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Neoplasms of the Pleura

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Pleural Neoplasms

In contrast to primary lung neoplasms, primary pleural neoplasms are uncommon. Pleural neoplasms may be difficult to diagnose and must be distinguished from metastatic carcinomas and sarcomas involving the pleura, and from benign reactive processes causing pleural thickening. A correct diagnosis is important so that appropriate therapy, although it may be only palliative, can be instituted.

The most common and most frequently referenced primary pleural neoplasm is mesothelioma, which is considered a signal tumor because of its etiologic relationship to asbestos exposure. Neoplasms such as metastatic carcinomas, sarcomas, leukemia, and lymphoma may occur primarily in the pleura and must be differentiated from mesothelioma.

Mesothelioma

Definitions, History, Incidence, and Epidemiology

Definition

Mesotheliomas are tumors derived from cells forming the serosal lining of the thoracic, abdominal, and pericardial cavities (see Chapter 30).[1,2] They exhibit a wide variety of histologic patterns and may be confused with many other types of neoplasms. Former pathologic "dogma" viewed mesothelioma as a diagnosis of exclusion that could be diagnosed only by postmortem examination. It is our opinion that immunohistochemical and ultrastructural analysis of pleural neoplasms can lead to an accurate diagnosis of mesothelioma and nonmesotheliomatous neoplasms in most cases, even with small biopsy specimens.

History

Mesotheliomas are rare tumors, accounting for less than 1% of all cancer deaths in the world.[3] Two pleural tumors possibly representing mesotheliomas, as noted by Chahinian,[4] were described by Joseph Lieutaud in 1767 in a study of 3000 autopsies. E. Wagner[5] recognized mesotheliomas as a pathologic entity in 1870, and concluded that only sarcomas could be classified as primary malignant pleural tumors and that all epithelial-appearing neoplasms were metastases from an unrecognized or latent primary site. In 1924 Robertson,[6] in an article titled "'Endothelioma' of the Pleura," provided a thorough account of early reports on the clinical and pathologic features of pleural neoplasms. Of interest, one case included in the evaluation of lung cancer related to asbestos by Doll[7] was referred to as an *endothelioma*, most likely indicating this case was a mesothelioma and not a lung cancer. In 1931 Klemperer and Rabin[8] described five primary pleural neoplasms—four were localized and had mesenchymal features and one was diffuse, encasing the lung with a mixed epithelial and mesenchymal histologic appearance. Klemperer and Rabin divided primary tumors of the pleura into localized and diffuse forms, stating localized tumors originated from subpleural "areolar" tissue and were low-grade malignancies usually causing death by interference with the pulmonary circulation, and were potentially curable by surgical removal. They concluded that diffuse neoplasms of the pleura arose from the mesothelial cells lining the serosal surface and could exhibit an epithelial or mesenchymal histologic pattern.

Most cases of mesothelioma reported between 1940 and 1960 were localized.[9,10] In 1943 Wedler[11] reported a case of a diffuse mesothelioma in a person with asbestos exposure. Wedler[12] and Merewether[13] referred to tumors of the pleura in discussing cases of lung carcinoma in patients with asbestosis. It is likely that these neoplasms referred to as "tumors of the pleura" represented mesotheliomas. In the United States the first report of a diffuse mesothelioma with asbestos exposure was in 1947.[14] Even as late as the mid-20th century, some pathologists, notably Willis,[15] denied the existence of mesotheliomas. A pleural and a peritoneal mesothelioma associated with asbestosis were respectively reported in

the German literature in 1953 and 1954,[16,17] and in 1960 Keal[18] reported the association of peritoneal mesotheliomas and asbestos exposure. Also in 1960 Wagner et al.[19] reported 33 cases of diffuse pleural mesothelioma in the North Western Cape Province of South Africa. Of these 33 patients, 32 had exposure to asbestos. Wagner[20,21] recounted his experience with the discovery of mesotheliomas in South Africa, and further suggested that all pleural mesotheliomas in the United States were caused by crocidolite asbestos, a suggestion with which we strongly disagree and which is not supported in the medical literature.[22,23] Smither et al.[24] and McCaughey et al.[25] recorded additional cases of asbestos-related mesothelioma in 1962, and for some of those cases the exposure appeared to have been minimal. In the same year, Wagner et al.[26,27] published studies on the mucin histochemistry of mesothelioma and on the induction of malignant mesothelioma (MM) in experimental animals by asbestos.

In 1964 and 1965 Selikoff and colleagues[28,29] linked mesotheliomas to asbestos exposure by finding that 10 of 307 consecutive deaths in asbestos insulation workers were caused by diffuse mesothelioma. Also in 1965 Newhouse and Thompson[30,31] recorded the occurrence of mesotheliomas as a consequence of domestic (household contact) asbestos exposure among those who shook out and laundered the asbestos-contaminated work clothes of their partners, and from neighborhood exposure acquired by residence in the vicinity of an asbestos factory. Most MMs reported since 1970 have been diffuse; the localized form is rare.[18,32]

By the late 1990s, the incidence of MM in some industrialized nations was comparable to that of cancer of the larynx,[33] with a death rate similar to that of renal cell carcinoma in males and uterine cancer in females.[33–37] Apart from lung cancer,[38] MM is now the most important occupational cancer among industrial workers, because of its prevalence, resistance to conventional cancer treatments, and its lethality.

The history of the medical-legal aspects of asbestos-related lung disease was discussed in detail by Motley[39] and Brodeur.[40,41] Information presented by these authors suggested that serious deleterious health effects of asbestos were known long before they were reported in the medical literature.

Incidence and Epidemiology

Mesotheliomas encountered in the early 21st century are most often a consequence of prior occupational exposure to asbestos from the 1940s through the 1970s, including end-uses of asbestos-containing materials and "bystander" (indirect) exposures.[36,42–44] The relationship between inhalation of asbestos fibers—especially one or more of the amphibole varieties—and MM is accepted by virtually all authorities as causal.[42] Because of the constancy and specificity of the asbestos–MM relationship, the incidence of mesothelioma is usually considered to reflect a society's past per capita usage of asbestos,[45–48] after allowance for a suitable latency interval between first exposure to asbestos and the subsequent rise in incidence of MM (Fig. 43.1 and Table 43.1).[47,49]

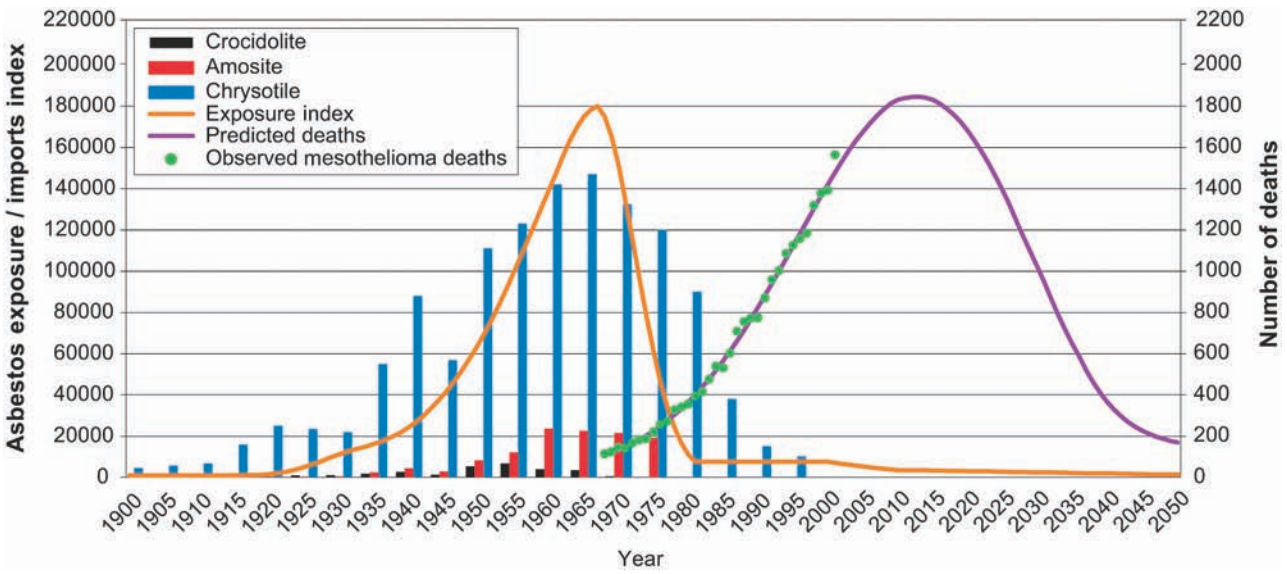


FIGURE 43.1. Observed and predicted deaths from mesothelioma in the United Kingdom, versus asbestos imports and estimated exposure indices, for men aged 20 to 89, for the years 1900 to 2050. (Modified from Health and Safety Executive [HSE]. Mesothelioma mortality in Great Britain: estimating the future burden, December 2003, with permission of the HSE.)

TABLE 43.1. Mesothelioma incidence for some countries relative to their historical per capita use of asbestos

Country	Mesothelioma incidence cases/10^6/yr	Use of asbestos in kg/capita (year)
Australia (1995)	33	4.4 (1968)
Netherlands (1995)	27	3.4 (1976)
United Kingdom (1991)	23	2.7 (1970)
Italy (1993)	22	2.5 (1975)
France (1996)	17	2.6 (1970)
Finland (1995)	15	2.2 (1970)
Germany (1997)	15	3.0 (1975)
Sweden (1995)	15	2.4 (1970)
United States (2000)	15	2.3 (1975)
Norway (1995)	14	1.9 (1970)

Source: Modified from Tossavainen.[47]

Because mesotheliomas are rare neoplasms, their exact incidence is unknown and varies among populations surveyed (Table 43.2).[50–59] The highest incidence in the world is currently in Australia.[58]

The incidence of mesothelioma in autopsy series is considerably lower. McDonald and McDonald[59] summarized the incidence in six series from eight cities between 1950 and 1970. They tabulated 165 cases in 69,302 autopsies (0.24%).

Several studies[54,56,57] documented an apparent increased incidence of MM, especially in men, during the last several decades. Hughes and Weill[60] estimated that 1500 new cases of mesothelioma were diagnosed in the United States in 1986. The increased incidence of MM is probably related to the delayed effects of an increase in occupational exposure to asbestos. Selikoff et al.[28] reported that 8% of 17,800 workers in the heat and frost insulation industry who were followed prospectively between January 1, 1967, and December 31, 1976, died of diffuse MM.[61] According to Huncharek,[62] the incidence of mesothelioma is increasing at a rate of about 10% per year for U.S. males.

The authors' experience has also suggested an increased incidence of MM that, in part, may reflect an increased awareness by pathologists of mesothelioma and of more accurate diagnostic methods such as electron microscopy and immunohistochemistry. In addition, many cases of mesothelioma in the United States come to litigation, which has made the general public more aware of mesothelioma and, in turn, has caused heightened physician awareness.

According to the Environmental Working Group,[63] there is an asbestos epidemic in America. This group reports that asbestos-related disease is responsible for the death of one in 125 American men over the age of 50, and that 10,000 Americans die each year—30 per day—from asbestos-caused diseases. At this time, the death toll is rising in nine of the 10 states with the highest number of mesotheliomas and asbestosis deaths. Between 1979 and 2001, more than 43,000 Americans died from MM. According to Price,[64] there are approximately 2500 new cases of MM annually in the U.S., 80% of which occur in men.[65] According to Price, the incidence of mesothelioma appears to be rising in men aged 45 years or older, with a maximum lifetime risk in the 1925 to 1929 cohort. The incidence of MM in women and in men less than 75 years of age is claimed to have been stable since 1983[64] (but see later discussion).

Peto et al.[66] predicted MM deaths would continue to increase for at least 15, and more likely 25, years. In the most affected cohort, men born in the 1940s, MM would

TABLE 43.2. Incidence of mesotheliomas[a]

Reference	Years surveyed	Location of population surveyed	Number of cases/million population/year
McDonald et al. [50]	1959–mid-1968	Canada	0.65 (males) 0.35 (females)
Theriault and Grand-Bois [51]	1969–1972	Quebec	1.56 (males) 0.74 (females)
Biava et al.[52]		Italy	21.4 (males)
Greenberg and Lloyd-Davies[53]	1967–1968	England, Wales, Scotland	1.88 (males) 0.42 (females)
McDonald and McDonald[54]	1960–1975 1972	Canada United States	2.8 (males) 0.7 (females)
Cutler and Young[55]	1969–1971	Metropolitan area[b]	1.5 (males) 0.7 (females)
Bruckman et al.[56]	1970–1972	Connecticut (U.S.)	1.7 (males) 0.9 (females)
Churg[57]	1982	British Columbia	17 (males) 1.9 (females)
McDonald and McDonald[59]	1950–1970	Eight cities	0.24% of 69,302 autopsies

[a]Incidence includes both pleural and peritoneal mesotheliomas, and in some instances mesotheliomas arising in ovary and male genital system.
[b]Atlanta, Birmingham, Dallas–Ft. Worth, Detroit, Pittsburgh, San Francisco–Oakland, Denver (U.S.).

account for around 1% of all deaths. In 2005 Hodgson et al.[67] stated there were 1848 mesothelioma deaths in Great Britain in 2001 and mesothelioma deaths were predicted to peak at around 1950 to 2450 per year between the years 2011 and 2015 (Fig. 43.1). The Health and Safety Executive Data[68] suggested the peak would occur earlier than originally predicted and the maximum would be approximately 2000 deaths in or around the year 2010. According to Treasure et al.,[69]

> one in every 100 men born in the 1940s will die of malignant pleural mesothelioma. . . . For a man first exposed as a teenager, who remained in a high-risk occupation such as insulation throughout his working life, the lifetime risk of mesothelioma can be as high as 1 in 5. . . . The disease is increasing in frequency. . . . We will see many more mesotheliomas in the next 25 years. In the developed world alone, 100,000 people alive will now die from it.

In Australia, mortality from MM was stated to have been increasing since 1975. Mesothelioma incidence rates are among the highest in the world, and the Australian Mesothelioma Registry received 6129 mesothelioma notifications between 1986 and 2000. Of the mesothelioma cases with past asbestos exposure, close to 89% were work-related, about 3% were not work-related, and about 8% could not be classified. Of the persons who developed work-related MM, one in three worked in the construction industry and one in five worked in the manufacturing industry.

In contrast, Roggli,[70] based on his experience, suggests that a mesothelioma epidemic was beginning to wane in the U.S. Lemen,[71] using Surveillance Epidemiology and End Results (SEER) data and International Classification of Diseases (ICD-10-TEM) coding that went into effect in 1999, stated the accuracy for reporting mesothelioma was about 80% effective, which would mean that in the U.S. there were over 6000 cases of mesothelioma per year.

Etiology

Asbestos

The association of asbestos exposure and the development of mesothelioma has been reviewed in detail.[72–74] The chronology of asbestos is shown in Box 43.1 (Figs. 43.2 and 43.3). Asbestos is the single most important causative agent of mesothelioma. Numerous

Box 43.1. The History of Asbestos

4000 BCE	Asbestos was used for wicks in lamps and candles. "Asbestos" means inextinguishable or unquenchable.
2000–3000 BCE	Embalmed bodies of Egyptian pharaohs were wrapped in asbestos cloths to offset the ravages of time.
2500 BCE	Used in Finland to strengthen clay pots.
800–900 AD	Anecdotal evidence of Charlemagne's tablecloth made from woven asbestos.
1000	Mediterranean people used chrysotile from Cyprus and tremolite from upper Italy for the fabrication of cremation clothes, mats, and wicks for temple lamps.
1300–1400	Marco Polo visited an asbestos mine in China in the latter half of the 13th century. He concluded that asbestos was a stone and lay to rest the myth that asbestos was the hair of a woolly lizard.
Early 1700s	Asbestos papers and boards were made in Italy.
1724	Benjamin Franklin brought a purse made of asbestos to England. The purse is now in the Natural History Museum.
1828	United States patent issued for asbestos insulating material used in steam engines.
1853	Asbestos helmet and jackets worn by Parisian Fire Brigade.
1866	Molded lagging material made from water, glass, and asbestos.
1896	First asbestos brake linings were made by Ferodo Ltd., in England.
1900	High pressure asbestos gaskets made by Klinger in Austria.
1913	First asbestos pipes developed in Italy.
1919	Standard corrugated sheet asbestos introduced in Australia by Hardies.
1939–1945	Wartime use included fireproof suits and parachute flares. In the film *The Wizard of Oz* in 1939, the Wicked Witch of the West appeared on a broom made of asbestos.
1945–1975	Postwar construction projects relied heavily on the use of asbestos, reaching an all-time high in 1973.
1990s	The solid fuel boosters of the space shuttle are insulated with asbestos, one of the few remaining current uses.

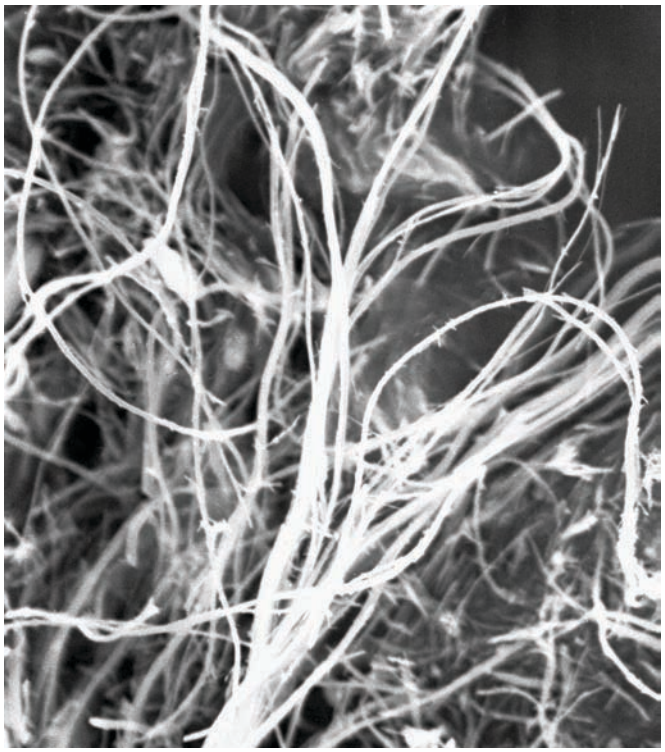


FIGURE 43.2. Canadian chrysotile fibers as visualized by scanning electron microscopy (SEM). The individual fibers are long and wavy (serpentine).

FIGURE 43.3. Scanning electron microscopy appearance of South African crocidolite fibers. In comparison to chrysotile fibers (Figure 43.2), these amphibole fibers are straight and show evidence of longitudinal splitting.

reports[31,59,75–86] have tabulated the percentage of mesothelioma cases associated with asbestos exposure (Table 43.3). The association between asbestos exposure and mesothelioma is stronger in men than in women and, in many series, very few women with mesothelioma have had a history of exposure to asbestos. The threshold amount of asbestos necessary to induce mesothelioma is unknown, although in most reports a dose–response relationship has been suggested[87,88]; that is, persons with a greater intensity and duration of exposure to asbestos have a higher incidence of mesothelioma. Small concentrations of asbestos may induce mesothelioma[89–97] (see

TABLE 43.3. Association of exposure to asbestos and incidence of mesothelioma

		Sex distribution			Cases associated with asbestos exposure		
Reference	Number of cases	Male	Unspecified	Female	Male	Unspecified	Female
Borow et al.[75]	72	64		8	55/55 (100%)		5/5 (100%)
Cochrane and Webster[76]	70		70[a]			60/70[a] (86%)	
Tagnon et al.[77]	61	61		0	45/56 (80%)		
Whitwell and Rawcliffe[78]	52	40		12	35/40 (87.5%)		8/12 (67%)
Hammar[79]	151	119		32	66/82 (80%)		10/22 (45%)
Taylor and Johnson[80]	30	23		7	17/23 (74%)		0/7 (0%)
Vogelzang et al.[81]	31	22		9	13/22 (59%)		2/9 (22%)
Newhouse and Thompson[31]	83	41		42	24/41 (59%)		17/42 (40%)
Peto et al.[82]	116	116		0	69/116 (59%)		
McDonald and McDonald[59]	557	395		162	188/344 (55%)		8/162 (5%)
Roggli et al.[83]	25	21		4	11/21 (52%)		0/4 (0%)
Oels et al.[84]	37	32		5	10/32 (31%)		0/5 (0%)
Brenner et al.[85]	123	84		39	16/84 (19%)		0/39 (0%)
Ratzer et al.[86]	31	21		10	4/31[a] (13%)		

[a]Gender not specified.

below). Malignant mesothelioma can occur via household exposure to asbestos.[98] Vianna and Polan[99] reported a relative risk of 10 for such situations compared to matched controls unexposed to asbestos. Kane et al.[100] reported 10 cases of MM in patients 40 years old or younger. In seven of the 10 cases, there was asbestos exposure—two occupational exposures and five household exposures. Cazzadori et al.[101] reported a case of pleural MM in a 37-year-old woman exposed to asbestos during childhood. From birth to age 10 she lived in a house next to an asbestos-processing factory. Asbestos exposure was confirmed by finding 0.3 asbestos bodies per milliliter in her bronchoalveolar lavage fluid. Huncharek[62] pointed out that exposure to asbestos was no longer confined to asbestos industry workers, and there were nonoccupational hazards such as household and building occupant exposures. Dodoli et al.[102] reviewed death certificates of 39,650 persons between 1975 and 1988 in Livorno, Italy and in 45,900 persons in La Spezia, Italy, between 1958 and 1988. A total of 262 cases of pleural mesothelioma were recorded, most of which occurred in persons occupationally exposed to asbestos in the shipbuilding industry. Thirteen cases of mesothelioma occurred in women who washed the asbestos-contaminated work clothes of their relatives, and six cases occurred in persons domestically exposed to asbestos, possibly from installing fireproof or nonconductive materials.

In 1997 Hammar et al.[103] reported on 103 women with mesothelioma of whom about 70 were exposed to asbestos, the most common source of asbestos exposure being domestic bystander exposure.

Proposed Nonasbestos Causes of Mesothelioma

Erionite

Theoretically, MM might develop at the site of pleural injury caused by almost any agent. Of particular interest has been a group of naturally occurring fibrous silicate minerals called zeolites. In 1975 and subsequent years, Baris and colleagues[104–108] reported that people living in Tuskoy and Karain (two small villages in central Turkey) had the highest incidence of mesothelioma in the world. In Karain, 21 of 50 deaths recorded in people over 20 years old during a 5-year period were caused by mesothelioma. People living in this region of Turkey were found to have very fine fibers of a zeolite called *erionite* in their sputum and lung tissue. These fibers were not found in similar specimens of people living in other areas of Turkey. A search for asbestos in soil, rock, and water samples was negative and it was hypothesized that airborne erionite fibers from building materials caused the mesotheliomas. Lillis[109] substantiated the findings of Baris et al. Sebastien and coworkers[110] demonstrated that 93% of ferruginous bodies from lung samples of two patients with MM from Tuskoy were formed on erionite cores. Wagner et al.[111] induced mesotheliomas in 38 of 40 rats inoculated with erionite. Rohl et al.,[112] however, were able to identify small amounts of tremolite and chrysotile in addition to erionite in environmental samples taken from Tuskoy and Karain (see Nonasbestos and Nonoccupational Mineral Fibers and Mesothelioma, below). They also reported that erionite was found in environmental samples taken from villages with no reported cases of mesothelioma. Recent studies have suggested a genetic susceptibility to mesothelioma in Turkey based on identification of mesothelioma in one village and not in another.[113,114]

Chronic Pleural Inflammation and Scarring

In 1985 Hillerdal and Berg[115] reported two patients who developed mesothelioma in regions of pleural scarring caused by tuberculosis that had been treated with pneumothorax. They reviewed the literature and found 20 additional cases of malignant tumors in pleural scars, 12 of which were found in areas of squamous carcinoma. They reported that squamous carcinoma was the most common tumor associated with scarring from chronic empyema and extrapleural pneumothorax. Malignant mesotheliomas have occurred years after chronic inflammatory lesions of the pleura; for example, chronic empyema or packing of the pleural cavity with leucite spheres as treatment for tuberculosis (so-called plombage therapy). Also, there are a few reports (about eight cases) of an association of peritoneal mesothelioma with familial Mediterranean fever (FMF), possibly related to recurrent FMF serositis.[116] Cases of this type are exceptional, and confounding factors for mesothelioma need to be addressed; for example, in relation to FMF, cases of mesothelioma have been reported in the Mediterranean littoral from white-washing of homes with tremolite-containing material, so that domestic and environmental tremolite exposure might represent a potential confounding factor for the association of FMF and mesothelioma.[117,118] In addition, most cases of postinflammatory mesothelioma with a short interval between inflammation and tumor are probably mesotheliomas that presented with a burst of inflammatory activity, perhaps related to production of cytokines or mediators of inflammation such as interleukin-8, before their final diagnosis as mesothelioma.[119,120]

Irradiation

The literature contains multiple reports of mesothelioma following exposure to ionizing radiation,[121–150] and excess rates of MM have also been reported among both Danish and German patients exposed to radio-active thorium dioxide (Thorotrast®) for radiologic procedures.[121,124,134,135]

Austin et al.[131] reported an ipsilateral malignant pleural mesothelioma in a 28-year-old woman who had a Wilms' tumor at age 4 that had been treated with nephrectomy followed by irradiation. This case is of further interest because asbestos analysis on the autopsy lung tissue found the asbestos content to be within the "normal" range (0–20 asbestos bodies/gram of wet lung tissue). Anderson et al.[132] reported a diffuse epithelial mesothelioma in a 16-year-old boy who at age 2 had received pulmonary irradiation for metastatic Wilms' tumor.

A case of mesothelioma was reported by Mizuki et al.[133] in a 75-year-old Japanese man who developed a left pleural mesothelioma 50 years after the atomic bomb was dropped on Nagasaki in 1945. However, this patient had a history of asbestos exposure at the munitions factory where he was employed as a shipbuilder for 2 years. This case emphasizes the dilemma that background asbestos exposure represents as a confounding factor for some cases associated with radiation (or other associations such as immunodeficiency); for example, in one report on mortality among 260 plutonium workers, all six mesotheliomas occurred in individuals who had also sustained asbestos exposure.[123] In the authors' files are three cases of MM following mantle irradiation for Hodgkin's disease, renal transplant, and radiotherapy for carcinoma of the vulva. Each patient, however, had background exposure to asbestos, including one patient with domestic exposure who laundered her husband's asbestos-laden work clothes.

Neugut et al.[130] carried out a retrospective study of 251,750 women with breast cancer (~25% of whom had been treated with radiation therapy [RT]) and 13,743 patients with Hodgkin's disease (~50% treated with RT), and found no evidence of an association with MM. Nonetheless, this study had two major weaknesses: (1) there appears to have been little or no pathologic verification or classification of recurrent tumors, so that given the past medical history for those patients (breast cancer, Hodgkin lymphoma), any mesotheliomas might have been misclassified as recurrent breast cancer or lymphoma; and (2) the follow-up for the patients in this study did not extend beyond 20 years, so that any mesothelioma cases developing thereafter would have been missed.

Teta et al.[151] found 26 patients with mesothelioma as second primaries based on an evaluation of 21,881 diagnoses of Hodgkin's lymphoma and 101,001 diagnoses of non-Hodgkin's lymphoma. There was stated to be a statistically increased incidence of mesothelioma, with a standardized incidence ratio (SIR) of 6.9 and a confidence interval (CI) of 1.79 to 16.87 among men with Hodgkin's lymphoma who received radiation, and a nonsignificant excess of mesothelioma among men with non-Hodgkin's lymphoma with an SIR of 1.91 and a CI of 0.77 to 3.93. Teta et al. concluded that mesothelioma rates for patients who received radiotherapy were increased for survivors of Hodgkin's lymphoma and non-Hodgkin's lymphoma. No increased incidence of mesothelioma was observed among the nonirradiated.

Travis et al.[129] carried out a study on second cancers among 40,576 testicular cancer patients with a focus on long-term survivors, and found a significantly elevated relative risk (RR) for pleural MM of 3.4 (95% CI, 1.7–5.9). The authors concluded that survivors of testicular cancer were at a statistically significantly increased risk of solid tumors for at least 35 years following treatment by either radiotherapy or chemotherapy. This study did not find any *peritoneal* mesotheliomas following radiation therapy; all of the MMs were *pleural* in location. The authors mentioned that the thorax can receive radiation as a consequence of radiotherapy for testicular cancer, but it is also worth emphasizing that the radiation field for testicular tumors is directed mainly to abdominal and paraaortic lymph nodes. Therefore, it is of interest that all the MMs in this study occurred *outside* the main radiation field, although there are at least two reports of peritoneal MM following radiotherapy for testicular cancer.[137,149]

It is well known that patients with one cancer have an increased risk of other cancers; for example, one strong risk factor for breast cancer is an antecedent cancer in the contralateral breast. The notion of innate (gene-mediated) predisposition to cancer/mesothelioma induction has also been debated by some of the authors addressing radiation and mesothelioma. For example, Shannon et al.[145] noted that the experimental data support a role for radiation in the development of pleural MM. Mesotheliomas were found in 65% of rats 1 year after intraperitoneal injection of plutonium 239 ($^{239}PuO_2$). Whether radiation acts as an independent carcinogen or whether it potentiates the effects of other carcinogenic factors such as asbestos is unclear. An overall increased incidence of pleural MM in rats exposed to irradiation and asbestos (11.8%) over those exposed to asbestos alone (3.8%) has been observed, suggesting that radiation may act as a cocarcinogen to induce MM.[139]

Shannon et al.[145] also reported the following:

> Other variables must be considered in cases negative for asbestos exposure. An obvious common denominator in each of the cases reported is a history of a previous malignancy. The incidence of metachronous multiple primary neoplasms varies from 0.2 to 12%, depending on the selection criteria for the study group. The excess rates of second neoplasms have been ascribed to a genetic predisposition for multiple cancers in several types of tumors. In particular, studies have found a two to three-fold increased incidence of second neoplasms in patients with colon, lung, breast and head and neck carcinomas as well as certain leukemias and Hodgkin's and non-Hodgkin's lymphoma. However, pleural MM as a second malignancy in cancer-prone patients does not appear to be increased in the absence of other predisposing factors. Hence, genetic predisposition is unlikely to be the sole factor in the development of MM as a second primary malignancy.

Travis et al.[129] also conclude that treatment (as opposed to genetic susceptibility to tumors) probably explains much of the observed excess tumors in testicular cancer patients, an interpretation supported by the lower risks in the first 10 years of follow-up.

