

United States and Canadian Academy of Pathology
94th Annual Meeting, San Antonio, Texas, 2005

Society for Ultrastructural Pathology
Companion Meeting
Sunday, February 27

Extrathoracic Mesothelial Proliferations and Their Mimics

Josep Lloreta-Trull

Department of Pathology

Hospital del Mar, Universitat Pompeu Fabra, Barcelona, Spain

INTRODUCTION

Mesothelial proliferations occurring in extrathoracic sites share many epidemiological and morphological features with their thoracic counterparts. However, they also have important clinical and pathological differences, relating to their anatomical location, the organs and tissues they may involve, and the spectrum of non-mesothelial tumors that may arise in these regions. The application of electron microscopy (EM) and immunohistochemistry (IHC) to their differential diagnosis must, therefore, take these peculiarities into consideration. This review is focused on mesothelial neoplasms arising in the peritoneum and in the testis and paratesticular structures, as well as on the main tumors that may be confused with them.

EM AND IHC IN THE IDENTIFICATION OF MESOTHELIAL DIFFERENTIATION: ADVANTAGES, PITFALLS, AND LIMITATIONS

EM has been the classic, gold standard tool used to confirm or identify mesothelial differentiation. Highly sensitive and specific diagnostic clues include the characteristically long and tortuous, occasionally dichotomized microvilli, with scanty filamentous cores, well-developed desmosomes, and tonofilament bundles [1-5]. In contrast, adenocarcinomas have much shorter, slender microvilli, with denser filamentous cores, forming core rootlets in the apical cytoplasm. These features are characteristic of gastrointestinal and other mucinous adenocarcinomas, but they can be less prominent in other sites, such as the breast, thyroid, urinary bladder, prostate or endometrium. Two well known disclaimers of EM are that it does not allow the identification of cells as neoplastic, nor does it distinguish between benign and malignant tumor cells. In addition, careful correlation with light microscopy is essential, because reactive mesothelial cells, admixed with metastatic tumor cells, may be erroneously identified as the neoplastic population. Keeping all this in mind, distinguishing between mesothelioma and adenocarcinoma by EM is a relatively easy task. However, prospective studies, which address the diagnostic accuracy of EM through a long series of unselected, clinically well-documented mesotheliomas, are lacking. Specific diagnostic, ultrastructural features are gradually lost in

less-differentiated, solid, or sarcomatoid varieties; thus, they require careful sampling and an extensive search. Nevertheless, and in spite of the lack of quantitative data, it can be stated that EM allows the identification of mesothelial differentiation, either focal or widespread, in most cases [1,4,6].

There is a plethora of reports dealing with the application of IHC to the differential diagnosis of mesothelial proliferations, however the ideal antibody or combination of antibodies has not yet been found [7-23]. Due to the great variation from one study to another, it is difficult to obtain precise figures on the sensitivity and specificity of the antibodies used in the differential diagnosis between mesothelioma and adenocarcinoma. A recently developed website, contains information on the application and sensitivity of many antibodies, pooled and averaged from many references (www.immunoquery.com). Table 1 is the result of a search on this website, regarding the overall sensitivity of the antibodies most commonly used in the diagnosis of mesothelioma. Some antibodies show a high sensitivity, but then their specificity tends to be less optimal. Thus, while AMAD-2 has 100% sensitivity, according to the laboratory where this antibody was originally synthesized, it also stains around 10% of the pleural metastases [24]. Currently, calretinin, thrombomodulin, WT1 gene product, and keratin 5/6 are considered the best antibodies for the identification of mesothelial differentiation [16]. It is generally advised that, in addition to positive “markers”, the panel should also include antibodies which should be negative in mesothelial cells and which may be positive in adenocarcinoma, particularly carcinoembryonic antigen (CEA), Ber EP4, Leu M1, MOC31 or B72.3 [13-15]. Many other antibodies have been used in the past, and new antibodies are being tested, but the definitive optimal markers are yet to be identified. Thus, D2-40, a recently developed antibody for germ cell neoplasia and lymphatic endothelium, has been shown to be positive in around 96% of the mesotheliomas and reactive pleural lesions, but it is also positive in 65% of the tested ovarian serous carcinomas [25]. Most studies, dealing with the diagnostic value of putative mesothelial markers, fail to include EM as their gold standard [26]. Thus, the diagnostic accuracy of new antibodies is compared with that of the old ones without an external control method to confirm the diagnosis. EM is not a generally available technique but it should be included, whenever

possible, in the work-up of peritoneal and testicular tumors, particularly in uncommon tumors or in cases with negative or paradoxical immunohistochemical results. As a general approach for the diagnosis of most peritoneal mesotheliomas, Ordóñez has suggested a panel of two positive (calretinin and cytokeratin 5/6) and two negative (CA19-9 and MOC-31) antibodies. When this panel fails to solve the case, he prefers EM, rather than a second panel of antibodies, to reach the correct diagnosis [16].

These general remarks apply to all mesothelial proliferations, but the situation is further complicated in peritoneal and testicular sites, due to the presence of embryologically related, but distinct, epithelial components. Thus, the so-called secondary Müllerian system and the epithelium covering the testicular appendages form a continuum with the respective mesothelial linings. This explains the occurrence of tumors with non-mesothelial features in these serosal surfaces. Some of the main antibodies used to identify mesothelial differentiation in the pleura may be positive in tumors derived from abdominal organs or the testis (Table 2), and therefore specific immunohistochemical strategies should be designed to address the differential diagnosis of extrathoracic mesotheliomas. In many of these cases, contribution of EM may be crucial to attain the correct diagnosis.

TABLE 1. SENSITIVITY OF ANTIBODIES COMMONLY USED FOR IDENTIFYING MESOTHELIAL DIFFERENTIATION

AMAD-2	100%
CD44H	91%
Mesothelin	90%
Cytokeratin 5/6	85%
Calretinin	84%
N-Cadherin	81%
HBME-1	80%
WT1	67%
Thrombomodulin	64%
EMA	56%

(Retrieved from www.immunoquery.com)

TABLE 2. MESOTHELIAL ANTIBODIES THAT MAY BE POSITIVE IN NON-MESOTHELIAL ABDOMINAL AND TESTICULAR TUMORS.

