Chapter

Mesothelioma: Overview of Technical, Immunochemical and Pathomorphological Diagnosing Aspects

Ave Minajeva and Diana Saranova

Abstract

For the clinicians with non-pathology background, first encountering the patients with pleural or peritoneal effusions, mesothelioma is only one statistically rare but clinically significant option of many differential diagnoses. This review aims to help the clinicians and broad life science audiences to understand step by step the possibilities and shortcomings of pathological diagnosing of mesothelioma, including the basic technical requirements. The first cytomorphology evaluation of pleural and peritoneal effusions in routinely stained smears enables in most cases only to identify cells suspicious for malignancy. The recent guidelines of epithelioid mesothelioma cytologic diagnosis and reporting emphasize immunochemistry (IC) in the cell blocks is mandatory whenever a diagnosis of malignancy is clinically entertained and/or cytologically suspected. The IC workup is challenging, since there is no fixed antibody panel, but multiple questions must be solved, such as 1) confirm the mesothelial or epithelial origin of isolated atypical cells and cell clusters; 2) delineate their benign or malignant nature; and 3) discriminate mesothelioma from other malignancies and metastatic disease. The rationale of the most widely clinically used IC markers is given and illustrated by the examples. The final confirmation of mesothelioma diagnosis and establishing its subtype and grade is possible only in the histological samples.

Keywords: mesothelioma, carcinoma, effusion, immunochemistry, cell block, cytology, histology

1. Introduction

Mesothelioma is a rare and malignant tumor arising from the mesothelial or submesothelial cells of the pleura, peritoneum, or pericardium. Until 2021, the term "malignant" had been used as a prefix for mesothelioma in order to distinguish it from the well-differentiated papillary mesothelioma. In the recently updated WHO Classification, this was renamed well-differentiated papillary mesothelial tumor (WDPMT), to highlight its differences from diffuse mesothelioma, the word "malignant" has been dropped [1]. Mesothelial tumor diagnoses according to the 2021 WHO Classification of

Benign and pre-invasive mesothelial tumors
Adenomatoid tumor
Well-differentiated papillary mesothelial tumor
Mesothelioma in situ
Mesothelioma
Localized mesothelioma
Diffuse mesothelioma
Epithelioid mesothelioma
Sarcomatoid mesothelioma
Mesothelioma, biphasic

Table 1.
Mesothelial tumors.

the tumors of the pleura and pericardium are summarized in **Table 1**. If not otherwise stated, most cases of mesothelioma in the literature refer to diffuse mesothelioma. There are rare benign mesothelial tumors such as adenomatoid tumor and WDPMT, only the latter will be briefly discussed in this review. Mesothelioma in situ refers to a flat noninvasive form of mesothelioma and localized mesothelioma is histologically identical to diffuse, but macroscopically solitary, circumscribed mass. Both of these are very rare, only a very few cases have been described [2, 3].

More than 80% of all diffuse mesotheliomas originate in the pleura and 10–15% are peritoneal [4, 5]. Clinical manifestations of mesothelioma are usually nonspecific and, due to a broad spectrum of differential options, can be difficult to diagnose especially in the early stage. The diagnosis of mesothelioma has to be made in the context of appropriate clinical, radiologic, and surgical findings. Because patients with mesotheliomas frequently present with effusions, sampling of pleural or peritoneal fluid for biochemical and cytological examination is often the first source of material [6–8]. The sampled diagnostic material bears limitations in pathological analysis. As cytological smear alone is insufficient for diagnosing mesothelioma, the utilization of immunochemistry (IC) must be applied to confirm both the mesothelial origin and its malignant nature, and exclude other potential mimickers such as metastatic carcinomas [8–11]. Final confirmation of the diagnosis and establishing the histological type, grade, and invasiveness of mesothelioma can be done in biopsy or operation material. Mesotheliomas are histologically divided into epithelioid, sarcomatoid, and biphasic varieties.

Current review aims to highlight the basic steps of the pleural and peritoneal mesothelioma pathological diagnosis along with most important technical handling details for clinicians and broad life science audiences. The sample figures of cytological and histological findings are from the archives of the North Estonian Medical Centre, the identity of patients remains unrevealed and the ethics committee permission is, therefore, unrequired.

2. Effusion fluid as a first-hand cytologic diagnostic material

2.1 Clinical conditions of differential significance

Mesothelioma is often but not always represented with effusion, the sampled fluid is typically exudate, yellowish, and often bloody [12]. It is reported to be thick and mucoid owing to hyaluronic acid or hyaluronan content. Notably hyaluronan and

N-ERC/mesothelin increase in effusion fluid predict mesothelioma with high specificity, prior to pathological examination. Pleural CEA increase can rule out mesothelioma with a high degree of certainty. Other soluble mesothelioma biomarkers such as C-ERC/mesothelin, osteopontin, fibulin-3, syndecan-1, syndecan-2, and thioredoxin are lacking sufficient accuracy for clinical use [13–15].