Accordingly, it is our view that ionizing radiation may play a causal-contributory role in the genesis of some mesotheliomas, probably as a cofactor along with innate susceptibility to cancer development (as demonstrated by one or more antecedent cancers), with or without past asbestos exposure, but the number of such radiation-related cases is small in comparison to the burden of asbestos-related MMs, for which radiation is not a co-factor.

Malignant Mesotheliomas in Children (and the Concept of Spontaneous Mesotheliomas)

In 1985 Talerman et al.[152] reported a case of a diffuse malignant deciduoid peritoneal mesothelioma in a 13-year-old girl and reviewed the literature identifying 41 previously reported cases of mesothelioma in children. Thirty-three of the 41 previously reported cases began in the pleura, and 40 of the 41 children died 2 weeks to 21 months after diagnosis, a clinical course similar to that in adults. In many reported cases of mesothelioma in children, a history of exposure to asbestos was not documented, and in Talerman et al.'s case and in two other cases reviewed, there was no history of exposure to asbestos.

Fraire et al.[153] independently reviewed slides available of 17 children previously diagnosed as having mesothelioma. Upon review, only three cases were confirmed as mesothelioma. Therefore, they concluded mesothelioma in children might be rarer than suspected. Fraire et al.[154] conducted an extended evaluation of 80 reported cases of mesothelioma in childhood. Of the 80 cases, tissue slides were available for review in 22 cases, of which 10 were considered MM, nine nonmesothelial malignant tumors, and three malignant neoplasms of uncertain type. The authors found no relationship between childhood MM and asbestos, radiation, or isoniazid therapy. Lin-Chu et al.[155] reported a confirmed case of MM in a 19-month-old girl. In their review of the literature, they found three other cases of MM in infants. In their case, there was no information concerning exposure to asbestos.

The occurrence of mesothelioma during infancy, childhood, and adolescence supports the notion of true spontaneous mesotheliomas. Diagnosis of mesothelioma during infancy and childhood poses greater difficulties than for adults, especially the distinction from pleuropulmonary blastomas of childhood[156] and perhaps desmoplastic small round cell tumors of the pleura,[37] but there is little doubt that childhood mesotheliomas do occur. From a review of three studies, McDonald and McDonald[157] suggest that the incidence of childhood mesothelioma may be within the range of 0.5 to 1.0 case/10^7/yr.

Background Exposure to Asbestos and Background or Spontaneous Mesotheliomas: Do They Exist? It is our perception that background asbestos exposure from the environment at large represents general environmental exposure unrelated to the use of asbestos-containing materials in the workplace or at home, or from significant point sources of asbestos such as factories. We consider background exposure to include exposures related to the passive weathering of in-place asbestos-containing materials, including asbestos-cement roofing materials with very low or unmeasurably low airborne fiber concentrations, and environmental exposure derived from the brakes of passing automobiles; we exclude from "background" any exposure arising from active disturbance of any asbestos-containing materials such as asbestos-cement building products or insulation materials.

It is also important to recognize that absence of a history of asbestos exposure does not equate to absence of exposure. Many cases of seemingly background MM can be attributed to long-past forgotten or unrecognized asbestos exposures. For example, many of the cases that are encountered in our everyday or referral practice are accompanied by a clinical statement that no asbestos exposure has been identified, but subsequent and more detailed history-taking usually does yield a history of brief exposure to asbestos, and in some of those cases the mesothelioma patient was unaware that the material used (e.g., fibrous cement building materials) did in fact contain asbestos. The problem of detailed and systematic history-taking is also exemplified by some of the data in the Australian Mesothelioma Surveillance Program, in which a substantial number of the cases initially classified as having no known exposure history in fact had asbestos exposure documented upon more detailed review.[43]

The often-cited background MM rate of 1 to 2 per million person-years, was derived partly from backward extrapolation of the incidence rates in men, to the point where the rates for men and women diverged from each other, based on a presupposition that the female incidence rate for mesothelioma has been stable, and that most MMs in women represent background cases.[158] In reality, there is persuasive evidence that both of these assumptions are false: (1) in the United Kingdom the death rate for MM in females increased from 4.67/10^6/yr in 1989–1991 to 5.77 in 1995–1997,[159] and to 9.75 in 2002–2004; (2) the female incidence rate in Australia rose about threefold over a period of ~20 years; (3) Strickler et al.[160]

also recorded a rising incidence of MM in the U.S. for women aged 45 to 54 years and above, for the period 1975–1997, based on SEER data, which cover about 14% of the U.S. population; and (4) among female MM patients, up to ~75% in some series[36,161] had a history of asbestos exposure, but the exposures were occupational in only a minority (~20%),[161] so that nonoccupational exposures such as domestic (household contact) exposure constitute a much higher proportion of MM cases among women than in men.[161] As foreshadowed in the preceding discussion, Roggli et al.[161] found that the lung tissue asbestos burden was elevated in 70% of a series of female MM patients in the U.S., and the main fiber type detected was amosite, followed by tremolite and chrysotile, and the lung tissue asbestos body and fiber concentrations as a consequence of such domestic exposure approached those found with some patterns of occupational exposure.[162]

The background environmental mesothelioma incidence rate and especially the true spontaneous rate is probably substantially less than one case/10^6/yr, but the true rate can only be guessed, because no significant control adult population without asbestos fibers in lung tissue can be assembled.[163]

Hereditary Factors and the Role of Genetic Susceptibility

Mesothelioma occurs in only a minority of asbestos-exposed individuals, even in those exposed heavily to amphibole asbestos.[36] This observation might be explicable by mesothelioma induction as a chance event; that is, mesothelioma is the outcome of a multistage process involving multiple mutational and epigenetic events, so that most of those exposed to asbestos simply do not strike the correct combination of a complex set of events necessary for development of mesothelioma. Alternatively, one of the mutations induced by asbestos may be lethal to the initiated cell, so that subsequent steps cannot occur (see Molecular Pathogenesis and Pathology of Malignant Mesothelioma, below). However, alternative explanations include (1) modulation of the asbestos-imposed risk by genetic or acquired susceptibility/resistance factors,[164] or (2) a combination of randomness and predisposition.

In 1985 Lynch et al.[165] described the occurrence of epithelial mesotheliomas in two brothers who had been exposed to asbestos, and reviewed the literature citing three other reports of familial mesothelioma. Ten of 11 family members in the four families reported had a definite history of exposure to asbestos. In 1984 Martensson et al.[166] reported two pairs of siblings, a brother and sister and identical twin brothers, who developed pleural MMs. Both pairs of siblings had exposure to asbestos. We reported three brothers who had an asbestos insulation business; two developed mesotheliomas that arose in the pleura and the other brother had peritoneal mesothelioma.[167] Subsequently, one male child and one female child in this family died from pleural MM.

Other studies have evaluated hereditary factors in mesothelioma. Huncharek et al.[168] studied 39 cases of pleural mesothelioma and 259 age-matched controls to assess the possibility of influence of family history on pleural MM risk. Twenty-eight (71%) cases reported a parental history of cancer versus 114 (44%) in the control group ($p < .01$), suggesting a possible role for a family history of cancer in the development of pleural mesothelioma.

Heineman et al.[169] evaluated mesothelioma, asbestos, and reported history of cancer in first-degree relatives. Specifically, they compared reported histories of cancer in first-degree relatives of 196 patients who had a pathologic diagnosis of mesothelioma, with those from 511 deceased controls. The authors found only limited suggestive evidence that a family history of cancer may be a risk factor for mesothelioma, possibly in conjunction with asbestos exposure. Studies of small family clusters, including that of Ascoli et al.[170] in relatives working in a confectionary shop highlighted the possibility that inherited factors might be involved in the development of MM. We have seen a number of other familial cases of mesothelioma where two or more family members developed mesothelioma, usually in a setting of occupational or domestic bystander asbestos exposure (Fig. 43.4).

A larger survey conducted by Bianchi et al.[171] included 610 pleural mesotheliomas of which 40 were found to be familial. Familial mesotheliomas included 31 men and nine women with an age range of 44 to 93 with a mean of 70.7 and a median of 71.0 years. In 15 families, there were blood relations between or among the members involved. However, all patients had reported exposures to asbestos, mostly in the shipyard.

Ohar et al.[172] tried to identify a more extensive set of traits that would define a mesothelioma phenotype for the purpose of genetic analysis. They found that compared to other asbestos-exposed groups, subjects with mesothelioma were younger at first asbestos exposure, had a greater risk of second cancer diagnosis, had a longer disease latency, and had a greater risk of cancer among first-degree relatives. The authors concluded that thoracic tumor location, work exposure, male gender, long latency, early age at first exposure, presence of a second cancer, and first-degree relative with cancer defined a phenotype that distinguishes mesothelioma patients with a short survival from other asbestos-exposed individuals. They proposed this phenotype could be applied to candidate gene analysis.

Several studies have attempted to determine a cytogenetic profile for MM. Ascoli et al.[173] performed genomic

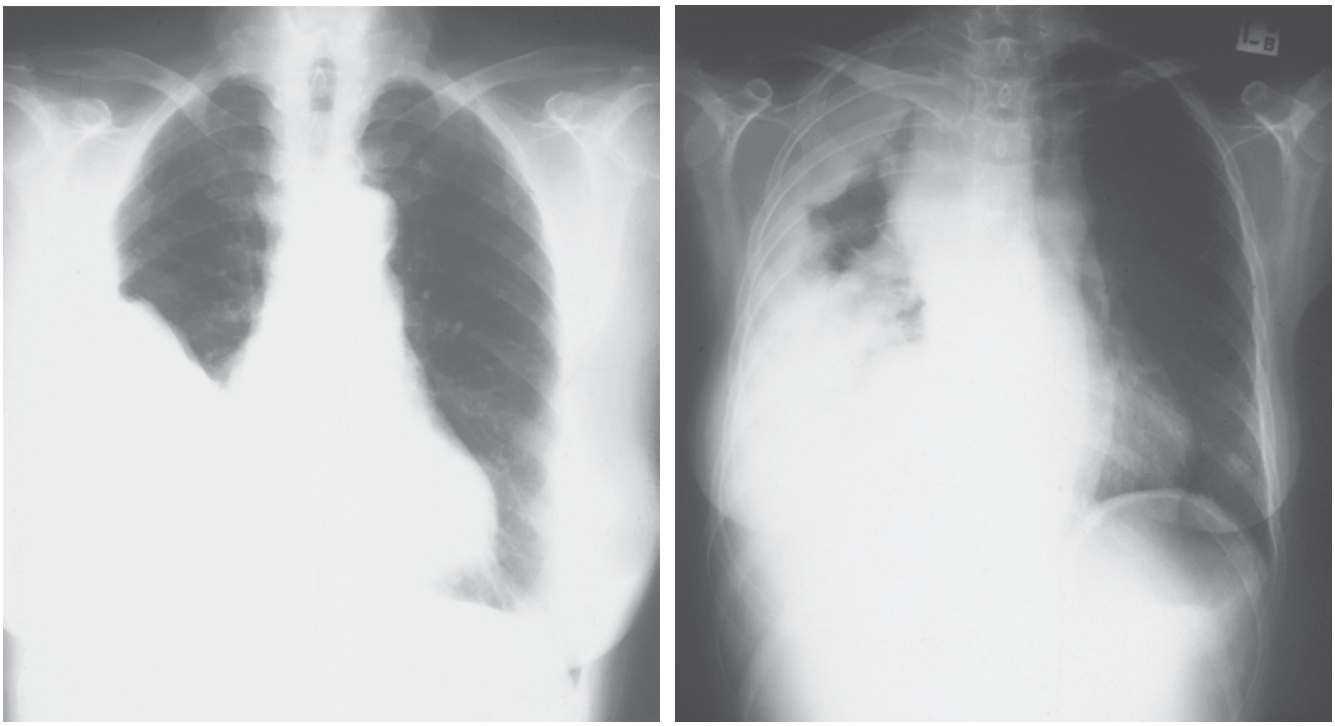


FIGURE 43.4. Familial pleural malignant mesothelioma (MMs) in a mother **(A)** and her daughter **(B)**, proven by surgical biopsy in each case. The mother often shook out and washed the asbestos-contaminated work clothes of the husband/father, and the daughter was often present in the laundry when her mother did so. The mother and daughter developed their mesotheliomas within 3 years of each other.

hybridization analysis on tumor samples from members of a family with MM of the pleura and a history of parental cancer. Their aim was to find a recurrent copy number loss indicating the chromosomal area to which a gene underlying the development of mesothelioma could be assigned according to the Knudson two-hit hypothesis. They found losses at 1p, 6q, 9p, 13q, and 14q. The copy number changes were stated to have been very similar to those reported in sporadic cases. Their findings and results from sporadic cases highlighted the importance of cloning of the genes in the loss sites at 1p, 6q, 14q, and 22q.

Musti et al.[174] described a family of three sisters affected by MM, two of which were pleural and one of which was peritoneal, and one brother who had pleural plaques. All family members were stated to have been subjected to previous asbestos exposure of environmental-residential type. DNA extracted from paraffin-embedded MM samples was used to search for chromosomal alterations by a comparative genomic hybridization (CGH) method. In two cases, a loss at 9p was found to be the only change. The loss at 9p was stated to be a frequent event in MM. The fact this anomaly was diagnosed in two sisters as the only alteration suggested this region could be the site of one or more oncosuppressor genes that could play an important role in the development of MM in inducing greater genetic susceptibility to the carcinogenic effect of asbestos.

Bianchi et al.[175] indicate that the most frequent cytogenetic abnormality in MM is loss of chromosome 22. Neurofibromatosis type 2 gene *(NF2)* is a tumor suppressor gene assigned to chromosome 22q that plays an important role in the development of familial and spontaneous tumors of neuroectodermal origin. Molecular studies have implicated *NF2* in the oncogenesis of MMs and possibly other nonneural tumors (see below).

Is There a Genetic Susceptibility to Mesothelioma Induction by Asbestos? Evidence for a component of genetic susceptibility to mesothelioma includes the following:

- There is an analogy with other cancers. From data in the Swedish Family-Cancer Database, Hemminki et al.[176] found evidence for a genetic component for a variety of cancers, among which mesothelioma is unlikely to be an exception.
- Familial clusters of MM[177,178] (Fig. 43.4) may be explicable mainly by the sharing of occupational, domestic,

environmental, and even recreational asbestos exposures among members of the same family,[179] but the development of MM among multiple different members of one family is unlikely, even when all the affected members did sustain asbestos exposure (see above discussion).

- The frequency of nonmesothelial cancers may be increased among first-degree relatives of MM patients; see above data of Huncharek et al.[168] and Heineman et al.[169] In contrast, Lynch et al.[180] found that the frequency of any cancers among the first-degree relatives of mesothelioma patients (43%) did not differ significantly from patients with lung cancer (41%) or patients with any cancers (40%), but their data did not include a control group of noncancer subjects. They also found that patients with epithelial MMs gave a stronger positive family history of cancer than other histologic types, but the numbers of cases were small and the results did not reach statistical significance.
- Sites of genomic instability affected by asbestos have been identified, and of genes liable to loss of heterozygosity (LOH) mutations inducible by asbestos, such as the fragile histidine triad *(FHIT)* gene.[181,182]
- Hirvonen et al.[183] carried out a molecular case-referent study on the glutathione-S-transferase M1 *(GSTM1)* gene and the *N*-acetyltransferase-2 *(NAT)* genotype (slow versus fast acetylators) among 145 Finnish asbestos insulators exposed to high levels of asbestos; 69 had no pulmonary disorders (controls), and 76 had either MM ($n = 24$), or benign pleuropulmonary disorders such as asbestosis or pleural plaques ($n = 52$). Hirvonen et al. found that the odds ratio (OR) for the development of either malignant or benign pulmonary disorders for individuals with a *NAT2* slow-acetylator genotype was more than double the OR for those with a *NAT2* fast-acetylator genotype (OR, 2.3; 95% CI, 1.1–4.7): for *NAT2* slow-acetylators, the OR_{MM} was 3.8 (95% CI, 1.2–14.3). Those who lacked the *GSTM1* gene and who had a *NAT2* slow-acetylator genotype had about a fivefold risk for both malignant and benign pulmonary disorders in comparison to those who had the *GSTM1* gene and a *NAT2* fast-acetylator genotype (OR, 5.1; 95% CI, 1.6–17.6). Subjects with a *GSTM1*-absent/*NAT2* slow-acetylator profile had an almost eightfold increased risk of MM (OR, 7.8; 95% CI, 1.4–78.7), although it is notable that the CI for this last result is very wide. Such findings are reviewed and discussed in greater detail by Puntoni et al.[184]
- There is evidence of species and strain susceptibility to mesothelioma among experimental animals used as models of mesotheliomagenesis. As examples, hamsters appear to be particularly susceptible to mesothelioma induction by a variety of factors, whereas rats are more resistant (and reportedly about 100-fold less susceptible to MM than humans[185]).

Nonetheless, it is worth emphasizing that it is unlikely that such genetic susceptibility would be expressed as mesothelioma in the absence of asbestos (in particular amphibole) exposure.

Simian Virus 40

Simian virus 40 (SV40) has been extensively evaluated with respect to the development of mesothelioma. The hypothesis has been that the development of the Salk polio vaccine used monkey kidney cells as a sole source of culturing the virus, and the monkey kidney cells were contaminated with SV40; therefore, individuals receiving the Salk vaccine were subjected to SV40. The issue of SV40 induction of mesothelioma is also discussed in Chapter 33. There are now numerous reports on the detection of SV40 DNA in human MMs and some other tumors such as osteosarcomas and brain tumors[186,187] (see Molecular Events in the Development of Malignant Mesothelioma VI, below). It could be argued that the presence of SV40 might explain (1) why MM only develops in a relatively small proportion of asbestos-exposed individuals, and (2) why no history of asbestos exposure is obtainable on a sizable minority of MMs. However, almost all the MMs in which SV40 DNA has been found were asbestos-associated. Existing data do not adequately address either of the two foregoing issues, for which there are alternative explanations. In other studies, SV40 or SV40 large T-antigen (Tag) could not be detected within MMs.[188] A statement on MM from the British Thoracic Society ranked the evidence for SV40 as a cofactor for mesothelioma induction as only "weak,"[189] and Lee et al.[190] argued that the relationship is unproven. In addition, an expert committee in the U.S. concluded that the evidence was insufficient either to assign or to exclude a contributory role for SV40 in the genesis of MM.[187] Two of the most recent studies suggest that there is no evidence that SV40 causes mesothelioma in humans.[191,192] Accordingly, SV40 might be regarded as a possible but unproven genetic susceptibility factor in the induction of MM by asbestos or a permissive factor for MM growth after its induction.

Immunodeficiency

Rare individual cases of MM have been recorded in association with immunodeficiency states, including HIV/AIDS, and in a renal transplant recipient.

Occupations at Risk

In national cancer registries, up to about 90% of male MM patients have a history of past asbestos exposure, especially for pleural MM, with a somewhat smaller percentage (about 60%) for patients with peritoneal MM.[193,194] Among female mesothelioma patients, about 40% to 75% have a history of asbestos exposure,[161] but

TABLE 43.4. Mesothelioma proportional mortality ratios (PMRs) in the United Kingdom, 1980–2000, by 5-year intervals, for men aged 16 to 74, according to last occupation, for the top 10 PMRs and the lowest five PMRs

Occupation	1980–1986 (excluding 1981)	1986–1990	1991–1995	1995–2000	Increased (↑) or decreased (↓) trend
Top 10 in 1995–2000					
Vehicle body builder	504	614	606	462	
Carpenter	361.5	373	361	395	
Electrical plant operator	405	163	255	295	
Metal plate worker	723	608.5	556	292	↓
Boiler operator	270	255.5	241	250	
Construction manager	180	226	185.5	195	
Metal, jewelry, electrical prod'n	105	84	167	165	↑
Construction worker	268	228	204	174	↓
Painter, decorator	137	146	168	173	
Technicians	182	124	170	158	
Lowest five in 1995–2000					
Lawyer	0.0	0.0	40	10	
Leather/shoe worker	34	39	34	11	
Clergy	46	48	60	20	
Doctor	0.0	25	37	32	
Farmer	15	28	25	32	

PMRs corrected to the nearest 0.5.
Source: HSE Statistics. Mesothelioma Occupation Statistics: Male and Female Deaths Aged 16–74 in Great Britain 1980–2000 (Excluding 1981): Table 3 in original.

the exposures are occupational in only about 20% of cases,[161] so that a higher proportion of MM cases among women is a consequence of nonoccupational exposure[161,193] (see previous discussion).

The occupations that account for the greatest absolute numbers of MMs have changed over the years from miners/millers, products manufacturers, and insulation workers, to other end-users of asbestos-containing products, including the building construction and demolition industries (Tables 43.4 and 43.5),[49] while ship construction and repair still account for substantial numbers of cases, especially in the U.S. (Table 43.5).[162]

The building construction workforce is large and comprises a heterogeneous collection of occupations and workers who vary from the self-employed, to employees of small or large corporations, and working conditions in

TABLE 43.5. Mesothelioma cases in the United States according to industry, among 1445 cases of malignant mesothelioma (MM)

Industry	Single pattern of exposure (No.)	Multiple patterns of exposure (No.)	Total (%)
Shipbuilding[a]	203	86	27.6
U.S. Navy/merchant marine	91	84	16.7
Building construction[b]	99	35	12.8
Insulation[c]	92	11	9.8
Oil/chemical	78	10	8.4
Power plant	50	10	5.7
Railways	37	16	5.1
Automotive/brake mechanic	24	27	4.9
Steel/metal/foundry/furnace	33	10	4.1
Asbestos products manufacture[d]	34	5	3.7
Paper mill	7	0	0.7
Ceramics/glass	6	0	0.6
Totals	754	294	1048

[a]Includes joiner, shipwright, rigger, sandblaster, shipfitter, electrician, painter, welder.
[b]Includes construction worker, laborer, carpenter, painter, plasterer.
[c]Includes pipe coverer (lagger), insulator, asbestos sawyer, asbestos sprayer.
[d]Includes textile and other products manufacture.
Source: Modified from Roggli et al.[162]

TABLE 43.6. Individual lifetime risk of mesothelioma (MM) in Australia by occupational groupings

Occupational group	Lifetime risk of MM (%)*
Wittenoom miner/miller	16.5
Power station worker	12
Railways laborer	6.5
Navy/merchant navy	5
Carpenter/joiner	2
Waterside worker/docker	2
Plasterer	2
Boilermaker/welder	2
Bricklayer	2
Plumber	1.5
Painter/decorator	1
Electrical fitter/mechanic/electrician	0.5
Vehicle/automobile mechanic	0.5
All Australian men	0.4
All Australian women	0.05

*To the nearest 0.5%, except for *all Australian men and women*.
Source: Modified from Leigh et al.[43]

the building industry have been poorly regulated.[42,159,195,196] In Australia, crocidolite miners/millers, power station workers, railway laborers, and naval, merchant naval, and shipyard personnel (in descending order of risk) have the highest estimated individual lifetime risks of MM (Table 43.6).[36] Even so, the number of personnel employed in each of those occupations is smaller than in the building and construction industry, so that carpenters/joiners, for example, contribute greater absolute numbers to the national MM toll, although their individual risk is less.[193]*

Statistical data for the U.K. published by the Health and Safety Executive (HSE)[44] also recorded significant numbers of mesotheliomas as a consequence of insulation materials in buildings (and elsewhere), the highest risks being the consequence of exposures related to shipbuilding, railway carriage and locomotive building, and the installation or maintenance of insulation materials in buildings or factories.

Substantial numbers of MMs—about 10% of the total, according to data from the HSE in the U.K.[49]—are now seen as a consequence of nonoccupational exposures, including occasional and transient "handyman"-type exposures related to home renovation, repairs and maintenance, and domestic exposure[197] (e.g., from shaking and laundering asbestos-contaminated work clothes[198]) and other types of occasional or nonoccupational exposures.[42,117,163,193] It is worth emphasizing, however, that not all such nonoccupational exposures necessarily represent low-dose exposures; for example, the shaking of asbestos-contaminated work clothes before laundering them can generate high peak concentrations of airborne asbestos fibers,[199,200] resulting in cumulative exposures that can approach or amount to some occupational exposures[162,201,202] (such as those recorded for electricians[162]), and some such cases have shown clinical or histologic evidence of asbestosis.[203,204] Roggli et al.[161] recorded asbestosis in three of 38 cases of mesothelioma that followed household contact exposure to asbestos (8%), and more than half had pleural plaques (Table 43.7).

Apart from some specific industries, such as former crocidolite miners/millers at the Wittenoom blue asbestos industry in Western Australia[205–207] (Figs. 43.5 to 43.8), those who assembled gas masks that contained crocidolite fibers during World War II,[208] and amosite factory workers, most asbestos exposures in the past (until about the early 1980s) involved mixtures of commercial amphibole and chrysotile fibers (e.g., in asbestos insulation and high-density asbestos-cement products), so that most mesotheliomas following end-use asbestos exposures are a consequence of mixed-fiber exposures.[42] There is also evidence that manipulations carried out on such materials resulted in preferential release of amphibole fibers as opposed to chrysotile, presumably because of differences in their physical properties. Accordingly, the proportional concentrations of the airborne fibers in the breathing zones of those exposed were not the same as the proportions in the products as manufactured; for example, in one report in Australia, the ratio of crocidolite/chrysotile fibers in the airborne dust produced by machining of asbestos-cement products was about 28:100 in comparison to 11:100 for the asbestos-cement as manufactured (about 2.5 times greater).[209]

Pleural/Peritoneal Mesothelioma Ratios

On theoretical grounds, one would expect the pleural/peritoneal ratio for true spontaneous MMs uninfluenced by any exogenous causal factor(s) to be about 1:1 or <1:1, taking into account the mesothelial surface areas for the pleural cavities combined versus the peritoneum. Although peritoneal mesotheliomas outnumber pleural MMs in some series—for example, in 86 deaths among Swedish insulation workers during the period 1970–1994, there were seven peritoneal mesotheliomas but no pleural MMs[210]—in most series and in national data, about 90% of MMs or more affect the pleura, about 9% the peritoneum, and about 1% or less the pericardium or tunica

*Data for Australia are discussed at various points in this chapter because the Australian Mesothelioma Register collated all cases of pathologically verified mesothelioma across the entire Australian population (~20 million), but following the introduction of privacy legislation, follow-up of the reported cases became more difficult and notifications to the register were suspended in 2006. However, it seems that mortality statistics and some other data will continue to be reported, from anonymous data sent from State Cancer Registries. The peak incidence of mesothelioma in Australia seems likely to occur in about 2020.

TABLE 43.7. Malignant mesothelioma (MM) pleura-to-peritoneum ratio, parietal pleural plaques and asbestosis, according to industry and occupational versus non-occupational exposures for 1445 cases of MM, in the United States

Industry/occupation	Pleura-to-peritoneum ratio	Parietal pleural plaques (%)	Asbestosis (%)
Industry			
Shipbuilding	52:1	81	26
U.S. Navy	54:1	21	11
Construction	8.6:1	34	17
Insulation	2.1:1	85	58
Oil/chemical	82:1	78	17
Power plant	17:1	85	19
Automotive	8:1	67	0
Railways	38:1	83	12
Steel/metal	9.3:1	93	27
Asbestos products mfg.	2.2:1	87	65
Paper mill	6.1	83	20
Ceramics/glass	6:0	50	0
Occupation			
Pipefitter	50:1	87	24
Boilermaker	30:1	81	24
Maintenance	26:1	80	20
Machinist	22:1	78	14
Electrician	74:1	83	27
Sheet metal	20:1	82	14
Other asbestos	5:1	33	0
Nonoccupational			
Domestic	4.3:1	57	7.9
Building occupants	1.8:1	43	0
Environmental	4:10	0	0
Other	8.5:1	46	9.5

Source: Modified from Roggli et al.[162]

FIGURE 43.5. Schematic map of Western Australia showing Wittenoom in relation to the Tropic of Capricorn, in the Pilbara-Hamersley region, together with other regional centers and Perth. WA, Western Australia; NT, Northern Territory; SA, South Australia; NSW, New South Wales; QLD, Queensland; VIC, Victoria; TAS, Tasmania.

FIGURE 43.6. The Wittenoom asbestos mine and mill. The mine was located slightly to the right of the uppermost white building, in the face of the gorge. The mill has a white roof and is located near the center of the photograph. The gray-blue material represents crocidolite tailings from the mine. For scale, note the parked white automobile on an access road, near the lower right portion of this view. (Courtesy of the Asbestos Diseases Society of Australia.)

FIGURE 43.8. A race at Wittenoom to determine who could fill a 44–gallon drum with crocidolite in the shortest time. All but two of the men in this field are thought to have died from asbestos-induced cancer. The man just to the left of the 44–gallon drum closest to the observers, from which blue-gray dust is streaming, was awarded compensation in 2004 for the emotional distress induced by his work at Wittenoom, in that he had seen his brother and many of his coworkers die from mesothelioma and other asbestos-related disorders. (Courtesy of the Asbestos Diseases Society of Australia.)

vaginalis testis.[162,193,211] Accordingly, it is thought that inhalation and deposition of asbestos fibers in the lung, with subsequent translocation of fibers (especially amphibole fibers) to the pleura, followed by lesser translocation to sites beyond the pleura, skews the anatomic distribution of MM toward the pleura. In a large series of 1445 mesotheliomas, Roggli et al.[162] tabulated the pleural/peritoneal ratios for MMs (Table 43.7) and found that the smallest ratio of 1.8:1 was for building occupants, which the authors suggested would have reflected, or nearly so, the "background rate of occurrence for these tumors" (but see Hemminki and Li[212]). Even so, the MM pleura/peritoneum ratio for insulation work was only 2.1:1, despite the finding of asbestosis as marker for substantial to heavy asbestos exposure in 58% of those MM cases (and pleural plaques in 85%),[162] so that insulation work appears to differ in some unknown ways from other occupational exposures. One might speculate that this reflects transport of a greater fraction of inhaled and deposited fibers from the lungs to the peritoneum than for other patterns of exposure.[213]

In general, peritoneal mesotheliomas tend to be associated with heavier asbestos exposures than pleural MMs,[214,215] with associated asbestosis in a higher proportion,[214] but the no-threshold model for mesothelioma induction by asbestos appears to apply to peritoneal as well as to pleural MM, as shown by (1) analysis of cases of peritoneal mesothelioma in the German Mesothelioma Registry,[214] where the asbestos exposures were sustained mostly in "metal industries, asbestos industries, and in the building trade"; and (2) the occurrence of mesothelioma (including peritoneal mesothelioma) relative to chrysotile-only exposures with analysis of the lung tissue asbestos fiber content, as reported by Rogers et al.[216] Furthermore, in an analysis of peritoneal MMs in Sweden using the Family-Cancer Database, Hemminki and Li[217] recorded an increasing incidence of peritoneal mesothelioma in women in Sweden (but not for men after 1985). They suggested this trend might be related "to nonoccupational exposure [to asbestos] or reasons other than asbestos." For men (among whom pleural mesotheliomas predominated), the occupational groups at greatest risk for peritoneal mesothelioma were bricklayers (SIR = 7.22) and plumbers (SIR = 5.12).