Calretinin	Steroid secreting tumors (adrenal, sex cord)
WT1	Serous carcinoma (ovary)
Thrombomodulin	Urothelial tumors
HBME-1	Serous tumors (ovary, peritoneum, testis)
CD10	Renal cell carcinoma. Endometrial tumors
D2-40	Serous carcinoma (ovary)

NORMAL MESOTHELIUM AND OTHER PERITONEAL AND TESTICULAR LINING CELLS

Normal Mesothelium

During embryonic development, cells of mesodermal origin line the celomic cavity. Similar to epithelial cells, they cover surfaces, contain keratins, and are joined by desmosomes and tight junctions. However, these are truly hybrid cells because, in addition, they secrete hyaluronic acid, an important component of the intercellular substance of connective tissue also produced by fibroblasts, and their cytoskeleton contains vimentin. The celomic cavity will later be divided into a pleural and pericardial cavity, a peritoneal cavity, and, in the male, a derivative of the latter that will give rise to the tunica vaginalis, upon migration of the testis to the scrotum.

In all of these serosal areas, there are two different populations: mesothelial and sub-mesothelial cells. The former show all of the characteristic features, the long, tortuous, often branching microvilli, well-developed desmosomes and tight junctions, tonofilaments, abundant cytoplasmic glycogen, and basal lamina. They express several keratin antibodies (5/6, 7, AE1-AE3), vimentin, HBME-1, calretinin, and thrombomodulin, among many others. Interestingly, normal and reactive mesothelial cells may also express desmin [27]. The sub-mesothelial connective tissue contains a mixture of fibroblasts (vimentin, CD34) and mesothelial spindle cells (keratin and vimentin).

Secondary Müllerian System

The female reproductive system arises from the müllerian or paramesonephric duct. Under the influence of the wolffian or mesonephric duct, the paramesonephric duct develops in both sexes from a placode-like thickening and deepening of the celomic epithelium. This celomic epithelium gives rise to the surface epithelium of the ovary and to the so-called extraovarian peritoneum [28-30]. Thus, the concept of the secondary müllerian system implies that a certain potential towards müllerian differentiation is retained by the peritoneum in the adult. This potential may be revealed under endogenous or exogenous

hormonal stimuli, mostly in women, but also, exceptionally, in men [29,30]. This notion has been considered a histogenetic link between the normal peritoneum and the peritoneal surface tumors, showing clinical and morphological features similar to their ovarian counterparts.

Testicular Appendages

The appendix testis or hydatid of Morgagni, the appendix epididymis, the vas aberrans, and the paradidymis constitute the so-called testicular appendages [31,32]. The appendix testis is a remnant of the müllerian duct found in more than 90% of normal individuals, and as such, it is covered by a cylindrical or cubic epithelium that may contain some ciliated cells. The appendix epididymis is present in around 25% of all testes, and it almost always has a cystic structure and a columnar epithelial lining. The paradidymis and the vas aberrans are related structures, also with a columnar lining around a cystic space. Out of all of these structures, the hydatid of Morgagni represents the normal counterpart that explains the occurrence of müllerian tumors in this area.

REACTIVE MESOTHELIAL HYPERPLASIA

Irritation of serosal surfaces, with or without a subsequent effusion, induces the proliferation of mesothelial, and often sub-mesothelial cells, giving rise to a variety of hyperplastic appearances. In some instances, the degree of hyperplasia may be so severe as to suggest a malignant process, particularly when examining small biopsies [28]. Criteria for this differential diagnosis have been dealt with extensively in the literature, and are mostly based on light microscopic features [33]. The invasion of intraperitoneal organs or fat is the most reliable indicator for malignancy, but this may be mimicked by mesothelial cells entrapped in organizing granulation tissue or between fat lobules. These benign cells usually arrange themselves in a more orderly fashion and parallel to the peritoneal surface, and they are associated with perpendicular blood vessels and abundant histiocytes, but all manner of exceptions can occur. The clustering of mesothelial cells on the peritoneal surface is usually observed in mesothelial hyperplasia, but when it is organized in papillary structures or cohesive, grossly apparent nodules, malignancy should be suspected. On the other hand, mesothelial cell clusters within the stroma, particularly if arranged in cords, nodules, papillae or gland-like structures, are highly suspicious for malignancy, but reactive, entrapped mesothelial cells may also focally present with this appearance. Necrosis and nuclear atypia are more often seen in mesothelioma, but atypical cells are quite common in reactive mesothelium, and necrosis of these benign cells may occasionally occur [33,34].

IHC or EM is of little help in this differential diagnosis. Although desmin expression is more often observed in reactive mesothelial cells, it may also, very rarely, be found in mesothelioma [27,35]. An admixture of mesothelial cells with CD68-positive macrophages is characteristic of reactive mesothelial hyperplasia, but the presence of macrophages cannot be taken as evidence for the benign nature of a mesothelial proliferation [36]. p53 overexpression and EMA positivity are more often found in malignant, rather than in reactive mesothelium, but they are not helpful in individual cases [28]. As stated above, EM is an excellent tool for the study of cell differentiation, but it cannot be used to determine the neoplastic, either benign or malignant, nature of cells [34].

MESOTHELIAL TUMORS AND TUMOR-LIKE CONDITIONS

Nodular Mesothelial Hyperplasia

This is a reactive condition presenting mostly, but not exclusively, in male children, as one or several nodules that may be grossly identified in hernia sacs [37]. The nodules are composed of polygonal cells, which may be moderately pleomorphic, showing low mitotic activity and accompanied by hyperplasia of the surface mesothelium. These lesions have been shown to be mostly made up of histiocytes, with a minor component of entrapped mesothelial cells. Due to this fact, it has been suggested that the term nodular histiocytic hyperplasia is more adequate [36]. The occasional presence of multinucleated and occasionally elongated cells may lead to confusion with embryonal rhabdomyosarcoma in pediatric cases. On the other hand, these nodules may contain a variable proportion of vacuolated histiocytic cells, which may be wrongly interpreted as signet-ring cell carcinoma.

Adenomatoid Tumor

Adenomatoid tumor is a benign, usually well-circumscribed proliferation of mesothelial cells, arranged in tubules, cords and gland-like spaces. It is the most common neoplasm in the epididymis [38-40]; it is also relatively common in the Fallopian tube and uterus [41], and has been reported in other locations, including the spermatic cord, ejaculatory duct, ovary, adrenal gland [42], pancreas [43], pleura[44], and even the heart [45].

