Diploma Thesis

Retrospective analysis of histologic characteristics of pleural mesothelioma

submitted by

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Dr.med.univ. Martin Zacharias

Graz, 18.01.2022

Declaration in lieu of oath

I, herewith, declare that I have written the following diploma thesis fully on my own and without any assistance from third parties. Furthermore, I confirm that no sources have been used in the preparation of this thesis other than those indicated in the thesis itself. Any thoughts directly or indirectly taken from somebody else's sources are made discernible as such.

Graz, 18.01.2022 *Bojan Stevic eh.*

Acknowledgements

First of all, I want to express my utmost appreciation and gratitude towards Univ. FA Priv.-Doz. Dr.med.univ. Dr.rer.nat. Luka Brcic. He provided me with his expertise in mesothelioma and encouraged my curiosity in other topics of lung pathology. I am very grateful to have found a mentor with whom I learned so much, and moreover, was able to talk in my native language.

Furthermore, I want to thank Dr.med.univ. Martin Zacharias who has always been there for me with advice and assistance and with whom I have not hesitated to ask for help. The level at which the statistical analysis is now has only been possible thanks to his experience.

And at last, I want to thank my parents, who made it possible for me to study medicine and follow my dream of becoming a doctor. Even in hard times, they did not put pressure on me but motivated me to keep going on.

Table of Contents

Glossary

BM	Biphasic Mesothelioma
EM	Epithelioid Mesothelioma
EPD	Extended Pleurectomy/Decortication
EPP	Extrapleural Pneumectomy
FISH	Fluorescence In Situ Hybridization
HIC	High Income Countries
ICC	Immunocytochemistry
IHC	Immunohistochemistry
IR	Incidence Ratio
LMIC	Lower-Middle Income Countries
PM	Pleural Mesothelioma
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SFT	Solitary Fibrous Tumor
SM	Sarcomatoid Mesothelioma
UMIC	Upper-Middle Income Countries

List of figures

List of tables

Zusammenfassung

Das Mesotheliom ist ein bösartiger Tumor, der aus Mesothelzellen hervorgeht und betrifft hauptsächlich die Pleura. Die Erkrankung ist sehr selten und betrifft hauptsächlich Männer im höheren Alter. Das ist darauf zurückzuführen, dass Mesotheliome hauptsächlich durch Asbest verursacht werden, welches vermehrt in Berufen, die männlich dominiert sind, wie Stahlindustrie und Schiffsbau, vorkommt und eine lange Latenzzeit besitzen. Histologisch lässt sich das Mesotheliom in einen epithelioiden, einen biphasichen und in einen sarkomatoiden Subtyp einordnen. Diese Einteilung hat auch prognostische Relevanz, da die Überlebensdauer vom ersten zum letzten Subtyp abnimmt. Aufgrund der Seltenheit und histologischen Mannigfaltigkeit, ist die Diagnose aufgrund von histologischer Morphologie allein schwierig und setzt die Zuhilfenahme von immunhistochemischen Markern voraus. Fortschritte im molekularbiologischen Verständnis des Tumors bringen hilfreiche Marker für die Diagnose für histologische Proben und auch für Zellblöcke und ermöglichen dadurch in manchen Fällen eine definitive Diagnose. Für die Therapie zeigen sich aber nur begrenzt Fortschritte und dementsprechend ist die Prognose nach wie vor ungünstig.

In dieser Analyse wurden 295 Fälle von Mesotheliomen des Brustfells von den Jahren 1989 bis einschließlich 2018 hinsichtlich Geschlechtsverteilung, Zeitpunkt der Diagnose und histologischen Subtyp untersucht. Mit 74% ist der überwiegende Anteil der Betroffenen männlich und hat ein medianes Alter von 68 Jahren. Die Anzahl der Fälle hat signifikant zugenommen, was sich mit den Ergebnissen von Statistik Austria deckt.

Obwohl das Verbot der Asbestnutzung in Österreich bereits 30 Jahre zurückliegt, wird die Zahl der Erkrankungen wahrscheinlich erst in den nächsten Jahren sinken. Daher ist es wichtig, diese Krankheit zu erkennen und zu melden, da es sehr wahrscheinlich eine Berufserkrankung ist und den Patient*innen Kompensationen zustehen. In Ländern mit niedrigem Einkommen wird aufgrund von uneingeschränktem Umgang mit Asbest mit steigenden Zahlen gerechnet.

Abstract

Mesothelioma is a tumor that mainly manifests in the pleura. It is a rare disease with a severe prognosis. Mesothelioma is primarily a consequence of asbestos exposure. As asbestos was commonly used in male-dominated industrial occupations, such as the steel industry and shipbuilding, and there is a long latency between exposure and outbreak of disease, mesothelioma mainly affects men of older age. Mesothelioma can be histologically classified into an epithelioid and biphasic, and sarcomatoid subtype with a better prognosis for epithelioid mesothelioma and decreasing survival rate for the ladder. Because of its rarity and histologic diversity, diagnosis based on histologic morphology alone is difficult and requires immunohistochemical markers. Markers that detect genetic alterations are also a helpful addition in diagnosis for histological and cytological samples. However, only limited progress in therapy was made, and the median survival rate remains short.

This analysis assessed the gender, age at the time of diagnosis, and histologic subtype of 295 cases with pleural mesothelioma from 1989 to 2018. At 74%, the majority of affected individuals are male and have a median age of 68 years. There has been a significant increase in the number of cases, which is consistent with the findings of Statistik Austria.

Although asbestos use was banned 30 years ago, the number of newly diagnosed mesothelioma will probably only decrease in the next few years. Therefore, recognizing and reporting this disease remains important as a classification as an occupational disease is very likely, and patients are entitled to compensation. In other high-income countries incidence of mesothelioma is expected to wear off, and conversely, in low-income countries, it is expected to rise.

1. Introduction

Mesothelioma is a rare disease arising from mesothelial cells with a high correlation to prior asbestos exposure. Asbestos is a mineral silicate, which reaches deep into the lung. In the alveoli, asbestos fibers induce inflammation, where macrophages try to digest the foreign material. Due to the resistance of asbestos, macrophages fail to degrade these fibers and die off, releasing intracellular material, which causes an ongoing activation of the immune system. Inflammation results in an increased production of reactive oxygen and reactive nitrogen species (ROS and RNS). ROS and RNS cause DNA alterations, which can result in cancer. (1)

Although in Austria, the usage of all asbestos forms and the most frequently used chrysotile was banned in 1990 (2), the number of newly diagnosed mesothelioma is not wearing off. Due to the long latency between exposure and disease outbreak, the number of newly diagnosed patients has yet to peak in many European countries. It is slowly declining in some US states, where earlier restrictions to asbestos use were established (3). The most common type of mesothelioma is the pleural mesothelioma (PM) and counts up to around 90%. The second most common form affects the peritoneum and comprises 6 to 10% (4, 5), followed by mesothelioma of the pericardium and the tunica vaginalis, making up less than 1% (4). However, there is a considerable difference in the distribution of mesothelioma types regionally and gender-related. In South Korea, for example, peritoneal mesothelioma makes up more than a quarter (27.1%) of all cases (5). In Sweden, 382 cases of the peritoneal mesothelioma were reported between 1961 and 2009, 208 (54.5%) among women (6). However, these numbers should be taken with caution. The specificity and sensitivity of the diagnosis of the peritoneal mesothelioma are poor and commonly misdiagnosed with other abdominal neoplasms in women like ovarian cancer (7).

1.1. Epidemiology

The latest data of the national statistical office in Austria, Statistik Austria, reported 91 cases of mesothelioma in 2018. During 1998 and 2018, annual cases vary from 65 to 123, whereas cases from 1998 to 2007 count up to 834 and from 2008 to 2017 rise up to 1070 cases showing an increase over the past decade. From 1998 to

2018, 1354 cases affected men, and 550 cases were diagnosed in women resulting in a male to female ratio of 2.47. A similar male-to-female ratio is seen in Italy, where the national register of mesothelioma ReNaM recorded 21 398 cases of mesothelioma from 1993 to 2012 with 6087 cases counting to women leading to a male to female ratio of 2.51(8). However, the distribution of female cases varies inland from an M/F-ratio of 1.75 (Lombardy) to 5.26 (Liguria). In Liguria and other municipalities where the M/F-ratio is similarly high, asbestos exposure mainly occurs in men working in shipyards and oil refinery plants. In male patients in Italy where asbestos exposure was assessed, 86.9% were exposed to asbestos, of which 95 % had occupational exposure. In females, 61.4% had positive asbestos exposure, while 54.3% had occupational exposure. Occupations predominantly carried out by women with asbestos exposure are found in the textile industry with direct use of asbestos fibers or without (asbestos being part of operating machines or in walls of factory halls). Some countries with a high mortality rate for PM, such as the UK and Australia, had widespread industrial use of asbestos between 1950 and 1980 and a high male to female ratio (9, 10).

In contrast, countries such as Turkey, Czech Republic, Romania, and Chile have a high rate of female mesothelioma-related deaths but a relatively low mortality rate. A low percentage of occupational-associated mesothelioma in women suggests other ways of exposure to asbestos (8). These non-occupational factors can be divided into three categories: naturally occurring asbestos, exposure due to proximity to factories where asbestos is mined or processed, and household exposure due to family members who are occupationally exposed to asbestos. South Korea has a relatively high proportion of women with mesothelioma. Due to rapid industrialization and urbanization in Korea, large-scale demolition took place, releasing environmental asbestos.

Additionally, between 1970 and 1980, many asbestos textile factories were active, where the primary workforce was composed of women (5). Camiade et al. conducted an explorative analysis of 318 female PM patients diagnosed between 1998 to 2009 in the French National Mesothelioma Surveillance Program, suggesting a correlation with living with an asbestos worker or washing their asbestos-contaminated laundry. They have named this para-occupational exposure.

Mesothelioma predominantly affects older people. The incidence ratio (IR) for persons under 50 years is ≤0.3 per 100 000 for males in the US and rises to 0.6 per 100 000 or 0.2 to 0.4 per 100 000 for females, respectively. The IR for those above 80 years is almost tenfold higher for women with an IR of 3,0 and even higher for males with 18,9 (3). According to Marinaccio et al. mean age at diagnosis was higher in women (70.5 years and 69.3 years) than in men (68.8 years and 66.4 years), both in occupational and non-occupational groups, respectively (8).

1.2. Histology

According to the WHO classification from 2021, PM is divided into an epithelioid (EM), biphasic (BM), and sarcomatoid subtype (SM) (11). This division is prognostically important, as biphasic and sarcomatoid subtypes have poorer prognoses than the epithelioid form (12, 13). EM contains polygonal, cuboidal, or oval cells (Figure 1), whereas SM consists of spindle cells with variable cytologic atypia (Figure 2). BM requires at least 10% of the tumor to contain sarcomatoid or epithelioid characteristics (Figure 3). This definition only applies to biopsy obtained from maximal cytoreductive therapy such as extended pleurectomy/decortication (EPD) and extrapleural pneumectomy (EPP). In smaller samples, diagnosis of BM can be suggested even without the rule of 10% of each component (11, 14). PM can be further subdivided according to its architectural pattern and cytological and stromal features. Identification of these features helps make the correct diagnosis, while specific patterns have a prognostic significance. Pleomorphic and transitional patterns, for example, have similar (bad) prognosis like biphasic and sarcomatoid subtypes.