FIGURE 43.7. Wittenoom crocidolite ore. The crocidolite was disposed as thin seams, enclosed by ironstone, as shown here.

In the German Mesothelioma Registry,[214] the mean age at the time of diagnosis of peritoneal MM was about 59 years for men, whereas women were on average 4 years younger. The mean survival time was about 1 year, but in six of 38 patients longer survival times of up to 7 years were recorded. The epithelial MMs predominated, but no effect on survival time was noticed. The average latency interval was 36 years.

Latency Intervals Between the Commencement of Asbestos Exposure and the Subsequent Diagnosis of Mesothelioma

In the Australian Mesothelioma Surveillance Program, the mean latency interval was 37 years and ranged up to 75 years,[218] and the corresponding latency interval for cases of mesothelioma certified by the Dust Disease Board (DDB) in New South Wales in 2001/2002 was approximately 42 years. In a study of 557 mesothelioma cases reported in 2001 by Bianchi et al.,[219] the latency intervals ranged from 14 to 75 years, with a mean of ~49 years and a median of 51 years. Some authorities and The Helsinki Criteria[220] specify a minimum latency interval of 10 years, whereas others require a minimum interval of 15 years.

Mineral Fibers and Mesothelioma

This section focuses on the relationship between exposures to mineral fibers and the resultant observations of the development of mesothelioma.[1,2] Pleural MMs are most common,[2] although a study of lung cancer and mesothelioma in the pleura and peritoneum among Swedish insulation workers[210] found that "mesothelioma in insulation workers seems to be situated in the peritoneum more often than in the pleura."

Mesothelioma is widely considered an asbestos "marker disease." The report of the Pneumoconiosis Committee of the College of American Pathologists and the National Institute for Occupational Safety and Health (NIOSH)[221] concluded, "malignant mesothelioma of the pleura and peritoneum either are exceptionally rare or never occur in persons not exposed to asbestos."

In fact, Henderson et al.[222] concluded in an overview of attribution of asbestos-related cohorts in Australia that "no threshold of exposure (in other words a level below which there is no effect) has been delineated for asbestos-related malignancies (mesothelioma and lung cancer), but there is some evidence for a threshold for asbestosis and perhaps diffuse pleural fibrosis."

Fiber Length and Mesothelioma

Mineral fibers other than asbestos have been of concern with regard to possible induction of MM in humans.[223] An appreciable concern for exposure to nonasbestos fibers and the risk of producing disease has been based on exposures using animal models where the exposure to the dust was via intraperitoneal route[224–227] or intrapleural implants.[228,229] The common conclusion in these models is that a comparison of risk for the induction of mesothelioma indicates that on a one-to-one basis, a short fiber is less carcinogenically active than a longer, thinner fiber of the same type. Stanton et al.[228,229] acknowledged that some tested fibers that were shorter or thicker also induced mesothelioma. Pott et al.[224–227] concluded the dimensions of fibers are only one factor that enables a fiber to have the ability of inducing mesothelioma.

The Stanton hypothesis[229,230] argues that carcinogenicity is expressed mainly by long thin asbestos fibers, with lengths >5 μm and especially >8 μm, and in the range of 10 to 20 μm, and diameters <0.25 μm. Shorter fibers appear to be less carcinogenic, although it is doubtful that carcinogenicity is restricted to a critical and precise fiber length or diameter.[38] The Stanton model is supported by evidence derived from animal experiments,[231–234] but it seems likely that biopersistence of amphibole fibers may be more important for MM induction than precise fiber dimensions, and data in humans concerning fiber length and mesotheliomagenicity are equivocal.[230,235] Even so, very short-length fibers (<1 μm) appear to have comparatively little carcinogenic activity.

The majority of the existing data from human studies indicates the fibers that are likely to be relocated from the lungs to extrapulmonary sites where mesothelioma develops are short or thin fibers.[236] In studies by Dodson et al.,[236] some longer fibers (>5 μm) were shown to reach the lymph nodes and pleural areas, but the shorter fibers of chrysotile were the predominant fiber type in pleural plaques. This same observation has been made by Sebastien et al.,[237] and by Suzuki and Yuen.[235,238] Suzuki and Yuen also reported short chrysotile fibers in mesothelial tissue. Dodson et al.[239] reviewed the content of omentum and mesentery tissue from occupationally exposed individuals. While there were some longer fibers in these sites where peritoneal mesothelioma develops, the majority of asbestos burden was found to be in the form of short fibers. Boutin et al.[240] did a comparative study of asbestos burden in lung tissue and "black spots" in the parietal pleura. Their finding in the study group was that there was a prevalence of amphiboles in all sites with 22.5% of the fibers ≥5 μm in the "black spots" of pleural tissue. The authors questioned whether this accumulation of fibers indicated a preferred site for mesothelioma and pleural plaques. In a companion paper, Mitchev et al.[241] evaluated the parietal pleura of 150 consecutive necropsies of urban dwellers. The size and intensity of spots were scored and recorded, as were pleural plaques. The report stated that 92.7% of cases had detectable black spots. The study concluded that "there was no relationship between the

predominant locations of black spots and hyaline pleural plaques" or the development of mesothelioma.

Nonasbestos and Nonoccupational Mineral Fibers and Mesothelioma

The risk of mesothelioma is not exclusively associated with occupational exposure to asbestos since there are reports of occurrence of mesothelioma in settings where there is no relationship to commercial asbestos exposure. These include reports from Southern Anatolia (Turkey) where causal exposures were suggested as being from environmental "asbestos most consistent with tremolite and actinolite."[242] One other famous internationally recognized area where there are appreciable environmentally induced MMs is in the Cappadocian region of Turkey.[243,244] The explanation for the causal agent in this region is environmental dust deposits of fibrous zeolite-erionite. Rohl et al.[112] found that environmental samples from the villages of Karain, Tuzkoy, and Sarihidir where mesotheliomas had been reported contained not only fibrous zeolite (erionite), but also trace (<3% by weight) to major (≥3% or more by weight) component of asbestos (chrysotile/tremolite). Fibrous outcrops of zeolite are also situated in the Western U.S. Johnson et al.[245] reported in a rat inhalation model that erionite (fibrous zeolite from the Rome, Oregon, area) could induce mesothelioma more rapidly and more frequently than asbestos.

Another example of exposure to a mineral that contains a component now recognized as a causal agent for MM is vermiculite that was mined in Libby, Montana.[246] This material was widely distributed across the U.S. to sites where it was processed into commercial products. This site and the surrounding areas are of concern, as is the "exposure pathway" from mined minerals, shipped minerals, processed minerals, and consumer exposures to asbestos- contaminated vermiculite.[247] Fibrous amphiboles, including tremolite asbestos, which contaminate vermiculite, have resulted in an appreciable loss of life due to asbestos-related diseases in Libby, not only among the miners and others working with processing and delivery of the mineral, but also within the town populace whose only contact was environmental. The previously described exposures reflect only a selected series of exposure to fibrous materials that may stimulate the development of mesothelioma once inhaled.

Identification of Tissue Markers of Past Exposure (Ferruginous Bodies and Uncoated Fibers)

Fibrous minerals in environmental or tissue samples can be assessed and quantitated by light or electron microscopy. To best interpret tissue burden of fibrous dust in individuals diagnosed with mesothelioma, it is imperative that one understands the limitations of detection with various instruments, magnifications, and preparative techniques used in such evaluation. The largest structures seen in tissue that reflect past exposure to fibrous dust (the causal agent for mesothelioma) are ferruginous bodies. These structures are representative of inhaled fibers (>10 μm) that accumulate surface deposits (to varying degrees) of iron coating along the fibrous core. A ferruginous body having a beaded structure with a clear, elongated, transparent, usually straight core is with a high degree of certainty an asbestos body (see Chapter 27).[248] Tissue sections are very insensitive indicators for determining asbestos content since random sampling and random orientation of ferruginous bodies in the plane of the sections require that many sections be reviewed before their presence can be detected, even when the tissue burden is at occupational levels.[249] Roggli and Pratt[249] stated that the sensitivity for quantitating ferruginous bodies increases greatly when the equivalent of many tissue blocks are digested. Several laboratories have defined a burden of asbestos bodies in tissue from the general population that falls in the range of 0 to 20 ferruginous bodies per gram of wet tissue.[250–253]

The use of light microscopy for the detection of uncoated asbestos fibers in tissue is of essentially no value since they are invisible with rare exception. Even when asbestos fibers are numerous only the larger fibers are seen. Langer et al.[254] stated, "The optical microscope delivers a select, biased population" (i.e., larger fibers thicker than 0.5 μm in diameter). The detection and identification of asbestos fibers isolated from tissue can be more readily done with the scanning electron microscope, but with inherent limitations when compared to the capability of the analytical transmission electron microscope (ATEM) as a counting tool,[255] which enables viewing the thinnest/shortest fiber and can confirm a fiber as asbestos based on morphology, elemental composition (chemistry), and crystalline structure (selected area diffraction). The following concepts may be helpful in interpreting data on tissue fiber content in individuals with mesothelioma:

1. The dust burden within a tissue sample represents that portion of the dust that has not been cleared by the time of evaluation. This skews the analysis toward larger inhaled structures, since the smaller ones are more easily cleared over time. This concept is highly relevant for chrysotile, since chrysotile is predominantly inhaled as a short fiber due to its innate physical curvature (Fig. 43.2).
2. The number of isolated ferruginous bodies (morphologically compatible with asbestos bodies) per gram of tissue, determined by tissue digestion, can be reasonably compared between different studies.

3. Low magnification counts of fibers by scanning electron microscopy (SEM) or transmission electron microscopy (TEM) potentially excludes long/thin asbestos fibers, particularly those of chrysotile.[255]

4. An exclusion of fibers <5 μm in a counting strategy, even when the resolution capability of the ATEM is used can result in exclusion of the vast majority of asbestos within tissue samples from lung and, even more dramatically, from extrapulmonary sites.

Much of the chrysotile burden in tissue can be missed due to items 3 and 4.

Studies Defining Mineral Fiber Content in Mesothelioma Patients

In a series of studies using tissue digestion, Roggli et al.[83] quantified ferruginous bodies using light microscopy and detected fibers (>5 μm in length) with SEM. In 25 cases of mesothelioma, Roggli et al. analyzed core material of ferruginous bodies and quantified their numbers per gram of tissue. They found the number of ferruginous bodies fell between the number in tissue from patients with asbestosis and controls. Those cases where ferruginous body counts overlapped with counts found in tissue from the general population often lacked an identifiable occupational exposure to asbestos. The cores of 88 of the 90 ferruginous bodies were found to be amphibole asbestos, with only two asbestos bodies having chrysotile cores.[256] A review of fiber exposures and disease by Roggli[257] concluded, "Mesothelioma may occur with fiber burdens considerably less than those necessary to produce asbestosis." Srebro and Roggli[258] reviewed the tissue burden of five cases with pleural mesothelioma and two with asbestosis. The study found that tremolite asbestos, although not commercially of interest, is a component of some commercially exploited chrysotile veins and vermiculite and talc veins. Their conclusion from the tissue evaluation was that "modest elevations of tremolite content in some of their mesothelioma cases suggest that at least for some susceptible individuals, moderate exposures to tremolite-contaminated dust can produce malignant pleural mesothelioma."

Srebro et al.[259] quantified ferruginous bodies and uncoated fibers in 18 mesothelioma cases in which the tissue burden of ferruginous bodies fell within a "control" population (0–20 asbestos bodies (AB)/g wet tissue). The findings indicated that "electron microscopic analysis of pulmonary mineral fibers may be required to differentiate asbestos-related mesotheliomas from non-asbestos-related cases when AB counts are within the range of background values."

In a synopsis of observations regarding tissue burden from 396 cases of MM, 28 of which were peritoneal, Roggli[260] concluded that the highest levels of fiber burden "occurred in patients who also had asbestosis, which was found in 12% of pleural and 43% of peritoneal cases." He concluded that the average lung fiber burden was higher in peritoneal cases than in pleural cases, a point that is not in agreement with data from our laboratories.[23,261] The observation was also made that approximately 70% of female mesothelioma cases had elevated fiber burden and many had exposure via household contact to an individual with occupational exposure to asbestos. The analysis strategy incorporating SEM included fibers that were detected and were >5 μm in length.

Paoletti et al.[262] reported a high number of pleural mesotheliomas in eastern Sicily. The study included residents who purportedly "never had any relevant exposure to asbestos during their professional lives." However, samples from quarries and building materials commonly used in the area yielded amphibole fibers, as well as the same type of tremolite-actinolite fibers as in lung tissue of a mesothelioma patient. In a similar environmental exposure, Langer et al.[263] reported that four small villages in northwestern Greece had levels of malignant pleural mesothelioma which accounted for "1% of the total mortality from 1981–1985." They reported fibers found in the lungs in individuals with so-called "Metsovo (Greece) lung" consisted of asbestiform tremolite that was identical to the fibers found in the whitewash once used in the area.

Howel et al.[264] reviewed the mineral fiber content and routes of exposure to asbestos associated with mesothelioma in a region of England. They concluded, "The study has confirmed previous results of higher concentrations of asbestos fibers in cases than controls, and has shown that this is still found in subjects with little evidence of occupational and para-occupational exposure. The overlap in concentrations of retained asbestos for different groups of subjects did not suggest a clear cut-off value."

One of the few places where anthophyllite has been mined for commercial utilization is in Finland. Karjalainen et al.[265] reviewed the clinical status of 999 Finnish anthophyllite miners. Three of the individuals died from pleural MM and one from peritoneal mesothelioma. The latency period from onset of employment until diagnosis was from 39 to 58 years. Such a long latency period is not unusual in asbestos-exposed individuals.[23,261] Tissue analysis was conducted on tissue from three individuals with the findings by ATEM being from 270 to 1100 million fibers per gram of dry tissue. This information is important in light of the discussions regarding the carcinogenicity of fibers based on a concept of long/thin fibers being the most dangerous, since individual anthophyllite fibers are among the thickest in diameter of all the amphiboles. Tuomi et al.[266] reported on tissue burden in 19 mesothelioma cases and 15 randomly selected autopsy cases from Finland. The technique used SEM analysis of lung tissue. The "fiber concentration ranged from 0.5 to 370 million fibers per gram of dry tissue in

the mesothelioma group and from <0.1 to 3.2 million fibers per gram of dry tissue in the autopsy group. . . . In the lungs of the six mesothelioma patients, anthophyllite was the main fiber type."

While most reports of individuals with MM involve a long period from first exposure, there are reports of mesothelioma developing in young people. Andrion et al.[267] reported a case of peritoneal mesothelioma in a 17-year-old boy. They analyzed lung tissue and found 510,000 asbestos fiber per gram of dry lung tissue, of which 62% were chrysotile and 38% were tremolite. It was suggested that "the tremolite fibres were probably due to environmental exposure to contaminated cosmetic talc."

Glickman et al.[268] reported a study of 18 histologically confirmed cases of canine mesothelioma. The "lung tissue from three dogs with mesothelioma and one dog with squamous cell carcinoma of the lung had higher levels of chrysotile asbestos fibers than lung tissue from control dogs." Such findings raise the question as to whether environmental/secondary exposures to mineral fibers in family members were similar to that of their pets.

It is appropriate to focus on publications that have reviewed mineral fiber content in mesothelioma cases from Canada since 90+% of asbestos used in commercial products in the U.S. came from mines in Canada. Canadian chrysotile has been reported to have a small component of fibrous tremolite asbestos. An evaluation for tremolite was conducted on a *Union Internationale Contre le Cancer* (UICC-B) sample of chrysotile. This sample was composed of chrysotile obtained from several mines in Canada with the percentage based on percent of total mined commercial product. Over 20,000 fibers were analyzed by ATEM and all asbestos fibers observed were chrysotile.[269] This finding is of considerable importance since chrysotile has been shown to induce mesotheliomas in animal models.[227,270] There is confusion as to the ore of which mines contain tremolite and what percent is tremolite.[271] Adding to the confusion is the doctoral dissertation by De[272] stating that crocidolite existed in the adjacent mineral formations to the mined veins of chrysotile.

There are several publications with the same theme regarding chrysotile and risk of MM. Churg[273] evaluated what he considered to be 53 "acceptable" cases of chrysotile-induced mesothelioma, 41 of which were in individuals exposed to chrysotile mine dust that was considered by Churg to be naturally contaminated with tremolite. Ten cases were in individuals who worked in industries where "suspicion of amosite or crocidolite contamination [was] high." His conclusion at that time was that "although chrysotile asbestos can produce mesothelioma in man, the total number of such cases is small and the required doses extremely large." He further concluded,

> The data [were] consistent with the idea that mesotheliomas seen in chrysotile miners and some secondary industry workers [was] produced by the tremolite contained in the chrysotile ore, but that the short length and low aspect ratio of the tremolite [made] its carcinogenicity quite low. However, these data are very indirect, and a role for the chrysotile fiber itself is still possible.

In another study from Churg et al.'s[274] laboratory, an evaluation of lung tissue from 94 long-term chrysotile miners and millers from the region of Thetford Mines, Quebec, was conducted. The conclusion was that "mesothelioma, airway fibrosis, and asbestosis were strongly associated with a high tremolite fiber concentration, whereas pleural plaques and carcinoma of the lung showed no relationship to tremolite burden." They stated,

> Total fiber size measures (total fiber length/g and others) showed differences similar to fiber concentration for mesothelioma, airways fibrosis, and asbestosis, but no one measure was clearly better than another or better than fiber concentration. We conclude that, in this population of heavily exposed chrysotile miners and millers, the presence of airways fibrosis and asbestosis and, probably, mesothelioma reflects high tremolite burden. Whether chrysotile fibers themselves play a role in disease induction remains uncertain.

Another report from Canada evaluated the fiber content in 50 workers seeking compensation from the Workers' Compensation Board of Quebec for pleural or peritoneal mesothelioma.[275] Twelve in the study group were from Asbestos Township (chrysotile mining region) and 11 were from the chrysotile mining region of Thetford Mines. The remaining 27 worked in various nonmining industries. The fiber types found in the three groups were different: "The lungs of workers from Thetford Mines [contained] only chrysotile and tremolite; those from Asbestos Township [contained] chrysotile, tremolite, amosite, and crocidolite; and those in other industries [contained] largely amosite and crocidolite."

Begin et al.[276] reviewed 120 cases referred to the Quebec Workman's Commission Board for work-related compensation of industrial disease. The individuals were divided into three groups. The first consisted of 50 cases from the manufacturing and industrial application sector (primary industry, group 1); 50 cases from the manufacturing industrial application sector (secondary industry, group 2); and 21 from industries where asbestos was not a major work material, often an "incidental" material (tertiary industry, group 3). They reported

> [the] incidence of new cases in each group documenting the general incremental trend in all groups, with the sharpest rises in group 3. In the mining towns of Thetford and Asbestos, the incidence of mesothelioma was proportional to the workforce, thus suggesting that the tremolite air contamination, which [was] 7× higher in Thetford, may not be a significant determinant of the disease in these workers. The incidence of the disease in these chrysotile miners and millers was 62.5 cases per million

per year for the 1980–1990 period in Quebec. The incidence of pleural mesothelioma in chrysotile miners and millers, although not as high as in crocidolite workers, [was] well above the North American male rate. Comparative analyses of incidence of the disease in the two mining towns suggest that tremolite contamination may not be a determining factor in these chrysotile workers.

Langer and McCaughey[277] analyzed lung tissue from an individual whose "sole exposure to asbestos was to chrysotile form during brake maintenance and repairs." Contrary to the concept that chrysotile clears from the lung, these investigators found unaltered chrysotile in the analysis in the form of chrysotile fibrils <1 μm and some >5 μm in length. There were no amphiboles found in the tissue; thus the data were consistent with the occupational history of exposure.

Nolan et al.[278] evaluated the fiber burden by ATEM in five lung cancer cases from Quebec, Canada, and one case of an American worker who developed pleural mesothelioma. Interestingly, the predominant fiber type in the tissue from the American worker was chrysotile, and it was present at a "concentration of 300 times that of the average total fiber content of the Canadian case." Furthermore, "the fiber length distribution of the chrysotile recovered from the U.S. mesothelioma case was indistinguishable from that of chrysotile specimens known to produce mesotheliomas in rats. It was also found that the characteristics of the calcium-magnesium-iron silicate fibers present in all six cases were not readily comparable to tremolite asbestos specimens known to induce mesotheliomas in animals." The longest chrysotile fiber found was 33 μm, with 99% of the fibers identified being chrysotile. No commercial amphiboles were found in the analysis and only 1.5% of the 883 fibers sized were reported as being ≥5 μm in length. An important observation was made that when studies report findings based on fibers ≥5 μm in length, a bias toward tremolite may be introduced since the fiber length distributions in this study indicate a difference between chrysotile and the CaMgFe fibers found in the samples. Eleven percent of the latter were ≥5 μm in length, and the mean of the three reference chrysotile specimens was 1.3%.[278]

Churg and Vedal[279] evaluated tissue samples from 144 shipyard workers and insulators in the Pacific Northwest. Amosite (the majority fiber type) was reported to be found in all lungs, while tremolite and chrysotile were found in most lungs. "No relationship was found between the concentration of chrysotile or tremolite and any disease. Analysis of fiber size measures (length, width, aspect ratio, surface, mass) showed that pleural plaques were strongly associated with high aspect ratio amosite fibers and suggested mesotheliomas were associated with low aspect ratio amosite fibers." They concluded that differences in fiber burden and disease exist when comparing mesothelioma in chrysotile miners and millers and shipyard workers, in that mesothelioma appears to occur at much lower amosite burdens than does asbestosis, "in contrast with the situation previously reported for chrysotile-induced mesothelioma."

McDonald et al.[280] reported on the fiber content of lung tissue from individuals with mesothelioma who were 50 years of age or younger at time of diagnosis. There were 69 males and four females. "Incremental risk examined in a linear model was as highly significant for all amphiboles together as individually. Short, medium and long amphibole fibers were all associated with increased risk in relation to length. In this young age group, amosite and crocidolite fibers could account for about 80% of cases of mesothelioma, and tremolite for some 7%." There was some increased risk with chrysotile, but that was determined to fall short of statistical significance.

Leigh and Driscoll[193] reviewed cases of MM in Australia. They reported that Australia had a history of asbestos mining extending over 100 years, and Australia was the world's highest user per capita of asbestos in the 1950s, with the highest reported national rates of mesothelioma in the world. A review of tissue burden in cases of mesothelioma without documented exposure to asbestos found asbestos in 80% of lung fiber burdens as determined by ATEM of >200,000 fibers >2 μm length per gram of dry lung. They noted the high rate of MM in Australia was related to high past use of asbestos, which was reflected in the findings of elevated tissue levels from previously unrecognized exposures.

Workplace exposures to asbestos often involve exposure to several types of asbestos. There are several reported settings where exposures are overwhelmingly limited to one type of asbestos. Such occurred in facilities where manufacturers were creating filters for cigarettes from crocidolite asbestos. In 1987 Talcot et al.[281] reported that mesotheliomas had been observed in three employees in such a facility. In 1989 Talcot et al.[282] reported that 15 of 33 deaths associated within the cohort were from cancer and five were due to MM. Tissue was referred to our laboratory from two individuals who worked in the facility and died from pleural mesotheliomas.[283] The lung tissue from each individual was found to contain large numbers of ferruginous bodies as well as asbestos fibers, the vast majority of which were crocidolite. Nearly all of the ferruginous bodies analyzed also had crocidolite cores. Dodson and Hammar[284] reported a case in which a housewife developed pleural mesothelioma and the only known contact with asbestos was a history of smoking crocidolite-filtered cigarettes. Crocidolite fibers were identified by ATEM in digested samples from this individual's lung and lymph node tissue, in which anthophyllite and tremolite fibers were also found.

Another rather isolated exposure to a type of mineral fiber (amosite asbestos) occurred in an asbestos pipe

insulation plant. The uniqueness of the exposure was that no other type of asbestos was ever documented as having been used in this isolated facility. Levin et al.[285] reviewed the status of former workers in the facility and determined that as of 1998, there were four deaths from pleural mesothelioma and two from peritoneal mesothelioma among a cohort of 1130 individuals. An interesting aspect of employment at the facility was that, historically, individuals often worked for only short periods of time before leaving the facility.

We have published findings in over 200 cases of mesothelioma referred to our labs for evaluation. Ferruginous body concentrations and uncoated asbestos fiber burden as defined on a count scheme by ATEM included fibers >0.5 μm in length. Dodson et al.[23] evaluated the asbestos content in 55 mesothelioma cases from the Northwestern U.S. The area has appreciable heritage in shipbuilding and repair, and thus it was not a surprise that the most common finding was amosite fibers in all but two lung samples (96.4%); 18 individuals had over one million amosite fibers per gram of dry tissue, and 46 of the 55 individuals had an average asbestos body burden of over 1000 asbestos bodies per gram of dry tissue. Analysis of the cores of ferruginous bodies indicated that most were formed on amphiboles: 92.9% were found to have amosite cores, 2.9% crocidolite cores, 1% tremolite cores, 0.4% anthophyllite cores, 0.4% actinolite cores, and 0.1% chrysotile cores. The common observation was that the positive lung samples often reflected a mixed asbestos exposure. The other commercial asbestos fibers were crocidolite in 40% of cases and chrysotile in 56.4% of cases. Five cases were diagnosed as having a primary mesothelioma of the peritoneum. Peritoneal mesotheliomas have traditionally been associated with a higher asbestos burden than pleural MMs. However, the five cases in this study did not follow this pattern, showing a range from high fiber burden to very low fiber burden. In another study by Dodson et al.,[261] cases of peritoneal mesothelioma did not follow the general rule of association with the highest fiber burdens.

A possible explanation for the relatively low fiber counts by Dodson et al.[261] may lie in the manner in which the counts were performed. Most asbestos fibers in human lung are less than 5 μm in length and are therefore not reported in many studies that include only the longer or thicker population of fibers in lung tissue. Both studies concluded that most fibers found in the lung tissue would not have been seen if screened by light microscopy or SEM.[23,261] The study from the Northwest cohort also found that 26 of the cases had appreciable ferruginous body and uncoated fiber burdens but did not have pathologically definable asbestosis.[23] All but three cases from the Northwestern cohort had levels of ferruginous bodies higher than that considered in our laboratory as representing general population levels (20 ferruginous bodies per gram of wet tissue). However, in the second study, 13 cases had ferruginous body levels within those considered as reflective of tissue from the general population.[261] This implies the importance of combining the data regarding uncoated fiber burden and ferruginous body burden when attempting to define past exposure and a causal relationship of that exposure to asbestos and mesothelioma.

A similar trend was seen in a study of tissue burden of ferruginous bodies and uncoated asbestos fibers in 15 cases of mesothelioma in women[286]; 13 of 15 samples contained ferruginous bodies and, as with the two previous studies, amosite was the most commonly found form (80% of cases). However, unlike the other studies, the second most commonly found form of asbestos was tremolite (60% of cases). There was a considerable drop in overall tissue burden of uncoated asbestos fibers in the lower half of the study group when compared with the levels found in the lower half of the other two mesothelioma study groups. Seven individuals had bystander exposure from contact with contaminated clothing of a spouse or family member.

The common findings in all three study groups were the presence of mixed types of asbestos. The lung tissue in some cases of mesothelioma in each group had low overall tissue burden of asbestos.

The transport and deposition of asbestos fibers in extrapulmonary sites was evaluated in another study from our laboratory.[239] These individuals resided in the shipyard building/repair areas of the Northwest. Ferruginous bodies were found in 18 lung samples, five mesentery samples, and two omentum samples. The common fiber type in the lung (95% of cases positive), mesentery (65%), and omentum (70%) was amosite. Chrysotile was found in 50% of lung samples. Chrysotile was the second most common form of asbestos found in the extrapulmonary sites; 25% of the mesentery and three omentum samples were positive for chrysotile. Crocidolite was found in 25% of lung samples, 15% of mesentery samples, and 5% of the omentum samples. In the amosite-exposed individuals, the predictors of the likelihood of finding an asbestos fiber in the extrapulmonary sites included the presence and numbers of ferruginous bodies and total asbestos fibers in the lung. The relevance of the findings was couched in the fact that the individual studies had appreciable amphiboles in the lung tissue and the parameters may well change in a heavily exposed chrysotile cohort.

Mesothelioma is a rare tumor that, based on the previous data, clearly is related to the exposure to fibrous minerals, and in most instances, Peto et al.[287] correctly observed, "the great majority of mesotheliomas are caused by asbestos" and a "country's mesothelioma rate is therefore a quantitative indicator of its population's past exposure—mainly occupationally—to asbestos."

Asbestos Fiber Types and Dose, and Mesothelioma Risk and Induction

It is well known that there exists a dose-response causal relationship between asbestos exposure and MM, for any fiber type or mixture[39] (Table 43.8).[288] In addition, the amphibole varieties of asbestos are substantially more potent for MM induction than chrysotile[42,288] (Table 43.9),[289] and an extensive review by Hodgson and Darnton[93] on the dose-response relationships between asbestos and mesothelioma risk estimated that the relative potencies for crocidolite, amosite, and chrysotile for mesothelioma induction are roughly 500:100:1, respectively. However, in a subsequent analysis from Australia, based on lung tissue amphibole fiber concentrations allowing for clearance half-lives, Leigh and Robinson[43] calculated the potency ratios to be 26:14:1, respectively, and another set of potency ratios cited in the literature is 30:15:1, respectively.[42]

The factors that determine these differential potencies are sometimes summarized as the three D's: dose, dimensions, and durability (i.e., biopersistence in tissue).[42] Because of their wavy characteristics, chrysotile fibers appear to be trapped more readily within the upper airways and central bronchi than amphibole fibers (Figs. 43.2 and 43.3).[290] In the circumstances of air flow through tubular airways, fibers tend to be concentrated in the central regions of the airway lumen where flow is laminar, with the long axes of fibers parallel to the direction of flow, and fractional deposition of fibers is determined by straight versus curly fiber characteristics and by the diameter of the fibers, rather than their length.[290] Accordingly, Middleton et al.[291] found that the fraction of chrysotile deposited in rats was in the range of 17% to 36% of crocidolite at varying inhaled concentrations, and the deposited fraction of amosite was 65% of crocidolite. Other studies did not detect such differences, but there appears to be general agreement that for exposures in experimental animals lasting for 6 weeks or longer, the relative retention of amphibole fibers is greater than for chrysotile.[290] Fibers and particles most likely to be deposited are those with an aerodynamic equivalent diameter in the range of about 1 to 5 μm, and the sites of greatest deposition are the bifurcations of terminal bronchioles.[290]

TABLE 43.8. Mesothelioma rates in groups exposed occupationally to asbestos, according to fiber types and duration

Fiber type	Industry	Duration (years since first employed)	Rate per 10^6 person-years
Mixed fiber exposure: crocidolite, amosite, and chrysotile	Textile manufacture and insulation	20–24	1520
		25–30	1710
		31+	3180
Mixed fiber exposure: mainly amosite	Insulation workers	20–24	290
		25–29	1550
		30–34	2760
		35–39	6300
		40–44	6330
		45+	8110
Mixed fiber exposure: crocidolite and chrysotile	Fibrous cement manufacture	20–24	2700
		25–29	6300
		30–34	9600
Chrysotile, some crocidolite	Textile manufacture	20–24	108
		25–29	143
		30–34	1156
		35–39	493
		40+	1774
Amosite	Insulation manufacture	20–24	744
		25–29	2623
		30–34	5078
		35+	1842
Mixed fiber exposure	Dockyards	20–24	120
		25–29	410
		30–34	220
		35–40	370
		40–44	1240
		45–49	1510
Crocidolite	Mining and milling	20–24	900
		25–29	2200
		30–34	3000
		35–39	7000

Source: Modified from de Klerk NH, Armstrong BK. The epidemiology of asbestos and mesothelioma. In: Henderson DW, Shilkin KB, Langlois SL, Whitaker D, eds. Malignant mesothelioma, pp. 223–250. Copyright 1992 by Hemisphere. Reproduced with permission of Informa Healthcare Books via Copyright Clearance Center. (See same reference for detailed reference listing.)