Initially considered to be of mesothelial origin by Pierre Masson, it was later the subject of some histogenetic controversy. The presence of gland-like spaces with very thin linings, mimicking lymphatic channels or early vascular structures, lead to the suggestion of a possible endothelial nature. EM and later IHC confirmed the mesothelial phenotype [46]. Considered a reactive lesion by some, there is now a general consensus on its benign, neoplastic nature.

Adenomatoid tumors present as relatively small, well-demarcated, non-encapsulated nodules with a mean diameter of 2 cm [38]. The cut surface is firm and solid with variable cystic spaces. Microscopically, cords, channels, and

microcystic spaces are lined by cuboidal or flattened cells, with vesicular nuclei and occasional nucleoli. Intracellular lumina may predominate, imparting a signet-ring appearance to the tumor, or may coalesce into larger spaces. Quite often, tumor stroma contains a prominent smooth muscle component and abundant elastic fibers [38-39]. This smooth muscle component may be particularly abundant in tubaric and uterine adenomatoid tumors [41]. In the uterus, they may be easily mistaken for leiomyomas with a peculiar, prominent blood vessel component. Although most uterine adenomatoid tumors are subserosal, some of them are totally intramural and even submucosal, and they often coexist with leiomyomas.

Ultrastructurally, the typical mesothelial features are recognized, with abundant, irregular microvilli projecting into the gland-like spaces, well-developed cell junctions, and tonofilaments [40,46]. As the correlate of these EM features, careful light microscopic examination of the luminal aspect of these cells reveals a prominent fuzzy border, often associated with the accumulation of a faint basophilic material. This may be shown to be hyaluronic acid by Alcian Blue stain with hyaluronidase digestion. The cells are positive with antibodies for cytokeratin 5/6, calretinin, EMA, AE1-AE3, and for most of the antibodies used for mesothelial cells. Antibodies for CEA, MOC-31, Ber-EP4, B72.3, and Leu M1 are usually negative. The differential diagnosis between adenomatoid tumors and endothelial cell neoplasms, particularly epithelioid hemangioendothelioma, is easily accomplished with EM, and also by showing its negative staining with antibodies for Factor VIII, CD31, and CD34 [38,39].

Benign Mesothelioma

Both in the peritoneum and in paratesticular locations, isolated papillary or multicystic proliferations of mesothelial cells are usually classified as benign mesotheliomas. The cystic cases must be distinguished from hydrocele in the testis or from cystic lymphangiomas in both the testis and the peritoneal cavity [15,38,47,50-52]. There is question regarding their true neoplastic nature. On the other hand, the benign papillary mesotheliomas must be single and relatively small lesions to be confidently classified as benign. Multifocality, the combination with solid areas, and atypia must raise the suspicion of a potentially malignant tumor. Actually, even some apparently benign lesions have evolved towards a more aggressive course [28,38,50,53].

Malignant Mesothelioma

It is defined as a proliferation of mesothelial cells with malignant morphological features and aggressive growth, arising in the peritoneal cavity, or in the tunica vaginalis and the tunica albuginea [28,38,50-53]. Patients with mesothelioma originating in one of these two sites may subsequently develop a tumor in the other. The latter are usually considered an extension of the primary lesion, rather than synchronous or metachronous tumors. When malignant mesothelioma simultaneously involves the scrotum and the peritoneal cavity, it is impossible to determine the origin of the process, although it is usually assumed to most probably be a primary peritoneal tumor. Association with asbestos exposure has been estimated in around 50% of the reported peritoneal tumors, and it is considered to be similar for the testicular cases [1,54,55].

Peritoneal mesotheliomas occur most often in men, usually over 40 years in age. Fibrous plaques are found in cases of peritoneal mesothelioma more often than in pleural mesothelioma. Clinical presentation depends on the areas and organs involved, and it is usually accompanied by ascites. Grossly, peritoneal mesotheliomas form multiple nodules or plaques, and they only occasionally present as isolated lesions. Again, microscopically, there is a wide range of appearances. The usual epithelioid mesothelioma may grow in tubules, papillae with variable psammomatous components, and solid areas. Nuclei may be

pleomorphic but are more often monotonous, either with dispersed or condensed chromatin [3,28,56-58]. Cell cytoplasm may be vacuolated, due to degenerative changes. They may also contain numerous lipid vacuoles, sometimes mimicking liposarcoma (lipid-rich mesothelioma) [59,60], or large glycogen pools, resulting in the clear-cell variety that may be confused with renal cell carcinoma [61]. Sarcomatoid mesothelioma, made up of diffusely growing spindle cells with variable degrees of pleomorphism, may predominate or occur as a component in a biphasic tumor [62,63].

Several clinicopathological varieties of peritoneal mesothelioma have been recognized. Well-differentiated papillary mesothelioma is mostly a superficial, multifocal tumor, most often found in women [28]. Thus, the main differential diagnosis will be made with peritoneal and ovarian serous carcinomas. A morphological variant of peritoneal mesothelioma that may also occur in the testis is the so-called deciduoid mesothelioma [64,65]. As the name implies, it is made up of cells with a decidualized appearance. In women, this may result in confusion with benign, sub-mesothelial decidualized nodules, and thus, the malignant nature of the lesion may be overlooked. In contrast with true decidualized cells that contain large amounts of glycogen, the appearance of these cells has been shown by EM to be the result of the prominent accumulation of a variety of organelles, including intermediate filaments, either dispersed or arranged in bundles, mitochondria, and rough and focally smooth endoplasmic reticulum [64,65]. Another variety, lymphohistiocytoid mesothelioma, is characterized by the combination of tumor cells along with a variable component of lymphocytes and histiocytes [66]. A rare variety, leiomioid mesothelioma, shows the co-expression of mesothelial markers with desmin and actin [67]. All of these varieties share the same ultrastructural and immunophenotypical profile as conventional mesotheliomas.