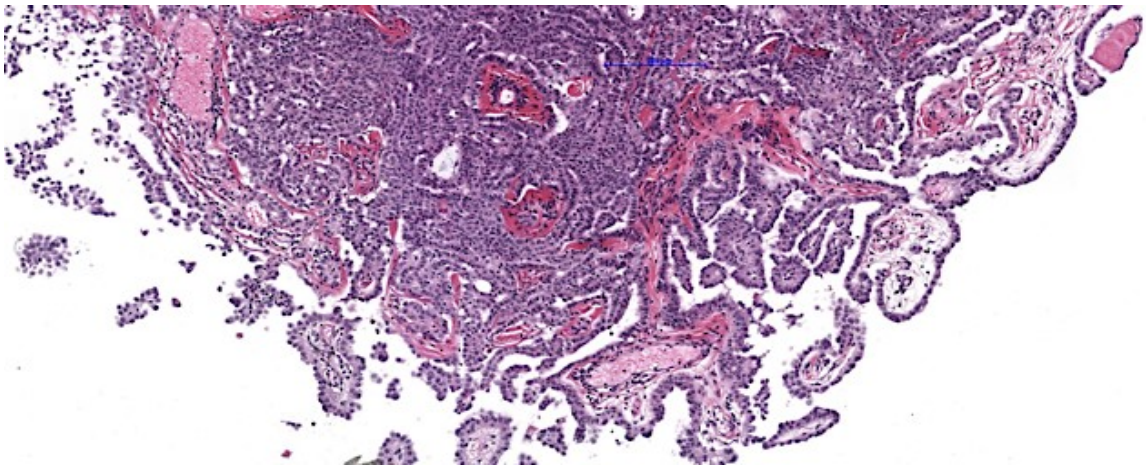

Figure 1 . Histologic presentation of epithelioid mesothelioma with tubulopapillary pattern (hematoxylin-eosin, original magnifications x100)

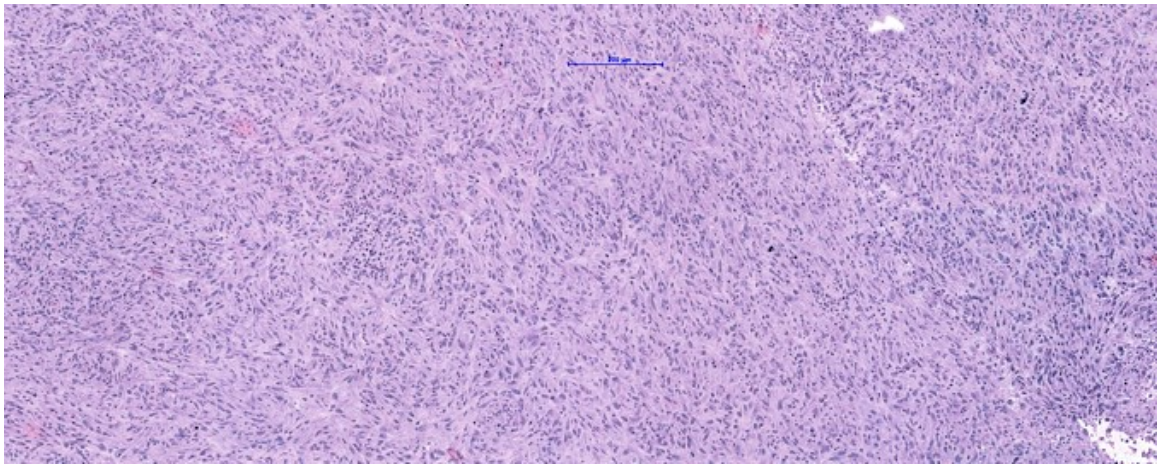

Figure 2. Histologic presentation of sarcomatoid mesothelioma with spindle cells (hematoxylin-eosin, original magnifications x100).

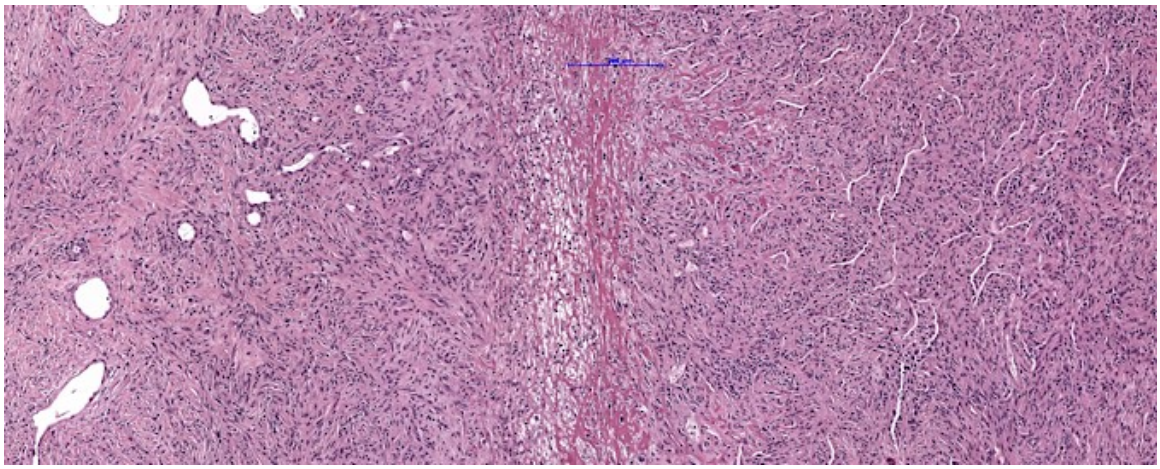

Figure 3. Histologic presentation of biphasic mesothelioma with sarcomatoid (left side) and epithelioid part (right side) (hematoxylin-eosin, original magnifications x100).

Architectural characteristics of epithelioid malignant mesothelioma include tubular (Figure 4), papillary (Figure 5), tubulopapillary (Figure 1), trabecular (Figure 6), solid (Figure 7), micropapillary (Figure 8), adenomatoid (Figure 9), and microcystic (Figure 10) patterns. The tubular pattern shows round to oval spaces that are encircled by a single layer of mesothelial cells. The papillary type consists of fibrovascular cores covered with mesothelial cells. The tubulopapillary form combines tubular and papillary characteristics. Sheets of mesothelial cells are features of the solid pattern. A micropapillary pattern resembles a papillary, but a fibrovascular core is absent. It is important to distinguish micropapillary growth from tubulopapillary as the former invades the lymphatic system more often (15). The adenomatoid pattern consists of similar characteristics to adenomatous tumors, such as gland-like structures overlayed by cuboidal malignant epithelioid cells. The microcystic pattern is a cribriform meshwork consisting of small acinar spaces full of round holes like a sieve (14). The most frequent architectural patterns are tubulopapillary, microcystic, adenomatoid, and solid (16).

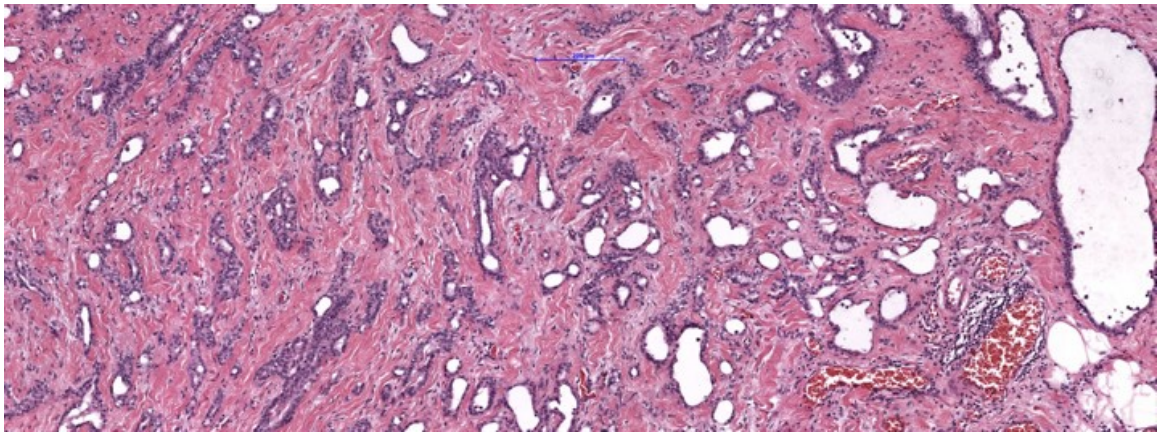

Figure 4. Epithelioid mesothelioma with tubular pattern (hematoxylin-eosin, original magnifications x50).

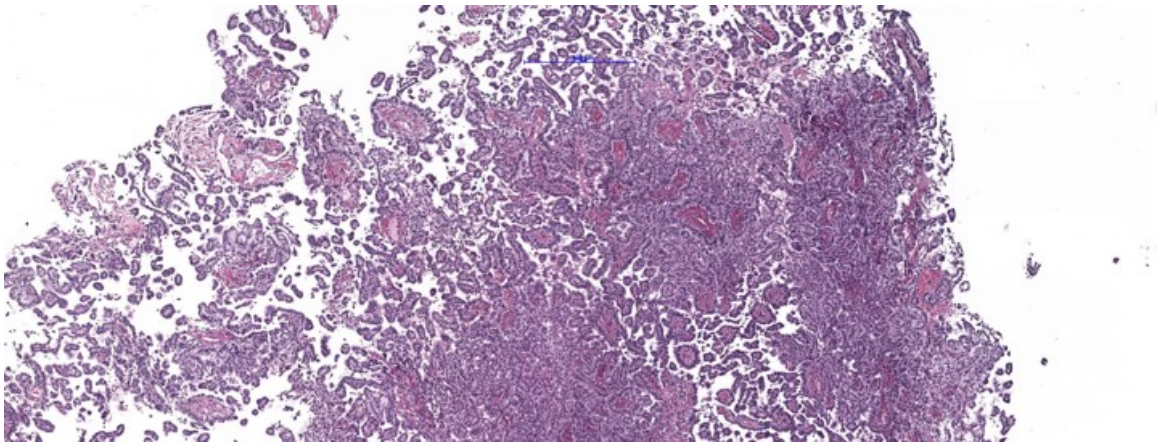

Figure 5. Epithelioid mesothelioma with papillary pattern (hematoxylin-eosin, original magnifications x100).

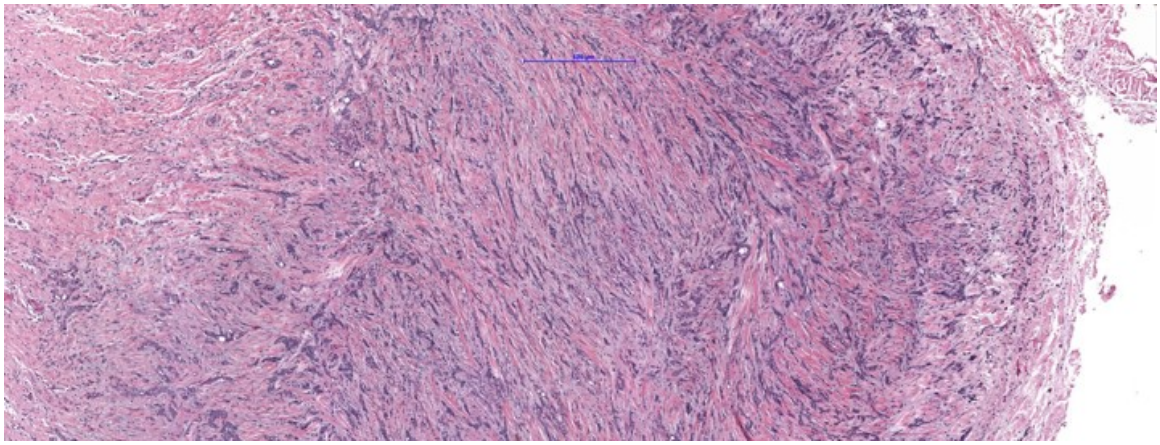

Figure 6. Epithelioid mesothelioma with trabecular pattern (hematoxylin-eosin, original magnifications x100).

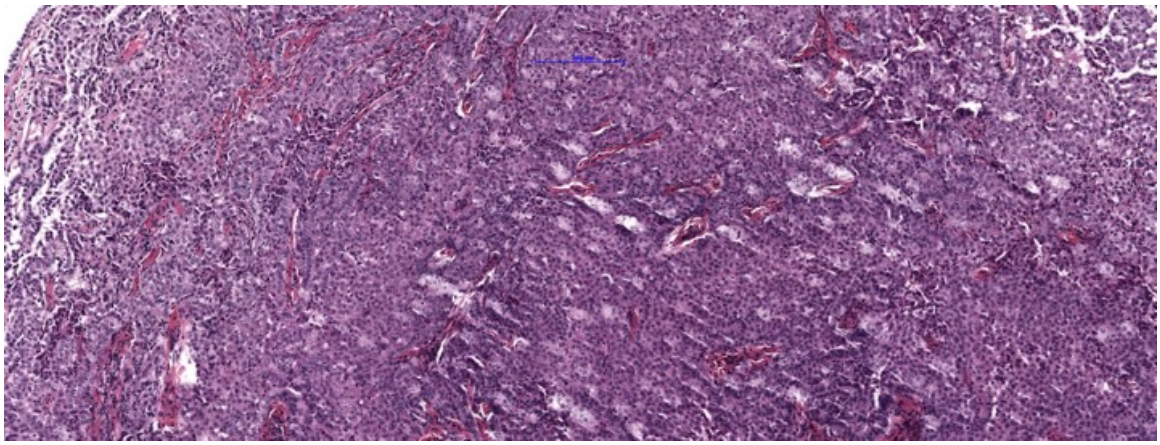

Figure 7. Epithelioid mesothelioma with solid pattern (hematoxylin-eosin, original magnifications x100).

