability, mainly through the downregulation of c-MET and AXL and the interference with the activation of downstream signaling. Interestingly, a persistent activation of ERK1/2 and AKT in miR-34a-reconstituted cells was found to counteract the anti-proliferative and pro-apoptotic effects of miRNA, yet not affecting its anti-invasive activity. Overall, these preclinical data strongly suggest the potential clinical utility of a miR-34a-replacement therapy for the treatment of DMPM and, on the other hand, provide the first evidence of a potential cytoprotective/resistance

mechanism that may arise towards miRNA-based therapies through the persistent activation of RTK downstream signaling [95].

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# 7 The Pathological Spectrum of Mucinous Appendiceal Tumours and Pseudomyxoma Peritonei

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## 7.1 Introduction

Pseudomyxoma peritonei (PMP) is a clinical syndrome that has intrigued surgeons and pathologists alike for the last four decades. Over this period, the management of the condition has undergone a radical change from an essentially palliative approach to a potentially curative one comprising of cytoreductive surgery and HIPEC. PMP is a clinical syndrome that comprises of a very heterogeneous group of tumours that have a similar clinical presentation but varied biological behaviour. The terminology used to classify these tumours has been changing constantly as the understanding of the disease process improves. Often different classifications and terminology are used by different pathologists and must be put in perspective by the treating surgeon. There has been progress on the molecular and genomic front as well. This chapter provides a simplified approach to diagnose and classify PMP of appendiceal origin which will be useful to both less experienced pathologists and surgeons. There is a tabulated comparison of the different classifications used for both the primary tumour and PMP. It also focuses on some 'grey areas' in classifying these tumours that need to be considered while treating these patients.

### 7.1.1 Definition

Pseudomyxoma peritonei (PMP) is defined as a clinical syndrome characterized by the presence of free or organized mucin with or without neoplastic cells in the peritoneal cavity and the typical pattern of redistribution [1]. This definition encompasses a spectrum of tumours with very bland ones with little or no epithelium at one end and the more aggressive ones akin to mucinous colorectal adenocarcinomas at the other. Though initially considered a benign or borderline condition, it is now considered to be a malignant one given its propensity for dissemination and recurrence [2].

### 7.1.2 Origin of PMP

The most common underlying cause of PMP is a mucinous appendiceal tumour (in approximately 94% of the cases) [3, 4]. Other primary sites in

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order of highest incidence are the ovaries, colon and rectum, pancreas, urachus, stomach, gall bladder, fallopian tubes, breasts and lungs [5]. It must be mentioned that an ovarian mucinous tumour giving rise to PMP is often a mucinous tumour developing in a mature teratoma [6, 7]. It is not uncommon for PMP to present as an ovarian mass. Primary ovarian mucinous tumours can closely mimic appendiceal metastases histologically (Fig. 7.1), although there are some morphologic features in the ovary that may point to the appendix as the source [8]. For all mucinous ovarian tumours, it is now recommended that a primary in the appendix should be considered, actively searched and ruled out. Either the appendix should be resected even if it appears normal or immunohistochemistry performed if the diagnosis has been established after surgery. Even in low-grade PMP with pushing invasion, ovarian metastases can occur (Fig. 7.2). In rare situations, patients present with mucinous peritoneal carcinomatosis with no evident primary tumour. These

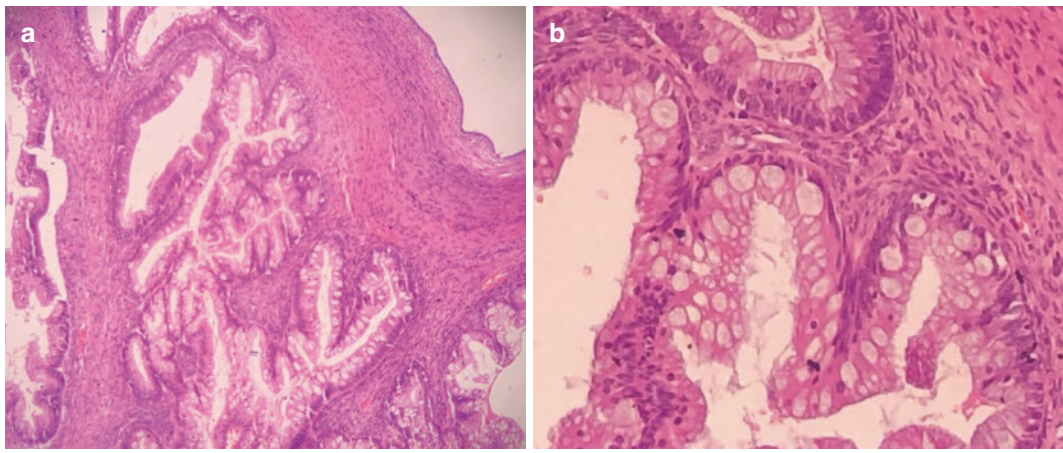


**Fig. 7.1** Low-grade mucinous tumour of the ovary. The tumour is characterized by low-grade epithelium with apical mucin and a pushing front. The appendix was normal and immunohistochemistry was suggestive of an ovarian primary tumour. (**a**) At 10× magnification. (**b**) At 40× magnification

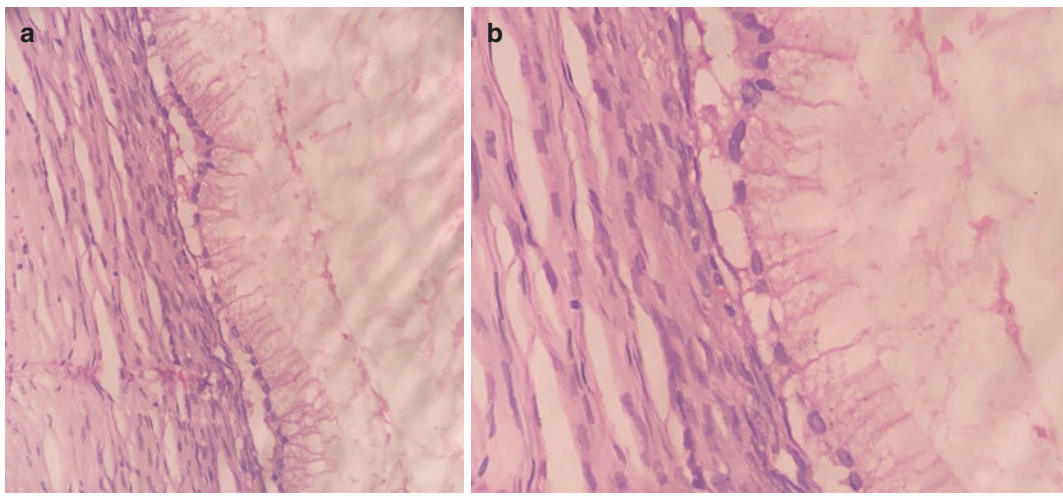


**Fig. 7.2** Ovarian metastases from a patient with LAMN. There is a single layer of low-grade mucinous epithelium lining the cystic tumour in the ovary. (**a**) At 10× magnification. (**b**) At 40× magnification

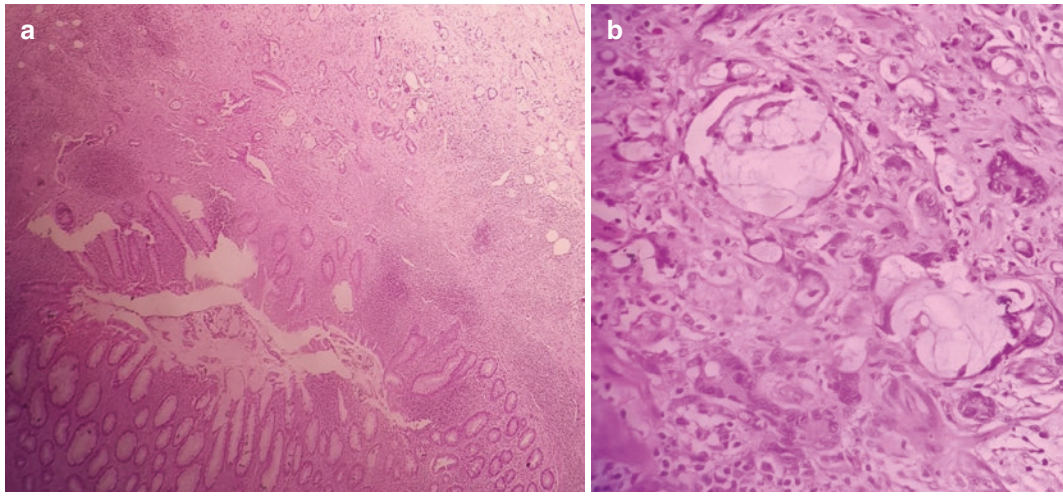


**Fig. 7.3** High-grade PMP with no identifiable primary tumour. (**a**) The appendiceal mucosa is normal and the tumour is infiltrating the appendix from the serosal side. (**b**) Mucinous adenocarcinoma in the peritoneal deposits. Immunohistochemistry showed positive staining for CDX2, SATB2, CK20, villin and was negative for CK7

are usually high-grade tumours and little is known about their origin and pathogenesis (Fig. 7.3).

### 7.1.3 Pathological Classification of PMP

The pathological grade of PMP is one of the most significant factors affecting treatment outcomes. Since the recognition of PMP as a clinical entity, several classifications have been developed for both the appendiceal primary tumour and PMP and have also gone out of use [9]. Classification systems do not just look at the grade but also the histological subtype of the tumours. There are different histological subtypes that can produce PMP-like signet ring cell carcinoma, mucinous adenocarcinoma and often the subtype and grade get incorporated into a continuum.

Initially, both the appendix primary and the peritoneal deposits were incorporated into one classification. More recently, different classifications are used for both. Due to the multitude of classifications proposed by different pathologists, there is still controversy regarding the most appropriate one and different centres and regions have different preferences. It may also partly be attributed to some limitations in each one and the disease heterogeneity which makes it almost impossible to classify every distinct entity into the right group. To add to this, there are non-expert pathologists who are often called upon to report such specimens and misclassification with its ensuing clinical consequences is not uncommon.

The appendiceal primary tumours underlying PMP and PMP itself are discussed separately here.

## 7.2 Mucinous Appendiceal Tumours Producing PMP

Mucinous appendiceal tumours which give rise to PMP are the second most common epithelial appendiceal tumours following carcinoids [10]. The incidence of non-carcinoid tumours is 0.9/100,000 per year and 25% of these are adenocarcinomas [11]. The biological spectrum ranging from seemingly benign tumours to frankly malignant adenocarcinomas that is seen in PMP is also seen in the appendix. The peculiarity is the ability of mucin to invade through the layers of the appendiceal wall leading to rupture and peritoneal dissemination with mucinous implants.

Mucinous tumours need to be distinguished from benign conditions and appropriately

classified. The classification of these tumours has prognostic and treatment implications and it is not just the pathologists but also the surgeons who should be well aware of the different classifications and their implications.

### 7.2.1 Diagnosis

An appendiceal tumour is usually an incidental finding in an appendectomy specimen. The indication of performing an appendectomy is usually the suspicion of a non-malignant condition. In patients undergoing CRS for PMP, an assessment of the primary tumour is performed as well. While evaluating the specimen, the whole appendix with the tumour must be embedded [12]. On examination, the appendix may be grossly dilated with or without rupture of the wall and extrusion of mucin or even appear extremely normal.

There is intraluminal mucin which may or may not be extruding through the wall and the lining epithelium which can have varying degree of cytological atypia. The diagnosis of a mucinous tumour is made by the finding of extracellular mucin exceeding 50%.

### 7.2.2 Classification, Staging and Grading

#### 7.2.2.1 History of Classification of Mucinous Appendiceal Tumours

Different aspects of current classifications of appendiceal tumours owe their origin to previous classifications and it is interesting to know how the classifications have evolved.

##### Ability to Produce PMP

The first classification developed by **Woodruff and McDonald** in 1940. In this classification, mucinous appendiceal tumours were either classified as benign mucoceles and cystadenocarcinomas [13]. Any tumour producing PMP was classified as a mucinous cystadenocarcinoma grade 1 in this classification. The difference between benign and malignant was the ability to produce PMP. However, it was observed that many a times the epithelium was a single strip of cuboidal cells or had features of low-grade dysplasia. Thus, PMP could have mucinous ascites resulting from the proliferation of benign-looking, mucin-secreting epithelium. Due to this some investigators termed it as benign condition while others argued that the ability to proliferate and produce mucin should be considered a malignant condition [14–18].

##### Tumours of Uncertain Malignant Potential

In 1994, **Carr et al.** reviewed 184 cases and classified these tumours as adenomas, carcinomas, and mucinous tumours of uncertain malignant potential (UMP) (Table 7.1) [19]. The tumours of uncertain malignant potential had histological features like microscopic rupture, diverticula, and other mural abnormalities that were not suggestive of frank malignancy. This terminology has been used in previous publications as well. The authors also observed that both benign and malignant tumours as well as those of UMP could produce PMP. Presence of mucin outside the right lower quadrant and epithelial cells in the peritoneal cavity were both associated with a poorer prognosis [19].

##### Cytological Atypia and Architectural Complexity

A similar classification was proposed by **Misdraji et al.** in which the appendiceal tumours were divided into two groups based upon their architectural complexity and degree of cytologic atypia [20]. Tumours that demonstrated low-grade cytologic atypia (nucleomegaly, nuclear stratification, rare mitotic figures, single cell necrosis) and minimal architectural complexity (villiform, flat epithelial proliferation, small papillary excrescences) were classified as low-grade appendiceal mucinous neoplasms (LAMNs). Contrary to this, if the tumours demonstrated destructive invasion of the appendiceal wall; high-grade cytologic atypia (extensive full-thickness nuclear stratification, vesicular nuclei, marked nuclear membrane irregularities, prominent nucleoli and brisk mitotic activity); or complex epithelial proliferation (complex papillary

**Table 7.1** Various classifications used for mucinous appendiceal tumours and PMP

| | Woodruff and McDonald [13] | Carr and Sobin [19] | Midraji [20] | Pai and Longacre [21] | Ronnett [22] | Bradley [24] | WHO (2010) [25] | PSOGI consensus classification [2] |
|---|---|---|---|---|---|---|---|---|
| *Tumour confined to the appendix* | | | | | | | | |
| Limited to the mucosa | | | | | | | | |
| Low-grade cytology | Mucocele | Adenoma | LAMN | Adenoma | | | Adenoma | LAMN |
| High-grade cytology | Mucocele | Adenoma | Non-invasive mucinous cystadenocarcinoma | Adenoma | | | Adenoma | HAMN |
| Positive surgical margin | Cystadenocarcinoma | Adenoma | LAMN | Uncertain malignant potential | | | Adenoma | LAMN/HAMN |
| Neoplastic epithelium/ mucin in the appendiceal wall | Cystadenocarcinoma | Tumour of uncertain malignant potential | LAMN | Uncertain malignant potential | | | Invasive mucinous adenocarcinoma | LAMN/HAMN |
| *Tumour beyond the appendix* | | | | | | | | |
| Low-grade epithelium in peritoneal mucin | Cystadenocarcinoma | Invasive mucinous adenocarcinoma | LAMN | High risk of recurrence | Disseminated peritoneal adenomucinosis | Low-grade mucinous carcinoma peritonei | Low-grade mucinous adenocarcinoma | Low-grade mucinous carcinoma peritonei |
| High-grade epithelium in peritoneal mucin | Cystadenocarcinoma | Invasive mucinous adenocarcinoma | Non-invasive mucinous adenocarcinoma | Invasive mucinous adenocarcinoma | Peritoneal mucinous carcinomatosis | High-grade mucinous carcinoma peritonei | Low-grade mucinous adenocarcinoma | High-grade mucinous carcinoma peritonei |

Abbreviations: *LAMN* low-grade mucinous neoplasm, *HAMN* high-grade mucinous neoplasm, *WHO* World Health Organization, *AJCC* American Joint Committee for Cancer

fronds, cribriform glandular spaces), they were classified at mucinous adenocarcinoma (MACA). Based on the survival outcomes in these patients, the authors concluded that the LAMNs or low-grade tumours had a significantly better long-term outcome even in the presence of peritoneal dissemination as compared to the MACAs. They recommended that both, the appendiceal primary tumour and peritoneal disease, should be classified separately. Secondly, patients who had mucin beyond the appendiceal wall had a greater propensity of developing peritoneal dissemination, whereas those without it seldom developed disease recurrence.

#### Probability of Recurrence

**Pai and Longacre** in 2009 classified these mucinous appendiceal tumours into four groups based on the probability of recurrence [21]. Tumours with low-grade columnar mucinous epithelium with flattened or villous architecture, with no invasion or extra-appendiceal mucin or cells were classified as adenomas. Only tumours excised with negative margins are included in this group. Tumours with similar low-grade epithelium but having presence of extra-appendiceal mucin without extra-appendiceal neoplastic epithelium or invasion were grouped as low-grade mucinous neoplasms and had a low risk of recurrence. Those with similar cytological and architectural features but having presence of extra-appendiceal neoplastic epithelium and not showing invasion were grouped as low-grade mucinous neoplasms having a high risk of recurrence. Group four comprised of mucinous adenocarcinomas that were characterized by the presence of invasion. Invasion was defined as the presence of irregular, jagged, neoplastic glands which were present beyond the mucosa. The tumours generally had high-grade cytological features though the architectural complexity varied.

What was common in all these classifications was that appendiceal tumours and PMP were classified in continuity. The basis of each classification was the presence of mucin within the wall, invading through the wall or presenting with extra-appendiceal dissemination. In 1996, Ronnett developed a three-tier classification that classified only the peritoneal disease and not the primary tumour (described later in this chapter). This was then replaced by a two-tiered classification [22–24]. Bradley et al. developed a similar classification [24].

### 7.2.2.2 Current Classification and Staging of Mucinous Appendiceal Neoplasms

Currently, the above classifications are not used for classifying these tumours. Instead, there are three classifications in common use—the WHO classification, the PSOGI consensus classification and the AJCC-8 classification.

There are two components to be looked at—epithelium and mucin. The tumour stage depends on both the epithelium and mucin, whichever is present at the farthest site. The tumour grade depends on the characteristics of the epithelium (both cytology and architecture) (Fig. 7.4).

The commonly used classification is the **WHO classification** [25]. In this classification, the presence of mucin/epithelium beyond the muscularis mucosae is considered invasion, and these tumours are classified as appendiceal adenocarcinomas (Table 7.1). Tumours (both cells and mucin) which do not breach the muscularis mucosae are classified as low-grade appendiceal mucinous neoplasms (LAMNs) or adenomas (Fig. 7.5).

In the classification by the American Joint Committee against Cancer, the **AJCC-8 classification**, LAMNs are considered Tis (Table 7.2) [26]. In this classification however, LAMN refers to tumours where neither the mucin nor the epithelium invades beyond the muscularis propria. There is no T1/T2 stage for a LAMN but it is there for mucinous and non-mucinous adenocarcinomas. T3 constitutes tumour breaching the muscularis and into the wall of the appendix but not breaching the serosa. The authors do not specify here whether it is epithelial cells alone that count or mucin alone is also considered to be tumour. T4a comprises of tumours with mucin and/or cells on the serosal surface. In T4b, the tumour directly involves adjacent organs or structures, including acellular mucin or mucinous epithelium (does not include luminal or mural spread into adjacent cecum). In this classification, adenocarcinomas

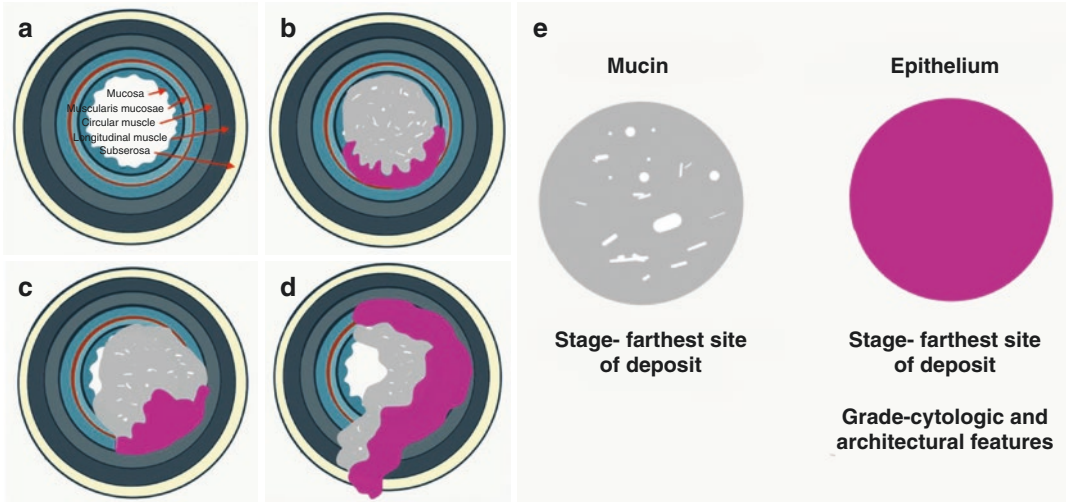


**Fig. 7.4** Diagrammatic representation to factors used to stage and grade mucinous appendiceal tumours: (**a**) layers of the appendiceal wall; (**b**) mucinous tumour with cells and mucin confined to the submucosa; (**c**) mucin alone invading the wall; (**d**) both mucin and cells invading the wall; (**e**) the stage is determined by the farthest deposit of mucin or epithelium and the grade is determined by the characteristics of the epithelium

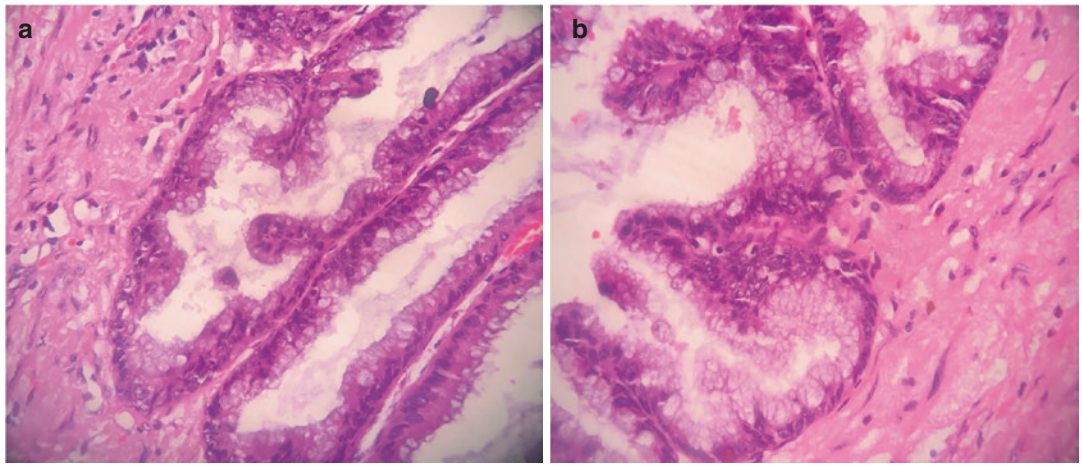


**Fig. 7.5** (**a**, **b**) Low-grade appendiceal mucinous neoplasm (LAMN)—single layer of epithelium with basal nuclei and apical mucin and a pushing invasive front

are grade 2 or 3 depending on the differentiation. What is of concern is that low-grade mucinous adenocarcinoma is used for LAMN interchangeably in this classification. The T3 and T4 tumours are termed as well, moderate or poorly differentiated adenocarcinomas.

In the **PSOGI classification** (Table 7.3), invasion is defined as the presence of infiltration and only tumours with infiltrative invasion are classified as adenocarcinomas [2]. Features of infiltrative invasion include tumour budding (discohesive single cells or clusters of up to five cells) and/or small, irregular glands, typically within a desmoplastic stroma characterized by a proteoglycan-rich extracellular matrix with activated fibroblasts/myofibroblasts with vesicular nuclei (Fig. 7.6). The presence of mucin and/or cells beyond the muscularis mucosa is not considered infiltrative invasion, and these tumours are classified as low-grade appendiceal mucinous neoplasms (if the

**Table 7.2** The AJCC-8 staging of appendiceal carcinoma and LAMN (from Ref. [26] with permission)

| TNM category | Description |
|---|---|
| TX | Tumour cannot be assessed |
| T0 | No evidence of primary tumour |
| Tis | Carcinoma in situ (intramucosal carcinoma; invasion of the lamina propria or extension into but not through the muscularis mucosae) |
| Tis (LAMN) | LAMN confined to the muscularis propria. Mucin or mucinous epithelium may extend into the muscularis propria |
| T1 (not applicable to LAMN) | Tumour invades the submucosa (through the muscularis mucosa but not into the muscularis propria) |
| T2 (not applicable to LAMN) | Tumour invades the muscularis propria |
| T3 | Tumour[a] extends through the muscularis propria into the subserosa or mesoappendix |
| T4 | Tumour penetrates the visceral peritoneum, ***including acellular mucin*** or mucinous epithelium involving the serosa of the appendix, and/or directly invades the adjacent structures |
| T4a | Tumour penetrates the visceral peritoneum, ***including acellular mucin*** or mucinous epithelium involving the serosa of the appendix |
| T4b | Tumour directly invades or adheres to adjacent organs or structures |
| M0 | No distant metastases |
| M1 | Distant metastases |
| M1a | Intraperitoneal acellular mucin, without identifiable tumour cells in the disseminated peritoneal mucinous deposits |
| M1b | Intraperitoneal metastasis only, including peritoneal mucinous deposits containing tumour cells |
| M1c | Metastasis to sites other than the peritoneum |

Abbreviations: *LAMN* low-grade appendiceal mucinous neoplasm

[a]It is not specified if tumour represents mucin alone as well

**Table 7.3** The PSOGI consensus classification of epithelial appendiceal tumours (from Ref. [2] with permission)

| Lesion | Terminology |
|---|---|
| Adenoma resembling the traditional colorectal type, confined to the mucosa, muscularis mucosa intact | Tubular, tubulovillous or villous adenoma with low- or high-grade dysplasia |
| Tumour with serrated features, confined to the mucosa, muscularis mucosa intact | Serrated polyp with or without dysplasia (low or high grade) |
| Mucinous neoplasia with low-grade cytological features and any of the following<br>• Loss of muscularis mucosa<br>• Fibrosis of submucosa<br>• Pushing invasion<br>• Dissection of acellular mucin<br>• Undulating or flattened epithelial growth<br>• Rupture of the appendix<br>• Mucin and/or cells outside the appendix | Low-grade appendiceal mucinous neoplasm (LAMN) |
| Mucinous neoplasm with architectural features of LAMN and no infiltrative invasion but high-grade cytological atypia | High-grade appendiceal mucinous neoplasm (HAMN) |
| Mucinous neoplasm with infiltrative invasion | Mucinous adenocarcinoma, well, moderately or poorly differentiated |
| Neoplasm with signet ring cells (<50% of the cells) | Poorly differentiated (mucinous) adenocarcinoma with signet ring cells |
| Neoplasm with signet ring cells (>50% of the cells) | Signet ring cell carcinoma |
| Non-mucinous adenocarcinoma resembling the traditional colorectal type | Adenocarcinoma, well, moderately or poorly differentiated |

cytological features are of low grade) (Fig. 7.7) and high-grade appendiceal mucinous neoplasms (HAMN), for tumour with high-grade cytological features and no infiltrative invasion. The invasion seen in these tumours is termed as 'pushing invasion' characterized by tongue-like protrusions, diverticulum-like structures or broad-front spread of epithelium. Acellular mucin alone also dissects into the appendiceal wall. A schema for classifying a tumour according to the PSOGI classification and its comparison with other classifications is provided in Fig. 7.8.

Essentially, the term adenoma is no longer used in any of these classifications and is reserved

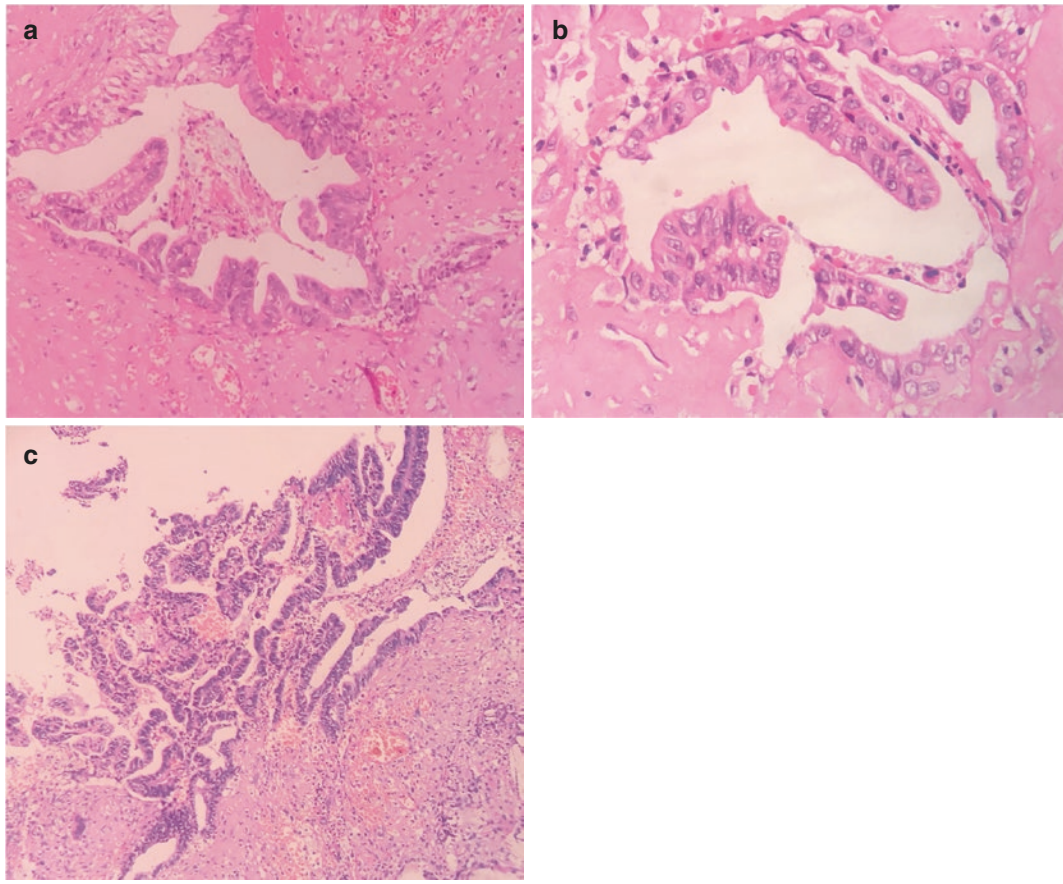


**Fig. 7.6** Mucinous adenocarcinoma of the appendix. Tumours show high-grade cytological features with infiltrative invasion. (**a**) At 10× magnification. (**b**) At 40× magnification. (**c**) Tumour infiltrating the wall of the appendix

for tumours resembling colorectal adenomas [27]. Adenomas do not have the potential to produce PMP. An adenoma can be tubular, villous or tubulovillous akin to colorectal adenomas. Though a low-grade mucinous tumour may have very bland epithelium, the features that distinguish it from an adenoma are the loss of muscularis mucosa and fibrosis of the submucosa with loss of submucosal lymphoid tissue. The term low-grade mucinous neoplasm (LAMN) is used in all three classifications to designate such a tumour. Sometimes, the muscularis mucosa appears intact in which situation the other features that are listed in Table 7.3 are needed to establish a diagnosis of LAMN. Presence of at least one of these features is required to make the diagnosis of a LAMN.

However, as discussed below what is included in the spectrum of a LAMN varies. An adenoma would essentially have neoplastic growth confined to the mucosa without any of these features. The appendix is not dilated in an adenoma. The PSOGI expert panel recommends that a villous lesion with conventional dysplasia and serration should be called a serrated polyp with dysplasia rather than a villous adenoma.

Some of the features of low-grade tumours are epithelium containing abundant mucin, a low nuclear-cytoplasmic ratio, with small, deeply stained, basally oriented nuclei and inconspicuous nucleoli [27]. Mitosis may be absent of few in number and even if the nuclei are mildly enlarged, polarity is preserved [20, 21, 28]. There is usually a single layer of epithelium with papillary, villous, undulating or flat architecture.

The mucinous adenocarcinomas are further classified as well, moderately or poorly differentiated. A mucinous adenocarcinoma with <50% signet ring cells is a mucinous adenocarcinoma

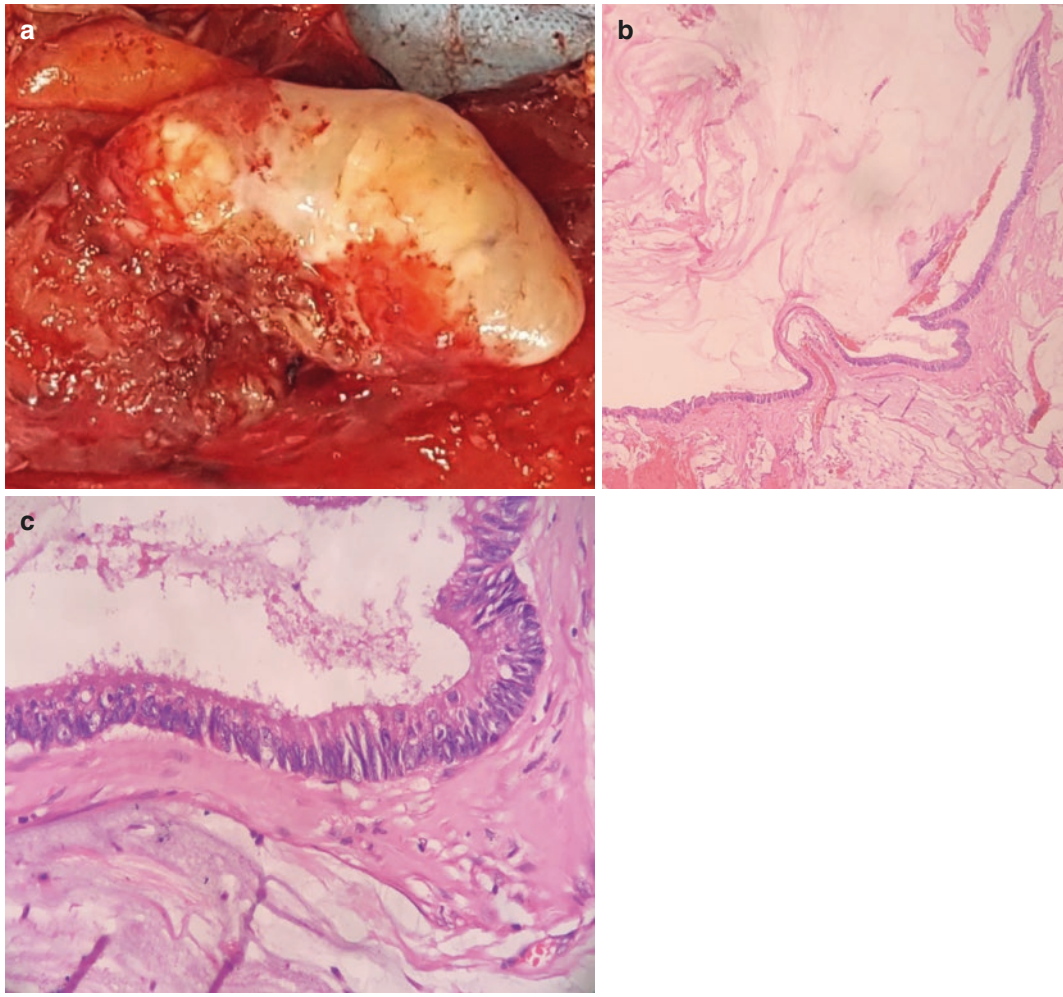


**Fig. 7.7** High-grade appendiceal mucinous neoplasm (HAMN) Tumour cells show high-grade cytological features with pushing invasion. (**a**) Gross appearance. (**b**) At normal magnification. (**c**) At 40× magnification

with signet ring cells and those with >50% signet ring cells is a signet ring cell carcinoma (Fig. 7.9) [19].

### 7.2.3 Comparison of the WHO, AJCC and PSOGI Classifications

In view of the existing systems of classification, the question is which one to use. And what to do with tumours reported at a different centre using a different classification. Expert pathologists are divided in their preferences. A comparison of the three classifications in the setting of different microscopic findings is provided in Tables 7.4, 7.5 and 7.6. A stepwise algorithm for classifying a mucinous appendiceal tumour according to each of the three classifications is provided in Fig. 7.8.

#### 7.2.3.1 Strengths and Limitations of the PSOGI Consensus Classification

Over two-thirds of the pathologists who participated in the PSOGI consensus classification recommended that the appendiceal tumours should be staged according to the AJCC-8 classification in addition. There are some strengths and weak-

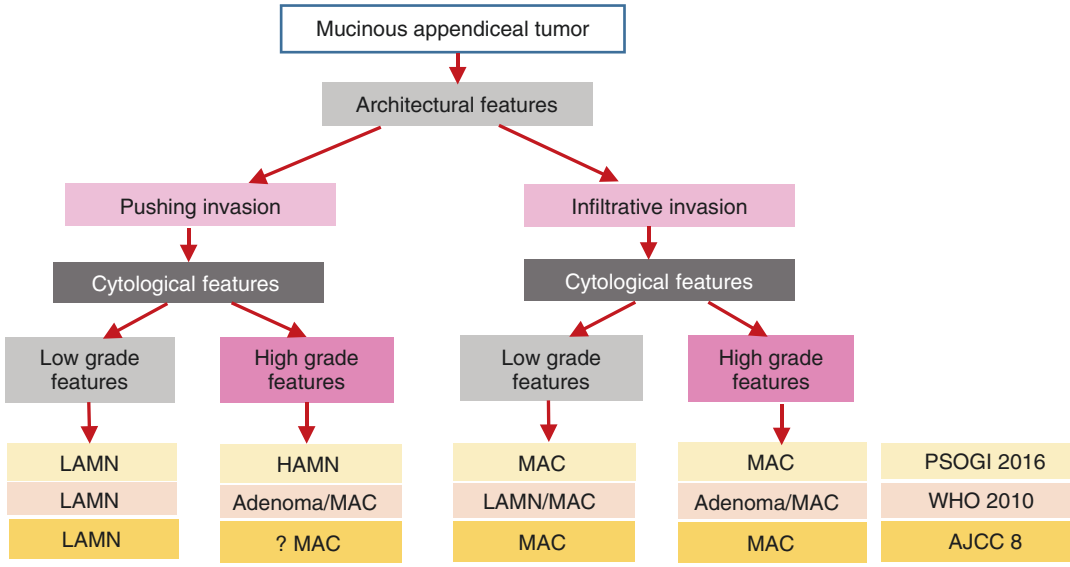


**Fig. 7.8** A stepwise algorithm for classifying mucinous appendiceal tumours according to the WHO, PSOGI and AJCC-8 classifications

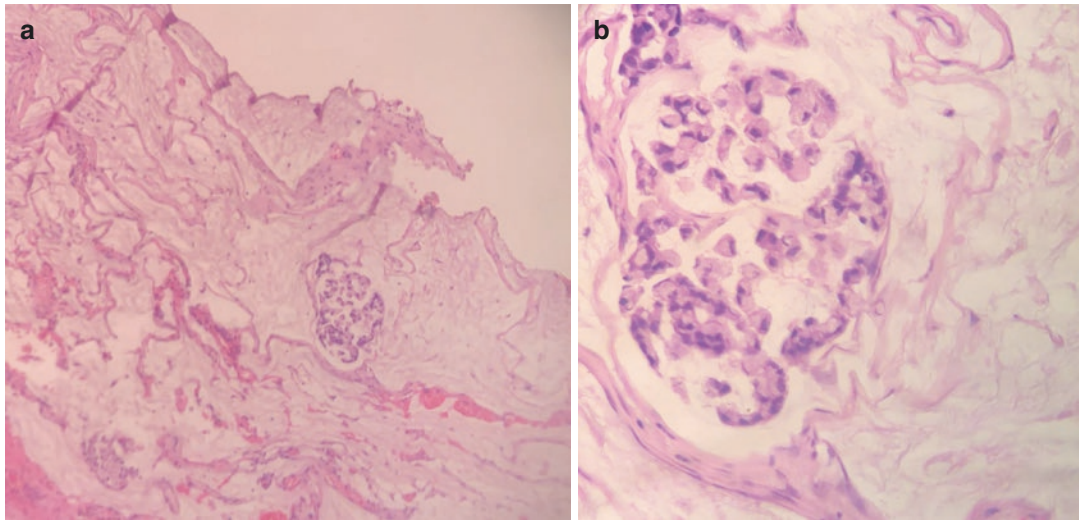


**Fig. 7.9** Mucinous adenocarcinoma with signet ring cells. The signet ring component is >50% of the epithelial component but not of the mucinous component. (**a**) Signet ring cells seen in pools of mucin. (**b**) At 40× magnification

nesses of each classification. In the PSOGI classification, it is only the features of the epithelium that are used to classify tumours. These features are the cytological and architectural features as described above. Thus, a LAMN could have epithelium or mucin or both dissecting through the layers of the appendiceal wall, but not breaching the serosa or breaching the serosa with or without intraperitoneal dissemination—all these are LAMN if the cytological features are low grade or HAMN if they are high grade and there is only pushing invasion.

In the AJCC, they would be LAMN, T3, T4a or b or M1a. For patients with neoplastic epithelium

**Table 7.4** Comparison of the PSOGI, WHO and AJCC classifications for mucinous appendiceal tumours with low-grade cytological atypia and pushing invasion

| Mucin and epithelium | | | Histological features and pathological classification | | | | | |
|---|---|---|---|---|---|---|---|---|
| Farthest distance at which mucin or epithelium is seen | | In the wall | + | + | + | + | + | + |
| | | Subserosal | – | + | + | + | + | + |
| | | Breaching the serosa | – | – | + | + | + | + |
| | | Adjacent organ involvement | – | – | – | + | + | + |
| | | In the right lower quadrant | – | – | – | – | + | + |
| | | Beyond the RLQ | – | – | – | – | – | + |
| Epithelium | Architectural features | Pushing invasion | + | + | + | + | + | + |
| | | Infiltrative invasion | – | – | – | – | – | – |
| | Cytological features | Low-grade atypia | + | + | + | + | + | + |
| | | High-grade features | – | – | – | – | – | – |
| | | High-grade features with signet ring cells | – | – | – | – | – | – |
| Classification | | PSOGI consensus | LAMN | LAMN | LAMN | LAMN | LAMN | LGMCP |
| | | WHO 2010 | LAMN | MAC | MAC | MAC | MAC | MAC |
| | | AJCC-8 | LAMN | WD MAC[a] (T3) | WD MAC[a] (T4a) | WD MAC[a] (T4b) | WD MAC[a] (M1a) | WD MAC[a] (M1a) |

Abbreviations: *RLQ* right lower quadrant, *LAMN* low-grade appendiceal mucinous neoplasm, *LGMCP* low-grade mucinous carcinoma peritonei, *MAC* mucinous adenocarcinoma, *WD* well-differentiated

[a]The term mucinous adenocarcinoma is used, as there is no distinction made according to the type of infiltration. Since the tumours will have low-grade cytological features, they would be classified as well-differentiated adenocarcinomas. It is not clear whether these should be called LAMN or well-differentiated mucinous adenocarcinomas

on the appendiceal surface, and disease confined to the right lower quadrant, the risk of overall recurrence is 42% [21, 28].

Though the PSOGI classification does not consider these features while classifying, there is an accompanying checklist in which the farthest distance of mucin and cells needs to be mentioned. When using the PSOGI classification, if this checklist is adhered to, all the information will be available and can be used to guide treatment decisions.

Using the PSOGI classification, a LAMN or HAMN could be perforated or non-perforated depending on the presence or absence of mucin and cells on the serosal surface or beyond [29, 30]. Honore et al. reported the occurrence of PMP in 65% of the patients with a perforated mucinous neoplasm [29]. A perforated LAMN is a T4a or T4b tumour if there is no free intraperitoneal dissemination of mucin and/or cells, and these patients can be managed with an appendectomy with free margins and complete resection of the adjacent mesentery and peritoneum [31]. Similarly, a M1a LAMN would undergo CRS and HIPEC if there is disease beyond the right lower quadrant [32]. For disease confined to the right lower quadrant that has been completely resected, further surgery is not recommended according to a recent report that found a low incidence of disease progression in these patients [31].