Testicular mesotheliomas present in a wide age range, from children to elderly men, with a mean age around 50 years. Although testicular mesothelioma is rare, it is the second most common malignancy in this location, after soft tissue tumors. It usually presents either as an incidental finding during hernia repair, in association with hydrocele, with or without a clinically

detectable mass, or primarily as a palpable tumor. Grossly numerous, small papillary lesions, multiple nodules or diffuse thickening of the vaginalis or albuginea are most often found. There may be obvious signs of infiltration into adjacent structures. Microscopically, 75% of the testicular mesotheliomas are epithelial and show combinations of tubular, papillary and solid areas. In the remaining cases, a variable proportion of sarcomatoid elements are found, resulting either in a biphasic or even purely spindle-cell tumor [38,51,53,68].

Clinically, peritoneal and testicular mesotheliomas are aggressive tumors. For those arising in the peritoneum, prognosis is extremely poor, and the majority of patients die two years after diagnosis. Progression is associated with extensive infiltration to adjacent organs, and with metastases to pelvic and retroperitoneal lymph nodes [69,70]. The exception to this rule would be the well-differentiated papillary mesotheliomas arising in women, which tend to show a more protracted course. Testicular mesotheliomas tend to recur after two years of diagnosis, infiltrating other testicular and paratesticular structures, the spermatic cord, and disseminating into inguinal and retroperitoneal lymph nodes, the peritoneum, mediastinum, lungs and pleura, bones, and the brain.

DIFFERENTIAL DIAGNOSIS OF PERITONEAL AND TESTICULAR MESOTHELIOMA

Common Epithelial Tumors of the Ovary and Uterus and Their Testicular Counterpart

This is an important differential diagnosis, with remarkable therapeutic and prognostic implications. Out of all the varieties, the main difficulties may be encountered with serous papillary carcinomas of the ovary. These tumors present with variable combinations of papillary and solid areas and may extend along the peritoneal surface, forming nodules and plaques, and making it difficult to establish their ovarian origin [28,71]. Similarly, serous carcinomas of the endometrium often disseminate to the peritoneum, with relatively little or no ovarian involvement [72]. Another finding that can be misleading is the presence of mesothelial hyperplasia, in association with some serous borderline

tumors [71]. To further complicate the situation, mesotheliomas may preferentially involve the ovarian surface [58]. When topographic data are not contributory, the differential diagnosis must be based on a combination of light microscopic, immunohistochemical, and ultrastructural features. Histologically, cells in mesothelioma tend to have more monomorphous and less atypical nuclei than those of carcinomas, and they may display a more prominent tubulopapillary pattern. Mucin stains such as mucicarmin are of little use in serous carcinomas, and, on the other hand, mucicarmin-positive mesotheliomas have been reported [3]. In the rare instances in which the differential diagnosis is a mucinous ovarian neoplasm, Alcian Blue stain, with and without hyaluronidase digestion, may be a more useful technique.

The immunohistochemical study of these tumors is even more problematic than the study of those in other sites (Table 2). Out of all of the mesothelial antibodies, calretinin shows the best sensitivity and specificity for mesothelioma in this setting. However, sex cord-stromal tumors (i.e., retiform Sertoli-Leydig cell tumor) may present with peculiar papillary areas, and calretinin is typically positive in most of these cases [71,73]. This may lead to an erroneous diagnosis, if calretinin is not used in combination with other antibodies. WT1 gene product is of little practical value, as it is typically expressed by most ovarian and surface serous carcinomas, and also by over 50% of the endometrial serous carcinomas [74,75]. This finding is related to the fact that the Wilms tumor gene plays complex roles in the development of the genitourinary tract and mesothelium, thus providing additional evidence for the histogenetic relationship between müllerian epithelia and mesothelial cells. Other antibodies used for mesothelioma or carcinoma are of limited value here: thrombomodulin is reported to be positive in 56% of the mesotheliomas and 30% of the serous carcinomas, cytokeratin 5/6 in 53% of the mesotheliomas and 25% of the carcinomas, and CD44H in 47% of the mesotheliomas and 25% of carcinomas [76]. HBME-1 is also expressed in serous carcinomas, although the membranous pattern of positivity is apical, compared to the more extensive apical and lateral pattern seen in mesothelioma. In addition, only 35% of the serous carcinomas express Leu M1, 10% polyclonal CEA and 5% monoclonal

CEA [13,16,77,78]. In fact, according to Ordóñez, CA19-9 is preferable to CEA in this setting [16].

In serous carcinomas, EM reveals the presence of shorter and straight microvilli, frequent ciliated cells, and junctional complexes, instead of the isolated, large desmosomes or tight junctions seen in mesothelioma [79,80]. Junctions between mesothelioma cells are most often located closer to the basal domain, resulting in a wide circumferential area covered by the characteristic microvilli [81]. In contrast, junctional complexes tend to be located closer to the apical domain in serous carcinomas, except in cells with a prominent hobnail appearance. This explains the different patterns of staining with antibodies against membranous or glycoliceal components of microvilli, such as HBME-1. Although EM may easily lead to the diagnosis, it is crucial to adequately select the areas of interest, in which all of the diagnostic features may be found, avoiding to erroneously identify entrapped or hyperplastic mesothelial cells as being part of the tumor. Both ovarian and mesothelial tumors tend to be large, and, in poorly differentiated cases, the diagnostic areas may be focal and, therefore, missed in the initial sampling for EM. However, they may be selected and retrieved from the paraffin block, without losing the essential information, provided that the previous fixation and paraffin embedding were adequate. In summary, the differential diagnosis between peritoneal mesothelioma and ovarian tumors is clinically relevant, and it may be achieved, in most cases, by paying close attention to morphology. Difficult cases will benefit from IHC and, if available, EM confirmation.

Tumors identical to those arising on the ovarian surface, benign, borderline or malignant, may arise in the testis and paratesticular structures. These are very uncommon neoplasms with a müllerian phenotype, mostly serous, but also of mucinous, endometrioid or Brenner types, and show an immunohistochemical profile and ultrastructural features similar to their ovarian counterparts. It is assumed that these tumors have a better prognosis than mesotheliomas in this location, but this is only based on single case studies or small series [38,51,53,82].