The diagnostic difficulty arises since there is a large diversity of other diseases, which can manifest with pleural or peritoneal effusions, creating an abundance of differential diagnoses to navigate in the cytological study. From a pathologist's perspective, benign infective, inflammatory, or other diseases are causing reactive changes in the mesothelial cells. Such reactive conditions manifesting predominantly with exudation can be related to tuberculous pleuritis or empyema or parapneumonic effusion caused by other bacteria, and collagen vascular diseases. Additionally, effusion can also be transudative because of hypoalbuminemia and heart or renal failure [16]. Among benign conditions causing peritoneal exudative effusion are infections such as tuberculosis or spontaneous bacterial peritonitis, whereas predominantly transudative effusion or ascites can be caused by portal hypertension due to liver cirrhosis, alcoholic hepatitis, or hepatic congestion, but also pancreatitis, hypoalbuminemia, or renal failure [17]. Reactive mesothelial cell changes can be extremely hard to distinguish from malignancy (see later). Therefore, another crucial question pathologist face is to confirm malignancy in the effusion cytology and to differentiate mesothelioma from other malignancies such as lung cancer and pleural metastasis from other organs, especially the breast [16]. In peritoneal effusions, other malignancies except mesothelioma to bear in mind are primary peritoneal papillary serous carcinoma, but more often hepatocellular carcinoma, metastatic liver disease, lymphoma with peritoneal involvement or the spread of other intra-abdominal malignancies such as pancreatic, gastric, colorectal, ovarian, or renal carcinomas [17–19]. Pathological differential diagnosis can help to identify the primary site of malignancy in a patient with a history of multiple malignancies or an unknown primary site.

2.2 Handling of material

Accuracy of pathological diagnosis heavily relies on high quality of material, which depends on its proper handling. The removed effusion is preferably sent to the laboratory fresh if possible with anticoagulants (heparin ethylenediaminetetraacetic acid or sodium citrate) present, but without added fixatives, and it should be refrigerated at 4°C until processing. When longer transportation times are needed, a volume of 50% ethanol can be added as a preservative [9].

Upon arrival in the laboratory, the fluid should be processed without delay. Refrigerated samples should be brought to room temperature, particularly when using preparation techniques associated with liquid-based cytology (LBC). To prepare a cell pellet, the material is centrifuged at 1000 g or more for 10 min. For the cytomorphological evaluation, smears are prepared from centrifuged deposits (preferably by cytospin method) and routinely stained with one of the Giemsa modifications (Romanowsky-Giemsa, Leishman-Giemsa or May-Grünewald-Giemsa kits), which enables well to examine cytoplasmic characteristics. Many labs are splitting the sample and use also Papanicolaou (PAP) stain preferably in liquid-based cytology to facilitate for nuclear evaluation [20].

The recent guidelines of mesothelioma diagnosis require additional IC studies (see later), which can be applied on smears, but the most popular technique is the cell block, obtained after the sediments from cytological specimens are processed,

formalin-fixed and embedded into paraffin blocks that can be serial sectioned and stained by the same methods used for histopathology [21].

3. Cytological diagnosis of mesothelioma

3.1 Cytological features of mesothelioma in routinely stained smears

Evaluating the cytomorphology of pleural and peritoneal effusions in routinely stained smears enables in most cases to identify malignant cells and suspicious for malignancy. In either case, to discriminate reactive proliferative mesothelium from mesothelioma and other malignancies, ancillary IC studies are required (see later). Some cases cannot be diagnosed by cytology like cases with minimal cell shedding, typically almost all sarcomatoid mesotheliomas. However, sarcomatoid mesothelioma can be overlaid by the reactive epithelioid mesothelial cells, which may readily shed into fluids and mislead the pathologist. Sarcomatoid mesothelioma can be successfully diagnosed only histologically by using core biopsy (or larger tissue samples) [21]. Since the cells in effusion are exfoliative from the tumor surface, and cytology material is lacking access to the deep structures, assessment of invasion of preexisting tissues and its correlation to the clinical and imaging findings are not possible.

Cytological features of mesothelioma are outlined in abundance for pathology specialists [9], but this information is based on histologically confirmed retrospective studies. There is significant overlap between mesothelioma, reactive mesothelial cells, and adenocarcinoma or anaplastic tumors [8, 22]. Also, a rare WDPMT has considerable cytological overlap with mesothelioma [23–25].

Figure 1 represents an example of the peritoneal fluid cytology with confirmed epithelioid mesothelioma by later histological studies. The basic general cytomorphological criteria indicating possible mesothelioma are: (1) material containing large numbers of mesothelial cells, including large ball-shaped or papillary cell aggregates with knobby outlines (scalloped borders) and (2) presence of overtly malignant cells, either as single cells or in tissue fragments [9].

The malignant mesothelial cells can be significantly larger than normal, and each of the components of the whole cell is enlarged: cytoplasm, nucleus, and nucleolus.

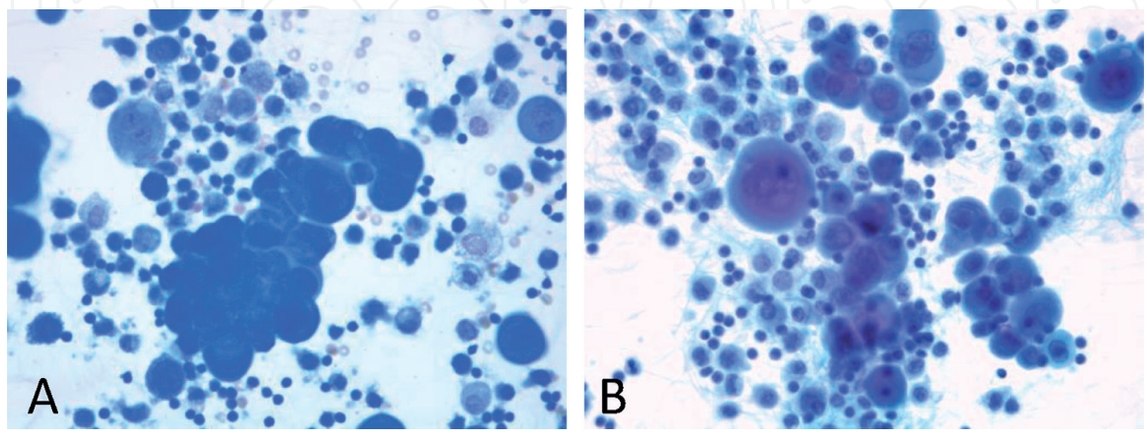

Figure 1.
Cytomorphology of the peritoneal epithelioid mesothelioma in effusion. Peritoneal effusion cytospin in epithelioid mesothelioma stained with Leishman-Giemsa (A) and Papanicolaou (PAP) stain (B), original magnification ×400. The specimen is highly cellular, containing large cell cluster (A) and papillary-shaped aggregates (B). Large mesothelial cells with macronucleoli and multinucleated cells (A and B).