Table 43.9. Different mineral fibers, their properties, and MM risks

Fiber	MM risk	Aspect ratio[a]	Biopersistence	Human exposure
Erionite (E)	High	High	Persistent	Environmental and residential (Turkey)
Amphibole asbestos				
Crocidolite (C)	High	High	Persistent	Occupational, nonoccupational
Amosite (A)	High but less than C, E	High but less than C	Persistent	Occupational, nonoccupational
Tremolite (T)	Probably high, ?≤C	As for A	Persistent	Environmental, some occupational
Anthophyllite	Low	Fairly low	Persistent	Environmental, formerly restricted occupational (Finland)
Chrysotile	Low, not zero (disputed)	Low	Poor; less than all above	Occupational, nonoccupational
Fiberglass	Zero	Low	Probably poor	Occupational
Ceramic/MMMF	Not documented in humans	High to low	Probably as for amphiboles	Experimental

[a]Length : diameter ratio.
MMMF, man-made mineral fibers.
Source: Modified from Hammar.[289]

Once deposited, amphibole fibers are more persistent in tissues than chrysotile. The clearance half-life in lung tissue has been estimated at 5 to 10 years for crocidolite[292,293] (clearance rate is about 10% to 15% per year) and up to 20 years for amosite fibers,[279] in comparison to 90 to 110 days for chrysotile (although one study[294] recorded a longer clearance half-life of about 8 years for long chrysotile fibers among chrysotile miners/millers in Quebec). Clearance appears to be more effective for short than long fibers—although de Klerk et al.[293] could find no difference between the clearance rates for long and short crocidolite fibers—so that the length of retained fibers increases with time after exposure.[290] Clearance for chrysotile appears to involve both longitudinal and transverse splitting and solubilization of fibers, so that such cleavage can increase the number of fibers per unit weight of lung even after cessation of exposure, before further clearance of fibers accompanied by a diminution in their numbers.[38,295]

To induce MM, deposited asbestos fibers presumably must first translocate to the pleura from the lung where they are deposited initially, but we know of no data on the precise mechanisms and rates at which translocation occurs in humans. However, Boutin et al.[240] demonstrated that asbestos fibers are concentrated in parietal pleural "black spots" located near stomata on the parietal pleura. Amphiboles outnumbered chrysotile in all samples, and 22.5% of fibers in black spots were ≥5 μm in length, which might explain in part why the parietal pleura seems to be the target site for both MM and plaques, and why chrysotile is less potent than the amphiboles (whereas chrysotile appears to be no less potent than the amphiboles when fibers are implanted directly into the pleural cavity of experimental animals). Other studies have demonstrated the presence or even a predominance of chrysotile fibers in human pleural tissue (e.g., see the World Health Organization monograph Environmental Health Criteria 203: Chrysotile Asbestos,[92] pp. 64–65).

Translocation may take place by either migration of naked amphibole fibers, or by ingestion of the fibers by macrophages followed by subsequent transport along lymphatic vessels to the subpleural lymphatic channels.[290] Nonetheless, it seems worth emphasizing that studies on the persistence and clearance of fibers discussed above have focused on lung tissue, obviously not the site where MMs develop, and there appear to be no systematic data for humans on the clearance rates for fibers translocated to the pleura.

The relationship between asbestos inhalation and the subsequent risk of mesothelioma can be expressed by the Peto model and its various modifications[288,296]:

$$I = k \cdot f \cdot (t^p - [t - d]^p)$$

where I is the incidence; k depends on fiber type, mix, size, and other site-specific variables; f is the intensity of exposure in fibers/mL; t is the time in years following exposure; and d is the exposure in years. For the purposes of modeling, variations of the basic equation have been proposed to account for latency period, multiple periods of exposure, weightings for different fiber types in the exposure history, and clearance rates.[297] From the Peto model and its modifications, the following deductions can be inferred:

- Early exposures to asbestos are more significant for MM induction than later exposures, other factors being equal.
- When there are multiple episodes of exposure, each increment of exposure within an acceptable latency interval produces a corresponding increment in the risk/incidence of MM, dependent on the time of the exposure, its magnitude, and the types of asbestos fiber involved. This issue was discussed at some length in the World Trade Organization (WTO) report on asbestos (specifically chrysotile),[42] and the dose-response relationship between asbestos and mesothelioma was illus-

trated in tabular form by de Klerk and Armstrong in 1992[288] (Table 43.8).

Is a Threshold or Minimal Level of Asbestos Exposure/Inhalation Required for Mesothelioma Induction?

No minimum threshold dose of inhaled asbestos has been delineated below which there is no increase in the risk of mesothelioma.[92,93,176,189,212,217] In a study on time trends and occupational risk factors for pleural mesothelioma in Sweden, based on the Swedish Family-Cancer Database, Hemminki and Li[212] found an increasing age-adjusted incidence of pleural mesothelioma over the period 1961–1998, not only for occupations expected to be associated with asbestos exposure (manual and blue-collar workers), but also in professional groups and even farmers.

In relation to the no-threshold model for mesothelioma induction by asbestos, reviews and several case-control studies from Europe are of particular relevance and include the following:

- A review by Hillerdal[163] on mesothelioma related to nonoccupational asbestos exposure was published in 1999. It is of particular interest in relation to mesotheliomas as a consequence of low-level exposures to asbestos.
- A review and meta-analysis by Bourdès et al.[298] of the risk of pleural mesothelioma from environmental exposure to asbestos was published in 2000. These authors identified eight relevant studies on the risk of pleural mesothelioma from household or neighborhood exposures to asbestos. These studies did not include the case-control studies outlined below. These authors found that the RRs of pleural mesothelioma for household exposure ranged between 4.0 and 23.7, with a summary risk estimate of 8.1 (95% CI, 5.3–12). For neighborhood exposures, the RRs ranged between 5.1 and 9.3 with a summary estimate of 7.0 (95% CI, 4.7–11). This analysis appears to be in reasonable agreement with the studies by Magnani et al.[299,300] and Rödelsperger et al.[301] (see below). Bourdès et al.[298] commented that their data were insufficient to estimate the magnitude of excess risk at the levels of environmental exposure commonly experienced by the general population in industrial countries (in other words, from the general environment).
- In a case-referent study reported from France by Iwatsubo et al.,[91] it was found that the odds ratio for mesothelioma (OR_{MM}) was 4.2 with low-dose exposures in the range of 0.5 to 0.99 fibers/mL-years (fiber-years). In this study, there was a clear dose-response trend from no exposure, through levels of 0.001 to 0.49 fiber-years, 0.5 to 0.99 fiber-years, 1.0 to 9.9 fiber-years, and >10 fiber-years with age and socioeconomic, class-adjusted ORs (RRs) of 1.0 (for no exposure), 1.2, 4.2, 5.2, and 6.7, respectively. Although the OR_{MM} of 1.2 at 0.001 to 0.49 fiber-years did not achieve statistical significance, further calculations show a highly significant trend. Furthermore, it has been suggested that this study lacked statistical power because the number of subjects was too small to detect an $OR_{MM} = 1.2$ at the usual scientific level of significance. Accordingly, this study[91] is not inconsistent with a no-threshold model.
- In a case-referent study reported from Germany by Rödelsperger et al.,[301] the OR_{MM} was >4.5 with lung tissue asbestos fiber concentrations in the range of 100,000 to 200,000 fibers longer than 5 μm per gram of dry lung tissue, and an OR_{MM} of about 2 or more was recorded for lower lung tissue asbestos fiber concentrations, in the range of 50,000 to 100,000 fibers longer than 5 μm per gram dry lung.
- In a meticulous case-referent analysis published in 2001 using individualized estimates of exposures, Rödelsperger et al.[94] found that the OR_{MM} was 7.9 with low exposures in the range of anything more than 0 to 0.15 fibers/mL-years (>0–0.15 fiber-years). Similar findings were reported by Magnani et al.[299]
- In a population-based study on the distribution of mesothelioma in California, after attempted allowance for occupational exposures, Pan et al.[302] reported an apparent direct correlation between the odds of mesothelioma and proximity of residence according to the distribution of ultramafic rocks in the general environment (serpentinite/ultramafic rocks in California contain mainly chrysotile, with some other forms of asbestos in some areas, such as tremolite). These authors found about a 6% reduction in the odds of mesothelioma for residence for every 10 km further away from the ultramafic rocks.
- As set forth in their review on dose-response relationships between asbestos and mesothelioma, Hodgson and Darnton[93] estimated that a cumulative exposure of 1.0 fiber/mL-year for crocidolite yields a lifetime risk "best" estimate of about 650 mesothelioma deaths/100,000 (range = 250–1500), 90/100,000 for amosite (range = 15–300), and 5/100,000 for chrysotile (range = 1–20). For a cumulative exposure of 0.1 fibers/mL-years, these authors set forth a best estimate of about 100 deaths per 100,000 exposed for crocidolite, with a highest arguable estimate of 350 and a lowest of 25; for amosite, the corresponding figures were 15 deaths per 100,000, with a highest arguable estimate of 80 and lowest of 2; at this level of exposure, the risk for chrysotile was "probably insignificant," with a highest arguable estimate of four deaths per 100,000. For a cumulative exposure of 0.01 fibers/mL-years, the best estimate was about 20 deaths per 100,000 exposed for crocidolite, with a highest arguable estimate of 100 and a lowest of two; for amosite, the corresponding figures

were three deaths per 100,000, with a highest arguable estimate of 20 and lowest that was "insignificant"; at this level of exposure, the risk for chrysotile was "probably insignificant," with a highest arguable estimate of 1 death per 100,000.

One point also worth emphasizing is that the estimated RRs, ORs, SIRs, or proportional mortality ratios (PMRs) for cohort and case-control studies on mesothelioma represent cases in excess of any background risk from background exposures; in all cohort and case-control studies, the control group represents a comparable group of individuals with background (or greater[303]) levels of asbestos in their lungs, so that the risks delineated by such studies represent risks in excess of no exposure and background exposure.[44]

In line with these considerations, the Industrial Injuries Advisory Council (IIAC) in the U.K. set forth in 2005 a comment concerning causation of mesothelioma,[304] similar to and reaffirming the criteria for causation originally set out in 1996:

Mesothelioma is a rare disease in the general population almost always caused by asbestos, so that attribution to occupation is far more straightforward [than lung cancer] and does not require epidemiological evidence. . . . The last IIAC review of asbestos-related diseases in 1996 . . . recommended that benefit for mesothelioma be awarded for claimants in any occupation involving asbestos exposure at a level above that commonly found in the environment at large. . . . The Council recommends that the prescription for [mesothelioma] should remain unchanged.

Commercial Chrysotile and Mesothelioma: Can Chrysotile-Only Exposure Induce Mesothelioma?

Chrysotile represented about 95% of past production and usage of asbestos, and it is still mined in particular in Russia (the world's largest producer), Canada (the world's largest exporter), Brazil, China, and Zimbabwe; small chrysotile mines also operated at some times in other nations, such as the U.S. and Australia.

There appears to be general but not universal agreement that commercial chrysotile as exemplified by the chrysotile mined and milled in Quebec has the capacity to induce mesothelioma, not only in experimental animals but also in humans. Nonetheless, Canadian chrysotile contains trace amounts of tremolite, including fibrous tremolite (a noncommercial amphibole), as a contaminant. The amount of tremolite appears to vary from one sample to another, but is generally <1%. Some authorities claim that the occurrence of mesotheliomas among the Quebec chrysotile miners and millers is a consequence not of the chrysotile per se but rather of the coexistent trace quantities of tremolite. The amphibole hypothesis,[305,306] which argues that chrysotile itself has little or no mesotheliomagenicity and that mesotheliomas following chrysotile exposure are a consequence of the admixed commercial or trace contaminant noncommercial amphibole fibers, remains the subject of dispute.[306–312]

Analysis of the asbestos fiber content of lung tissue from the cohort of Quebec chrysotile miners/millers has consistently demonstrated disproportionately high concentrations of tremolite in comparison to chrysotile (Table 43.10).[313] This appears to represent a bioaccumulation phenomenon whereby chrysotile is cleared from lung tissue more rapidly than the tremolite, so that the tremolite not only persists but increases in proportional concentration. In this respect, the tremolite content of the lung tissue can be used as an index of past chrysotile-only exposures, and some claim that the incidence of mesotheliomas in the same cohort can be related directly to the tremolite content.[313,314]

Mesotheliomas related to the use of tremolite in whitewash or stucco have been reported in Turkey,[315,316] Greece,[118] Cyprus, Corsica,[317] and New Caledonia[318,319] (see also Schneider and Woitowitz[117]). Tremolite has also been implicated in lung cancer and mesothelioma induction among vermiculite miners in Montana,[320,321] who were exposed only to tremolite-actinolite fibers.

TABLE 43.10. Asbestos fiber concentrations in lungs at autopsy from 21 mesothelioma cases among Quebec chrysotile miners and millers (fibers per microgram [μg]; geometric means)

Place of employment	No. of cases	Chrysotile	Tremolite	Crocidolite	Amosite
Mines and mills					
Thetford Mines	14	12.8	104.1	0	0
Asbestos	5	4.3	7.5	1.7	0.3
Factory					
Asbestos	2	2.1	0.5	6.4	0.3

Source: Modified from McDonald et al.,[313] Table 2 in the original reference; see also Table 1 in the original. In calculating geometric means, a zero count has been replaced by half the detectable limit. For crocidolite and amosite, all counts were zero; i.e., below the detection limit. For fiber counts/g lung tissue, multiply the raw figures by 10^6.

Case[322] has extensively reviewed the biohazards of tremolite, including epidemiologic investigations in humans and experimental data on animal models. He also favored the expression chrysotile/tremolite for Quebec chrysotile, but is of the opinion that it is the tremolite component that causes mesothelioma.

The Quebec Chrysotile Cohort

In an analysis of mesotheliomas among the Quebec chrysotile miners and millers, up to 1997, McDonald et al.[313,314] reported 38 mesotheliomas, most of which occurred after prolonged and heavy exposure, especially at the mine where the greatest concentrations of trace tremolite occurred (Thetford). In comparison to the Thetford main complex, relatively few mesotheliomas occurred among workers at the Asbestos mine and mill (23 versus eight), despite nearly equivalent person-years of observation. In addition, asbestos fiber analysis on lung tissue demonstrated crocidolite and amosite in five of the eight cases from the mine and mill at Asbestos and in two out of the five mesotheliomas from the Asbestos factory (Table 43.11).[313]

The clear implication of this study is that the risk of MM was related strongly to years of service in the central area at Thetford where geologic factors "would probably result in tremolite, some in fibrous form, being mined with the ore."[313] In addition, the MM rate for miners and millers was >2.5 times higher at Thetford mines (excluding the smallest mines) than at Asbestos, and this difference was also attributed to differences in the amount of fibrous tremolite in the ores. Despite these differences within the cohort for the distribution of MM related to chrysotile and tremolite (and also to crocidolite and amosite at the Asbestos factory and the Asbestos mine and mill), the results clearly indicate that Quebec chrysotile has the capacity for mesothelioma induction. The abstract describes 25 MMs from the Thetford mines,[313] representing a mesothelioma rate of 337 per million person-years, substantially (almost 20-fold) higher than the incidence rate of about 17 cases/10^6/yr for men in British Colombia and the U.S. in 1982 and 1973–1984, respectively, and well above the often-cited MM "background" rate of 1 to 2 cases/10^6/yr.

In the final two paragraphs of the paper, McDonald et al.[313] commented, "The tremolite hypothesis, if correct, has several important implications. First, it supports the widely but not universally held view that most, if not all, asbestos-related mesotheliomas are caused by amphibole fibers. This in turn points to fiber durability and biopersistence as critical factors in aetiology."

A report from the *Institut National de Santé du Québec* pointed out that the average annual rate of increase in the incidence of MM in Quebec during the period 1982–1996 was 5% for men, and that work in the (chrysotile) mines was associated with 35% of a total of 691 cases of asbestos-related diseases (MM, asbestosis, and lung cancer).[323] An earlier report from the same institute found that average adjusted incidence rates for pleural MM were 32% and 92% higher for men and women, respectively, in Quebec "than those of Canadian men and women in all other provinces combined."[324] The second (2005) institute report also commented that multiple criteria for causation "show that chrysotile is carcinogenic" and that "safe use of asbestos is difficult, perhaps impossible, in industries such as construction, renovation, and asbestos processing."[323]

Mesotheliomas have also been produced in experimental animals by implantation and inhalation of chrysotile (presumably also containing trace amounts of tremolite). Mesotheliomas can also be induced in rats by intraperitoneal injection of chrysotile, with evidence of a dose-response effect.[227]

Other Chrysotile-Exposed Cohorts and Studies

In addition to the Quebec chrysotile miners and millers, mesotheliomas have also been reported among other workforces apparently exposed to chrysotile only, with much smaller amounts of contaminant tremolite.

Even so, it is doubtful whether chrysotile exists in the complete absence of contaminant amphiboles. For example, Yano et al.[325] reported a 25-year longitudinal cohort study on male asbestos workers exposed to

Table 43.11. Mesotheliomas among Quebec chrysotile miners and millers, 1997

	Number of mesothelioma deaths	Person-years (thousands)	Mesothelioma rate (per million person-years)
Thetford Mines:			
Main complex and the oldest of the smaller mines	23	65.14	353
The five smallest mines	1	6.01	266
Asbestos:			
Mine and mill	8	60.64	132
Factory	5	10.84	462

Source: Modified from McDonald et al.[313]

Table 43.12. Mesotheliomas according to types of exposures to asbestos in Saxony-Anhalt

	Amphiboles	Amphiboles and chrysotile	Chrysotile; possible amphiboles	Chrysotile	Mean values
Age at beginning of exposure	25	28	28	34	28
Duration of exposure	16	21	19	14	19
Latent period (years)	40	40	41	31	38
Age of person dying of mesothelioma	65	68	69	65	66
Number of mesotheliomas	135	279	331	67	Total = 812

Note: All types of application of asbestos with common addition of chrysotile fall under the heading *Chrysotile*; *possible amphiboles* when previous admixture of amphiboles could not be definitely excluded.
Source: Modified from Sturm et al.[336,337]

chrysotile in Chongqin, China. The factory used only Sichuanese chrysotile that was claimed to be virtually amphibole-free (<0.001% tremolite, below the detection limit of the assays). Nonetheless, subsequent investigations reported by Tossavainen et al.[326,327] using acid-alkali digestion of the bulk samples of chrysotile[328] or from analysis of the lung tissue asbestos fiber types have demonstrated that tremolite or anthophyllite is in fact present in both Russian and Chinese chrysotile (including chrysotile from the two Sichuanese mines that apparently supplied the factory studied by Yano et al.[325]).

Russia

Although precise figures for the mesothelioma incidence in the Urals region (Uralasbest) in Russia, where chrysotile is mined,[329–331] are difficult to procure, Kogan[332] commented in a textbook on occupational lung diseases published in 1998, that in the Middle Ural mountains, the main asbestos mining region in Russia, the mortality from mesothelioma over a 10-year period was sixfold higher than the average rate in the Sverdlovsk region, an area where there was negligible asbestos mining. Most of those with mesothelioma had worked at the asbestos mining and milling plants, or had lived in an adjacent town near old and very "dusty" mills.

Other Central and Eastern European Nations

One might expect data on mesothelioma incidence in Central and Eastern European countries to be of interest, from an assumption that some of them would have imported mainly chrysotile from Russia until the breakup of the Soviet Union. Unfortunately, it is difficult to evaluate national mesothelioma statistics because a number of these countries also imported amphibole asbestos.[333–335]

The Former German Democratic Republic (GDR)

Sturm et al.[336,337] have published data on asbestos-related diseases and asbestos types in the German State of Saxony-Anhalt. They report that the asbestos used in the GDR was essentially "pure" chrysotile from the Soviet Union, with a small amount (approximately 7%) of long-fiber chrysotile from Canada. In addition to these imports of chrysotile asbestos, smaller quantities of amphibole asbestos were imported.

Between 1960 and 1990, a total of 1082 mesotheliomas was recorded in Saxony-Anhalt, and these included 843 "proven asbestos-accepted mesotheliomas." Table 43.12, as modified from Sturm et al.[336,337] gives a breakdown of 812 cases for which adequate data were available: 67 were said to follow exposure to chrysotile only, and 331 were associated with "chrysotile; possible amphiboles."

China

Yano et al.[338] reported on lung cancer incidence in a Chongqin cohort of 515 male asbestos workers heavily exposed to chrysotile claimed to contain <0.001% tremolite (see preceding discussion in this chapter); two mesotheliomas over 11,850 person-years of observation occurred in this cohort. Assuming this rate to be representative, it would amount to 170 mesotheliomas/10^6/yr (about half the rate for the Quebec chrysotile miners/millers at the Thetford mines main complex[313]).

In a retrospective cohort mortality study of 1227 men employed at a chrysotile mine in Hebei Province of China before 1972, there were three deaths from mesothelioma.[92]

Other Countries

A few isolated cases of mesothelioma in chrysotile textile workers or in asbestos miners and millers have been reported from the U.S.[339,340] and Zimbabwe,[92] respectively.

Chrysotile Content of Human Lung Tissue from Mesothelioma Patients

Morinaga et al.[341] detected asbestos fibers in 19 of 23 cases of mesothelioma studied. Amphibole fibers were found in 13 cases, but six were found to have only chrysotile fibers (five pleural mesotheliomas and one peritoneal mesothelioma). Nonetheless, the methodology for this study was unimpressive, with relatively small numbers of fibers analyzed.

TABLE 43.13. Distribution of fiber concentrations: transmission electron microscopic analysis, chrysotile only (all lengths)

Fibers/gram (F/g) dry lung		Mesothelioma cases		Controls		Odds ratio (95% confidence interval)
		No.	%	No.	%	
F/g	0–200,000	12	48.0	26	83.9	
Log_{10} (F/g)	5.3–5.5	1	4.0	2	6.5	1.08 (0–17.95)
	5.5–6	7	28.0	3	9.7	8.67 (1.77–48.14)
	6–6.5	3	12.0			
	6.5–7	1	4.0			
	7–8	1	4.0	$\chi^2_1 = 9.80$ ($p < .0005$)		

Source: Modified from Rogers et al.[216]

A 1991 paper by Rogers et al.[216] recorded a substantial number of mesothelioma patients in whom the only detectable type of asbestos was chrysotile (Table 43.13), with evidence of a dose-response effect as reflected in a trend to an increasing OR_{MM} at a relatively low fiber concentration of $\leq 10^6$ fibers per gram dry lung tissue ($log_{10} = 5.5–6$; OR = 8.67).

More recently, Yarborough[342] has argued that chrysotile fibers found in the lung tissue of MM patients are unrelated to causation of the MM; the implication is that because of rapid clearance of chrysotile fibers, with a correspondingly short half-life, and the known long latency between first exposure to asbestos and the subsequent clinical development of the mesothelioma, any parenchymal fibers must have been deposited more proximately in time, after mesotheliomagenesis began; that is, neither the presence nor the absence of chrysotile fibers would be considered as evidence of causation. This argument overlooks the fact that chrysotile fibers *are* found in the parenchymal tissue of asbestos-exposed individuals, years and even decades after cessation of asbestos exposure. In this regard, it must be remembered that the clearance times represent *half-lives*, not absolute clearance times (see also the preceding discussion on mesotheliomas in Quebec).

Chrysotile-Only Exposure: Asbestos and Mesothelioma Among Automotive and Brake Mechanics

Before bans in many countries in the 1990s and early 2000s on the use of any type of asbestos, but on a continuing basis in some nations, vehicular brake blocks and linings contained substantial amounts of commercial chrysotile (within the range of about 30% to 70% by weight[343]), mostly from Canada, bound in a resinous matrix.[343] Since the 1970s there have been concerns over the potential for dust derived from the brake materials[344,345] to be inhaled by automotive mechanics, with the potential for mesothelioma induction, and individual case reports of MM among automotive/garage mechanics have been published,[277,346] yet workers in the friction products manufacturing industry appeared to have a low risk of MM.[42,347–350]

Braking of automobiles generates high temperatures in the brake drums/linings, up to about 700°C or more, and at this temperature a high proportion (up to about 98%) of the chrysotile undergoes degradation and recrystallization to form the mineral forsterite,[344,345] which is not implicated in mesothelioma induction. Nonetheless, asbestos fibers, mainly short fibers[351] but including a small proportion of long fibers,[92] remain within the dust created within worn brake linings. In addition, it is a truism that heat-related changes do not apply to work on or with new brake linings/pads.

In August 1975, NIOSH in the U.S. Department of Health, Education and Welfare issued a communication to alert the country to "recently gathered information indicating a potential health hazard for persons exposed to asbestos during the servicing of motor vehicle brake and clutch assemblies." This communiqué indicated that average peak airborne fiber concentrations for "blow-out of automobile drum brake assemblies, grinding of used truck brake linings and bevelling of new truck brake linings" were 10.5, 3.75, and 37.3 fibers/mL, respectively (for fibers longer than 5 μm). Analysis of brake drum dust (worn linings) demonstrated that almost all of the fibers were shorter than 0.4 μm in length. The same communiqué stated that the "present findings indicate that enough asbestos is preserved to produce significant exposures during certain brake servicing procedures."

A 1998 monograph from the World Health Organization/International Programme on Chemical Safety (WHO/IPCS), entitled "Environmental Health Criteria 203: Chrysotile Asbestos,"[92] reviewed studies on airborne dust concentrations produced by blowing out worn brake linings with a compressed air hose or from grinding new brake blocks/linings, and commented that recent findings are probably not applicable to the airborne fiber concentrations from these types of work in the past. For example,

the WHO/IPCS monograph stated that during "early" years when poor or no control measures were used, there was "high total dust exposure," especially during grinding of brakes and the use of compressed air to blow off dust, but lower levels "were measured when engineering controls were introduced."

The same WHO/IPCS monograph set forth the mean airborne asbestos fiber concentrations measured during maintenance and replacement of brakes. Studies carried out in 1976 revealed mean concentrations of 3.8 fibers/mL for grinding truck brakes and 15.9 fibers/mL for blowing out brakes. Different studies carried out in the same year also found a mean airborne fiber concentration of 3.8 fibers/mL for grinding brake blocks, 16 fibers/mL for blowing out the brakes, and 2.5 fibers/mL for "dry brushing." Subsequent studies have generally found lower airborne fiber concentrations, but one investigation carried out in 1985 found that blowing off and grinding brakes produced a mean airborne fiber concentration of 6.25 fibers/mL. Other investigations also recorded elevated airborne fiber concentrations from such maintenance and replacement work on brakes,[344,345,352] whereas later studies recorded lower[353,354] or no significant[351] elevations of airborne fiber concentrations.[343] Furthermore, in Germany, Rödelsperger et al.[355] recorded the presence of long fibers 5 μm or more in length in the airborne dust.

A study by Butnor et al.[356] on lung tissue fiber analysis for 10 cases of MM among brake mechanics found that the individuals with elevated fiber counts had "excess" commercial amphibole fibers in their lung parenchyma and that elevated levels of noncommercial amphibole fibers—such as tremolite as a marker for chrysotile, or anthophyllite or actinolite—were found only in those who also had elevated levels of commercial amphibole fibers, leading to the conclusion that those subjects had "unrecognized" exposures other than the brake dust exposure. However, this study concerned only a small number of MM cases associated with exposure to brake dust, with no analysis of MM risk relative to parenchymal asbestos fiber concentrations.

In addition, one of these cases was evaluated by Dodson et al.[357] by ATEM, which found high concentrations of chrysotile in parenchymal lung tissue and two chrysotile asbestos-cored asbestos bodies.

Several reviews have argued that there is no increased risk among automotive mechanics,[343,358–360] These publications have been funded by the automotive industry, related to litigation in the U.S.[343,361] Those same reviews have also been criticized by Egilman and Billings[361] on a number of grounds and, in generic sense, by Egilman and Bohme[362] and Gennaro and Tomatis.[363] These latter three reviews can be regarded as adversarial or polemical, but they do raise substantive issues of risk assessment such as stratifying cumulative exposures within the group being studied in order to avoid underestimating the risk for those exposed or, conversely, overestimating the risk for those not, or only minimally, exposed.[364]

In addition, to evaluate any risk cogently, a distinction should be made between work on worn (heat-altered) versus work with new brake linings and, perhaps, between those who worked with brake materials for passenger cars as opposed to those who worked with brake materials for heavy vehicles (trucks).