Peritoneal Serous Tumors

There is a group of serous papillary neoplasms, benign, borderline or malignant, that present in the absence of ovarian involvement. They can be considered the equivalent of serous ovarian tumors in an extraovarian location [83,84]. Their histological features and ultrastructural appearance are identical, including cilia, short microvilli and junctional complexes [30]. The vast majority of cases occur in women, although there are isolated reports in men. It has been hypothesized that their origin could be traced through serous metaplasia of the mesothelium or endosalpingiosis, in the context of the so-called secondary müllerian system [29,30,83,84]. It is important to distinguish between a primary peritoneal serous tumor and an implant or metastasis from an ovarian primary lesion, because of obvious differences in staging. Apparently these tumors behave in a similar fashion and are susceptible to similar treatment strategies with their ovarian equivalents, and therefore, it is even more important to differentiate between a primary serous peritoneal lesion and a malignant mesothelioma. Again, a judicious combination of clinical and histological features, IHC and EM will allow the diagnosis. Also, a special note of caution applies to these tumors, as many of the antibodies that show moderate to low positivity in the ovarian tumors tend to be completely negative, or only focally and faintly positive, in many of the peritoneal ones. This is the case with CEA, either mono or polyclonal, Leu M1, or B72.3 [13,29,74,76]. In spite of their serous phenotype, many of these tumors are reported to be positive with stains for epithelial mucin, but, in the absence of other supporting features, this is not enough evidence for the diagnosis, since mucin stains may be positive in mesothelioma [3].

Rare Epithelial Testicular and Epididymal Tumors

Testicular mesotheliomas must be distinguished from a group of very uncommon neoplasms, arising in the epididymis or rete testis. Some of them are benign, and others tend to be more aggressive than conventional testicular mesothelioma. Because of their rarity, the diagnosis may be easily missed.

Papillary cystadenoma of the epididymis

This is a benign cystic tumor, arising in the epididymis and showing variable, aborting papillary structures, that are made up of glycogen-rich, clear cells, in

which light or EM shows occasional cilia. These tumors are bilateral in about half of the cases, and particularly in patients with the Von Hippel-Lindau syndrome, in which they are much more prevalent. The main differential diagnoses for these tumors are the malignant counterpart, clear-cell carcinoma of the epididymis, and clear-cell renal cell carcinoma, which may rarely metastasize to the testis and paratesticular structures. Some areas in papillary cystadenoma of the epididymis may show features strongly reminiscent of mesothelioma, and, on the other hand, mesotheliomas may on occasion show a prominent accumulation of glycogen [61]. Therefore, all of the previously discussed immunohistochemical and ultrastructural features may be helpful in this differential diagnosis, but it is obviously essential to be aware of the entity in order to recognize it [38,48,51,53].

Adenocarcinoma of the epididymis

This is an uncommon, malignant glandular proliferation, arising in the epididymis, which presents as an occasionally painful mass, associated with hydrocele in about half of the cases. It occurs between the third and ninth decade, and may attain a variable size. It shows a tubular, tubulopapillary, or cystic growth pattern, and is made up of cuboidal or columnar cells, often containing cytoplasmic glycogen. Usually, there is no associated, desmoplastic stromal response. The main differential diagnoses include, in addition to the benign epididymal variety already discussed, adenomatoid tumor, mesothelioma, serous carcinoma, and tumors of the rete testis [38,48,51,53]. For rete testis and serous tumors, topography is important, as rete testis tumors must be seated in this structure to assume that they originate within it, and serous tumors tend to arise in the testiculo-epididymal groove. However, when the tumors are large, topography may be difficult to assess. Relatively, the easiest differential is for mesothelial neoplasms, as they may be excluded or confirmed by histological features, plus the usual ancillary techniques. Again, IHC must be used with care. Epididymal adenocarcinoma shows strong luminal staining with antibodies for EMA, and CEA has shown contradictory results, according to different authors; also, Leu M1, B72.3, Ber-EP4, as well as alpha-fetoprotein, prostatic acid phosphatase, prostatic specific antigen, and vimentin are reported to be negative in most cases [38,51]. EM reveals the classic

features of adenocarcinoma, with occasional cilia [51]. The long, branching microvilli of normal epididymis, also known as stereocilia, have not been reported in these tumors. In addition to anatomical location, the distinction between epididymal carcinoma and serous and rete testis tumors will mainly rely upon subtle histological features. There is little information on prognosis, due to the small number of published cases, but around 50% of the patients with epididymal carcinoma are reported to die with disseminated tumors, in spite of several methods of treatment.

Adenoma of the rete testis

This is also a rare tumor, with a solid, cystic or mixed macroscopic appearance, arising in the hylum of the testis. Characteristically, nodular aggregates of tumor cells project into cystic spaces. The cells form papillae, slit-like spaces, and tubules reminiscent of Sertoli cell tumors. The term sertoliform cystadenoma has been applied to those cases in which the latter component predominates. In addition, when there is stromal proliferation, the term adenofibroma is preferred [52,53,85].

Adenocarcinoma of the rete testis

This is a tumor reported exclusively in older Caucasian men, and, mainly due to its location in the posterior aspect of the testis, it is often missed in its initial stages. Related in part to this fact, adenocarcinoma of the rete testis has a poor prognosis, with frequent local and lymphatic spread, and with an average survival of 8 months after diagnosis [85,86]. Strict requirements for the diagnosis of adenocarcinoma of the rete testis, proposed by Nochomovitz and Orenstein, include the absence of an extrascrotal tumor with a similar morphology, a location centered in the testicular hylus, a morphology different from any other testicular or paratesticular tumor, microscopic evidence of transition between the rete testis and the tumor, and a predominantly solid growth, although focal cystic change is allowed [52,85,86]. Tumor cells arrange themselves in nodules and form slit-like spaces, combined with papillary, tubular, and solid areas. Cells are relatively small, lack overt pleomorphism, and have molded or grooved nuclei. Serous tumors and mesothelioma are the main differential diagnostic considerations [52,85].

EMA is characteristically positive in rete testis adenocarcinoma, Leu M1 is negative, and CEA gives contradictory results. Positivity with HBME-1 and thrombomodulin antibodies has been reported in one case that was classified as an adenocarcinoma of the rete testis [87]. As stated above, HBME-1 is known to be positive in many adenocarcinomas, and it is the pattern of positivity that helps in its distinction from mesothelioma. On the other hand, thrombomodulin is not exclusive for mesothelioma. Similar to the ovary, calretinin must be used with great caution in the differential diagnosis between mesothelioma and these testicular neoplasms, which may mimic one of the many histological patterns of sex cord tumors, in which calretinin is also positive. Ultrastructurally, rete testis adenocarcinoma is characterized by variable proportions of short microvilli, devoid of core rootlets, complex lateral interdigitations with abundant desmosomes, and characteristically indented nuclei. The cytoplasm contains variable amounts of lipid and glycogen, but lacks secretory granules. These features are similar to those of normal rete testis epithelium [88].