**Table 7.5** Comparison of the PSOGI, WHO and AJCC classifications for mucinous appendiceal tumours with high-grade cytological features and pushing invasion

| Mucin and epithelium | | | Histological features and pathological classification | | | | | |
|---|---|---|---|---|---|---|---|---|
| Farthest distance at which mucin or epithelium is seen | | In the wall | + | + | + | + | + | + |
| | | Subserosal | – | + | + | + | + | + |
| | | Breaching the serosa | – | – | + | + | + | + |
| | | Adjacent organ involvement | – | – | – | + | + | + |
| | | In the right lower quadrant | – | – | – | – | + | + |
| | | Beyond the RLQ | – | – | – | – | – | + |
| Epithelium | Architectural features | Pushing invasion | + | + | + | + | + | + |
| | | Infiltrative invasion | – | – | – | – | – | – |
| | Cytological features | Low-grade atypia | – | – | – | – | – | – |
| | | High-grade features | + | + | + | + | + | + |
| | | High-grade features with signet ring cells | – | – | – | – | – | – |
| Classification | | PSOGI consensus | HAMN | HAMN | HAMN | HAMN | HAMN | HGMCP |
| | | WHO 2010 | MAC | MAC | MAC | MAC | MAC | MAC |
| | | AJCC-8 | MD[a] MAC (T1–2) | MD[a] MAC (T3) | MD[a] MAC (T4a) | MD[a] MAC (T4b) | MD[a] MAC (M1a/b) | MD[a] MAC (M1a/b) |

Abbreviations: *HAMN* high-grade appendiceal mucinous neoplasm, *HGMCP* high-grade mucinous carcinoma peritonei, *MAC* mucinous adenocarcinoma, *MD* moderately differentiated

[a]In absence of frank infiltrative invasion, these tumours may be classified as MD MAC

#### 7.2.3.2 Strengths and Limitations of the WHO Classification

The main problem with the WHO classification is that tumours that would be classified as a mucinous adenocarcinoma by the WHO classification would be a LAMN or HAMN by the PSOGI classification. Similarly, lesions with high-grade cytological features without infiltrative invasion would be classified as adenocarcinomas by the AJCC-8 and HAMN by the PSOGI classification. The terminology used must be put into perspective while deciding the patient's treatment.

For example, for a LAMN, an appendectomy with free margins is sufficient, whereas for an adenocarcinoma, a hemicolectomy is recommended. Similarly, for a M1a mucinous adenocarcinoma, CRS and HIPEC may be recommended, whereas for a LAMN, just a complete resection may be enough if the disease is confined to the right lower quadrant.

Secondly, the term mucinous adenocarcinoma and LAMN are used interchangeably. This could lead to confusion amongst clinicians and lead to patients getting systemic chemotherapy where it is not indicated. The pathologist must mention the classification used and describe the microscopic features in detail. This could help in extrapolating the nomenclature to the different classification where required.

**Table 7.6** Comparison of the PSOGI, WHO and AJCC classifications for mucinous appendiceal tumours with infiltrative invasion

| Mucin and epithelium | | | Histological features and pathological classification | | | | | |
|---|---|---|---|---|---|---|---|---|
| Farthest distance at which mucin or epithelium is seen | | In the wall | + | + | + | + | + | + |
| | | Subserosal | – | + | + | + | + | + |
| | | Breaching the serosa | – | – | + | + | + | + |
| | | Adjacent organ involvement | – | – | – | + | + | + |
| | | In the right lower quadrant | – | – | – | – | + | + |
| | | Beyond the RLQ | – | – | – | – | – | + |
| Epithelium | Architectural features | Pushing invasion | – | – | – | – | – | – |
| | | Infiltrative invasion | + | + | + | + | + | + |
| | Cytological features | Low-grade atypia | + | + | + | + | + | + |
| | | High-grade features | – | – | – | – | – | – |
| | | High-grade features with signet ring cells | – | – | – | – | – | – |
| Classification | | PSOGI consensus | MAC | MAC | MAC | MAC | MAC | MAC |
| | | WHO 2010 | LAMN | MAC | MAC | MAC | MAC | MAC |
| | | AJCC-8 | MAC[a] (T1–2) | MAC[a] (T3) | MAC[a] (T4a) | MAC[a] (T4b) | MAC[a] (M1a) | MAC[a] (M1a) |

Abbreviations: *RLQ* right lower quadrant, *LAMN* low-grade appendiceal mucinous neoplasm, *MAC* mucinous adenocarcinoma

[a]May be termed as well, moderately or poorly differentiated

#### 7.2.3.3 Strengths and Limitations of the AJCC-8 Classification

Using the AJCC-8, the grade and the stage get combined as one continuum. Hence, a tumour with low-grade cytological features with either pushing or infiltrative invasion would be classified as a LAMN or mucinous adenocarcinoma. Though this classification defines a LAMN as a Tis, there is no clarity on whether tumour extending beyond the muscularis mucosa should be called a LAMN or mucinous adenocarcinoma if there is only pushing invasion. A low-grade mucinous adenocarcinoma can be interchangeably used with the term LAMN in this classification.

The PSOGI classification is more objective and easy to use as demonstrated by some investigators [33, 34]. However, it does not provide staging information. The purpose of staging is to determine the prognosis of the tumour and select the right treatment. The PSOGI classification can be used to classify the tumour and then the AJCC-8 stage applied to take treatment decision. Alternatively, the PSOGI checklist can be used to enumerate all features and the treatment selected based on the recommendations for each type as described above.

The PSOGI classification also identifies distinct histological entities (HAMN) which by the WHO classification could fall into either of the groups. The exact incidence and prognostic information about such tumours is not available, but proper identification and classification is the first step toward it.

### 7.2.4 Differential Diagnosis

A LAMN must be distinguished from benign conditions like cystic dilatation of the appendix and other inflammatory conditions [35, 36]. Sometimes due to distension of the appendix with mucin, there is extensive ulceration with loss of epithelium [27]. If neoplastic epithelium is not seen on microscopic examination, the diagnosis of a retention cyst or inflammatory

mucocele is made and prognosis will be completely different. However, these lesions are usually <2 cm in size [27].

## 7.3 Pseudomyxoma Peritonei (PMP)

PMP is a clinical condition and not a pathological diagnosis. However, the term continues to be used in absence of a suitable alternative. The clinical picture of mucinous ascites/peritoneal implants beyond the right lower quadrant should be present for a diagnosis of PMP to be made [2].

### 7.3.1 Pathogenesis of PMP

To begin with there is neoplastic transformation of the goblet cells leading to the formation of a mucinous tumour [37]. The tumour cells maintain their level of mucin expression while proliferating. This leads to intraluminal accumulation of mucin and the formation of a mucocele. Either the mucin alone or epithelium can dissect into the wall of the appendix leading to rupture and intraperitoneal dissemination [38]. An alternative mechanism of intraperitoneal dissemination may be rupture through the lesion as a consequence of appendicitis [27]. Any amount of peri-appendiceal mucin should be completely processed to look for the presence of neoplastic epithelium, the presence of which entails a poorer prognosis [28].

These tumour cells lack cell surface adhesion molecules, exfoliate easily and passively circulate with the peritoneal fluid and redistribute throughout the peritoneal cavity, a phenomenon that has been termed as complete redistribution (CRD). This is seen in the low-grade tumours. High-grade tumours undergo widespread cancer distribution (WCD), in which there is presence of adhesion molecules on the surface of cancer cells that produce a great amount of mucus, interfering with early cell adhesion [39]. As a result, the tumour implants and mucin collections form at the peritoneal fluid reabsorption sites and at dependent portions within the abdomen and pelvis, to create characteristic pattern of PMP (Fig. 7.10). Extensive

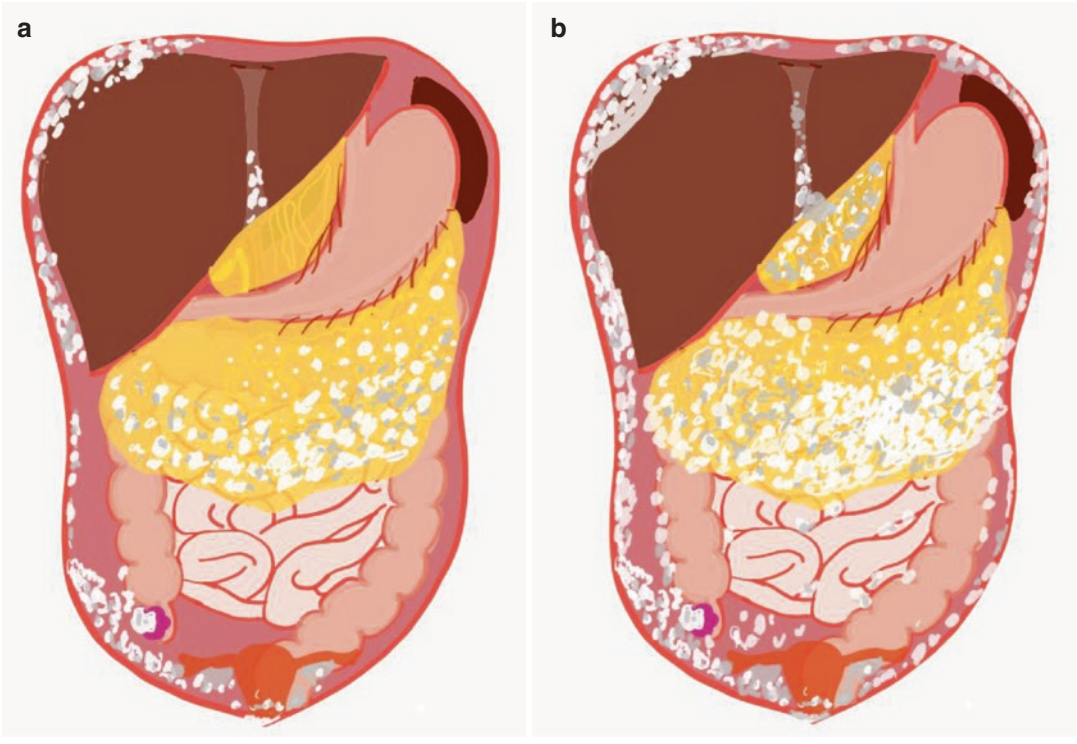


**Fig. 7.10** Redistribution phenomenon in PMP. (**a**) A less extensive disease. (**b**) More extensive disease

disease is seen in the pelvis, greater omentum, beneath right hemi diaphragm, right retro hepatic space and in the paracolic gutters [40, 41].

### 7.3.2 Diagnosis

The concerns for a pathologist are performing a proper diagnosis and classification. It is not uncommon to receive a sample of mucin aspirated percutaneously from the peritoneal cavity or a trucut biopsy performed from an omental cake. Often, these samples contain only acellular mucin and few or no epithelial cells.

Many pathologists continue to report this situation as 'adenomucinosis'. The term adenomucinosis is no longer used. Sampling is an important concern in PMP as many of these tumours are pauci-cellular. Hence, not just the aspirated sample but even a trucut biopsy many contain only mucin and no epithelium. Secondly, different regions may have a different grade and thus the diagnosis made on such samples is always of limited value. The report should mention the gross and microscopic findings in detail, the limitations of a diagnosis made in this manner, and recommend further evaluation.

### 7.3.3 Classification of PMP

#### 7.3.3.1 History and Evolution of the Classification of PMP

As described above, early classification only looked at the appendiceal primary tumour and the same grade or subtype was applied to the peritoneal disease. Some classifications have classified them in continuity. The first classification to consider the peritoneal disease alone was developed by Ronnett et al. [22] This is a three-tiered classification in which the term 'diffuse peritoneal adenomucinosis' (DPAM) is used for tumours that are quintessential of PMP with pauci-cellular mucinous ascites with bland epithelium and no invasion. At the other end of the spectrum is peritoneal mucinous adenocarcinoma (PMCA) comprising of frankly malignant cells invading the underlying tissues. There was an intermediate group comprising of hybrid tumours with PMCA comprising not more than 5% of the tumour. The three groups correlated with survival (Fig. 7.11). In a subsequent analysis, there was not much difference in the intermediate and PMCA groups and they were grouped together [23]. Contrary to this, Bradley et al. found no survival difference between DPAM and intermediate grade tumours and thus developed a two-tiered classification.

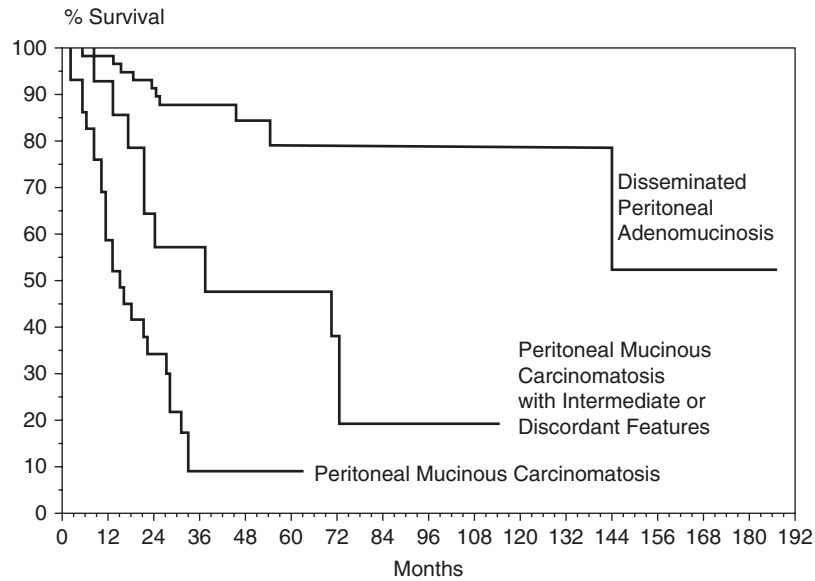


**Fig. 7.11** Impact of the grade of PMP on survival as first demonstrated by Ronnett et al. in 1995. (From Ref. [22] with permission)

They considered DPAM as a very well differentiated mucinous adenocarcinoma and classified PMP into two groups—mucinous carcinoma peritonei low grade and mucinous carcinoma peritonei high grade [24].

#### 7.3.3.2 Currently Used Classifications

The Ronnett classification went out of use as it described DPAM as a benign condition.

In 2010, the American Joint Committee on Cancer (AJCC) and WHO proposed a two-tiered classification in which low-grade PMP comprised of tumours having mucin pools with low cellularity (<10%), bland cytology and non-stratified cuboidal epithelium and high-grade PMP comprises of tumours having more cellular mucin pools, moderate/severe cytological atypia and cribriform/signet ring morphology with desmoplastic stroma (Table 7.7) [9].

The term 'PMP' is retained in the WHO classification. Involvement of the surface of organs like the ovaries and the spleen is classified as high-grade PMP.

The WHO, defining PMP as a malignant condition, gave the following explanations.

- Some carcinomas are extremely well differentiated with minimal cytological atypia and hence low-grade cytology does not exclude the diagnosis of a malignant condition. Parenchymal invasion of solid organs like the ovaries and spleen is seen in PMP which is a feature of malignancy.
- Some tumours have a broad growth pattern and invade with pushing margins which explains the absence of infiltrative glands and desmoplasia.
- Mucinous tumours that have spread beyond the appendix often lead to progressive mucinous ascites, disease recurrence and death in at least 50% of the patients, which is a feature of malignancy [9].

**Table 7.7** The WHO-2010 classification of PMP (from Ref. [9] with permission)

| Terminology | Histological features |
|---|---|
| Low-grade PMP | Tumours with mucin pools with low cellularity (<10%), bland cytology and non-stratified cuboidal epithelium |
| High-grade PMP | Tumours having more cellular mucin pools, moderate/severe cytological atypia and cribriform/signet ring morphology with desmoplastic stroma |

The 7th edition of the American Joint Committee on Cancer adopted a 3-tiered in which the low-grade tumours were classified as grade 1 (G1) or well-differentiated mucinous adenocarcinoma and high-grade tumours as grade 2 (G2), the moderately differentiated carcinomas or grade 3 (G3), poorly differentiated carcinomas [42]. However, the histopathological criteria were not defined. This grading is similar to that followed for other gastrointestinal tumours and low-grade tumours with bland features and pushing invasion were classified as a well-differentiated mucinous adenocarcinoma. Davison et al. in their study of 219 patients defined the histological features of each of these groups and showed that they correlated with survival (Table 7.8) [43]. The histological features that were evaluated included cytologic grade, tumour cellularity, destructive invasion, histologic pattern of destructive invasion, signet ring cell component, lymph node involvement, angiolymphatic invasion and perineural invasion.

Low grade was defined by the presence of flat strips of cells with mildly enlarged, hyperchromatic nuclei with nuclear stratification and maintenance of cell polarity without significant mitotic activity or prominent nucleoli. High grade was defined by the presence of any enlarged, vesicular nuclei with full-thickness stratification, loss of nuclear polarity, prominent nucleoli, cribriform or micropapillary growth, and increased mitotic figures, which often extended to the luminal aspect of the epithelial cell. Any tumour with signet ring cells was classified as high cytological grade. The histological patterns of infiltrative invasion were further defined as (1) infiltrating, haphazard, irregular, jagged neoplastic glands or single cells associated with desmoplastic stromal reaction, (2) expansile and confluent cribriform glandular growth, or (3) small nests, glands, or single neoplastic cells floating within small pools of mucin with or without desmoplastic stromal reaction.

**Table 7.8** AJCC-7 classification of PMP with histological features as described by Davison et al. (adapted from Refs. [42, 43] with permission)

| Terminology | Histological features |
|---|---|
| Well-differentiated mucinous adenocarcinoma (G1) **(disseminated low-grade mucinous neoplasms)** | Characterized by the presence of flat strips of cells with mildly enlarged, hyperchromatic nuclei with nuclear stratification and maintenance of cell polarity without significant mitotic activity or prominent nucleoli.<br>Includes patients with acellular mucin alone **(disseminated low-grade mucinous neoplasms with acellular mucin alone)**<br>Includes patients with small areas of high-grade features like nuclear enlargement and stratification, insufficient to be designated as high grade and not comprising of >10% of the entire tumours. Tumours lacking frank invasion but demonstrating questionable and focal areas of invasion involving at most a single low-power (40×) field **(disseminated low-grade mucinous neoplasms with increased proliferation)** |
| Moderately differentiated mucinous adenocarcinoma (G2) **(High-grade mucinous adenocarcinoma)** | Characterized by the presence of any enlarged, vesicular nuclei with full-thickness stratification, loss of nuclear polarity, prominent nucleoli, cribriform or micropapillary growth, and increased mitotic figures, which often extended to the luminal aspect of the epithelial cell.<br>The histological patterns of infiltrative invasion are further defined as (1) infiltrating, haphazard, irregular, jagged neoplastic glands or single cells associated with desmoplastic stromal reaction, (2) expansile and confluent cribriform glandular growth, or (3) small nests, glands, or single neoplastic cells floating within small pools of mucin with or without desmoplastic stromal reaction |
| Poorly differentiated mucinous adenocarcinoma (G3) **(Mixed high-grade and pure signet ring cell adenocarcinoma)** | Any tumour with signet ring cells was classified as high cytologic grade.<br>This group includes patients with any number of signet ring cells. Tumours with >50% signet ring cells are classified as signet ring cell carcinomas. |

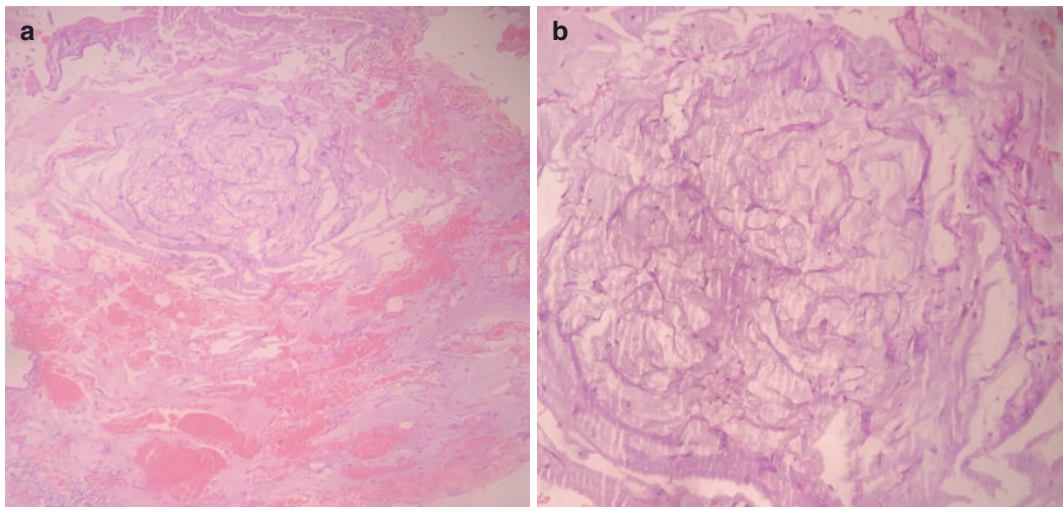


**Fig. 7.12** Acellular mucin in PMP. (**a**) At 10× magnification. (**b**) At 40× magnification

Patients with acellular mucin were classified as G1 (Fig. 7.12). They defined another subgroup called tumours with 'increased proliferation', which were characterized by small areas of high-grade features like nuclear enlargement and stratification, insufficient to be designated as high grade and not comprising of >10% of the entire tumours. This subgroup included tumours that lacked frank invasion but demonstrated questionable and focal areas of invasion involving at most a single low-power (40×) field (Fig. 7.13). These were included in G1 and corresponded to the intermediate group of the Ronnett classification.

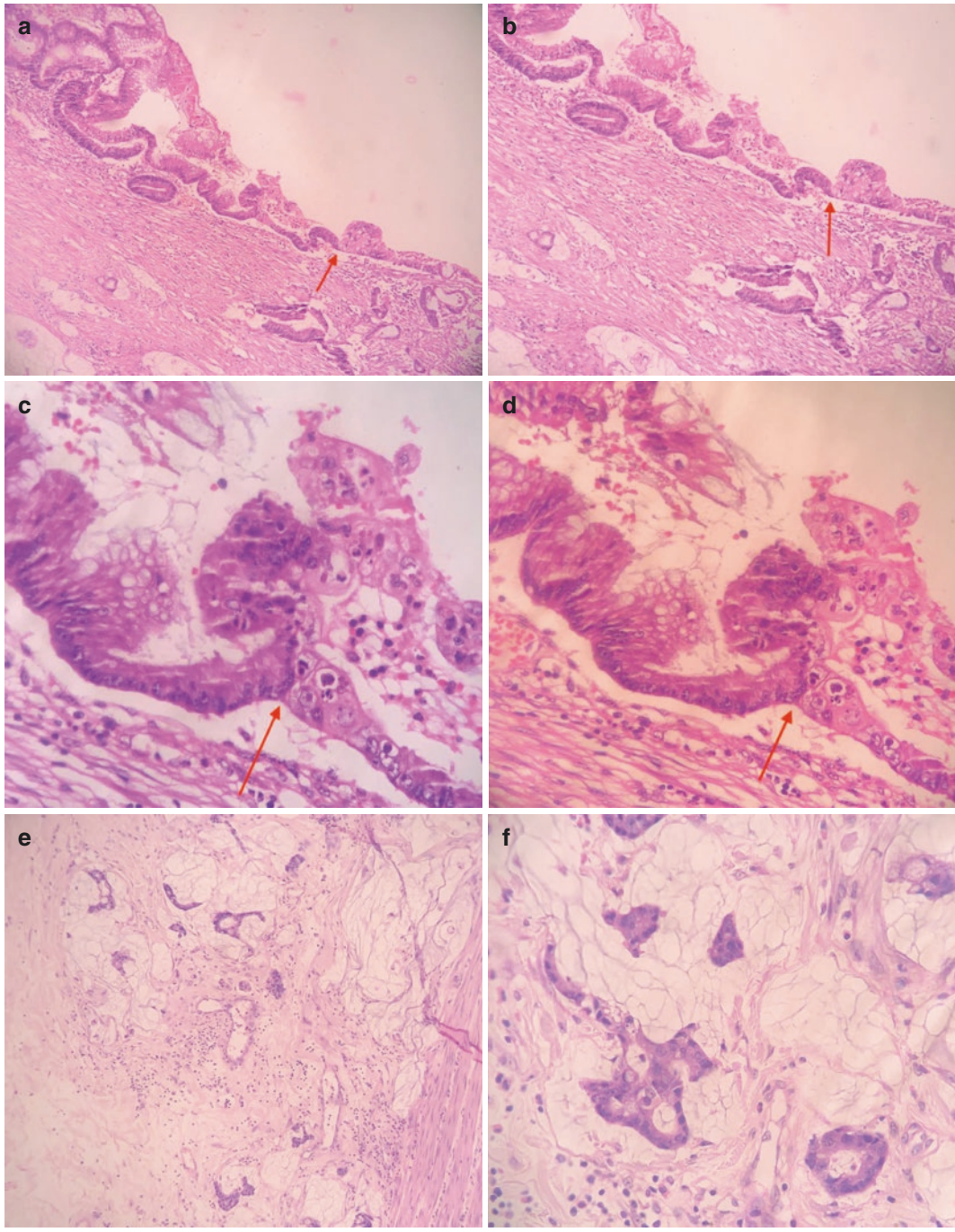


**Fig. 7.13** Low-grade mucinous neoplasm with increased proliferation or intermediate grade tumour (according to Ronnett's classification). (**a**, **b**) Low-grade epithelium on the left with pushing invasion and the transition to high-grade epithelium (arrow) seen in the appendix. (**c**, **d**) Same findings seen at high power (arrow). (**e**) Infiltrative peritoneal implants in the same patient (10× magnification). (**f**) Infiltrative peritoneal implants at 40× magnification

**Table 7.9** The PSOGI consensus classification for pseudomyxoma peritonei arising from an appendiceal primary tumour (from Ref. [2] with permission)

| Lesion | Terminology |
|---|---|
| Mucin without epithelial cells | Acellular mucin (a descriptive diagnosis followed by a comment is likely to be appropriate depending on the overall clinical picture. It should be stated whether the mucin is confined to the vicinity of the organ or origin or distant from it, i.e. beyond the right lower quadrant in the case of the appendix. The term PMP should normally be avoided unless the clinical picture is characteristic) |
| PMP with low-grade histologic features[a] | Low-grade mucinous carcinoma peritonei or disseminated peritoneal adenomucinosis (DPAM) |
| PMP with high-grade histologic features[a] | High-grade mucinous carcinoma peritonei or peritoneal mucinous carcinomatosis (PMCA) |
| PMP with signet ring cells | High-grade mucinous carcinoma peritonei with signet ring cells OR peritoneal mucinous carcinomatosis with signet ring cells (PMCA-S) |

[a]Omental cake and ovarian involvement can be consistent with a diagnosis of either low-grade or high-grade disease

The PSOGI classification divides PMP into four groups, namely acellular mucin, low-grade mucinous carcinoma peritonei (LGMCP), high-grade mucinous carcinoma peritonei (HGMCP) and high-grade mucinous carcinoma peritonei with signet ring cells (HGMCP-S) (Table 7.9) [2]. Figure 7.14 shows a stepwise approach to classifying tumours according to the PSOGI classification. When mucin shows no epithelial cells in all the areas sampled, the diagnosis of acellular mucin is made. Classification of other implants is based on the cytological and architectural features of the epithelium and the type of invasion—those with predominantly low-grade cytological features and no desmoplasia are classified as LGMCP and those with high-grade cytological features with or without desmoplasia are classified as HGMCP. Low-grade cytological features include cuboidal or columnar epithelium with or without pseudostratification, few nucleoli, presence of apical mucin, and few or no mitosis. Papillary pattern with low-grade cytological features is classified as low grade. High-grade cytological features include architectural complexity, stratification, high nuclear-cytoplasmic ratio and

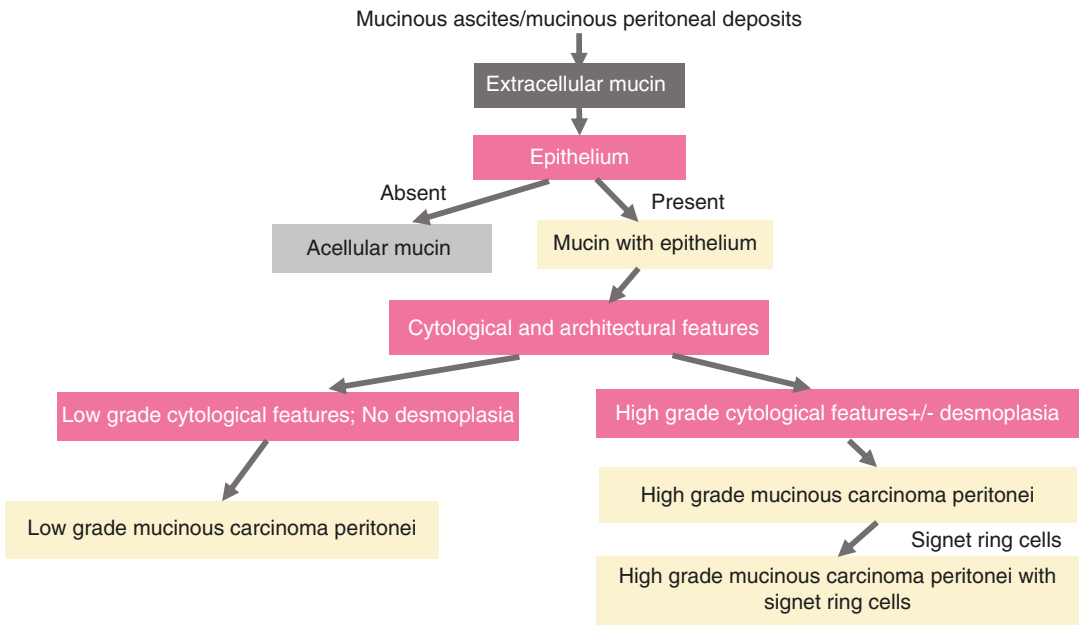


**Fig. 7.14** A stepwise approach to classifying tumours according to the PSOGI classification

mitosis. Lesions with any percentage of signet ring cells are classified as HGMCP-S.

Signet ring cells can be present occasionally floating in pools of mucin or there may be numerous cells in mucin or they could be infiltrating the tissue [44]. The occasional cells floating in mucin pools are usually degenerative cells and the expert panel recommends discounting these cells. But numerous signet ring cells and those invading tissues are not disregarded [43, 45]. The exact percentage of these cells should be mentioned. Extensive signet ring cell differentiation in an appendiceal tumour should raise concern for an underlying goblet cell neoplasm [27, 46].

In the AJCC-8 classification, M1a stands for acellular mucin alone, M1b is for mucinous peritoneal implants with neoplastic cells and M1c for non-peritoneal metastases. The grading system of G1, 2 and 3 is further applied to this.

#### 7.3.3.3 Which Classification to Use?

**A Pathologist's Perspective**

Though the PSOGI classification has been brought together by a group of expert pathologists with the largest experiences in evaluating these tumours, not all agree with the system of classifying the primary and peritoneal disease separately. Some recommend grading the appendiceal tumour and applying the same grade to the peritoneal disease. The proportion of patients with discordant features between the primary and peritoneal disease is very small.

Several others believe that no staging information is provided by the PSOGI classification and should be provided which is the benefit of the AJCC-8 classification. Hence, the AJCC stage can be applied to the PSOGI grade.

**A Surgeon's Perspective**

With the WHO classification, low-grade tumours would get classified as high grade solely on the basis of organ involvement and this has treatment implications. The terms LAMN and low-grade mucinous adenocarcinoma are used interchangeably; this creates confusion, especially amongst clinicians who treat PMP occasionally. It would also make it difficult to compare the results from various centres/studies.

The PSOGI classification is preferable for the fact that it does not classify tumours with non-infiltrative invasion as adenocarcinomas.

Both the AJCC and PSOGI classifications have been validated though in one study, the survival did not correlate with the PSOGI grade [34]. The plausible reason may be the small number of patients in the first and last subgroups.

An important aspect of classification is reproducibility. It is not just at expert centres where pathologists see these patients on a regular basis but at non-expert centres with limited experience where such tumours are diagnosed and reported. The PSOGI classification scores over the AJCC-8 and WHO classifications in being less ambiguous and more reproducible [33, 47].

The expert panel has derived a correlation between the PSOGI classification and the TNM grading system for PMP in which G1 corresponds to low-grade mucinous carcinoma peritonei, G2 corresponds to high-grade mucinous carcinoma peritonei, and G3 corresponds to high-grade mucinous carcinoma peritonei with signet ring cells. The PSOGI panel has produced a checklist for histopathological reporting of PMP and its precursor lesions (Fig. 7.15) [2].

The reporting format for PMP proposed by the expert panel looks at many other features, which have prognostic value but no therapeutic implications—like cellularity, type of epithelium and percentage of signet ring cells [2, 47]. This will make reporting uniform across centres and lead to capturing of valuable clinical information.

### 7.3.4 The Spectrum of PMP: The Grey Areas

The division of all tumours into low and high grade is very broad. Though the PSOGI classification has made two groups in addition acellular mucin and HGMCP-S, with the low-grade and high-grade mucinous carcinoma peritonei, the pathological findings vary.

**a**

PSOGI check list

| Peritoneal disease (includes omentum) | |
|---|---|
| Mucinous disease involving peritoneum<br>☐Yes ☐No | If yes, overall classification:<br>☐ Low grade mucinous carcinoma peritonei/DPAM<br>☐ High grade mucinous carcinoma peritonei/PMCA<br>☐ High grade mucinous carcinoma peritonei with signet ring cells/PMCA-S<br>Percentage of signet ring cells, if present ☐ <10% ☐ 10–50% ☐ >50%<br><br>For appendiceal primaries<br>• Spread of acellular mucin:<br>☐ Acellular mucin confined to the vicinity of the appendix<br>☐ Acellular mucin beyond the right lower quadrant<br><br>• Spread of epithelial cells:<br>☐ Epithelial cells confined to the vicinity of the appendix<br>☐ Epithelial cells beyond the right lower quadrant |
| Other neoplasm involving peritoneum?<br>☐Yes ☐No | If yes, type<br>☐ Goblet cell carcinoid<br>☐ Adenocarcinoma ex-goblet cell carcinoid<br>☐ Neuroendocrine tumor grade 1<br>☐ Neuroendocrine tumor grade 2<br>☐ Non-mucinous adenocarcinoma<br>☐ Other_____ |

**b**

| **Additional cellular features, if cells are present (optional):** | | |
|---|---|---|
| Cytologic atypica:<br>☐ None<br>☐ Minimal<br>☐ Moderate<br>☐ Marked (high grade)<br><br>Mitotic activity<br>☐ Rare (0–2/10hpf)<br>☐ Occacional (3–5/10hpf)<br>☐ Abundant (>5/10hpf)<br>☐ Not accessible | Architectural pattern<br>☐ Flat strip<br>☐ Villiform/papillary<br>☐ Serrated<br>☐ Cribriform<br>☐ Single cells or small clusters of cells in mucous | ☐ Signet ring cells-discohesive<br>☐ Signet ring cells in confluent sheets<br>☐ Signet ring cells infiltrating stroma<br>☐ Infiltrating glands with irregular profiles<br>☐ Other_____ |
| | Cellularity<br>☐ Acellular (no epithelial cells)<br>☐ Scant (<2% of mucinous component consists of cells)<br>☐ Moderate (2–19% of mucinous component consists of cells)<br>☐ High (>20% of mucinous component consists of cells)<br>☐ Not assessable | |
| Invasion of other organs | | |
| Organ invasion?<br>(This includes any spread into the wall/parenchyma of the organ whether infiltrative or not)<br>☐ Yes<br>☐ No<br>☐ Not assessable | If yes, list organs involved<br>☐ Ovary<br>☐ Spleen<br>☐ Large intestinal wall<br>☐ Small intestinal wall<br>☐ Stomach wall<br>☐ Myometrium<br>☐ Any other _____ | Invasion pattern (indicate the most aggressive pattern)<br><br>☐ Acellular mucin only<br>☐ Pushing, broad front invasion by epithelium<br>☐ Infiltrative invasion by irregular glands or single cells with desmoplasia; includes tumor budding and discohesive cells at the invasion front |
| Neoadjuvant therapy | | |
| Neoadjuvant therapy given<br>☐Yes ☐No ☐Not known | Result of neoadjuvant therapy, if applicable<br>☐ No significant histological response<br>☐ Response _____ | |

TNM stage _____
Comments _____

**Fig. 7.15** (**a**, **b**) PSOGI reporting checklist for PMP

The epithelial component in low-grade PMP can vary from the finding of a single layer of cuboidal epithelium, pseudostratification (Fig. 7.16) to more aggressive features like papillary and cribriform patterns (Figs. 7.17 and 7.18) [33]. Both these would be classified as LGMCP, but the biological behaviour is likely to be different [33]. Several investigators have shown that LGMCP is a heterogeneous group with a small proportion of patients having an aggressive clinical course despite a very bland looking tumour [6, 48]. The treatment recommendations for these variants are not different and it is not clearly known what factors portend a more aggressive behaviour. Levine et al. used gene expression profiling to identify two prognostic subgroups amongst the low-grade tumours but such stratification has not been used in clinical practice [19]. Similarly, though an increased expression of markers like KRAS and GNAS is seen in mucinous appendiceal neoplasm, there are no prognostic or treatment implications derived from them [20, 21].

Patients with few foci of high-grade tumour are classified as high grade based on the recommendation of the expert panel but not all patholo-

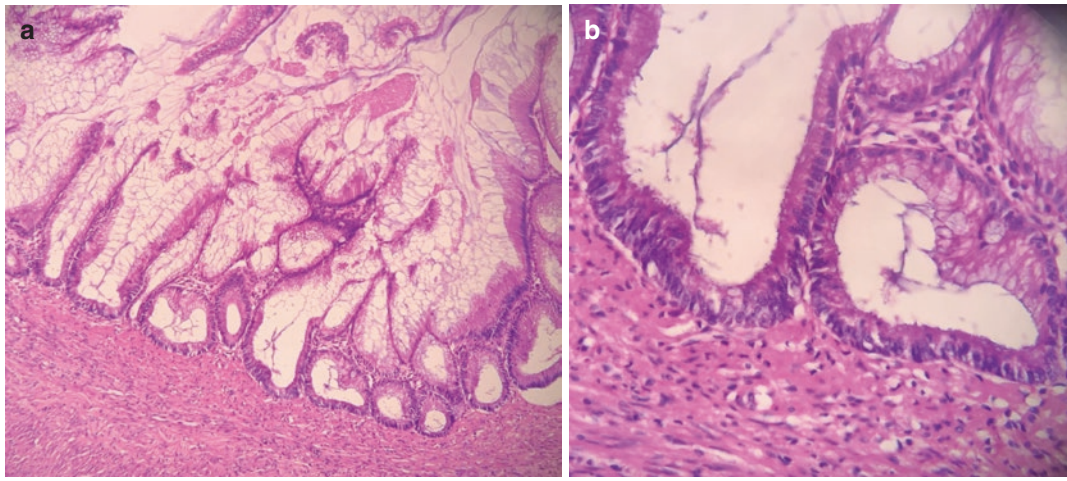


**Fig. 7.16** Pseudostratified columnar epithelium in a case of LAMN. (**a**) At 10× magnification. (**b**) At 40× magnification

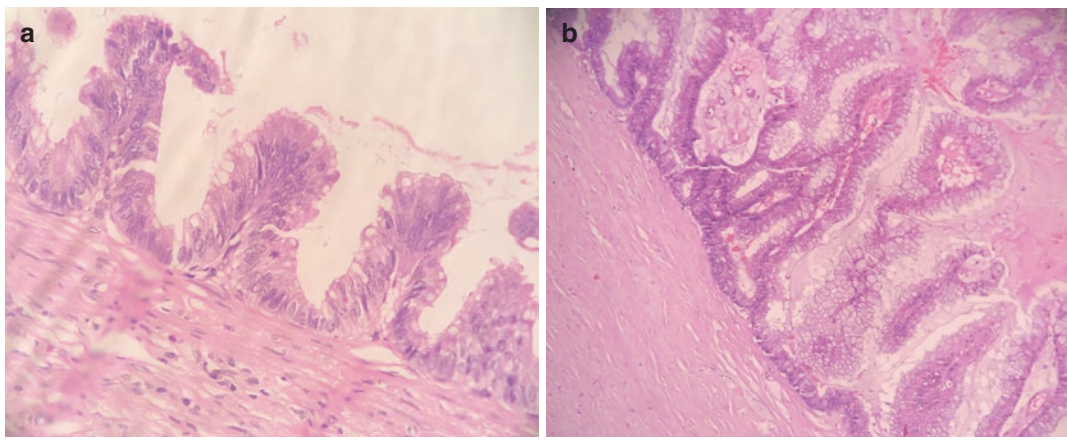


**Fig. 7.17** (**a**, **b**) Papillary pattern seen in low-grade mucinous carcinoma peritonei (LGMCP)

gists prefer to classify them as high grade. Some of these may not progress to high-grade disease and the recurrent disease can have a low-grade histology throughout. Figure 7.19 shows the peritoneal disease in a patient with PMP of appendiceal origin with foci of high-grade features in less than <5% of the resected areas. The recurrent disease that occurred 3 years later showed low-grade features in all the involved regions (Fig. 7.20). Thus, the biological behaviour of these 'intermediate grade' tumours may be different from those with a high-grade tumour with infiltrative invasion in all regions. The recurrent disease can be of a higher grade than the initial disease. Figure 7.21 shows peritoneal deposits with signet ring cells in a patient with appendiceal PMP. The recurrence that occurred 1 year later showed a higher proportion of signet ring

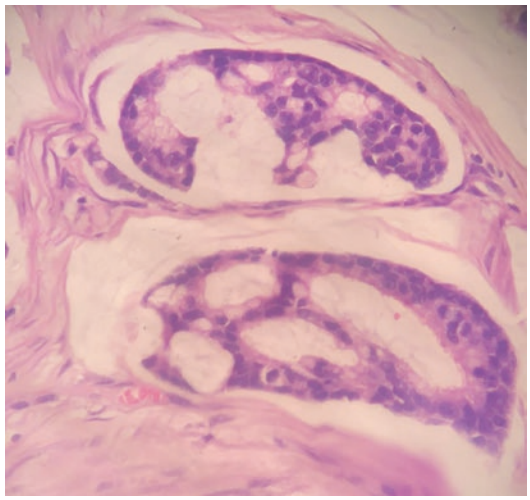

**Fig. 7.18** Cribriform pattern seen in low-grade mucinous carcinoma peritonei (LGMCP)

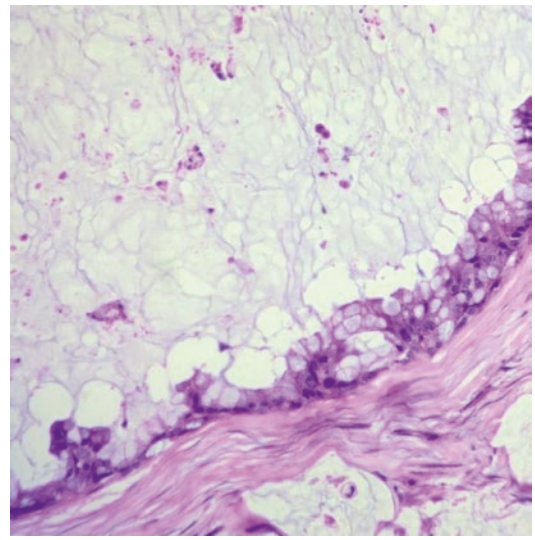

**Fig. 7.20** Low-grade mucinous carcinoma peritonei was seen in all regions in the recurrent disease presenting 3 years after the first surgery

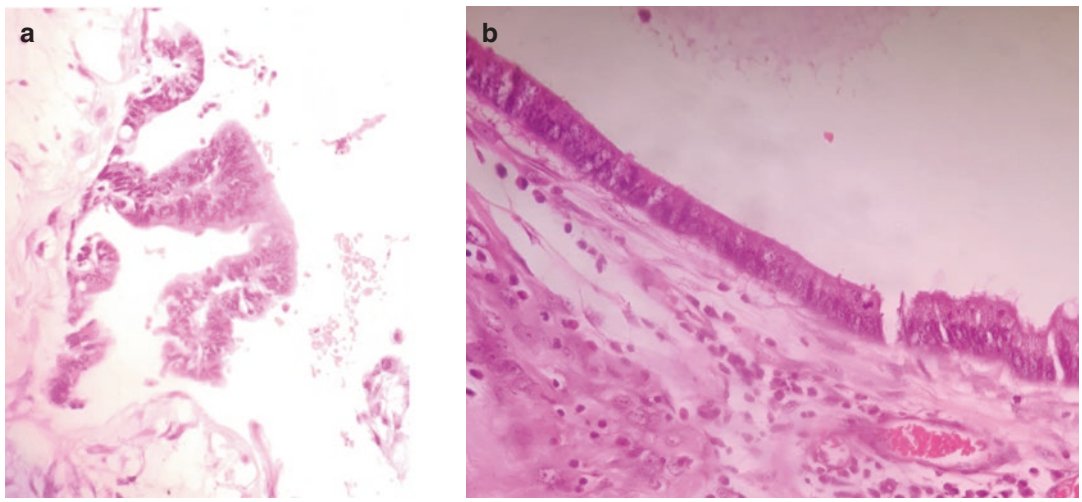


**Fig. 7.19** Focus of high-grade tumour (**a**) in a patient with low-grade mucinous carcinoma peritonei (**b**)

cells (>50%) (Fig. 7.22). With high-grade tumours as well, a disease spectrum may exist. For example, Figs. 7.23 and 7.24 show the histological findings in two different areas in the same patient—there are areas of high-grade cytology with pushing invasion and other areas of high-grade cytology with infiltrative invasion.