It is well known that death certificates are a poor measure of disease outcome because of their inherent limitations, and studies that rely on death certificate diagnoses are subject to error as was pointed out by Paustenbach et al.[343] relative to the Connecticut friction products study. Death certificates simply may not list the disease under investigation (e.g., mesothelioma). It is also essential that all cases of the disease in question are captured by the study: this is a major problem when the duration of the study is short and cannot allow for the long latencies that underpin MM induction by asbestos. Another issue that must be taken into account is ensuring that the control reference population is truly unexposed in order not to underestimate the risk of disease in the exposed group.[303] A further question is whether the individual studies reviewed had the statistical power to detect small increments in risk if they did exist.[38,365]

Data in Australia point to an increased risk of mesothelioma among brake mechanics. The Australian Mesothelioma Register (AMR) Report for 2002 lists 59 cases of mesothelioma for the exposure category *brake linings–made/repaired* (single exposure only) and a further 19 cases for the same class of exposure but with multiple patterns of exposure, giving a total of 78 cases.[36,43,193,366] Taking into account census data for automotive mechanics in Australia, it has been estimated that brake mechanics have a MM rate of at least 20 cases per million person-years, as discussed in the Dispute Settlement Report for the WTO[42] (i.e., a risk that is up to about 20-fold greater than the background risk of mesothelioma). In addition, it has been noted that the increase in the number of cases of mesothelioma apparently related to work on brake linings roughly parallels the increase in the number of cases of mesothelioma related to other occupations that involved asbestos exposure.[367]

As of 2007, the AMR data constitute the strongest evidence for an increased risk of mesothelioma among brake mechanics who ground and chamfered new brake pads/linings/blocks, but those figures have been criticized as inferior in probative value to formal epidemiologic studies (an issue debated at some length in the WTO report on chrysotile[42]). In terms of science, the question of whether automotive mechanics—and especially dedicated brake mechanics with protracted exposures to dust derived from the grinding/chamfering of new brake

blocks/linings—have an increased risk of MM remains unresolved and contentious.

Summary

- The association between asbestos inhalation and the development of MM fulfills all of the Bradford Hill criteria[368] for the establishment of causality, in terms of the strength, consistency and specificity of the association, biologic gradient (dose-response), relationship in time, experimental evidence, reasoning by analogy, biologic plausibility, and coherence of the evidence (and its apparent resistance to falsification[369,370]).
- All forms of asbestos have the capacity to induce MM, but the commercial amphiboles crocidolite and amosite are substantially more potent on a fiber-for-fiber basis than chrysotile (white asbestos). The exact ratio of potencies for crocidolite, amosite, and chrysotile remain somewhat uncertain, with different ratios being cited in the literature.
- No lower (minimum) threshold level of exposure to asbestos has been delineated below which there is no increase in the risk of MM, and most authorities approach causation of mesothelioma by asbestos from the perspective of a no-threshold model.
- From the Peto model and its modifications, the risk of MM can be related to cumulative asbestos exposure (assessed from the intensity, frequency, and duration of exposure) multiplied by time in years raised to about the cubic or 4th power), so that other factors being equal, the time elapsed following commencement of exposure is a major probability factor for risk; that is, early exposures are more significant for MM risk than later exposures, other factors remaining constant.
- Epidemiologic studies indicate that there is no increase in the risk of MM for at least 10 years following the commencement of exposure, and the Helsinki criteria,[220] for example, adopt a minimal 10-year latency interval in order to assign causation of MM to asbestos; other authorities require a minimum latency interval of 15 years.
- One factor that emerges from the Peto model and its modifications is that when there are multiple asbestos exposures, each contributes to cumulative exposure and hence to the risk and causation of MM, within an appropriate latency interval.
- Asbestos alone appears capable of acting as a *complete* carcinogen for the mesothelium. As such, asbestos and the secondary reactions associated with its inhalation are apparently sufficient over time to elicit malignant transformation of the mesothelium.
- Only a minority of those exposed even heavily to asbestos develops MM, even after heavy exposures to amphibole asbestos. This has given rise to the notion that there may be a possible genetic predisposition to MM.

The Molecular Pathogenesis and Pathology of Malignant Mesothelioma

The mechanisms whereby asbestos fibers induce malignant transformation of mesothelial cells have long remained elusive, despite extensive investigation.[371–374] Nonetheless, there have been substantial advances in uncovering some of the mechanisms for the induction of MM, and these appear comparable to the multiple steps implicated in the development of other cancers. It is now recognized that asbestos fibers themselves are carcinogenic,[37] mainly by indirect mechanisms, and that malignant transformation is a multistage process, correlating with the known long latency interval between the first exposure to airborne asbestos fibers and the subsequent diagnosis of the MM (see later discussion). However, no single molecular event or series of events can explain all MMs, and most studies have investigated only single steps in what appears to be a highly complex sequence of cellular and molecular events (Fig. 43.9).

Malignant mesotheliomas do not commonly show mutations in oncogenes, but rather multiple alterations in

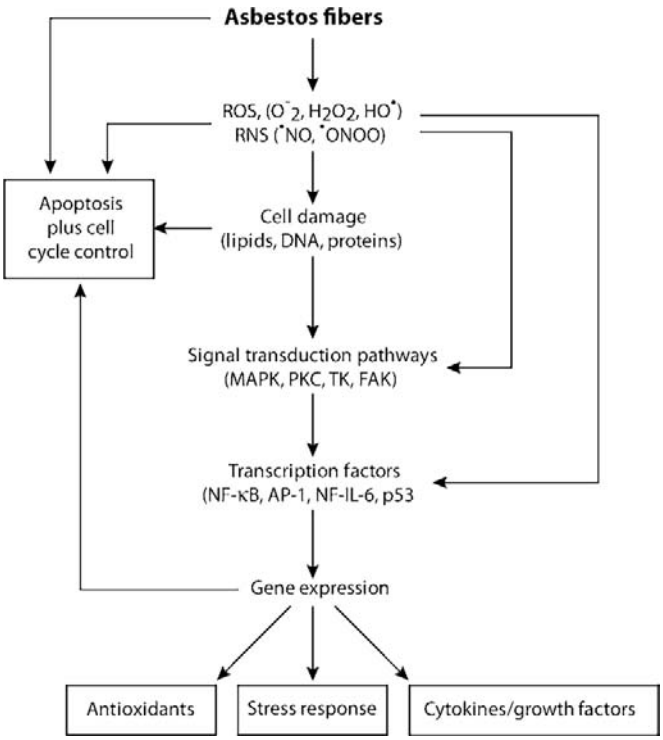


FIGURE 43.9. Mechanisms of asbestos-induced pleuropulmonary toxicity. Schematic illustration of the likely pathways involved in asbestos-induced damage. ROS, reactive oxygen species; RNS, reactive nitrogen species; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; TK, tyrosine kinase; FAK, focal adhesion kinase; NF, nuclear factor; IL, interleukin. See text. (Modified from Kamp and Weitzman.[371])

Box 43.2. Important Definitions (see also Chapter 33)

Oncogenes: Genes that stimulate cell growth under normal conditions, to allow for continuous turnover of tissues such as skin and gastrointestinal epithelia. They are analogous to the accelerator in a car. A mutation of these genes is comparable to a "stuck accelerator" that is independent of the driver's action: forward motion continues, even if the driver removes his foot from the accelerator. Cells with defective oncogenes continue to grow, even in the absence of valid growth stimuli. Examples of classical oncogenes are *bcl-2* and *ras*. In malignant mesotheliomas, mutations of oncogenes are rare.

Tumor suppressor genes: A car with a "stuck accelerator" may still be stopped using the brakes; because the ability to brake is vitally important, there are several to choose from (brake pedal, hand brakes, gears). Similarly, cells possess multiple mechanisms that regulate cell proliferation and restrict cell numbers, either by promoting programmed cell death (apoptosis) or by inhibiting progression through the cell cycle, and slowing mitotic activity. Examples of classical tumor suppressor genes are *p53*, *pRb* (the gene inactivated in retinoblastoma), and $p16^{INK4a}$, which inhibits cyclin-dependent kinases, therefore preventing completion of the cell cycle. Many of the mutations found in malignant mesotheliomas affect tumor suppressor genes.

DNA repair genes: Even a car with functional brakes and accelerator needs to be serviced regularly. Repair genes themselves do not control cell proliferation directly; they simply fix mutations in all genes. If repair genes are defective, there is an increased rate of mutations in all genes.

tumor suppressor genes, most of which are regulated by a complex network of regulators with several backup loops. This type of mutation interferes with the regulatory mechanisms that normally restrict cell numbers and is therefore akin to "defective brakes" (Box 43.2; see Chapter 33).[375] There are multiple regulatory backups, so that there is a requirement for a number of mutations in several genes, and this type of mutation initially produces little increase in cell growth rate. Instead, there is a lack of cell death, resulting in a net increase in cell numbers. This may help to explain in part the long latency interval between exposure and clinically evident disease.

Molecular Events in the Development of Mesothelioma I: Physical Interaction Between Fibers and Cells

Asbestos fibers may exert their carcinogenic effects on mesothelial cells by direct and indirect mechanisms. Direct effects are related to the physical interaction of fibers with target cells or by the generation of free radicals and reactive oxygen species (ROS) at the surface of fibers. Indirect effects are related to an inflammatory response to fibers, including the generation of factors, such as ROS and cytokines as a consequence of attempted but incomplete phagocytosis of fibers by macrophages ("frustrated phagocytosis").[376] There is now substantial scientific evidence for the indirect model, as discussed in several reviews.[371,372,376–378]

Direct genotoxic effects following exposure to asbestos fibers include chromosome mis-segregation, disruption of the mitotic spindle, the formation of aneuploid and polyploid cells, and disruption of nuclei. The formation of micronuclei as a result of DNA disruption is also common. There is experimental evidence, based on in vitro cell culture experiments, that asbestos fibers can interact directly with the mitotic spindle, resulting in aneuploidy.[379,380] Asbestos also has been shown to induce structural and numerical chromosomal alterations in cultured human mesothelial cells[381] (see Molecular Events in the Development of Mesothelioma III). In some of these processes, the particle state and fiber dimensions are considered important parameters in the generation of the genotoxic effects.[382] According to the Stanton hypothesis,[229,383] long, thin fibers appear to be more carcinogenic than short fibers (see above).

Molecular Events in the Development of Mesothelioma II: Free Radicals

Indirect Toxic Effects

Some of the very early steps in the malignant transformation of mesothelial cells are related to oxido-reduction processes generated by fibers.[382] It is now widely accepted that a key process in the development of MM is the production of free radicals, including ROS[371,372,376–378,382] (Fig. 43.9; Box 43.3). This process is neither unique nor specific to MM, and free radicals are implicated in carcinogenesis of many tumors; for example, some carcinogenic polycyclic aromatic hydrocarbons (PAHs) in cigarette smoke are known to generate showers of free radicals,[384] and the mutagenicity of ionizing radiation is related predominantly to the generation of free radicals in tissues (see Chapter 33).

As reviewed by Kamp and Weitzman[371] (Fig. 43.9; Box 43.3), there is abundant evidence that free radicals such as ROS, including hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), the hydroxyl radical ($HO^\bullet$), and singlet oxygen (O), as well as reactive nitrogen species (RNS), are important mediators of asbestos-induced tissue injury, including MM induction. The RNS include nitric oxide ($^\bullet NO$) and peroxynitrite ($^\bullet ONOO$). The ROS (notably $HO^\bullet$) and RNS (notably $^\bullet ONOO$) can affect a variety of macromolecules, with multiple genotoxic effects and

Box 43.3. Reactive Oxygen Species (ROS)

ROS, including free radicals, are thought to play an important role in the molecular pathogenesis of a number of tumors, including MM. ROS include hydrogen peroxide (H_2O_2), the hydroxyl radical (HO^-), and superoxide anion (O_{2-}). Reactive nitrogen species (RNS) are also thought to play a role.

All types of asbestos contain iron cations, either as part of their crystalline lattice structure (crocidolite and amosite), or as a surface impurity (chrysotile). ROS may be generated at the surface of asbestos fibers by chemical reactions catalyzed by the iron component of the fibers or they may be released by macrophages that have partially engulfed the fibers. Cell damage may be related to the peroxidation of phospholipids, such as those present in cell membranes, or by direct damage to DNA and other macromolecules.

The Fenton reaction is the primary reaction involved in OH^- formation, but free radicals may be produced by the Haber-Weiss reaction in the presence of iron (as present on chrysotile), resulting in generation of hydrogen peroxide (H_2O_2).

1. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$ (Fenton reaction)
2. $Fe^{3+} + O_{2-} \leftrightarrow Fe^{2+} + O_2$
3. $2O_{2-} + 2H^+ \rightarrow H_2O_2 + O_{2-}$
4. $H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^\bullet + Fe^{3+}$ (iron-catalyzed Haber-Weiss reaction)

activation of signaling cascades. The free radicals may originate either at the surface of the asbestos fibers or they may be released by macrophages that have partially phagocytosed long fibers.[378]

All varieties of asbestos have iron either as a component of the crystalline lattice (crocidolite and amosite) or as a surface impurity (chrysotile has a low but significant surface iron component as a contaminant). The iron associated with asbestos is thought to generate ROS partly by the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet$). In addition, H_2O_2 can be converted to $HO^\bullet$ by the iron-catalyzed Haber-Weiss reaction (Box 43.3), and iron is also thought to catalyze alkoxyl radical production from inorganic hydroperoxides. The process of free radical production can also involve other highly reactive molecules such as ferryl or perferryl species. In relation to mutations inducible by iron ions, it has been found that Fe^{2+}-treated DNA shows a 20- to 80-fold greater frequency of mutations, and these mutations appear to include G→C transversions, C→T transitions, and G→T transversions.[385] Such observations may also account in part for the greater carcinogenicity of crocidolite and amosite than chrysotile for the mesothelium. The importance of iron contaminants for cytotoxicity and mutagenic potential has also been demonstrated for erionite in an in vitro system.[386] Even so, the total amount of breakage of plasmid DNA in a cell-free system was not directly associated with the amount of iron released by the fibers, and iron reactivity alone cannot explain all the DNA damage observed.[382] Instead, fiber characteristics such as size, availability of calcium, and the state of cells nearby appear to be important for malignant transformation.

Most studies on free radicals and ROS have been carried out on in vitro systems, such as cell cultures or cell-free systems, and this approach cannot examine the role of secondary ROS released by macrophages during phagocytosis. Reactive oxygen species have an extremely short half-life. and therefore physical proximity of DNA and cell membranes susceptible to the damage by ROS is a prerequisite for damage to occur. As instructive as those studies are, it is likely that a significant proportion of the cell damage in vivo is actually induced by oxidoreduction secondary to inflammatory cellular processes, and phagocytosis in particular. The damage is likely to be transmitted via secondary molecules that are more stable than ROS. This is supported by the fact that asbestos fibers can induce the release of ROS from neutrophils and macrophages.[387] When incubated with neutrophils in vitro, crocidolite, amosite, and chrysotile fibers induce greater release of lactate dehydrogenase than rockwool, glasswool, or ceramic fibers.[387] Experimental studies have also shown that crocidolite, amosite, and chrysotile fibers appear to produce significantly greater amounts of HO^- from mixtures of neutrophils and asbestos fibers than from mixtures of such cells and man-made fibers such as rockwool, glasswool, and ceramic fibers. It appears that asbestos fibers are more efficient for stimulation of ROS from phagocytic cells than are nonfibrous mineral dusts.[387]

In this context it seems worth reiterating that small fibers can be successfully phagocytosed, whereas large fibers are resistant to complete phagocytosis because of their dimensions, and such "frustrated phagocytosis" yields abundant ROS. This partly supports the Stanton hypothesis, but it seems to be the biopersistence of fibers resistant to clearance by inflammatory or other processes that is important for MM induction, rather than a precise and critical fiber dimension per se.

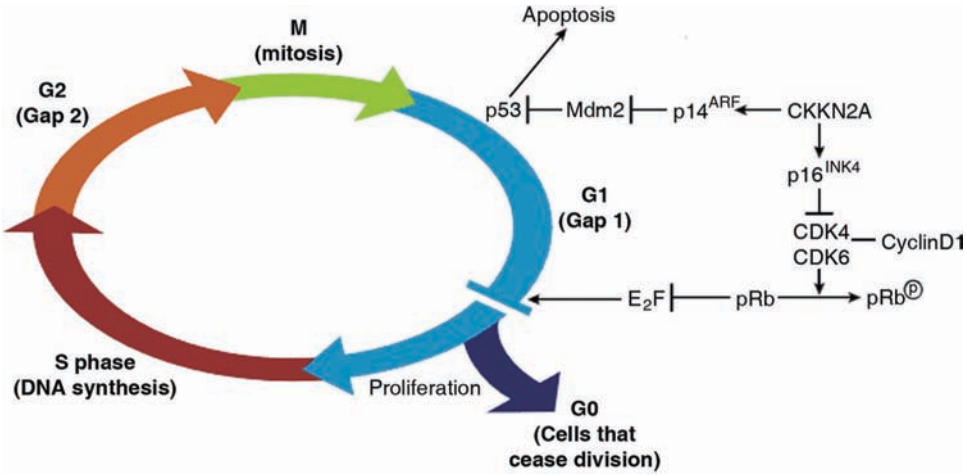
Interference with Apoptosis

Asbestos fibers and ROS induce apoptosis in cultured normal mesothelial cells (Fig. 43.9).[388–391] One function of apoptosis is the elimination of severely damaged cells, including cells that may have undergone some of the steps potentially leading to malignant transformation. Apoptosis is therefore one of the protective mechanisms against

Box 43.4. Control Mechanisms of the Normal Cell Cycle and Loss of Control in MM

The cell cycle is a tightly controlled process, with the greatest level of control being exerted at the transition from the G_1 to the S phase. The S, G_2, and M phases are largely autonomous ,and the only opportunity for DNA repair or induction of apoptosis in the case of irretrievable damage is during the transition from G_1 to S. Two of the major pathways altered by mutations in MM are involved in the regulation of transition from G_1 to S phase: these are the retinoblastoma gene product (pRb) pathway and the p53 pathway. (See diagram below and The Cell Cycle in Chapter 33).

The regulatory proteins $p14^{ARF}$ and $p16^{INK4}$ are each encoded by CDKN2A/ARF at 9p.21, a locus commonly mutated in MM, resulting in loss of control of cell cycle progression and loss of an initiating stimulus of apoptosis. However, in a normal cell, there are numerous regulatory interactions and backups between these two major pathways of growth control. For example, the transcription factor E2F-1 also induces transcription of $p16^{INK4}$, resulting in stabilization of pRb and inhibition of E2F-1 itself in a classical negative feedback loop. Therefore, deletion or mutation of both $p14^{ARF}$ and $p16^{INK4}$ in the same cell is likely to have a synergistic disruptive effect, rather than simply an additive effect, on cell cycle and growth control.



the development of tumors (Boxes 43.4 and 43.5). In contrast, MM cell lines are highly resistant to asbestos-induced and ROS-induced apoptosis.[391] This effect is not related to expression of Bcl-2, an important regulator of apoptosis that is mutated in many tumors, but the mechanism underlying this resistance is not understood at present.

Direct Activation of Transcription Pathways

Induction of the mitogen-activated protein kinase (MAPK) signaling pathways occurs in response to exposure to asbestos and appears to be related to ROS. This cascade includes signal transcription factors such as nuclear factor κB (NF-κB),[371] which triggers activation of a number of genes involved in cell proliferation and apoptosis, including cytokines, growth factors, and adhesion molecules as well as proto-oncogenes such as c-*myc*. Reactive oxygen species also induce expression of the AP1 transcription factors c-*fos* and c-*jun*, both of which are also proto-oncogenes[392] implicated in malignant transformation. However, recent experiments investigating protein expression and phosphorylation status (activity) of the extracellular-regulated kinase (ERK), the c-*jun* amino-terminal kinase (JNK), and the high-osmolarity glycerol response kinase (p38) in fresh frozen reactive mesothelium and MM specimens did not detect significant differences between reactive mesothelium and MM.[393] Although there is undoubtedly upregulation of these genes, there is insufficient experimental evidence at present to conclude that MAPK activation contributes significantly to malignant transformation.

Molecular Events in the Development of Mesothelioma III: Chromosome and Gene Alterations, and Disruption of Cell Pathways

The capacity of asbestos to induce mesothelioma in experimental animals was established as early as the 1960s by inhalation/installation and direct implantation experiments, where chrysotile was found to be about equipotent with the amphiboles when implanted directly

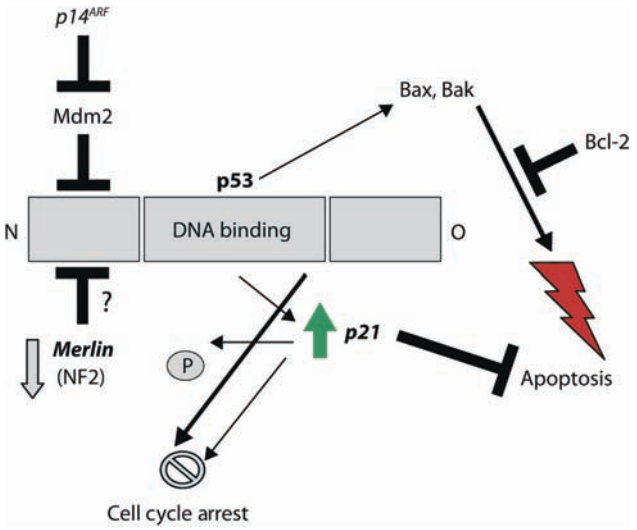
Box 43.5. Apoptosis

The term *apoptosis* is of Greek origin, meaning "falling off" and is used to describe the process that leads to controlled self-induced cell death. Apoptosis plays an important role in the morphogenesis of developing organisms, as well as maintaining homeostasis in adult organisms. In addition, apoptosis allows deletion of damaged and potentially dangerous cells, such as cells that contain irreparable DNA damage, infected cells or autoreactive immune cells. (See sections Cell Death and Survival, and Apoptosis in Chapter 33.)

There are many potential pathways that may lead to apoptosis, and some of those that have been investigated in MM are illustrated below.

The role of p53 is central. Activated p53 may arrest the cell cycle, via upregulation of p21, a cyclin-dependent kinase, which has been found to be altered in some MMs. Depending on the cell type, p21 may not only induce cell cycle arrest, but also initiate apoptosis directly. In addition, p21 phosphorylates merlin, the gene product of the *NF2* gene at chromosome 22, which has been found mutated in a significant number of MM. Phosphorylation decreases function of merlin, which is thought to act as a tumor suppressor gene, although the exact mechanism is not well understood as yet.

In addition, there are alternative pathways to apoptosis via the mitochondrial-bound proteins Bax and Bak, which are opposed by the antiapoptotic protein Bcl-2. This pathway is commonly affected in malignant tumors, but there is currently insufficient evidence to suggest that it plays a major role in the development of MM.



into the pleural cavities. More recently, in vitro studies have yielded significant information, and it has been established that asbestos has clastogenic and genotoxic effects in cells.[37] Asbestos fibers have been shown to induce chromosomal aberrations, anaphase-telophase abnormalities, and sister chromatid exchanges in cultured rodent and human cells. Both crocidolite and chrysotile have been shown to disturb cell division, resulting in aneuploidy or polyploidy. However, although asbestos was found to induce clonally aneuploid cells with abnormal banding patterns in vitro, these alterations were insufficient to render the cells tumorigenic.[394]

Studies on the chromosomal profile of MMs have demonstrated multiple abnormalities, usually more than 10 clonal abnormalities in any one case, although no consistent or specific chromosomal abnormality has been identified. Nonetheless, recurrent chromosomal abnormalities are common in MM, deletions being the most common chromosomal alterations,[395–402] including deletions in chromosome arms 1p, 3p, 4q, 6q, 9p, 13q, 14q, 15q, and 22q where the neurofibromatosis 2 *(NF2)* gene is located.[403–407] The most frequent numerical change is monosomy of chromosome 22[395]; gains are less common, but gains of chromosomes 5, 7, and 20 have been described.[37] Comparative genomic hybridization (CGH) studies have shown multiple chromosome abnormalities in most of the tumors analyzed, with no consistent or specific abnormality.[397,402]

Combinations of such cytogenetic abnormalities can be found in most MMs, and all are present in about 25%.[37] Loss of heterozygosity has also been demonstrated on chromosome 1, as have allelic deletions on chromosomes 3, 4, and 6 as well as 15 (where *RAD51*, a tumor suppressor gene that participates in the repair of breaks in double-stranded DNA, is located at 15.q.15.1[37]). Deletion of $p16^{INK4A}$ has also been demonstrated at 9p.21, in about 85% of MM cell lines and about 22% of primary MMs.[37] In a study on transgenic mice carrying the *lacI* reporter gene, Rihn et al.[408] also found evidence suggestive of a decrease in DNA repair in crocidolite-treated animals.

It is worth noting that most of the studies investigating the molecular basis for MM were carried out using epithelial mesotheliomas, although some studies did not distinguish between the different types of mesotheliomas. This may affect some of the results and may explain some contradictory results in different studies.

Most of the recurring mutations seem to affect tumor suppressor genes and growth factors, rather than oncogenes. Although no specific chromosomal or genomic abnormality has been demonstrated in MM, it has been recognized that the disruption of certain cellular *pathways* is a recurring event. Therefore, it is useful to think of MMs as being characterized and unified by disruption of those pathways, rather than by mutations of specific genes.

Molecular Events in the Development of Mesothelioma IV: Interference with Cell Cycle Control and Apoptosis: *p53*

Because the induction of MM represents a multistep process that requires progressive accumulation of mutations, the pathways that prevent this occurrence in healthy cells play a pivotal role. The tumor suppressor gene *p53*, sometimes termed "the guardian of the genome," initiates cell cycle arrest or programmed cell death in response to cellular damage and stress (Boxes 43.4 and 43.5; also see The Cell Cycle in Chapter 33).

Mutations of the gene encoding *p53* (usually point mutations leading to an inactive form of p53) are well characterized and are known to play a role in the carcinogenesis of many tumors, but are rarely identifiable in mesotheliomas. For example, mutations in *p53* itself or the tumor gene *RAS*, which is known to interfere with p53 concentrations and which is commonly mutated in lung cancer,[179] are not common in mesotheliomas.[409,410] Even so, whereas mutations in *p53* itself are rarely described in mesotheliomas, the p53 pathway is commonly affected. The high frequency of deletions at the 9p.21 locus corresponds to loss of functional activity of a number of critical proteins involved in the p53 and pRB pathways, namely $p14^{ARF}$, as well as the CDK inhibitors $p16^{INK4a}$ (and, to a lesser extent, $p15^{INK4b}$).

The protein $p14^{ARF}$ induces p53. The level of p53 in unstressed cells is low, maintained by degradation of p53 and suppression of its transcriptional activity by binding of Mdm2. Mdm2 effectively counteracts p53 tumor suppressor activity. Mdm2 activity is blocked by $p14^{ARF}$, and $p14^{ARF}$ acts as a positive regulator for *p53*. Therefore, functional loss of $p14^{ARF}$, as seen in many MMs, results in lack of functional p53 because of unopposed Mdm2. This results in a loss of the ability of the cell to arrest the cell cycle and or undergo apoptosis in response to cell damage sustained, for example, by ROS. Unless the cell damage is lethal, the mutated cell undergoes uninhibited growth.

This has been exploited in several experimental models, where transfection of cultured human mesothelioma cell lines with an adenovirus vector expressing $p14^{ARF}$ resulted in increase of functional p53, and therefore cell cycle arrest and slowing of tumor growth.[411–414]

Molecular Events in the Development of Mesothelioma V: Cell Cycle Control: *pRb*

The gene product of the retinoblastoma gene, *pRb*, is the prototypical tumor suppressor gene. It is part of the cyclin-dependent kinase-cyclinD1/INK4/pRb/E2F cascade, and mutations in this cascade have been identified in more than 80% of human neoplasms.[415] Active pRb is hypophosphorylated and binds to transcription factors, E2F-1 in particular, and inactivates them. Phosphorylation renders pRb inactive, so that the transcription factors become active and DNA synthesis is initiated. Members of the INK4 family (CDK inhibitors $p16^{INK4a}$ and $p15^{INK4b}$) inhibit phosphorylation of *pRb*, by interaction with cyclin-dependent kinases, maintaining the binding of transcription factors to *pRb* and preventing transcription. The cells remain in G_1 phase and do not progress through the cell cycle (Box 43.4). Cyclin-dependent kinases act as checkpoints that prevent transition into the next cell cycle phase, and loss of CDK inhibitors results in uncontrolled cell proliferation (see The Cell Cycle in Chapter 33).

Mutations in this cascade can occur within the effector proteins, such as pRb itself, as in the case of retinoblastoma. In MM and MM cell lines, expression of the wild-type pRb is mostly maintained. In contrast, there is deletion or mutation of the upstream regulators, $p16^{INK4a}$ and $p15^{INK4b}$. When $p16^{INK4a}$ is replaced in human mesothelioma cells by adenovirus gene transfer, and functional protein expressed, arrest of the cell cycle occurs via inhibition of pRb phosphorylation. The end result is diminished cell growth, and, eventually, death of the transfected cells.[411,414] This approach may have some therapeutic potential.

It becomes clear at this stage that there are many interconnections of the pathways that are commonly altered in MM. The intimate association of p53 and pRb pathways does not end with the shared site that expresses $p16^{INK4a}$, $p15^{INK4b}$, and $p14^{ARF}$. In addition, transcription of p21WAF/CIP1 is induced by p53, and p21 then may act as an inhibitor of cyclin-dependent kinases involved in the pRb pathway, causing cell cycle arrest (rather than apoptosis). The expression of p21 appears to have prognostic significance in MM, and increased expression of p21 is associated with improved survival.[416]

Molecular Events in the Development of Mesothelioma VI: Interference with Cell Cycle Control: pRb and Simian Virus 40

Simian virus 40 (SV40) DNA (see above) has been found in many human mesothelioma samples: in the U.S., some studies have reported SV40 DNA in at least 40% to 60% of human mesotheliomas, but other studies did not detect SV40 DNA in any tumors[417] or in cell lines established from human MM,[192,418] raising the possibility that the positive results represented laboratory contamination.[418,419] Simian virus 40 DNA has also been found in a number of other tumors, including osteosarcomas, brain tumors, and papillary thyroid carcinomas.[420,421]

Two epidemiologic studies published in 1998 found no evidence of an increased rate of bone or brain tumors, or

mesothelioma, 30 years after the use of polio vaccines contaminated with SV40.[422,423] In a later study stratified for age, Strickler et al.[160] found that the incidence of pleural mesothelioma remained stable or declined in younger age groups with a high probability of having received the SV40-contaminated vaccine, whereas the incidence rose in the oldest age groups with a low probability of inoculation with the contaminated vaccine. The evidence on SV40 and human cancer, including four epidemiologic studies, has been reviewed systematically by Shah.[424]

Simian virus 40 encodes two tumor antigens, large T and small t; SV40 causes interference with cell cycle regulation, in part by the blocking of p53 via its SV40 large T antigen (SV40 LT), but SV40 LT also interacts with pRb. These interactions result in inactivation of proteins with tumor suppressor activity, via two pathways that are commonly disrupted "upstream" by other mechanisms in MM. Interference with either pathway is sufficient to induce tumors, because a mutant SV40 LT which cannot bind p53 is still capable of transforming cells lacking p53.[192] The t antigen has also been implicated in the oncogenic activity of SV40 via binding to phosphatase 2A, but its role is less well established.[192]

There is no doubt that SV40 can be oncogenic under certain conditions. In particular, this DNA virus has been shown to induce mesotheliomas when injected into the heart or pleura of hamsters.[425] In addition, SV40 also transforms human cells in tissue culture, and these cells show extensive DNA damage. Although it appears certain that SV40 can induce tumors in animal models and in vitro, this does not seem to contribute to an understanding of MM carcinogenesis in humans.