In summary, the use of IHC in the diagnosis of mesothelioma is a difficult and unsettled issue. In both the peritoneum and the testis and paratestis, it is further complicated by the sometimes paradoxical or unexpressive immunohistochemical phenotypes of the many tumors which enter the differential diagnosis. There is no single clue or magic marker, and therefore EM may be particularly helpful in this setting. As in many other areas of Pathology, the careful evaluation of clinical and histopathological data, along with the judicious application of IHC and EM, are required to reach the correct diagnosis.

ACKNOWLEDGEMENTS

To Dr. Patricia Troncoso, Dr. Anais Malpica and Dr. Pheroze Tamboli, from the Department of Pathology at the University of Texas M.D. Anderson Cancer Center, for their help with the preparation of this presentation.

REFERENCES

1. Comin C, de Klerk NH, Henderson DW. Malignant mesothelioma: current conundrums over risk estimates and whether electron microscopy for diagnosis? *Ultrastruct Pathol* 1997; 21:315-320.
2. Dardick I, Al-Jabi M, McCaughey WTE, et al. Ultrastructure of poorly differentiated diffuse epithelial mesotheliomas. *Ultrastruct Pathol* 1984; 7:151-160.
3. Hammar SP, Bockus DE, Remington FL, Rohrbach KA. Mucin-positive epithelial mesotheliomas: a histochemical, immunohistochemical, and ultrastructural comparison with mucin-producing adenocarcinomas. *Ultrastruct Pathol* 1996; 20:293-325.
4. Ordóñez NG, Mackay B. Electron microscopy in tumor diagnosis: indications for its use in the immunohistochemical era. *Hum Pathol* 1998; 29:1403-1411.
5. Oury TD, Hammar SP, Roggli VL. Ultrastructural features of diffuse malignant mesotheliomas. *Hum Pathol* 1998; 29:1382-1392.
6. Turbat-Herrera EA, Knowles K. Cytology: screening or diagnostic tool? *Hum Pathol* 1998; 29:1356-1366.
7. Brown RW, Clark GM, Tandon AK, Allred DC. Multiple-marker immunohistochemical phenotypes distinguish malignant pleural mesothelioma from pulmonary adenocarcinoma. *Hum Pathol* 1993; 24:347-354.
8. Comin C, Novelli L, Boddi V, et al. Calretinin, thrombomodulin, CEA, and CD15: a useful combination of immunohistochemical markers for differentiating pleural epithelial mesothelioma from peripheral pulmonary adenocarcinoma. *Hum Pathol* 2001; 32:529-536.
9. Han AC, Peralta-Soler A, Knudsen KA et al. Differential expression of N-cadherin in pleural mesotheliomas and E-cadherin in lung adenocarcinomas in formalin-fixed, paraffin-embedded tissues. *Hum Pathol* 1997; 28:641-645.
10. Khor A, Whitsett JA, Stahlman MT, et al. Utility of surfactant protein B precursor and thyroid transcription factor 1 in differentiating adenocarcinoma of the lung from malignant mesothelioma. *Hum Pathol* 1999; 30:695-700.
11. Ordóñez NG. The immunohistochemical diagnosis of mesothelioma. Differentiation of mesothelioma and lung adenocarcinoma. *Am J Surg Pathol*. 1989; 13:276-291.
12. Ordóñez NG. The value of antibodies 44-3A6, SM3, HBME-1, and thrombomodulin in differentiating epithelial pleural mesothelioma from lung adenocarcinoma. A comparative study with other commonly used antibodies. *Am J Surg Pathol* 1997; 21:1399-1408.
13. Ordóñez NG. Role of immunohistochemistry in distinguishing epithelial peritoneal mesotheliomas from peritoneal and ovarian serous carcinomas. *Am J Surg Pathol* 1998; 22:1203-1214.

14. Ordóñez NG. Value of the MOC-31 monoclonal antibody in differentiating epithelial pleural mesothelioma from lung adenocarcinoma. *Hum Pathol* 1998; 29:166-169.
15. Ordóñez NG. The immunohistochemical diagnosis of epithelial mesothelioma. *Hum Pathol* 1999; 30:313-323.
16. Ordóñez NG. Immunohistochemical diagnosis of epithelioid mesotheliomas: a critical review of old markers, new markers. *Hum Pathol* 2002; 33:953-967.
17. Ordóñez NG. Value of E-cadherin and N-cadherin immunostaining in the diagnosis of mesothelioma. *Hum Pathol* 2003; 34:749-755.
18. Ordóñez NG. The diagnostic utility of immunohistochemistry in distinguishing between mesothelioma and renal cell carcinoma: a comparative study. *Hum Pathol* 2004; 35:697-710.
19. Riera JR, Astengo-Osuna C, Longmate JA, Battifora H. The immunohistochemical diagnostic panel for epithelial mesothelioma. A reevaluation after heat-induced epitope retrieval. *Am J Surg Pathol* 1997; 21:1409-1419.
20. Robb JA. Mesothelioma versus adenocarcinoma: false-positive CEA and Leu-M1 staining due to hyaluronic acid. *Hum Pathol* 1989; 20:400.
21. Sheibani K. Increasing role of immunopathology in diagnosis of malignant mesothelioma. *Hum Pathol* 1997; 28:639-640.
22. Weiss LM, Battifora H. The search for the optimal immunohistochemical panel for the diagnosis of malignant mesothelioma. *Hum Pathol* 1993; 24:345-346.
23. Wick MR. Immunophenotyping of malignant mesothelioma. *Am J Surg Pathol* 1997; 21:1395-1398.
24. Donna A, Betta PG, Chiodera P, et al. Newly marketed tissue markers for malignant mesothelioma: immunoreactivity of rabbit AMAD-2 antiserum compared with monoclonal antibody HBME-1 and a review of the literature on so-called antimesothelioma antibodies. *Hum Pathol* 1997; 28:929-937.
25. Chu AY, Litzky LA, Pasha TL, et al. Utility of D2-40, a novel mesothelial marker, in the diagnosis of malignant mesothelioma. *Mod Pathol* 2005; 18:105-110.
26. Lloreta-Trull J, Ferrer L, Ribalta T, et al. Electron microscopy in pathology articles: a retrospective appraisal. *Ultrastruct Pathol* 2000; 24:105-108.
27. Hurlimann J. Desmin and neural marker expression in mesothelial cells and mesotheliomas. *Hum Pathol* 1994; 25:753-757.
28. Rosai J. *Rosai and Ackerman's Surgical Pathology*. Edinburgh: Mosby, 2004.
29. Khoury N, Raju U, Crissman JD, et al. A comparative immunohistochemical study of peritoneal and ovarian serous tumors, and mesotheliomas. *Hum Pathol* 1990; 21:811-819.