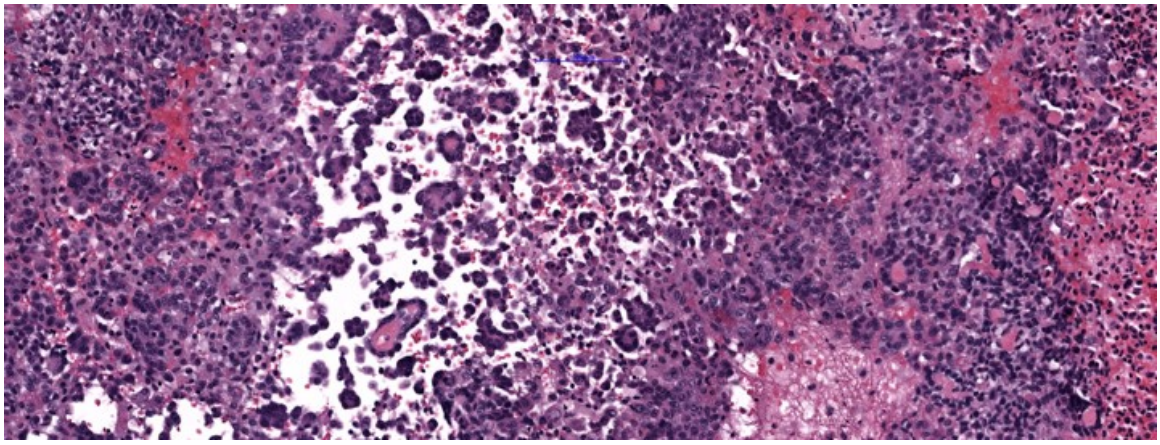

Figure 8. Epithelioid mesothelioma with micropapillary pattern (hematoxylin-eosin, original magnifications x100).

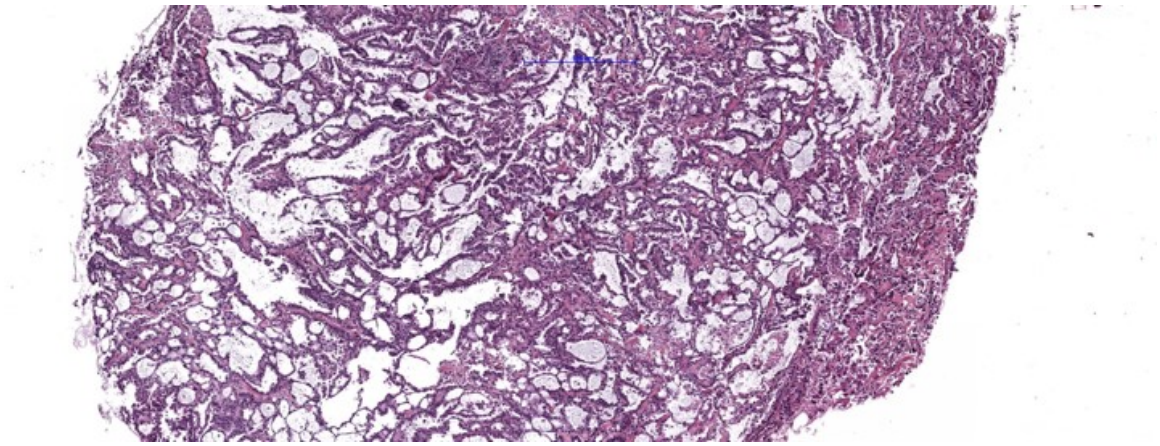

Figure 9. Epithelioid mesothelioma with adenomatoid pattern (hematoxylin-eosin, original magnifications x100).

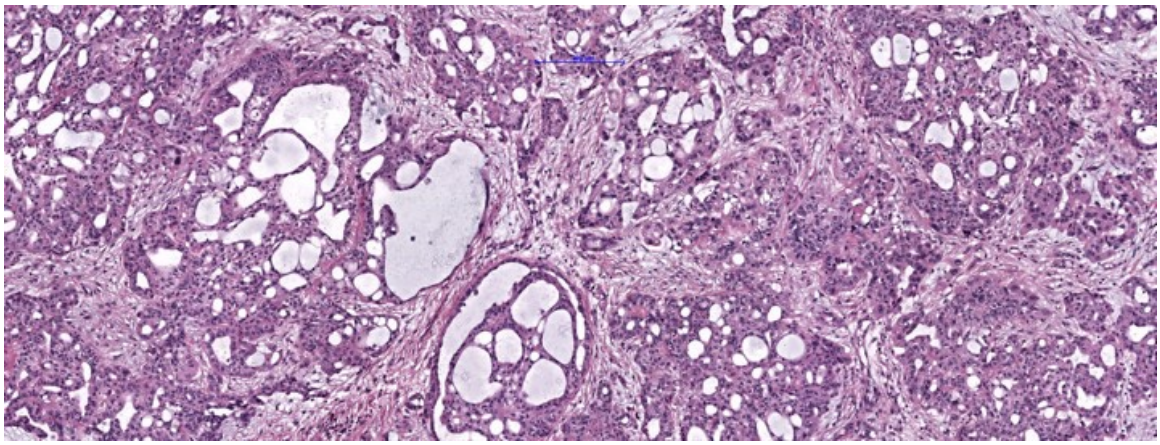

Figure 10. Epithelioid mesothelioma with microcystic pattern (hematoxylin-eosin, original magnifications x100).

Cytologic features are described as follows: pleomorphic cells (Figure 11) are large cells with pronounced nuclear atypia and often bizarre nucleoli. Transitional cells are between epithelioid and sarcomatoid morphology, missing a round shape but not being excessively sarcomatoid. Due to the cohesive property of the transitional pattern, it was classified as an epithelioid subtype, although the prognosis is more similar to that of biphasic and sarcomatoid mesothelioma. It was recently included as a pattern in both epithelioid and sarcomatoid subtypes until ancillary data will be published. Rhabdomyoid cells have eosinophilic globules in the cytoplasm that are positive for cytokeratins but generally negative for muscle markers. Deciduoid cells (Figure 12) resemble cells of the decidual placenta showing excessive eosinophilic cytoplasm. Small cells resemble cells of a small cell carcinoma but with a mesothelial phenotype. Clear cells (Figure 13) have clear cytoplasm, and signet ring (Figure 14) cells contain intracytoplasmic vacuoles that push the nucleus to the side. Deciduoid and small cells carry no prognostic value but are important to identify to avoid misdiagnosis (such as metastatic carcinoma). Lymphohistiocytoid features now described in both epithelioid and sarcomatoid mesothelioma and consist of histiocytoid tumor cells and an intense lymphoplasmacytic infiltration.

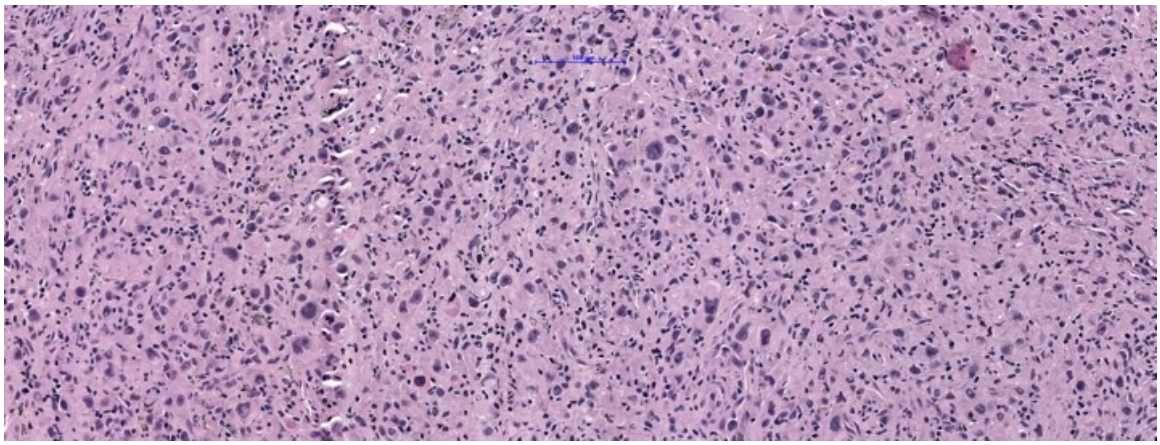

Figure 11. Epithelioid mesothelioma with pleomorphic cells (hematoxylin-eosin, original magnifications x200)

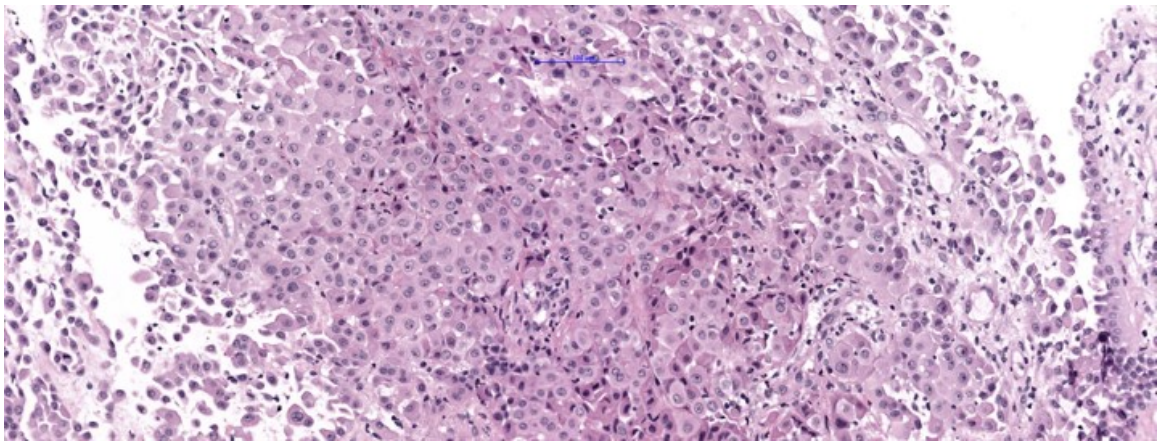

Figure 12. Epithelioid mesothelioma with deciduoid pattern (hematoxylin-eosin, original magnifications x200)

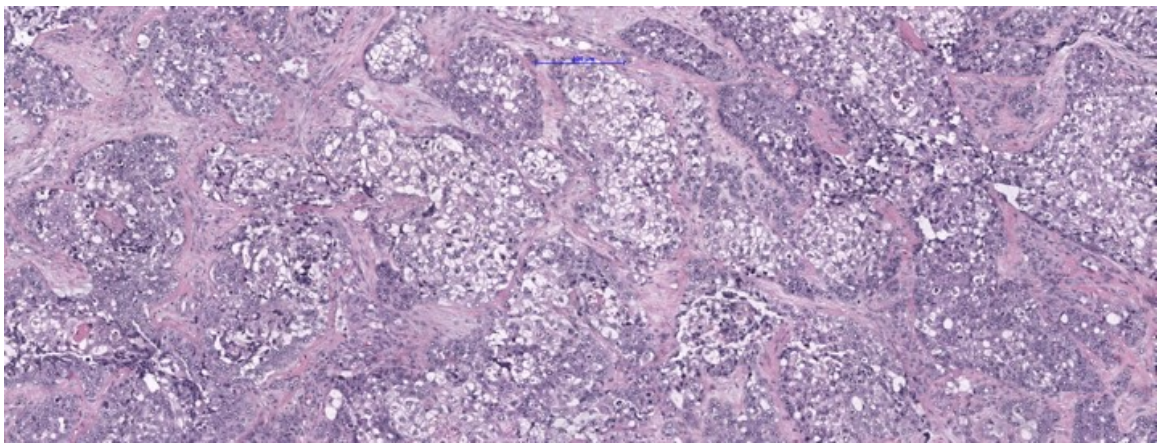

Figure 13. Epithelioid mesothelioma with clear cells (hematoxylin-eosin, original magnifications x100)