The ATCC cell line Met-5A (www.atcc.org) comprises nonneoplastic human mesothelial cells that have been immortalized by transfection with a plasmid containing the SV40 early region DNA. Met-5A cells, one of the standard human mesothelial cell lines used in many experiments investigating MM, have a single copy of SV40 early region DNA integrated in their genome. They express SV40 large T antigen, and they maintain mesothelial cell characteristics, such as sensitivity to the cytotoxic effects of asbestos fibers. However, when injected into nude mice, these cells are nontumorigenic, providing evidence that SV40 alone is insufficient to induce MM.[426]

Asbestos alone fails to induce the transformation of these human mesothelial cells in vitro, but if interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are added (simulating the release of these major cytokines by macrophages after inhalation of asbestos), they contribute to erionite-induced transformation of the MeT-5A cells in vitro. These cells could only be transformed when exposed to a combination of cytokines and erionite, or at least two cytokines together without erionite, for at least 4 months in vitro. The findings presented here suggest that IL-1β and TNF-α play a significant role in the pathogenesis of mesothelioma, and that it might be desirable to block or inhibit cytokine secretion in high-risk populations to prevent mesothelioma.[427]

As discussed above, both of the pathways that are subject to inactivation by SV40 are usually already mutated further upstream in MM. For example, loss of effective pRb control in human MM already results from the near-universal deletion of $p16^{INK4a}$; therefore, an argument can be adduced that SV40 LT is not necessary for inactivation of pRb in MM, and that it may not contribute to tumor development.[428] Also, since SV40 LT acts downstream from both $p16^{INK4a}$ and $p14^{ARF}$, the effect would be expected to be more akin to a point mutation in these major tumor suppressor proteins. Therefore, one would expect much faster tumor growth, and the long latency of MM argues against a significant role for SV40 in human MMs.

Finally, other polyomaviruses, such as JC virus, which have been shown to be oncogenic in animal models could not be detected in significant numbers of MMs.[429]

Molecular Events in the Development of Mesothelioma VII: SV40: Other Effects

There is evidence that SV40 may induce some growth factors, including vascular endothelial growth factor (VEGF),[430,431] which has been found to play an important role in the growth of MM.[432] It is therefore possible that SV40 creates a favorable environment for the accelerated growth of MM. Some authors believe that VEGF shows potential as a prognostic indicator,[433] whereas others deny that VEGF predicts prognosis.[434]

At present, a significant role for SV40 in the induction of MM is far from accepted and appears unlikely, despite the undisputed fact that this virus has oncogenic capacity in some models. In particular, epidemiologic data make it unlikely that SV40 can act as the single causative agent inducing MM. Rather, it appears likely that SV40 may contribute to a permissive environment that may favor tumor growth. Finally, crocidolite asbestos has been shown to mediate transfection of human mesothelial cells by plasmid DNA containing SV40 sequences, and it is possible that exposure to asbestos simply facilitates entry of SV40 into affected cells, and that in fact the frequent finding of SV40 sequences in MM is a consequence of exposure to asbestos.[394]

Molecular Events in the Development of Mesothelioma VIII: Interference with the p53 Pathway: The Role of Wilms' Tumor 1 (*WT1*)

WT1 is a tumor-suppressor gene expressed in the developing kidney, whose inactivation leads to the

development of Wilms' tumor, a pediatric kidney cancer. *WT1* is expressed in normal mesothelium and in most epithelial mesotheliomas. *WT1* mutations have been found to be expressed in mesothelioma,[428,435] although this is disputed by others who believe mutations to be exceptional.[37] In contrast, lung carcinomas rarely express WT1, and this has led to the use of WT1 antibodies for diagnosis of epithelial MM, although there is some debate in the literature about their usefulness.[436,437]

WT1 encodes a transcription factor that binds to the early growth response gene 1 *(EGR1)* consensus sequence and suppresses transcription of early growth response genes including insulin-like growth factor-I (IGF-I) receptor and epidermal growth factor receptor (EGFR).[438] It is therefore conceivable that mutation of *WT1* could lead to increased growth factor release, creating a favorable environment for tumor growth. WT1 also interferes with the p53 pathway, because the tumor-suppressor gene *p53* physically associates with WT1. The interaction between WT1 and p53 modulates their respective capacity to transactivate their respective targets. Unexpectedly, in the absence of p53 (as would be the case in MM cells), WT1 acts as a potent transcriptional *activator* of the EGFR-1 site,[439] so that even normal WT1 could potentially lose its tumor-suppressant attributes in this environment. Nonetheless, no correlation between WT1 expression and expression of growth factors has been demonstrated so far in MM.[440] Even so, the interaction among p53, WT1, and growth factors appears to play a role in the growth of MM, and we have found antibodies against WT1 to be useful for diagnosis in many cases.

Molecular Events in the Development of Mesothelioma IX: NF2 Inactivation and Mesothelioma

The neurofibromatosis 2 (NFS)-encoded protein belongs to the ERM (ezrin-radixin-moesin) family of cytoskeleton-membrane linkers.[438] The protein encoded by NF2 is a tumor suppressor protein called merlin (for mesosin-ezrin-radixin-like protein) or schwannomin, which functions as a negative growth regulator, and it is known that inactivating mutations in *NF2* predispose humans to tumors. Some of its tumor suppressor properties are probably associated with contact-mediated growth inhibition. Mutations of the *NF2* gene or reduced expression of the gene product are an extremely common finding in MM,[405,407,441] but not in lung cancers.[37] There are several connections of merlin with the p53 pathway (Box 43.5). First, merlin increases p53 stability by inducing degradation of the p53 inhibitor Mdm2. In addition, merlin appears to mediate an increase of p53-mediated transcriptional activity. As mentioned in Box 43.5 and above, there is already a connection of *NF2* with the p53 pathway, as p53 induces p21, a cyclin-dependent kinase, which phosphorylates merlin.[442] This diminishes the function of merlin and acts as a negative feedback loop. However, there is nearly ubiquitous loss of $p14^{ARF}$ in MM, resulting in lack of p53 and lack of induction of p21, so that this pathway is unlikely to play a major role.

Furthermore, patients with NF2 appear to have no increase in the risk of MM. This implies that the tumor suppressor gene *NF2*, despite the common presence of mutations or deletions in MM, is likely to play a permissive or supportive role in the development of MM, rather than being an initiator of tumorigenesis (similar to *WT1* and SV40). Similar observations have been made in other hereditary cancers where tumor suppressor genes are affected; for example, *Rb-1* is commonly mutated in nonhereditary small cell carcinomas of lung, but patients with hereditary retinoblastoma do not have an increased risk for developing small cell carcinoma of lung. It has been proposed that, depending of the tissue type, further pathogenetic stimuli are required.[443]

Molecular Events in the Development of Mesothelioma X: *FHIT*

The fragile histidine triad *(FHIT)* tumor suppressor gene located at 3p14.2 appears to represent a site of genomic fragility relevant to carcinogenesis,[181,444–446] including the pathogenesis of MM.[182] FHIT protein is expressed in most nonneoplastic tissues, and the highest levels of expression occur in epithelial cells. FHIT appears to be subject to deletion or LOH by cigarette smoke and asbestos.[181,182,444,445] Diminished expression of FHIT has been recorded in up to 80% of cigarette smoke–associated lung cancers,[444] and in both asbestos-associated lung cancers (69%) and nonexposed cases (59%) in one study,[181] and in 54% of mesotheliomas.[182] The limited data available suggest a frequent decrease of FHIT protein expression, thus supporting the significance of FHIT inactivation in development of MM.

Molecular Events in the Development of Mesothelioma XI: Growth Factors/Cytokines

There is now a large body of evidence that growth factor signaling, and in particular EGFR signaling, plays a key role in tumor growth. Consisting of complex cascades of interactions, the EGFR signaling system is one of the most extensively studied signaling pathways (Fig. 43.10). As discussed above, disruption of regulation of apoptosis plays a major role in MM development, and there are complex interactions between growth factor signaling and apoptosis control. The intracellular mechanisms of interactions between EGF and apoptosis pathways are incompletely understood, but many of them involve the

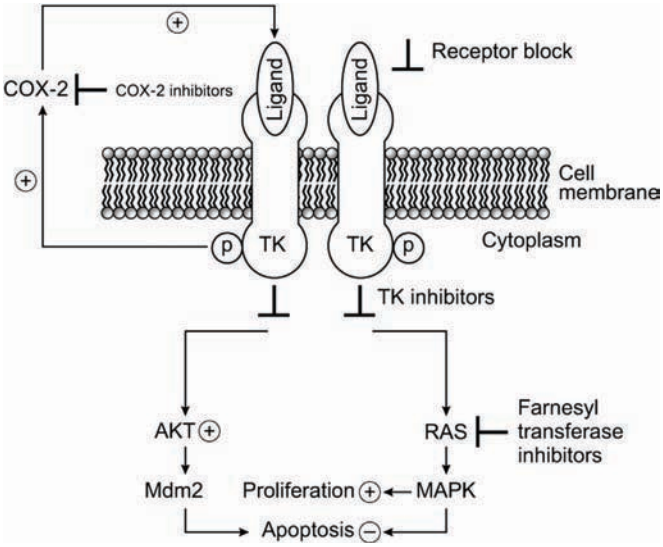


FIGURE 43.10. The epidermal growth factor receptor domain and its interaction with signaling pathways and cyclooxygenase-2 (COX-2), and potential targets for therapeutic intervention.

kinase Akt (see PI3K/Akt/mTOR Pathway in Chapter 33), which is activated downstream of many growth factors not limited to EGF. Another pathway involves RAS signaling (Box 43.6). These signaling pathways have been the focus of targeted treatment attempts (see also Chapter 33, section on Ras/Raf-1/Mitogen activated protein kinase pathway).

Epidermal growth factor receptor signaling has been recognized as a key step in MM growth and it has been suggested that control of cell survival through EGFR activation is conditional, in the sense that it is crucial for tumor cell survival but not for survival of normal mesothelial cells. Specifically, normal epithelial cells are provided with a full complement of physiologic cell-cell contacts and cell-matrix interactions that lessen their dependence on survival signals provided by the EGFR. In contrast, malignant tumor cells faced with inadequate cell-matrix contacts are thought to depend critically on EGFR activation for survival, making them more susceptible to apoptosis induction by EGFR blockade. This was the basis for focusing research efforts on developing potential clinical treatments for MM, based on blocking EGFR signaling, but it now appears that redundant control of cell survival by the EGFR and extracellular matrix/cell adhesion receptors remains, to a degree, enabled in tumor cells. This is at least in part the result of shared signal transduction pathways controlling apoptosis (Fig. 43.9), and these complex interactions are discussed below.

In addition, growth factors are also involved in the regulation of matrix metalloproteinases (MMPs), a group of enzymes involved in dissolution of extracellular matrix that enable cell growth and vascularization under normal circumstances, and in tumors play a key role in cell invasion and metastasis.

The Epidermal Growth Factor Receptor Transforming Growth Factor-α Loop

Epidermal growth factor receptor belongs to the ErbB family of receptor tyrosine kinases, which has recently gained prominence because of the mutations found in a group of breast carcinomas, which then may be selectively treated with specific tyrosine kinase inhibitors, such as Herceptin. This family of receptors includes EGFR (ErbBHer1), ErbB2-Her2/neu, Her3, and Her4. Epidermal growth factor receptor is a transmembrane glycoprotein that consists of the extracellular ligand-binding domain, a transmembrane component, the intracellular tyrosine kinase functional domain, and a COOH-terminal region containing autophosphorylation sites (Fig. 43.10). Phosphorylation at the COOH-terminal tail initiates a cellular signaling pathway that regulates fundamental cellular processes such as proliferation, migration, differentiation, and survival.

Epidermal growth factor receptor on the cell surface presents as an inactive monomer that is activated by binding of specific ligands, including EGF and transforming growth factor-α (Fig. 43.10). The activated EGFR monomer can pair with another EGFR to form an active homodimer, or an EGFR receptor monomer may pair with another member of the ErbB receptor family, such as Her2/neu, to create a heterodimer.

Ligand binding induces the intrinsic protein-tyrosine kinase activity of EGFR, initiating a signal transduction

Box 43.6. Cell Signaling Pathways and RAS

RAS pathways are involved in cell signaling pathways that control cytoskeletal integrity, cell proliferation, cell–matrix interactions, apoptosis, and cell migration. RAS is a G protein (a small guanosine triphosphatase [GTPase]) that alternates between two conformations: activated or inactivated. Mutations in the *ras* family of proto-oncogenes (e.g., H-*ras*, N-*ras*, and K-*ras*) are present in 20% to 30% of all human tumors, but are not common in MM.[47] Despite this, the RAS pathway may still be affected indirectly (e.g., by increased EGFR expression), and downstream modulation and inhibiting of RAS signaling may inhibit growth and promote apoptosis. Farnesylation is necessary to attach RAS to the cell membrane. Without this attachment to the cell membrane, RAS cannot transfer signals from membrane receptors, and this is the rationale for treatment attempts with farnesyltransferase inhibitors.

cascade. This involves the MAPK, Akt, and JNK pathways, among others (see relevant sections in Chapter 33). Increased proliferation is achieved by promoting cell cycle progression at the level of the G_1-phase, and inhibiting apoptosis, and the net effect is tissue growth. The kinase activity can also result in autophosphorylation of the COOH terminal region, as mentioned above, resulting in activation of proteins distinct from those activated by the kinase signaling cascade directly. These proteins include regulatory proteins involved in cell matrix continuity and play a role in maintaining cell-cell and cell-matrix interaction, disturbance of which may lead to loss of contact inhibition and increased invasiveness.

There has been convincing evidence that expression of EGFR at the protein and transcriptional level is increased in MM in comparison to reactive mesothelial proliferations or normal mesothelial cells.[447–449] The EGFR ligands that have been shown to play a role in the pathogenesis of MM include EGF and TGF-α. Binding of TGF-α induces an autocrine feedback loop resulting in increased EGFR expression and increased proliferation. Phosphorylation of EGFR[450] and an increase in expression of TGF-α is observed early after exposure to asbestos,[451] and cell growth can be inhibited under those circumstances by antibodies to TGF-α. In addition, there appears to be a correlation between the expression of EGFR and the carcinogenicity of the fibers used.[452] Furthermore, autophosphorylation of EGFR can be induced by asbestos fibers directly in vitro, with long fibers being more effective than short fibers.[453] It can be argued that the ongoing inflammatory response directed at the asbestos fibers in vivo provides an ongoing source for TGF-α, and in effect delivers a continuous growth stimulus.

Selective inhibition of EGFR signaling by the small molecule inhibitor gefitinib (ZD1839) in models using mesothelioma cell lines in vitro results in reduced growth of tumor cells of some cell lines. In most cell lines this effect appears to be cytostatic, rather than cytotoxic, as evidenced by a lack of increase in the apoptotic fraction,[454] although there was an increase in apoptosis in another cell line.[455] However, the main effect of EGFR blockade appears to be arrest of the cells in the G_1/S phase.[456] A similar effect resulting in reduction of tumor volume has also been reported in an in vivo murine model of mesothelioma. Inhibition of EGFR signaling was effective in reducing tumor size if used alone, with an increased effect if used in conjunction with radiation.[457] It appears that selective blockade of the EGFR pathway at the ligand level in solid tumors limits tumor cell survival rather than survival of normal tissues, as alternative pathways of apoptosis control and cell proliferation are intact in the normal tissues, making EGFR pathway blockade an attractive potential treatment modality for MM.[458] More recently, use has been made of lapatinib, which blocks both EGFR (Erb1-Her) and Erb2 receptors, but inhibition of growth has been found in only some tumor cell lines.[456] Therefore, although redundancy in regulatory pathways may protect nontumor tissue and minimize side effects of treatment, it may also mean that treatment may not be sufficiently effective because alternative pathways can also be utilized by tumor cells. Because of the redundancy in regulatory pathways, combining EGFR (or more generally, ErbB-family) inhibitors with signal transduction inhibitors in mesothelioma might enhance their effectiveness. However, if EGFR signaling is blocked further downstream by farnesyltransferase inhibitors, no or only minimal growth inhibition was seen in in vitro models.[449] However, if the lapatinib is combined with intracellular signaling inhibitors, such as rapamycin, the net effect on inhibition of cell growth in the sensitive cell lines is greater than that with either drug alone.[456]

Only some of the mesothelioma cell lines tested in vitro were sensitive to treatment targeting the EGFR pathways, and this is reflected in the results of recent clinical trials. Use of alternative pathways appears to occur in vivo, and to date, clinical trials with oral gefitinib have been disappointing.[459,460] This may have been expected, because EGFR status has not been identified as an independent prognostic factor, presumably due to this redundancy in regulatory pathways. In addition, EGFR expression in MM seems to correlate with epithelioid histology, and it would be desirable to differentiate clinical treatment groups according to the histologic subtype of MM. Combination of EGFR inhibitors and intracellular signaling inhibitors has been proposed for future clinical trials.[456]

The Epidermal Growth Factor Receptor–Cyclooxygenase-2 Loop

Apart from these fairly direct effects on growth, EGFR is also involved in a second autocrine feedback loop via cyclooxygenase-2 (COX-2), with EGFR increasing COX-2 transcription, and COX-2 increasing EGFR transcription, in turn (Fig. 43.10). Cyclooxygenase-2 expression has been proposed as an independent negative prognostic factor,[461–463] although other investigators claim that COX-2 expression indicated improved survival.[464] Inhibition of COX-2 may be achieved by nonspecific nonsteroidal antiinflammatory drugs (NSAIDs) such as acetylsalicylic acid or indomethacin, or more selectively by the COX-2 inhibitor NS-398 or celecoxib (Fig. 43.10).

Cyclooxygenase-2 has been implicated in carcinogenesis by way of downregulation of cell-mediated immunity and promotion of angiogenesis, and COX-2–expressing cancer cell lines are associated with increased proliferation and invasive potential. Cyclooxygenase-2 overex-

pression has been noted in many solid tumors, and expression has recently also been shown in MM,[462,465,466] as well as reactive mesothelial proliferations.[466] Selective inhibition of COX-2 with the COX-2 inhibitor NS-398 in vitro revealed dose- and time-dependent antiproliferative activity,[466] and similarly, the selective COX-2 inhibitor celecoxib reduced in vitro proliferation of several MM cell lines obtained from previously untreated patients. In addition, there was increased MM cell apoptosis that involved decreased Akt phosphorylation, loss of bcl-2, survivin protein expression, and caspase-3 activation.[467] Simultaneous application of VEGF rescued apoptosis and Akt phosphorylation, but if anti-VEGF antibodies were also given, this effect was abrogated. This finding highlights the complex interaction and cross-regulation between the different growth factors, all leading to tight control of apoptosis.

Vascular Endothelial Growth Factor

Vascular endothelial growth factor is a potent angiogenic factor, involved in the growth and metastasis of neoplasms by stimulating stromal vascular growth. There is overexpression of VEGF and VEGF-C in MM,[430,434,468–470] but this also occurs in some reactive conditions. Although some studies claim negative prognostic significance associated with VEGF expression, this has not been confirmed by all investigators.[471] In MM, VEGF is expressed along with the VEGF receptor flt-1.[472] The production of the growth factors by tumors is a widespread phenomenon, but the coexpression of receptors and formation of an autocrine loop, as seen in MM, is less common. This pathway appears to be effective in promoting tumor growth, as VEGF also has been shown to increase proliferation of MM by directly stimulating tumor growth in a dose-dependent manner.[432] Blocking of the autocrine loop by antibodies against VEGF receptor or antisense oligonucleotides that act as inhibitors of VEGF and VEGF-C has been shown to inhibit MM cell growth in vitro.[473] Also, if there is a role for SV40 as a driving agent for MM development, it may be through VEGF activation.[430,474]

Tumor Necrosis Factor-α

Tumor necrosis factor-α is a potent initiator of apoptosis, but paradoxically, in some cases, it can inhibit apoptosis by upregulation of survival-inducing proteins, including members of the so-called inhibitors of apoptosis (IAP) family. Interestingly, raised serum levels of TNF-α have been found in those individuals exposed to asbestos who would eventually develop a thoracic malignancy.[475] The secretion of TNF-α may also aid in explaining a paradox: crocidolite asbestos is cytotoxic, and in isolation fails to transform primary human mesothelial cells, causing extensive cell death instead. In in vitro experiments, treatment with TNF-α significantly reduced crocidolite cytotoxicity and promoted cell survival, thus increasing the pool of asbestos-damaged cells susceptible to malignant transformation.[476] In vivo, macrophages are a potential source of TNF-α, and secretion of TNF-α has been linked with fiber length, with longer more carcinogenic fibers inducing higher levels of secretion.[476,477]

Inhibitors of Apoptosis Proteins and Tumor Necrosis Factor-α

The family of IAPs includes the proteins IAP-1, IAP-2, livin, and survivin. These are proteins that can block apoptosis. There is increased expression of survivin in MM (but also in some inflammatory conditions),[478,479] and this appears to have some prognostic significance in predicting poorer outcome.[479] Anti-survivin oligonucleotides could inhibit survivin activity in vitro in cell lines expressing survivin, resulting in apoptosis, whereas apoptosis could not be induced in the survivin-negative cell line LRK1A by antisense oligonucleotides. Therefore, down-regulation of survivin by a targeted antisense oligonucleotide could represent an effective gene therapy approach to the treatment of mesothelioma. Tumor necrosis factor-α has been shown to increase expression of IAP-1, IAP-2, and XIAP in MM in vitro.[480] Inhibitors of apoptosis may therefore represent an additional target for treatment attempts in clinical trials.

Growth Factors and Extracellular Matrix Interaction

Malignant mesotheliomas express a wide range of MMPs in comparison to normal pleura[481–484] (Box 43.7). Matrix

Box 43.7. Extracellular Matrix and Matrix Metalloproteinases

Extracellular matrix proteins and interaction play an important role in maintaining tissue integrity. Mutations and activations of some of these enzymes that can dissolve extracellular matrix are essential steps for a tumor to promote angiogenesis, and acquire invasiveness and metastatic potential. Numerous mutations of matrix proteins have been described in MM. Matrix metalloproteases (MMPs) are a family of zinc-dependent enzymes that dissolve extracellular matrix and seem to play a particularly important role in tumor cell invasion and metastasis. Most of the enzymes are secreted as inactive proenzymes and activated by cleavage of the N-terminal sequence. They are directly negatively regulated by tissue inhibitors of metalloproteinases (TIMPs), and growth factors and angiogenic factors such as TGF-α, EGF, and COX-2 activate MMPs.

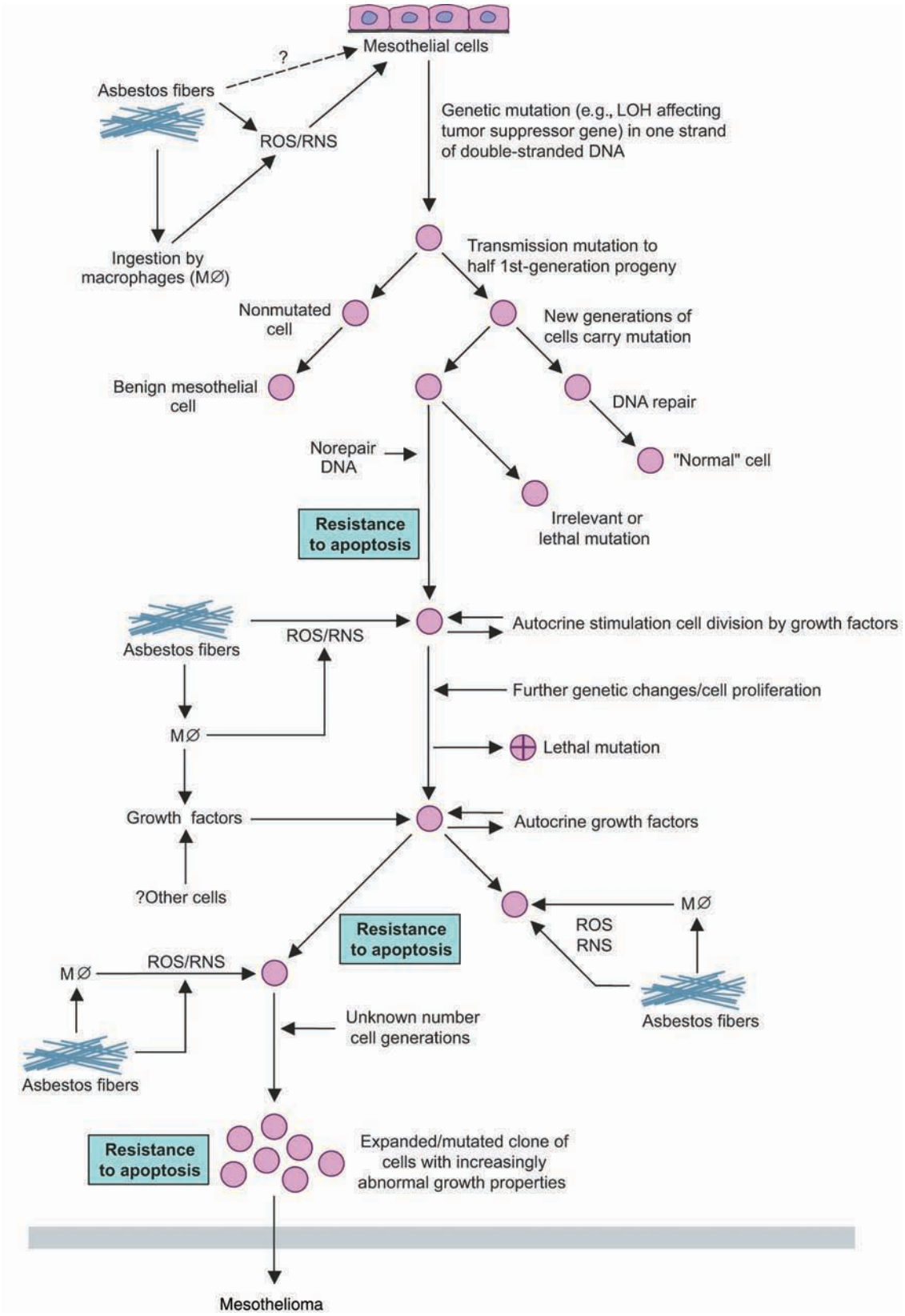


FIGURE 43.11. Schematic overview of possible or likely events leading to the development of MM, extending over multiple generations of mesothelial cells.

metalloproteinase-2 was found to be the predominant gelatinase in a study of 16 tumors,[483] but MMP-2 expression was not induced by the growth factors EGF or TGF-α,[485] and there was no correlation with expression of COX-2. Instead, ligation of EGFR increases MMP-3 and MMP-9 production,[482,485] and the increase in MMP could be blocked in vitro by the tyrosine kinase inhibitor genistein.[485] This increase in MMP expression was associated with enhanced cell motility, and this may play a role in acquiring invasive potential. In addition, MMP-1, which may be induced by platelet-derived growth factor (PDGF) and TGF-α, has been shown to increase mesothelial cell motility and possibly play a role in invasiveness.[482]

Hoang et al.[486] found an 826-fold increased expression of matriptase, a trypsin-like protease, in epithelioid MM cells. Matriptase messenger RNA (mRNA) has been "characterized as an extracellular matrix-degrading protease system that may function as an epithelial membrane activator for other proteases and latent growth factors involved in cancer cell growth, invasion, and metastasis."[164] Hoang et al. also found upregulation of insulin-like growth factor exon I (IGF-I), which has also been found to act as an autocrine growth factor for normal and neoplastic mesothelial cells, and IGF-I also drives mesothelial cell differentiation toward a fibroblast-like morphology.[164] Strong expression of the c-*sis* gene (PDGF B-chain) has also been recorded in comparison to normal mesothelial cells.[487,488]

Molecular Events in the Development of Mesothelioma XII: Mesothelial Cell Kinetics and Proliferation

Although some authorities have invoked a multipotential subserosal cell as the stem cell for repair of mesothelial injury and for the histogenesis of MM, studies on the repair of mesothelial cell damage that does not include disruption of the basal lamina or other submesothelial tissues indicate that repair is effected by mesothelial cells themselves by a process of proliferation, migration, and probably detachment and reimplantation. The concept of mesothelioma in situ[489,490] has also redirected attention to the mesothelium itself as the target site for mesothelioma induction.

It is known that in the resting mesothelium in the rat, about 1% of the mesothelial cells are in the S-phase of the mitotic cycle (0.5–3.0%, and about 0.16–0.25% in the mouse); however, about 60% to 80% of the cells go into the S-phase within 1 to 2 days of a superficial injury that denudes the mesothelium, with proliferation of mesothelial cells that then move across the denuded surface to reestablish continuity of the mesothelial layer in about 8 to 10 days.[491–494] (A single mesothelial cell has been observed by time-lapse cinephotography to travel a distance of up to 75 μm within the space of 3 hours).[492,493,495] It appears that about 30% of resting mesothelial cells turnover about every 10 days, and "inflammatory" stimuli and asbestos fibers have the effect of increasing the rate of turnover. Suppose, however, that the resting rate remains unchanged after asbestos fibers reach the pleural membrane, and that the turnover rate is 10 to 20 days for 30% of the mesothelial cell population, so that the time for the entire population to be renewed is about 35 to 65 days; this means that the pleural mesothelium renews itself about six to 10 times each year.

The mean lag time between first exposure to asbestos and the diagnosis of MM is about 35 to 45 years (rounding off to the nearest 5 years). Suppose also that a mesothelioma comes into existence as such about 5 years before diagnosis. During the preceding 30 years there would be some 180 to 300 generations of mesothelial cells for an average MM case. Even if the first mesothelioma cell came into existence only 5 years after exposure, one can calculate that some 30 to 50 generations of mesothelial cells would have passed before the MM would have come into existence. Figure 43.11 presents a schematic overview of the types of events that are considered likely in the development of MM, over multiple generations of mesothelial cells.

Pathologic Features of Malignant Pleural Mesothelioma

Macroscopic Features of Pleural Mesothelioma

Most patients with pleural mesotheliomas present with shortness of breath due to a pleural effusion on the side of the tumor. When these patients are evaluated by video-assisted thoracoscopic surgery or by an open thoracotomy, the visceral and parietal pleura are often found to be studded by multiple nodules ranging in size from less than 1 mm to about 1 cm. (Fig. 43.12). As time progresses, the small nodules coalesce to form a solid tumor that encases the lung and obliterates the pleural cavity (Fig. 43.13). In most instances, the tumor is thicker at the base of the thoracic cavity than at the apex. The tumor not infrequently invades the lung parenchyma and chest wall (Fig. 43.14). Mesotheliomas frequently become nodular and sometimes can present as large nodules within the lung parenchyma (Fig. 43.14). Mesotheliomas frequently metastasize to lymph nodes, causing their enlargement. Occasionally, metastatic mesothelioma to bronchopulmonary, hilar, and mediastinal lymph nodes produces a hilar mass that can be mistaken for a primary lung cancer (Fig. 43.15).