The cells may be multinucleated, contain prominent macronucleoli or there are vacuoles overlapping with cell nuclei. Protrusions from the cell membrane or blebbing and prominent degree of cell-within-cell arrangements are also characteristics. Background may be acidophilic due to large amounts of hyaluronan and contain granular extracellular matrix fragments of collagen and basement membrane cores, as well as multinucleated giant cells and small pyknotic eosinophilic cells [9].

3.2 General aspects of immunochemistry

Effusion cytology work-up mostly faces discrimination of epithelioid mesothelioma since sarcomatoid subtype rarely exfoliates in the fluids. The recent guidelines of epithelioid mesothelioma cytologic diagnosis and reporting emphasize the role of IC in conjunction with the cytomorphologic evaluation because it substantially increases diagnostic accuracy [9, 21]. IC on cell blocks is mandatory whenever a diagnosis of malignancy is clinically entertained and/or cytologically suspected [21].

There is no fixed IC panel or absolute number of antibodies that can be recommended for the diagnosis of mesothelioma. Workup can be done in stages. It is recommended that a panel of at least four antibodies should be used, two in favor and two against mesothelioma. The diagnosis should never be based on one single IC reaction. Numerous antibodies for mesothelioma are commercially available, but most are not entirely specific and may show cross-reactivity with other tumors [9]. It has to be emphasized that only validated antibodies should be used for clinical diagnosis and different antibody clones have to be carefully tested with appropriate controls in the labs. If possible, antibodies should be chosen with a sensitivity or specificity of at least 80% [9]. The staining patterns (i.e., nuclear, cytoplasmic, and membranous) are important for most antibodies, and since these may differ with the new antibody clones, up-to-date information has to be followed and the tests performed with appropriate controls. There is no standard for the percentage of tumor cells that should be positive, but some have used a 10% cutoff for membranous and cytoplasmic staining [9]. IC results should be interpreted in complexity and in the context of morphological and clinical data. Of notice, the cell blocks can be also used for molecular studies, which is beyond the scope of this review.

3.3 Immunochemical workup of mesothelioma

The antibodies used for mesothelioma IC workup are largely similar in effusion cell blocks and in histological tissue blocks, however, some extra advice is added for antibody application in tissues.

The diagnosis in effusions is more challenging, comprising the following tasks: 1) confirm the mesothelial or epithelial origin of isolated atypical cells and cell clusters; 2) delineate their benign or malignant nature; and 3) discriminate mesothelioma from other malignancies and metastatic disease, which can show diffuse pleural or peritoneal spread.

Summary of the most widely clinically used IC markers will be given and illustrated by the examples in **Figure 2**. For the rest of markers, only brief references are given [8]. The paraffin-embedded cell blocks are sectioned and stained similarly to the histological specimen and, therefore, a routine hematoxylin and eosin (H&E) staining is also applied, which provides additional cytomorphological evaluation (**Figure 2A**).