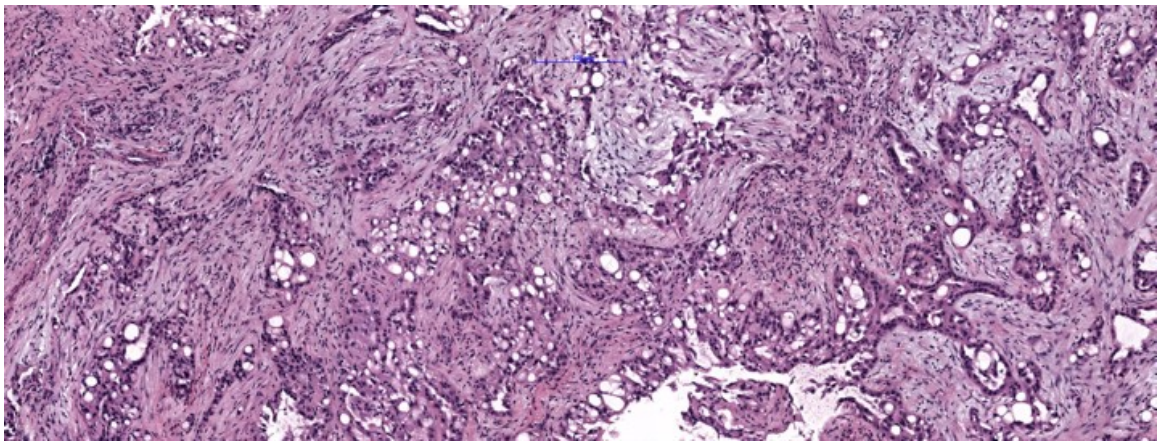

Figure 14. Epithelioid mesothelioma with solid pattern and signet ring cells (hematoxylin-eosin, original magnifications x100)

At last, stromal features of PM should also be taken into account (Figure 15, 16). Myxoid stroma is characterized by tumor cells in a pale hematoxyphilic mucoid stroma and should be noted when present in more than 50% of the tumor, which has less than 50% of solid growth pattern. Desmoplastic stroma is found in sarcomatoid mesothelioma and consists of prominent dense hyaline fibrous tissue, slit-like spaces, bland necrosis, and cellular proliferation nodules. The last stromal feature-heterologous elements- are found in less than 3% of sarcomatoid mesotheliomas and encompasses osteosarcoma, chondrosarcoma, or rhabdomyosarcoma characteristics.

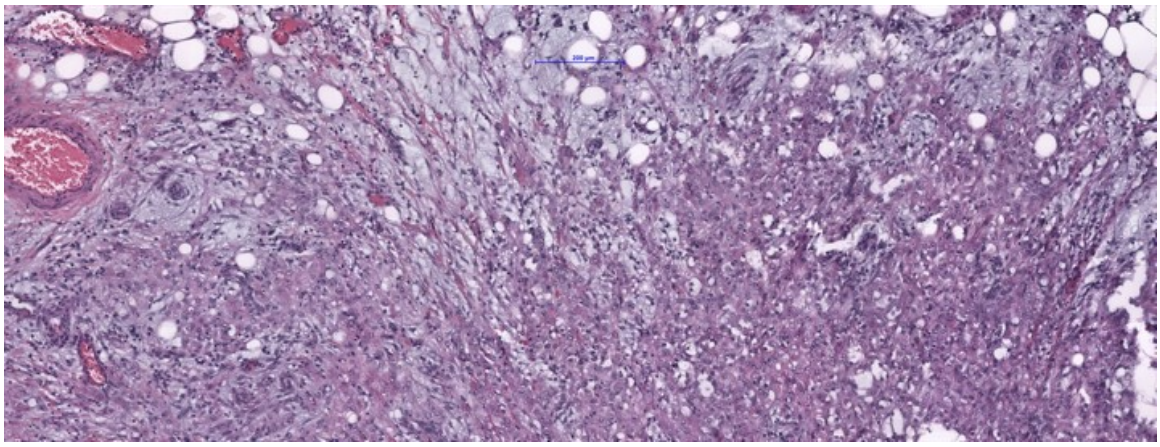

Figure 15. Epithelioid mesothelioma with solid pattern and myxoid stroma (hematoxylin-eosin, original magnifications x100)

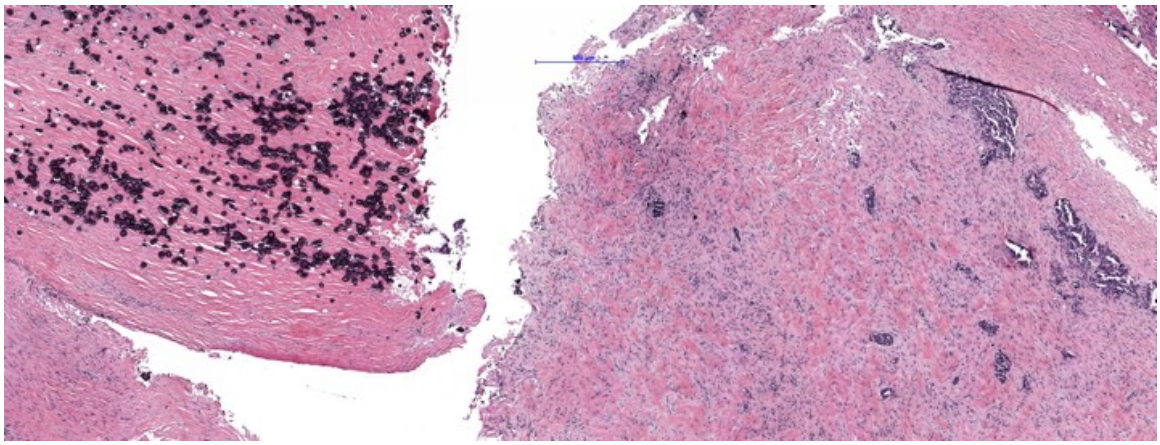

Figure 16. Epithelioid mesothelioma with hyalinized pleura and calcification (hematoxylin-eosin, original magnifications x40)

1.2.1. Grading

Although it is not traditionally performed, recent studies showed prognostic value in the grading of EM. Kadota et al. showed that stratification into three nuclear grades, composed of nuclear atypia and the mitotic count, had significant prognostic value (17). A recent study proposed the additional importance of the necrosis for grading system, differentiating four categories: nuclear grade I tumor without necrosis, nuclear grade I tumor with necrosis and nuclear grade II without necrosis, nuclear grade II with necrosis, nuclear grade III(18). Based on this study, a two-level grading system (low vs. high grade) was proposed (14).

1.2.2. Biopsy

A reliable diagnosis of PM requires an adequate biopsy. An ideal biopsy should include subpleural fat or lung parenchyma as invasion is a good diagnostic lead. It is essential to have enough biopsy specimens as histologic subtyping can be flawed when certain areas are not covered. Chirieac et al. showed that the accuracy of histologic subtyping increases with the number of examined biopsy specimens (19). To determine the ideal number of biopsies for the accurate diagnosis, further studies are required. Before that happens, the EURACAN expert group recommends samples from at least three different pleural areas, including areas of interest according to pre-surgical imaging (14). Procedures that allow maximal cytoreduction, such as EPD and EPP (20), should be pathologically staged(14).

1.2.3. Benign vs. malignant process

1.2.3.1. EM vs. reactive pleural hyperplasia

Indicative features for EM, such as high cellularity, mitoses, nuclear atypia, and entrapment of mesothelial cells resembling invasion, may also occur in reactive mesothelial proliferation. As long as there is no apparent tumor mass, invasion is the most reliable criterion. Staining with pan-cytokeratin or calretinin can be helpful for the detection of invasion since it is often subtle. Features indicating benign mesothelial proliferation are zonation, absent stromal invasion, capillaries perpendicular oriented to the surface, and uncommon necrosis and, if present, associated with acute inflammation. Zonation describes the highest cellularity and often shows marked cellular atypia right under the pleural effusion and decreasing cellularity from the effusion towards deeper structures. On the other hand, in the mesothelioma cellularity increases or is the same throughout the whole pleura thickness. Surface proliferation is typical for a benign process. Still, there are cases where invasive mesothelioma demonstrates a similar growth pattern imitating benign surface proliferation. In these cases, detecting the loss of *CDKN2A* by fluorescence in situ hybridization (FISH) or BAP-1 by immunohistochemistry can be helpful (see below) (21).

1.2.3.2. Desmoplastic variant of SM vs. fibrous pleurisy

Stromal invasion of SM can often be challenging to detect as the malignant cells tend to look bland and resemble fibroblasts. Mesothelial cells should not be present in adipose tissue, skeletal muscle, visceral pleura, or lung parenchyma. Pan-cytokeratin (Figure 26) staining is remarkably useful to detect invasion in these areas. When assessing invasion, fake fat is a misleading phenomenon that should be taken into consideration. It occurs due to the biopsy or sampling of fibrotic pleura and consists of round to oval spaces resembling fat. It can contain horizontally interspersed keratin-positive mesothelial cells mimicking invasion. In such cases, S100 staining will be helpful to demonstrate true fat. Furthermore, desmoplastic mesothelioma grows rather downwards than horizontally. These features help to separate a benign from a malignant process (22). Fibrous pleurisy shows bland

spindle cells forming regular layers and parallel fascicles remaining within mesothelial boundaries. On the other hand, SM shows a disorganized pattern with random proliferations. Another feature speaking for the SM is expansive nodules that vary in size, show a sharp margin and difference in cellularity to the surrounding area (16).

1.3. Immunohistochemistry

Significant improvements in the usage of immunohistochemistry (IHC) to distinguish malignant mesothelioma from other carcinomas have been made since the 2004 WHO classification, where only general carcinoma markers were mentioned. Which IHC markers to use depends on localization, histologic subtype, and thus the considered differential diagnosis. The most common application for IHC takes place in the differentiation of EM with other carcinomas. As no marker has 100 % sensitivity and specificity, the usage in an immunohistochemical panel of at least two mesothelial markers and two carcinoma markers is recommended.

For EM, the most common differential diagnosis is adenocarcinoma of the lung. In this regard, cytokeratin 5/6 (Figure 17), calretinin (Figure 18), D2-40 (Figure 19), thrombomodulin (Figure 20) and WT-1 (Figure 21) have shown high sensitivity and specificity for mesothelial cells, while claudin 4, MOC31, BerEP4, and TTF-1 proved to be the best markers for adenocarcinoma. Occasionally, EM can resemble squamous cell carcinoma of the lung. In such cases, the usage of cytokeratin 5/6 and D2-40 is not recommended as they are positive in up to 100% and 50% of squamous cell carcinomas, respectively.

IHC markers that are often positive in EM such as calretinin (Figure 22), Cytokeratin 5/6 (Figure 23) and WT-1, scarcely show positivity in SM but these cases are often positive for D2-40 (Figure 24). GATA3 shows positivity in 50 % of mesothelioma cases with the highest amount in the sarcomatoid subtype (Figure 25). For distinction of soft tissue sarcomas from SM cytokeratin IHC staining is useful, as a large part of SM (95% or 90% of SM with heterologous elements) is keratin positive. Detecting tumor cells that invade into fat can be facilitated with cytokeratin staining (Figure 26) (21). Carcinomas that often metastasize to the pleura and come in a differential diagnosis with EM, with recommended markers for their distinction, are summarized in Table 1.