The third problem is the finding of low-grade cytological features with infiltrative invasion. An inexperienced pathologist would classify it as G1, whereas if the grade proposed by Davison et al. is followed, it would be G2 (Fig. 7.25).

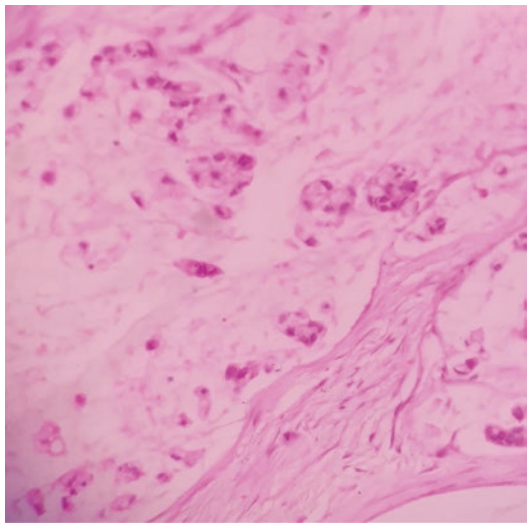

**Fig. 7.21** Mucinous adenocarcinoma with signet ring cells producing PMP

### 7.3.5 The Mucins in PMP

PMP is characterized by the intraperitoneal accumulation of mucin. The mucin that is seen in PMP has certain characteristic features that differentiate it from mucinous peritoneal deposits arising from mucinous adenocarcinomas. Acellular mucin is seen in patients with PMP. It can be seen irrespective of the grade of the primary tumour. Figure 7.26 shows acellular mucin alone in extensive peritoneal deposits in a patient with a mucinous adenocarcinoma. In another patient with mucinous adenocarcinoma with signet ring cells, mucin alone was seen in the lymph nodes (Figs. 7.27 and 7.28). The mucin in PMP is associated with an inflammatory reaction and has fibrosis and ingrowth of blood vessels [49]. The mucin progressively accumulates as it cannot be degraded or drained. It coats the free tumour cells, preventing them from adhering to the peritoneal surfaces and thus redistributing freely in the peritoneal cavity. The mucin prevents immune recognition and leads to resistance to chemotherapy [50].

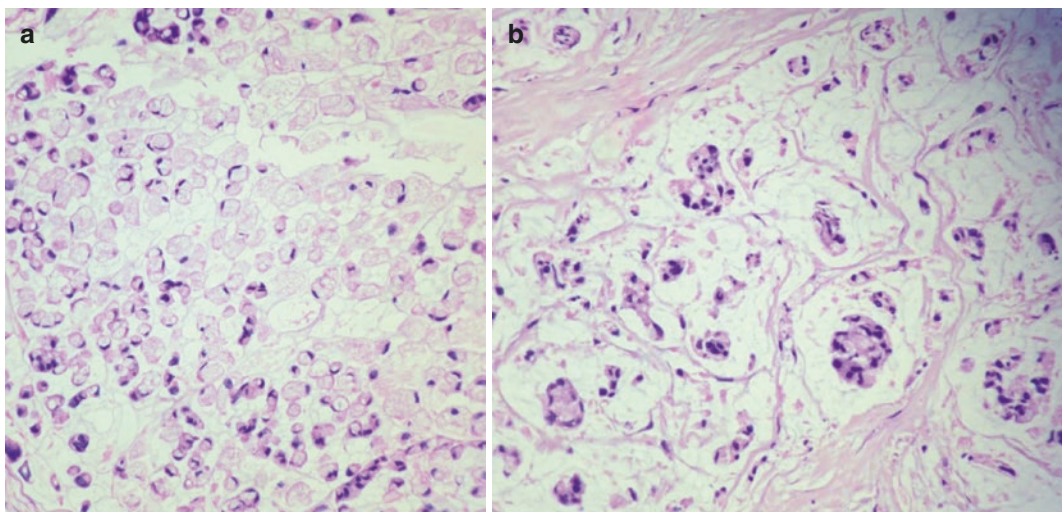


**Fig. 7.22** (**a**, **b**) Progressive disease in the same patient as Fig. 7.21 1 year later shows increase in signet ring cell component to >50%

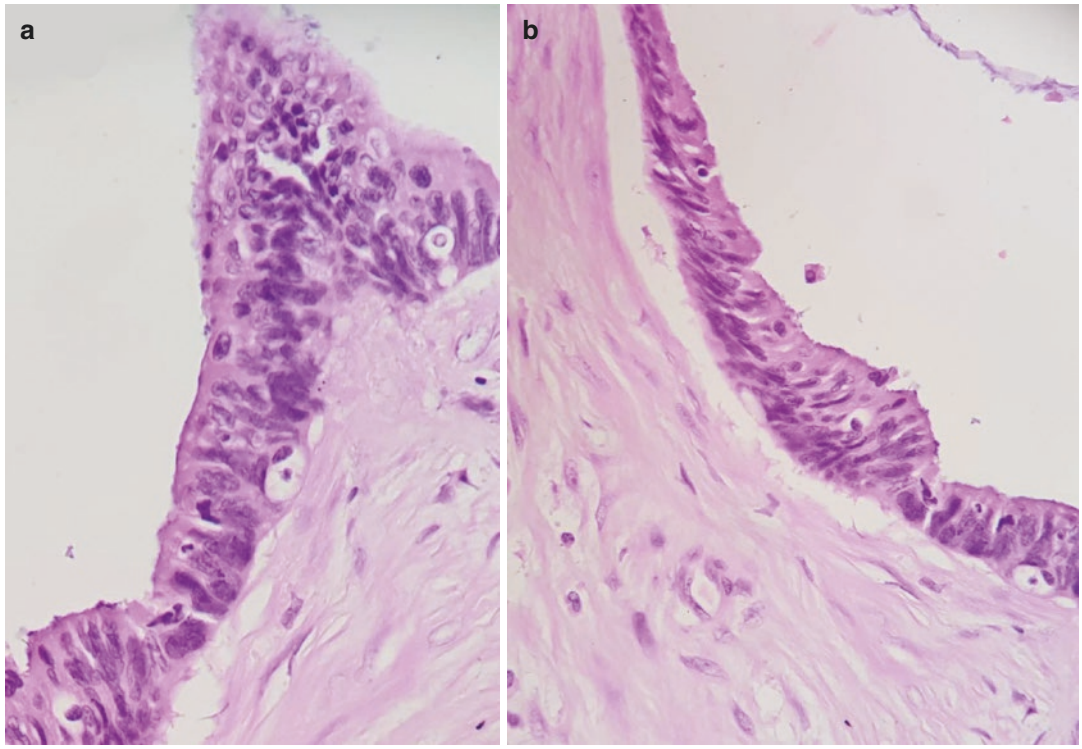


**Fig. 7.23** (**a**, **b**) Area of high-grade cytology with pushing invasion (HGMCP)

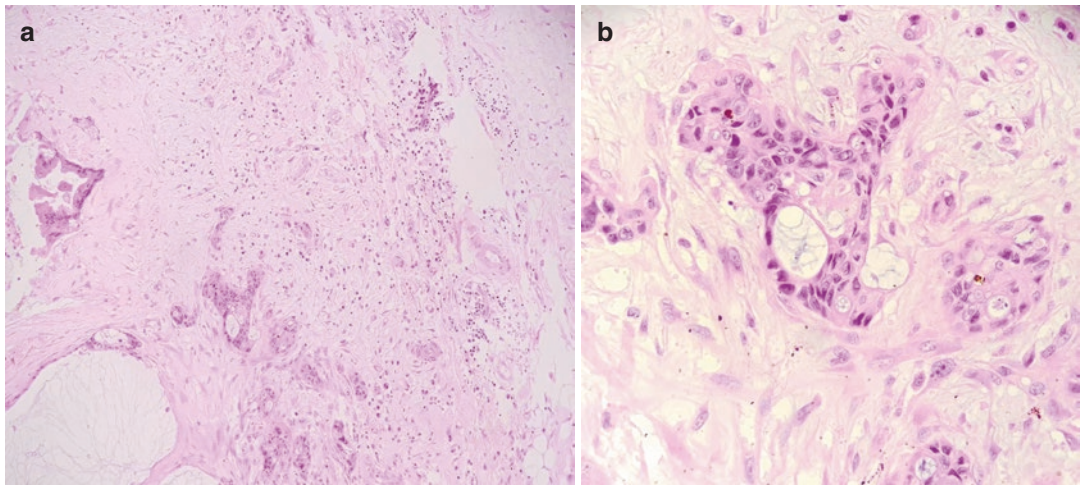


**Fig. 7.24** In the same patients as Fig. 7.23, area of high-grade cytology with infiltrative invasion (HGMCP). (**a**) At 10× magnification. (**b**) At 40× magnification

Mucins are secreted by normal epithelial cells in the body and line the mucosal surfaces. There are two types of mucins—membrane-associated mucins and secreted mucins. These may or may not be gel forming [51, 52]. Colonic mucin is a gel-forming mucin, and this is the mucin that is secreted in PMP. There are various gel-forming mucins of which MUC2 is specifically secreted in the small intestine and colon [50].

MUC2 is extensively glycosylated and produced in large volumes, and hence there is abun-

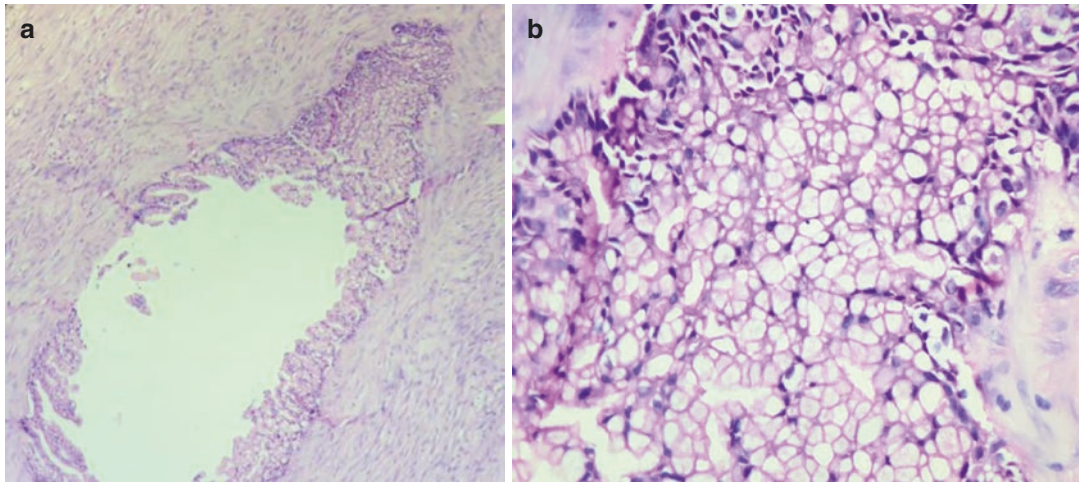


**Fig. 7.25** Low-grade mucinous adenocarcinoma falsely classified as low-grade PMP (LGMCP). (**a**) At 10× magnification. (**b**) At 40× magnification

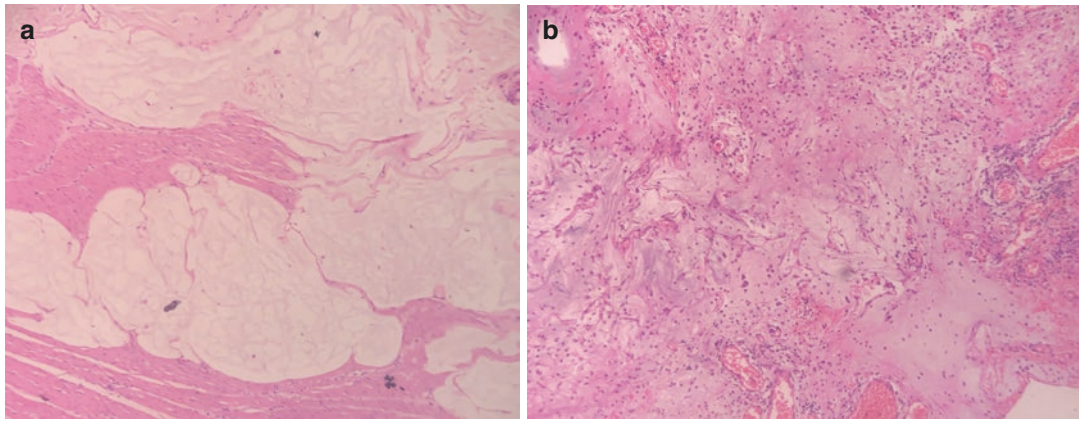


**Fig. 7.26** Acellular mucin in the peritoneum in a patient with a mucinous adenocarcinoma of the appendix. (**a**) At 10× magnification. (**b**) At 40× magnification

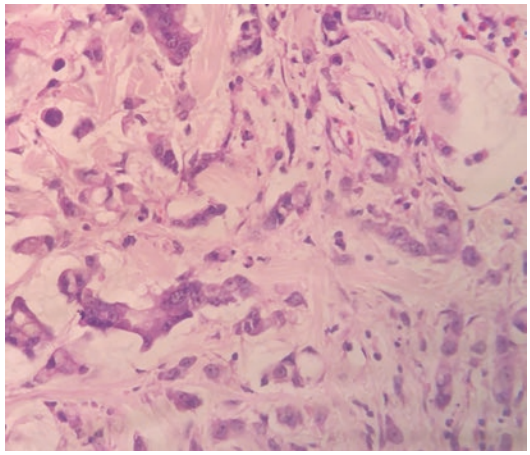

**Fig. 7.27** High-grade mucinous carcinoma peritonei with signet ring cells

dant mucin collection with an average mucin/cell ratio of >10:1. O'Connell showed that primary ovarian mucinous tumours express MUC5AC, while solitary appendiceal mucinous tumours express MUC2 and MUC5B. MUC2 is a molecular marker for PMP [53, 54].

The mucin can have a varied physical appearance. It can be in the form of mucinous fluid or firm or hard deposits. Generally mucinous fluid is seen in low-grade tumours though its presence does not rule out high-grade disease. Similarly, firm to hard deposits are usually seen in high-grade tumours but some low-grade tumours can also have such a presentation.

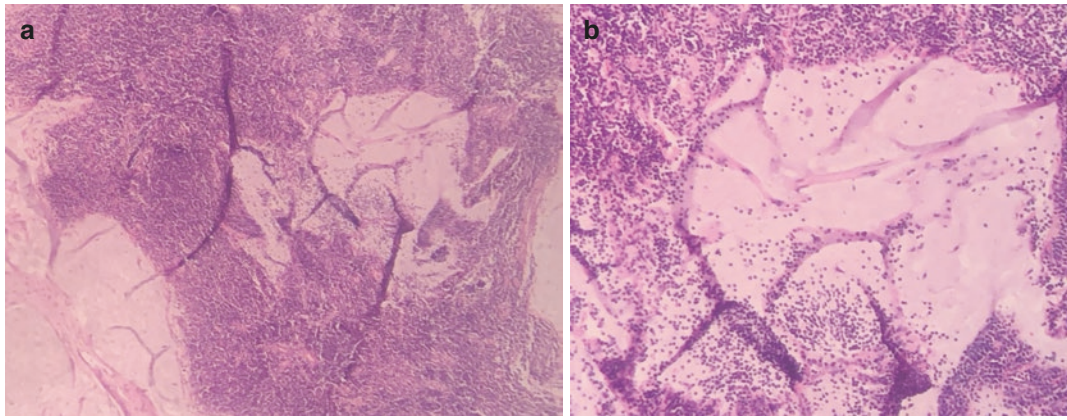


**Fig. 7.28** Acellular mucin alone is seen in the regional nodes in the same patient as Fig 7.27. (**a**) At 10× magnification. (**b**) At 40× magnification

### 7.3.6 Histopathological Evaluation of CRS Specimens

Many patients with PMP present with high volume disease and have extensive surgery comprising of peritonectomies and visceral resections. This results in large specimens sent to the pathologist for evaluation. Currently, there are no standard guidelines for reporting of such specimens. It is uncertain if all resected regions need to be extensively sectioned and evaluated. PMP is a heterogeneous disease. It is not uncommon to find low- and high-grade disease in the same patient and even the low- and high-grade tumours are heterogeneous. Till all regions are evaluated, the final grade cannot be assigned. Hence, every region sent to the pathologist should be evaluated separately.

The appendix should be completely embedded irrespective of the tumour size and extent. The tumour often dissects into the wall at one point only, which is difficult to determine on gross examination and if the entire specimen is not embedded, it may be missed. Sections are taken at 5 mm to 1 cm [12].

The evaluation of the rest of the specimen should be performed as described elsewhere in this book. Our prospective study showed the presence of microscopic disease in 12.2% of the patients in normal peritoneal regions and in 50% of the patients there was microscopic disease in the normal peritoneum between tumour nodules [55]. At present the clinical implications of such findings are not known, but this may be a rationale for more extensive surgery in these patients. Acellular mucin in patients with PMP is considered to be the presence of disease and should be distinguished from acellular mucin in adenocarcinomas following chemotherapy that is considered a pathological complete response [56, 57].

## 7.4 Conclusions

The correct diagnosis and classification of mucinous appendiceal tumours and PMP is challenging for the most pathologists. A combination of the PSOGI consensus classification and AJCC-8 stage may be the most comprehensive way of reporting these tumours. Pathologists must mention the classification used in their reports and other details that are not essential for classification according to the PSOGI checklist which can make clinical decision making and comparison of studies easier. Some nuances and rare clinical situations must be kept in mind by both the surgeon and pathologist while classifying and treating these tumours.

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# 8 Genomics in Pseudomyxoma Peritonei

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## 8.1 Background

Cytoreductive surgery (CRS) plus Hyperthermic IntraPEritoneal Chemotherapy (HIPEC) is, nowadays, known as the "gold standard" treatment for Pseudomyxoma Peritonei (PMP) from mucinous appendiceal tumors. Appendiceal mucinous tumors generically show a favorable biologic behavior due to a pattern of late or non-invasive superficial spread into tissues, with low risk of hematogenous dissemination [1–8]. Regarding the appendiceal tumors, the treatment by CRS + HIPEC permits a high 10 and 15 years overall survival rate (OS) in several literature reports [7–12]; however, clinical outcomes such as Disease-Free Survival (DFS) show a significant and often unpredictable variability, with a non-negligible relapse rate in patients treated [12–17]. The relapse of disease is sometimes not amenable with a complete surgical cytoreduction or debulking surgery [11, 13–15]. Relapse is so therefore treated by systemic chemotherapy with drug regimens commonly used on advanced colonic cancer [9, 10, 13], with poor results. A number of criteria and parameters have been recognized to have an impact on survival (e.g., completeness of cytoreduction, previous surgical score, neoadjuvant systemic chemotherapy, center of treatment's experience) [9–17], but even in leading referral centers' reports, the prediction of outcome in patients treated, revealed to be very difficult. To better predict patients' outcome, a number of histopathologic classification systems have been proposed, as reported in previous chapters [18–20]. Unfortunately those histopathological classifications revealed to be insufficient to predict the disease history. The heterogeneity of PMP is difficult to be classified by histopathology alone. This is probably the reason why, for example, expected good outcomes on low-grade disease are often disregarded. In the last decades, the characterization of tumor specimens with genome-wide analysis provided novel insights in the tumor biology which were reflected in better stratification of tumor outcome, as well as prediction of response to therapies in different tumor types. Genomic profiling of PMP is reported by few authors and recently some evidence of a link between gene expression profile (GEP) and outcome of PMP patients has been demonstrated.

In this chapter, we review literature reports and briefly describe our recent findings about this topic.

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## 8.2 Literature Review

Literature reports may be grossly divided in two groups: the first one is focused on the overexpression or suppression of single or few oncogenes, while few and latest papers are, instead, focused on global expression profiling (GEP), identifying clusters of genes that have been directly linked to prognosis of PMP patients.

The first report found in literature dates 2001 by Shih et al. [21]. The paper was based on the molecular genetic analysis performed on two twins, both with an appendicular adenoma, one of them associated with PMP. The analysis was focused on APC and K-RAS mutations. The authors observed that identical *K-RAS* mutations were detected in the appendiceal adenoma and peritoneal tumor from the twin with PMP, whereas the adenoma from the other twin harbored a different mutation. A loss of heterozygosity of *APC* only in the adenoma from the twin without PMP but not from the appendiceal or peritoneal tumors of the twin with PMP was found. The *K-RAS* mutational analysis supported the view that PMP was clonally derived from the associated appendiceal mucinous adenoma. ***The lack of loss of heterozygosity of APC in the adenoma and peritoneal tumor from the twin with PMP suggested that loss of heterozygosity of APC was not necessarily involved in the development of all appendiceal adenomas or PMP. The different types of mutations in K-RAS from both twins suggested that mutation in K-RAS and loss of heterozygosity of APC occurred somatically in adenomas and was independent from the identical genetic background of the twins.***

Maheshwari et al. [22] found an association between tumor loss of heterozygosity markers and histopathology of PMP. On 23 mucinous appendiceal tumors, the loss of heterozygosity by a panel of 15 allelic loss microsatellite markers and KRAS-2 point showed a mutational damage. The fractional mutational rate (FMR), the number of mutated markers divided by the total number of informative markers, was calculated by using the six most informative markers and the KRAS-2 gene. Statistics were calculated to test the association between FMR and the histopathologic classification.

An association between tumor loss of heterozygosity markers and histopathologic classification ($p < 0.05$) was found. There was also an association between FMR and pathological classification as well as between the FMR and survival ($p < 0.05$). An FMR less than 0.25 indicated low-grade disease, an FMR of 0.25–0.50 indicated intermediate grade, and an FMR greater than 0.5 indicated a high-grade tumor. ***The authors concluded that mutational profiling of accumulated allelic loss and point mutational damage correlated strongly with histopathologic definitions of PMP and was useful to predict the prognosis of patients.***

Nishikawa et al. [23] reported the results obtained analyzing 35 appendiceal mucinous neoplasms for GNAS and KRAS mutations. A functional analysis of mutant GNAS was performed using a colorectal cancer cell line. They observed that a mutational analysis identified activating GNAS mutations in 16 of 32 Low-grade Appendiceal Mucinous Neoplasms (LAMNs) but in none of three Mucinous Adenocarcinomas (MACs). KRAS mutations were found in 30 LAMNs and in all MACs. The authors reported that the introduction of the mutant GNAS into a colorectal cancer cell line markedly induced MUC2 and MUC5AC expression, but did not promote cell growth in vitro or in vivo. ***The authors' conclusion was that activating GNAS mutations are a frequent and characteristic genetic abnormality of LAMN. Mutant GNAS might play a direct role in the prominent mucin production that is a hallmark of LAMN.***

An interesting paper by Liu et al. [24] underlined that multiple mutations were found among different subgroups of PMP and that may be considered for targeted therapies. In fact, a single *JAK3* mutation was detected in the mucocele group while, among the PMPs, 6 mutations were detected in the *KRAS* gene and also in the *GNAS*, *TP53*, and *RB1* genes. Appendiceal cancers showed mutations in the *APC*, *ATM*, *KRAS*, *IDH*, *NRAS, PIK3CA*, *SMAD4*, and *TP53* genes. ***The results suggested a high molecular***

***heterogeneity among epithelial tumors of the appendix, and sequencing mutational spectra in several subtypes of these tumors may suggest, by the authors, a phenotypic heterogeneity showing mutations that are relevant for targeted therapies.***

Noguchi et al. [25], to elucidate the molecular mechanisms underlying PMP, analyzed 18 PMP tumors comprising 10 Diffuse Peritoneal AdenoMucinosis (DPAMs) and 8 Peritoneal Mucinous CArcinomatosis (PMCAs) by Ronnett's classification [18]. DNA was extracted from tumor and was sequenced using a Cancer Panel containing *50 cancer-related genes.* The authors identified 35 somatic mutations in 10 genes, and all mutations were judged pathological mutations. Mutations were frequently identified in *KRAS* (14/18) and *GNAS* (8/18). Interestingly, *TP53* mutations were found in three of the eight PMCAs, but not in the DPAMs. *PIK3CA* and *AKT1* mutations were also identified in two PMCAs, but not in the DPAMs. ***These results suggested that KRAS and/or GNAS mutations are common genetic features of PMP and that mutations in TP53 and/or genes related to the PI3K-AKT pathway may render malignant properties to PMP.***

Nummela et al. [26] explored the molecular features of mucin-producing appendiceal neoplasm. The authors extracted DNA from 19 appendix-derived PMP tumors and nine corresponding normal tissues and analyzed the mutational hotspot areas of 48 cancer-related genes by next-generation sequencing (NGS).

They further analyzed the protein expression of V600E mutated *BRAF, MLH1, MSH2, MSH6*, and *p53* from a larger set of PMP tumors (74 patients), using immunohistochemistry. With NGS, activating somatic *KRAS* mutations in all of the tumors studied was found. *GNAS* was mutated in 63% of the tumors with no marked difference between low-grade and high-grade tumors. Only one tumor showed oncogenic *PIK3CA* mutation, one showed oncogenic *AKT1* mutation, three showed *SMAD4* mutations, and none showed an *APC* mutation. *P53 protein was aberrantly expressed in higher proportion of high-grade tumors* as compared with low-grade ones (31.3% vs. 7.1%, respectively; $p = 0.012$) *and aberrant expression was an independent factor for reduced overall survival ($p = 0.002$).* *BRAF* V600E mutation was only found in one high-grade tumor. All the studied tumors expressed mismatch repair proteins *MLH1, MSH2*, and *MSH6*. The authors' conclusions were that KRAS mutations were evident in all and *GNAS* mutations in most of the PMPs, but *BRAF V600E, PIK3CA*, and *APC* mutations were rare. ***Aberrantly expressed p53 was associated with high-grade histology and reduced survival.***

Pietrantonio et al. [27] performed on 40 patients with mucinous appendiceal tumors and PMP a next-generation sequencing (NGS) of 50 gene's hotspot regions.

*KRAS* and *GNAS* mutations were found in 72% and 52%, and their allelic frequency was below 10% in 55% and 43% of samples, respectively. *KRAS* and *GNAS* mutations were associated with worse progression-free survival (PFS) at univariate analysis ($p = 0.006$ and 0.011, respectively). At multivariate analysis, only *KRAS* mutations were independently associated with PFS ($p = 0.012$); *GNAS* mutations were not significantly associated with other poor prognostic features such as incomplete cytoreduction or *KRAS* mutations. Validation of results was carried out in an independent bi-institutional cohort of 25 patients and the prognostic effect of *KRAS* mutations was again confirmed in the multivariate model ($p = 0.029$). NGS approach allowed the discovery of other potentially "druggable" mutations such as those in *PI3K, AKT, LKB1, FGFR3*, and *PDGFRA*. ***The authors demonstrated a poor prognostic role of KRAS mutations in PMP.***

Borazanci et al. [28] performed a large analysis on 588 samples with appendix primary tumor sites and related results to therapeutic options. Sixty-two percent of samples were adenocarcinomas (used for analysis); the rest consisted of 9% goblet cell, 15% mucinous; 6% pseudomyxoma, and less than 5% carcinoids and 2% neuroendocrine. Profiling across all appendiceal cancer histological subtypes for IHC revealed: 97% *BRCP*, 81% *MRP1*, 81% *COX-2*, 71% *MGMT*, 56% *TOPO1*, 5% *PTEN*, 52% *EGFR*,

40% *ERCC1*, 38% *SPARC*, 35% *PDGFR*, 35% *TOPO2A*, 25% *RRM1*, 21% *TS*, 16% *cKIT*, and 12% for *TLE3*. NGS revealed mutations in the following genes: 50.4% *KRAS*, 21.9% *P53*, 17.6% *GNAS*, 16.5% *SMAD4*, 10% *APC*, 7.5% *ATM*, 5.5% *PIK3CA*, 5.0% *FBXW7*, and 1.8% *BRAF*. ***The authors concluded that appendiceal cancers show considerable heterogeneity with high levels of drug resistance proteins (BCRP and MRP1) and suggested a potential link to therapeutic options: "the incidence of low TS (79%) could be used as a backbone of therapy (using inhibitors such as 5FU/capecitabine or newer agents). Therapeutic options include TOPO1 inhibitors (irinotecan/topotecan), EGFR inhibitors (erlotinib, cetuximab), PDGFR antagonists (regorafenib, axitinib), and MGMT (temozolomide).***" The last, someway amazing, consideration done was that appendiceal cancers have similar patterns, in their molecular profile, to pancreatic cancers and have differential expression from colorectal cancers.

Saarinen et al. [29] also tried to better understand the genetic background of PMP and to find a correlation between gene expression and targeted treatments. The authors underlined that while RAS signaling pathway is affected in most if not all PMP cases and over half of them also have a mutation in the *GNAS* gene, other genetic alterations are poorly known. In this study, *the authors sequenced whole coding genome of nine PMP tumors* and paired normal tissues in order to identify additional, commonly mutated genes and signaling pathways affected in PMP.

Seven genes that contribute to the protein kinase A (PKA) pathway were found. PKA pathway, which also contains *GNAS*, revealed to be a major player of overproduction of mucin, which is the main feature of PMP. In addition to PKA pathway, mutations in six genes that belong to the transforming growth factor beta (TGF-β) pathway which is a key regulator of cell proliferation were found. ***GNAS mutation or an alternative mutation in the PKA pathway was identified in 8/9 patients, so the authors' hypothesis may be summarized as: "the inhibition of the PKA pathway may reduce mucin production in most of the PMP patients and potentially suppress disease progression."***

Gleeson et al. [30] analyzed a total of 54 patients with appendiceal-derived PMP with gene sequencing, protein expression (immunohistochemistry), and gene amplification. The authors found that targeted sequencing of 47 genes detected variants in *KRAS* (81%), *GNAS* (74%), *SMAD4* (16%), and *ATM* (16%). Mutations were found at low frequencies in *APC*, *BRAF, PIK3CA, MLH1*, and *TP53*. *GNAS and KRAS co-occurrence was found in 87%.*

Protein overexpression was found in epidermal growth factor receptor (83%), cyclooxygenase-2 (73%), *c-MET* (63%), *cKIT* (58%), and platelet-derived growth factor receptor alpha (58%). Immune checkpoint expression was found in 36% (programmed cell death protein 1) and 18% (programmed death-ligand 1). Surrogate markers of cell proliferation were found at low rates (*TLE3* 23%, *TOP2A* 22%), consistent with the slow-growing biology of PMP. *Patients exhibited stable microsatellite status and mismatch repair proficiency (93%).* Importantly, multidrug resistance protein expression was elevated (100% *BCRP*, 94% *MRP1*, 88% *PGP*). Markers for gemcitabine (*RRM1*), fluorouracil (*TS*), oxaliplatin (*ERCC1*), and irinotecan (TOPO1) chemosensitivities were detected at favorable rates: 93%, 87%, 77%, and 65%, respectively. ***The authors concluded that "molecular profiling by multiple platforms identified potential therapies for the nontargetable KRAS-mutated population. The role of c-MET-targeted therapeutics and immune checkpoint inhibitors merits further investigation. Biomarker-guided selection of cytotoxic chemotherapies may facilitate efficacy to systemic treatment."***

Sio et al. [31] hypothesized that next-generation exomic sequencing would identify recurrent mutations that may have prognostic or therapeutic implications. Ten patients were selected on the basis of availability of tissue and adequate follow-up. Using next-generation exomic sequencing, the authors tested for mutations in 236 cancer-related genes. MCL1 amplification was additionally tested with

immunohistochemical staining. Detectable mutations were found in 8 patients (80%). Seven patients harbored a KRAS mutation, most commonly involving codon 12.

Four *GNAS* mutations were also detected. *MCL1* and *JUN* were concurrently amplified in three patients. One patient with *MCL1* and *JUN* amplification had concurrent amplification of *MYC* and *NFKBIA*. *ZNF703* was amplified in one patient. Patients with *MCL1* amplification were also found to express *MCL1* with immunohistochemistry, but *MCL1* expression was also detected in some patients without amplification. The authors first reported *MCL1* and *JUN* coamplification in *PMP* and hypothesized that expression of *MCL1* may not be completely dependent on amplification. The prognostic and therapeutic implications of these recurrent mutational were unknown at the time of the study.

An innovative study was published by Roberts et al. [32]; in fact, the study was drawn to develop immortalized PMP cells line for preclinical testing and PMP oncogene discovery. The authors performed an exon array analysis from laser microdissected PMP tissue and normal colonic epithelia. *The array analysis identified 27 upregulated and 34 downregulated genes:* candidate upregulated genes included *SLC16A4, DSC3, Aldolase B, EPHX4*, and *ARHGAP24*; candidate downregulated genes were *MS4A12, TMIGD1*, and *Caspase-5*. Then the authors established *two primary PMP cell lines: N14A and N15A* and immortalized with an SV40 T-antigen lentiviral vector. They crosschecked for expression of the candidate genes (from the array analyses) using qPCR in the cell lines and demonstrated that the gene profiles were distinct from those of colorectal tumor libraries and commonly used colon cell lines. *N14A and N15A were responsive to mitomycin and oxaliplatin.* ***This study characterized the global gene expression in PMP, and, interestingly, the parallel development of the first immortalized PMP cell lines for pre-clinical testing and PMP oncogene discovery.***

**The two main papers published about the relationship between clusters of genes found by Gene Expression Profiling (GEP) and outcome of PMP were published by Levine et al. in 2012 and 2016.**

In the paper published in 2012, Levine et al. [33] described the first use of gene expression profiling (GEP) for appendiceal cancer and demonstrated that the genomic signatures of PMP were distinct from colorectal cancer. In fact from a prospective database and tissue bank, 41 snap frozen samples of peritoneal metastases (26 appendiceal, 15 colorectal) from patients undergoing HIPEC (with complete cytoreduction and >3 years of follow-up) underwent global GEP analysis. Distinct phenotypes were identified using unsupervised hierarchical clustering based upon differential gene expression. Survival curves restratified by genotype were generated. The results were that three distinct phenotypes were found, two consisting of predominantly low-grade appendiceal samples (10/13 in Cluster 1 and 15/20 in Cluster 2) and one consisting of predominantly colorectal samples (7/8 in Cluster 3). Cluster 1 consisted of patients with good prognosis and Clusters 2 and 3 consisted of patients with poor prognosis ($p = 0.006$).

***Signatures predicted survival of low- (Cluster 1)* vs. *high-risk (Cluster 2) appendiceal (p = 0.04) and low-risk appendiceal (Cluster 1)* vs. *colon primary (Cluster 3) (p = 0.0002).***

## Highlights

(a) All the patients considered ***underwent complete cytoreductive surgery + HIPEC*** and had, at least, 3 years of follow-up before the analysis.

(b) From an initial number of 113 (57 peritoneal colon samples and 56 appendiceal samples) cases analyzed, the exclusion of neuroendocrine tumors, contaminated samples, missing follow-up data reduced the number to 41 samples fit for the analysis ***(the initial number of samples available was half-reduced!).***

(c) ***All but 2 of the appendiceal cancers (24/26 cases) were low histologic grade.***

(d) The authors report that gene associated with worse prognosis in the appendiceal tumors included mucin-related genes such as mucin

5, mucin 2, and Trefoil factors 1 and 2. Another interesting observation is that using gene set enrichment analysis (GSEA) between the low-risk appendiceal (Cluster 1) and high-risk appendiceal (Cluster 2) to identify biological processes and pathways associated with the poor prognosis. ***This revealed multiple pathways known to be involved in advanced disease (immune pathways, oncogenic pathways such as src and myc, TGF-β, and resistance to chemotherapy).***

(e) The most interesting observation done by authors into the discussion of this paper is that histologic examination of appendiceal tumors has long been known to have great prognostic value. Grading of the lesions clearly stratifies prognosis; however, even with low-grade lesions there were a minority of patients who failed quickly. The gene expression profiles had a prognostic value and were found to be prognostic without stratification by grade, as 24 of the 26 appendiceal cases were low grade. ***The authors identified, through the first genetic analysis of this disease, a prognostic signature for appendiceal cancer. This breaks low-grade appendiceal disease (by histology) into 2 separate groups with a 5-year survival difference of nearly 50%!*** In addition to pure prognostication, those observations have a potential value in selecting patients most likely to benefit from emerging adjuvant therapies.

***Clearly, not all low-grade appendiceal disease have a good prognosis.***

In 2016, Levine et al. [34] published the "evolution" of the 2012 study:

In this new study, the authors focused their attention on low-grade appendiceal tumors and used *an oncogenomic cassette of 139 genes* which demonstrated to be predominant for the 3 clusters classification described in their previous 2012 study.

In fact, the results of this new study empowered the observation of the 2012 paper and showed a strong relationship between gene expression and patients' outcome *on low-grade PMP*. The author reported that: "*unsupervised hierarchical clustering analysis of tumor expression profiles revealed a 139-gene cassette that distinguished 2 molecular subtypes (based on low vs high expression of the gene cassette)* ***with statistically significant survival differences*** *(disease-specific survival, p = 0.0075; progression-free survival, p = 0.0072).* In a second appendiceal cohort, the 139-gene cassette reproducibly partitioned tumors into subtypes with significant survival differences. Tumors showing high relative expression of the genes comprising the cassette associated with poor survival outcomes (disease-specific survival, $p = 0.047$; progression-free survival, $p = 0.0079$) and exhibited gene expression patterns enriched for oncogenic processes and pathways. ***The prognostic value*** of the molecular subtypes ***was specific for low-grade appendiceal tumors*** (disease-specific survival, $p = 0.028$; progression-free survival, $p = 0.0016$) ***and remained significant in the presence of conventional prognostic markers***, including grade, surgical resection score, ECOG status, and age."

**Highlights**

(a) The validation cohort consists of 39 PMP from low-grade appendiceal tumors patients who underwent cytoreductive surgery plus HIPEC, while the 24 patients considered for the analysis were the same low-grade appendiceal tumors considered for GEP (24/26 PMP patients) in the 2012 study.

(b) The validation cohort (39 samples) confirmed that ***the prognostic subtypes found by the 139 genes-cassette are reproducible in an independent cohort***.

(c) Another interesting finding is that ***prognostic power of the molecular subtypes is independent from conventional prognostic variables*** (e.g., ECOG score, surgical score, age, grade!).

(d) The study also confirmed a lot of data listed in previous chapter: biologic hallmark gene sets representing glycolysis, epithelial to mesenchymal transition, and E2F target genes were found to be highly significantly enriched in the poor prognosis subtype. Oncogenic signatures of genes overexpressed in the context of

p53 mutation, AKT activation, *HER2* (*erb-B2*) overexpression, and cancer stem cells isolated from hepatocellular carcinomas were also found to be significantly enriched in the poor prognosis subtype.

(e) The last interesting observation that can be found in this study is that the authors *cross-referenced the genes of the 139-gene cassette with the Drug Gene Interaction Database.* This analysis revealed ***a number of cancer-associated genes***, the products of which are ***targets of existing or emerging anti-neoplastic drugs***, including *erb-B3* (*HER-3*), *c-MET, FGFR3, CDH1, GPRC5A, DDR1, CA2, CA9, CEACAM5, MST1R, MUC1*, and *SLC2A1*.

## 8.3 *IRCCS (Candiolo Cancer Institute)* Experience

After Levine published his first paper in 2012, we were inspired to explore if in our casuistry we were going to find the same results. Our experience consists in over 20 years of treatment of peritoneal carcinomatosis by surgery associated to locoregional treatments like HIPEC. We operated on over 1500 patients for peritoneal carcinomatosis and performed over 500 CRS + HIPEC procedures. In more than 160 cases, we performed CRS + HIPEC for PMP. After the first Levine's paper was published, in 2012, we started the collection of tissue samples of PMP patients. Thirty five PMP samples of patients treated by CRS + HIPEC were collected and oncogenomic expression according to Levine first study was performed. The control group consisted in 10 patients with peritoneal carcinomatosis from colorectal cancer. The samples of those 10 patients were collected and analyzed by gene expression profiling too. The 35 PMP patients enrolled showed a high-grade PMP (PMCA) in 14 cases and a low-grade PMP (DPAM) in 21 cases according both to Ronnett's [18] and WHO [19] histopathological classification.

In the first part of our experience, we analyzed the samples by the oncogenes indicated by Levine in his 2012 paper. In fact we had, grossly, the same clusters distributions, for PMP and colon cancer, as shown in Fig. 8.1. PMP were divided in two groups labelled high risk and low risk, while colon cancer showed a third genomic profile, according to Levine's findings.

As shown in Fig. 8.1, we found 2 PMP clusters (Cluster 1 and Cluster 2) characterized by low (Cluster 1) and high (Cluster 2) risk to develop recurrence and have a different overall survival (the majority of PMP patients with a disease-free survival lower than 2 years are

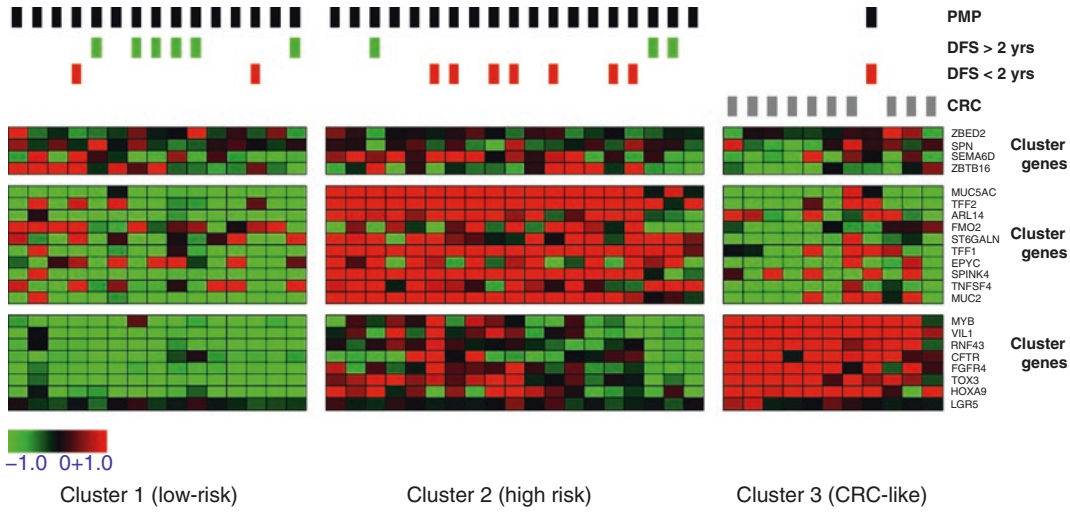


**Fig. 8.1** IRCCS oncogene clusters of 45 samples of peritoneal metastases (35 appendiceal, 10 colorectal). Cluster 1: low-risk PMP; Cluster 2: high-risk PMP, Cluster 3: colorectal-like

included in Cluster 2). Only one PMP patient showed Cluster 3 (CRC-like) oncogenomic expression (related to poorer prognosis). Cluster 3 remarkably showed a different signature towards PMP Cluster 1 and 2, confirming ***that PMP from appendicular tumors and colorectal cancer are two different diseases.***

The second paper published by Levine in 2016, summarized in the previous chapter, inspired the next step of our work. From the initial number of 35 PMP patients, 11 were excluded due to pathology (signet ring cells, neuroendocrine tumors, ovarian PMP patients) or lost at follow-up. The remaining 24 appendix-related PMP patients underwent the 139 oncogenes cassette analysis purposed by Levine (Fig. 8.2). Sixteen patients had low-grade PMP histopathology, while eight patients had high grade or intermediate disease by Ronnett's classification [18]. All the patients underwent complete CRS (CC-0/1) and HIPEC and had a follow-up higher than 24 months.

As shown in Fig. 8.3, a statistical significant relation between the two clusters and disease-free survival (DFS, $p = 0.022$) and overall survival (OS, $p = 0.005$) was found (Cluster 1 showed better prognosis).