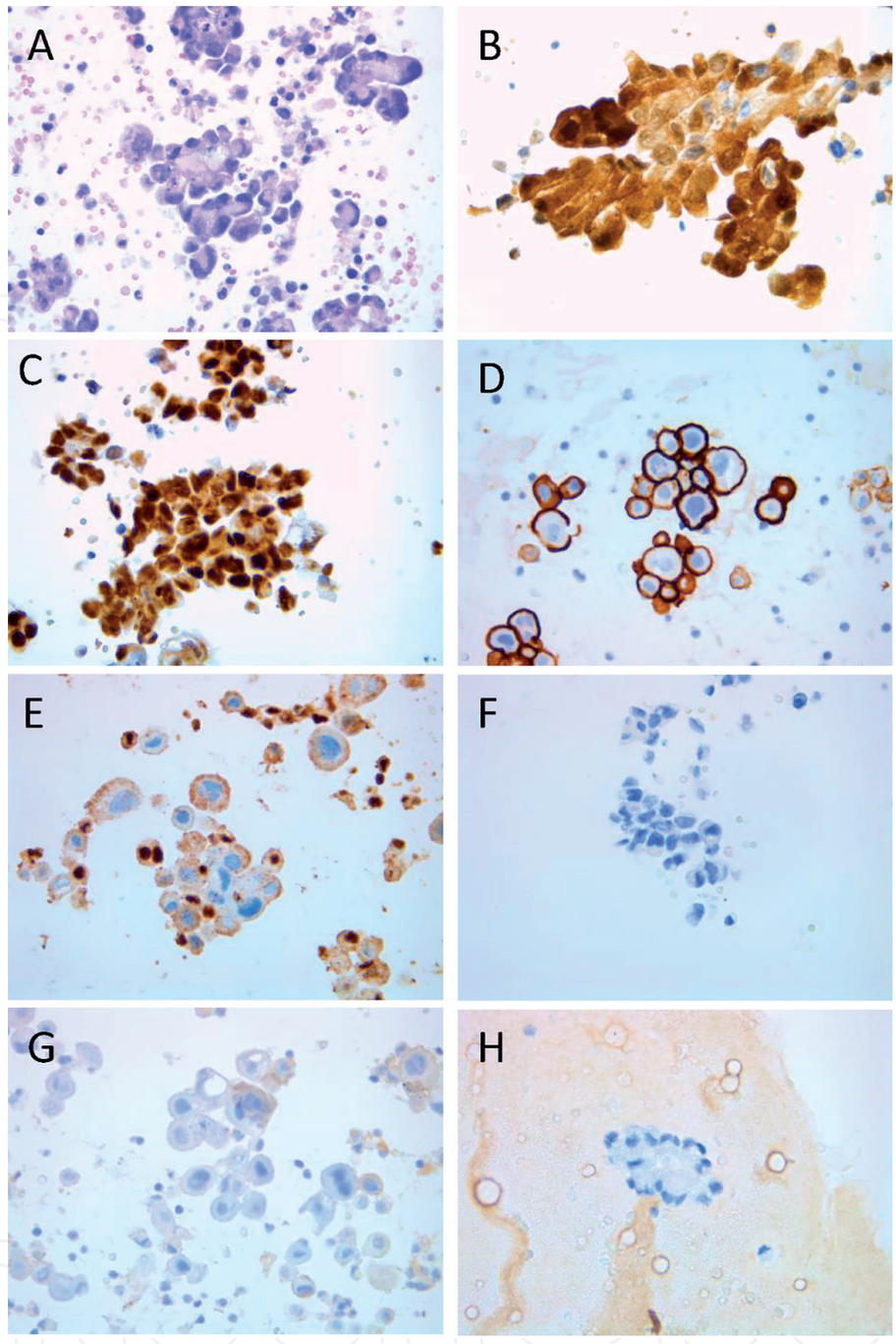

Figure 2.
Malignant mesothelioma in peritoneal fluid cytoblock. A staining panel confirming mesothelial origin, malignancy, and discriminating from gastrointestinal and gynecologic tumors. All antibodies are applied as ready-to-use (RTU) solutions, the producers are shown in the brackets. A, H&E stain to assess cytomorphology: Highly cellular specimen, enlarged atypical cell aggregates, with hyperchromatic pleomorphic nuclei and vacuolated cytoplasm could be seen (original magnification ×400). B, Calretinin expression both in nuclei and cytoplasm (Ventana, RTU, ×400). C, WT1 specific staining is nuclear (Ventana, RTU, ×400). D, D2–40 strong membranous expression (Dako, RTU, ×400). E, BAP-1 shows nuclear loss of expression in mesothelioma cells, whereas reactive mesothelial cells and background lymphocytes retain nuclear staining (BioSB, RTU, ×400). F, CEA negative (Dako, RTU, ×400). G, Ber-Ep4 negative with minimal nonspecific stain (Dako, RTU, ×400). H, CDX2 negative in mesothelioma cells (nonspecific background stain) (Dako, RTU, ×400).

3.3.1 Markers used to confirm mesothelial origin

Markers of mesothelial cells are immunoreactive with both benign and malignant cells.

3.3.1.1 Calretinin

The recent Calretinin antibodies (**Figure 2B**) require both nuclear and cytoplasmic staining to support a diagnosis of mesothelioma [26]. There are earlier reports of only nuclear staining with "fried egg appearance" [27, 28]. Cytoplasmic staining alone should be interpreted negatively [27]. In effusions, the sensitivity of calretinin in detecting mesothelioma ranges from 81 to 100% [26, 29, 30].

Calretinin can be expressed in breast carcinomas [31], and a weak cytoplasmic staining is reported in variety if other adenocarcinomas [27, 28]. Some studies have shown calretinin positivity in squamous cell carcinoma (SCC) of the lung ranging from 40 to 100% [27, 32].

3.3.1.2 Wilms tumor-1 (WT1)

Specific WT1 staining in mesothelioma is only nuclear (**Figure 2C**). WT1 frequently cross-reacts with cytoplasmic proteins in a variety of benign and malignant entities [33]. WT1 nuclear reactivity was reported in more than 90% of mesothelioma effusion specimens versus 20–30% of metastatic adenocarcinomas, particularly of pulmonary and breast origin [34–36]. In contrast, WT1 is not useful to distinguish peritoneal mesothelioma from ovarian/Mullerian tumors in effusions, since it is expressed in 80%–90% of ovarian malignancy [35, 37], and of notice, not recommended as a carcinoma-specific marker of these tumors either [8].

3.3.1.3 D2-40/podoplanin

D2–40 and podoplanin are specific lymphatic endothelial markers [38].

D2–40 immunostain shows strong membranous staining pattern in mesothelial cells (**Figure 2D**), with reported sensitivity of 83–100% and specificity of 49–100% [30, 39, 40].

Podoplanin has been shown to be even more specific than D2–40, but the number of studies is limited. Podoplanin is expressed in 94% of mesothelioma, 97% of reactive mesothelial cells, and 7% ovarian adenocarcinoma, while it is nonreactive in lung and breast adenocarcinoma, with an overall sensitivity and specificity of 94% and 97%, respectively, for mesothelioma [38]. While podoplanin showed strong membranous reactivity in mesothelioma cells, ovarian adenocarcinoma exhibited weak membranous staining [38].