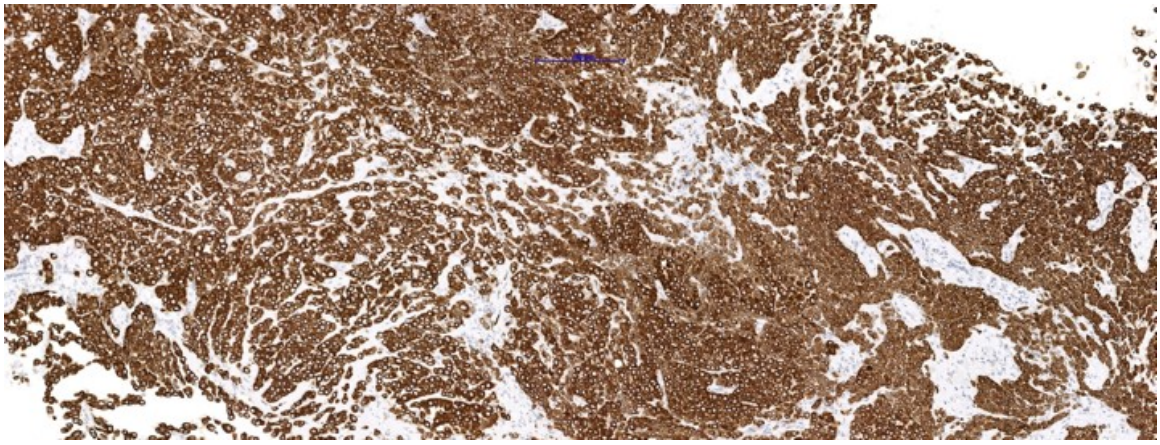

Figure 17. Epithelioid mesothelioma, deciduoid pattern with CK 5/6 staining (original magnifications x100)

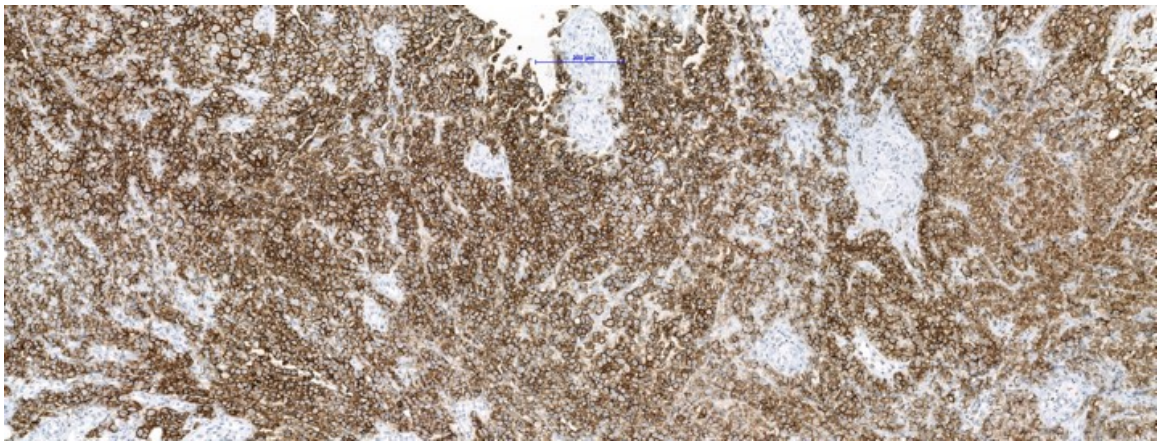

Figure 18. Epithelioid mesothelioma, deciduoid pattern with D2-40 staining (original magnifications x200)

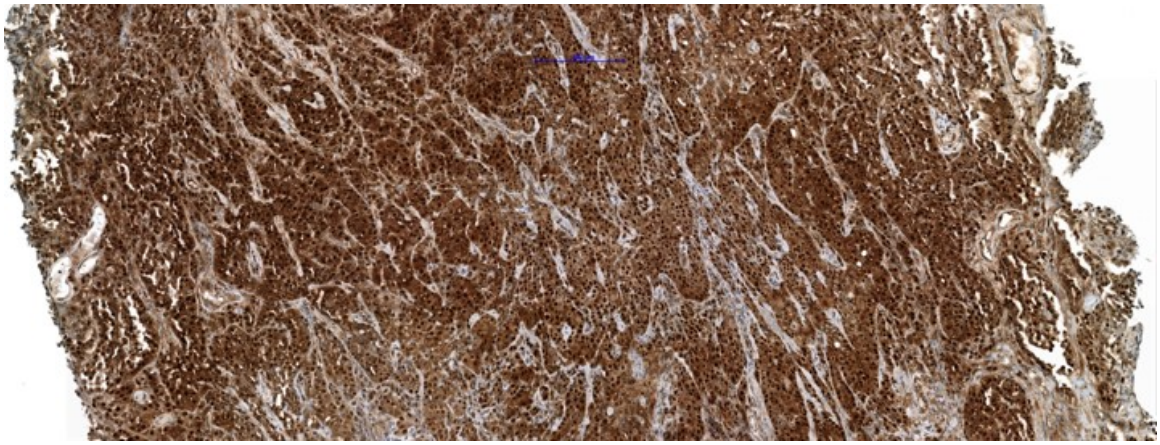

Figure 19. Epithelioid mesothelioma, deciduoid pattern with calretinin staining (original magnifications x100)

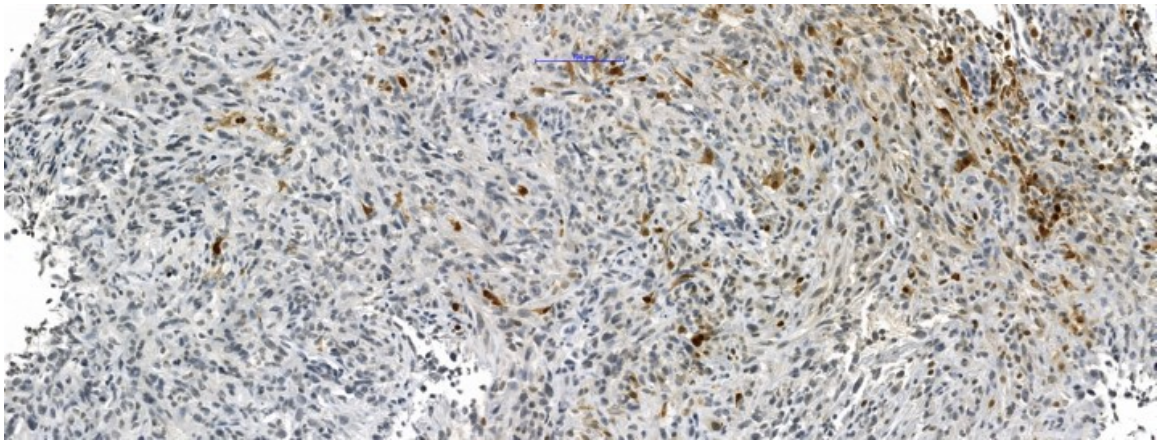

Figure 20. Sarcomatoid mesothelioma with calretinin staining (original magnifications x200)

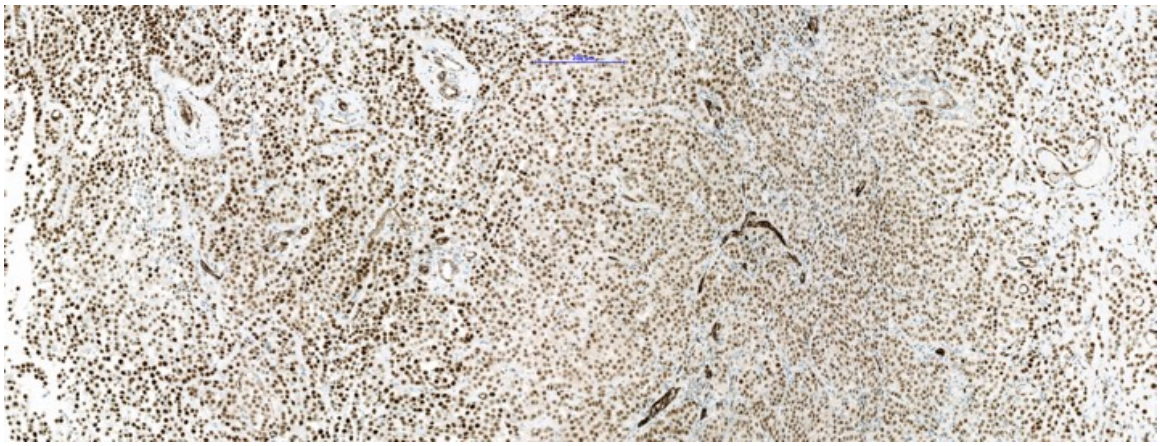

Figure 21. Epithelioid mesothelioma, deciduoid pattern with WT-1 staining (original magnifications x100)

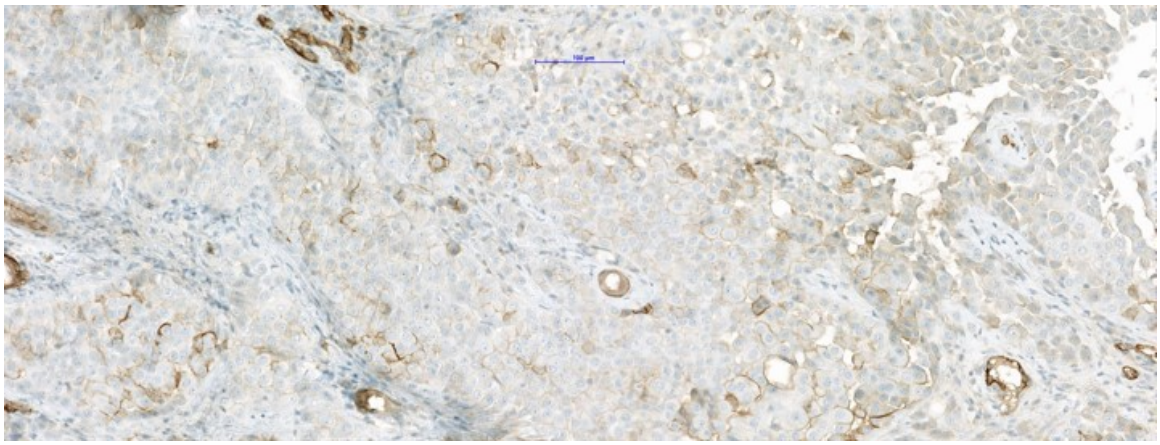

Figure 22. Epithelioid mesothelioma, deciduoid pattern with Thrombomodulin staining (orginal magnifications x200)

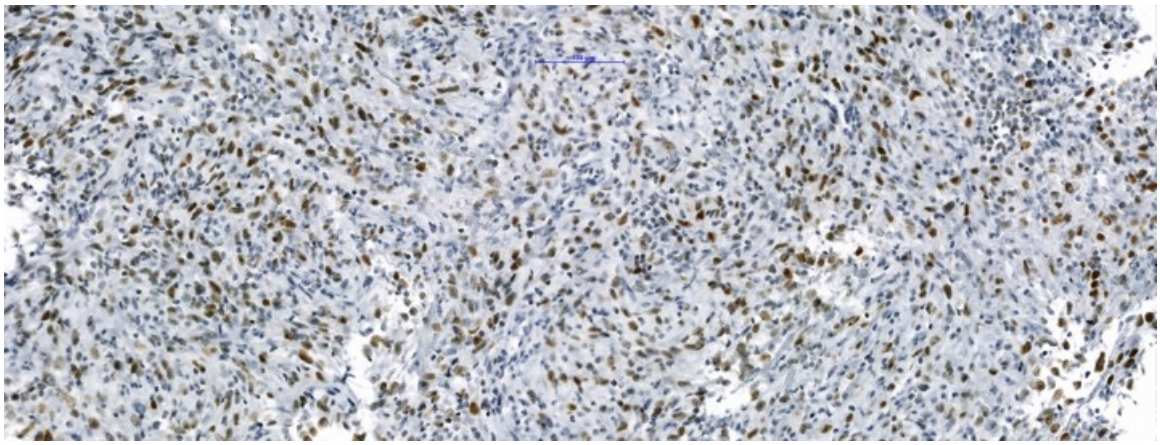

Figure 23. Sarcomatoid mesothelioma with GATA 3 staining (original magnifications x200)

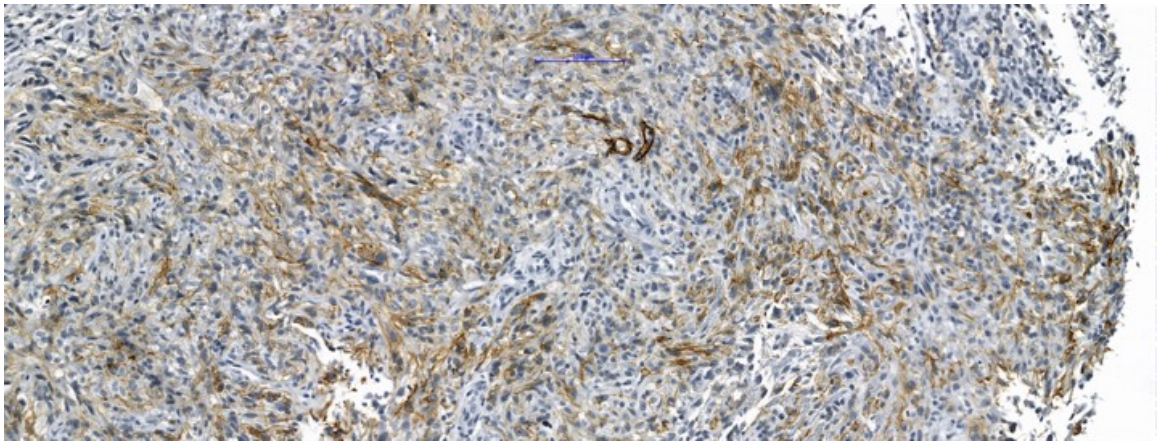

Figure 24. Sarcomatoid mesothelioma with D2-40 staining (original magnifications x200)