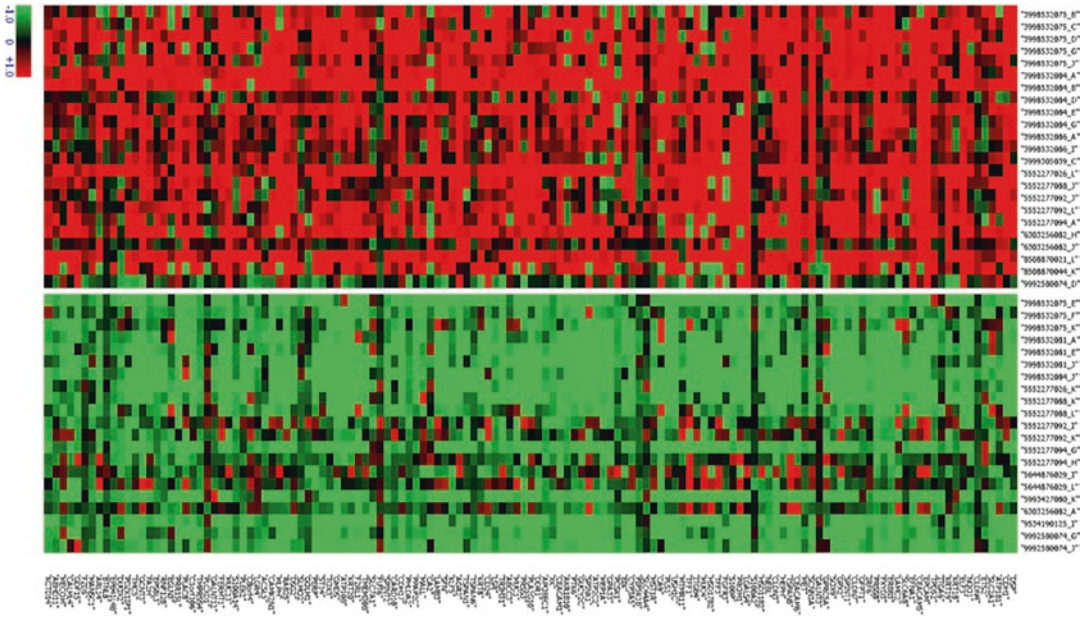

**Fig. 8.2** IRCCS prognostic tumor clusters on 139-gene cassette purposed by Levine on 24 PMP patients (16 low-grade, 8 high-grade/intermediate PMP). Cluster 1: low-risk (green field), Cluster 2: high risk (red field)

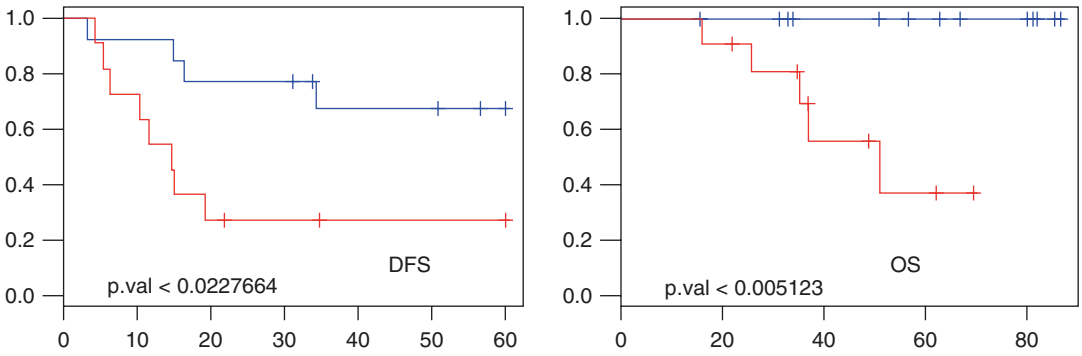


**Fig. 8.3** IRCCS DFS (disease-free Survival) and Overall Survival (OS) of 24 PMP patients analyzed by 139-gene cassette purposed by Levine. Blue line: Low-risk patients (Cluster 1); Red line: High-risk patients (Cluster 2)

## 8.4 Discussion

All the abovementioned papers show that oncogenomics may play an important role in refining and better defining the features of an heterogenous disease like PMP.

*GNAS* and *KRAS* are reported to be common genetic features of PMP and showed a direct involvement in the prognosis of the disease. While *GNAS* (and PKA pathway, which also contains *GNAS*) seems to play a role in the prominent mucin production that is a hallmark of LAMN, *KRAS* mutations were found in variable number of low-grade PMP but nearly in the totality of high-grade PMP. Both *KRAS* and *GNAS* mutations are often reported to be associated with worse progression-free survival of PMP patients [21–30].

*TP53* and/or genes related to the *PI3K-AKT* pathway may render malignant properties to PMP; in fact, aberrantly expressed p53 is associated with high-grade histology and reduced survival [26].

In some reports mentioned above, a direct link between oncogene expression and therapy is suggested:

As appendiceal cancers show considerable heterogeneity with high levels of drug resistance proteins (*BCRP* and *MRP1*), the incidence of low *TS* (79%) could be rationale to consider inhibitors such as 5FU/capecitabine or newer agents. Therapeutic options may also include TOPO1 inhibitors (irinotecan/topotecan), EGFR inhibitors (erlotinib, cetuximab), PDGFR antagonists (regorafenib, axitinib), and MGMT (temozolomide) [28].

*GNAS* mutation or an alternative mutation in the PKA pathway was described, so a hypothesis done is that the inhibition of the PKA pathway may reduce mucin production in PMP and potentially suppress disease progression [30].

The results reported by Levine and in our series of patients show that, even in small numbers, the relationship between outcome and GEP is statistically significant. The GEP shows that there is a significant oncogenomic difference between PMP and colonic cancer, so the treatment of the two diseases must be someway different. Histopathology, even considering recent advances purposed by WHO [19] and PSOGI [20], in PMP patients, treated in referral centers, by complete CRS and HIPEC, is not the best tool to predict the outcome of the patients (low-grade disease, with favorable histology and biology, associated to poor outcome remains someway unexplained).

Levine papers [33, 34] are focused on low-grade PMP, in our second part of the study, 16 on 24 patients were low-grade PMP too; despite this histopathologic definition, a cluster of patients (Cluster 2) in both studies showed a poor prognosis.

***The observation to be done is that "low-grade PMP" definition may lead to a misinterpretation of the expected prognosis both on surgeons and patients.***

On further investigation ongoing in our institute, in a larger series of patients, we observed that, given the superiority of GEP to predict the outcome of low-grade PMP patients towards histopathology or other tools, on high-grade tumors the sensitivity and specificity of the analysis resulted lower than we expected. This observation brought to explore some other tool, useful both on low- and high-grade PMP. On the basis of a paper published by Isella et al. [35] about the stromal contribution to the colorectal cancer transcriptome, the idea was to explore (on an heterogenous, myxoid, tumor, like PMP) the eventual relationship between outcome, stromal score, and cellularity of PMP.

The preliminary results seem to encourage the direction we have chosen; in fact, while merged together, GEP, stromal signature, and cellularity of the tumor seem to predict in a better way the outcome of patients, with a higher impact of GEP in low-grade disease, while stromal score and cellularity seem to be more important in high-grade PMP.

The results of those observations are near to be published and will hopefully clarify some shadows around PMP disease.

## 8.5 Future Directions

An obvious observation, considering the papers analyzed in this chapter, is that all the studies reports are based on a relative small number of

patients. This may be due both to the rarity of the disease and the economic charge of the analysis purposed. Even if in a number of studies, the results reported have a statistical impact, those findings need to be applied in larger scale. In fact, the power of those reports is insufficient, nowadays, to "reconsider the state of the art" of PMP treatment approach. We think that the only way to reach an organic and useful redefinition of PMP, of its clinical management and treatment, is to build a network worldwide to standardize analysis procedures, data collection and at least, fundraising to reach the target of a complete comprehension of this challenging disease.

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# 9 Peritoneal Regression Grading Score (PRGS) for Therapy Response Assessment in Peritoneal Metastasis

Wiebke Solass

## 9.1 Background

The introduction of multimodal therapeutic strategies has improved the outcome of patients with peritoneal metastasis (PM). For example, a phase I study in ovarian cancer showed a survival advantage by combining intraperitoneal and intravenous chemotherapy [1]. The combination of cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) in colorectal cancer has improved the outcome and survival of these patients [2]. And new ways of administration of drugs like Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC) might be a promising approach in the palliative setting [3].

## 9.2 Challenge of Therapy Response Assessment in PM

However, therapy response assessment in PM remains a challenge in modern oncology. Neither computed tomography nor magnetic resonance imaging are reliable predictors, especially in the case of small bowel or mesenteric involvement [4]. Therefore PM are often classified as non-measurable and are not eligible for therapy response assessment which results that these patients are not included in clinical trials [5].

## 9.3 Role of Histology in Therapy Response Assessment

Despite the progress in molecular techniques like liquid biopsy for cancer screening, prognostic stratification, therapy selection, and disease surveillance [6], conventional morphological investigation by histology remains the gold standard in diagnosing malignancies and assessing therapy response. Under chemotherapy, malignant tumors undergo regressive changes which can be various such as fibrosis, hyalinosis, infarct like necrosis, infiltraion of foamy histiocytes, foreign body reaction, acellular mucin pools, inflammation, changes in vessel structure and most importantly loss of vital tumor cells [7], etc. Tumor regression grading scores (TRG) are routinely used in pathology mainly in the neoadjuvant setting and for primary tumors.

For example, the TRG according to Mandard in esophageal squamous cell carcinoma [8], TRG according to Dworak in rectal cancer [9], or the TRG according to Becker in gastric cancer [10] only to name few examples. These scoring systems are similar but not identical regarding the specific criteria, ranking, and categories.

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The absence of a standardized TRG is an essential issue because it prevents comparison between different research studies from different primary tumors and therefore delays the assessment of novel therapeutic strategies regarding efficacy and other outcome criteria.

In the metastatic disease, only few TRG are existing like in colorectal liver metastasis the TRG according to Rubbia-Brandt [11]. The major histological response has been recognized as a beneficial prognostic factor after induction therapy [12, 13].

The applicability of the existing TRG in PM remains uncertain and only little is known.

In a retrospective study on patients with colorectal cancer and PM having received induction chemotherapy before CRS and HIPEC, the pathological response had a prognostic significance [14]. This observation has been confirmed by a French study on 142 patients [15]. The cumulative 5-years survival rates were 75% and 57% for patients with complete and major response, respectively, and histological tumor response was the sole independent predictor of survival in multivariate analyses.

## 9.4 Presentation of the Peritoneal Regression Grading Score (PRGS)

Against this framework, a group of European pathologists has proposed a novel regression grading score for therapy response assessment in peritoneal metastasis, the Peritoneal Regression Grading Score (PRGS).

The PRGS defines four categories, based on the presence of residual tumor cells and the extent of regressive features (Fig. 9.1). Major histological features of regression are fibrosis, inflammation, hyalinosis, acellular mucin pools, necrosis, accumulation of macrophages/multinucleated giant cells, and granulomas.

PRGS 1 corresponds to a complete regression with absence of tumor cells; PRGS 2 to a major histological response with regressive features predominant over residual tumor cells; PRGS 3 to a minor histological response with predominance of residual tumor cells over regressive features; and PRGS 4 to a lack of histological response to therapy where the tumor cells are not accompanied by any regressive features.

| Peritoneal Regression Grading System (PRGS) | | |
|---|---|---|
| **Grade** | **Tumor cells** | **Regression features** |
| **PRGS 1 – complete response** | No tumor cells | Abundant fibrosis<br>and/or acellular mucin pools<br>and/or infarct-like necrosis |
| **PRGS 2 – major response** | Few tumor cells (isolated or small clusters) | Fibrosis<br>and/or acellular mucin pools<br>and/or infarct-like necrosis<br>predominant over tumor cells |
| **PRGS 3 – minor response** | Predominant tumor cells | Tumor cells predominant over fibrosis<br>and/or acellular mucin pools<br>and/or infarct-like necrosis |
| **PRGS 4 – no response** | Visible tumor cells (at lowest magnification) | No regressive changes |

**Fig. 9.1** Peritoneal regression grading score. (From Ref. [21])

## 9.5 Methodological Requirements for PRGS Assessment

The peritoneal sampling during laparotomy or laparoscopy should be standardized and well documented (PCI, video-documented, and protocol).

### 9.5.1 Peritoneal Biopsies

It is recommended to take at least four biopsies from macroscopic tumor suspect lesions and if possible one biopsy out of each abdominal quadrant (right upper quadrant, right lower quadrant, left upper quadrant, left lower quadrant).

The peritoneal biopsies should have a diameter of at least 3 mm, ideally 5 mm. In analogy to state-of-the-art practice in dermatology, the use of a punch biopsy device is recommended to generate standardized samples. The sample morphology is decisive for proper histological analysis. The biopsy should contain both the mesothelial and the submesothelial layers. Peritoneal samples are directional and the relationship surface/depth can influence results, in particular for quantitative pharmacological analyses. Additionally, a local peritonectomy of several square centimeters should be taken: a larger sample is needed in order to increase the accuracy of negative (tumor-free) biopsies for documenting complete tumor regression.

### 9.5.2 Representative Samples Should Be Taken from Surgical Specimen

In the case of cytoreductive surgery, representative samples should be taken from each resected organ. The analysis of all tumor nodules is not feasible in clinical routine and is not required to assess diagnosis, extent, and PRGS. Only appropriate selection is mandatory.

### 9.5.3 Cytology

In the cases a negative peritoneal histology is suspected, a peritoneal cytology is recommended. After induction therapy or palliative chemotherapy, no vital tumor cells might be documented in the peritoneal biopsies and in the local peritonectomy sample. In this case, another three-step section is recommended to confirm complete response. In the presence of tumor scarring or in the absence of macroscopic peritoneal lesions, sampling of peritoneal fluid for cytological analysis is recommended. Cytology can give additional information but is so far not able to replace the conventional biopsy in response assessment [16].

## 9.6 Interpretation of Tumor Regression in PM

### 9.6.1 Macroscopy

Under therapy, the macroscopic aspect of PM nodules changes. Before chemotherapy, nodules appear ill delineated with a soft consistence or might have marmalade-like aspect when mucinous or signet-ring histology is diagnosed. Under therapy, the nodules develop a glassy aspect and a harder consistence and flatten progressively (Fig. 9.2). This renders the documentation of the PCI difficult, but should still be done.

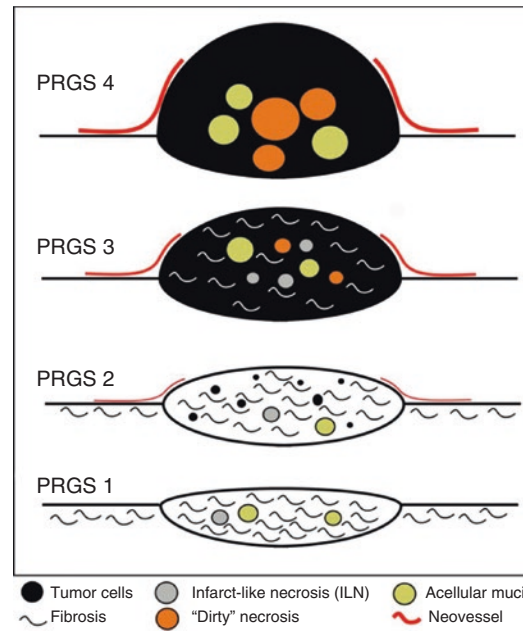


**Fig. 9.2** Macroscopic and microscopic changes of peritoneal nodules during time and therapy. (From Ref. [21])

### 9.6.2 Microscopy

According to the 4-tiered PRGS, the pathologist should assess tumor response to therapy. Multiple biopsies might reveal different scores. In this case, the mean PRGS should be calculated.

### 9.6.3 Role of Immunohistochemistry

Most regression systems published so far do not require complementary immunohistochemical analysis. However, immunohistochemistry is an important adjunct in routine practice of clinical pathology. In the setting of PRGS, immunohistochemistry might allow identification of isolated tumor cells in scar tissue that could not be visualized by HE-staining, in particular for differentiating between PRGS 1 and 2. So far no recommendations regarding the choice of staining has been published and remains an individual decision to each pathologist [17].

## 9.7 Reproducibility of PRGS

Most regression scores have not been validated; in particular, the reproducibility of these scores between different pathologists has not been tested extensively. However, recently, a six-tiered chemotherapy response score (CRS) for tubo-ovarian high-grade serous carcinoma after neoadjuvant chemotherapy and interval debulking surgery has been proposed. After condensation to a 3-tiered system, CRS proved high reproducibility with a Kappa coefficient of 0.76 [18, 19].

Therefore, we evaluated reproducibility of PRGS in various tumor histologies in an observational, retrospective, longitudinal, single-blinded study [17].

A total of 331 quadrant biopsies obtained from 33 patients with PM taken at three different time points were evaluated. In this study, reproducibility of the PRGS for assessing histological response of PIPAC of PM was found to be substantial.

The intraobserver agreement was good to excellent/almost perfect. We found no training effect when comparing the agreement at the first 33% of the scored biopsies with the remaining 67%.

The interobserver agreement was moderate to good/substantial. When comparing the agreement between groups, residents had a slightly better agreement than senior consultants. Agreement between pathologists was slightly better regarding the assessment of the mean PRGS per biopsy set compared to the maximum PRGS per biopsy set. This might be explained by the fact that, when using the maximal PRGS, most information (3 out of 4 biopsy results) available on the intraperitoneal tumor is discarded.

Although the mean PRGS decreased from PIPAC 1 to PIPAC 3, there was no change in the accuracy during the course of therapy.

## 9.8 Clinical Interpretation of PRGS

Regardless of the approach used to quantify tumor response, there is an urgent need for an objective, practical, reproducible, and clinically relevant regression grading system for PM with acceptable interobserver and intraobserver variability [20]. To our knowledge, PRGS is the first biopsy-based scoring system focusing on the assessment of histological response in the palliative setting in PM. Due to methodological facts, PRGS has been used for evaluating response to intraperitoneal therapy but might also be indicated in the future for determining response of PM to systemic chemotherapy. The score has moderate to good/substantial interobserver variability and good to excellent/almost perfect intraobserver variability for the assessment of response to treatment of PM. It can be used by younger pathologists without loss of accuracy which is important in everyday routine in pathology. The inclusion of a wide range of different primary malignancies in the reproducibility study is certainly a strength. The mean PRGS has a better interobserver reproducibility than the maximal PRGS. However, the clinical significance of this result remains unclear. Future studies should now address the prognostic and predictive role of PRGS in peritoneal metastasis.

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# 10 Rare Peritoneal Tumours: Histopathological Diagnosis and Patterns of Peritoneal Dissemination

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## 10.1 Introduction

Rare peritoneal tumours comprise of rare tumours arising de novo from the peritoneum or metastasizing to the peritoneum. Many of these tumours are extremely rare with few hundred cases reported in literature, sometimes even lesser. The natural history of many of these tumours is not known. Some tumours are so rare that only few cases are seen by a surgeon or a pathologist in their entire career. The pathological features may overlap with other more common tumours and it may be a diagnosis of exclusion or the tumour may have peculiar features that a pathologist is unfamiliar with. Some of the rare tumours have already been discussed in other parts of the book.

In this chapter, we look at the diagnostic challenges and peculiarities of the remaining rare tumours.

## 10.2 Classification

Broadly, rare peritoneal tumours could be divided into primary and secondary peritoneal tumours. The rare primary peritoneal tumours include peritoneal mesothelioma, primary peritoneal serous carcinoma, disseminated peritoneal leiomyomatosis, and desmoplastic small round cell tumours. Though these tumours have a common origin from the peritoneum, the pathobiology and clinical behaviour differs significantly. The secondary tumours could be further classified according to the site of origin or the histology or both. A list of rare peritoneal tumours is provided in Table 10.1.

## 10.3 Pathological Evaluation

The history and clinical findings should be kept in mind when performing a pathological evaluation. Histopathological findings should not be considered in isolation. Immunohistochemistry is used liberally both to confirm and arrive at the

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**Table 10.1** Rare primary and secondary peritoneal tumours

| |
|---|
| *Primary peritoneal tumours* |
| Mesothelial tumours<br>• Peritoneal malignant mesothelioma<br>• Well-differentiated papillary mesothelioma<br>• Multi-cystic mesothelioma<br>• Adenomatoid tumour |
| Epithelial tumours<br>• Primary peritoneal serous carcinoma<br>• Primary peritoneal serous borderline tumour |
| Smooth muscle tumour<br>• Leiomyomatosis peritonealis disseminata |
| Tumours of uncertain origin<br>• Desmoplastic small round cell tumour<br>• Fibromatous tumour |
| *Rare secondary tumours* |
| Rare ovarian tumours<br>• Malignant germ cell tumours<br>• Granulosa cell tumours |
| Sarcomas<br>• Endometrial stromal sarcoma<br>• Uterine leiomyosarcoma<br>• Others |
| Gastrointestinal stromal tumours |
| Neuroendocrine tumours |
| Small bowel adenocarcinoma |
| Fibrolamellar hepatocellular cancer |
| Papillary serous carcinoma of the endometrium |
| Mucinous urachal tumours |

diagnosis. Molecular tests can be used for confirmation or to determine the subtype where mutations are known and histopathology is pointing towards a given diagnosis. Laboratories should consider storing the tissue samples for future research. The RENAPE registry, for example, is associated with a biorepository that collects and stores all such samples and there is system for referral to expert pathologists of the RENEPATH group for establishing the diagnosis of some of these rare tumours. The pathological evaluation of specimens should be as described elsewhere in this book.

## 10.4 Mesothelial Tumours

Peritoneal mesothelioma is described as a separate chapter and in this section we discuss an extremely rare variant mucinous mesothelioma or epithelial mesothelioma with intracellular mucin.

### 10.4.1 Mucinous Peritoneal Mesothelioma

Malignant peritoneal mesotheliomas are rare and aggressive tumours of the peritoneum. The commonest variety is the epithelioid variety and the less common ones are the sarcomatoid and biphasic varieties [1]. Occasionally, intracellular mucin is seen in epithelioid mesotheliomas that is rich in hyaluronic acid and mimics signet ring cells. In very rare instances, such cells are numerous giving the appearance of a mucinous peritoneal tumour or signet ring cell carcinoma and there is production of mucinous ascites. These tumours are termed as mucinous mesotheliomas or signet ring cell mesotheliomas. Less than ten cases have been reported in existing scientific literature [2, 3]. In this section, we look at the diagnostic aspects of mucinous mesothelioma which every pathologist dealing with peritoneal metastases should be aware of.

#### 10.4.1.1 Clinical Presentation

There is no data on the age and incidence of these tumours. In the pleura, the incidence is 2–5% [4, 5]. The presentation is of peritoneal metastases and ascites with or without obstructive symptoms [6]. All the cases reported so far have advanced disease at the time of presentation [7]. Symptoms are non-specific. There are no radiological features specific for mesothelioma and diagnosis is made on histopathological and immunohistochemistry evaluation [8].

#### 10.4.1.2 Gross Features

When ascites is present, it is mucinous (Fig. 10.1). The gross appearance of these deposits has not been described but in our experience of 1 case (unpublished report) the deposits were larger than those seen in epithelioid mesotheliomas and had a bosselated appearance (Fig. 10.2). They resembled the deposits of malignant mesothelioma rather than mucinous carcinomas. The peritoneum in between the tumour nodules was thickened.

#### 10.4.1.3 Microscopic Findings

Cells are arranged in sheet-like structures with occasional tubules and papillae (Fig. 10.3).

The cells have the appearance of anaplastic epithelioid cells [7].

They are round or polygonal with eosinophilic cytoplasm, single prominent nucleoli, and frequent mitosis. The characteristic feature is the presence of vacuolated or signet ring cells (Fig. 10.4) [7]. Cell vacuolization, including the presence of signet ring cells, is not an infrequent feature of mesotheliomas. Ordóñez described the mechanism of formation of these cells [9]. There is a single enlarging lumen within the cytoplasm or multiple intracytoplasmic lumina that coalesce to form a larger one that progressively displaces the nucleus towards the periphery of the cell, resulting in the characteristic signet ring-like features seen on light microscopy. Sometimes these cells appear binucleated [9]. Such cells are seen in isolation in most of the common subtypes of epithelioid mesotheliomas and also the uncommon ones like small cell and deciduoid varieties [10, 11]. These findings are better appreciated on electron microscopy. Mesothelin and podoplanin are commonly expressed along the membrane limiting these lumina [9]. The cells can be positive for neutral mucin, as demonstrated by Mayer's mucicarmine stain or periodic acid-Schiff with diastase pre-treatment [2]. This positivity has been attributed by some authors to the presence of large amounts of proteoglycans [4]. This proteoglycan (hyaluronic acid) can be lysed with hyaluronidase leading to reduced positivity with these stains [7].

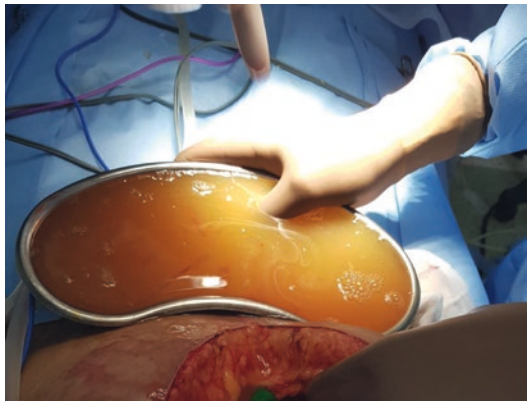

**Fig. 10.1** Mucinous ascites in a patient with peritoneal mesothelioma

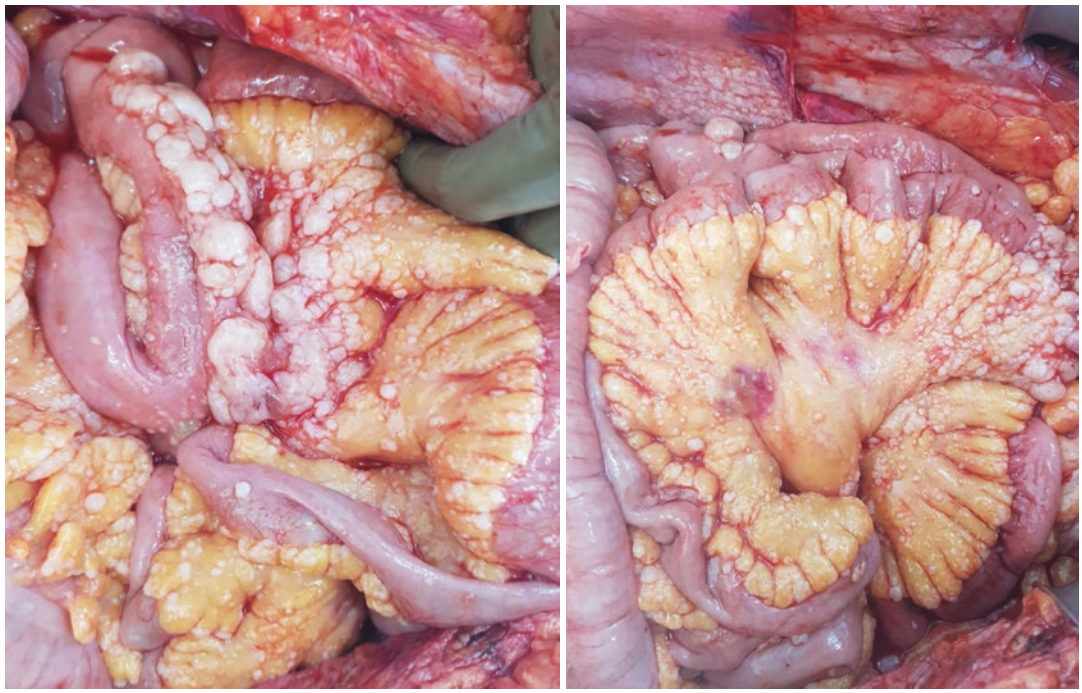

**Fig. 10.2** (**a**, **b**) Morphological appearance of peritoneal deposits in a patient with mucinous mesothelioma

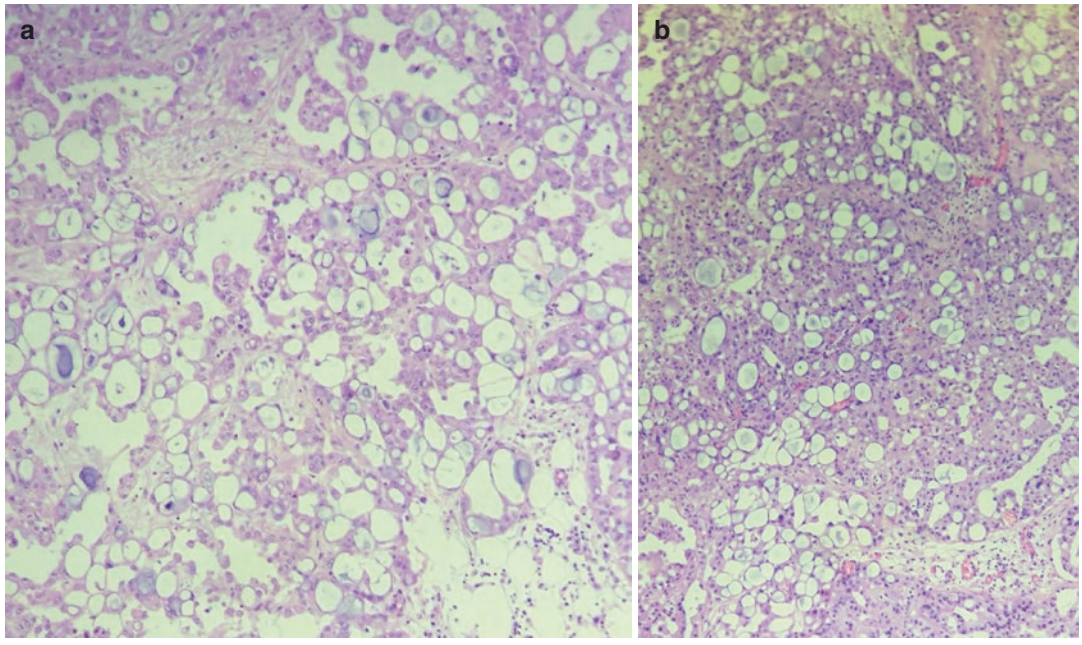


**Fig. 10.3** (**a**, **b**) Histological appearance of peritoneal deposits of mucinous mesothelioma. The cells have intracytoplasmic mucin but the cellular features are of mesothelial cells

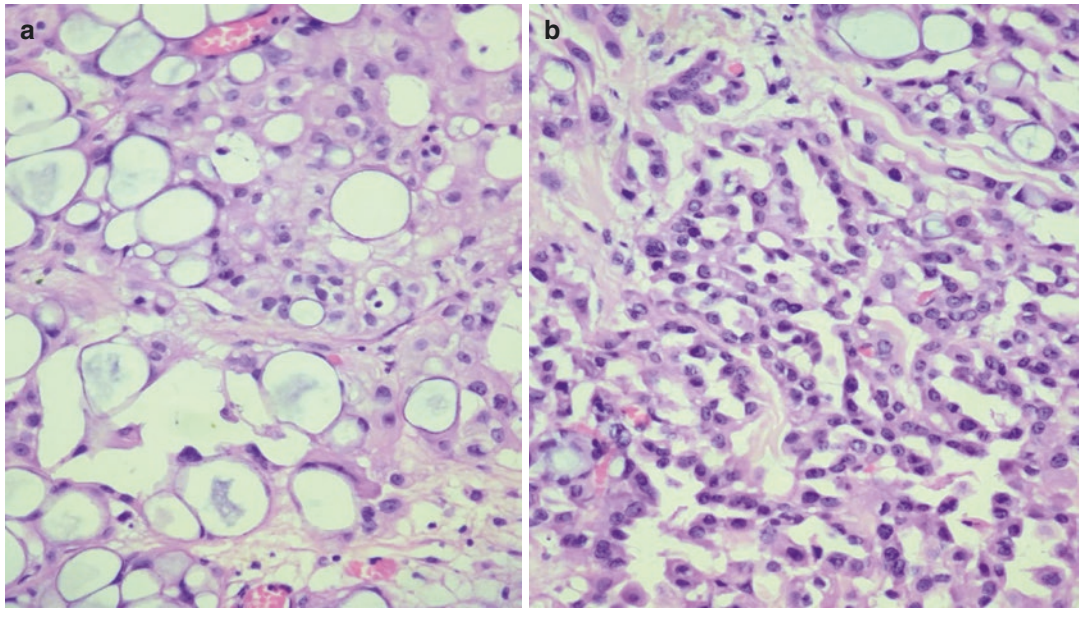


**Fig. 10.4** (**a**, **b**) Mesothelial cells with intracellular mucin seen on high power giving the appearance of signet ring cells

Infiltration of the submesothelial layer is present. Ki-67 is high. Vascular invasion and lymph node involvement have been reported [7].

#### 10.4.1.4 Immunohistochemistry

The diagnosis of peritoneal mesothelioma is established on immunohistochemistry using both positive and negative markers. Malignant peritoneal mesotheliomas are generally positive for calretinin, D2-40, podoplanin, CK 5/6, thrombomodulin, EMA, mesothelin, WT1 gene product, HBME-1, vimentin, and CD146. They are generally negative for Ber-EP4, MOC-31, B72.3, BG-8, Leu-M1, and CEA [9]. However, hyaluronic acid may produce false positive immunoreactivity with various adenocarcinoma markers (CEA, Leu-M1, Ber-EP4, MOC-31, BG-8, and B72.3) [12, 13]. These stainings are abolished or diminished in intensity in most cases by pretreatment with hyaluronidase before immunohistochemistry as mentioned above.

#### 10.4.1.5 Electron Microscopy

Electron microscopy is a confirmatory test for establishing the diagnosis of mucinous mesothelioma. Usually, this test is not performed and the diagnosis is made on the histopathological findings and immunohistochemistry. The most common ultrastructural findings of mesotheliomas are the presence of long, slender, often branching and undulating microvilli on the apical surface of the cuboidal cells lining the tubules or papillae, dilated intercellular spaces, and intracellular lumens [4, 5, 14]. The tumour cells have desmosomes and prominent intercellular junctional complexes. Intracytoplasmic tonofilaments are present. The characteristic feature of mucinous mesotheliomas is the presence of extracellular and at times intraluminal crystalloid structures that are unique to these tumours. Hyaluronic acid is seen as a medium electron dense material covering the microvilli [4, 12]. Formalin-fixed tissue is usually used for the diagnosis of mesothelioma on transmission electron microscopic (TEM) and scanning electron microscopic (SEM) examination. Paraffin-embedded blocks are adequate for diagnostic examination of malignant mesothelial cells on SEM especially when additional tissues for TEM are not available. The long microvilli of the mesothelial cells are easily recognizable by SEM examination in paraffin-embedded tissue sections [14].

#### 10.4.1.6 Disease Distribution

Due to the paucity of cases in literature, there is no information on the patterns of peritoneal spread. In all the cases reported so far, the presentation is of advanced disease involving all the peritoneal surfaces.

#### 10.4.1.7 Section Summary

Both pathologists and peritoneal surface oncology units should be aware of the existence of mucinous mesotheliomas. The presence of mucin with atypical peritoneal implant should raise a suspicion of this condition. These tumours can be confused with signet ring cell carcinomas and immunohistochemistry should be used to correctly establish the diagnosis where doubt persists. Electron microscopy can be used to better appreciate the morphological features that may not always be appreciable of light microscopy and study the characteristic features of mesotheliomas.

### 10.4.2 Disseminated Peritoneal Leiomyomatosis

#### 10.4.2.1 Introduction

Diffuse peritoneal leiomyomatosis (DPL) is characterized by the proliferation of multiple benign nodules comprising of smooth muscle cells in the peritoneal cavity. Fewer than 150 cases have been reported in history [15].

DPL is usually found in females of reproductive age, but cases have been described in both postmenopausal and foetal periods [16–19]. These extra-uterine smooth muscle proliferations have been associated with the altered hormonal environment of pregnancy, steroid-secreting ovarian tumours, or oral contraceptive use, and

potential hormonal responsiveness is suggested by the presence of estrogen and progesterone receptors [20–23].

Despite having the appearance of disseminated malignancy with multiple nodules over all the peritoneal surfaces simulating peritoneal carcinomatosis, most of these tumours pursue an indolent clinical course. The nodules may, in some instances, even regress partially or completely following withdrawal of the hormonal stimulus [24–27]. Alternatively, DPL may progress, recur, or undergo malignant transformation [28–31].

DPL is a rare presentation to a peritoneal surface malignancy unit but one that is challenging both for establishing a diagnosis and conferring the right treatment.

#### 10.4.2.2 Aetiology and Pathogenesis

The pelvic peritoneum especially in female has the ability to differentiate at any time into different epithelia and stroma giving rise to various pathological conditions both in childhood and adult life. This phenomenon rarely occurs in males as well and is referred to as Mullerianosis [32–34]. The presence of smooth muscle cells expressing estrogen and progesterone receptors in the subperitoneal regions has been demonstrated in biopsies performed for benign conditions like endometriosis and chronic pelvic pain [34, 35]. DPL is the nodular proliferation of smooth muscle cells in the subperitoneal mesenchyme. The expression of ER and PR distinguishes it from other retroperitoneal smooth muscle tumours that do not arise from this secondary Mullerian system and therefore do not express these receptors. There are several theories about the pathogenesis of DPL.

Parmley et al. proposed that DPL is a benign reparative process in which benign smooth muscle cells replace decidual cells, also called the "fibrosing deciduosis" theory. This theory is not accepted anymore [36, 37].

The second theory and more widely accepted theory is that DPL results from Mullerianosis, due to the transformation of pluripotent stem cells in the subperitoneal mesenchyme [24, 38]. This development can be triggered by various stimuli, the most common being the hormonal stimulus. DPL can be brought on and aggravated by high oestrogen states like pregnancy, long-term use of oral contraceptives or hormone replacement therapy, tamoxifen and oestrogen producing ovarian tumours [39–42]. It is also hypothesized that these tumours have an increased association with endometriosis which makes the mesenchymal cells more sensitive to hormonal stimuli [43].

DPL can also occur secondary to morcellation of uterine fibroids. Such procedures can lead to implantation and proliferation of benign smooth muscle cells from a uterine leiomyoma and are more common with laparoscopic morcellation [44, 45]. The pneumoperitoneum facilitates the distribution throughout the peritoneal cavity. Iatrogenic dissemination has been reported in literature [46, 47]. It is also possible that cells are shed from a leiomyoma as in the case of borderline ovarian tumours.

#### 10.4.2.3 Pathological Diagnosis

The pathologist should have this diagnosis in mind as a rare peritoneal tumour. If the clinician does not have this condition in mind, the clinical findings could be confused with lymphomas or ovarian cancer. Radiologically, these conditions can be distinguished by an experienced radiologist. A biopsy of the nodules is essential to establish the diagnosis even if the clinical and radiological picture is suggestive of this condition.

#### 10.4.2.4 Gross Features

The tumours are multiple, small and round varying in size from 1 to 5 cm though there is no cut off of size. Larger tumours should raise the suspicion of malignant transformation.

The tumours are homogenous in consistency and have an expanding growth pattern [48]. There is no extension along blood vessels or tissue septa. The presence of such tumours subperitoneally points towards a metaplastic origin rather than intraperitoneal dissemination [48]. Even when tumour deposits are on the bowel mesentery, they are subperitoneal, thus ruling out a hematogeneous or lymphatic spread. Other gross findings are similar to those seen in other leiomyomas.

#### 10.4.2.5 Microscopic Features

These tumours comprise of fusiform spindle cells arranged in compact fascicles oriented perpendicular to each other. The spindle cells are bland and have few or no mitosis [49]. The mitotic index is less than 3/10 HPF. High-grade features are absent. The nuclei are ovoid or elongated with rounded ends, and hyperchromasia and atypia are not seen. The nodules may contain fibroblasts, myofibroblasts, decidual cells, and, sporadically, endometrial stromal cells in addition to smooth muscle cells. Evaluation of the ER and PR status should be performed in all patients.

#### 10.4.2.6 Genetic Alterations

The molecular, genetic, and cytogenetic features of DPL suggest that each tumour deposit is monoclonal. Quade et al. analysed multiple nodules of DPL from four patients and found the same pattern of X chromosome inactivation in all patients, which was contrary to the expectation that the inactivation would be random and polyclonal [50]. It is uncertain at present whether DPL tumourlets are metastatic deposits of unicentric disease or multicentric deposits having inactivation of the same X chromosome [50]. Similar findings were reported by Miyake et al. [51]. DPL can arise from a single uterine leiomyoma.

#### 10.4.2.7 Progression to Malignant Disease

In patients whom DPL occurs without exogenous or endogenous oestrogen exposure, in those which it is not secondary to uterine leiomyomas, and those not expressing ER and PR, the risk of developing malignancy is high. Malignant degeneration can occur within months of the diagnosis [29–31]. In some cases, a low-grade leiomyosarcoma may be misdiagnosed as DPL or is already present in one of the nodules that was not biopsied. In one study, progression to sarcoma occured in 10% of the 49 cases [52].

Women who do not respond to antiestrogen therapy should undergo radical surgery to remove all the tumour nodules. An alternative would be to perform periodic laparoscopic evaluations with biopsy of suspicious nodules [52]. Such an approach has not been validated.

#### 10.4.2.8 Section Summary

DPL is a rare peritoneal tumour that should be kept in mind in patients having peritoneal deposits comprising of smooth muscle cells. Histopathological evaluation and immunohistochemistry can yield the diagnosis in most cases. Malignant change should be considered and ruled out in the submitted specimens.

### 10.4.3 Desmoplastic Small Round Cell Tumours

#### 10.4.3.1 Introduction

Desmoplastic small round cell tumour (DSRCT) is rare malignant tumour that mainly affects young boys and arises from the peritoneum lining the pelvis or other parts of the abdominal cavity. Uncommon sites of origin include the paratesticular region (arising from the membranes covering the testes), the pleura, posterior cranial fossa, bones and soft tissues, ovaries and parotid gland [53–57]. This entity was first described in 1989 by Gerald and Rosai and Ordóñez and Zirkin and less than 500 cases have been reported so far [58, 59].

These tumours comprise of small uniform round cells lying in variable amounts of fibroblastic stroma and are characterized by a nested growth pattern and marked desmoplastic reaction [58]. The tumours express epithelial, smooth muscle, and neural markers in differing combinations, making this tumour heterogeneous both morphologically and immune-phenotypically. Thus, they need to be distinguished from a large number of other tumours that can have a similar presentation and overlapping pathological features.

DSRCT has a highly aggressive clinical course with multiple local recurrences but few distant metastases.

Both clinical suspicion and pathological expertise are needed to establish the diagnosis correctly, not only because of the difference in prognosis between DSRCT and other round cell neoplasms but also developing therapies directed towards the molecular targets of the *EWSR1-WT1* fusion gene which is characteristic of this tumour. This is crucial as conventional chemotherapeutic

agents have only shown very limited efficacy in treating both local and metastatic disease [60].

#### 10.4.3.2 Origin of DSRCT

The cell or origin and pathogenesis of DSRCT are unknown. It is considered to be a distinct entity due to the presence of a specific chromosomal abnormality.

A specific translocation, t(11; 22) (p13; q12), is seen in almost all cases, juxtaposing the Ewing sarcoma (EWS) gene to the Wilms tumour (WT)-WT1 tumour suppressor gene [61–63].

DSRCT, WT, and EWS share a chimeric relationship with one another. DSRCT is caused by the translocation of the *EWSR1* gene from chromosome 22 to chromosome 11, resulting in a fusion product *EWSR1/WT1* [64]. *EWSR1-WT1* codes for a chimeric protein that acts as a novel transcription factor, which modulates transcription at *WT1* target sites and deregulates several target genes [65, 66].

There is heterogeneity of the *EWSR1-WT1* fusion transcripts generated, including differences in the combinations of *EWSR1* exons that fuse with *WT1* (including use of *EWSR1* exons 7, 8, and 9, as well as variant transcripts due to aberrant splicing resulting in loss of *EWSR1* exon 6 or *WT1* exon 9) [67, 68]. Fish and RT-PCR can be used to detect these mutations.

While *EWSR1-WT1* fusion was thought to be specific for DSRCT, it has also been described in a clinically indolent low-grade small round cell tumour of the cauda equina, which was composed of nests and cords of small round cells with some rosette-like structures, infrequent mitotic figures and a low Ki-67 proliferation index, and immunophenotypic features of smooth muscle differentiation, as well as focal CD99 and Neu-N expression [69]. It is therefore important to note that as the *EWSR1-WT1* fusion is no longer specific to DSRCT, the documentation of these fusion transcripts by RT-PCR needs to be correlated with the clinical and histopathologic findings for each case.

#### 10.4.3.3 Pathological Findings

There are several challenges for a pathologist. The diagnosis is often to be made on small biopsy samples which may neither be adequate nor representative of the entire tumour. In addition, there is heterogeneity between different areas of the same tumour and between different tumours in cellularity, architecture, stromal components, and immunoreactivity. Thus, a core biopsy may not bear the characteristic features of the tumour.

#### 10.4.3.4 Gross Features

In surgically resected specimens, DSRCT comprises of multiple firm white peritoneal nodules having varying amounts of necrosis of haemorrhage [70]. Nodules are often confluent producing larger masses. The cut surface is fleshy and may have cystic degeneration.