3.3.2 Markers differentiating benign from malignant mesothelial proliferations

Many of the markers supposedly differentiating mesothelioma from benign reactive mesothelial cells have limited sensitivity or a too broad spectrum of reactivity. For example, relevance of EMA, p53, IMP-3, CD146, or glucose transporter 1 in defying benign and malignant cases is questioned, especially in histology materials [21].

3.3.2.1 BRCA1-associated protein (BAP1)

BAP1 is a nuclear ubiquitin hydrolase involved in various cellular processes, including chromatin remodeling. *BAP1* behaves as a true tumor suppressor gene.

BAP1 double-hit inactivation is a key driver event in about half of all mesotheliomas [41, 42]. Loss of BAP1 expression by IC can be a useful adjunct to distinguish mesothelioma from reactive mesothelial proliferations in some cases [43]. However, BAP1 is not very sensitive, with a reported loss of nuclear staining only in 27–57% of mesothelioma but in none of the reactive mesothelial cells [41, 42]. For correct interpretation, only nuclear loss of staining is accepted as true loss of expression [8]. Reactive mesothelial cells and background lymphocytes should express nuclear staining and can serve as internal control (**Figure 2E**).

BAP1 use has more limitations since it is preserved in many non-mesothelial malignancies, frequently encountered in effusion cytology, and BAP1 loss may be also encountered in other malignancies rarely seen in effusions such as malignant melanoma and urothelial carcinoma [44].

3.3.2.2 Enhancer of zeste 2 homolog (EZH2)

EZH2 is a member of the family of polycomb group genes (PcGs), which is a group of important epigenetic regulators that repress transcription. BAP1 loss can promote cell proliferation *in vitro* through up-regulation of EZH2 [45]. High EZH2 expression was observed in 66% of malignant mesothelioma cases, whereas none of the benign lesions showed high EZH2 expression. The combination of BAP1 loss and high EZH2 expression as markers to differentiate epithelioid/biphasic malignant mesothelioma from benign mesothelial lesions was highly sensitive (87–90%) and specific (100%) [46, 47]. Using IC alone for EZH2 also yielded a good sensitivity of 86.9%; this level is high enough for routine diagnostics [47].

3.3.2.3 Methylthioadenosine phosphorylase (MTAP)

MTAP is located in the 9p21.3 locus and is often deleted with p16. Detection of homozygous deletion of the 9p21.3 region by p16-fluorescence in situ hybridization is a reliable marker for malignancy in mesothelial effusions. MTAP IC has been suggested as a good surrogate marker for 9p21.3 deletion in surgical and cytology specimens [48]. The association of MTAP and BAP1 IC staining loss can reportedly detect mesothelioma with 78% sensitivity [49]. Only cytoplasmic loss of MTAP should be interpreted as a true loss of expression [48, 49].

3.3.2.4 Desmin

Since benign mesothelial cells express desmin, reactive proliferative mesothelial cells also express desmin in 84%–92% cases, whereas mesothelioma cells only in 0%–6% [30, 50]. Mesothelial cells tend to lose their cytoplasmic desmin expression as they transition to malignancy [22]. Attention has to be paid that any malignant effusion with mesothelioma still has few background reactive mesothelial cells which still are expressing desmin.

3.3.2.5 Epithelial membrane antigen (EMA)

EMA is expressed in adenocarcinoma with a very high sensitivity 91%–100% and a specificity of 86%–100% in differentiating adenocarcinoma from reactive mesothelial cells in effusions [51, 52]. EMA has distinctive staining of the cytoplasmic

membrane brush border in mesothelioma, while it exhibits a diffuse cytoplasmic staining pattern in carcinomas [53].

3.3.3 Carcinoma markers

Due to close morphological resemblance, mesothelioma most often has to be differentiated from adenocarcinoma, but depending on location, many other types of carcinoma may be considered diagnostically important. The IC markers are serving two purposes: 1) distinguish broadly carcinoma cells from mesothelial malignancy and 2) differentiate carcinomas of a specific type or location.

3.3.3.1 Carcinoembryonic antigen (CEA)

CEA is a recommended marker for discriminating between mesothelioma and adenocarcinoma in effusions [54] (**Figure 2F**). It has a high reported specificity (90%–100%) and variable sensitivity (43%–100%) [54, 55] in detecting adenocarcinoma in effusions and exhibits a strong membranous staining pattern [55]. Monoclonal CEA antibody is more commonly used in effusions and generally preferred over polyclonal antibody to avoid the nonspecific staining in background inflammatory cells [8]. CEA is less specific in tissue sections as carcinomas of various origins and well-differentiated neuroendocrine tumors are negative with monoclonal CEA antibodies on tissue sections [56].

3.3.3.2 Claudin-4 (CL-4)

CL-4 belongs to a family of tight junction-associated proteins expressed in most epithelial cells but absent in mesothelial cells. CL-4 is a useful pan-carcinoma marker for serous effusion specimen, showing strong diffuse membranous expression pattern in 84%–96% adenocarcinomas and being negative in most mesotheliomas [57, 58]. CL-4 is useful also in tissue sections, where it has been expressed in 91% of carcinomas of different types and negative in mesothelioma [57]. CL-4 has a sensitivity of 85%–99% and specificity of 99%–100% in distinguishing carcinoma versus mesothelioma [57–61]. CL-4 is also very useful in detecting single tumor cells dispersed among heavy inflammatory reactions [61] or metastatic epithelial cells in serous effusions [8, 57, 61].