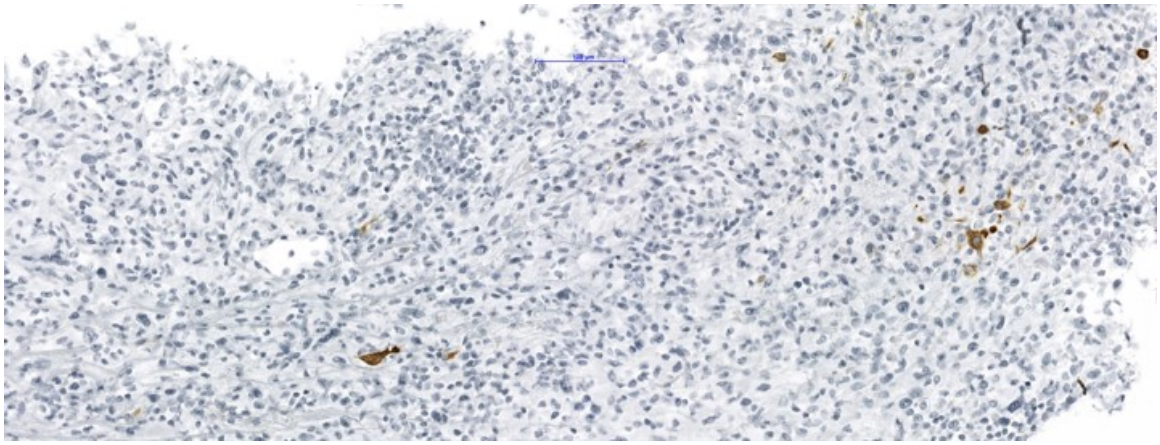

Figure 25. Sarcomatoid mesothelioma with CK 5/6 staining (original magnifications x200)

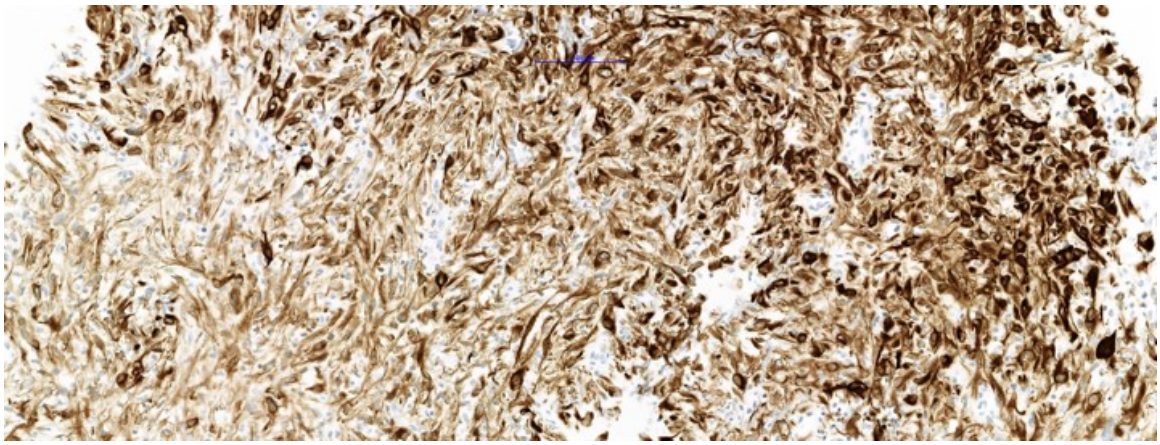

Figure 26. Sarcomatoid mesothelioma with strong positivity with keratin staining (original magnifications x200)

Table 1.. Carcinomas often occurring in a differential diagnosis with EM with recommended markers for their distinction (according to the Guidelines for Pathologic Diagnosis of Malignant Mesothelioma (16)).

Considerable differential diagnosis to malignant mesothelioma that originate from distant organs		
Tumor type	Marker	Comments
Adenocarcinoma of the lung	Claudin 4, MOC31, CEA, B72.3, Ber-EP4, BG8, TTF-1, Napsin A	Claudin 4, MOC31, and BER-EP4 are the best positive carcinoma markers
Squamous cell carcinoma of the lung	p40 or p60, Claudin 4, MOC31, BG8	p40 is especially useful as it is not only strongly expressed in squamous cell carcinoma and absent in mesothelioma but also helps to differentiate between adenocarcinoma
Breast Cancer	Estrogen receptor (ER), gross cystic disease fluid protein 15, mammaglobin, progesterone receptor (PR)	A relatively new marker GATA3 for carcinoma of breast and bladder shows positivity in up to 50% in mesothelioma
Renal Cell Carcinoma	PAX8, PAX2	Expressed in most renal cell carcinoma but not found in PM
Adenocarcinoma of the prostate	Prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA)	
Adenocarcinoma from the gastrointestinal tract	CDX2, cytokeratin 20	
Serous papillary carcinoma of ovary, endometrium, or peritoneum	PAX8, PAX2, ER, claudin 4, MOC31	

1.4. Molecular pathology

The most common genetic aberrations in mesothelioma affect the tumor suppressor gene *NF2*, the 9p21 locus, and *BAP1*. The 9p21 locus contains *p16INK4A*, *p14ARF, MTAP*, and *p15INK4B* (11). A deletion on this locus results in a decrease or loss of the corresponding proteins. Loss of *p16INK4A* or, as it is also called, *CDKN2A* can be detected by FISH. On the other hand, BAP1 expression is detected by immunohistochemistry (Figure 27). It proves to be an excellent tool, especially in combination with *CDKN2A* FISH, in distinguishing mesothelioma from a benign process. Sheffield et al. examined the utility of BAP1 IHC and *CDKN2A* FISH in the separation of benign mesothelial proliferations from malignant. They have shown that benign processes do not demonstrate loss of BAP1 and *CDKN2A* (23). Additional studies showed that *CDKN2A* loss detected by FISH, and loss of BAP1 by IHC, were not found in benign proliferations emphasizing the benefit of these methods in distinguishing benign proliferations and mesothelioma (24-28). Loss of BAP1 is found in 40-60% of EM and substantially lower (<20%) in SM. On the other hand, *CDKN2A* deletion is found in 70% of EM and BM and up to 90-100% of SM (25, 29, 30). Separating a benign proliferation from mesothelioma in cytology specimen has low accuracy compared to tissue biopsies as the important feature of malignancy, invasion, cannot be determined. Therefore, the detection of *CDKN2A* deletion by FISH is also essential in favoring the diagnosis of mesothelioma. Savic et al. analyzed 52 pleural effusions of histologically confirmed mesothelioma and demonstrated a sensitivity of 79% for FISH *CDKN2A* with a positive predictive value of 100% (31). Since the sensitivity of *CDKN2A* FISH and BAP1 IHC is relatively low, negative results with these methods do not rule out the possibility of mesothelioma. It is known that there is a considerable discrepancy between the *CDKN2A* deletion detected by FISH and the expression of p16 detected with IHC. Some cases show no deletion of *CDKN2A* but an expression of the related protein and vice versa (24). Hence, IHC staining is not useful as a surrogate marker for *CDKN2A* deletion. P16 IHC shows prognostic significance, and its expression is associated with shorter survival (32).

As *BAP1* mutations and 9p deletions occur in other carcinoma and sarcoma, these markers are not useful to differentiate between mesothelioma and other

malignancies. Until now, no molecular marker is helpful in this matter. However, certain tumors that can come in differential diagnostic considerations with mesothelioma, such as the solitary fibrous tumor (SFT), have specific molecular markers. Generally, SFT is diagnosed due to its distinctive histologic morphology, but in some cases, especially when biopsy specimens are small, a definite diagnosis can be difficult. Whole-genome sequencing led to the discovery of NAB2-STAT6 fusion, a characteristic mutation for SFT that can be detected via STAT6 IHC. Although a proportion of desmoid tumors and unclassified sarcomas – potential diagnostic pitfalls to SFT – can express STAT6, it is still a highly sensitive and specific marker for SFT (33, 34). Epithelioid hemangioendothelioma, another tumor that can grow in the pleura and cause diagnostic problems, has a highly specific mutation. This is a translocation resulting in a WWTR1-CAMTA1 fusion that can be detected with FISH. This can also be used in small biopsies and is a helpful diagnostic addition (35). Another mutation with diagnostical importance is CTNNB1, and it occurs in 85% of sporadic desmoid-type fibromatosis. This locally high aggressive neoplasm can grow in the pleura and infiltrate the surrounding soft tissue of the chest wall. CTNNB1 mutation leads to the expression of β-catenin, and 75% of desmoid-type fibromatosis shows nuclear reactivity by IHC(36).

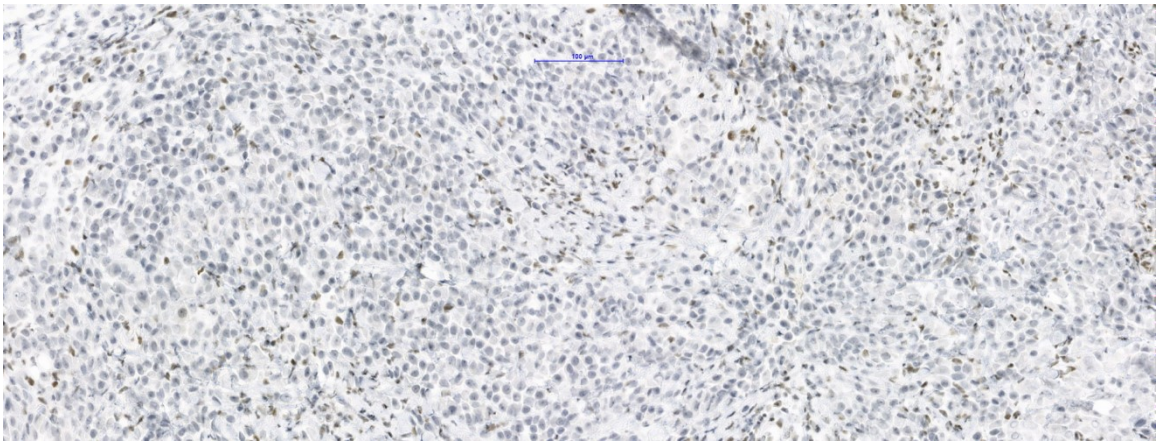

Figure 27. Epithelioid mesothelioma, deciduoid pattern with BAP-1 immunohistostaining (original magnifications x200)

1.5. Cytopathology

As mesothelioma often causes pleural effusions that undergo cytological examination, a definite diagnosis based on cytopathologic features alone is a great benefit, especially for patients where invasive procedures display a high health risk. But as invasion is the most reliable criteria for differentiating mesothelioma from a

benign reaction, this feature cannot be evaluated in a cytologic specimen. Therefore, sensitivity is relatively low and has a broad range from 30% to 75%(37). Sampling error probably leads to a considerable amount of false-negative results. However, it should be emphasized that many features typically found in mesothelioma are similar in benign reactions: scalloped borders of cell clumps, intercellular windows with lighter, dense cytoplasm edges, and low nuclear to cytoplasmic ratio (16). Another limitation of a cytologic specimen is that spindle cells rarely shed into pleural effusions, which potentially leads to false-negative results when an SM is present.