#### 10.4.3.5 Microscopy

Histologically, DSRCTs are composed of uniform small round cells with round or ovoid hyperchromatic or vesicular nuclei, inconspicuous nucleoli, minimal amounts of cytoplasm, and indistinct cell borders [70]. These cells are laid out in hypercellular nest and islands of varying size. Mitosis are numerous. Necrosis is seen often in the tumour islands and cells.

The islands and nests are separated by varying quantities of desmoplastic stroma that in some cases is inconspicuous [70]. The stroma is very vascular and contains spindle-shaped fibroblasts and smooth muscle actin (SMA)-positive myofibroblasts in collagenous or looser extracellular matrix [71].

There are several other cellular and architectural variations that are seen in these tumours. The cells themselves may have cytological atypia or giant and bizarre nuclei [72]. Alternatively, majority of the cells may have a spindle cell appearance or large cell variety that comprises of large epithelioid cells with anaplastic areas like metastatic carcinomas [73, 74]. Some neoplasms contain greater amounts of cytoplasm, which can show clearing or vacuolation. The architectural variations include rosette formation or tubule formation with intracytoplasmic eosinophilic inclusions composed of intermediate filament bundles giving the appearance of rhabdoid tumours. The tumour

can also have the appearance of lobular breast carcinoma comprising of glands or pseudoglands or single rows of cells [75].

#### 10.4.3.6 Immunohistochemistry

These tumours express epithelial, smooth muscles and neural antigens in varying combinations which means that not all antigens are expressed by each tumour. Moreover, it should be borne in mind that even the commonly expressed antigens like desmin and cytokeratin may be negative in a given biopsy specimen due to tumour heterogeneity [75]. The list of commonly expressed antigens is provided in Table 10.2. The smooth muscle antigens include desmin which is expressed by a large majority of the tumours. The epithelial antigens commonly expressed are cytokeratins AE1/AE3 and CAM5.2 and less commonly CK5/6 and CK 20 and epithelial membrane antigen (EMA) [76]. The commonly expressed neural markers are neuron-specific enolase (NSE) and CD57 and those expressed less commonly are chromogranin, synaptophysin, CD56, neurofilament protein, and S100 protein [73, 75]. The desmin expression is paranuclear and termed as paranuclear dot distribution which may also be seen with keratin. Expression of desmin and cytokeratins can be diffuse. The most sensitive myogenic marker is desmin and epithelial marker is CAM5.2 [76, 77].

Other markers expressed by these tumours include muscle-specific actin or α-SMA.2, the carboxy terminus of Wilms tumour (WT1) protein in nearly 90%, CD99 in a third to half of the cases [76–78]. INI1 is seen in the nuclei though it may be lost in tumours lacking a rhabdoid morphology [79, 80]. Some of the less commonly expressed markers are CD15, MOC-31, and Ber-EP4 and the uncommonly expressed ones are CD117, calretinin, and NB84 [77]. DSRCT may very occasionally express NKX2-2, a homeodomain transcription factor which is involved in neuroendocrine/glial differentiation and is a downstream target of the *EWSR1-FLI1* fusion oncogene, and which is a sensitive (but not wholly specific) immunohistochemical marker of Ewing sarcoma [81, 82]. Early myogenic regulatory nuclear transcription factors myogenin and MyoD1 are consistently not expressed. DSRCT is typically negative for CK5/6, CK20, glial fibrillary acidic protein, peripherin, CA19-9, thrombomodulin, α-fetoprotein, carcinoembryonic antigen, TAG-72 (B72.3), placental alkaline phosphatase, S100 protein, HMB45, and myoglobin [76].

**Table 10.2** Common immunohistochemical markers expressed in desmoplastic small round cell tumours

| Antibody | Nuclear (N)/cytoplasmic (C)/membranous (M) |
|---|---|
| *Epithelial* | |
| Pancytokeratin | C |
| CAM5.2 | C |
| Cytokeratin AE1/AE3 | C |
| Epithelial membrane antigen | C, M; sometimes dot distribution |
| WT1 (carboxy terminus) | N |
| INI1 | N |
| *Neuronal* | |
| Neuron-specific enolase | C |
| Chromogranin | C |
| Synaptophysin | C |
| CD56 | C, M |
| S100 protein | N, C |
| *Smooth muscle* | |
| Desmin | C, typically paranuclear dot distribution |
| Muscle-specific actin | C |
| CD 99 | C, M |

#### 10.4.3.7 Differential Diagnosis

Common differential diagnosis of DSRCT includes Ewing's sarcoma, rhabdomyosarcoma, carcinoma, and small cell mesothelioma.

##### Ewing's Sarcoma

Ewing's sarcoma can arise from the retroperitoneum and thus be mistaken for DSRCT. The cytological features of the two tumours are similar. The stroma of DSRCT may be less conspicuous and Ewing's sarcoma may have surrounding desmoplastic, fibrotic, or sclerosing hyalinized stroma [83]. The adamantinoma-like variant displays nests of cells with peripheral palisading and a desmoplastic response. CD99 expression

can be there in DSRCT but it is different from the diffuse membranous positivity that is seen in Ewing's. Ewing's sarcoma lacks WT1 expression [83]. Most of these tumours contain characteristic translocations involving EWSR1 and the ETS family of transcription factors, particularly those generating EWSR1-FLI1 and EWSR1-ERG fusions. They can express cytokeratins and rarely even desmin [83].

### Undifferentiated Round Cell Tumours

There is a recently discovered group of primitive round cell tumours that commonly have the *CIC-DUX4* gene fusion. This genetic abnormality is most commonly associated with these tumours that lack *EWSR1* rearrangements and occur in young males. The commonest site for such tumours is not the peritoneum but the limbs [84–86]. These tumours are usually strongly positive for WT1 and may occasionally be focally positive for desmin, cytokeratin, EMA, and S100 protein, but these may be associated with a slightly greater degree of morphologic heterogeneity and pleomorphism, greater prominence of nucleoli, spindle cell elements, and myxoid changes and generally have minimal or absent intervening collagen [82–84, 87]. Other gene rearrangements like *FOXO1*, *DDIT3*, or *SS18* that are commonly seen in small round cell tumours are also not seen in these.

Other uncommon undifferentiated tumours that are Ewing's like and can be confused with DSRCT are those harbouring *CIC-FOXO4* fusions and *BCOR-CCNB3* gene fusions [88, 89]. The former has a morphology similar to DSRCT, whereas the latter express bcl-2, CD117, CD99, the more specific marker being CCNB3 [90, 91].

### Synovial Sarcoma

Poorly differentiated synovial sarcomas (SS) can be morphologically confused with DSRCT. These tumours can present with bulky disease in the peritoneum or retroperitoneum. These tumours are composed of monotonous sheets or fascicles of relatively uniform ovoid to rounded cells with focal expression of cytokeratin and EMA [92–94]. Diffuse, moderate to strong nuclear expression of TLE1 is commonly expressed by these tumours, but is not associated with DSRCT [95, 96]. The distinguishing feature is the t(X; 18) (p11.2; q11.2) translocation, in which the *SS18* gene on chromosome 18 fuses with one of the *SSX* genes located on the X chromosome (usually *SSX1* or *SSX2*).

### Rhabdomyosarcomas

These can be confused morphologically and immumophenotypically with DSRCTs. The alveolar variety that has round cells and the embryonal variety with spindle or ovoid cells are the varieties that are commonly confused. The stroma in the former is separated from cell nests by septa which may be mistaken for the stroma of DSRCT. But these tumours are seldom confined to the abdominal cavity alone. Common origins are the limbs and head and neck. Desmin expression is typically diffuse and strong, and there is nuclear expression of myogenin and MyoD1 which are negative in DSRCT. The characteristic mutation is the *PAX3/7-FOXO1* gene fusion [97].

### Clear Cell Sarcoma

Clear cell sarcoma (CCS) and clear cell sarcoma-like tumour of the gastrointestinal tract (CCSLGT) are both associated with *EWSR1* rearrangements and can mimic DSRCT. Most CCS are associated with *EWSR1-ATF1* fusion transcripts and EWSR1-CREB1 in smaller numbers, whereas *CCSLGT* typically harbours *EWSR1-CREB1* or sometimes *EWSR1-ATF1* fusions; neither of these gene fusions are associated with DSRCT.

Both present with disseminated intra-abdominal disease and are seen in young adults [98, 99]. These are characteristically centred in the muscularis propria of the stomach or bowel, with secondary extension into the submucosa and subserosa. The characteristic differentiating feature is the abundant cytoplasm as opposed to the scanty cytoplasm in DSRCT. An additional distinctive feature is CD68-positive, multinucleated osteoclast-like giant cells [100–102]. CCSs diffusely express S100 protein and are positive for HMB45, MelanA, and MiTF. CCSLGTs express S100 protein but are negative for HMB45 and MelanA [98].

**Rare Differentials**

- *Neuroblastomas*: Though these occur in infancy and are rare in young adults, some DSRCTs express the neuroblastoma marker NB84 and can lead to confusion. The other findings like site of origin, secretion of catecholamines, and expression of NB84, neurofilament protein, chromogranin, synaptophysin, and CD56 are used to distinguish them from DSRCTs [60].
- *GIST*: Epithelioid GISTs occur in intra-abdominal, retroperitoneal or pelvic sites, and are usually composed of sheets of rounded cells that may be sometimes relatively small and uniform, resembling DSRCT [60]. Epithelioid GIST may show larger cells with clear cytoplasm, or more spindled cells in other areas, without intervening collagenous stroma. While this is patchier in epithelioid GIST, these still typically express CD117 and DOG1 at least focally, along with CD34 and sometimes h-caldesmon, none of which are typically positive in DSRCT [60]. Most GISTs also contain KIT or less frequently PDGFRA mutations that are not described in DSRCT.
- *Malignant peripheral nerve sheath tumour (MPNST)*: These tumours have a varied morphology and rarely have small cells but are distinguished by their clinical features. They occur more frequently in patients with neurofibromatosis type-1 and may be seen to originate from a pre-existing benign nerve sheath neoplasm (most often neurofibroma) or from a nerve. Histologically, MPNSTs show at least focal atypia, and tend to display at least focal areas of cells with "nerve sheath" morphology, with elongated, buckled, or tapered hyperchromatic nuclei [97].

#### 10.4.3.8 Section Summary

DSRCT is a rare and aggressive tumour and the diagnosis requires a high index of suspicion especially when the presentation is of less common histological features. A combination of morphology, immunohistochemistry, and mutation studies is required to correctly establish the diagnosis. There is little information about patterns of peritoneal dissemination but most patients present with disseminated disease.

### 10.4.4 Germ Cell Tumours of Ovary

#### 10.4.4.1 Introduction

Germ cell tumours are heterogeneous group of tumours reflecting ability of stem cells to differentiate into one or more lineages. Majority of these neoplasms originate at different stages of development from germ cells that colonize ovary.

Germ cell tumours constitute approximately 20% of all ovarian neoplasms. Most of them are seen in children and young adults, and approximately 95% are benign cystic teratomas. The dictum is that the younger the patient, the more likely the germ cell tumour will be malignant [103, 104]. Malignant ovarian germ cell tumours are predominantly unilateral, are diagnosed at an early stage, are chemosensitive, and have a high cure rate [105]. For early-stage disease, the cure rate approaches 100% and is approximately 75% for those with advanced tumours [105].

The peritoneum can be involved by these tumours though the exact incidence is not known. There are few reports of cytoreductive surgery being performed for these tumours.

#### 10.4.4.2 Classification

The simplest classification is to divide these tumours into two groups—dysgerminomas that are the most common type and a counterpart of testicular seminomas in male and non-dysgerminomatous tumours. The most common types of non-dysgerminomatous tumours are yolk sac tumours, immature teratomas, and mixed germ cell tumours, with embryonal carcinomas, nongestational choriocarcinomas, and polyembryomas being much less common [106].

#### 10.4.4.3 WHO Classification of Ovarian Germ Cell Tumours

In the most recent version of the WHO classification system, ovarian germ cell tumours are divided into three categories: primitive germ cell tumours, biphasic or triphasic teratoma, and monodermal teratoma (Table 10.3) [107]. Dysgerminomas and low-grade immature teratomas have a good prognosis. Endodermal sinus tumours, choriocarcinomas, and high-grade

**Table 10.3** WHO 2014 classification of ovarian germ cell tumours

| |
|---|
| *Primitive germ cell tumours* |
| Dysgerminoma, yolk sac tumour, embryonal carcinoma, polyembryoma, choriocarcinoma, mixed germ cell tumours |
| *Bi- or triphasic teratoma* |
| Immature teratoma, mature cystic teratoma with malignant transformation |
| *Monodermal teratoma* |
| Malignant struma ovarii, malignant carcinoid, other malignant lesions |

immature teratomas are the more aggressive tumours.

Morphologically, the different tumour types present in this group of ovarian tumours represent in a distorted, grotesque form various stages of embryonal development from early transient structures to mature adult tissues that in turn may also be capable of undergoing malignant change.

#### 10.4.4.4 Dysgerminoma

Dysgerminoma constitutes less than 1% of all ovarian tumours [108]. Most patients are young, majority occurs in less than 30 years of age. Around 5% tumours occurs in abnormal gonads like in gonadal dysgenesis or testicular feminization syndrome [109]. These tumours are bilateral in 15% cases and more common in right side ovary [110].

##### Gross Features

A dysgerminoma is usually large, encapsulated, with a smooth bosselated surface. Cut section is solid, uniform, lobular creamy white with focal haemorrhage (suggestive of trophoblastic component) and necrosis. Cystic change or macroscopic calcification may be seen.

##### Microscopic Features

Tumour cells are arranged in well-defined nests separated by fibrous strands infiltrated by lymphocytes. This infiltration by lymphocytes is characteristic of the tumour. Few cases may show cords, clumps, and pseudoglandular spaces. Cells are monotonous polygonal in shape with regular round nucleus with elongated nucleoli and abundant pale cytoplasm with distinct cell membrane. Focal necrosis, hyalinized blood vessels, and granulomatous reaction are noted [111, 112].

Immunohistochemically, the tumour cells are consistently reactive for placental alkaline phosphatase (PLAP) with membranous positivity for CD117, variably positive for keratin, and sometimes for GFAP and desmin but not for CD30 [113–115].

SALL4 is positive. SALL4 is a nuclear factor and a member of the family of SALL genes, which are also involved in totipotency and are expressed at an early stage of embryogenesis [116]. SALL4 is strongly expressed by dysgerminomas. However, since it is a pluripotency marker, it can show positivity in embryonal carcinoma, yolk sac tumours, and primitive areas of immature teratoma. Hence it is a good but broad marker for ovarian germ cell tumours [117].

OCT3/4 is particularly useful in demonstrating the primitive germ cell when the tissues are poorly fixed, and there are microcysts or marked fibrosis and inflammation. This marker differentiates these tumours from small cell tumours and struma ovarii. Furthermore, it is particularly useful in the identification of the primary tumour in distant metastases with occult primary [118].

The expression of cytokeratins can be focal or diffuse and does not exclude the diagnosis of dysgerminoma. It is also seen in those with trophoblastic differentiation [119, 120].

Like testicular seminoma, ovarian dysgerminoma may exhibit signs of early differentiation towards other types of germ cell elements. These include:

1. Scattered hCG-positive syncytiotrophoblastic cells, often in close proximity to blood vessels or to hemorrhagic foci. This change, seen in approximately 3% of all dysgerminomas, may be accompanied by serum elevation of hCG and tissue immunoreactivity for this marker.
2. Abortive yolk sac elements are associated with serum elevation of alpha-fetoprotein and tissue immunoreactivity for this marker.

Diagnostic problems occur with dysgerminoma often related to poor fixation and unusual growth patterns, and in both situations immuno-

histochemistry enables a correct identification of the proliferating germ cells. These findings should be considered together with classic histologic dysgerminoma features such as lymphocytic infiltrates.

Metastases of dysgerminoma occur more commonly in the contralateral ovary, retroperitoneal nodes, and peritoneal cavity.

#### 10.4.4.5 Yolk Sac Tumour (Endodermal Sinus Tumour)

These tumours are heterogeneous, primitive teratoid neoplasms differentiating into multiple endodermal structures. These tumours have epithelial patterns and are typically alpha-fetoprotein immunoreactive. Serum alpha-fetoprotein is invariably raised. In rare cases, a yolk sac tumour is found in the pelvis (in close proximity to the uterus), omentum, or mesentery, unattached to the ovary [121–123].

##### Gross Features

Average tumour dimension is 15 cm with smooth bosselated surface. Cut section is variegated, partially cystic and often contains large foci of haemorrhage and necrosis. Cysts at periphery show honeycomb-like appearance.

##### Microscopic Features

Appearance is extremely variable. There are reticular or microcystic areas formed by a loose meshwork lined by flat or cuboidal cells (Fig. 10.5), rounded or festooning pseudopapillary processes with central vessels (Schiller–Duval bodies), and solid areas. Mesenchyme may be in the form of spindle cells in a well-vascularized myxoid background and may contain heterologous elements such as skeletal muscle. Intracytoplasmic eosinophilic globules are PAS positive.

Histological variants are polyvesicular vitelline tumour (vesicular structures with eccentric constrictions surrounded by a dense spindle cell stroma), solid, parietal, glandular and hepatoid (composed of masses, nests, and broad bands of large polyhedral cells with occasional glandular formations and numerous hyaline bodies) variants [124–126]. Areas of luteinization may be responsible for virilization.

##### Immunoprofile

These tumours are immunoreactive for AFP, SALL4, glypican 3, and pankeratin, but not CK7. CD 30 is focally positive. OCT4 is typically negative.

Glypican 3 is a useful marker in hepatocellular carcinoma and is a complementary antibody for the diagnosis of yolk sac tumours. It is also secreted by the early secondary yolk sac and liver [127]. The staining is cytoplasmic, and less often membranous, and is almost, but not exactly, parallel to that of AFP [128, 129]. There are AFP-negative tumours that are GLP3 positive. The combination of the two markers is very specific for yolk sac tumours.

SALL4 has a consistently strong expression in the nuclei of yolk sac tumours regardless of their germ cell or somatic origin.

Different areas of endodermal differentiation from yolk sac tumours express their characteristic markers: hepatic areas are positive for hepatocyte paraffin antigen 1 (HepPar-1) and intestinal areas for CDX2 and villin. Glands differentiating into foregut express thyroid transcription factor 1 [124, 125, 130, 131].

#### 10.4.4.6 Embryonal Carcinoma and Polyembryoma

Embryonal carcinoma are tumours composed of epithelial cells resembling the embryonic disc and growing into different patterns like glandular, tubular, papillary, and solid. These tumours arise in the dysgenetic Y chromosome containing gonads and sometime 46 XX gonads.

Polyembryoma is rare tumour composed of embryoid bodies resembling early embryos.

##### Gross Features

The average size of the ovarian tumour is 17 cm. The external surface is smooth and glistening, and the cut surface is predominantly solid and variegated, with extensive areas of necrosis and haemorrhage.

##### Microscopic Features

Tumours are composed of solid sheets and nests of large primitive cells, occasionally forming papillae and abortive glandular structures (Fig. 10.5).

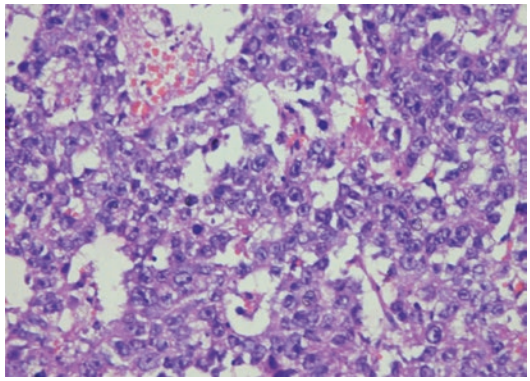

**Fig. 10.5** Histological features of an embryonal carcinoma

Syncytiotrophoblastic cells are scattered within tumour cells and are positive for beta HCG [132].

#### Immunoprofile

Embryonal carcinoma shows immunoreactivity for pan-keratin, CD30, OCT4, and SALL4 [133, 134].

All the tumours are cytokeratin positive. CD30 membrane expression remains one of the most reliable and accessible markers for embryonal carcinomas [135]. Anti-CD30 is an antibody against a surface glycoprotein corresponding to a cytokine receptor, and CD30 is a member of the superfamily of tumour necrosis factors. CD30 is expressed by many other tumours, including anaplastic lymphomas, and by Reed–Sternberg cells [136]. Some reactive inflammatory conditions may also show CD30-positive immunoblasts [137].

SOX2 is another nuclear transcription factor also involved in totipotency. It is also responsible for neuronal differentiation and useful, together with CD30, in the differentiation of solid areas of embryonal carcinoma with dysgerminoma. SOX2 and OCT3/4 co-expression in the papillary areas of embryonal carcinoma contrasts with these markers' negativity in Schiller–Duval sinuses of yolk sac tumours [138].

Glypican 3 shows patchy positivity in embryonal carcinoma, especially in areas of early endodermal differentiation, such as the organoid areas (primitive yolk sac endodermal cavities) of embryoid bodies in the rare polyembryoma [129].

### 10.4.4.7 Choriocarcinoma

Most choriocarcinomas involving the ovary represent metastases from uterine tumours. The exceedingly rare primary ovarian choriocarcinomas can develop from an ovarian pregnancy (gestational type, which is the most common) or as a form of germ cell neoplasm (nongestational) [139]. Most commonly it is associated with mixed germ cell tumours; however, if it is associated with the pure form, then a DNA test may be necessary to determine the paternal or maternal germ cell origin in case of a nongestational choriocarcinoma.

#### Gross Features

These tumours are large, haemorrhagic with large luteinized nodules or cysts.

#### Microscopic Features

Choriocarcinoma shows admixture of fenestrated, plexiform pattern or pseudopapillae of syncytiotrophoblastic elements rimmed by cytotrophoblasts in a necrotic and haemorrhagic background. Vascular invasion is common.

#### Immunoprofile

Tumour cells are positive for beta HCG. Trophoblast stains strongly for cytokeratin, human chorionic gonadotropin, α-inhibin, CD10, and GLP3 [129, 140]. Human placental lactogen can identify the intermediate (extravillous) trophoblastic component [141].

It is important distinguish the gestational from the nongestational type since later is associated with poor prognosis and needs aggressive management.

### 10.4.4.8 Immature Teratoma

Immature teratomas are teratomas composed of immature embryonal type of tissue. The embryonal elements are derived from all three germ layers. The tumour is largely composed of neuroepithelial elements, but mesodermal elements can also be seen.

#### Gross Features

These tumours are solid, solid with a microcystic component, or entirely cystic. The cut sur-

face is solid with few cystic spaces filled with mucinous or serous fluid or haemorrhagic fluid or hair. Solid areas are usually composed of neural tissue which is soft, fleshy, grey to pink with focal haemorrhage. Areas of bone and cartilage may be visible. Bilateral involvement is rare [142].

#### Microscopic Features

The immature embryonic type tissue varies from small foci to being the predominant component and is composed of neuroectodermal elements (Fig. 10.12). It consists of neuroepithelial rosettes, tubules and foci of mitotically active glia and occasionally glioblastoma multiforme or neuroblastoma. Immature elements of ectodermal or endodermal origin show immature cartilage and skeletal muscle [142].

Immature teratomas are graded from 1 to 3 based on amount of immature neural tissue as follows [143, 144].

- Grade 1: Rare foci of neural tissue less than one low power field in any slide.
- Grade 2: Foci of immature neural tissue from more than one to less than 4 low power field.
- Grade 3: Foci of more than 4 low power field.

#### Immunophenotype

Markers such as SOX2 and SALL4 are strongly expressed by immature neuroepithelium but are only weakly expressed or absent in well-differentiated neural areas [144].

### 10.4.4.9 Mature Teratoma

#### Gross Features

Mature teratomas are almost always dermoid cysts. Dermoid cysts are globular to ovoid, white or grey, and usually measure less than 15 cm in maximum diameter. Fifteen percent of the tumours are bilateral. The cut section shows yellow to brown sebaceous material and hair filled cysts. There are single or multiple polypoidal masses known as Rokitansky's protuberances, composed of fat. Teeth are seen in one-third cases [145].

#### Microscopic Features

Dermoid cysts are composed of adult-type tissue. Ectodermal derivatives predominantly comprise of epidermis, pilosebaceous structures, sweat glands, and neural tissue which is often glial. Mesodermal derivatives include smooth muscle, bone, cartilage, and fat. Endodermal component includes respiratory and gastrointestinal structures and thyroid tissue. One percent cases may show malignant change. Figure 10.6 shows two different malignancies arising from a germ cell tumour. The diagnosis of a mature teratoma was made on the gross appearance comprising of pilosebaceous structures and cartilage.

Gliomatosis peritonei (GP) is a rare condition whereby immature and, less often, mature teratomas become associated with a myriad of peritoneal nodular or miliary implants composed of mature glia. Despite its clinical stage III, its behaviour is benign, since mature glial cells are not aggressive and remain stable for long periods of time [146]. However, on rare occasions, GP can induce a florid vascular proliferation that may result in peritoneal haemorrhage and shock and can even develop a secondary malignant glial tumour [147].

Benign and malignant ovarian mucinous tumours associated with mature cystic teratomas may show massive mucin secretion, goblet cells, carcinoid-like patterns, pseudomyxoma ovarii and peritonei, and signet ring cells characteristic of a gastrointestinal phenotype. These tumours express markers like CDX2, HepPar-1, and villin, and also have the cytokeratin 7-negative/cytokeratin 20-positive profile [148]. All these features would point towards a teratoid origin for this mucinous component, which should be differentiated from a metastasis from a gastrointestinal primary tumour. Demonstration of teratomatous foci may be difficult in rare cases when they are small and escape sampling or become overgrown by the mucinous neoplasm [148]. A comparison of different immunophenotypic markers expression

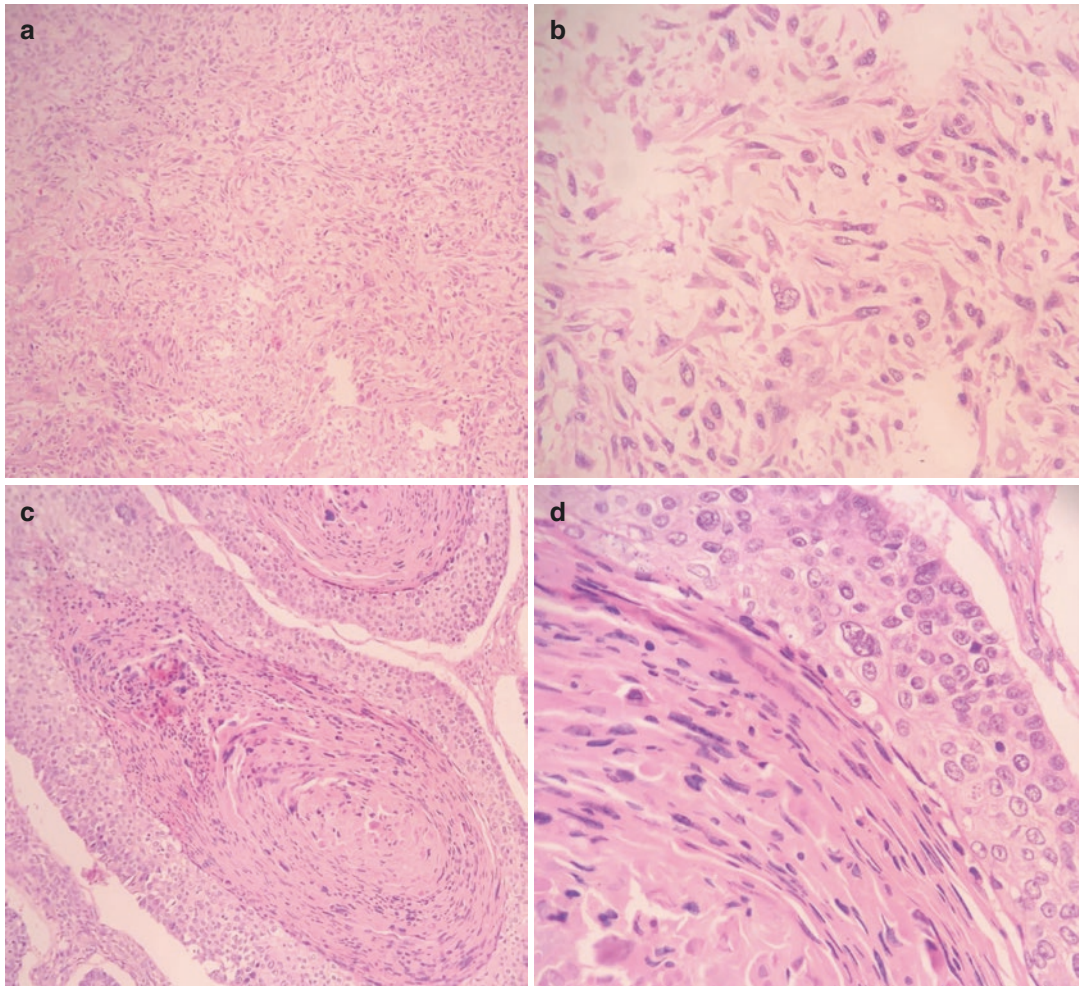


**Fig. 10.6** Mature teratoma giving rise to an osteosarcoma (**a**, **b**) and squamous cell carcinoma (**c**, **d**). The diagnosis of mature teratoma was made on the gross appearance of the tumour which showed elements of a mature teratoma. (**a**, **c**) At 10× magnification. (**b**, **d**) At 40× magnification

between different germ cell tumours is provided in Table 10.4.

#### 10.4.4.10 Monodermal Teratomas

**Struma Ovarii**

Struma ovarii is defined as an ovarian goitre which comprises either entirely or predominantly of thyroid tissue (>50%). This also includes cases of mature teratoma with less than 50% thyroid tissue but harbouring thyroid-associated malignancy [149].

Gross Features

The thyroid tissue is brown solid and gelatinous. It is also found in wall of mucinous cystadenomas or Brenner's tumour [149].

Microscopic Features

Struma may include normal thyroid tissue or adenoma of macrofollicular or microfollicular type. Areas of thyroiditis and colloid-filled follicles are seen. Malignant thyroid tumours are rare and most are papillary type. Criteria for defining capsular invasion in a follicular neoplasm are unclear [149].

**Table 10.4** Comparative immunohistochemical expression in malignant ovarian germ cell tumours (from Ref. [148] with permission)

| | Immunohistochemical markers | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Classic | | | | | Pluripotency | | | Somatic differentiation | | | |
| Tumour | PLAP | CD30 | AFP | GLP3 | D2-40 | OCT3/4 | SOX2 | SALL4 | Villin | CDX2 | HepPar-1 | TTF-1 |
| Dysgerminoma | + | – | – | – | + | + | – | + | – | – | – | – |
| Yolk sac tumour | +/– | – | + | + | +/– | – | – | + | +INT | +INT | +HEP | +FRG |
| Immature teratoma | – | – | – | – | – | – | – | + | – | – | – | – |
| | – | | +END | +NEP | +STR | | +NEP | | +INT | +END | | |
| Embryonal carcinoma | + | + | – | +Focal | +/– Apical | + | + | + | NA | – | – | – |
| Choriocarcinoma | – | – | – | – | – | – | – | – | – | – | – | – |
| | +SYNC | | | +SYNC | | | | | | | | |

Abbreviations: *AFP* α-fetoprotein, *END* endodermal; *FRG* foregut, *GLP3* glypican 3, *HEP* hepatic, *INT* intestinal, *NA* not available, *NEP* neuroepithelium, *PLAP* placental alkaline phosphatase, *STR* stroma, *SYNC* syncytiotrophoblast, *TTF1* thyroid transcription factor 1

#### Carcinoids

Ovarian carcinoid tumours are monodermal teratomas occurring in a pure form (15%) or combined with other teratomatous components (85%), such as a dermoid cyst or a struma ovarii [150]. They are the second most common form of monodermal teratoma. They can also be a component of mucinous and Brenner tumours. Carcinoid tumours of the ovary can be metastatic from gastrointestinal tumours. Primary ovarian carcinoids are mostly confined to a unilateral ovary and behave in an indolent fashion, whereas metastatic tumours tend to be aggressive and are associated with poor outcome. Ovarian carcinoids can be confused with other primary ovarian tumours, particularly Brenner tumours, granulosa cell tumours, and Sertoli or Sertoli–Leydig cell tumours [151–156]. Metastatic carcinoids more often show bilateral distribution, multinodular growth, extra-ovarian tumour nodules, lymphovascular invasion, and absence of teratomatous elements.

The majority of primary ovarian carcinoids occur in association with either cystic teratoma or ovarian epithelial tumours, in which enterochromaffin cells give rise to the carcinoids [157] However, a small portion of ovarian carcinoids are present in pure form. The origin of these tumours is still unclear, but enterochromaffin cells have been observed within normal ovarian tissue [158]. Studies have shown that insular carcinoids represent tumours of midgut derivation, while trabecular carcinoids are tumours of foregut and hindgut derivation, which may help to explain that only the insular subtype is associated with the carcinoid syndrome [153, 158, 159].

##### Gross Features

Pure carcinoids are divided into insular, trabecular, stromal, and mucinous subtypes. The insular subtype is the most common one followed by the strumal subtype [160]. Carcinoids are typically firm tan to yellow. Cysts filled with clear fluid may be present.

##### Microscopic Features

Seventy-five percent of the carcinoids are mixed with teratomatous elements. Insular carcinoids comprise of discrete cellular masses and nests. Trabecular carcinoids comprise long wavy ribbons of cells. The ribbons are composed of columnar cells with moderate amount of eosinophilic cytoplasm [160].

Carcinoid tumours are immunoreactive to neuroendocrine markers, such as chromogranin, synaptophysin, and CD56. Chromogranin and synaptophysin are specific discriminatory neuroendocrine markers for a carcinoid tumour [160].

Mucinous or goblet cell carcinoids resemble analogous appendiceal tumours.

Other variants include neuroectodermal tumours, pituitary adenomas, sebaceous and other rare varieties.

#### 10.4.4.11 Mixed Malignant Germ Cell Tumours

Around 8% germ ell tumours are mixed type. Combination of dysgerminoma with yolk sac tumour is most common. Choriocarcinomas are noted in 20% of cases [110].

#### Clinical Presentation

The diagnosis of a germ cell tumour may be an incidental finding or it may present with an ovarian mass with its associated symptoms. Rarely, peritoneal disease is present at diagnosis. There is no published literature on which types of germ cell tumours produce peritoneal metastases.

#### 10.4.4.12 Section Summary

Germ cell tumours of the ovary are uncommon. Some tumours have a propensity for peritoneal dissemination; however, there is very limited published literature on the same. In young female patients, with an ovarian tumour, this diagnosis should be kept in mind. The pathological features can overlap between the different histological types and immunohistochemistry markers including the newer pluripotency markers can be used to establish the diagnosis.

### 10.4.5 Granulosa Cell Tumours

#### 10.4.5.1 Introduction

Granulosa cell tumours of the ovary (GCTs) come under the category of sex cord-stromal tumours of the ovary. Ovarian sex cord-stromal

tumours are rare, comprising only 1.2% of all primary ovarian cancers [161]. Ovarian sex cord-stromal tumours are derived from ovarian matrix which consists of cells from embryonic sex cords and mesenchyme [161].

Granulosa cell tumours are the most common type of malignant ovarian sex cord-stromal tumour with the potential to metastasize and recur. They comprise 2–5% of all ovarian malignancies and 70% of malignant sex cord-stromal tumours [162].

There are two subtypes of GCTs, adult and juvenile. The adult subtype, which occurs most commonly in middle-aged and older women (median age, 50–54 years), comprises 95% of these neoplasms [163].

The juvenile type comprises 5% of all granulosa cell tumours. They typically develop before puberty, and are more common among children and young women [164, 165].

There are some specific molecular aberrations that are associated with each subtype. FOXL2 C124W mutation is commonly seen in the adult type, and G protein (gsp) mutations are commonly seen in the juvenile type [166]. The risk factors for granulosa cell tumours appear to be similar to epithelial ovarian carcinomas and these tumours are more common in women who are nonwhite, obese (body mass index >30), and nulliparous [166].

These tumours have a long natural history and tend to relapse even after more than 10 years of the initial diagnosis. Reported 5-year OS for patients with stage I disease ranges from 75 to 95%, with many studies demonstrating survival rates in excess of 90%; it drops to 55–75% for patients with stage II disease and 22–50% for patients with stage III/IV disease [167, 168].

Peritoneal cancer spread is more common in patients with recurrent adult GCT. Fotopoulou et al. analysed 45 patients with adult GCT, of which 18 had primary and 27 recurrent GCT [169]. Peritoneal involvement was more common in recurrent tumours as compared to primary tumours (52% vs 15.8%; $p = 0.027$), and involvement of the middle (48.1% vs 15.8%; $p = 0.05$) and upper abdomen (33.3% vs 0%; $p = 0.006$) was also higher in recurrent tumours [169].

#### 10.4.5.2 Clinical Features

Granulosa cell tumours have a propensity to remain localized and demonstrate an indolent growth. Therefore, many women present with asymptomatic, abdomino-pelvic masses which have reached large sizes before they are diagnosed. Peritoneal metastases are often present at the time of diagnosis.

Many patients present with abnormal uterine bleeding. This is attributed to hyperestrogenism on account of excessive production of oestrogen and/or progesterone by GCTs [170]. Increased production of oestrogen may also cause breast tenderness, postmenopausal bleeding, menstrual abnormalities, and, in children, sexual precocity, which may be the presenting complaints [170].

There is a well-documented association between granulosa cell tumours and endometrial neoplasms (complex endometrial hyperplasia and adenocarcinoma) [171]. For this reason, preoperative endometrial biopsy is suggested in all women with abnormal uterine bleeding, and an adnexal mass and/or postmenopausal bleeding with a thickened endometrium (≥5 mm) on ultrasound evaluation [171]. Non-specific symptoms or signs associated with these neoplasms include ascites, increasing abdominal girth, abdominal pain due to torsion, intra-tumoural haemorrhage, or tumour rupture and rarely hemoperitoneum.

Besides granulosa cell tumour, the differential diagnosis of a woman who presents with bilateral adnexal masses and abnormal vaginal bleeding should also include ovarian metastasis from a primary endometrial carcinoma, an endometrial metastasis from a primary ovarian malignant neoplasm, and separate primary ovarian and endometrial carcinomas.

The hormonal activity of granulosa cell tumours permits the use of a variety of serum tumour markers in the diagnostic evaluation. These markers include:

- Inhibin—Clinically, the most useful serum marker for granulosa cell tumours is inhibin, a peptide that is produced by the ovaries in response to follicle stimulating hormone and luteinizing hormone. Inhibin usually becomes undetectable after menopause, unless produced by certain ovarian tumours, mostly

mucinous epithelial ovarian carcinomas and granulosa cell tumours [172–174].

Inhibin exists as two different isoforms, inhibin A and inhibin B.

An elevated inhibin level in a premenopausal woman presenting with amenorrhea and infertility or in a postmenopausal woman is suggestive of the presence of a granulosa cell tumour, but is not specific. Epithelial ovarian tumours especially the mucinous variety may also secrete inhibin. Thus, inhibin is not specific for GCT. Inhibin levels fall to normal range around 1 week after tumour removal which implies that inhibin is secreted either by the tumour tissue or surrounding normal ovarian tissue [171].

Conversely, both inhibin A and B may be negative in patients with active granulosa cell tumours. Loss of inhibin expression may be associated with poor prognosis as these tumours are usually poorly differentiated tumours [175].

- Anti-Müllerian hormone (AMH, also known as Müllerian inhibiting substance [MIS])—AMH is produced by granulosa cells in the developing follicles and has emerged as a potential tumour marker for granulosa cell tumours. As with inhibin, AMH is typically undetectable in postmenopausal women. An elevated AMH level appears to be highly specific for ovarian granulosa cell tumours [176, 177]. One observational cohort study of 123 women has demonstrated that monitoring both AMH and inhibin was superior to inhibin alone in detecting macroscopic disease [178].
- Estradiol is one of the first markers identified in the serum of patients with granulosa cell tumours though it is not a sensitive marker. Approximately 30% of these neoplasms do not produce estradiol, perhaps related to the lack of theca cells, which produce androstenedione, a necessary precursor for estradiol synthesis [171].
- Androgens—In rare cases, granulosa cell tumours may produce androgens, such as testosterone, which may result in the patient presenting with virilization [171].

Diagnosis of a granulosa cell tumour is made by histology at the time of surgical excision. Preoperatively, a granulosa cell tumour should be suspected when a large adnexal mass presents with features of hyperestrogenism. Ultrasonographic findings (an echogenic, septated cystic or solid mass related to the ovary) are typically non-specific and so are the CT scan and MRI findings. Evaluation essentially consists of serum tumour markers which include CA125, serum inhibin A and B and an imaging such as CT scan/MRI of the abdomen and pelvis. For patients with peritoneal dissemination, the primary may be present or removed previously. The presentation of recurrent disease after a decade or more after initial treatment raises the suspicion of an alternative diagnosis and a biopsy or tumour markers can be performed to confirm the diagnosis.

#### 10.4.5.3 Pathology

##### Gross Features

These tumours are encapsulated with a smooth lobulated surface that is grey tan or yellow in colour. They may grow large in size or may the just few centimetres in size and non-palpable. On cut section there are mixed solid-cystic areas with straw coloured or mucoid fluid. Grossly they may resemble benign cystadenoma. Peritoneal deposits tend to be few in number and are often large in size.

##### Microscopic Features

On microscopy, the tumour comprises of small, bland, cuboidal, or polygonal cells arranged in various patterns. Well-differentiated GCTs have a microfollicular, macrofollicular, trabecular, insular, solid-tubular, or hollow tubular pattern (Figs. 10.7 and 10.8).

Characteristic of granulosa cell tumours is Carl–Exner bodies which are small follicle-like structures filled with acidophilic material [179]. The poorly differentiated forms (39%) may have a watered silk appearance in which cells are arranged in an undulating parallel pattern or a zigzag (gyriform) pattern or diffuse (sarcomatoid) pattern characterized by a monotonous

appearance [165]. GCTs with a diffuse pattern can be mistaken for a poorly differentiated carcinoma on frozen section. The nuclear pattern is diagnostic.

Cells may be luteinized (plump with ample cytoplasm), or may have a theca cell component. Cells may also have coffee bean nuclei with folds or grooves, or floret giant cells, indicative of degeneration [164]. Rarely focal hepatic cell differentiation (large cells with abundant eosinophilic, slightly granular cytoplasm) may be found.

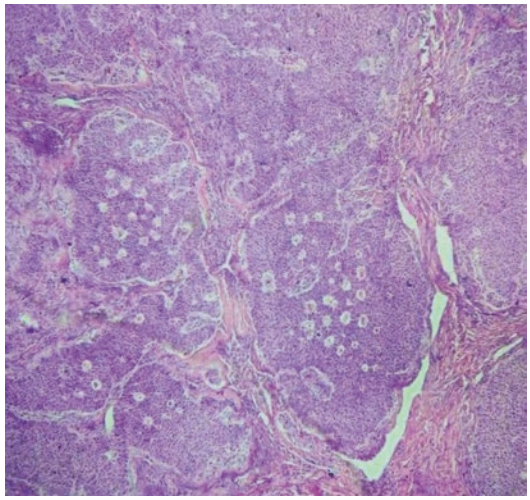

**Fig. 10.7** Typical histological features of a granulosa cell tumour of the ovary. Numerous Carl–Exner bodies are seen

On microscopic examination, two characteristics distinguish juvenile from adult granulosa cell tumours: the nuclei of juvenile granulosa cell tumours are rounded, hyperchromatic, and ungrooved with moderate to abundant eosinophilic or vacuolated cytoplasm, and the theca cell component is luteinized [171].

Occasional tumours can show mesenchymal differentiation into smooth muscle and osteoid components. The spindle cells component could be another presentation of this tumour (pseudosarcomatous pattern) (Figs. 10.9 and 10.10) or it might represent a fibrothecomatous component, taking into consideration that most granulosa cell tumours have at least some theca cells focally [180, 181].

### Immunohistochemistry

Immunohistochemistry helps in consolidating the diagnosis especially in the absence of classical features or above-mentioned histological variants.