Approximately 25% to 30% of pleural mesotheliomas invade the parietal and, occasionally, visceral pericardium, and sometimes there is massive involvement of the heart

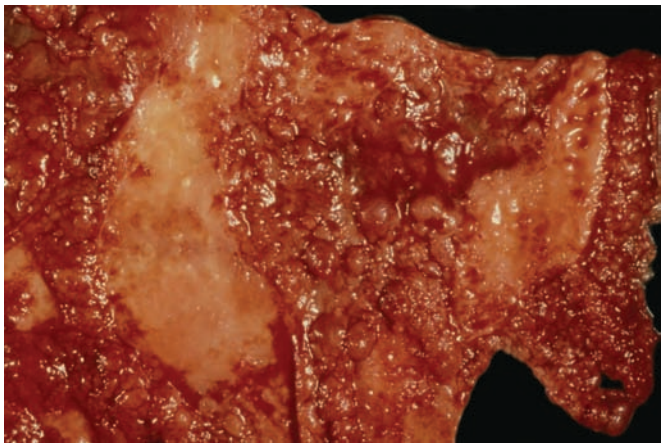


FIGURE 43.12. The parietal pleura is studded by <1- to 5-mm tumor nodules of mesothelioma. The pleura is also involved by larger hyaline pleural plaque characteristic of plaque caused by asbestos.

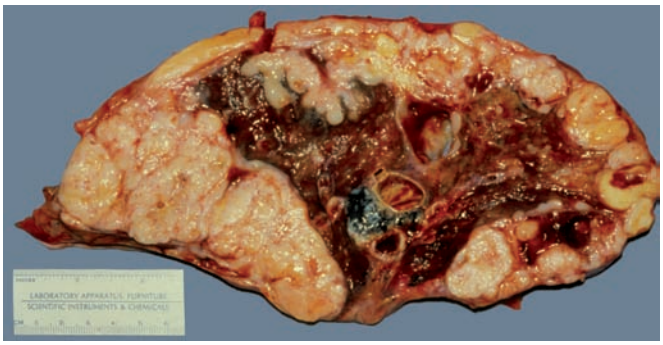


FIGURE 43.14. Pleural mesotheliomas frequently invade lung parenchyma and chest wall. Note also the nodular growth pattern, which is typical of most mesotheliomas.

with replacement of a sizable portion of the myocardium by tumor (Fig. 43.16). Rarely secondary tumor encasement of the heart is so thick as to simulate a primary pericardial mesothelioma. In this situation it may be difficult to determine whether a tumor showing both pleural and pericardial involvement is a primary pericardial mesothelioma or a primary pleural mesothelioma (Fig. 43.16).

Some epithelial mesotheliomas produce excess amounts of hyaluronic acid that can result in cyst formation within the tumor (Fig. 43.17). Occasionally, such tumors will invade the lung to the point that one cannot recognize normal pulmonary parenchyma. Outward growth into the mediastinal fat with metastasis to mediastinal lymph nodes is characteristic (Fig. 43.15).

The most common site of intrathoracic metastasis of pleural mesothelioma is to bronchopulmonary, hilar, and mediastinal lymph nodes. The next most common metastatic site is the contralateral pleural surface. Sometimes, mesotheliomas metastasize outside the chest cavity, such as to the adrenal gland. On the other hand, peritoneal mesotheliomas may metastasize to the pleural surfaces, producing a relatively thin, whitish film that encases the lung (Fig. 43.18).

Because most mesotheliomas are caused by asbestos, it is common to see mesotheliomas in association with hyaline pleural plaques that involve the lateral and diaphragmatic parietal pleura (Fig. 43.12). Mesotheliomas can directly invade or encase the plaque.

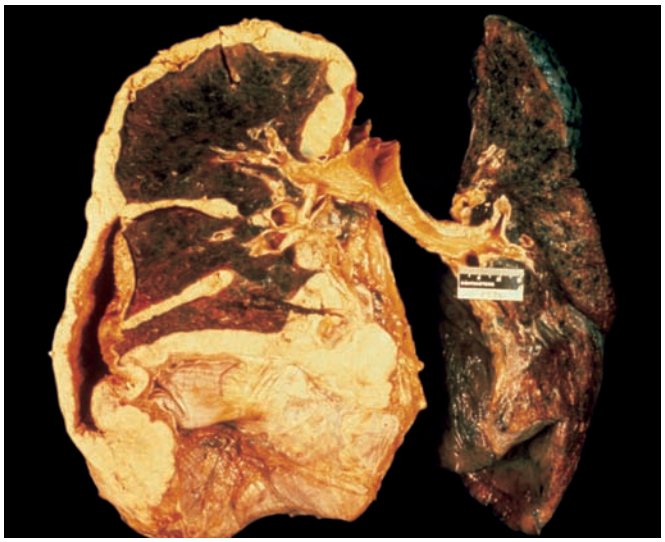


FIGURE 43.13. Right pleural mesothelioma showing encasement of lung by rind of tumor, which, like most mesotheliomas, is thicker at the base (diaphragmatic surface) than at the apex.

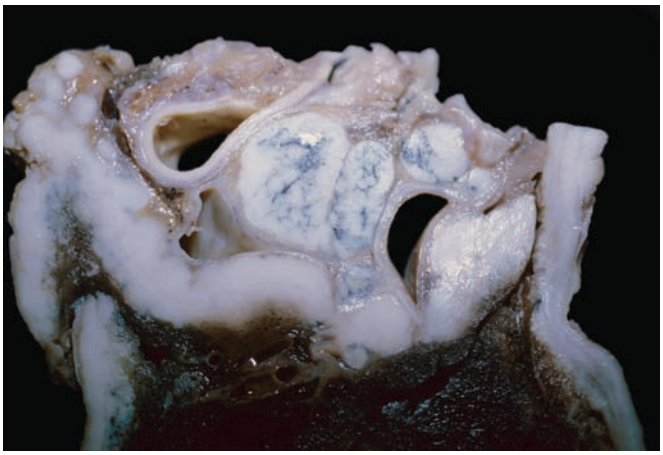


FIGURE 43.15. Pleural mesotheliomas not infrequently metastasize to hilar, bronchopulmonary, and mediastinal lymph nodes producing what is seen radiographically as a hilar mass.

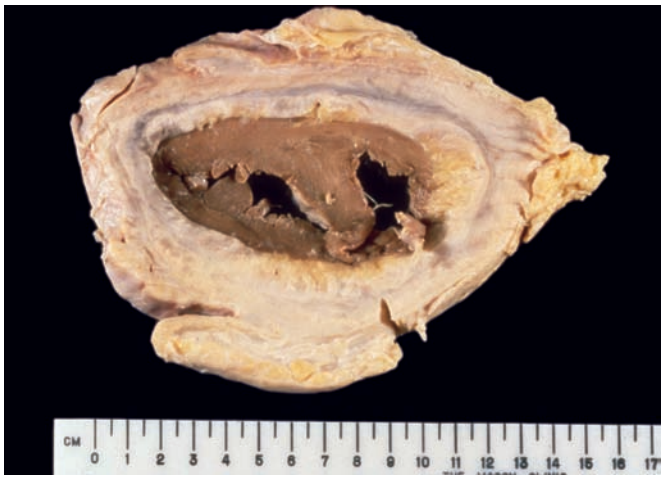


FIGURE 43.16. Pleural mesotheliomas may directly invade the pericardium and myocardium and may replace a significant portion of the myocardium. In some instances, it is difficult to tell if the pericardial involvement is an invasion or metastasis of a pleural mesothelioma or a primary pericardial mesothelioma.

FIGURE 43.18. The lungs are coated by a thin rind of grayish-white tissue that represents a metastasis from a peritoneal mesothelioma.

Histologic Features and Classification of Pleural Mesothelioma

Mesotheliomas show a wide variety of histologic patterns and can resemble many other types of malignant neoplasms.[38,495–503] The application of immunohistochemistry and electron microscopy to percutaneous and open pleural biopsy-obtained tumor specimens or neoplastic cells in pleural fluid is often necessary to render a diagnosis of mesothelioma versus some other type of neoplasm.

The simplest histologic classification of mesothelioma encompasses three general categories: epithelial (epithelioid) mesothelioma, sarcomatoid (fibrous, sarcomatous) mesothelioma, and biphasic (mixed epithelial-sarcomatoid) mesothelioma. Desmoplastic mesothelioma, a form of sarcomatoid mesothelioma, is sometimes put into a separate subtype because it has such a unique morphology. A more detailed, expanded classification includes epithelial mesothelioma, sarcomatoid mesothelioma, biphasic mesothelioma, transitional mesothelioma, and pleomorphic mesothelioma. Within each of these categories, especially that of epithelial mesothelioma, there are additional histologic variants. Some of the more recognizable variants are listed in Box 43.8 and are discussed separately below.

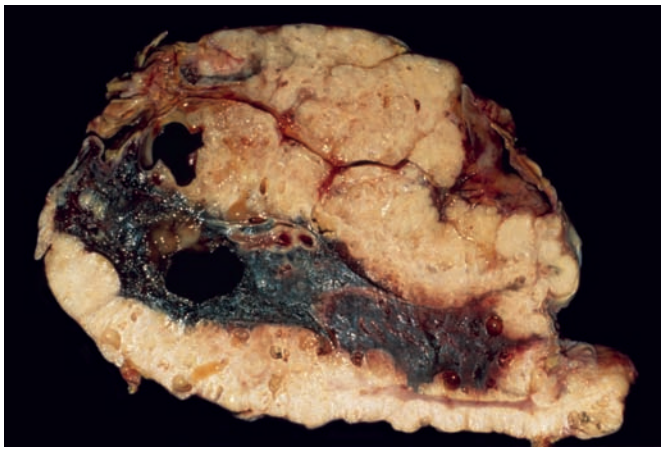


FIGURE 43.17. Some epithelial mesotheliomas produce excess amounts of hyaluronic acid/proteoglycan, producing cysts filled with this material.

Epithelial Mesothelioma

Epithelial mesotheliomas are the most frequently diagnosed histologic type of mesothelioma and show a wide variation in histologic patterns (Box 43.8). It is not uncommon to see more than one histologic pattern (subtype) of epithelial mesothelioma in any given mesothelioma. The more tissue one has to evaluate, the more likely one will see additional subtypes or a biphasic pattern.

The tubulopapillary pattern is the most common epithelial subtype, being composed of relatively uniform cuboidal to rectangular cells with centrally located round nuclei that form distinct papillary structures containing a fibrovascular core or small tubular structures when cut in cross section (Figs. 43.19 and 43.20). They may be associated with psammomatous calcification (Fig. 43.20), which is a nonspecific histologic feature and can be seen in any papillary neoplasm. Occasionally, individual tubulopapillary epithelial mesotheliomas are composed of large,

Box 43.8. Epithelial Mesothelioma (Histologic Subtypes)

Adenoid cystic
Adenomatoid
Bakery roll
Clear cell
Deciduoid
Diffuse—not otherwise specified
Gaucher-like
Glandular/acinar
Glomeruloid
Histiocytoid/epithelioid
In association with excess amounts of hyaluronic acid or proteoglycan
In situ
Macrocystic
Microcystic
Mucin positive
Placentoid
Pleomorphic
Poorly differentiated
Rhabdoid
Signet ring
Single file
Small cell
Tubulopapillary
Well-differentiated papillary

more pleomorphic, cells with large nuclei and prominent nucleoli (Fig. 43.21).

Epithelial mesotheliomas may form predominantly glandular/acinar structures that vary in size and shape and be histologically identical to adenocarcinomas

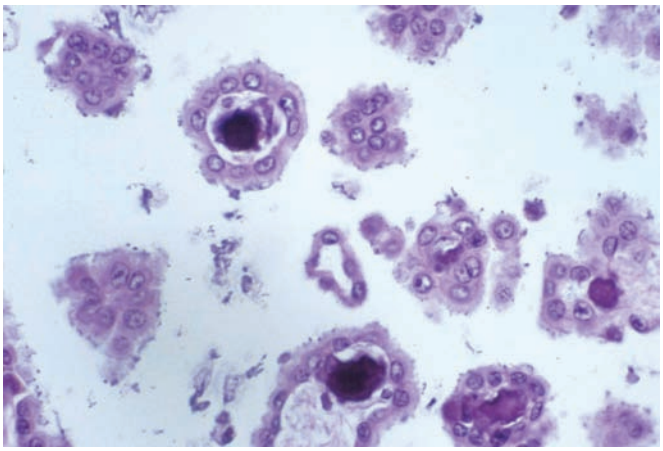


FIGURE 43.20. This tubulopapillary epithelial mesothelioma was associated with numerous psammoma bodies.

(Fig. 43.22). Sometimes the glandular/acinar epithelial mesotheliomas are composed of large columnar cells and resemble mucus-producing adenocarcinomas (Fig. 43.23).

Mesotheliomas are not infrequently composed of round histiocytoid cells that vary in size. The smaller-sized cells have an epithelioid/histiocytoid morphology resembling alveolar macrophages (Fig. 43.24). These cells have round nuclei and often large nucleoli and have abundant glassy eosinophilic cytoplasm on hematoxylin and eosin (H&E)–stained sections (Fig. 43.25). They not infrequently show periodic acid-Schiff (PAS)-positive staining that is sensitive to diastase, indicating glycogen in the cytoplasm of these cells. As we have reported,[504] round cell mesothelioma encompasses a spectrum based on cell size, the large-cell end of which is referred to as a *deciduoid mesothelioma*. Deciduoid mesotheliomas are com-

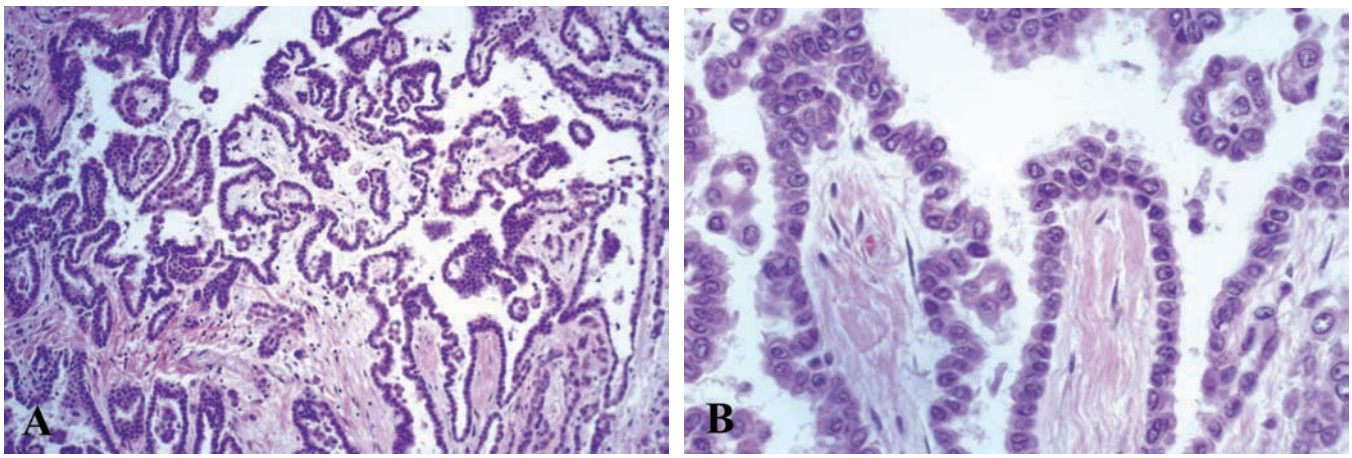


FIGURE 43.19. **(A)** This epithelial mesothelioma shows a tubulopapillary pattern. **(B)** Greater magnification showing fibrovascular cores that are covered by fairly uniform cuboidal epithelial cells.

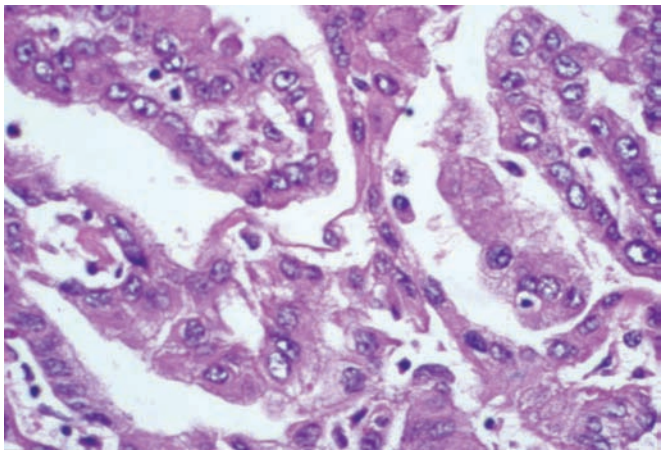


FIGURE 43.21. Some tubulopapillary epithelial mesotheliomas are composed of large cells with large nuclei and prominent nucleoli.

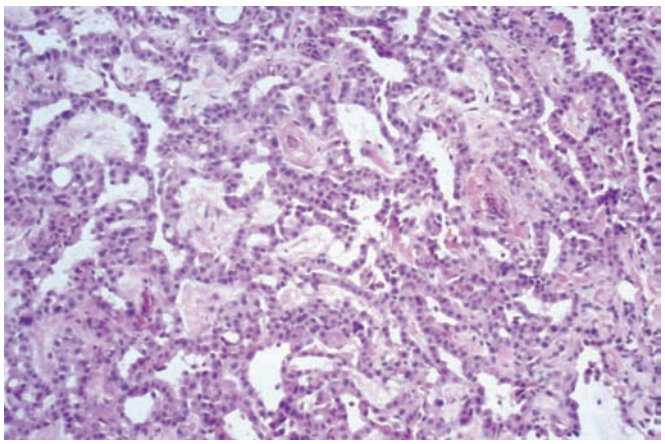


FIGURE 43.22. This epithelial mesothelioma shows a complex glandular (acinar) structure, resembling an adenocarcinoma.

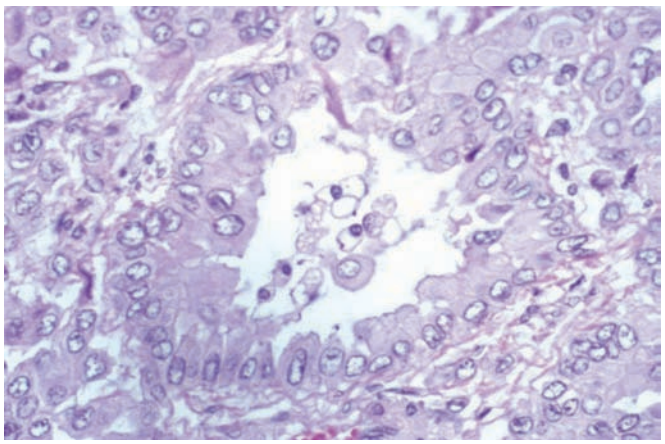


FIGURE 43.23. This epithelial mesothelioma is composed of tall cells suggestive of mucus production.

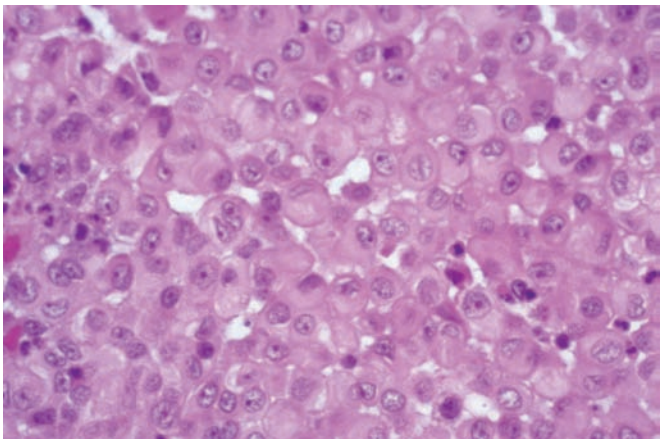


FIGURE 43.24. Diffuse sheets of uniform round tumor cells resemble histiocytes.

posed of cells that resemble progestationally stimulated endometrial stromal cells or cells seen in placental tissue (i.e., deciduoid cells). Occasionally, round cell mesotheliomas exhibit a rhabdoid morphology with the nucleus of the cell toward the cell membrane with intracytoplasmic eosinophilic inclusions that represent intermediate filaments (Fig. 43.26).

Not infrequently, epithelial mesotheliomas are composed of cystic structures ranging from an adenoid cystic morphology (Fig. 43.27) to cells organized as microcystic or macrocystic structures. The microcystic morphology appears as small cysts usually formed by somewhat attenuated squamoid-appearing cells (Fig. 43.28A). The same type of cell also forms the larger macrocystic structures (Fig. 43.28B). Some mesotheliomas are formed by cells that contain intracytoplasmic vacuoles that may impart a signet ring morphology (Fig. 43.29).

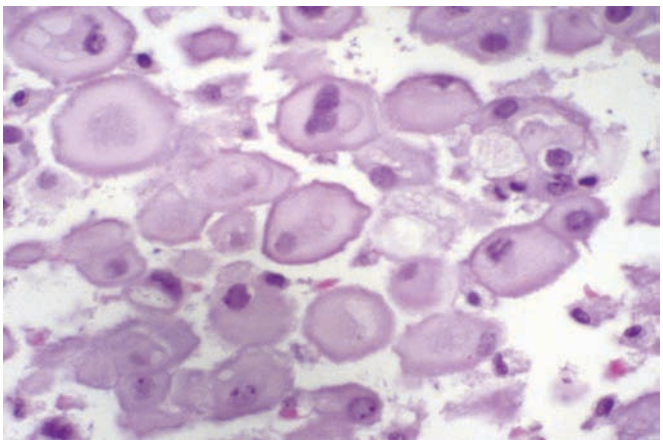


FIGURE 43.25. This mesothelioma is composed of large round cells with mostly centrally located nuclei and abundant, glossy eosinophilic cytoplasm. This is referred to as a *deciduoid mesothelioma* because of its resemblance to decidualized endometrial stromal cells.

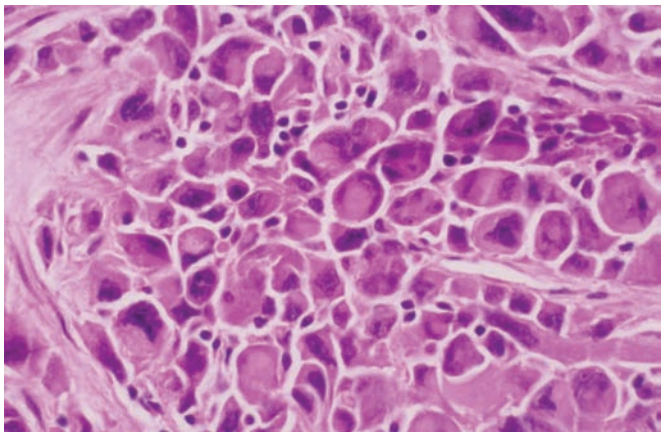
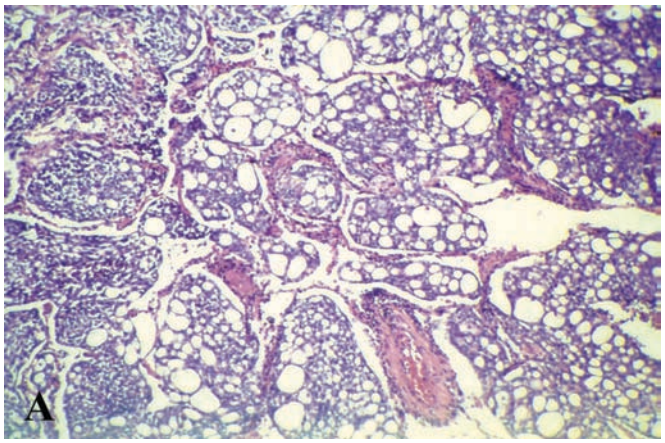


FIGURE 43.26. Some round cell mesotheliomas are composed of cells exhibiting a rhabdoid morphology with nuclei at the edge of the cell in association with nodular-appearing eosinophilic cytoplasm.



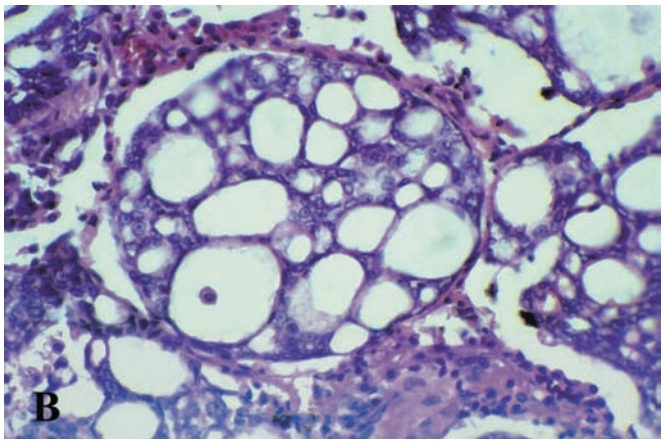
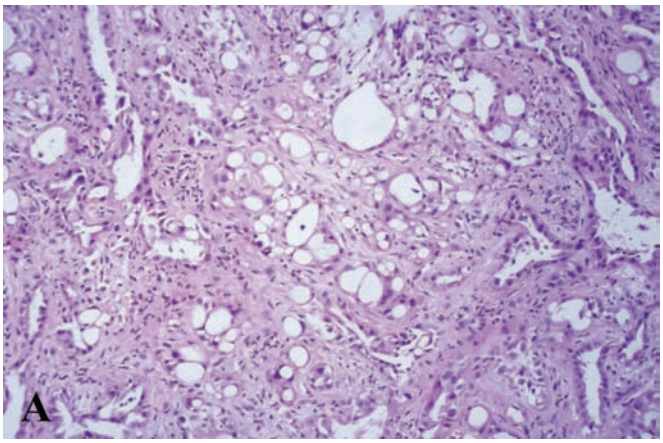


FIGURE 43.27. **(A,B)** Some epithelial mesotheliomas produce an adenoid-cystic pattern resembling adenoid-cystic carcinoma.



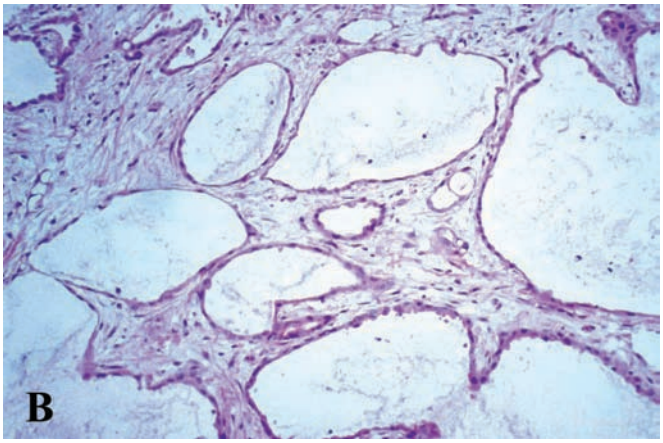


FIGURE 43.28. Epithelial mesotheliomas exhibit a wide-range of cystic patterns. **(A)** In this example, the mesothelioma is composed of relatively small cystic structures formed by flattened, somewhat squamoid cells and by cuboidal cells. **(B)** This epithelial mesothelioma is composed of flattened cells that form larger cystic structures. These cysts often contain a basophilic material in them that represents hyaluronic acid or proteoglycans.

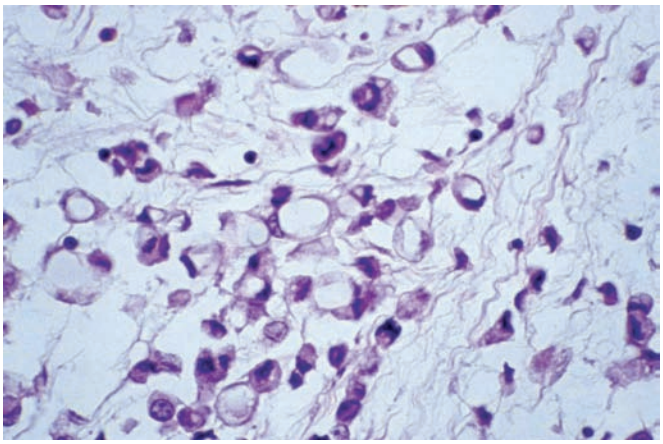


FIGURE 43.29. Occasional mesotheliomas are formed by cells that contain intracytoplasmic vacuoles. Some cells have a signet-ring morphology.

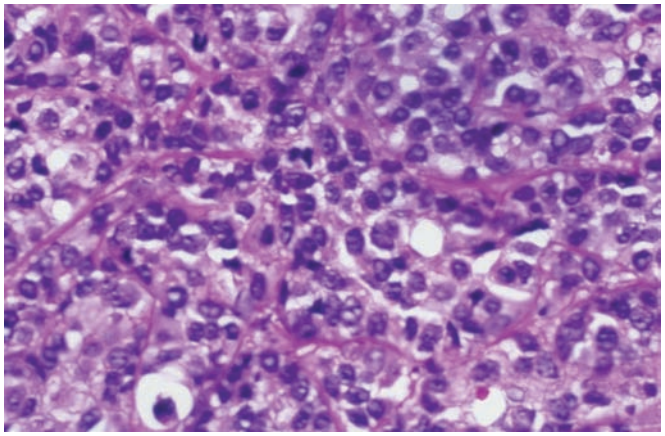


FIGURE 43.30. An uncommon epithelial mesothelioma is composed of small cells that resemble cells of neuroendocrine carcinomas.

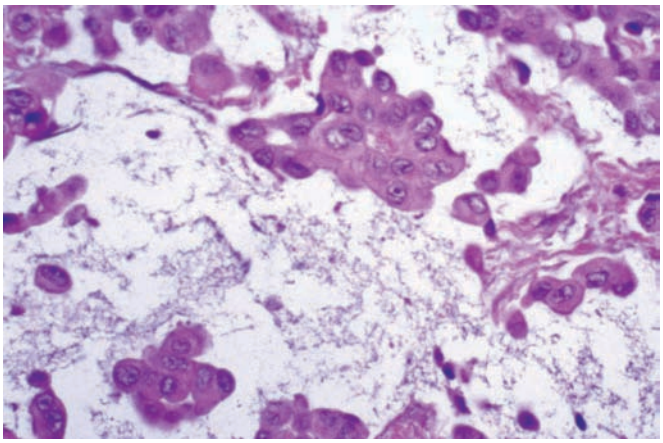


FIGURE 43.32. This epithelial mesothelioma produced large excess amounts of hyaluronic acid/proteoglycan that separates the neoplastic cells. Granular gray material surrounds the tumor cells.