In differentiation reactive vs. malignant cells in cytology, it helps when a specimen is densely populated with mesothelial cells. Malignant mesothelial cells are bigger and form clusters that are greater in size and number. The protruding cytoplasm of these cohesive groups leads to a flower-like appearance, and nuclear atypia and prominent nucleoli, without a second population, are other characteristics of malignant mesothelial cells (Figure 28). Another indication for mesothelioma are parakeratotic or orangeophilic cells with pyknosis as keratinizing squamous cells rarely occur in body fluids and are more common in mesotheliomas than in benign proliferation (38). Matsumoto et al. showed that certain gene alterations such as homozygous deletions on the 9p21 locus are associated with cell-in-cell engulfment, multinucleation (more than two nuclei), and larger multicellular clusters composed of more than ten cells (39). Cytologic features of reactive mesothelial cells can be a pleomorphic pattern, artifactual vacuoles that are smaller compared to malignant cells as they do not cover up the entire cells. Furthermore, these vacuoles have a blurred margin, and a targetoid pattern, as seen with mucin, is absent. Mitoses can be commonly seen in benign proliferation but without atypical mitotic figures.
To improve the accuracy of diagnosis, immunocytochemical (Figure 29) and molecular techniques similar to that of tissue samples can be applied on smears or cell blocks (40-43). As mentioned previously, the detection of gene alterations of *BAP1* and *CDKN2A* is a beneficial addition in diagnosing mesothelioma in tissue samples and cytologic specimens. Initial workup in terms of a step-by-step approach with validated BAP1 antibodies is recommended. The next step consists of FISH testing for homozygous *CDKN2A* deletion when BAP1 is intact, but morphology and clinic are highly suspicious of PM. The *MTAP* gene is located close to *CDKN2A* on

the 9p21 locus and is co-deleted in >90%. Therefore, MTAP loss detected by immunocytochemistry (ICC) is a reliable surrogate marker for CDKN2A homozygous deletion. On the contrary, p16 ICC is not recommended as a surrogate marker due to low sensitivity and specificity for detecting *CDKN2A* deletion(44). As mentioned in molecular advantages, BAP1 ICC and *CDKN2A* deletion by FISH shows 100% specificity, which was demonstrated in various other studies; therefore, a definite diagnosis based on cytology is possible (16, 23, 27, 45-48). Chevrier et al. showed that BAP1 ICC and *CDKN2A* by FISH detected mesothelioma in pleural effusions even when morphology was negative, suggesting that these methods are a considerable option for elderly patients with unexplained recurrent pleural effusions (45). Cases where some cells display intact BAP1 and others BAP1 loss require further testing with MTAP ICC or *CDKN2A* detection via FISH as a small tumor cell population can be hiding with normal, reactive cells. If additional tests are normal, these cases are considered suspicious for malignancy, and tissue sampling is recommended.

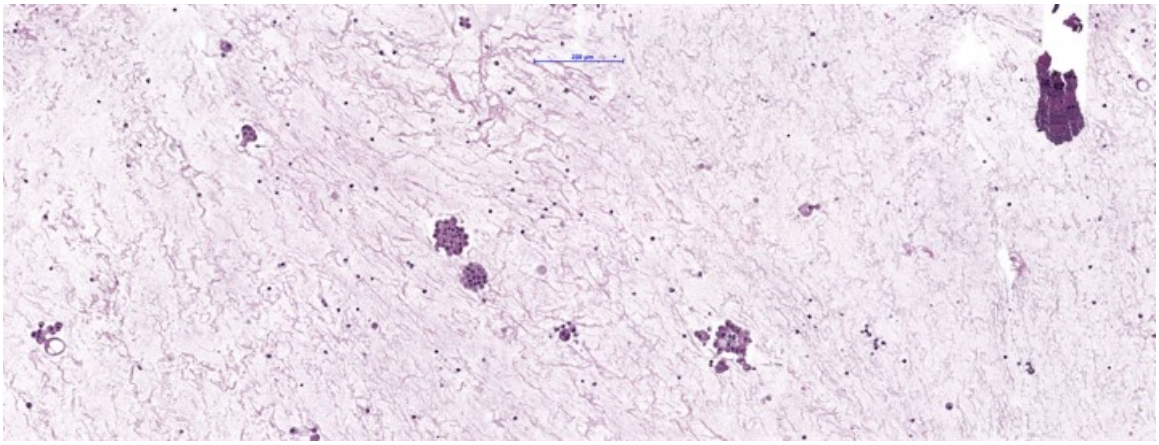

Figure 28. Cell block of an epithelioid mesothelioma with hematoxylin-eosin staining (original magnifications x100)

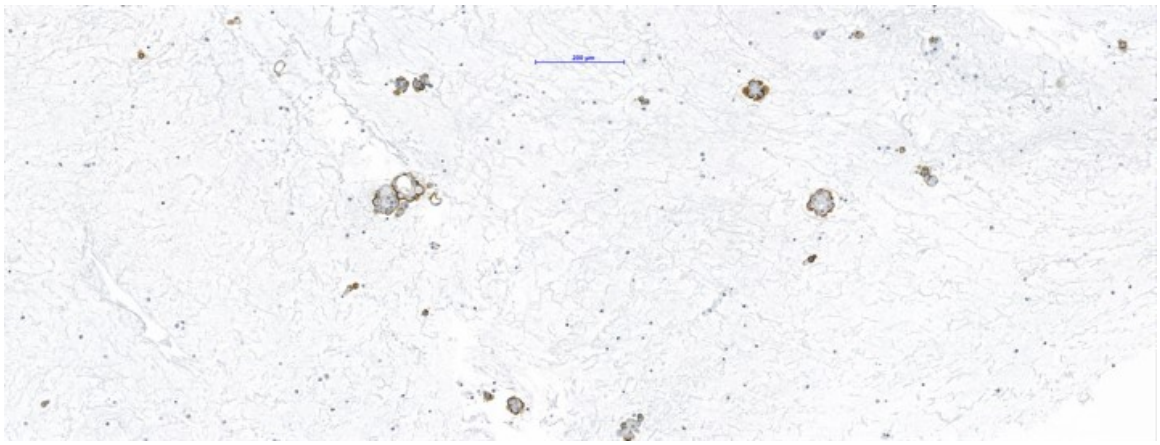

Figure 29. Cell block of an epithelioid mesothelioma with D2-40 immunohistostaining (original magnifications x100)

1.6. Mesothelioma in situ (MIS)

A small case series in 1992 examined seven patients without evidence of tumor in the first biopsy but who afterward developed a PM. The previously acquired tissue showed a single layer of mesothelial cells with cellular atypia. The possibility of underlying invasive mesothelioma that was not recognized or ascertained by biopsy could not be excluded (19). Invasion is an important criterion for differentiating between reactive mesothelial proliferation and malignant mesothelioma. Therefore, the distinction between MIS and reactive mesothelial proliferation based on histology alone is difficult. Common molecular alterations in PM, such as loss of BAP1 expression or homozygous *CDKN2A* deletion, could contribute to diagnosing MIS.

1.7. Symptoms

PM is often accompanied by pleural effusions, which lead to breathlessness. Over the course of the disease, the pleural effusions tend to diminish, and pleural space is infiltrated by the tumor, restricting lung movement and causing breathlessness again (49, 50). Chest wall pain is another common symptom caused by pleural effusion or the tumor itself. As chest wall invasion progresses, chest wall pain tends to increase. Bone pain or neuropathic pain can result from rib invasion or intercostal nerve involvement, respectively (51). Compared to carcinoma of the bronchus that frequently presents with cervical lymphadenopathy, cough, hemoptysis, and symptoms due to distant metastasis, these are less commonly found in mesothelioma. The tumor grows locally and then spreads hematogenous. This local tumor growth can affect mediastinal structures such as the vena cava superior or the laryngeal nerve. However, severe symptoms due to this are unlikely to occur, and dysphagia because of laryngeal palsy indicates a pre-terminal stage. Apparent weight loss can occur as the disease progresses (49).

1.8. Patient Perspective

Due to high mortality and limited therapy options, patients confronted with mesothelioma diagnosis often feel uncertain and hopeless regarding their future (52-54). Compared to other tumors, depression, and anxiety more commonly affect

patients with mesothelioma (52, 55-57). PM being classified as an occupational disease enables a claim for compensation, which can be complex and stressful for both patients and their families and generates anger towards the previous employer(53, 54, 57). Others are concerned that colleagues or family members exposed to asbestos may face the same fate(58). On the other side, those who learn about asbestos-related death of colleagues experience anticipatory anxiety, also known as “Damocles Syndrome,” as they are at similar risk (57, 58).

1.9. Prognosis and Therapy

There is no curative treatment for PM, and management of the disease often requires a multimodal concept. Surgery is limited for patients in an early stage of disease (tumor is confined to the pleura, no N2 lymph node invasion) with favorable histology. Based on histology, a pulmonary reserve of the patient and adjuvant/intraoperative strategies extended pleurectomy decortication (EDP) or extrapleural pneumonectomy (EPP) are carried out. The latter is the more radical and, therefore, riskier procedure, although it is unclear which procedure is oncologically better (59-62). Depending on preoperative therapy and pathological staging, adjuvant chemotherapy or radiotherapy should be performed. Unresectable patients should be treated with systemic therapy. In 2003 and 2005, two-phase III studies assessed the effectiveness of third-generation anti-folate agents in combination with platinum in PM treatment (63, 64). They showed prolonged survival compared to monotherapy with cisplatin, leading to the implementation as first-line therapy for PM. Since then, only marginal progressions were made, as this combination is still in the first-line therapy regiment. The median overall survival is around 13 months with standard first-line chemotherapy (63, 64). In the last years, advancement in understanding the biology of PM was made, and currently, various studies assess the effectiveness of anti-angiogenetic and targeted therapy (65-69).

2. Materials and Methods

2.1. Cases

For this retrospective study, the Lung Archive Dataset from Prof. Popper and data from the BioBank of the Medical University of Graz through the Institute of Statistics of the Medical University of Graz were used. We have obtained two lists, and after correlation, assembled one cumulative list containing all mesotheliomas diagnosed at the Diagnostic and Research Institute of Pathology from 1989 till the end of 2018. This list included all diagnosed and suspected mesothelioma cases. Applying our inclusion criterium (pleural mesothelioma) and excluding mesothelioma arising in the peritoneum, pericardium, or tunica vaginalis testis, the final list was made. All

available paraffin blocks and matching slides from the Lung Archive and BioBank were obtained based on this list. For each patient/sample, the number of available blocks and slides was recorded, and the histology was re-evaluated to confirm the diagnosis. In the Excel datasheet, the following parameters were inserted: gender (M, F), age at the time of diagnosis, diagnosis with histologic type, number of blocks, and number of slides (including hematoxylin-eosin- and IHC-slides).
This retrospective study was approved by the Ethics Committee of the Medical University of Graz (30-475 ex 17/18).

2.2. Statistics

Descriptive statistics were performed on the whole study cohort and then separately for the samples diagnosed from 1989-1998, 1999-2008, and 2009-2018. The number of diagnosed PM, gender, age at diagnosis, and histologic type were compared between groups http://www.graphpad.com/using the Mann-Whitney test or the Fisher´s exact test, as appropriate. A p-value of ≤ 0.05 was regarded as statistically significant. The statistical analyses were performed with GraphPad Prism version 9.1 for Mac, GraphPad Software, San Diego, California, USA, www.graphpad.com.

3. Results

Out of all 295 cases, 218 (74%) were male, and 74 (25%) were female, while for the remaining 3 cases (1%), the gender could not be determined. Most of the patients had epithelioid mesothelioma (211 cases, 71.1%), and only 53 (18%) and 31 (10.5%) were biphasic and sarcomatoid respectively. The number of patients increased substantially over the years: 57 in 1989-1998 (cohort 1), 87 in 1999-2008 (cohort 2), and 151 in 2009-2018 (cohort 3). The mean age at the point of diagnosis was 68 years (Table 2).

Table 2. An overview of the collected data and performed statistical analysis.

	1989-1998 (n=57)	**1999-2008 (n=87)**	**2009-2018 (n=151)**	***p*-value ***
Basic characteristics				
Age - median (range)	60 (33-94)	66 (33-81)	71 (34-94)	0,0005
Female gender - no. (%)	13 (22.8%)	26 (29.9%)	35 (23.2%)	<0.999 9
Male gender - no. (%)	44 (77.2%)	61 (70.1%)	113 (74.8%)	0,8569
Histology - no. (%)				
Epithelioid	33 (57.9%)	62 (71.3%)	116 (76.8%)	0,0095
Biphasic	19 (33.3%)	18 (20.7%)	16 (10.6%)	0,0003
Sarcomatoid	5 (8.8%)	7 (8.0%)	19 (12.6%)	0,627
* p-values for the comparison of 1989-1998 vs 2009-2018. Kruskal-Wallis test for age Fisher´s exact test for all other parameters (gender, histology)				

The comparison of the three cohorts regarding their age showed that in more recent cohorts, there was a significant (p=0.0005) increase of age at the point of diagnosis. The median age from 2009-2018 was 71 years compared to 66 years and 60 years from 1999-2008 and 1989-1998, respectively. There was a broad spectrum of age, as the youngest patients in the cohorts were 33, 33, and 34 years old and the oldest 94, 81, and 94 years, respectively. Figure 30 shows the age distribution over the examined years.

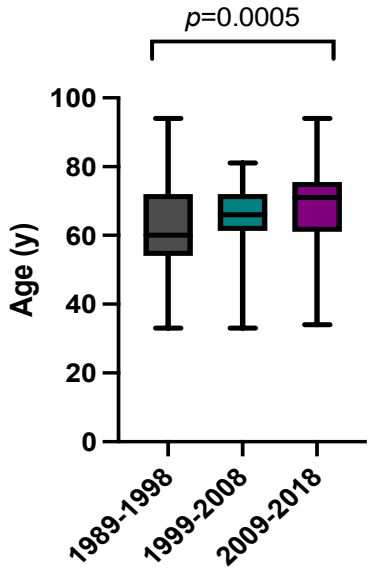

Figure 30. Comparison of age between the different subgroups over the years.