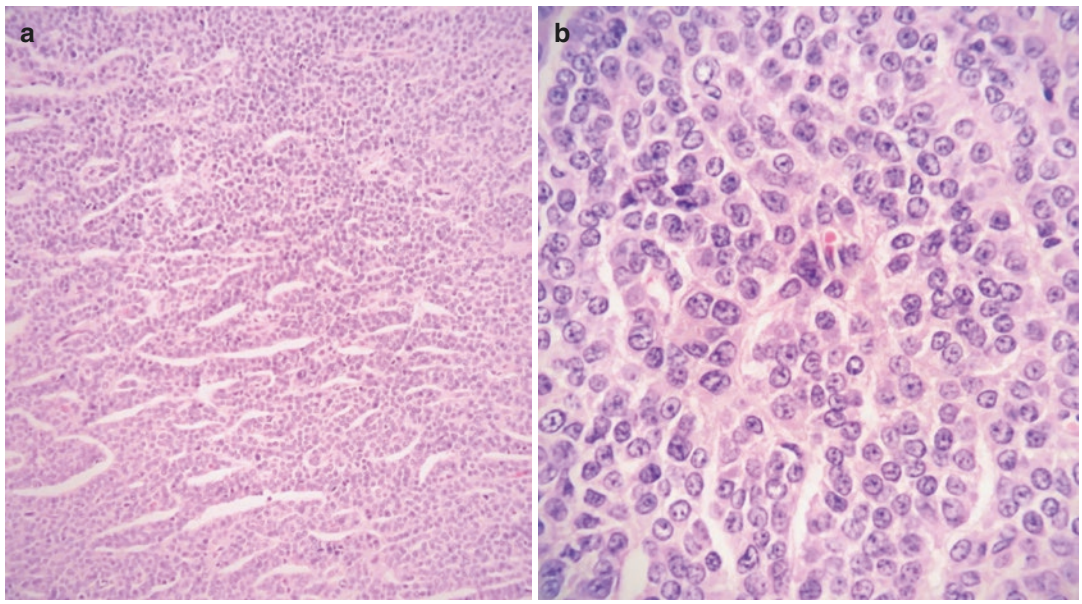


**Fig. 10.8** Peritoneal deposits in a recurrent granulosa cell tumour. (**a**) At 10× magnification. (**b**) At 40× magnification

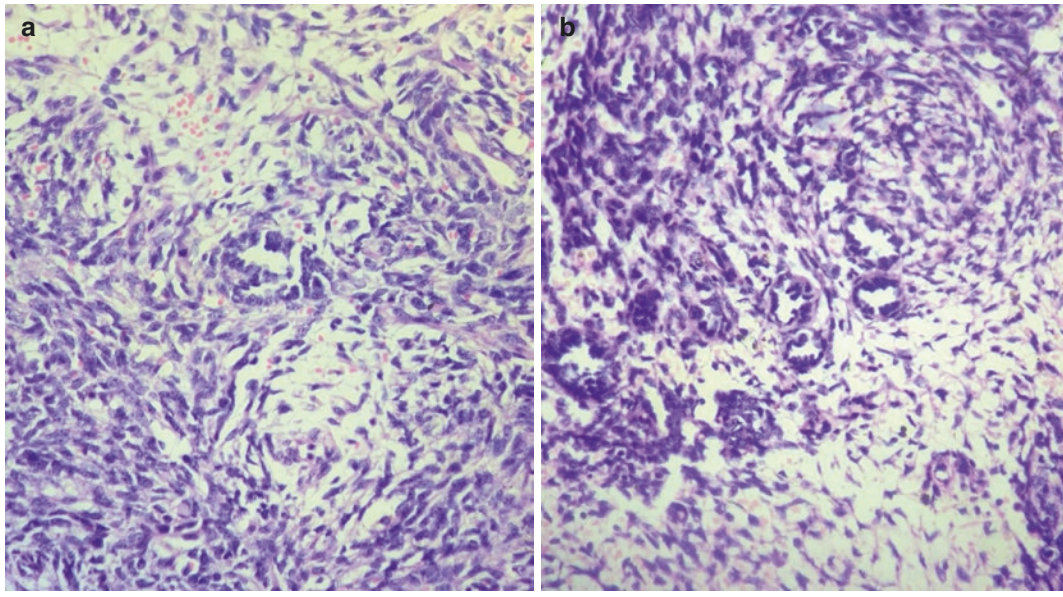


**Fig. 10.9** (**a**, **b**) Atypical presentation of a granulosa cell tumour with sarcomatous change. The immunohistochemistry profile favoured a granulosa cell tumour with diffuse inhibin expression and also marked elevation of serum inhibin

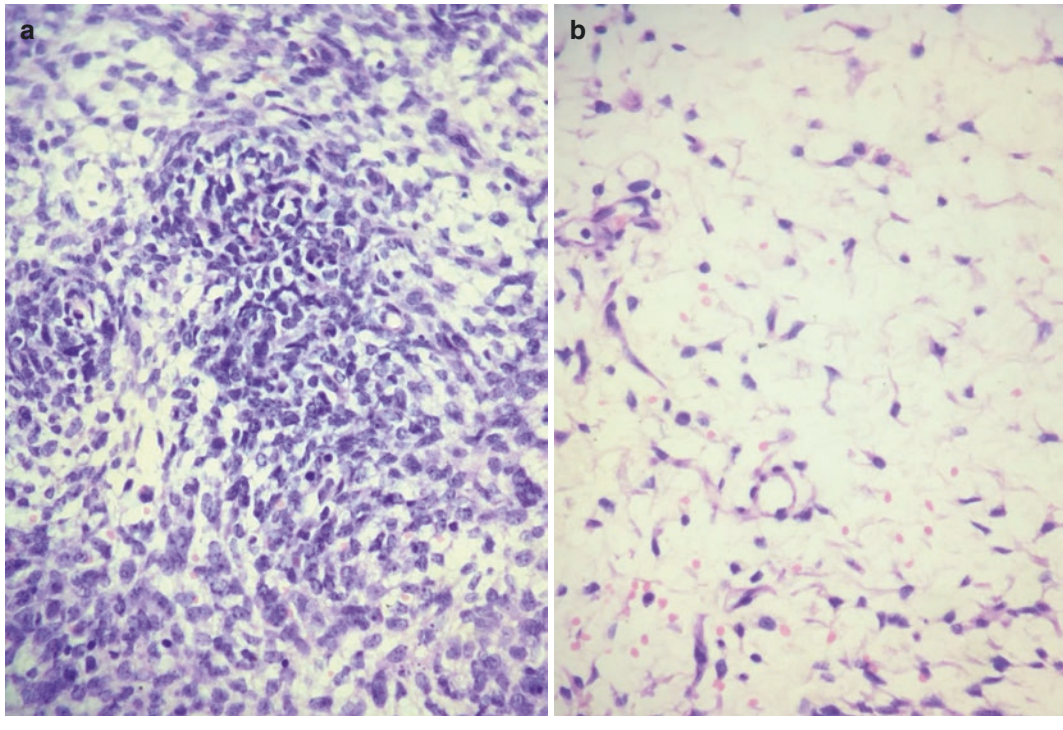


**Fig. 10.10** High power images of the same patient showing sarcomatous cells (**a**) in immature oedematous mesenchyme (**b**)

Granulosa cell tumours stain positive for inhibin alpha (Fig. 10.13), vimentin, calretinin, CD99, smooth muscle actin, desmoplakin, S100 (in 50% of cases), keratin (dot-like in 30–50%, primarily low molecular weight), focal positivity for anti-Müllerian hormone and desmin positivity in 35% of cases. Silver stains demonstrate reticulin surrounding clusters of cells [182–187]. Special attention should be paid when low-molecular-weight keratin is used as part of a panel differentiating granulosa cell tumours from carcinomas, as a significant proportion of the former are positive [188]. Cells are negative for EMA [183, 186]. Juvenile granulosa cell tumours are positive for inhibin, calretinin, FOXL2, and SF1. WT-1, CD10, S-100, CD56, and smooth muscle actin are also frequently positive, but EMA is typically negative (with rare exceptions) [189, 190]. It should be borne in mind that AE1/AE3 and less commonly CAM5.2 may be positive [189, 190].

IHC plays an important role as granulosa cell tumours may closely resemble endometrioid adenocarcinoma and mixed Mullerian tumour (MMT) of the ovary. Endometrioid adenocarcinomas are strongly positive for CD10 with patchy vimentin positivity [188, 191, 192]. On the other hand, MMTs, because of their presence of mesenchymal and epithelial elements, show EMA, CEA, and vimentin positivity [191–193].

#### Genomic Changes

The cytogenetic changes in GCT differ from those in epithelial ovarian cancer, which is characterized by gains at 3q, 8q, and 20q, often displaying high level amplification. In particular, gains of chromosome 14 and loss of chromosome 22 seen in GCTs are rarely found in ovarian carcinomas [194].

On chromosome 14, a number of important genes have been identified that are involved in the regulation of cell proliferation and cell death, such as *FOS,* the major component of the activator protein-1 (*AP-1*) transcription factor complex; *BCL2L2* (BCL2-like2), a regulator molecule of apoptotic cell death; and *TGF3*, which controls cell proliferation and differentiation [195]. Moreover, cytogenetic studies have showed trisomy 12 to be a frequent abnormality in granulosa cell tumours. Several important genes have been identified on chromosome 12 (e.g. *KRAS 2, KRAG, MDM2*); however, it is not clear whether they play a role in GCT. p53 protein accumulation is found in 95% of GCT studied; however, correlation with tumour stage, quantity, or type of chromosomal aberrations and survival has not established [196]. Studies have shown that p53 may not have a major role to play as it does in epithelial ovarian carcinoma.

#### 10.4.5.4 Section Summary

Granulosa cell tumours are rare and often associated with peritoneal disease in the primary and recurrent setting. A combination of histopathological features and immunohistochemistry can establish the diagnosis in most situations. Serum markers like inhibin A and B are useful adjuncts, especially in atypical cases.

### 10.4.6 Peritoneal Sarcomas

Peritoneal sarcomas are usually secondary to primary tumours arising at different sites. After the lungs and bones, the peritoneum is a common site of spread from soft tissue sarcomas. Nearly 30% of the patients with a sarcoma will have intra-abdominal disease which is either peritoneal dissemination or locoregional recurrence [15]. The commonest sarcomas metastasizing to the peritoneum are retroperitoneal liposarcomas, uterine leiomyosarcomas, and low- and high-grade endometrial stromal sarcomas [15]. Low-grade uterine sarcoma (LGUS) can arise from the ovaries and the peritoneum itself, usually, in the setting of endometriosis [197]. The omentum can be the site of origin in rare scenarios for a variety of soft tissue tumours, the commonest being leiomyosarcoma [198]. Diffuse peritoneal leiomyomatosis (DPL) is a rare tumour that arises de novo from the peritoneum. It is usually caused due to a hormonal stimulus and resolves after the stimulus is removed [37]. If left untreated, leiomyosarcoma can arise in the setting of DPL. Gastrointestinal stromal tumours can arise from the omentum or peritoneum itself [199].

The pathological aspects of diagnosis of peritoneal sarcomas are discussed here.

#### 10.4.6.1 Endometrial Stromal Sarcoma

Endometrial stromal sarcoma is a rare malignancy that constitutes <1% of all uterine malignancies and 15% of the uterine sarcomas [200]. ESS can spread to the peritoneum or in very rare cases arise de novo from the peritoneum.

##### Pathological Features

Endometrial stromal sarcoma (ESS) has been divided into low and high grades in the world health organization (WHO) 2014 classification [197]. Each of these is characterized by a specific chromosomal mutation. High-grade sarcomas are characterized by the presence of a recurrent chromosomal translocation—t(10;17) (q22;p13), resulting in *YWHAE-NUTM2A* or *YWHAE-NUTM2B* genetic fusions (collectively referred to as *YWHAE-NUTM2*) [201].

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

###### Low-Grade ESS

Low-grade sarcomas are characterized by 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 (Fig. 10.11) [202–205]. Tumour cells are concentrically arranged around the vascular channels and mitotic activity is usually low (usually <5 Mitotic Figures F/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 [206–208]. This patchy, cytoplasmic, and membranous pattern is seen in stromal sarcomas that mimic ESS, including 50% of hemangiopericytomas and over 60% of solitary fibrous tumours [13]. CD34 expression, however, is nearly universal in hemangiopericytomas and solitary fibrous tumours, but is not seen in endometrial stromal sarcoma [209]. Therefore, one should test for CD10 expression along with ER and CD34. Even if only focally CD10+, essentially all stromal sarcomas express ER, and almost none expresses CD34. Solitary fibrous tumours and hemangiopericytomas, in contrast, express CD34 but not ER. PR expression is not used in this setting as it can be expressed in both stromal sarcomas and in nearly 50% of solitary fibrous tumours and hemangiopericytomas [209].

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* rearrangement showing smooth muscle differentiation. The ki-67 proliferation index (<5%) is low and nuclear cyclin D1 expression is typically weak and focal (<5%) [210]. There are also reports of KIT expression in a subset of low-grade ESSs, though the staining tends to be weak and very focal [211–214]. DOG1 expression is consistently absent in low-grade ESS [215].

###### High-Grade ESS

High-grade ESS on the other hand has characteristic diffusely positive staining for cyclin D1 and is negative for CD10, ER and PR [197]. There is strong cytoplasmic c-KIT staining. Areas of low-grade ESS are seen in YWHAE-NUTM2 ESS. The term undifferentiated uterine sarcoma is not used for endometrial stromal sarcomas as it can arise from smooth muscles as well [197].

###### Undifferentiated Uterine Sarcoma

Undifferentiated uterine sarcoma includes not just endometrial stromal sarcomas but sarcomas arising from other areas of the uterus as well [216]. The characteristic features are severe nuclear atypia, a high mitotic rate, and tumour necrosis. The majority of UUS show prominent nuclear pleomorphism, though a subset of cases displays more uniform albeit high-grade nuclear features

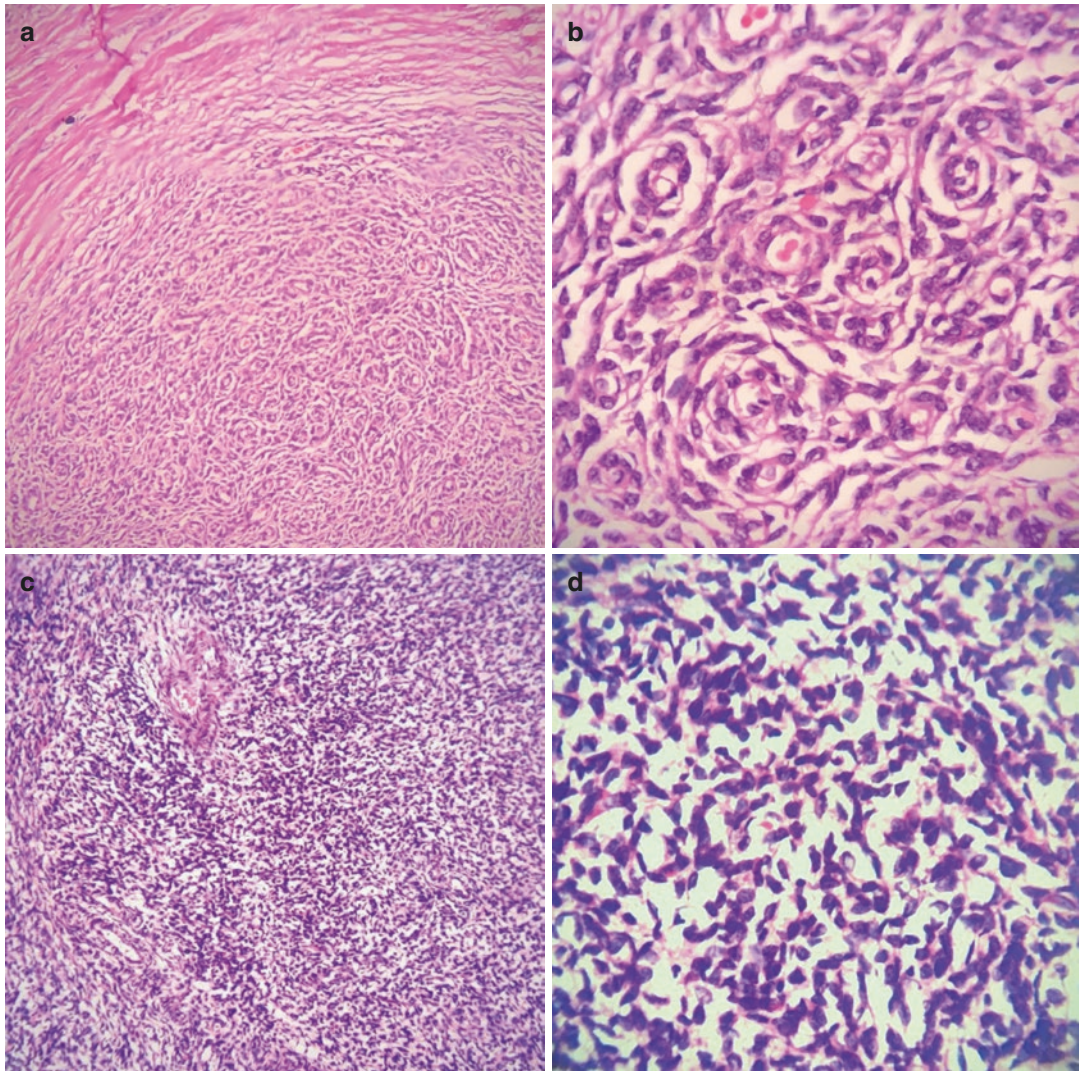


**Fig. 10.11** (**a**, **b**) Histological features of peritoneal deposits from low-grade endometrial stromal sarcoma. (**c**, **d**) Area of high-grade morphology in the same patient. A final distinction can be made only on immunohistochemistry. (**a**, **c**) At 10× magnification. (**b**, **d**) At 40× magnification

[197]. Varying amount of rhabdoid morphology may be present. Undifferentiated uterine sarcomas lack specific lines of mesenchymal differentiation and thus, it is a diagnosis of exclusion [197]. And thus, other sarcomas like 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 should be excluded [197].

UUS is often positive for CD10. CD10 itself is not a marker for stromal differentiation. The presence of focal staining for smooth muscle actin may be present but also raises the suspicion for leiomyosarcoma or malignant PEComa [197]. 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 [197].

#### Extra-uterine Endometrial Stromal Sarcoma

The exact cause of extra-uterine ESS is not known but majority of them arise in the setting of endometriosis [217, 218]. Malignant transformation of implants in endometriosis is known. In majority of the cases this occurs in the ovarian implants, giving rise to a primary ovarian tumour [219]. Malignant transformation of extra-ovarian implants is rare. And even more rare is a sarcomatous transformation. The commonest sites of extra-uterine ESS are the ovaries, rectum, vagina, and pelvic peritoneum [219].

The pathological findings are of ESS arising in the background of endometriosis. Many of these tumours are low-grade ESS and show stromal proliferation without glands. Changes peculiar to endometriosis like fibrosis and cyst formation are common. The immunohistochemistry profile is similar to the other ESS but the specific genetic mutations may not be present. Peritoneal ESS needs to be distinguished from other common peritoneal sarcomas and GIST [220]. Significant overlap in staining for CD10 and desmin can occur in ESS and smooth muscle tumours; hence, a panel that includes additional smooth muscle markers, such as h-caldesmon, calponin, or SMMS-1, which are usually negative in ESS, should be used [24]. Gastrointestinal stromal tumour can be excluded with the combined use of c-Kit, CD34, and DOG1 stains [220].

Most studies show that extra-uterine ESSs are usually of low grade and have an indolent behaviour [221, 222]. Involvement of single versus multiple extra-uterine sites does not have an impact on the outcomes. Clinical and histologic features, such as tumour size, tumour location, mitotic index, and vascular invasion, do not appear to correlate with clinical outcome [217, 221, 222]. The only histologic feature that suggests a worse prognosis is synchronous or metachronous development of high-grade cytologic features (dedifferentiation) [217, 223]. However, literature on dedifferentiated tumours arising from low-grade tumours is sparse.

Favourable outcomes may be obtained with CRS with or without HIPEC for low-grade ESS [224]. There is no evidence to support performing surgery for high-grade ESS with peritoneal dissemination. These are aggressive tumours that are difficult to control with systemic chemotherapy.

### 10.4.6.2 Uterine Leiomyosarcomas

Uterine leiomyosarcomas are the commonest uterine sarcomas though they constitute only 1% of all the uterine neoplasms. There are two hypothesis of the origin of these tumours. The first is that these tumours arise de novo and this is supported by the fact that only 1 in 800 uterine smooth muscle tumours is malignant [225]. Recent studies have shown that the microRNA expression profiles of leiomyomas and leiomyosarcomas are different. The second hypothesis is that these tumours arise from pre-existing leiomyomas and are supported by the presence of leiomyoma-like areas in LMS (specifically, cellular and symplastic areas), with discovery of additional genetic aberrations in the sarcoma, differences in immunohistochemical profile between the benign and sarcomatous areas within the same tumour, and demonstration of identical pattern of X chromosome inactivation between benign and malignant tumours [226, 227]. This suggests that a minority of tumours indeed originate from pre-existing leiomyomas [228–230].

#### Gross Features

When arising de novo, these tumours are solitary reaching up to 10 cm in size or more and have an infiltrative margin [225]. The tumour lacks a whorled appearance but is fleshy due to areas of haemorrhage and necrosis. When it arises in the setting of leiomyomatosis, the LMS is the largest of the tumours. Solitary tumours usually always arise in the uterine wall though few can arise from the cervix [225].

#### Microscopic Features

Most clinically malignant tumours usually show a combination of diffuse moderate-to-severe nuclear atypia, greater than10 mitotic figures (MF) per 10 high power fields (HPF), and pres-

ence of (coagulative) tumour-cell necrosis**.** Tumours that have any two of these features are also sufficient to be diagnosed as LMS [231].

Tumour-cell necrosis, the "bad necrosis", is a common finding in 80% of LMS, characterized by an abrupt transition from viable to necrotic cells. Infarct-type (or hyaline) necrosis, the "good necrosis", may be seen in benign leiomyomas and LMS and is characterized by the presence of a zone of reparative granulation tissue separating the viable and devitalized tissue [231]. Tumours that appear histologically benign, with only tumour-cell necrosis, can occasionally be clinically malignant. It is therefore important to determine the type of necrosis though this may not always be possible in early cases that have minimal granulation tissue [232, 233]. In these difficult situations, when there is tumour without atypia or a high mitotic count, it is classified as a smooth muscle tumour of uncertain malignant potential (STUMP).

LMS have four histological subtypes, spindle cell variety, that is the commonest and followed by the epithelioid, myxoid, and rare variants. Epithelioid LMS do not differ grossly from those of the spindle cell subtype. Histologically, they are defined by the presence of rounded or polygonal cells that have a microscopic appearance of "epithelial cells" in at least 50% of the tumour [234].

### Immunohistochemistry

Diagnosis of most LMS can usually be made by light microscopic examination. Immunohistochemical study is sometimes necessary to confirm the smooth muscle nature.

In tumours with uncertain or poor differentiation, or when distinction from other neoplasms is required, a combination of markers, including desmin, smooth muscle actin, and h-caldesmon, may be used to confirm the smooth muscle origin [235]. The epithelioid variety may express cytokeratins in nearly a fourth of the patients and may not express myogenic markers like desmin. Histone deacetylase 8 and myocardin are more specific for this variant as compared to desmin or h-caldesmon [236–239].

The overexpression of p16 was identified in 86.7%, 86%, and 51% of uterine LMS in three studies [240–242]. The frequency of overexpression of p53 protein in uterine LMS has been variable and has ranged from 13% to 56.5% [240–244].

Estrogen, progesterone, and androgen receptors are expressed in about 30–40% of LMS [245–247]. Even though some LMS show immunoreactivity for CD117 (C-KIT), there is no underlying KIT oncogenic mutation or KIT phosphorylation and targeted treatment with imatinib is ineffective [248–250].

Histologic grading of LMS nonetheless is still controversial, with no universally accepted grading system. Tumour size greater than 5 cm was a major prognostic indicator in two studies [251, 252]. This last parameter has now been incorporated into the 2009 International Federation of Gynecology and Obstetrics staging for uterine LMS [253].

ULMS have a high propensity for hematogeneous spread most commonly to the lungs. The peritoneum is the next common site of metastases [254].

### Clinical Presentation

Uterine sarcomas can arise in patients who have undergone morcellation of a fibroid as an early tumour may be missed in some of these patients, and the surgical procedure can lead to peritoneal tumour dissemination. Current estimates are that approximately 1 in 350 patients will be exposed to this risk of widespread sarcomatosis [254]. Sugarbaker et al. based on their experience proposed that a prophylactic CRS and HIPEC should be performed in patients who have undergone morcellation of a ULMS since most of these patients will eventually develop sarcomatosis [255]. A similar strategy has been recommended by other investigators as well [256].

### Extra-uterine Leiomyosarcomas and Rare Omental Tumours

Leiomyosarcomas arising from the greater omentum have been reported [257–259]. The embryologic origin of these tumours is variable because of the different tissues that can be found in the omentum, namely vessels, lymphatics, and fat [260].

Reported primary tumours of the omentum include leiomyosarcoma, fibrosarcoma, hemangiopericytoma, spindle cell sarcoma, liposarcoma, leiomyoma, lipoma, desmoid tumour, fibroma, mesothelioma, and others [261–265]. They derive from different elements in the greater omentum which is composed mainly of fat but contains various tissues—such as vessels and lymphatics.

In a review by Branes et al., the median age of patients with leiomyosarcoma of the omentum in the cases published in the literature was 51 years [260]. The tumour was slightly more common among males (16 patients, 59.2%) and females (11 patients, 40.7%).

Pathological findings are the same as that in uterine leiomyosarcomas. The epithelioid variety has been seen more commonly in the omentum. Like the uterine LMS, these tumours can express CD117 without an underlying genetic mutation and hence, targeted therapies specific for this mutation are ineffective.

### 10.4.6.3 Carcinosarcoma

Carcinosarcomas are a group of rare and aggressive malignancies that comprise of a mixture or carcinomatous and sarcomatous elements and can arise from various primary sites.

These tumours were first described by Virchow in 1863. Several hypotheses have been considered to explain their occurrence. Initially, it was believed that one cell type gave rise to the other, it is the carcinomatous element that gives rise to the sarcomatous counterpart. The more modern theory is that both arise from the same precursor [266–269].

The phenomenon of epithelial-mesenchymal transition (EMT) has been used to explain the pathogenesis of these tumours. Carcinomas in the process of developing metastases lose signs of epithelial differentiation and give rise to stem cell-like precursors which can be the source of the sarcomatous component [270]. Carcinosarcomas, thus, retain the features of carcinomas as demonstrated by the expression of keratins and also have the features of sarcomas demonstrated by the expression of vimentin. The existence of biphasic tumours suggests a fluid process of differentiation that can be modified or selected for during a cancer's evolution, which one only observes as a snapshot when a tumour is biopsied or removed [271].

Carcinosarcomas have also been called a stable disruption of the epithelial-mesenchymal transition. The common sites of origin of carcinosarcomas are the uterus and adnexae, lung breast, and head and neck sites.

In the Mullerian system, the uterus is the commonest site for development of these tumours, followed by the vagina, cervix, and adnexa, and in very rare cases, they arise de novo from the peritoneum [272–276].

Uterine and ovarian carcinosarcomas have a propensity for peritoneal spread and are discussed in this section.

#### Uterine Carcinosarcomas

Uterine carcinosarcomas are rare tumours that account for less than 5% of all uterine malignancies [277].

##### Definition and Aetiopathogenesis

These tumours are grouped together as (malignant) mixed Müllerian tumours (MMMTs), which encompass carcinosarcomas, adenosarcomas, and carcinofibromas [278].

The nomenclature depends on the sarcomatous component that can be homologous or heterologous. Homologous sarcomatous components arise from typical uterine cell types (i.e. leiomyosarcoma, endometrial stromal sarcoma, or undifferentiated pleomorphic sarcoma). Heterologous elements include cartilage (chondrosarcoma), skeletal muscle (rhabdomyosarcoma), or sometimes osteosarcoma, liposarcoma, or other entities [278].

Risk factors for the development of carcinosarcoma are similar to those of endometrial carcinoma and include nulliparity, advanced age, obesity, exposure to exogenous oestrogens, and long-term use of tamoxifen [279, 280].

There are three theories that explain the pathogenesis of these tumours.

1. The collision theory suggests that the two components have separate points of origin prior to their "colliding" together to form a single tumour. This mode of development is seen only

in a minority of the patients and is explained by the p53 staining which is similar in the two elements in nearly 80% of the patients and differs in the remaining 20% [281].

2. The combination theory postulates that a common stem cell precursor undergoes bidirectional differentiation that results in the creation of the two histological types.
3. In conversion theory, a single epithelial component is hypothesized to undergo metaplastic differentiation from which the mesenchymal component is derived.

Both the second and third theories suggest a monoclonal origin [282]. Though epithelial markers are expressed in more than 60% of the sarcomatous component, mesenchymal markers are seldom expressed in the carcinomatous component which supports the conversion theory.

Clinical, pathological, and molecular observations suggest that these neoplasms are derived from the Müllerian epithelium's single stem cells, with metaplasia or dedifferentiation resulting in the sarcomatous elements [283]. Cell cultures, ultrastructural studies, and immunohistochemical analyses all support the conversion theory for the tumourigenesis of this neoplasm [284].

### Clinical Presentation

The clinical presentation is often non-specific and similar to that of other pelvic malignancies. The patient may be asymptomatic or have anaemia. The typical symptoms are vaginal bleeding, bloody or watery discharge, and abdominal pain. There may be a polypoidal mass in the uterus that may or may not protrude through the cervix or pyometra leading to uterine enlargement in over 50% [285, 286].

The "symptom triad" indicative of carcinosarcoma rather than endometrial adenocarcinoma includes pain, severe vaginal bleeding, and the passage of necrotic tissue per vaginum [287].

### Pathological Features

#### Gross Features

The characteristic finding is a polypoidal mass arising from the posterior wall [286]. It can grow to fill the entire uterus and cause it to enlarge, protrude through the cervix, and extend beyond the uterus [285]. When the sarcomatous component is more, the tumours are bulkier and fleshier [288, 289]. Cut surface shows areas of haemorrhage and necrosis [290]. Areas of osseous or cartilaginous differentiation may be seen [288].

#### Microscopic Features

Microscopically, carcinomatous and sarcomatous components may be intermittently mixed or be seen as two distinct components [291]. The epithelial component is often a high-grade carcinoma such as papillary serous carcinoma (66%) or endometrioid carcinoma (42%) [211]. Other uncommon histological subtypes include squamous cell carcinoma, basaloid squamous carcinoma, adenocarcinoma, adenosquamous carcinoma, adenobasal carcinoma, adenocystic carcinoma, or an undifferentiated carcinoma [273]. These tumours have more high-grade features like areas of marked pleomorphism, bizarre cells, embryonal glandular growth patterns, and lace-like arrangement of cells in comparison to conventional adenocarcinomas [288]. The mesenchymal element may be (a) homologous, containing cells native to the uterus including stromal sarcoma, fibrosarcoma, undifferentiated sarcoma, or leiomyosarcoma (2%) or (b) heterologous with mixed components including rhabdomyosarcoma (18%), chondrosarcoma (10%), osteosarcoma (5%), or liposarcoma (1%). One-third of carcinosarcomas have two or more sarcomatous elements, with high-grade stromal sarcoma being the most common type [282]. Choriocarcinoma and melanocytic differentiation are unusual [291, 292]. Sometimes a tumour may have non-malignant mesenchymal elements (Fig. 10.12) and such tumours may be called carcinomas with mesenchymal differentiation based on the mesenchymal element that is present.

#### Immunohistochemistry

Carcinosarcomas are diagnosed based on morphological features alone and immunohistochemistry is used for confirmation. Commonly expressed epithelial markers are epithelial membrane antigen and pancytokeratin. The commonly expressed stromal markers are desmin in areas of

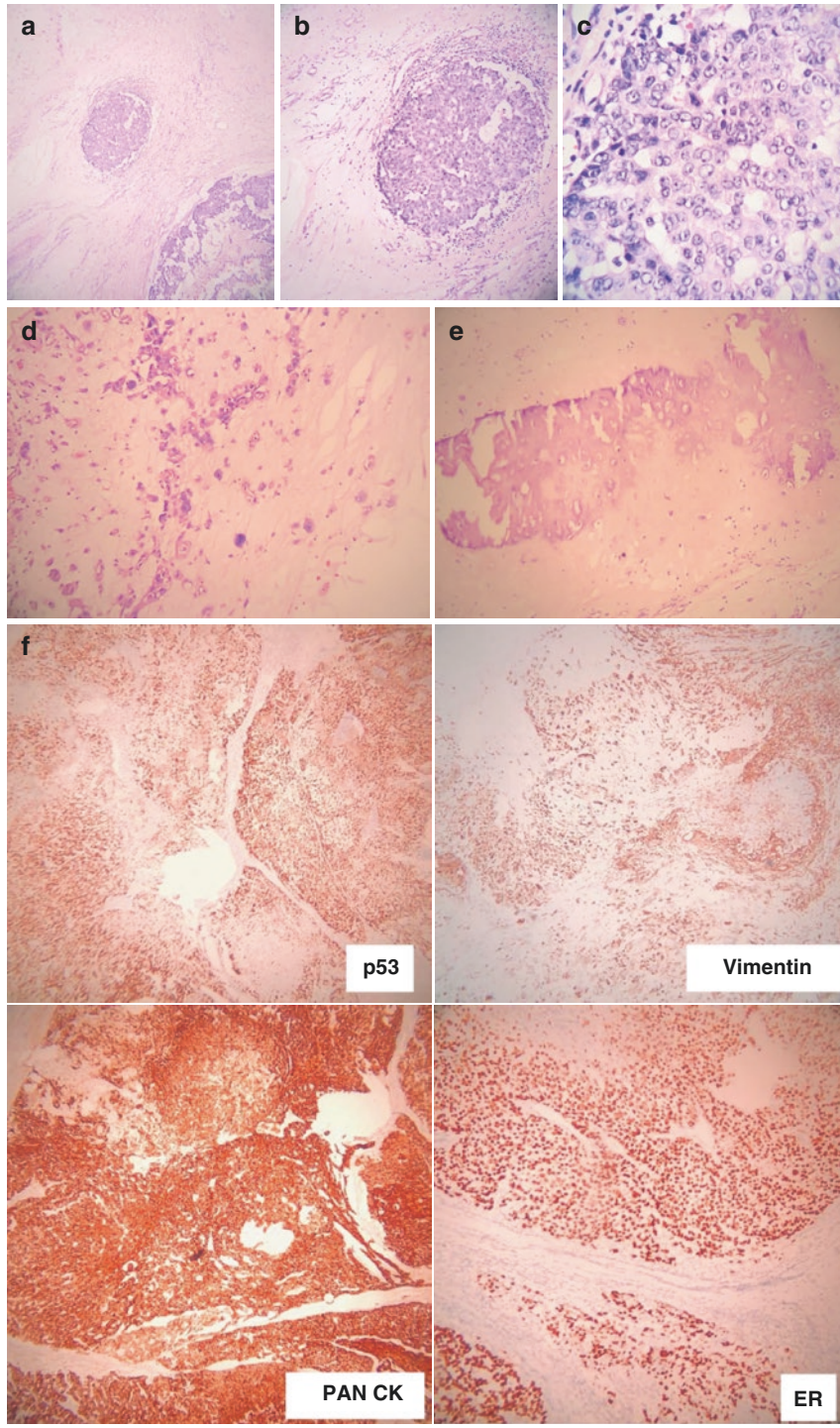


**Fig. 10.12** Histological features of peritoneal deposits in a uterine endometrioid carcinoma. Areas of chondroid differentiation were seen that constituted less than 10% of the tumour volume. In absence of frank sarcomatous element, the diagnosis of endometrial carcinoma with chondroid differentiation was made. (**a**) At normal magnification. (**b**) At 10× magnification. (**c**) At 40× magnification. (**d**) Heterologous chondroid elements. (**e**) Area of osteoid differentiation. (**f**) Positive IHC markers

smooth muscle differentiation and S100 in areas with chondroid or lipomatous differentiation.

There are some markers that are used to establish the origin and diagnosis of a carcinosarcoma. Besides these, there are several other markers of cell cycle proliferation and regulation of apoptosis that can be developed as prognostic markers or potential therapeutic targets [293, 294]. Overexpression of tyrosine kinase receptors such as *HER-2, EGFR, and KIT* suggests potential targets for therapeutic use in subgroups of carcinosarcoma [295–299].

### Ovarian Carcinosarcoma

Like its uterine counterpart, ovarian carcinosarcoma is also known as a mixed malignant Mullerian tumour (MMMT) of the ovary. Ovarian carcinosarcomas (OCSs) constitute around 1–4% of all ovarian tumours [300]. The epithelial component may be endometrioid, clear cell, serous or squamous epithelium. The mesenchymal component may be homologous: fibrosarcoma and leiomyosarcoma, or heterologous: osteosarcoma, rhabdomyosarcoma, liposarcoma, or chondrosarcoma [300]. The average age of presentation is 65–70 years and most present in locally advanced stages [301]. The prognosis is poor and the median overall survival is around 20 months [301].

#### Origin

The same theories apply to ovarian carcinosarcomas; however, there is evidence supporting all the theories and it is possible there are different mechanisms working in different patients. In a study of comparative genomic hybridization and fluorescence in situ hybridization of 30 ovarian carcinosarcomas, chromosome amplification of the c-myc protooncogene on chromosome 8q and 20q was noted, supporting the monoclonal theory [302]. The conversion theory was supported by this study too as genetic changes similar to those found in ovarian serous carcinomas were seen implying that the tumours could have developed due to metaplasia. Another study showed that there was clonal loss of *BRCA2* allele and a somatic mutation in *p53* in both the carcinomatous and sarcomatous elements, thus supporting the combination theory [303]. Several oncoproteins were studied in three cases of primary peritoneal carcinosarcoma and showed expression of *p16* in all three cases. There was no difference in the expression of other markers like p53, BCL2, Cerb-B2, E-cadherin, P-cadherin, and N-cadherin between the two elements, thus favouring a monoclonal origin [304].

The conversion theory finds support in a study of two ovarian serous epithelial carcinomas recurring as *OCS*. Evaluation of loss of heterozygosity, p53 mutation, and microsatellite analysis revealed identical findings in both the primary and recurrent tumours [305].

#### Clinical Features

There are no specific symptoms attributed to ovarian carcinosarcoma and most symptoms are similar to epithelial ovarian cancer. When present, symptoms may include pain in the abdomen or pelvic area, bloating or distention of the abdomen, and early satiety [306, 307].

#### Pathology

Ovarian MMMT are always high-grade tumours. Extra-ovarian spread (high stage), tumour rupture, high grade, and presence of high-grade sarcomatous elements are features associated with poor prognosis.

Although previous studies reported worse outcomes in patients with OCS whose tumours had heterologous elements, in more recent reports histology (homologous vs heterologous elements) has no clear influence on patient outcome [308–310].

The presence of greater than 25% sarcoma composition as well as a high number of small vessels in the primary tumour have also been linked to worse outcome [311, 312].

#### Gross Features

On gross examination, these tumours appear as large, bulky, fleshy, necrotic, and haemorrhagic tumours, which may show cartilage or bony tissue. They are mostly unilateral and are usually solid with occasional small cystic areas.

### Microscopy

Of particular mention is that the combination of epithelial and sarcomatous elements can have low-grade features. Mullerian adenosarcomas are an example in which the epithelial component is benign or has only atypia and the sarcomatous component is low grade [313]. These tumours are not included in the spectrum of carcinosarcomas. Carcinosarcomas by definition have high-grade epithelial and stromal components [314].

In carcinosarcoma of the ovary, the mesenchymal component may comprise of native ovarian stroma and its homologous malignant counterpart like undifferentiated sarcoma, leiomyosarcoma, or endometrial stromal sarcoma [315, 316]. When the mesenchymal component is non-native of ovarian stroma, the sarcomatous elements may have component derived from skeletal muscle, bone, or cartilage and is characteristically heterogeneous (Fig. 10.13). Epithelial component may be glandular or non-glandular [316]. Squamous or undifferentiated carcinoma most commonly represents the non-glandular elements [316].

Hyaline globules may be seen in ovarian carcinosarcomas and may be reactive to alpha-1-antitrypsin. If seen in a small biopsy sample, this morphological finding may aid in preoperative diagnosis of carcinosarcoma [317].

### Immunohistochemistry

Imunohistochemistry is widely used to identify the epithelial and mesenchymal components. Epithelial component usually stains for cytokeratins, while sarcomatous elements stain for vimentin, although sarcomatous elements may show cytokeratins occasionally. p53 frequently shows positive staining in both elements [318, 319]. This is indicative of a common molecular pathway and monoclonality of tumourigenesis. p16 is also commonly expressed in both elements [320]. Desmin and myoglobin 1 stain the rhabdomyosarcomatous component, while cartilageneous elements are positive for S100 [321]. CD34 staining may help distinguish OCSs from epithelioid sarcomas, which strongly express CD34 [322]. PAX-8 which is a marker of Mullerian origin is expressed in the epithelial component but not in the sarcomatous one [315, 323].

### Clinical Perspective

Carcinosarcomas are rare and aggressive malignancies that can present with peritoneal dissemination. In the setting of peritoneal carcinomatosis, these tumours can be confused with endometrial or ovarian carcinoma especially when the diagnosis is made on a small biopsy specimen. Resection of the primary tumour itself or peritoneal disease may reveal the diagnosis. Progression

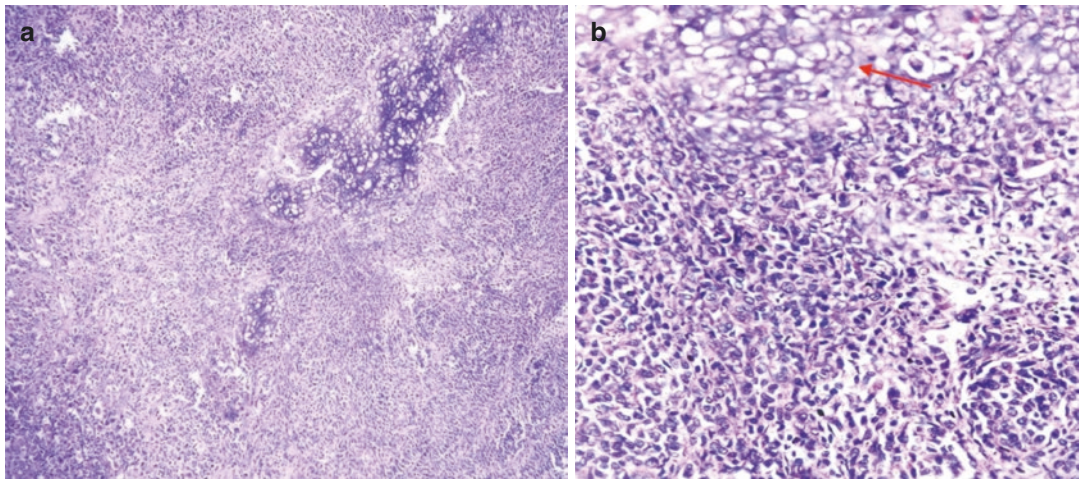


**Fig. 10.13** (**a**, **b**) Carcinosarcoma of the ovary

to carcinosarcoma in patients with serous carcinoma has been reported.

#### Molecular Mechanisms and Therapeutic Targets

Carcinosarcomas are aggressive tumours with a 5-year overall survival rate of <40% for all stages and a 50–80% recurrence rate [324–326]. There are several known mutations which can in future serve as therapeutic targets. Frequent mutations have been reported in *TP53, PTEN, PIK3CA, PPP2RIA, FBXW7,* and *KRAS* genes, which is similar to endometrioid and serous carcinomas [327]. There are several genes and pathways implicated in epithelial-mesenchymal transition. Amplification of *CCNE1* and *RB1* loss have been implicated [328]. Mutations in the chromatin remodelling genes like *CHD4* and *ARID1A* have also been implicated [329]. One study found increased incidence of mutations in genes encoding histone H2A and H2B, as well as significant amplification of the segment of chromosome 6p harbouring the histone gene cluster containing these genes [330].

#### Patterns of Peritoneal Dissemination

We did not find any studies looking at pathways of peritoneal dissemination and disease distribution in the peritoneal cavity. Whether uninvolved regions like the omentum should be resected or not in absence of visible disease is not known. It is possible that a large proportion of the peritoneal dissemination is due to intraoperative tumour spillage caused during the first procedure. Retroperitoneal implantation at the surgical site is common and may preclude a curative resection, especially in high-grade tumours.

#### Section Summary

Peritoneal sarcomas are rare tumours, each having its peculiar features that pathologists dealing with peritoneal surface oncology should be aware of. Molecular tests can be used to confirm or establish the diagnosis where histology and immunohistochemistry do not clearly point to a particular diagnosis. Pathological reporting should capture disease distribution to better understand the biology of these tumours that will help guide surgical decisions in future.

### 10.4.7 Gastrointestinal Stromal Tumours

Gastrointestinal stromal tumours are rare tumours arising from the gastrointestinal tract. They are the commonest mesenchymal tumours of the gastrointestinal tract. These tumours can have peritoneal dissemination with or without spread to other sites [331]. Rarely, GIST arises from the omentum or the peritoneum [199]. The histopathological features of GIST are characteristic and confirmed with immunohistochemistry.