30. Raju U, Fine G, Greenawald KA, Ohorodnik JM. Primary papillary serous neoplasia of the peritoneum: a clinicopathologic and ultrastructural study of eight cases. *Hum Pathol* 1989; 20:426-436.
31. Srigley JR. The paratesticular region: histoanatomic and general considerations. *Semin Diagn Pathol* 2000; 17:258-269.
32. Trainer TD. Testis and excretory duct system. In: Sternberg SS, ed. *Histology for Pathologists*. Philadelphia: Lippincott-Raven; 1997:1019-1037.
33. Churg A, Colby TV, Cagle P, et al. The separation of benign and malignant mesothelial proliferations. *Am J Surg Pathol* 2000; 24:1183-1200.
34. Bolen JW, Hammar SP, McNutt MA. Reactive and neoplastic serosal tissue. A light-microscopic, ultrastructural, and immunocytochemical study. *Am J Surg Pathol*. 1986; 10:34-47.
35. Kung ITM, Thallas V, Spencer EJ, Wilson SM. Expression of muscle actins in diffuse mesotheliomas. *Hum Pathol* 1995; 26:565-570.
36. Ordóñez NG, Ro JY, Ayala AG. Lesions described as nodular mesothelial hyperplasia are primarily composed of histiocytes. *Am J Surg Pathol* 1998; 22:285-292.
37. Rosai J, Dehner LP. Nodular mesothelial hyperplasia in hernia sacs. A benign reactive condition simulating a neoplastic process. *Cancer* 1975; 35:165-175.
38. Davis CJ, Sesterhenn IA, Woodward PJ. Tumours of paratesticular structures. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA eds. *Tumours of the Urinary System and Male Genital Organs*. Lyon: IARC Press; 2004:267-276.
39. Pérez-Ordóñez B, Srigley JR. Mesothelial lesions of the paratesticular region. *Semin Diagn Pathol* 2000; 17:294-306.
40. Sidhu GS, Fresko O. Adenomatoid tumor of the epididymis: ultrastructural evidence of its biphasic nature. *Ultrastruct Pathol* 1980; 1:39-47.
41. Srigley JR, Colgan TJ. Multifocal and diffuse adenomatoid tumor involving uterus and fallopian tube. *Ultrastruct Pathol* 1988; 12:351-355.
42. Raaf HN, Grant LD, Santoscoy C, et al. Adenomatoid tumor of the adrenal gland: a report of four new cases and a review of the literature. *Mod Pathol* 1996; 9:1046-1051.
43. Overstreet K, Wixom C, Shabaik A, et al. Adenomatoid tumor of the pancreas: a case report with comparison of histology and aspiration cytology. *Mod Pathol* 2003; 16:613-617.
44. Kaplan MA, Tazelaar HD, Hayashi T, Schroer KR, Travis WD. Adenomatoid tumors of the pleura. *Am J Surg Pathol* 1996; 20:1219-1223.
45. Natarajan S, Luthringer DJ, Fishbein MC. Adenomatoid tumor of the heart: report of a case. *Am J Surg Pathol* 1997; 21:1378-1380.

46. Mackay B, Bennington JL, Skoglund RW. The adenomatoid tumor. Fine structural evidence for a mesothelial origin. *Cancer* 1971; 27:109-115.
47. Sawh RN, Malpica A, Deavers MT, Liu J, Silva EG. Benign cystic mesothelioma of the peritoneum: a clinicopathologic study of 17 cases and immunohistochemical analysis of estrogen and progesterone receptor status. *Hum Pathol* 2003; 34:369-374.
48. Nistal M, Mate A, Paniagua R. Cystic transformation of the rete testis. *Am J Surg Pathol* 1996; 20:1231-1239.
49. Marshall RM, Gould VE, King ME, et al. Multicystic abdominal peritoneal tumor presenting as an enlarging incisional hernia. *Ultrastruct Pathol* 1985; 8:249-256.
50. Ulbright TM. Neoplasms of the testis. In: Bostwick DG, Eble JN, eds. *Urologic Surgical Pathology*. St. Louis: Mosby; 1997:567-645.
51. Henley JD, Ferry J, Ulbright TM. Miscellaneous rare paratesticular tumors. *Semin Diagn Pathol* 2000; 17:319-339.
52. Jones EC, Murray SK, Young RH. Cysts and epithelial proliferations of the testicular collecting system (including rete testis). *Semin Diagn Pathol* 2000; 17:270-293.
53. Bostwick DG. Spermatic cord and testicular adnexa. In: Bostwick DG, Eble JN, eds. *Urologic Surgical Pathology*. St. Louis: Mosby; 1997:647-671.
54. Andrion A, Bosia S, Paoletti L, et al. Malignant peritoneal mesothelioma in a 17-year-old boy with evidence of previous exposure to chrysotile and tremolite asbestos. *Hum Pathol* 1994; 25:617-622.
55. Attanoos RL, Gibbs AR. Primary malignant gonadal mesotheliomas and asbestos. *Histopathology* 2000; 37:150-159.
56. Eyden BP, Banik S, Harris M. Malignant epithelial mesothelioma of the peritoneum: observations on a problem case. *Ultrastruct Pathol* 1996; 20:337-344.
57. Talerman A, Chilcote RR, Montero JR, Okagaki T. Diffuse malignant peritoneal mesothelioma in a 13-year-old girl. Report of a case and review of the literature. *Am J Surg Pathol* 1985; 9:73-80.
58. Clement PB, Young RH, Scully RE. Malignant mesotheliomas presenting as ovarian masses. A report of nine cases, including two primary ovarian mesotheliomas. *Am J Surg Pathol* 1996; 20:1067-1080.
59. Shimazaki H, Aida S, Iizuka Y, et al. Vacuolated cell mesothelioma of the pericardium resembling liposarcoma: a case report. *Hum Pathol* 2000; 31:767-770.
60. Chang HT, Yantiss RK, Nielsen GP, et al. Lipid-rich diffuse malignant mesothelioma: a case report. *Hum Pathol* 2000; 31:876-879.