3.3.3.3 Ber-EP4

Ber-EP4 is an epithelial cell adhesion molecule (TACSTD1) that shows a predominantly membranous pattern [55]. Mesothelial cells are shown negative for Ber-EP4 in most studies (**Figure 2G**) [8]. Ber-EP4 has a sensitivity of 76%–94%, and specificity of 84%–100% in detecting adenocarcinoma [8, 51, 54, 55]. It is also reportedly positive in 87%–100% of SCC cases [8, 32].

3.3.4 Additional markers for organ/differentiation specific differentiation

In addition to general carcinoma markers, many antibodies can be helpful for detecting specific differentiation of cells and distinguishing mesothelioma from other malignancies in specific settings. **Table 2** summarizes some of their most common applications [7, 8, 62].

	Antibodies for organ-specific differentiation of mesothelioma
Lung adenocarcinoma	TTF1, Napsin A
Breast	GATA3, ER, PR, mammoglobin, GCDFP15
Thyroid	TTF1, Pax8, thyroglobulin
Squamous cell carcinoma	p40, p63, CK5/6
Renal cortical	Pax8, Pax2, CA9, RCC
Mullerian/ovarian origin	Pax8, Pax2, WT1, BerEP4, ER
Colorectal	SATB2, CDX2
Liver	HepPar1, Arginase-1, AFP
Prostate	NXK3.1, PSMA, PSA
Urotelial	p63, p40, GATA3
Malignant melanoma	SOX10, HMB45, S100, MART1, MITF
Hematopoietic	CD45, CD43, CD3, CD20, CD34, CD117, TdT

Table 2.
Additional immunostains used for organ-specific differentiation of epithelioid mesothelioma.

4. Histological sampling and typing of mesothelial tumors

4.1 General considerations of histological diagnostic material

Tissue sampling is currently achieved either by image-guided/thoracoscopic-guided or surgical biopsy, both of which are recommended by major guideline committees. Surgical biopsies in principle generate more tissue materials, occasionally as much as pleural decortication and extrapleural pneumonectomy.

Biopsies comprise too little tissue and are known to suffer from sampling bias. Microscopically, tissue fields from pleural and peritoneal cavities are often obscured by inflammation and fibrinous debris. Subpleural or intraperitoneal fat sampling, important in the assessment of invasion, may be absent in cases of significantly thickened pleura or peritoneum. False-positive immunostaining may be seen in tiny needle biopsy specimens with crushed artifacts and at the edges of biopsy samples [21].

Larger materials give better overview, especially of intra-tumoral heterogeneity and invasion, but to get these results, the materials should be sampled extensively. The histologic diagnosis should be based on both the appropriate morphology and on IC findings.

4.2 Well-differentiated papillary mesothelial tumor (WDPMT)

WDPMT is a relatively uncommon subtype of mesothelial neoplasm with a distinct molecular profile [63] and histological appearance [25, 64]. It arises most commonly in the peritoneal cavity, but can also be found in the pleural cavity, pericardium, and tunica vaginalis [25, 64, 65]. WDPMT typically exhibits indolent behavior and is generally considered of low malignant potential [64].

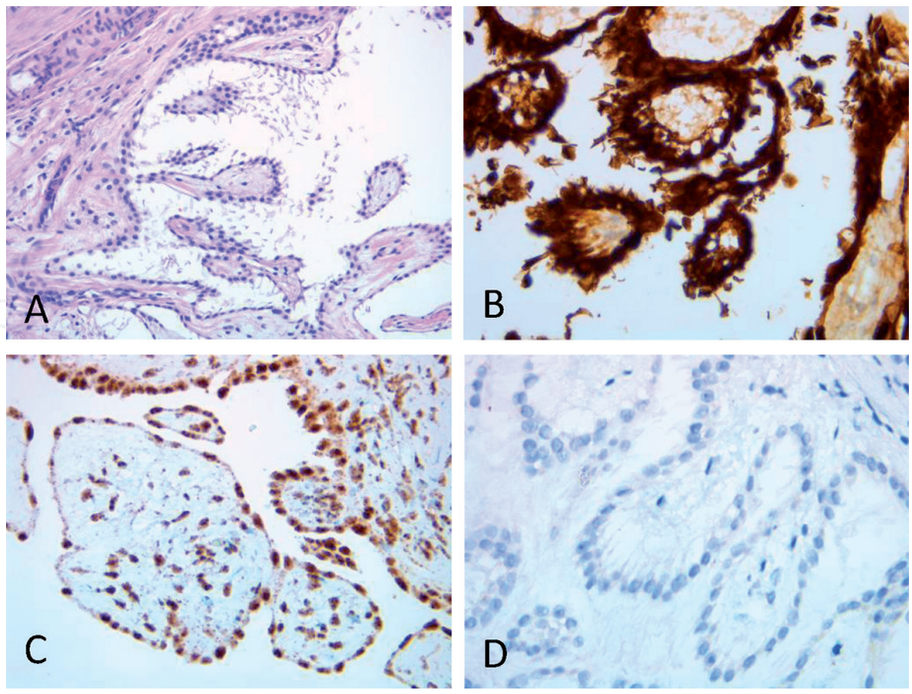

Figure 3.
Peritoneal well-differentiated papillary mesothelial tumor histology. A, H&E stain shows fibrovascular papillae lined by a simple uniform cuboidal epithelium, without nuclear atypia or mitoses (original magnification ×400). B, Calretinin expression both in nuclei and cytoplasm of lining epithelium confirms its mesothelial origin. The lining epithelial cell has enlarged appearance due to very intense staining (Ventana, RTU, ×400). C, BAP-1 expression is retained and shows uniform nuclear expression confirming benign nature of lining mesothelial cells (BioSB, RTU, ×400). D, PAX8 negativity helps to differentiate the serous neoplasms of ovaries and peritoneum (Abcam, 1:200, ×400).