#### 10.4.7.1 Gross Features

Gastrointestinal tumours are known to form large, bulky intramural masses. The cut surface shows fish flesh or tannish brown surface with areas of haemorrhage, necrosis, and cyst formation [332]. Peritoneal deposits also form masses of variable size with similar features.

#### 10.4.7.2 Microscopic Features

There are three main varieties of GIST—the spindle cell variety is the commonest and seen in about 70%, the epithelioid variety is next, constituting 20% of the tumours and the remaining are of the mixed variety [333].

The spindle cell variety comprises of cells with pale eosinophilic cytoplasm that is paler than other smooth muscle tumours. The cells have indistinct margins giving rise to a fibrillary or syncytial appearance [333]. The nuclei are uniform and may have vesicular chromatin. The cells are arranged in short fascicles or whorls (Fig. 10.14). The characteristic feature is the presence of stromal haemorrhage. Thin walled stromal vessels are common. Cytoplasmic vacuoles adjacent to the nucleus are seen in 5%. Other common features are nuclear palisading, stromal lymphocytes, and microcystic stromal degeneration (as in schwannomas) [333].

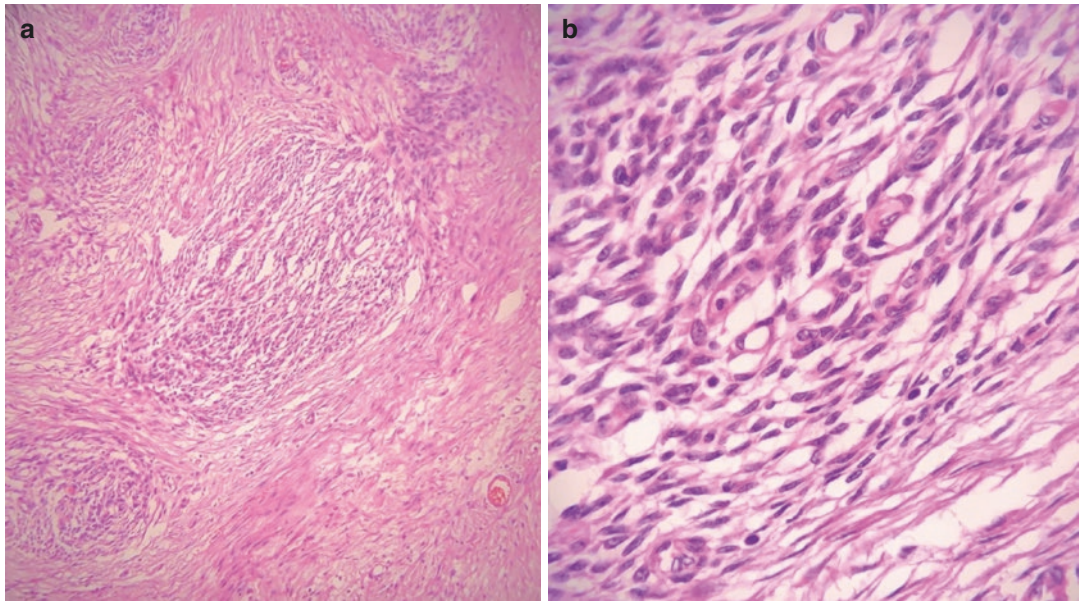


**Fig. 10.14** (**a**, **b**) Histological features of peritoneal deposits in a GIST arising from the greater curvature of the stomach

GISTs of epithelioid type are composed of rounded cells with variably eosinophilic or clear cytoplasm [333]. Cells with clear cytoplasm show areas of retraction of eosinophilic cytoplasm resembling inclusions adjacent to the nuclei. The nuclei are similar to the spindle cell variety containing vesicular chromatin. Lesions of mixed cell type may exhibit an abrupt transition between spindle cell and epithelioid areas (necessitating careful sampling if both patterns are to be recognized) or may have a complex comingling of these cell types throughout, leading to an "intermediate" ovoid cytologic appearance [333].

Roughly 5% of lesions show a variably prominent myxoid stroma, or a paraganglioma or carcinoid type of growth pattern. Approximately 2–3% show notable cytologic pleomorphism [333].

GIST may be further divided into eight different subtypes. (1) Spindle cell subtypes: sclerosing, palisading-vacuolated, hypercellular, and sarcomatous spindle cell; and (2) Epithelioid cell subtypes: sclerosing, discohesive, hypercellular, and epithelioid spindle cell [334].

The distinction between benign and malignant depends on the presence of nuclear atypia and presence of necrosis, haemorrhage, and mitotic activity. It is necessary to determine mitotic rate, grade of dedifferentiation, size, location, tumour infiltration, grade of necrosis and haemorrhage, surgical margins, and whether a tumour ruptures because these factors are implicated in the risk of relapse [335].

#### 10.4.7.3 Immunohistochemistry

The characteristic finding is CD117 (KIT) positivity (Fig. 10.15). In addition to this, about 60–70% of GISTs express CD34, 30–40% express smooth muscle actin (SMA), and around 5% show immunopositivity for S-100 protein. None of the latter antigens are specific for GIST [336]. Desmin positivity in true KIT-positive GISTs is extremely uncommon (1–2% of cases) and is invariably focal, with positivity in only a small number of tumour cells [336].

Almost 85% of GISTs have a mutation in KIT or PDGFRA that induces a KIT activation, which is a tyrosine kinase receptor that stimulates the growth of tumour cells. Mutational analysis is acquiring a growing importance and should be performed when adjuvant and/or neoadjuvant therapy show possible mutations with a tendency towards imatinib mesylate-resistance [337].

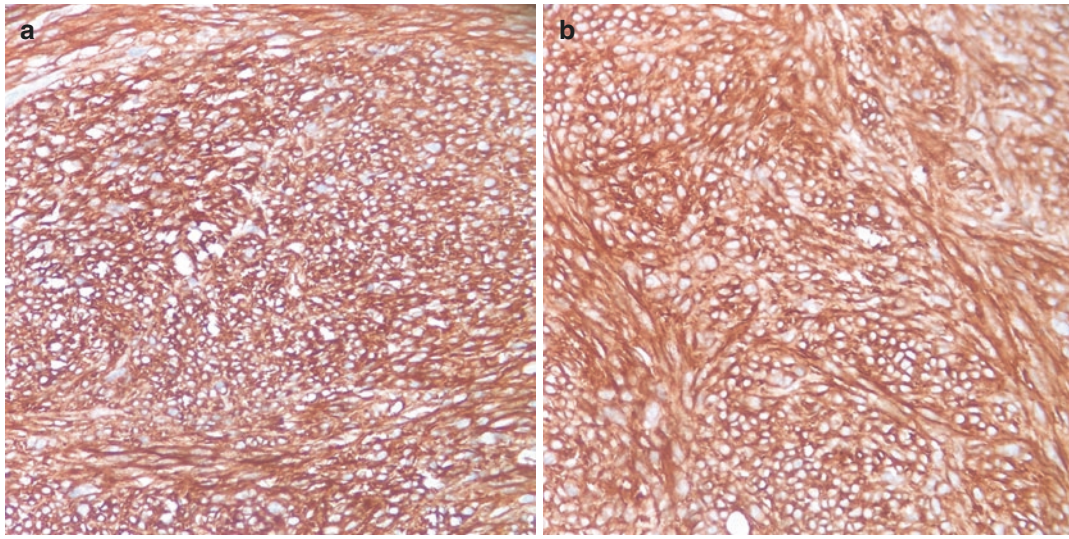


**Fig. 10.15** CD117 (**a**) and DOG1 (**b**) expression in the same patient as Fig. 10.13

**Table 10.5** Genetic mutation and selection of tyrosine kinase inhibitor in GIST

| Gene | Mutation | TKI |
|---|---|---|
| KIT | Exon 11<br>Exon 13<br>Exon 17<br>Exon 9<br>Exon 18. D842V mutation | Imatinib-Mesylate |
| PDGFRA | Exon 12<br>Exon 14<br>Exon 18. D842V mutation | Imatinib-Mesylate<br>Sunitinib<br>Regorafenib |
| Wild-type | | Sunitinib<br>Regorafenib |

Tyrosine kinase inhibitor selection based on gene mutations is described in Table 10.5.

- *KIT gene mutations (80%)*: KIT exon 11 is the most common mutation and may be observed in approximately 75% of all mutation-positive tumours primarily affecting codons 557–559. Exon 11 mutations are more common in GIST of gastric origin and portend a poor survival and high risk of developing metastatic disease. However, tumours carrying these mutations are also more responsive to imatinib [337–339]. Exon 9 mutations are seen in approximately 10% of the patients. Mutations in exons 8, 13, and 17 are infrequent and seem to be <3% [340].
- *PDGFRA gene mutations (5–8%)*: GISTs with *PDGFRA* mutations are regularly located in stomach [337]. The *D842V* mutation in PDGFRA exon 18 is the most common mutation found (65%–75% of PDGFRA mutations); this mutation is associated with imatinib and sunitinib resistance [339, 341, 342]. *Non-D842V* exon 18, 12, and 14 mutations are rare and sensitive to imatinib.
- *Wild-type GISTs (12–15%; 90% of paediatric GISTs)*: In these cases, there are no detectable mutations in KIT or PDGFRA genes that are resistant to treatment with imatinib although tyrosine kinases are still activated. Wild-type GISTs represent a heterogeneous group that includes several oncogenic mutations such as *BRAF V600E* substitution, *NF1* mutation, and defects in the succinate dehydrogenase complex [343–345].
- *KIT-negative GISTs (CD117-negative)*: Approximately 5% of GISTs do not express CD117 on immunohistochemistry but 30–50% of these have KIT or PDGFRA mutations [346].

Ki67 is an important prognostic factor that has been implicated in recurrence and survival and should be included in pathologist's report [335, 347].

#### 10.4.7.4 Section Summary

GIST can metastasize to the peritoneum or arise de novo from the omentum and rarely the peritoneum. Mutational studies should be performed both to predict response to tyrosine kinase inhibitors and distinguish these tumours from those that express CD117.

## 10.5 Conclusions

Every surgeon and pathologist treating peritoneal surface malignancies should be familiar with these rare peritoneal tumours and their diagnostic evaluation. Genomic studies should be used both to establish and confirm the diagnosis and identify therapeutic targets. Details of disease distribution should be captured while evaluating surgical specimens to develop a better understanding of the clinical behaviour and patterns of dissemination that can guide surgical treatment in future.

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

Aditi Bhatt, Loma Parikh, Suniti Mishra, and Olivier Glehen

## 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

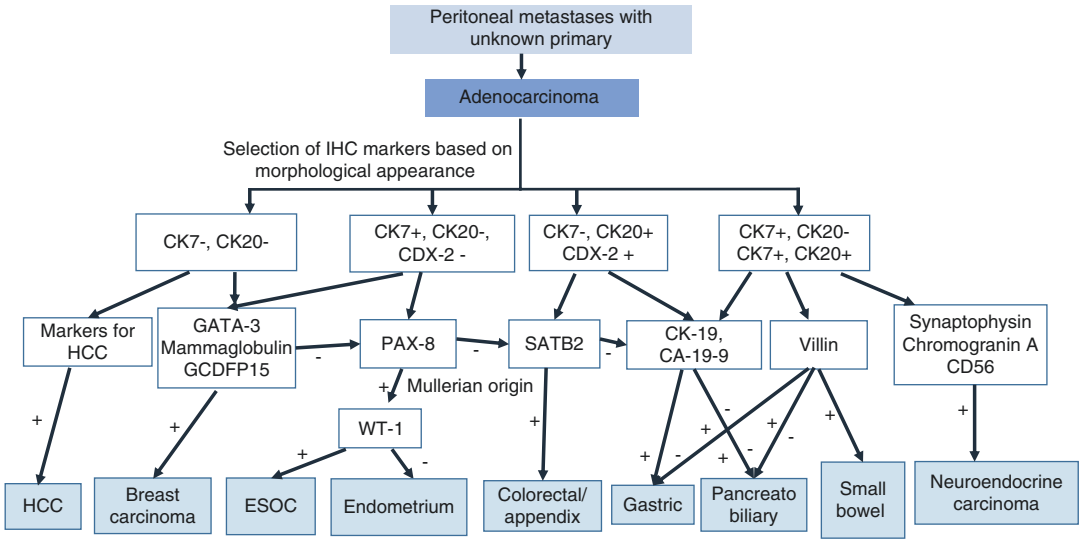


**Fig. 11.1** Algorithm for determining the primary site in adenocarcinoma

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

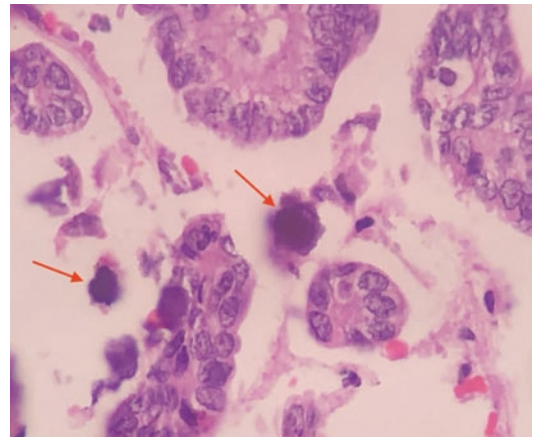

**Fig. 11.2** Psammoma bodies which are characteristic of serous carcinomas

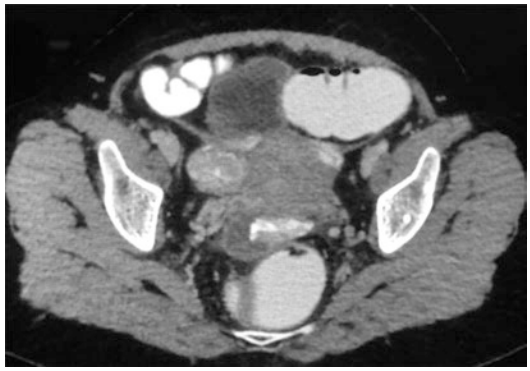

**Fig. 11.3** Pelvic mass in a patient with serous carcinoma. The site of origin could be the ovary or the uterus. The prognosis is significantly worse in serous endometrial carcinomas

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

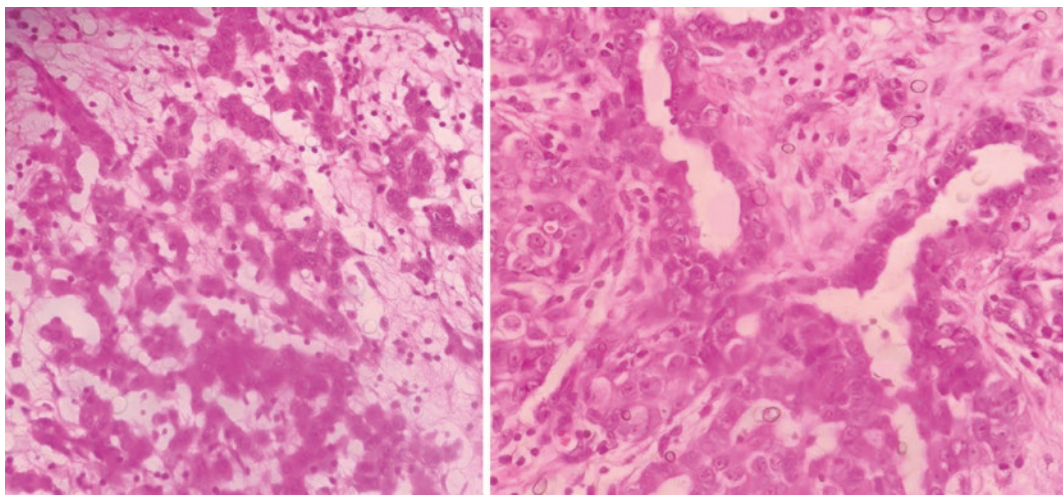

**Fig. 11.4** Histological findings in the peritoneal biopsy suggestive of peritoneal mesothelioma several years after the initial diagnosis of breast cancer. The immunohistochemistry profile was in favour of a peritoneal mesothelioma

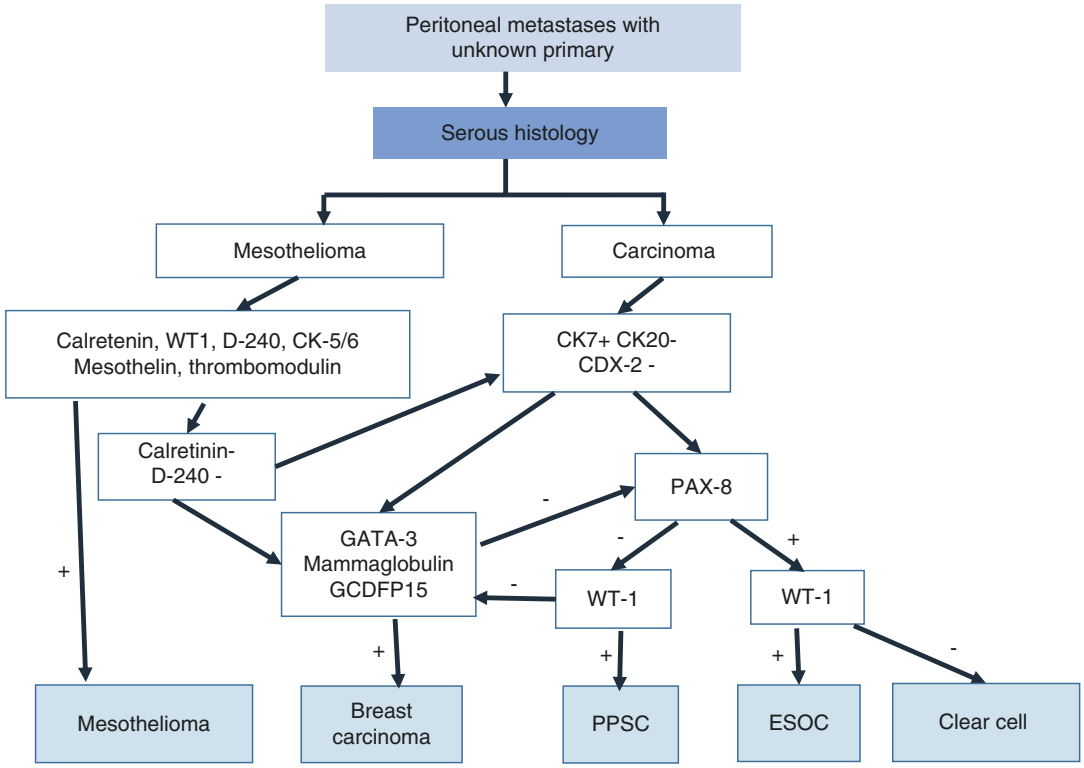


**Fig. 11.5** Algorithm for determining the primary site in peritoneal metastases with serous histology. *PPSC* primary peritoneal serous carcinoma, *ESOC* epithelial serous ovarian carcinoma

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.

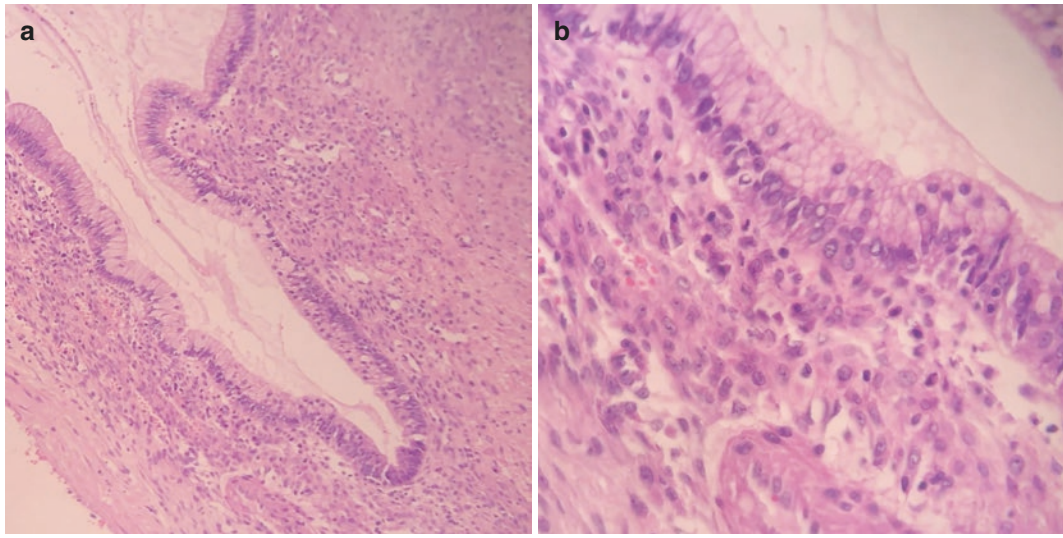


**Fig. 11.6** (**a**, **b**) Peritoneal deposits from low-grade mucinous neoplasm of the ovary

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

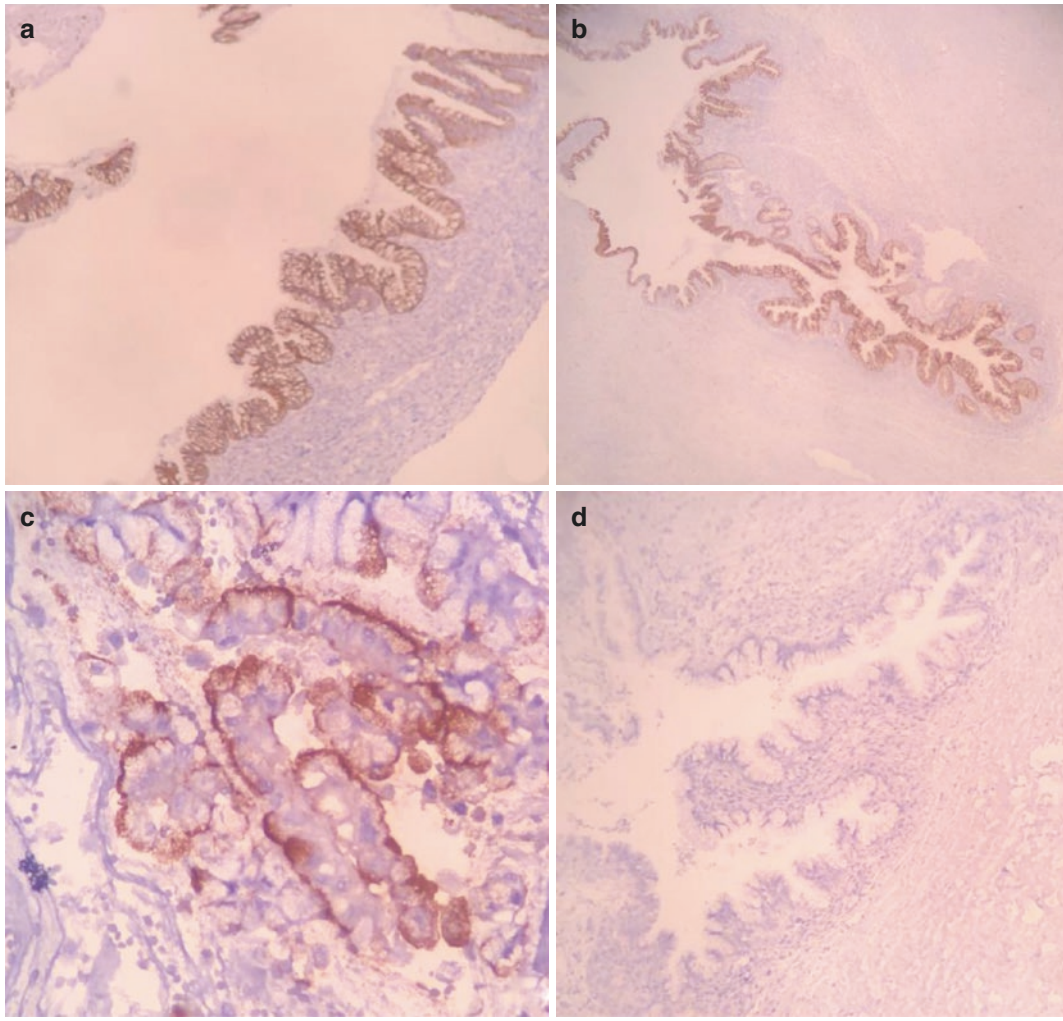


**Fig. 11.7** Tumour cells express CK-7 (**a**), CA-125 (**b**), PAX-8 (**c**) and are negative for CDX-2 (**d**), CK-20 (not shown) and WT1 (not shown)

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].

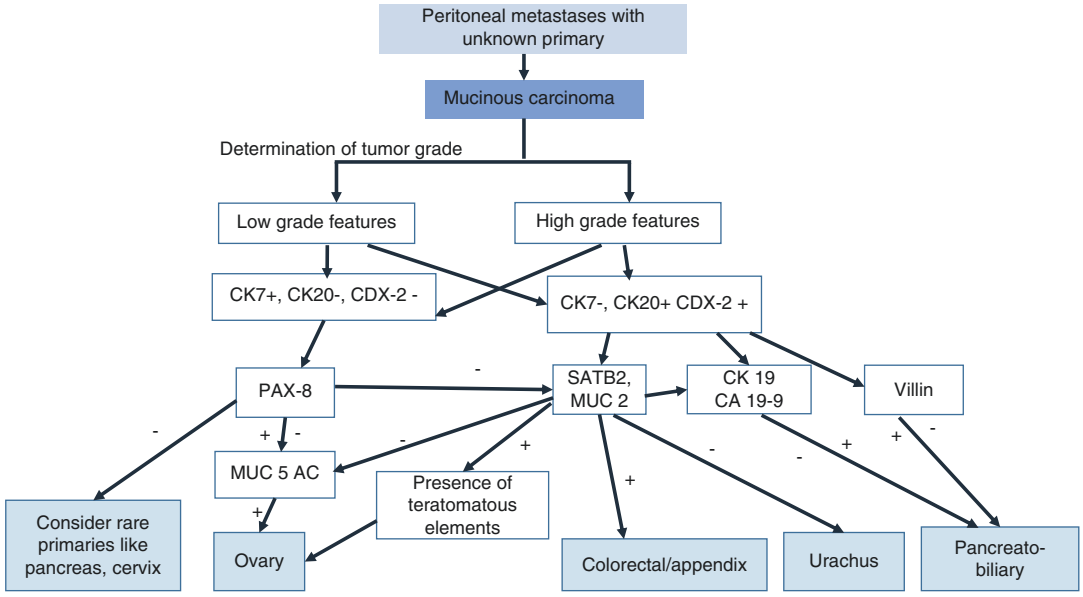


**Fig. 11.8** Approach to a patient with mucinous peritoneal carcinomatosis

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.

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# 12 Biomarkers in the Management of Peritoneal Metastases

Ninad Katdare, Aditi Bhatt, and Olivier Glehen

## 12.1 Introduction

Selected patients with peritoneal metastases are treated with a curative intent, the backbone of which is cytoreductive surgery (CRS) with or without hyperthermic intraperitoneal chemotherapy (HIPEC) [1]. Despite this aggressive and morbid treatment, a large majority of the patients develop recurrence and/or progressive disease [2]. Surgical, disease-related and patient-related variables have been identified and validated and are used to select patients for such procedures but the rates of recurrence have improved only marginally [3]. The search for new prognostic markers is an ongoing process to identify patients most likely to benefit from the treatment [4]. In the era of molecular oncology, molecular markers have been gaining increasing focus as they not only have prognostic value but can also serve as therapeutic targets for developing systemic therapies. Whole genome sequencing has accelerated the process of biomarker discovery. A molecular marker or biomarker is defined as "a biological molecule found in blood, other body fluids, or tissues, that is a sign of a normal or abnormal process, or of a condition or disease" [5]. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. As the field of peritoneal oncology is evolving, many studies have been done and are ongoing, to assess the utility of biomarkers in various aspects of management of peritoneal metastases. This chapter provides a review of the known and emerging biomarkers related to some common peritoneal tumors.

## 12.2 Classification

Biomarkers are used across the spectrum of medical sciences [6]. For malignancies, the older term was 'tumor markers' but this referred only to circulating substances in blood like CA125, CEA, etc. In the era of molecular oncology, biomarkers can be measured at various levels [7].

Specific descriptors are thus used to define the types of biomarkers.

- *Cells or Tissue*: Presence of cells outside their milieu, e.g., in circulation, demonstration of neovascularization in tissue specimen.
- *Protein*: Overexpression, underexpression, or qualitative abnormalities.

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- *RNA*: MicroRNA (miRNA), mRNA.
- *DNA*: Gene mutations, deletions, amplifications, or methylation.
- *Epigenetic Markers*: Heritable changes in gene expression that do not cause permanent alteration of the underlying DNA sequences, and include DNA methylation, histone modifications, and noncoding RNAs.

Alternatively, based on their clinical utility, they can also be divided into the following groups [8]:

(a) *Prognostic biomarkers*: These are disease-related biomarkers. They give an idea regarding the course of the disease irrespective of the treatment chosen.
(b) *Predictive biomarkers*: These are drug-related biomarkers. They give an idea regarding the response to treatment.
(c) *Diagnostic biomarkers*: These help diagnose a cancer, perhaps before it is detectable by conventional methods.

As with tumor markers, an ideal biomarker should have the following properties:

- It should be specific to the tumor.
- The levels should change in response to tumor size.
- Abnormal level should be obtained in presence of micrometastases.
- Level should not have large fluctuations that are independent of changes in tumor size.
- Levels in healthy individuals are at much lower concentrations than those found in cancer patients.
- Predict recurrences before they are clinically detectable.
- Test should be cost-effective.

Given the heterogeneity of tumors and the multitude of treatments available, it is not possible for one single test to be able to address all these issues. Often there are different markers for different outcomes or a combination of markers is used. Many markers are used for more than one tumor and address different clinical end points.

Biomarkers in peritoneal surface oncology can have the following roles:

- Diagnosis and classification of rare tumors
- Early detection of PM
- Predicting benefit from surgery
- Detecting early recurrence/identifying patients prone to recurrence
- Predicting response to systemic therapies
- Targets for drug development

## 12.3 Ovarian Cancer

Ovarian cancer is the fifth frequently occurring cancer among women and the leading cause of death among gynecological cancers. On the basis of histological classification, there are different types of ovarian cancers. Epithelial ovarian cancers (EOC) is the most common, whereas stromal and germ cell tumors are of lower abundance [9]. Epithelial tumors are classified into two types: type 1, with mutations in genes such as *PTEN*, *KRAS*, and *BRAF*, which ultimately increase the expression of *Mitogen-Activated Protein Kinase (MAPK)* and phosphatidylinositide 3-kinase (PI3K) signaling pathways which lead to proliferation and metastasis of ovarian cancer cells, and type 2 which involves mutations in *P53* [10–12]. Type 2 tumors are the high-grade serous cancers and type 1 includes low-grade serous carcinomas and other epithelial subtypes.

There are several challenges in the treatment of ovarian cancer. Early detection still remains a problem with most of the tumors being detected in stage 3 or above. Use of rising CA125 to trigger transvaginal ultrasound may produce as much as a 20% reduction in mortality in the latter trial with additional follow-up [13]. As outcomes are improved when primary surgery is performed by a gynecologic oncologist, preoperative discrimination of benign and malignant pelvic masses can facilitate appropriate referral. CA125, HE4, apolipoprotein A1, transthyretin, transferrin, and β2-macroglobulin have contributed to the RMI, ROMA, or OVA1 algorithms to distinguish benign from malignant disease [14, 15]. Patients with advanced disease are subjected

to cytoreductive surgery. When the disease is not amenable to surgery upfront, few cycles of neoadjuvant chemotherapy are given before performing CRS. Recently, HIPEC combined with CRS showed a survival benefit in the interval setting. There is a need to define subsets of patients who benefit from this treatment [16]. This is more relevant in light of recent clinical trials showing prolonged survival with PARP inhibitors [17].

Despite maximal surgical efforts, a large proportion of the patients develop disease recurrence that is defined based on the time interval from the last dose of platinum-based chemotherapy. Treatment of recurrent disease remains a challenge and the need for newer systemic therapies persists.

Thus, ovarian cancer remains a key area for biomarker development. Some potential areas for biomarker development in ovarian cancer are:

- Early detection
- Response to platinum agents
- Response to other therapies
- Benefit of HIPEC

### 12.3.1 Early Detection Biomarkers

At present, 75–80% of ovarian cancer patients are diagnosed with advanced stage (III/VI) where the cure rate is less than 20% [13–15, 18]. If, however, the disease is diagnosed in Stage I or II, 70–90% of patients can be cured with conventional surgery and chemotherapy. Early detection could significantly improve clinical outcomes in ovarian cancer since computer models suggest that detection of early stage disease could improve cure rates by 15–30% [13].

1. *CA125*: CA125 (carbohydrate antigen 125 or MUC16) is a high-molecular-weight glycoprotein found on the surface of epithelial cells. It is overexpressed in EOC and is a widely used serum biomarker for the monitoring of patients with ovarian cancers. The expression of CA125 is usually low in normal ovaries, but a proteolytic site presented in the structure of CA125 is believed to cause the formation of high invasive characteristics of ovarian cancer cells [19]. The interaction of CA125 with mesothelin on the surface of mesothelial cells mediates cell adhesion. Therefore, it is proposed that CA125 may contribute to the metastasis of ovarian cancer [12, 20]. Levels of CA125 correlate with response to treatment and can rise 4.8 months prior to clinical disease recurrence. Response to treatment is generally associated with a decrease in CA125 values by half, whereas a doubling of CA125 indicates drug resistance and disease progression. Persistent elevation of CA125 in patients after primary chemotherapy predicts persistence of residual disease with 90% accuracy [21]. Values higher than 35 U/mL are considered significant and indicate the need for follow-up. An important point to remember is that serum CA125 levels also increase in some physiological conditions such as pregnancy, and certain diseases such as uterine fibroids, endometriosis, and pelvic inflammation [22]. The use of CA125 as a biomarker results in a 47% likelihood of detecting ovarian cancer in its early stage and a likelihood of 80–90% in late stages. The sensitivity of CA125 is about 50–60%, and its specificity is about 90% [23]. In view of the low sensitivity of CA125 and average specificity, newer biomarkers are increasingly used in early diagnosis of ovarian cancers.
2. *HE4*: HE4 has a molecular weight of 25 kDa and is encoded by the WFD2 gene. It circulates in the blood stream and can be assessed by an enzyme immunoassay [24]. In 2009, the Food and Drug Administration (FDA) approved the use of HE4 for monitoring of women to diagnose EOC. Scientists demonstrated that HE4 is overexpressed in EOC but not in other types of ovarian cancer [12, 25]. The reference range of serum HE4 in normal conditions is less than 140 pmol/L. Serum HE4 levels also increase with pregnancy, aging, and menopausal status [24]. HE4, in early ovarian cancer detection, has a sensitivity of about 90% and a specificity of 72.9%; the combination of HE4 and CA125 can

distinguish between benign and malignant conditions and improve the early detection of cancer.

3. *Mesothelin*: Mesothelin is a 40 kDa glycoprotein expressed on mesothelial cell surface. Serum and urine levels of mesothelin can be elevated in some cancers, such as mesothelioma, ovarian cancer, and pancreatic cancer [26]. Mesothelin alone has a 60% sensitivity and a 98% specificity in cancer detection, but in combination with CA125, the sensitivity is improved [27]. The measurement of mesothelin in urine is more effective than performing a serum assay; urine assays exhibit 95% specificity in early stage detection of ovarian cancer [28]. However, costs and limited availability of the assay limit its use in routine clinical practice.
4. *Kallikrein-related peptidases (KrP)*: KrP are a group of serine proteases. This is the largest continuous cluster of proteases on the human genome [12]. KrP exhibit low sensitivity in the early detection of ovarian cancers when used alone, but a 90% specificity and a 72% sensitivity have been reported in combination with CA125 [29]. Serum levels more than 4.4 mg/L indicate poor prognosis in patients [30]. They are measured in serum through ELISA. Cost and availability have limited its widespread use.
5. *Osteopontin*: It is an adhesive glycoprotein synthesized by vascular endothelial cells and osteoblasts. Its function is related to bone remodeling and immunity. It was first identified in bones, but immune cells can also express this protein [31]. It has a high sensitivity of around 83.3% especially when combined with CA125. However, it lacks specificity [32].
6. *Apo-A1*: ApoA1 is part of the family of high-density lipoproteins. Contrary to the other biomarkers, a reduction in the levels of Apo-A1 is associated with detection of ovarian cancers. In combination with CA125, the sensitivity is around 93.9% with a specificity of 95%. It is used in conjunction with other biomarkers for the OVA1 test discussed below [33].
7. *ROMA*: As seen above, a single biomarker often lacks the sensitivity and specificity to qualify as a good biomarker. This has led to development of algorithms and combination tests which can improve early detection and treatment. ROMA stands for Risk Of Malignancy Algorithm. In studies carried out by Moore et al., in which various biomarkers were tested, it was observed that a combination of CA125 and HE4 had the best area under the curve in the receptor operating characteristic (ROC) curve of 91.4% [34]. This was then subsequently integrated with menopausal status to develop the ROMA. Sensitivity of the ROMA for detecting malignancy has been lower in premenopausal women than in postmenopausal women [35]. Another community trial by Moore et al. revealed 94% sensitivity at 75% specificity [36]. The sensitivity was 100% in premenopausal patients and the negative predictive value reached 98%. This led to approval of the algorithm by FDA. However, future studies showed conflicting results with some concluding combining CA125 and HE4 with the ROMA provides no more discrimination than either biomarker alone [37–42]. Hence, the use of this algorithm is not widespread.
8. *OVA1*: The OVA1 is a multivariate index calculated by combining data from imaging, menopausal status, and CA125 with four other protein biomarkers including apolipoprotein A1, transthyretin, transferrin, and β2-macroglobulin [43]. It was approved by FDA in 2009. Validation in various studies showed that the OVA1 panel demonstrated higher sensitivity, but lower specificity than physician evaluation. Addition of the OVA1 panel enhanced the sensitivity for detection of malignant pelvic masses from 78% to 98%, but reduced specificity from 75% to 26%. A high negative predictive value of 98% was, however, achieved with OVA1 [44].

Neither ROMA nor OVA1 should be used as a screening test for ovarian cancer. They only help in defining the benign ovarian masses from the malignant one. At present only Ca125 and HE4

are used in regular clinical practice for early detection of ovarian cancers.

### 12.3.2 Newer Biomarkers

1. *Autoantibodies*: The expression of antigen, the rate of antigen-shedding, and the volume of cancer before it becomes capable of metastasis often limit the detection of various biomarkers in the early stage of the disease. This has led to the hypothesis that small volumes of cancer may not release adequate amounts of antigen to elevate serum levels, but could induce a human immune response [45]. The *TP53* tumor suppressor gene is mutated in virtually all high-grade serous ovarian cancers. Recent studies suggest that titers of anti-TP53 autoantibodies are present in as many as 25% of ovarian cancer patients and can rise 8–12 months prior to CA125 and more than 2 years before clinical presentation of CA125 negative cases [13, 46].
2. *MicroRNAs (miRNA)*: miRNAs are small noncoding RNAs which downregulate protein expression of target genes by degrading their messenger RNA (mRNA) or interfering with translation of specific proteins. Several miRNAs have been proposed as biomarkers for early detection, diagnosis, and prognostication of ovarian cancers, but none has been established to date [47–49].
3. *Circulating tumor DNA (ctDNA)*: Since its discovery in cancer patients in 1977, the evolution of interest and research has been slow. In recent times, with improvement in techniques of detection and isolation the interest has rekindled. ctDNA has been isolated in pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers [50]. ctDNA had comparable sensitivity and specificity to CA125 and detected persistent cancer in 6 cases with negative CT scans [51].
4. *Exosomes*: Exosomes are highly stable membrane vesicles released from variety of normal cells as well as malignant cells. As the content of the exosomes is similar to the cells from which they are released, it is hypothesized that they will provide the same unique signatures from which they are released [52]. Exosomes derived from normal cells were larger than those derived from malignant cells.
5. *New Protein Biomarkers*: Using proteomics, at least 11 candidates, namely IGFBP2, IGFBP3, KLK6, KLK7, KLK9, MDK, CA125, PROS1, SLPI, TIMP1, and HE4, have been discovered in ovarian cancer cell lines, and five candidates, namely GRN, IGFBP2, RARRES2, TIMP1, and CD14, have been identified in plasma from ovarian cancers including early stage patients [53, 54]. The Early Detection Research Network (EDRN) website lists more than 200 ovarian cancer biomarker candidates. Large-scale clinical studies are required for evaluating those candidates as potential biomarkers.

### 12.3.3 Prognostic Biomarkers

Many of the diagnostic markers also guide the prognosis of the disease in ovarian cancer. Most of the markers are described in the above section. A list of biomarkers with their clinical relevance in prognosis is mentioned in Table 12.1 (adapted from Ref. [55] with permission).

### 12.3.4 Predictive Biomarkers

One of the most important prognostic factors in ovarian cancer has been sensitivity to platinum agents. There is a large body of literature highlighting a number of biomarkers as potential candidates for predicting resistance or sensitivity to treatment.

#### 12.3.4.1 Ceruloplasmin

Ceruloplasmin, a plasma glycoprotein, transports copper throughout the body. High serum levels of ceruloplasmin have been demonstrated in various cancers such as thyroid, prostate, and colon cancer, and microarray analysis has linked this gene to tumor invasion and metastasis in breast cancer [67–69].

**Table 12.1** Biomarkers and their clinical relevance in epithelial ovarian cancer

| Biomarker [ref.] | Clinical relevance |
|---|---|
| CA 125 [56] | Postoperative levels of CA125 >35 U/mL (no residual) or >65 U/mL (residual) are independent prognostic factors for survival |
| HE4 [57] | High serum level predicts unfavorable prognosis. A change in HE4 level of ≥25% is considered significant (an increase of this magnitude suggests recurrence or disease progression; a decrease suggests therapeutic response) |
| M-CSF [58] | Markedly elevated levels of M-CSF1 in serum and ascites are associated with a poor prognosis |
| Bikunin [59] | There is a 2.2-fold increased risk of death for patients with preoperative lower plasma bikunin levels |
| Plasma cell-free DNA [60] | Levels correlate with increasing tumor burden and decline following therapy |
| VEGF [61] | Higher serum expression of VEGF is associated with a shorter overall survival; elevated VEGF ascites levels negatively correlate with patient survival |
| EphA2 expression [62] | Overexpression is associated with poor prognosis |
| Claudin family members [63] | Claudin-3 and claudin-7 expression in effusions independently predicts poor survival |
| EGFR and HER2 [64, 65] | Tumors with increased EGFR protein tend to grow more aggressively, are more likely to metastasize, and are more resistant to chemotherapy HER-2/neu expression does not appear to be an important prognostic factor in patients with advanced epithelial ovarian cancer |
| Serum sFas levels [66] | Survival rates decrease as serum sFas levels increase; serum sFas level is also a useful biomarker for predicting response to platinum-based chemotherapy |

One study found a significantly upregulated level of ceruloplasmin in the ascitic fluid of intrinsic chemoresistant serous EOC patients, thus suggesting its potential as a prognostic biomarker for response to chemotherapy [70].

#### 12.3.4.2 Cancer Stem Cells (CSCs)

CSCs are a relatively small subset of cancer cells that indefinitely self-renew, initiate, and maintain tumor growth and may remain quiescent for prolonged periods [71, 72]. In ovarian cancer, these stem cells remain quiescent during chemotherapy and thus promote platinum resistance [73, 74]. Though the exact mechanism has not been elucidated, this seems to be the most plausible one. Chemotherapy acts on dividing cells and thus, these cells escape getting destroyed by it [75].

Known immunohistochemical markers of CSCs are ALDH, CD133, and BMP2 [76–78].

#### 12.3.4.3 Epithelial Mesenchymal Transition

Epithelial mesenchymal transition plays a pivotal role in the development of ovarian cancer. It is a complex process that involves apoptosis, metabolism, cell proliferation, angiogenesis, and cell growth [79]. A distinctive mesenchymal gene expression profile has been identified [80]. Marchini et al. in their study of 46 patients identified a resistance gene expression signature for TGF-β-mediated EMT that was further validated in 52 patients [81].

PI3K-AKT-mTOR inhibitors are the most promising therapeutic targets for EMT reversal, but it is difficult to ascertain if the disease control is a result of EMT reversal or suppression of the other processes [82]. Another approach to reversing EMT is targeting the epigenetic alterations that drive the transition and specific microRNAs for epithelial serous ovarian cancer, DNA methylation, and histone acetylation patterns [82].

#### 12.3.4.4 MicroRNAs

There are over 1000 human miRNAs and most have been associated with regulation of mRNA in normal and disease processes. There are many miRNAs that have been implicated in platinum resistance and can serve as future therapeutic targets. The most significant ones are miR-622, which targets the Ku pathway and downregulates non-homologous end joining (NHEJ); miR-484 that targets *VEGFB* and *VEGFR2* pathways and tumor vasculature; and

a miRNA profile of 9 miRNAs that are involved in regulation of EMT and TGF/WNT signaling [83–85]. Overexpression of miR-27a, miR-23a, miR-30c, Let-7g, miR-199a-3p, and miR-141-3p have also been associated with cisplatin resistance [86, 87].