The distribution of age regarding gender shows that male patients were slightly older at the time of diagnosis (median 69 vs. 66), although the difference is not significant (Figure 31).

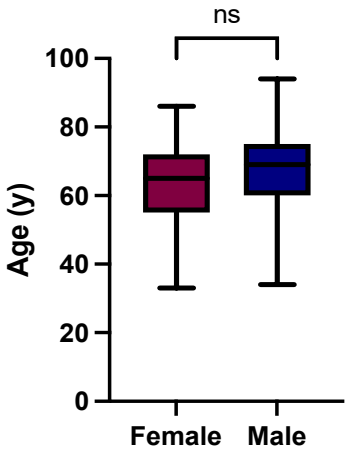

Figure 31. Comparison of age between female and male patients.

In terms of histologic subtype, epithelioid mesothelioma was by far the most common one in all three cohorts. However, the group from 2009-2018 has, with nearly 80%, the highest amount of epithelioid mesothelioma. There is a stepwise decrease in the proportion of epithelioid samples in the previous cohorts with around 70% from 1999-2008 and just under 60% from 1989-1998, respectively (p=0.0095). Conversely, the smallest proportion of biphasic mesothelioma could be observed in 2009-2018 (10.8%), whereas the highest amount of biphasic mesothelioma in 1989-1998 (30%; p=0.0003). The distribution of sarcomatoid cases in the cohorts was roughly the same, with a slightly higher proportion in the 2009-2018 group (12.8% vs. 8.0% and 8.7%); however, differences were not significant (Figure 32).

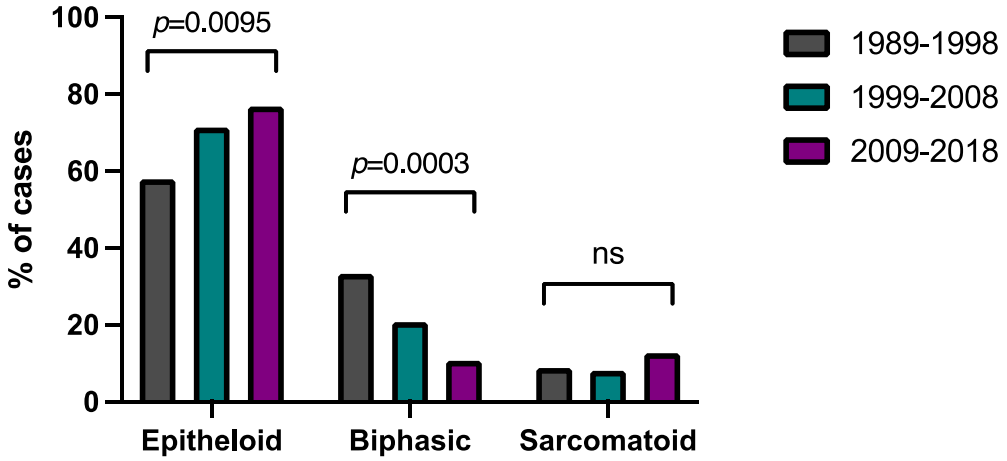

Figure 32. Comparison of histological subtypes between the different subgroups over the year.

In terms of gender, only little differences could be observed between histologic subtypes. The proportion of biphasic mesothelioma was higher in males, and conversely, the number of sarcomatoid samples was higher in females. However, differences in epithelioid, biphasic, and sarcomatoid subtypes were not significant (Figure 33).

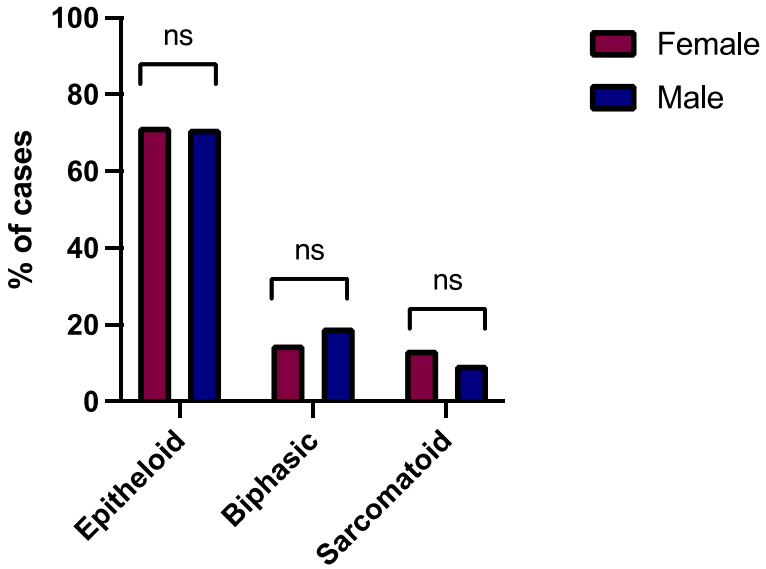

Figure 33. Comparison of histological subtypes between female and male patients.

Furthermore, an assessment of the distribution of histologic subtypes regarding age was conducted. The analysis shows that no subtype preferably affects younger or older people (Figure 34).

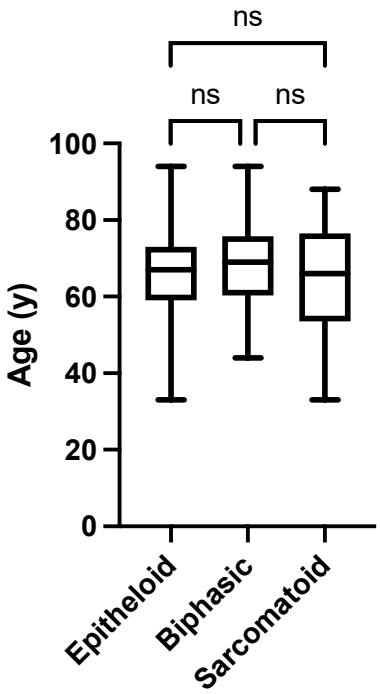

Figure 34. Comparison of age between the different histological subtypes.

4. Discussion

This work analyzed age and gender distribution and histologic subtypes of PM in the period of 30 years at a single institution (Medical University of Graz) in Austria. Our data clearly showed the increase in the number of diagnosed cases over the years, with a high predominance of males and older patients.

In our cohort, the male to female ratio was 2,95, which is slightly higher than that reported from Statistik Austria (2.47). A high proportion of male patients might indicate that occupational asbestos exposure is likely the root of the disease in most cases since males were predominant workers in the asbestos-associated industry. However, additional clinical data is required to explore further how many of the analyzed cases are actually the result of asbestos exposure and the source of this exposure. In Austria, every diagnosed mesothelioma has to be reported to the AUVA (Austrian Workers Compensation Board) via a completed form where a physician documents the presence or absence of asbestos exposure. An occupational medicine report indicates that mesothelioma is underreported because only 40% of all mesothelioma cases covered in Statistik Austria were recognized as an occupational disease compared to 65% in Germany with similar social security law (70).

Surveillance systems that assess mesothelioma cases with sufficient clinical data and asbestos exposure, such as the ReNaM in Italy, are rare; comparable registries are found in France, Australia, and South Korea. The Australian Mesothelioma Registry (AMR) assesses asbestos exposure with a postal questionnaire or a telephone interview after receiving mesothelioma reports from the states/territories (Australian methodology paper). In comparison, data for the ReNaM are obtained through a trained interviewer that uses a standardized questionnaire to ask about occupational history, lifestyle habits, and residential history (8). Unfortunately, such an approach, although highly efficient, still does not exist in Austria.

As for the distribution of histologic subtypes, an accurate comparison with other epidemiologic studies is challenging as most studies that assess the quantity of different histology samples consist only of small cohorts. Two studies obtained PM samples from the Memorial Sloan Kettering Cancer Center. Muller et al. assessed VISTA expression in PM, examining 319 cases that were immunotherapy-naïve.

254 (79.6%) were EM, 24 (7.5%) were BM and 41 (12.9%) were SM. Kadota et al. analyzed CD10-Expression of PM and evaluated 176 cases from the MSKCC with 148 (84.1%) epithelioid, 14 (8.0%) biphasic and 14 (8.0%) sarcomatoid subtypes. The Italian register ReNaM collected 19955 cases of PM, of which 14495 were classified according to the WHO histology subtypes. 10771 (74.3%) accounted to EM, 2167 (14.9%) cases were BM and 1557 (10.7%) were SM. As histology subtypes are of prognostic importance and may affect therapeutic options, this highlights the need to document subtypes accurately.

Our analysis showed an increase of PM cases over time, which corresponds to the data of Statistik Austria as well as to data from other European countries that refrained from asbestos use at the same time. In Germany, a significant decrease in the incidence rate from 2000 to 2014 was observed in women and men below 75 years (71). However, incidence increased in men over 75 years, similar to our observation about the age distribution in the different cohorts (Figure 30). Besides the long latency, the expected decline is probably also delayed due to higher diagnostic accuracy because advancements in IHC and molecular pathology lead to better detection of PM cases. However, some of the cases can still be misdiagnosed as non-neoplastic or another neoplastic process.

In our cohort, male patients were slightly older at diagnosis compared to female patients (Figure 31). One explanation for this might again be differences in occupational asbestos exposure between genders. Opposing to this, a large-scale study in Italy shows a higher mean age in females than in men (8). However, to reliably determine if there are significant regional age differences between genders, higher case numbers in our region would be needed, with adequate clinical data (or minimal data about asbestos exposure).

Interesting developments regarding the distribution of histologic subtypes were observed in our analysis, as seen in Figure 32. The proportion of EM has significantly increased over time, whereas the proportion of BM has significantly decreased. No significant differences in SM proportions could be observed. To our knowledge, there is currently no convincing study that systematically examined histological subtype proportions over time. However, as documentation of PM improves, more data in this regard can be expected in the future. A possible explanation for the described inverse developments of EM and BM might be

changes in the diagnostic workup of PM (e.g., immunohistochemistry) and the addition of new growth patterns to EM and SM, rather than changes in epidemiology. Figure 33 shows that female patients were proportionally slightly more commonly diagnosed with EM compared to males, whereas BM were more common in men and SM more common in women. In our analysis, these differences were not significant, so one could assume that variations observed in our cohorts are based on chance, and with higher case numbers, this distinction will adjust. However, a comparison with data from the ReNaM shows a similar distribution with more EM in female patients (78.6% vs. 72.7%) and more BM in male patients (15.6% vs. 13.3%), but less SM in the female group (11.7%vs 8.1%). Although again no systematic studies exist in this regard, a possible explanation might lie in the association with the influence of some other toxic agents (like nicotine, radiation) and/or genetic changes.

Before widespread ban or regulations of asbestos use, high-income countries (HIC) had the highest asbestos consumption (72). Around this time, upper-middle-income countries (UMIC) increased their asbestos production, and the global volume of asbestos consumption peaked in 1980 with 6 million metric tons. A few years later, at around 1985, UMIC overtook first place in asbestos consumption, and they hold their share until now. The global volume decreased over the turn of the millennium and stabilized at 2 million metric tons. Asbestos consumption continuously decreased until 2010, where global asbestos consumption is only shared between UMIC and lower-middle-income countries (LMIC). Based on this, the burden of PM is currently highest in HIC but is expected to decline in the next few years, as it is already seen in the US (3). Conversely, the incidence of PM will likely rise in UMIC and LMIC. Diagnosis of PM can be challenging, especially in LMIC, where histopathologists are rare, the disease is uncommon, and capacities for sufficient IHC are not present. Data from LMIC regarding PM are often declared unreliable because underreporting and/, or underdiagnosing is a common problem. Therefore, assessing the actual impact of the current asbestos consumption in these countries will be difficult (72).

Although in most countries the biggest sources of asbestos production closed after the introduction of restrictions for asbestos usage, other countries seem to accept the accompanying health risks. PM burden has very likely reached its peak in HIC such as Austria but will still play a role in the next few years. This is particularly

important as affected patients are entitled to compensation. As therapy options and prognosis are very limited, the best option against PM remains to refrain from asbestos usage. To ensure the detection of asbestos sources and evaluate the effectiveness of asbestos bans, surveillance with epidemiologic studies is an important measure against PM.

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