Histologically, WDPMT usually has an architecture of fibrovascular papillae, lined by a simple uniform cuboidal epithelium, with little to no nuclear atypia or mitoses (**Figure 3A**). Areas of invasion are typically not seen [64, 66]. The lining epithelium bears immunochemical profile of mesothelium, showing nuclear and cytoplasmic positive expression of calretinin (**Figure 3B**). BAP-1 staining is particularly helpful as retained nuclear expression shows benign nature of lining epithelial cells (**Figure 3C**). Great care should be taken to differentiate WDPMP from serous neoplasms of the ovaries and peritoneum, where IC markers, for example PAX8, are highly useful (**Figure 3D**) [23].

4.3 Diffuse mesothelioma histological diagnosis

Examples of diffuse mesothelioma histological types are illustrated in **Figure 4**. Epithelioid mesothelioma comprises approximately 80% of all pleural mesotheliomas and is defined as being composed of epithelioid, rounded, or polygonal cells [1, 62, 67]. Epithelioid mesothelioma can have various architectural patterns depending if the cells are located in solid sheets or form tubular, papillary, adenomatoid, and trabecular patterns [62, 67]. Sarcomatoid mesothelioma is the second most common subtype, composed of elongated spindle cells arranged in solid sheets or within fibrous stroma [62, 67]. Biphasic mesotheliomas are composed of both epithelioid and sarcomatoid components and at least 10% of each component is required for definite diagnosis in resection specimen. Regardless if a diagnosis is made in biopsy or extended operation material, sarcomatoid components should be reported and quantified in the pathology report, because it influences the treatment and prognosis.

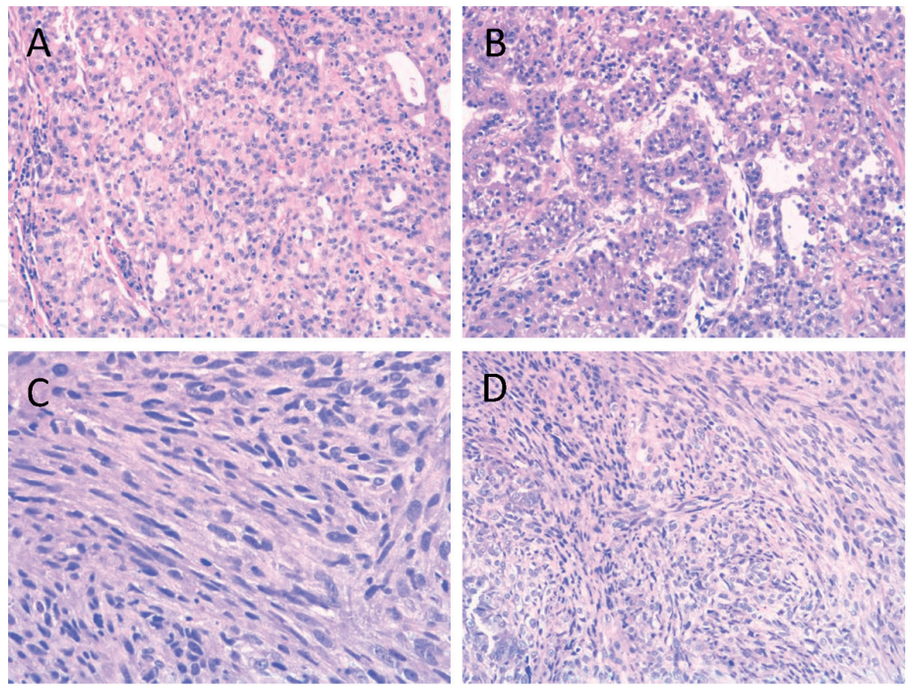

Figure 4.
Diffuse pleural mesothelioma histological subtypes. A, epithelioid mesothelioma is composed of rounded cells with eosinophilic cytoplasm and round nuclei with small nucleoli. In this tumor, the cells are located mostly in solid sheets with few gland-like structures (H&E stain, original magnification ×200). B, epithelioid mesothelioma architectural patterns may comprise trabecular, tubulopapillary, and gland-like structures (H&E, ×200). C, Sarcomatoid mesothelioma pattern is characterized by malignant elongated spindle-shaped cells (H&E, × 400). D, diffuse biphasic mesothelioma, which shows both epithelioid and sarcomatoid malignant areas (H&E, ×200).

IC is essential in establishing a diagnosis, and the choice of antibodies, particularly carcinoma markers, depends on histological architecture, and also whether the tumor has a pleural or peritoneal location. In pleural location, lung adenocarcinoma, SCC, and breast carcinomas are the most frequent differential diagnoses, but metastases from a variety of other organs could be confused with epithelioid mesothelioma. The case of pleural epithelioid mesothelioma presented in **Figure 5**, presence of psammoma bodies along with few papillary areas required an extended panel for testing ovarian serous carcinoma and gastrointestinal carcinomas (not shown), all of which were negative. Peritoneal mesotheliomas most often need to be distinguished from gastrointestinal, renal, and ovarian malignancies.

Epithelioid mesotheliomas are graded using a two-tiered system (low and high grade), combining nuclear grade (mitotic count and nuclear atypia) and presence of necrosis, because these features have been demonstrated to be strongly predictive of survival in patients with epithelioid mesothelioma [1, 62].