### 12.3.5 Genetic Mutations and Targets for Drug Development

*BRCA1/BRCA2* germline mutations are observed in 8–10% and somatic mutations in 3% of the patients with ovarian cancer. Other genes commonly mutated in ovarian cancer include *RB1, NF1, FAT3, CSMD3, GABRA6*, and *CDK12* [88]. Additional somatic gene mutations include *BRAF, PIK3CA, KRAS*, and *NRAS* mutations and amplifications in *CCNE1, MYC*, and *MECOM* [89]. Some mutations unique to ovarian cancer include mutations at loci 3q28, 4q32.3, 8q21.11, 10q24.33, 18q11.2 and 22q12.1, 2q13, 8q24.1, and 12q24.31 [90].

The microenvironment of peritoneal metastases (PM) can also serve as a potential therapeutic target. During the development of PM, the peritoneum becomes thickened with an increase in the vascular permeability which is attributed to the ensuing inflammatory response [91]. These events are associated with increased macrophage infiltration, specifically M2 CD68 cells. M2 cells are commonly associated with increased Tregs in the tumor microenvironment and decreased CD8, CD4, and M1 macrophages [92]. TIE2 is a receptor for Ang2 that promotes angiogenesis and the Erk 1/2 and Akt pathways [92]. Expression of TIE2 by M2 macrophages has been identified in epithelial ovarian cancer and TIE2 expression and the increased expression of TIE2 by M2 macrophages may be a diagnostic marker for metastasis [92]. Other established immunologic markers also include macrophage-specific induction of nuclear factor-kB and c-Jun kinase signaling pathways resulting in increased interleukin-6, interleukin-8, and vascular endothelial growth factor-C which promote metastasis [93, 94].

### 12.3.6 Newer Approaches

The composition of ascites, which includes cellular and acellular components, constantly adapts during the course of the disease in response to various cellular cues originating from both tumor and stromal cells [95]. Increasing evidence now supports an active role of ascites in the progression of ovarian cancer. Although much work is still needed to fully understand the contribution of ascites to ovarian cancer aggressiveness, this tumor environment potentially provides a wealth of opportunities for translational research including biomarker discovery and novel therapeutic target identification [96].

## 12.4 Pseudomyxoma and Appendiceal Tumors

Pseudomyxoma peritonei is a clinical syndrome comprising of a wide spectrum of tumors ranging from very bland benign looking tumors to frank adenocarcinomas [97]. Cytoreductive surgery and HIPEC have drastically improved the survival in these patients over systemic and palliative therapies alone. There still remains a lot of scope for improvement. Following appendectomy for mucinous neoplasms at high risk for peritoneal dissemination, the search for ideal surveillance markers persists. A large number of patients who are subjected to prophylactic procedures have no disease on exploration. There is a subset of patients with acellular mucin alone in the peritoneal implants that develop recurrence after CRS and HIPEC. Similarly, for both low- and high-grade tumors following complete CRS and HIPEC, there are no predictors of recurrence. For patients not eligible for complete cytoreductive surgery, there is a need to identify targetable mutations as systemic chemotherapy alone has limited efficacy.

### 12.4.1 Pathophysiology of PMP and Related Biomarkers

Numella et al. studied the glycomic profiling in PMP and found that altered glycosylation

especially in the form of fucosylation is linked to the characteristic mucin production of PMP. Glycomic data of this study are available via ProteomeXchange with identifier PXD010086 [98].

Mucinous appendix cancers/PMP are characterized by abundant extracellular MUC2 protein and have distinct molecular profiles compared to their non-mucinous counterparts [99–103]. The extracellular mucin in PMP is abundant and results from *GNAS* mutations which are associated with upregulation of the *PGE2/EP4/cAMP/PKA/CREB* signaling pathway [104–106]. COX-2 is also overexpressed in PMP.

Dill et al. showed that MUC2 expression could be reduced in vitro by small molecule inhibitors targeting *EP4/PKA/CREB* molecules and celecoxib (COX-2 inhibitor), and this was mediated by reduced *CREB* transcription factor binding to the *MUC2* promoter. In their study, celecoxib (5–40 μM) reduced MUC2 expression in vitro in a dose-dependent fashion but only high-dose celecoxib (≥20 μM) decreased cell viability and induced apoptosis. Chronic oral administration of celecoxib decreased mucinous tumor growth in their in vivo PMP model via a combination of MUC2 inhibition and induction of apoptosis [107].

Grizzi et al. studied the microenvironment of PMP and found increased expression of *PTTG1*, *SCCA1*, and *2TAAs* previously associated with progression and recurrence in various human malignancies. They also studied the distribution of B- and T cells in the microenvironment and identified these as areas for future research [108].

The team led by David Morris at St. George's hospital Sydney has demonstrated that bromelain (Brom) and acetylcysteine (Ac) have synergistic activity resulting in dissolution of tumor-produced mucin (MUC1, MUC2, and MUC5AC) both in vitro and in vivo. The investigators recently published the results of their phase 1 study showing the considerable mucolytic activity of this combination as demonstrated by the volume of mucin extracted and radiological appearance. The authors concluded that this treatment should be further investigated in patients with unresectable disease [109].

### 12.4.2 Detection of Early Disease

Following appendectomy, patients with low- and high-grade mucinous neoplasms and mucinous adenocarcinomas are at a risk of developing peritoneal recurrence. There are some pathological features like tumor perforation that are predictive of peritoneal recurrence but even with these indicators, nearly half the patients who have no disease are subjected to prophylactic or second look procedures. Circulating tumor makers like CEA, CA-19-9, and CA-125 are used as surveillance markers in combination with abdominal imaging. These markers are good indicators of peritoneal disease as any amount of peritoneal disease is almost always associated with elevation of at least one of these markers. However, there are no studies looking at the sensitivity or specificity of these markers for detecting peritoneal disease. Moreover, all the three markers can be elevated in a large number of both benign and malignant conditions [110–112].

Song et al. screened expression of 2549 miRs in 3 pooled PMP patient serum samples and 3 pooled healthy controls with Agilent microarrays [113]. Their results showed a statistically significant difference in the expression levels of miR-423-5p and miR-6728-5p between the two groups and these two emerged as promising biomarkers for screening or early detection of PMP. miR-423-5p has been reported to be a promising biomarker for colorectal carcinoma, bladder cancer, heart failure, and some other diseases, but mechanisms remain unclear. MiRs are highly conserved noncoding RNAs that negatively regulate gene expression post-transcriptionally by binding to the 3′-untranslated region of target mRNAs, resulting in either mRNA degradation or translational repression [114–119]. Target genes of miR-6728-5p and miR-423-5p will be targets of research in the future.

### 12.4.3 Predictors of Recurrence and Survival in PMP

Typically, high-grade disease and high PCI are the strongest predictors of disease recurrence and lower survival in PMP.

Carbonic anhydrase (CA) II is highly expressed in most organs. In contrast, malignant cells typically express no or only low levels of CA II [120, 121]. A loss in the expression of CA II is reported to be linked to the process of malignant transformation and the progression of colorectal, hepatocellular, gastric, and pancreatic cancers [122–124].

Järvinen et al. studied CA II expression in 89 PMP patients. Positive CA II expression was found in 58 patients (65%) and absent in 31 patients (35%). High-grade (HG) morphology was associated with a loss of CA II expression ($p = 0.048$). The 5-year overall survival (OS) for those patients with CA II expression was 80% and 59% for those without ($p < 0.001$). The 5-year OS rate for those patients with high-grade morphology and positive CA II expression was 72% and 31% for those with negative CA II expression ($p = 0.044$). This study concluded that the expression of CA II acts as independent prognostic biomarker for survival in PMP [125].

### 12.4.4 *KRAS* Mutations

*KRAS* mutations are the commonest, in both appendiceal tumors (53–100%) and PMP (57–100%) [105, 126–128]. It is more frequently seen than in colorectal cancers. Some series report a higher incidence of these mutations in patients with peritoneal mucinous carcinomatosis (PMCA), whereas others have reported a similar incidence in both diffuse peritoneal adenomucinosis (DPAM) and PMCA. Most of mutations are found on codons 12 and 13 of exon 2. The difference between the lowest and highest frequencies could be due to a variation in the sensitivity of the detection method and the tumor cell percentages in the samples; most of these tumors have a low cellularity which makes analysis difficult [126, 129–132].

Shetty et al. studied the mutation of *KRAS* and P53 in patients with PMP. Of the 221 patients in the study, 64 had analysis of *KRAS* mutations. There was a higher frequency of *KRAS* mutations in patients with low-grade PMP which is associated with greater mucin production. Codon 12 was affected in 88.6% (31 of 35) of the mutations versus 11.4% (4 of 35) seen in codon 13. These findings are similar to other studies which suggest that *KRAS* mutations, specifically mutations in codon 12, appear to be associated with mucin production [127, 133].

The frequency of mutations was lower in signet ring cell carcinomas which is also seen in colorectal signet ring cell carcinomas and was not seen in patients with goblet cell carcinoids/adenocarcinoids [134–137]. The authors proposed that the mutation in these more aggressive tumors may be seen on codons other than 12 and 13 which were the only ones looked for in their study and in the commonly used commercial tests [138].

Survival analysis did not show a difference in overall survival between patients with and without *KRAS* mutations at a median follow-up of 39 months. The difference was not observed in both high-and low-grade tumors [139].

Similarly, Austin et al. reported no difference in patients with and without *KRAS* mutations though the results were not stratified according to completeness of cytoreduction [130]. Overexpression of *p53* was associated with a significantly worse overall survival.

Contrary to the above, Pietrantonio et al. reported a negative prognostic impact of *KRAS* mutation on both disease free and overall survival in 40 patients with appendiceal PMP following CRS and HIPEC [140]. Seventy-two percent of the patients had a *KRAS* mutation. *KRAS* mutation was also an independent predictor of disease free survival. Notably, next-generation sequencing was used in this study and in patients with mutations, the allelic frequency was below 10% in 55% of the patients. The *KRAS* mutant allelic fraction did not have a prognostic impact unlike colorectal cancer [141].

### 12.4.5 *GNAS* Mutations

*GNAS* encodes the α-subunit of a stimulatory G-protein (Gαs) responsible for the production of adenylyl cyclase. *GNAS* mutations cause the constitutive activation of adenylyl cyclase and an elevated cyclic AMP (cAMP) level, regardless of the presence or absence of receptor agonists [142,

143]. *GNAS* mutation promotes tumorigenesis only, not cell growth, thus leading to the indolent behavior of mucinous tumors. But it increases the expression of *MUC2* and *MUC5AC* implying the role of this pathway in mucin overproduction. However, this is not the only pathway responsible for mucin overproduction [144]. *GNAS* mutations are also seen in other tumors of the gastrointestinal tract like villous adenomas of the colorectum, pyloric gland adenomas of the stomach and duodenum, and intra-pancreatic mucinous neoplasms suggesting a preferential association with tumors having a benign or indolent behavior [145, 146]. They are rare or absent in adenocarcinomas arising from these organs [145, 147].

*GNAS* mutations are typically codon 201 mutations and are found in 40–70% of the patients with PMP and 40–77% of the patients with LAMN [148].

Nishikawa et al. reported *GNAS* mutations in 50% of their patients but none of the patients with high-grade PMP had these mutations [144]. Contrary to this, Singhi et al. found these mutations in 31% in their series of 41 patients with no difference in the incidence between high- and low-grade tumors [149]. None of the patients in this series had either *GNAS* or *KRAS* mutations in the pure signet ring cell carcinomas, which indicates that a different molecular pathway is involved in their pathogenesis. In the study by Pietrantonio et al., GNAS mutations were found in 52% of the patients and their allelic frequency was below 10% in 43% of the patients. GNAS mutations were more common in low-grade PMP and did not have a significant impact on either disease free or overall survival [140].

### 12.4.6 TP53

Mutations in *TP53*, a tumor suppressor gene on chromosome 17p, and overexpression of the protein have been described in various malignancies including CRC [150, 151].

Microsatellite instability and *TP53* overexpression are reported to be infrequent. In a retrospective study by Shetty et al., *TP53* mutation was associated with high-grade histology and a reduced survival [139, 152].

There was a significantly higher rate of *p53* overexpression (54.4%) in high-grade PMP compared with low-grade PMP (35.6%), which correlates with the comparatively aggressive behavior of the former. Other studies report a lower rate of overexpression [152]. Data regarding the overexpression or loss of *TP53* are variable in colorectal cancer. Some studies show negative impact of overexpression while others show a negative impact of loss of *TP53* [153–159].

### 12.4.7 Other Mutations and Potential Therapeutic Targets

Deregulation of *PI3K-AKT* pathway has also been implicated in the progression to PMCA [127].

Mutations of p53 gene and overexpression of the protein p53 are frequent in CRC and range from 5% to 30% in colorectal adenomas and to 50% to 75% in adenocarcinomas [126, 136]. Appendiceal neoplasms have infrequent *TP53* gene mutations and a lower rate of loss of the allele 17p, which is the location of the *TP53* gene [160].

*BRAF V600E, PIK3CA, AKT1, SMAD4,* and *APC* mutations are rare in PMP tumors, and they express mismatch repair enzymes.

Gleeson et al. performed NGS, IHC, and FISH in 54 patients with PMP and found *KRAS* mutations in 79%, *GNAS* in 73%, *SMAD4* in 18% and a low frequency of mutations in *APC, ATM, BRAF, PIK3CA, MLH1,* and *TP53* [161]. *GNAS* and *KRAS* were concurrently expressed in 38%. There was increased protein expression of *EGFR* in 83%, *cMET* in 59%, *cKIT* in 58%, and *PDGFRA* in 58%. Immune checkpoint expression was found in 36% (PD1-positive tumor infiltrating lymphocytes) and 9% (PDL1 tumor expression). There was a low rate of expression of surrogate markers of cell proliferation consistent with the slow growing nature of the tumor. *PTEN* mutations were uncommon as was microsatellite instability. Multidrug resistance protein

expression was found at high level and markers for gemcitabine, fluorouracil, oxaliplatin, and irinotecan chemosensitivity had favorable levels.

Sio et al. identified *MCL1* and *JUN1* amplification in 30% of PMP cases using next-generation sequencing assay with Illumina HiSeq2000 platform. *MCL1* is a *BCL2* family anti-apoptotic gene, and its overexpression may contribute to chemotherapy resistance to 5-fluorouracil, which is given commonly for PMP during HIPEC. *JUN* is a proto-oncogene commonly expressed in gastroenteropancreatic neuroendocrine tumors and squamous cell lung cancers. Both of these may represent novel targets for treatment in the future but require further study [148]. The common genetic mutation seen in PMP of appendiceal origin is listed in Table 12.2.

### 12.4.8 Gene Expression Profiles

Roberts et al. used exon-array analysis to study differential gene expression in tumor samples from patients with PM and compared it with the expression in normal colonic mucosa [162]. They identified 27 upregulated and 34 downregulated genes in the PMP samples which was not seen in the normal colonic mucosa, thus demonstrating that the gene profiles in PMP are different from colorectal cancer. Although their sample population was small (4 PMP samples, 3 normal colonic mucosa), their data demonstrated that gene profiles in PMP are distinct from colon cancer. For the first time, they also developed two immortalized PMP cell lines (N14A and N15A).

Levine et al. used microarray analysis to study gene expression in low-grade appendiceal primary tumors and were able to segregate patients into two risk groups based on the expression of 139 genes. The high-risk group had a significantly shorter survival compared to the low-risk group following complete cytoreductive surgery. In this work, a gene signature (139 gene cassette) was established which could prognosticate patients based on their likelihood of benefit from CRS and HIPEC [163]. Gene expression profiles in PMP are described in more detail elsewhere in this book.

**Table 12.2** Common mutations in PMP of appendiceal origin

| | Genetic mutation | Frequency |
|---|---|---|
| PMP of appendiceal origin | *KRAS* | 53–100% |
| | *GNAS* | 40–70% |
| | *MCL1 and JUN1* | 30% |
| | *SMAD4* | 18% |
| | *BRAF* | Uncommon |
| | *APC, ATM* | Uncommon |
| | *PIK3CA* | Uncommon |
| | *MSI and P53* | Uncommon |

## 12.5 Colorectal Cancer

Colorectal peritoneal metastases (CPM) are now treated aggressively with cytoreductive surgery and HIPEC. Despite this aggressive treatment, more than 70% of the patients will experience disease progression and/or recurrence. Many prognostic scores and molecular markers are known in addition to the conventional prognostic indicators in peritoneal oncology. Colorectal tumorigenesis has been well studied and follows one of three defined pathways. Many studies have been evaluating the role of biomarkers in the diagnosis, prognosis, and treatment of CPM and the effort to integrate these into clinical practice continues.

### 12.5.1 Molecular Mechanisms Underlying Colorectal Cancer (CRC)

There are three main pathways underlying colorectal tumorigenesis [164].

1. *Chromosomal instability*: This pathway correlates with loss of APC, which is typically seen in familial adenomatous polyposis (FAP).
2. *Mismatch repair defect pathway*: Inactivation of mismatch repair genes occurs in Lynch

syndrome (inherited mutation) as well as approximately 15% of patients with sporadic colorectal cancer [165]. Tumors with MSI tend to be right, are poorly differentiated, show mucin production and signet ring cells and have a better overall prognosis. These tumors are less likely to metastasize to the peritoneum, unlike microsatellite stable tumors with poor differentiation/mucin production/signet ring cells [166, 167]. These tumors are unresponsive to 5-flurouracil (5-FU) based treatment but may benefit from irinotecan [168]. Interestingly, the presence of MMR mutations is also predictive of response to pembrolizumab (programmed death-1 blocker) in colorectal cancer [169]. The frequency of MSI in peritoneal carcinomatosis from colorectal cancer is usually low but should be tested for mucinous tumors [170].

3. *Aberrant DNA methylation*: Epigenetic modifications such as aberrant DNA methylation of the CpG-rich *CpG islands* (a cytosine base is followed immediately by a guanine base) in the promoter regions are also commonly seen leading to silencing of gene expression. In sporadic colorectal cancer with microsatellite instability (MSI), epigenetic silencing blocks the expression of *MLH1* leading to mismatch repair. These patients often also have a concordant mutation in *BRAF* [171]. Also, subsets of CRC including those with MSI may have concordant methylation of multiple genes called the CpG island methylator phenotype [165, 166, 172].

The *Ras/Raf/MEK/ERK* signaling cascade is used by growth factors (e.g., EGFR) and mitogens to transmit signals from their receptors to regulate gene expression and prevent apoptosis. *RAS* and *BRAF* are two components of these pathways that are mutated or aberrantly expressed in CRC in 40% and 10% of the patients, respectively [173]. EGFR signaling is closely related to *Ras/Raf/MEK/ERK* pathway and mutation in *KRAS*, or *BRAF* downstream leads to lack of response to anti-EGFR antibodies which are known to improve survival in metastatic colorectal cancer [174, 175]. The various epigenetic changes seen in colorectal cancer are listed in Table 12.3.

**Table 12.3** Epigenetic changes associated with colorectal cancers

| Gene/miRNA | Function |
|---|---|
| *Expression inhibited by hypermethylation* | |
| *APC* | Tumor suppressor |
| *MGMT* | DNA damage repair |
| *CDKN2A/p16* | Tumor suppressor |
| *RASSF1A* | Tumor suppressor |
| *CHFR* | Tumor suppressor/checkpoint inhibitor |
| miR-34a | Tumor suppressor |
| *Expression increased by hypermethylation* | |
| COX2 | Metastases |
| SOCS1 | Signal transduction |
| ADAM23 | Metalloprotease |
| miR-21 | Invasion/metastases |

### 12.5.2 Hypermutated and Non-hypermutated Tumors

The Cancer Genome Atlas (TCGA) project analyzed 276 samples of colorectal cancer with exome sequencing, DNA copy number, methylation analysis as well as RNA and microRNA expression revealed that 16% of CRC were hypermutated (mutation rates of >12 per $10^6$). Seventy-five percent of these were MSI-H tumors, usually with hypermethylation and *MLH1* silencing, and 25% had somatic *MMR* gene and polymerase e (*POLE*) mutations. Among non-hypermutated tumors, colon and rectum cancers were found to have similar patterns of genomic alteration. A total of 24 genes were identified that had one or more mutations. Ninety-three percent of non-hypermutated and 97% of hypermutated cases had a mutation at one or more points in *WNT* signaling pathway, with *APC* gene mutation being the most common. Additional common pathways altered include *TGF-β*, *RTK-RAS*, and *PI3K* signaling pathways. New findings included recurrent mutations in *FAM123B*, *ARID1A*, and *SOX9*. Mutations and amplifications of *ERBB2* were observed in a significant percentage of patients. These discoveries carry translational significance

as *ERBB2* (*HER-2*) is a significant cancer therapeutic target with antibody trastuzumab [176].

Preclinical studies further showed that dual targeted therapy using trastuzumab plus tyrosine kinase inhibitors is effective against CRC xenografts with HER-2 amplification [177]. Based on these findings, the HERACLES trial was conducted, which showed that that the combination of trastuzumab and lapatinib is active in patients with HER2-positive metastatic colorectal cancer refractory to chemotherapy and anti-*EGFR* antibodies. Thirty percent of patients achieved an objective response in this trial [178].

## 12.5.3 Biomarkers for CPM

### 12.5.3.1 Diagnostic Biomarkers

The role of CEA, CA125, and CA19-9 as diagnostic biomarkers in colorectal PM has been evaluated in several studies [179–183]. One study showed higher levels in patients with CPM and a correlation with tumor volume. The expression differed according to the primary site and sex; however, this study compared tumor markers levels with positive imaging findings as a confirmatory finding which itself is not the gold standard for diagnosis of CPM [184].

Five studies were identified assessing the value of CEA, CA125, and CA19-9 as diagnostic biomarkers in CPM; evaluating their sensitivity and specificity for CPM diagnosis; and comparing them to conventional imaging.

Two studies were identified that evaluated the predictive value of CEA and CA19-9 (cut-off used 37.0 U/mL) in diagnosing synchronous peritoneal metastases in patients with CRC [180, 183]. Both demonstrated that elevated levels of CA19-9 were significantly associated with the presence of CPM, whereas CEA did not retain its significant value in multivariate analysis. Lee et al. also found that intraperitoneal CEA levels were significantly correlated with recurrence and peritoneal metastasis, in patients with negative peritoneal cytology, allowing for a measurement of a marker that could aid in developing stratified follow-up regimens for early detection of CPM in high-risk patients [179].

Bhullar et al. performed a systematic review and meta-analysis looking at the genetic concordance between the primary colorectal tumor and the metastatic sites and found a high concordance rate between primary colorectal cancers and their liver/lung metastases. Despite peritoneal metastases being the third most common site, they found little information on the concordance of genetic composition with the primary. Their study concluded that there was no evidence to show a benefit of performing biopsies at multiple sites versus one site provided adequate samples were taken for lung and liver metastases [180].

### 12.5.3.2 Prognostic Biomarkers

#### Tumor Markers

Seven studies found CEA to be a prognostic marker of overall survival [179, 181, 183, 185–189]. Cut-off thresholds for CEA showed marked variability between the different studies and ranged from >5 to >70 ng/mL. Despite these variable cut-offs, a CEA level higher than the specified cut-off had a negative impact on survival.

Contrary to these reports, Ozawa et al. failed to demonstrate the prognostic value of CEA in multivariate analysis but found that preoperative CEA correlates with likelihood of complete cytoreduction (CR0) [186].

#### *BRAF* Mutation

The *BRAF* phenotype in association with CPM has been evaluated in several studies [190–192]. Tumors with the *BRAF* mutation are more likely to present with peritoneal metastases and aggressive biology [193]. Sasaki et al. observed that *BRAF V600E* mutation was more prevalent in patients with CPM as compared to those without [191].

#### Platelet-to-Lymphocyte Ratio

Bong et al. and Ihemelandu et al. evaluated the platelet-to-lymphocyte ratio (PLR), and this marker was shown to be an independent prognostic factor of poor overall survival, in levels exceeding 200 and 300, respectively [188, 194]. Patients with a PLR of 150–300 had a

median overall survival (OS) of 36 months, and those with a lower value had an OS of 47 months. PLR was established to be a significant prognostic factor in predicting 5-year OS [195].

### Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) was found to be evaluated as a prognostic factor in three studies [187, 196, 197]. Chia et al. showed that lower levels of intraperitoneal VEGF at the time of abdominal cavity exploration were associated with improved overall survival in patients with CPM [187]. Two other studies showed a significant association between high tissue VEGF expression and reduced overall survival in CPM [196, 197]. Sluiter et al. also identified epithelial and stromal VCAN expression as a potential marker of improved overall survival [197].

### Molecular Phenotype

To resolve inconsistencies among the reported gene expression-based colorectal cancer classifications and facilitate clinical translation, Guinney et al. formed an international consortium dedicated to large-scale data sharing and analytics across expert groups [198].

They showed marked interconnectivity between six independent molecular classification systems that could be coalesced into four consensus molecular subtypes (CMS) with distinguishing features (Fig. 12.1):

- *CMS1 (MSI Immune, 14%)*: hypermutated, microsatellite unstable, strong immune activation
- *CMS2 (Canonical, 37%)*: epithelial, chromosomally unstable, marked *WNT* and *MYC* signaling activation
- *CMS3 (Metabolic, 13%)*: epithelial, evident metabolic dysregulation
- *CMS4 (Mesenchymal, 23%)*: prominent transforming growth factor-β activation, stromal invasion, and angiogenesis

Thirteen percent of the samples showed mixed features and were considered to represent a transition phenotype or intra-tumoral heterogeneity. The authors concluded that the CMS groups could be the most robust molecular classification and should be the basis for future clinical stratification and subtype-based targeted interventions.

Further to this, a study by Ubink et al. showed the relationship between consensus molecular subtype (CMS) and occurrence of CPM. In their study, CMS4-positive tumors were more likely to present with peritoneal metastases. The CMS-4 positive tumors were associated with a poorer response to common chemotherapy drugs like oxaliplatin [199].

## 12.5.3.3 Therapeutic Biomarkers

For a long time, different studies have been performed looking at biomarkers that can predict response to chemotherapy. Koumpa et al. in their systematic review identified eight studies evalu-

| CMS 1<br>MSI Immune | CMS 2<br>Canonical | CMS 3<br>Metabolic | CMS<br>Mesenchymal |
|---|---|---|---|
| MSI, CIMP high, hypermutation | SCNA high | Mixed MSI status, SCNA low, CIMP low | SCNA high |
| BRAF mutation | | KRAS mutation | |
| Immune infiltration and activation | WNT and MYC activation | Metabolic deregulation | Stromal infiltration, TGF-B activation, angiogenesis |
| Worse survival after relapse | | | Worse relapse-free and overall survival |

**Fig. 12.1** The consensus molecular subtypes of colorectal cancer and their characteristics. (From Ref. [198] with permission)

ating biomarkers for therapeutic monitoring in CPM [190, 196, 200–205]. The biomarkers evaluated were *ERCC1, TS, VEGF, CTGF*, and *CRC* gene expression.

In one study looking at ERCC1 and TS expression levels, there was some prediction of response but no clear relationship between response and resistance to 5FU and oxaliplatin containing therapeutic regimens [203]. This study also showed that in vitro chemosensitivity testing was more effective in predicting clinical response to treatment than these biomarkers [204].

Another study demonstrated a significant correlation between high *BRCA2* gene expression and *BLM* gene and protein expression with resistance to mitomycin C (MMC) therapy in peritoneal carcinomatosis [190].

Varghese et al. demonstrated distinct gene upregulation of *IGF1, HIF1, TIMP2, mTOH, COH17*, and *MSLN* in CPM compared to other metastatic sites. These can be used as targets for developing drugs specific to CPM [201].

Logan-Collins et al. and de Cuba et al. evaluated VEGF expression levels in patients undergoing CRS and HIPEC and found that high VEGF expression was associated with poor overall survival following treatment. VEGF emerged as a potential therapeutic target and a useful marker for identifying patients at risk for early failure [196, 202]. Chia et al. demonstrated that low preoperative intraperitoneal (IP) VEGF levels were associated with improved survival and suggest that bevacizumab, which selectively targets the VEGF receptor, could be selectively used in these patients to improve disease control [187].

Data in a study by Lin and colleagues demonstrated that connective tissue growth factor (CTGF) has a role in inhibiting colorectal cancer cell adhesion (a crucial step in peritoneal seeding), highlighting the potential to use CTGF for the development of targeted therapies that dampen cell adhesion and mitigate peritoneal seeding [203].

Shannon et al. aimed to identify preoperative predictive molecular markers that can be assessed in tumor biopsy samples as a surrogate for chemosensitivity to mitomycin C in CPM before HIPEC is performed. Three potential biomarkers were identified and optimized for IHC. Patients exhibiting lower expression of *PAXIP1* and *SSBP2* had poorer survival than those with higher expression ($p$ = 0.045 and 0.140, respectively). No difference was observed in patients with differing *DTYMK* expression. Combining *PAXIP1* and *SSBP2* in a set, patients with two dysregulated protein markers had significantly poorer survival than one or no dysregulated marker ($p$ = 0.016). This set independently predicted survival in a Cox regression model [206].

### 12.5.4 Prognostic Scores

The peritoneal surface disease severity score has been used as a method for determining the prognosis of patients undergoing CRS and HIPEC preoperatively. This score incorporates clinical symptom severity, extent of disease as peritoneal cancer index (PCI) calculated on CT scan or laparoscopy, and primary tumor histology [207]. This score was validated by a study evaluating 1013 patients with PM and showed that PSDSS was capable of defining populations with a high or considerably lower likelihood of long-term survival after CRS/HIPEC but some studies did not show a correlation with survival [208, 209]. Aronja-Sanchez et al. combined this score with the RAS mutation status. In their study of 77 patients, both RAS mutation status and PSDSS were independent predictors of survival. The combined score is shown in Fig. 12.2. Early PSDSS stages I and II associated to RAS mutations impaired their overall survival with no significant differences with PSDSS stage III overall survival ($p < 0.05$). These results were supported by the international multicenter validation [210].

Schneider et al. developed a simple point-based risk score termed BIOSCOPE (BIOlogical Score of COlorectal PEritoneal metastasis) based on PCI, nodal status, tumor grade, and RAS/RAF status, which showed good discrimination and allowed categorization of patients into four groups with strongly divergent survival outcomes [211]. The four risk groups based on points are as follows.

| Clinical symptoms | PCI | Histology | Points |
|---|---|---|---|
| None | <10 | G1; G2 N-, L-,V- | 1 |
| Mild | 10–20 | G2 N+ and/or L+ and/or V+ | 2 |
| Severe | >20 | G3; signet ring | 3 |

Clinical symptoms: Mild symptoms- weight loss <10%, mild pain, some ascites; Severe symptoms- weight loss >10%, bowel obstruction, symptomatic ascites
PCI is determined preoperatively on imaging or staging laparoscopy or surgery performed for the primary tumor in case of synchronous PM

Score stage
Stage 1- (2–3 points) + KRAS wild type
Stage 2- (4–7 points) + KRAS wild type
Stage 3- (8–10 points) + KRAS wild type
(2–10 points) + KRAS mutated
Stage 4- (>10 points) + KRAS any type

**Fig. 12.2** The modified PSDSS score combining KRAS mutation status with the PSDSS score. (From Ref. [210] with permission)

- **BIOSCOPE A** (0 risk points) that represents patients with absent risk factors (PCI <10, N0, G1–2, RAS/RAF wt). The median cancer specific survival (mCSS) in these patients in the study was 70 months and median recurrence free survival 65 months in the study.
- **BIOSCOPE B** (1–3 risk points) reflects patients with moderate risk factors; these patients had a mCSS of 50 and median RFS of 39 months, respectively.
- **BIOSCOPE C** patients (4–7 points) profit from CRS/HIPEC with a mCSS of 33 and mRFS of 25 months, which is still superior to the mCSS of 16.9 months in patients with PM treated systemically with modern targeted chemotherapy only.
- In contrast, **BIOSCOPE D** patients (>8 points) show a dismal survival of 13 (development cohort of the study) and 7 (validation cohort) months only. The authors recommended that CRS/HIPEC in these patients should be evaluated critically regarding possible complications and time for convalescence, and the decision for CRS/HIPEC should be made carefully on an individual basis.

These scores show that biomarkers alone even if associated with poor prognosis cannot preclude surgery in any patients. Common clinical and pathological prognostic indicators like PCI, complete cytoreduction, and tumor histology and grade will still have an impact and remain the main criteria for selecting patients for surgery.

## 12.6 Gastric Cancer

Over half the patients with gastric cancer (GC) develop peritoneal metastases at some time-point in the course of their disease. Given the aggressive nature of the tumor, it is often presumed that the predominant mode of spread is through the hematogenous route, transcoelomic spread has been demonstrated in gastric cancer [212]. Peritoneal metastases usually present in an advanced stage precluding aggressive surgical treatment. Neoadjuvant approaches that employ the intraperitoneal route of drug delivery like PIPAC and port directed normothermic chemotherapy have shown promising results and are being developed in addition to newer systemic therapies. Unlike colorectal cancer, the systemic chemotherapy regimens and targeted therapies are less effective in gastric cancer. Serosal involvement results in downregulation of intracellular adhesion molecules such as E-cadherin leading to

shedding of free cancer cells in the peritoneal cavity [213]. These free malignant cells can then adhere to distant peritoneal sites through adhesion molecules such as selectins and CD44, with subsequent local invasion via matrix metalloproteinases and other motility factors [214]. The genomic alterations that enable this invasive transformation have only recently been explored.

### 12.6.1 Molecular Mechanisms in Gastric Cancer

Whole genome sequencing of primary and metastatic gastric cancer has identified several somatic variations associated with peritoneal metastases [215]. Mutations in retinitis pigmentosa 1-like 1 gene (*RP1L1*), *PRB1* (BstNI subfamily), dynactin (*DCTN1*), and *HS6ST3* were all observed in both primary lesions and metastatic tumor deposits. Although *RP1L1*, *PRB1*, and *DCTN1* have no known oncologic association, *HS6ST3* is implicated in proliferation, differentiation, adhesion, and migration, and is highly expressed in chondrosarcomas [216]. In another study, more than 50% of patients with gastric peritoneal metastases had mutations in Rho-ROCK pathway components (*RHOA*, *ROCK1*, *ROCK2*, *FYN*, and *MYO9B*), which are involved in actin cytoskeleton formation, focal adhesion, and the Rho-protein signaling, suggesting that these mutations could play a role in peritoneal spread [212].

### 12.6.2 Biomarkers Related to Gastric Peritoneal Metastases

#### 12.6.2.1 Early Detection of PM: Identifying Patients at Risk

Ohi et al. studied 493 patients undergoing surgery for gastric cancer in absence of any prior therapy and identified risk factors for developing PM. Specific clinical factors, including tumor size, histopathology of biopsy sample, and tumor morphology, were significantly correlated with peritoneal metastasis. CA19-9, lymphocyte count, and NLR were also predictive factors for peritoneal metastasis. Multivariate analysis identified the clinical factors tumor morphology and histopathology, and laboratory markers CA19-9 and lymphocyte count as independent factors predictive for peritoneal metastasis. A combination of independent predictive factors achieved high predictive accuracy (0.882) for peritoneal metastasis preoperatively [217].

Chemokine genes, such as *CXCL12* and *VEGF*, have been reported to be elevated in the development of PM [218]. Takeno et al. identified a 22-gene expression profile which is associated with PM [219]. Zhang et al. reported a case of GC with matched primary cancer and peritoneal metastatic tissue, and identified several genes especially mutated in PM from gastric cancer [215].

A study by Zeng et al. demonstrated that lysophosphatidic acid levels in plasma and ascites may be useful diagnostic biomarkers for PM of gastric cancer and that higher levels are associated with poor prognosis [220].

Progastrin assay is a simple and inexpensive blood test exhibiting high diagnostic accuracy in patients with GI carcinomas, along with promising therapeutic longitudinal changes across sequential managements. Assessment of progastrin value as a multi-tumor screening assay, and as a monitoring test, is ongoing in the setting of a clinical trial in France (ClinicalTrials.gov Identifier: NCT03787056).

Chen et al. compared the mutation pattern of genes between patients of gastric cancer with PM group and without PM and identified three genes (*CDC27*, *MACF1*, and *HMCN)*, which showed moderate enrichment in patients PM group [221]. Among them, *MACF1* is a pan-cancer driver gene, which is related to cell adhesion function. Another gene *PDZD2*, which has been associated with the early stages of prostate tumorigenesis, was also altered in patients with PM.

### 12.6.3 Prognostic Markers in Patients with PM

Wang et al. sequenced the whole exome and transcriptome of peritoneal deposits from 43 patients with gastric cancer and integrated these findings

with clinical and histopathological data [222]. They found that there are some common genetic mutations seen in both the primary tumor and peritoneal metastases, whereas there are some mutations that are specific to peritoneal metastases like *CDH1*, *TAF1* mutations. Patients with peritoneal metastases had an increased proportion of "clock-like" mutational signature and decreased levels of signatures associated with defective DNA mismatch repair and *POLE* mutations. They also had an increased frequency of 6q loss and chromosome 19 gain.

This study also demonstrated the clonal and subclonal genomic architecture of PM, which revealed intra-tumor heterogeneity. Four distinct clonal patterns were identified.

Novel genomic and transcriptomic features that are associated with aggressive PM phenotypes, including higher frequency of *TP53, CDH1, TAF1* and *KMT2C* mutations, increased proportion of "clock-like" mutational signature, increase in whole genome doubling events, and chromosomal instability, particularly copy number losses, reprogrammed PM microenvironment (tumor and immune cell contents and composition), enriched signaling pathways related to cell cycle, *MYC* activation and impaired immune response were identified.

The authors further divided PM of gastric origin into two groups based on their immune profile—the T-cell "exclusive" and T-cell "exhausted" subtypes. The T-cell "exhausted" subtype showed high levels of immune checkpoint TIM-3, its ligand galectin-9, VISTA and transforming growth factor-β (TGF-β1), while other classical checkpoints were low, suggesting potential therapeutic immune targets.

Novel molecular subtypes (the "mesenchymal-like" and the "epithelial-like") by integrative clustering of the genomic/transcriptomic/immune features were identified. The "mesenchymal-like" subtype was associated with resistance to therapy, while no association was observed between the traditional histopathology-based subtypes and therapy response. The authors recommend that the molecular and not the histological subtype should be considered while making systemic therapy related decisions. The results also provided a rationale for developing targeting therapies and conducting clinical trials looking at the benefit of immune checkpoint inhibitors.

Lim et al. compared matched pairs of whole-exome sequences between primary tumors and malignant ascites of patients with gastric cancer, and demonstrated an unusually high rate of C-to-A transversions in the exomes of metastatic cells (59.4%) when compared with their primary tumors (39.3%) [212]. Patients who received systemic chemotherapy (5-fluoracil, leucovorin) for their peritoneal disease exhibited a higher proportion of C-to-T transitions (43%) when compared with their non-treated counterparts (22.2%). As a control, cirrhosis-derived benign ascites was analyzed and also demonstrated high rates of C-to-A transversions, leading to around 10 and 21 somatic mutations per patient. The authors concluded that there could exist a yet-to-be-defined process within ascitic fluid that may promote C-to-A transversions and thereby mutagenesis.

### 12.6.4 Predicting the Effectiveness of IP Chemotherapy

Kitayama et al. looked for prognostic factors for predicting response to IP chemotherapy in a study of 243 patients [223]. They compared samples of ascitic fluid or peritoneal lavage with ascitic fluid samples drawn from patients with liver cirrhosis. Cells were recovered by centrifugation of peritoneal fluids and immunostained with monoclonal antibodies to CD45 and to CD326 (EpCAM). Using flow cytometry, the number of CD326 positive and CD45 negative cells (tumor cells) and CD45 positive and CD326 negative cells (leukocytes) were calculated in $10^4$–$10^5$acquired cells, and the tumor/lymphocyte ratio (TLR) was calculated.

Median (M) of TLR of the GC patients with PM was significantly higher with those without PM and also that in patients with liver cirrhosis. Cytology positive patients with PM had a significantly higher ratio than cytology negative patients. In 37 patients who underwent repeated IP chemotherapy, TLR was markedly

decreased after chemotherapy and the response was more sensitive than the changes in Cy or mRNA of CEA. Moreover, TLR of the patients with PM before chemotherapy was significantly associated with their outcome. Median survival times (MST) of the patients whose initial TLR were <1.0%, 1.0–10%, and <10% were 765, 394, and 271 days, respectively ($p < 0.001$). The authors concluded that TLR measured with flow cytometry well reflects the relative volume of living tumor cells in peritoneal cavity and thus could be a useful biomarker to predict the prognosis as well as the effectiveness of IP chemotherapy in patients with PM.

## 12.7 Mesothelioma

Malignant mesothelioma (MM) is rare cancer that arises from the mesothelial cells that line the serous surfaces (pleura, peritoneum, pericardium, and tunica vaginalis). Only 20–33% of all mesotheliomas arise from the peritoneum itself; the pleura is the most common site of origin [224]. It is an aggressive tumor with a historically reported median survival of 10 months [225].

There is a survival benefit of CRS and HIPEC; however, eligible patients are on the lower side and recurrences are common. There is need for early diagnosis and development of effective systemic therapies.

### 12.7.1 Biomarkers for Early Diagnosis

Biomarkers for early diagnosis have been identified for malignant pleural mesothelioma. It is not known whether the same can be used for malignant peritoneal mesothelioma too.

Several markers have emerged that may have a potential role and include osteopontin, fibulin-3, Soluble Mesothelin-Related Proteins (SMRP), High Mobility Group Box 1 (HMGB1), microRNAs, peripheral blood-based markers, and Slow Off-rate Modified Aptamer (SOMAmer) proteomic assays [226].

Soluble mesothelin, also known as soluble mesothelin-related peptide (SMRP), is a glycoprotein encoded by the *MSLN* gene [227]. It is expressed on the surface of normal mesothelial cells in limited amounts and overexpressed by tumor cells in most MPM tumors and other cancers. The regulation of mesothelin expression is not fully understood; however, it has been observed that mesothelin can be shed from the cell surface and can be detected in the blood [227]. SMRP is currently the only blood-based biomarker that has been clinically validated and FDA-approved for mesothelioma [228]. Although SMRP has been validated as a diagnostic biomarker, its clinical utility is limited by its apparent poor sensitivity, with meta-analysis reporting sensitivity of 32% at 95% specificity [226]. The clinical utility of SMRP is also limited by its apparent high false-positive results.

Other markers that have been investigated are neutrophil-lymphocyte ratio, integrin, BAP-1, calretenin, caveolin-1, and P16-CDKN2A [226]. However, even for pleural mesothelioma, no marker has shown a conclusive benefit in early detection.

### 12.7.2 Molecular Mechanisms in Peritoneal Mesothelioma

*BAP1* (*BRCA1* associated protein 1) is the most commonly altered gene in peritoneal malignant mesothelioma. It is a tumor suppressor gene. The effects are mediated through chromatin modulation, transcriptional regulation, and possibly via the ubiquitin-proteasome system and the DNA damage response pathway. Individuals with germline mutations in the gene are predisposed to malignant mesothelioma and other tumors like uveal and cutaneous melanoma [229]. *BAP1* mutations can be diagnosed by loss of nuclear staining on immunohistochemistry and is used as a diagnostic tool as well. The mutational load is only a median of 1.3 mutations per million base pairs, which is much lower than other adult solid tumors. Copy number alterations are rare [230]. Other less common mutations involve *NF2*, *SETD2*, and *DDX3X* genes. *CDKN2A*

mutation which is common in pleural mesothelioma is uncommon in peritoneal mesothelioma (>60% pleural vs 8% peritoneal mesothelioma, respectively) [231]. *BAP1*, *SETD2*, and *DDX3X* genes play an important role in epigenetic regulation. These represent potential therapeutic targets using pharmacologic inhibition of epigenetic modifier enzymes like histone deacetylases (HDAC) and the histone and the histone methyltransferase *EZH2* [231].

## 12.8 Conclusions

Known and emerging biomarkers have yielded important information about disease biology and response to therapy and are being increasingly incorporated into clinical practice. The identification and clinical utilization of biomarkers specific to peritoneal metastases is increasing. Though the currently known biomarkers can determine the prognosis of patients before surgery and predict to an extent the benefit of surgery, the importance of clinical and pathological prognostic factors is not undermined as most surgical decisions are still based on these factors. Thus, biomarkers are not considered in isolation but in addition to these factors. The development of targeted therapies specific for peritoneal metastases based on biomarker expression holds promise for the future for further improving the outcomes in patients with peritoneal metastases.

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