61. Ordóñez NG, Mackay B. Glycogen-rich mesothelioma. *Ultrastruct Pathol* 1999; 23:401-406.
62. Flint A, Weiss SW. CD-34 and keratin expression distinguishes solitary fibrous tumor (fibrous mesothelioma) of pleura from desmoplastic mesothelioma. *Hum Pathol* 1995; 26:428-431.
63. Klima M, Bossart MI. Sarcomatous type of malignant mesothelioma. *Ultrastruct Pathol* 1983; 4:349-358.
64. Shanks JH, Harris M, Banerjee SS, Eyden BP, et al. Mesotheliomas with deciduoid morphology. A morphologic spectrum and a variant not confined to young females. *Am J Surg Pathol* 2000; 24:285-294.
65. Shia J, Erlandson RA, Klimstra DS. Deciduoid mesothelioma: a report of 5 cases and literature review. *Ultrastruct Pathol* 2002; 26:355-363.
66. Khalidi HS, Medeiros J, Battifora H. Lymphohistiocytoid mesothelioma. *Am J Clin Pathol* 2000; 113:649-654.
67. Mayall FG, Goddard H, Gibbs AR. Intermediate filament expression in mesotheliomas. Leiomyoid mesotheliomas are not uncommon. *Histopathology* 1992; 21:453-457.
68. Jones MA, Young RH, Scully RE. Malignant mesothelioma of the tunica vaginalis. A clinicopathologic analysis of 11 cases with review of the literature. *Am J Surg Pathol* 1995; 19:815-825.
69. Masangkay AV, Susin M, Baker R et al. Metastatic malignant mesothelioma presenting as colonic polyps. *Hum Pathol* 1997; 28:993-995.
70. Shah IA, Somsin A, Wong SX, et al. Malignant mesothelioma presenting as colonic tumor. *Hum Pathol* 1998; 29:657.
71. Prat J. *Pathology Of The Ovary*. Philadelphia: Saunders, 2004.
72. Soslow RA, Pirog E, Isacson C. Endometrial intraepithelial carcinoma with associated peritoneal carcinomatosis. *Am J Surg Pathol* 2000; 24:726-732.
73. Baker PM, Oliva E. Immunohistochemistry as a tool in the differential diagnosis of ovarian tumors: an update. *Int J Gynecol Pathol* 2005; 24:39-55.
74. Acs G, Pasha T, Zhang PJ. WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the peritoneum, fallopian tube, ovary, and endometrium. *Int J Gynecol Pathol* 2004; 23:110-118.
75. Hwang H, Quenneville L, Yaziji H, Gown AM. Wilms tumor gene product: sensitive and contextually specific marker of serous carcinomas of ovarian surface epithelial origin. *Appl Immunohistochem Mol Morphol* 2004; 12:122-126.
76. Attanoos RL, Webb R, Dojcinov SD, Gibbs AR. Value of mesothelial and epithelial antibodies in distinguishing diffuse peritoneal mesothelioma in females from serous papillary carcinoma of the ovary and peritoneum. *Histopathology* 2002; 40:237-244.

77. Wauters CC, Smedts F, Gerrits LG, Bosman FT, Ramaekers FC. Keratins 7 and 20 as diagnostic markers of carcinomas metastatic to the ovary. *Hum Pathol* 1995; 26:852-855.
78. Cibas ES, Corson JM, Pinkus GS. The distinction of adenocarcinoma from malignant mesothelioma in cell blocks of effusions: the role of routine mucin histochemistry and immunohistochemical assessment of carcinoembryonic antigen, keratin proteins, epithelial membrane antigen, and milk fat globule-derived antigen. *Hum Pathol* 1987; 18:67-74.
79. Fenoglio CM. Ultrastructural features of the common epithelial tumors of the ovary. *Ultrastruct Pathol* 1980; 1:419-444.
80. Ghadially FN, Rippstein PU, Cavell S, Venance SL. Giant desmosomes in tumors. *Ultrastruct Pathol* 1995; 19:469-474.
81. Posalaky Z, McGinley D. Occluding junctions in an epithelioid mesothelioma: a freeze-fracture study. *Ultrastruct Pathol* 1984; 6:177-183.
82. Jones MA, Young RH, Srigley JR, Scully RE. Paratesticular serous papillary carcinoma. A report of six cases. *Am J Surg Pathol* 1995; 19:1359-1365.
83. Weir MM, Bell DA, Young RH. Grade 1 peritoneal serous carcinomas. A report of 14 cases and comparison with 7 peritoneal serous psammocarcinomas and 19 peritoneal serous borderline tumors. *Am J Surg Pathol* 1998; 22:849-862.
84. Truong LD, Maccato ML, Awalt H, et al. Serous surface carcinoma of the peritoneum: a clinicopathologic study of 22 cases. *Hum Pathol* 1990; 21:99-110.
85. Nochomovitz L. Tumours of collecting ducts and rete. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA eds. *Tumours of the Urinary System and Male Genital Organs*. Lyon: IARC Press; 2004:265-266.
86. Nochomovitz LE, Orenstein JM. Adenocarcinoma of the rete testis: review and reagrupping of reported cases and a consideration of miscellaneous entities. *J Urogenital Path* 1991; 1:11-40.
87. Skales GE, Menasce L, Banerjee SS, et al. Adenocarcinoma of the rete testis. *Clin Oncol (R Coll Radiol)* 1998; 10:401-403.
88. Mrak RE, Hussain MM, Schaefer RF. Ultrastructure of metastatic rete testis adenocarcinoma. *Arch Pathol Lab Med* 1990; 114:84-88.