Sarcomatoid mesothelioma should be distinguished from metastatic sarcomatoid carcinomas from lung and other sites, particularly renal carcinomas [62]. Differential diagnosis can be challenging because markers can overlap, and will not be fully reviewed here. Immunochemical profile of sarcomatoid mesothelioma is different from the epithelioid. Sarcomatoid mesotheliomas are at least focally positive for cytokeratins AE1/AE, pan-cytokeratin (OSCAR), and anti-cytokeratin clone 1(KL1), as well as cytokeratin CAM5.2 [62, 68]. But sarcomatoid mesotheliomas can be cytokeratin-negative. Sarcomatoid mesotheliomas are positive for mesothelial

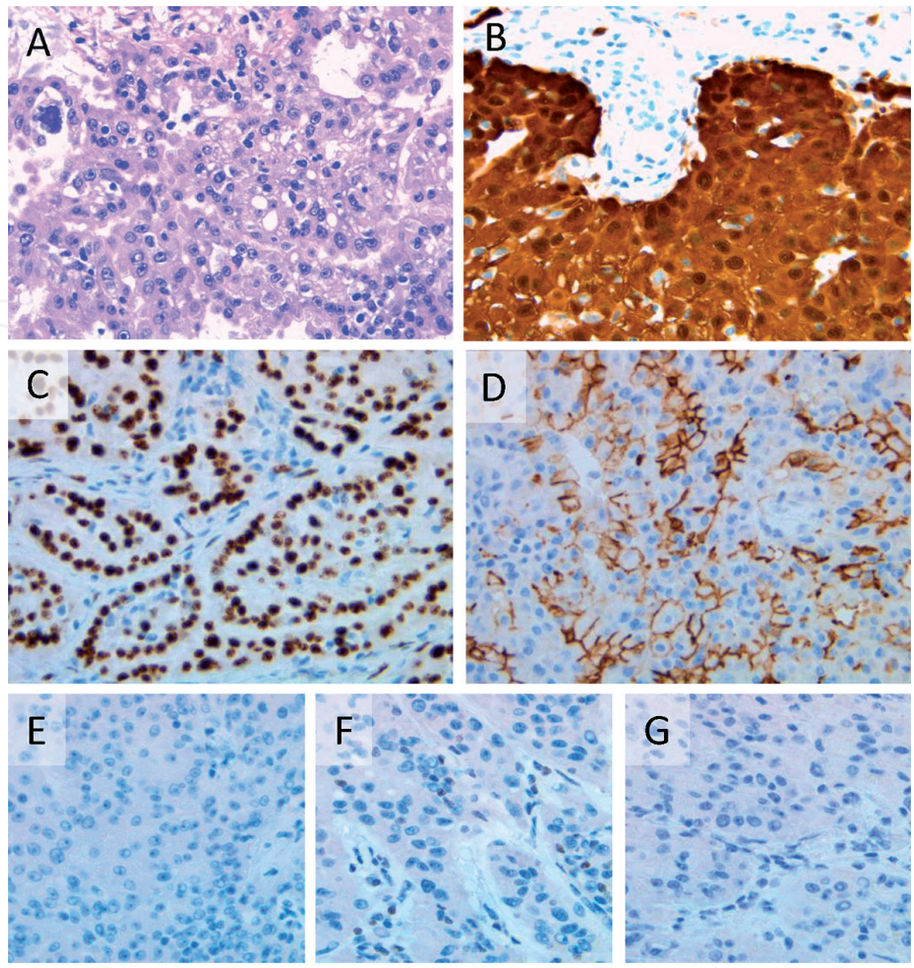

Figure 5.
Pleural epithelioid mesothelioma histology. A, H&E stain shows tubulopapillary mesothelioma structures. Tumor cells display moderate eosinophilic cytoplasm, mostly round nuclei with vesicular chromatin and small nucleoli. Psammoma body is seen in upper left corner (original magnification ×400). If concentrations are not indicated, antibodies are applied as ready-to-use (RTU) solutions. B, Calretinin diffuse expression both in nuclei and cytoplasm of malignant cells (Ventana, RTU, ×400). C, WT1 positive expression in all mesothelioma cell nuclei, but negative in fibrous stroma (Ventana, RTU, ×400). D, D2–40 strong membranous expression in most of the mesothelioma cells (Dako, RTU, ×400). E, TTF1 negativity in mesothelioma cells differentiates it from adenocarcinoma of the lung (Ventana, RTU, ×400). F, GATA3 negativity in mesothelioma cells differentiates it from breast carcinoma. Weak positivity is seen in the nuclei of lymphocytes (Ventana, RTU, ×400). G, PAX8 negativity in mesothelioma cells to differentiate from serous ovarian carcinoma (Abcam, 1:200, ×400).

markers such as calretinin, WT1, and D2–40 in limited cases [62, 68]. Sarcomatoid mesotheliomas are often vimentin-positive, whereas epithelioid mesotheliomas are often negative to vimentin. Occasionally, sarcomatoid mesotheliomas express actin, desmin, or S100 [62].

5. Conclusions

Diagnosing mesothelioma is a stepwise process, requiring complex orientation in a vast spectrum of clinical conditions and their corresponding pathological morphological criteria along with immunochemical proof. It needs careful individual decisions for applying ancillary studies and drawing proper conclusions considering the limitations of each diagnostic specimen.

Conflict of interest

The authors declare no conflict of interest.

Author details

Ave Minajeva[1*] and Diana Saranova[2]

1 Department of Pathological Anatomy and Forensic Medicine, University of Tartu, Institute of Biomedicine and Translational Medicine, Tartu, Estonia

2 Pathology Department of the North Estonia Medical Centre, Tallinn, Estonia

*Address all correspondence to: ave.minajeva@ut.ee

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