An uncommon type of epithelial mesothelioma referred to as a small cell mesothelioma closely resembles small cell neuroendocrine lung cancers. These mesotheliomas are usually arranged in diffuse solid sheets of small cells (Fig. 43.30) and are discussed in detail below (see Rare/Unusual Mesotheliomas or Mesothelial Proliferations). A probable subtype of small cell mesothelioma is what we describe as glomeruloid mesothelioma, in which the small cells are arranged into structures that resemble renal glomeruli (Fig. 43.31).

Approximately 10% to 20% of epithelial mesotheliomas produce excess amounts of hyaluronic acid or proteoglycan (Figs. 43.32 and 43.33) that can be identified with an Alcian blue or colloidal iron stain. Pretreatment of the tissue sections with hyaluronidase usually decreases the intensity of, but often does not completely eliminate, the colloidal iron and Alcian blue staining (Fig. 43.33). Hyaluronic acid frequently crystallizes, which is best seen ultrastructurally (see Ultrastructural Features of Mesotheliomas, below). Histologically, this material is grayish-blue and sometimes forms distinct crystalloid structures (Fig. 43.34).

In contrast to epithelial mesotheliomas that produce hyaluronic acid and proteoglycan, pulmonary adenocarcinomas contain intracellular mucosubstances that usually stain with a neutral mucosubstance stain such as PAS-diastase stain or with a slightly acidic mucosubstance stain such as Mayer's mucicarmine. We found that pulmonary adenocarcinomas that stain positive with PAS-diastase and mucicarmine also stain intensely positive with an Alcian blue or colloidal iron stain.[505]

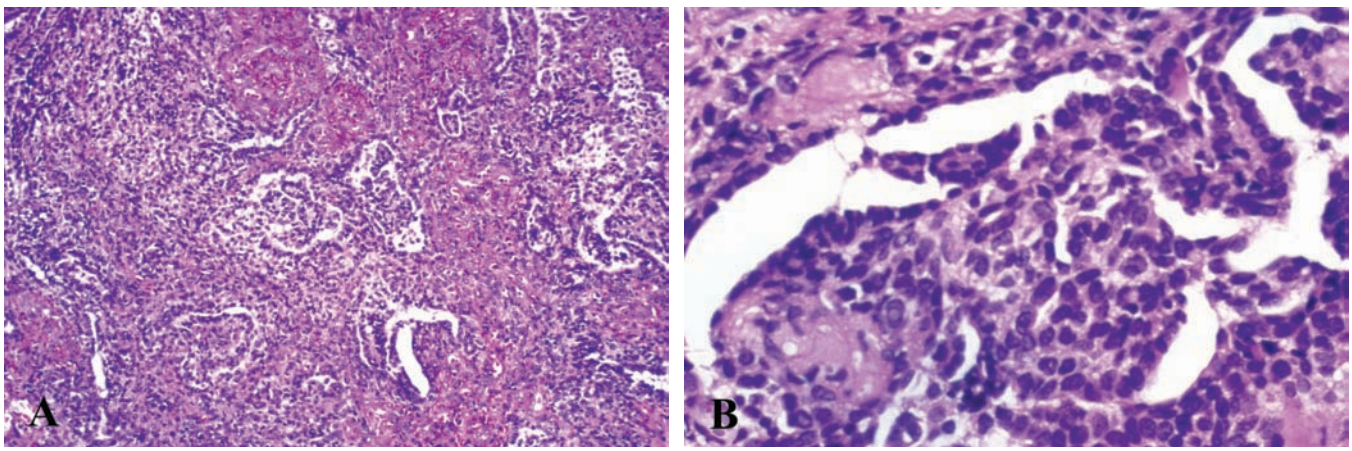


FIGURE 43.31. **(A,B)** A variant of small cell mesothelioma is composed of small cells that produce structures that resemble glomeruli.

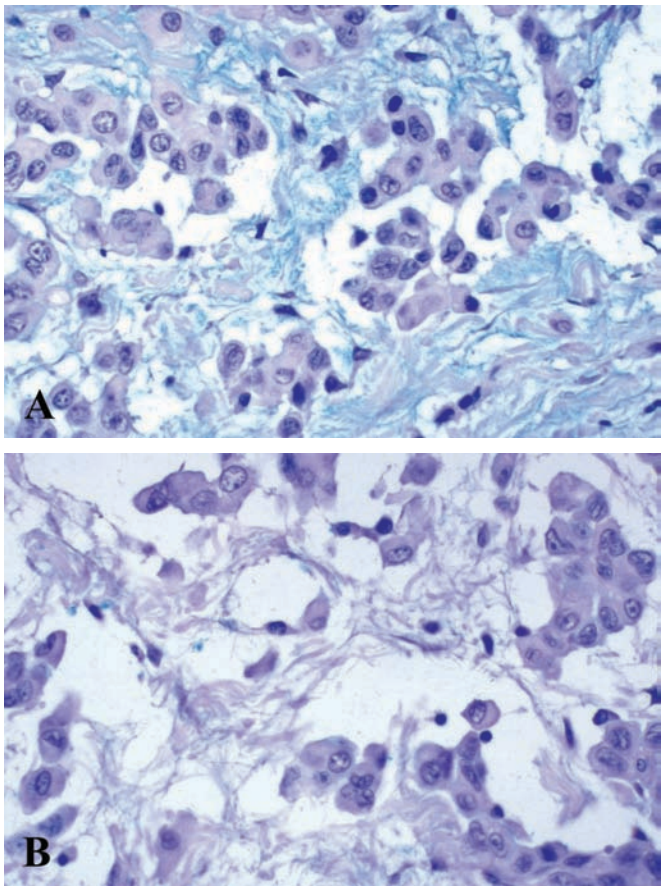


FIGURE 43.33. **(A)** Alcian blue–stained section shows intense bluish staining of the hyaluronic acid/proteoglycan. **(B)** When pretreated with hyaluronidase, the Alcian blue staining material is decreased in intensity or totally abolished.

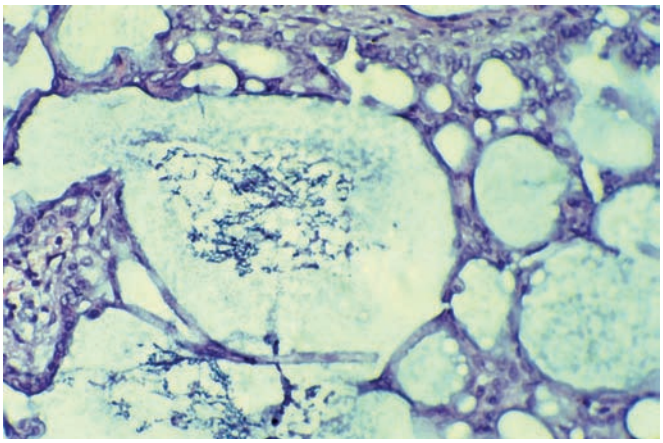


FIGURE 43.34. Crystallized proteoglycan in the cystic structures of an epithelial mesothelioma.

Some mesotheliomas are composed of relatively small, uniform cells that infiltrate in a single file arrangement and resemble lobular breast carcinomas. This type of pattern can be extensive (Fig. 43.35). Rare epithelial mesotheliomas are composed of relatively uniform cells that form concentric rolls (bakery roll pattern) (Fig. 43.36) or resemble chorionic villi (placentoid mesotheliomas) (Fig. 43.37). Rarely focal areas of squamous differentiation (Fig. 43.38) occur, which perhaps is not surprising given that reactive nonneoplastic mesothelial cells show squamous metaplasia. Some epithelial mesotheliomas are composed of cells that have clear cytoplasm (clear cell mesotheliomas) (Fig. 43.39). This clearing is usually caused by glycogen, but has been reported by Ordóñez

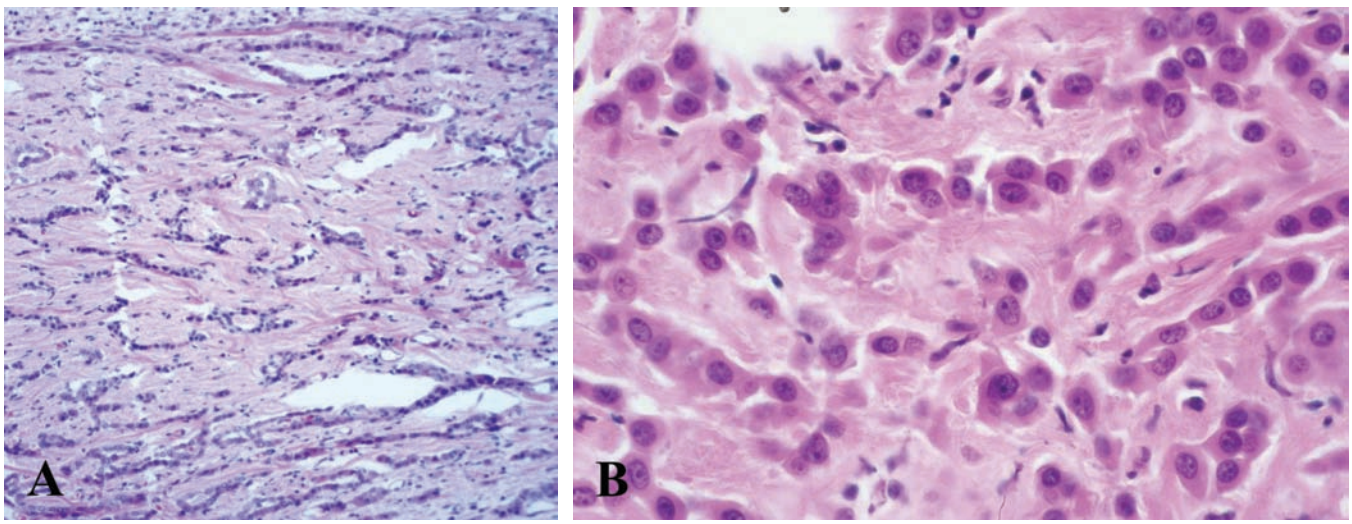


FIGURE 43.35. **(A,B)** Some epithelial mesotheliomas are composed of cells that infiltrate stroma in a single file arrangement reminiscent of infiltrating lobular carcinoma of breast.

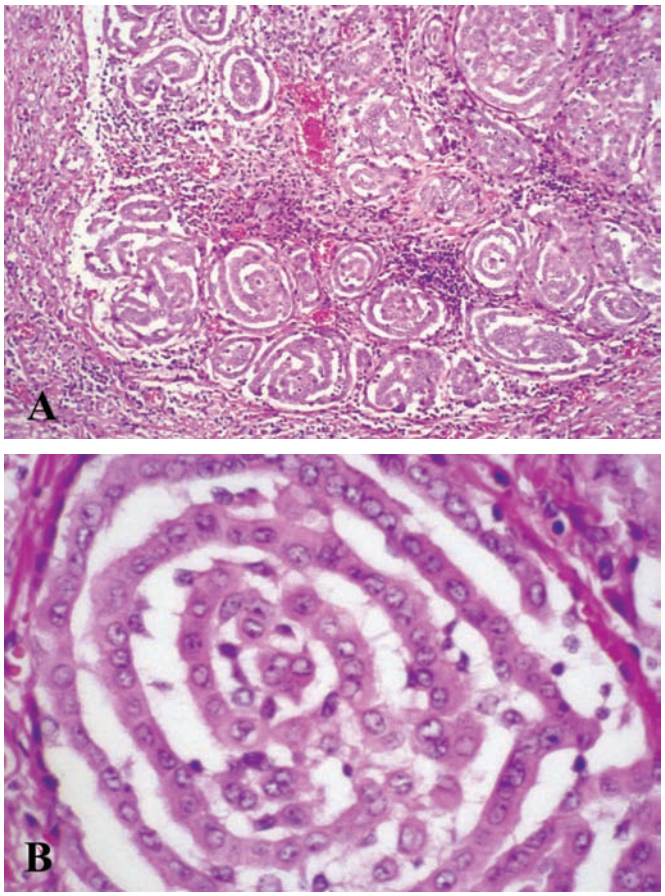


FIGURE 43.36. **(A,B)** Epithelial mesothelioma composed of uniform cells may arrange themselves in a circular pattern resembling a bakery roll.

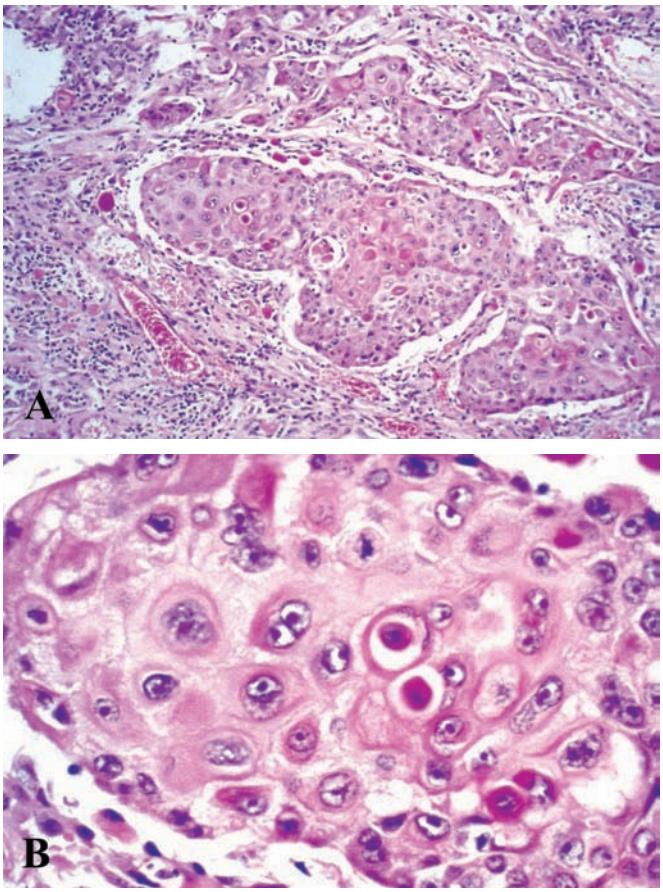


FIGURE 43.38. **(A,B)** This epithelial mesothelioma shows focal squamous differentiation. Finding squamous epithelium does not necessarily indicate metastatic squamous carcinoma.

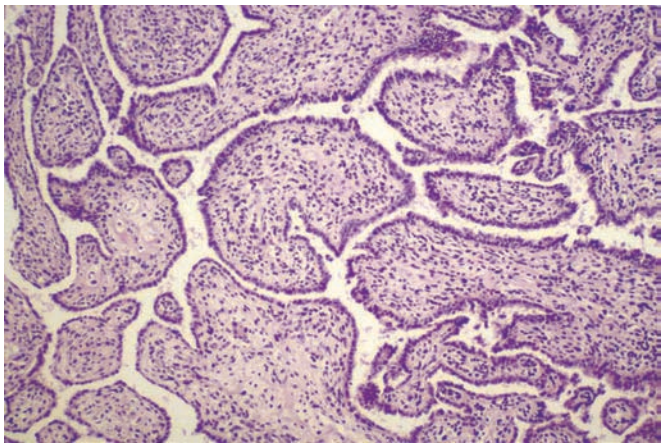


FIGURE 43.37. Occasional epithelial mesotheliomas are composed of cells that form structures that resemble chorionic villi.

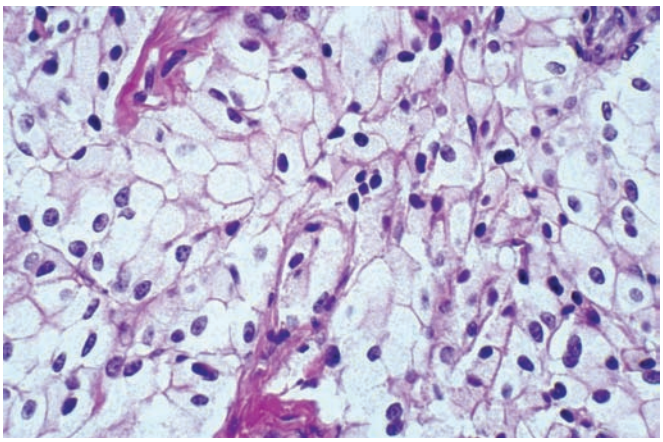


FIGURE 43.39. Some epithelial mesotheliomas are composed of cells that have clear cytoplasm usually due to glycogen accumulation. These may resemble metastatic clear cell carcinoma of the kidney.

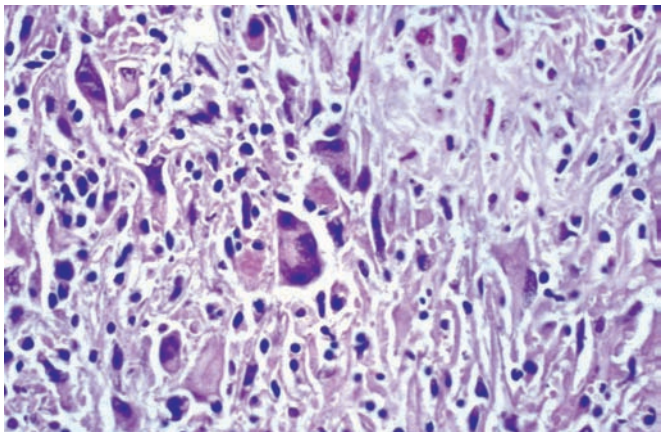


FIGURE 43.40. This mesothelioma is composed of poorly differentiated epithelial and spindle cells.

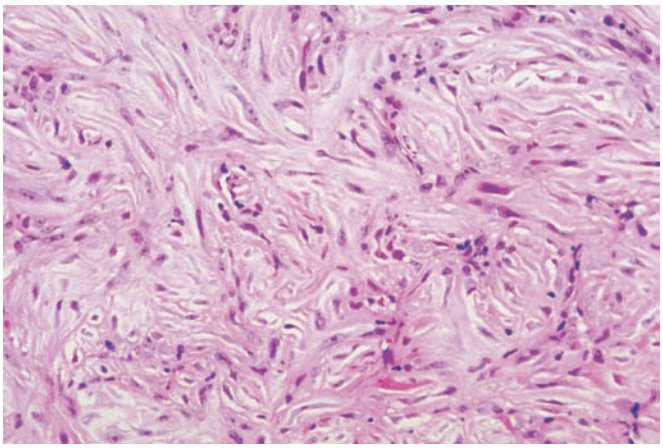


FIGURE 43.42. Fibrocollagenous tumor tissue in a pleural sarcomatoid mesothelioma.

et al.[506] as being due to large numbers of cytoplasmic vesicles, the source of which is unknown. Finally, some mesotheliomas are composed of solid sheets of epithelioid cells that are poorly differentiated (Figs. 43.40 and 43.41). These can be difficult to prove as having a mesothelial origin since they may not express immunohistochemical mesothelial markers other than broad-spectrum keratin and vimentin.

Sarcomatoid Mesothelioma of the Pleura

Pleural sarcomatoid MMs, as defined by either complete absence of epithelial tissue in an adequate biopsy or less than 10% of epithelial tissue,[37] represent about 10% of pleural MMs, within a reported range of about 7% to 22%.[37,211,503,507] The usual histologic pattern of sarcomatoid MM resembles that of a soft tissue fibrosarcoma or malignant fibrous histiocytoma (MFH).[211] Some tumors may be extremely pleomorphic,[503] whereas others are deceptively "bland" in appearance, posing difficulty in the distinction from benign fibrous pleuritis (Figs. 43.42 and 43.43). Other histologic patterns characteristic of sarcomatoid MM include leiomyoid differentiation[508,509] (resembling leiomyosarcoma), and chondrosarcomatoid and osteosarcomatoid differentiation on rare occasions.[38,211,503] Patterns resembling neurogenic sarcoma and rhabdomyosarcoma have also been described,[211] as has a focal hemangiopericytic architecture (which requires distinction from a localized fibrous tumor of the pleura and from a pleural synovial sarcoma; see later discussion). In

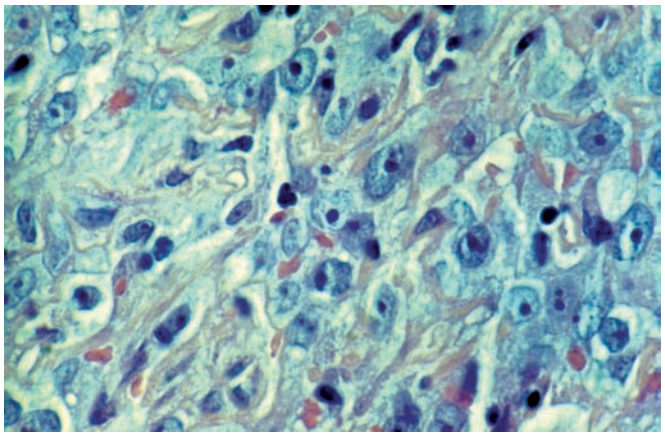


FIGURE 43.41. This mesothelioma is composed of plump, somewhat spindle-shaped cells with large nuclei and prominent nucleoli. (Alcian blue stain.)

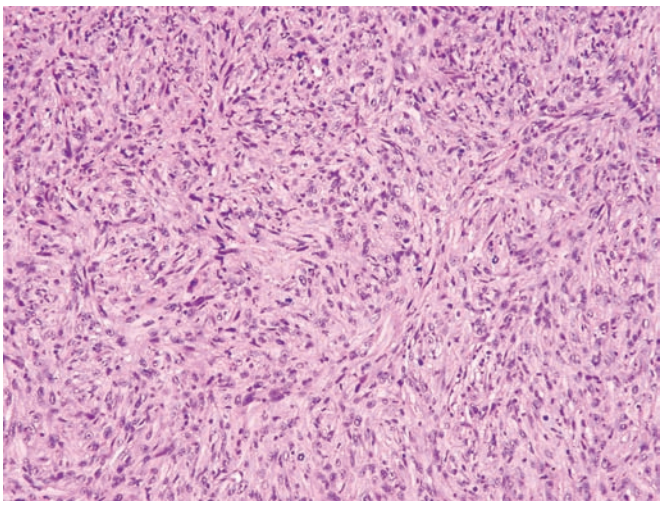


FIGURE 43.43. Pleural sarcomatoid mesothelioma. The neoplastic tissue has a focal storiform architecture and the overall appearances resemble those of malignant fibrous histiocytoma.

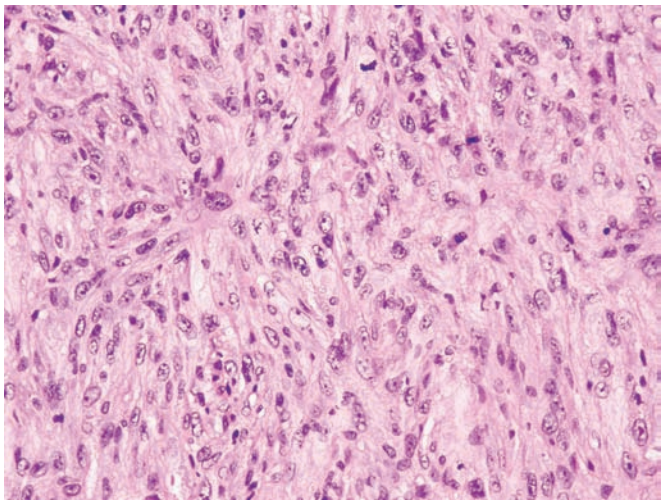


FIGURE 43.44. Pleural sarcomatoid mesothelioma. (Same case as in Fig. 43.43.) The storiform architecture of the tumor is shown at higher magnification. Mitotic figures are also evident.

the Australian Mesothelioma Surveillance Program, an MFH-like appearance was the most common histologic pattern, and cytokeratin expression by the tumor cells was usually detectable (Figs. 43.44 and 43.45).

The immunohistochemical repertoire of sarcomatoid MMs is usually more restricted than epithelial MMs, and immunohistochemistry is less decisive in diagnosis.[507] It is unusual for the positive markers of mesothelial differentiation—useful for the diagnosis of epithelial and biphasic MMs—to be expressed. In this regard, the most valuable and common pattern of antigen expression is that of strong cytokeratin expression (which also aids in the important assessment of invasion),[119,507] but cytokeratin-negative sarcomatoid MMs are well described.[37,211,503,510] Attanoos et al.[511] identified calretinin expression in only 39% of a series of 31 sarcomatoid MMs (usually focal and patchy in distribution in our experience), cytokeratin (CK) 5/6 expression in only 29%, and pan-CK expression in about 75%. Nonetheless, the combination of calretinin and CK expression was highly specific for mesothelioma in their series of 31 cases, and was not found in nonmesothelial sarcomas.

Hinterberger et al.[512] performed a tissue microarray-based analysis for calretinin and podoplanin (D2-40 antigen) expression in 341 MMs (112 epithelioid MMs, 46 sarcomatoid MMs, and 183 biphasic tumors): 91% of the epithelial MMs showed calretinin expression, as opposed to 57% of sarcomatoid tumor areas; for D2-40, the figures were 66% and 30%, respectively. The combination of calretinin and D2-40 increased the sensitivity in epithelioid areas to 0.96, and to 0.66 in sarcomatoid areas.[512]

We encounter numerous referred cases of sarcomatoid mesotheliomas where the diagnosis has been considered doubtful because of failure to demonstrate expression of calretinin, CK5/6 or with Hector Battifora Mesothelial Epitope (HBME-1), whereas this is far from unusual with sarcomatoid MMs. It is also worth emphasizing that CK expression in some sarcomatoid mesotheliomas is patchy in distribution, with areas of intense CK expression interrupted by extensive regions where CK expression is undetectable. The confidence index for a diagnosis of sarcomatoid MM is roughly proportional to the size of the biopsy and is least when the biopsy is small (for example, a core biopsy).

It is also worth emphasizing that considerable and sometimes high-grade cytologic atypia can be found superficially in some cases of benign fibrous pleuritis, presumably representing reactive atypical myofibroblasts. In some cases this pattern of atypia poses considerable diagnostic difficulties, but in our experience such cytologic atypia restricted to the superficial (subsurface) zone of pleural fibrous lesions is of little or no significance for a diagnosis of MM.[503,513] On the other hand, in our experience the most cellular and atypical areas of sarcomatoid MMs are usually found at the deep advancing margin of the tumor, as opposed to the superficial zone (in other words, a reversal of the zonal pattern found in benign fibroinflammatory disorders of the pleura).[503]

In our experience, the following criteria, not all of which need be encountered in any one case, are useful for the diagnosis of sarcomatoid mesothelioma:

- A confluent growth pattern of the tumor along the pleura, whether the lesion shows CK expression or not, although localized sarcomatoid MMs do occur. In limited biopsy specimens the anatomic distribution and

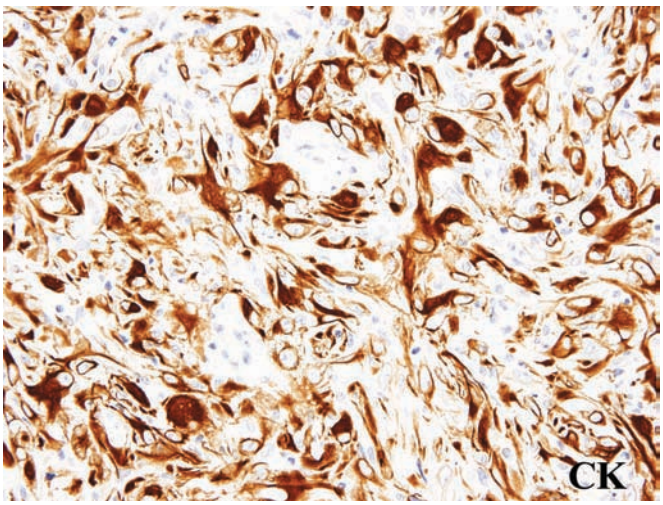


FIGURE 43.45. Pleural sarcomatoid mesothelioma. (Same case as in Figs. 43.43 and 43.44. Strong expression of low molecular weight cytokeratins (CK) by the fibroblastoid tumor cells.

localization of the lesion may not be readily apparent. In this circumstance, the findings on radiologic investigation, including computed tomography (CT) scans, can substitute as a useful surrogate for gross assessment.
- Sarcomas of extraserosal soft tissue or bone, sarcomatoid renal cell carcinoma, and amelanotic spindle cell melanoma should be excluded on clinical grounds, including organ imaging studies such as ultrasound or CT scans, or (importantly) from consideration of the past medical history of the patient in question.
- Cellularity, cytologic atypia and pleomorphism, and a mitotic index that are excessive for a benign fibrous lesion of the pleura; in other words, tissue that is overtly sarcomatoid in the context of lesions of the pleura, with exclusion of reactive serosal fibrosis (benign fibrous pleuritis), from the histologic appearances of the lesion in question, including the zonal pattern.
- Focal tumor necrosis.
- The presence of invasion[119,503,513]: it is our experience that most sarcomatoid mesotheliomas show an insinuative pattern of invasion into subpleural adipose tissue (and occasionally deeper structures), whereby the spindle-shaped cells insinuate between individual adipocytes, splaying them apart and incorporating them into the advancing margin of the tumor (see discussion of desmoplastic sarcomatoid mesothelioma of the pleura).
- In the case of localized tumors in particular, a malignant solitary fibrous tumor (SFT) requires exclusion, from the gross morphology of the lesion, or by immunohistochemical studies for CKs, CD34, bcl-2, and CD99, but on rare occasions it may be impossible to distinguish between a localized sarcomatoid MM and SFT because of discordant immunohistochemical staining (see later discussion).
- Usually, intense CK expression by the tumor, as revealed by immunostaining using either a pan-CK cocktail such as AE1/AE3 or on staining for low molecular weight CKs, which also aids in the assessment of invasion,[38,119,507,514–516], but a diagnosis of sarcomatoid MM remains tenable in the absence of detectable CK expression, provided that the other criteria are fulfilled.

Electron microscopy is of limited usefulness for the diagnosis of sarcomatoid MM in our experience. Although occasional cases show evidence of mesothelial differentiation in the form of serpentine microvilli, desmosomal intercellular junctions, or tonofibrils, many other cases comprise only fibroblastoid or myofibroblastoid cells without differentiating features.[211]

Heterologous Differentiation in Sarcomatoid Malignant Mesotheliomas

The distinction between pleural sarcomatoid MM with osseous differentiation and osteogenic sarcoma arising in relation to the rib or chest wall soft tissues (extraosseous osteosarcoma) can pose difficulties (Fig. 43.46). Analogous considerations apply to chondroid tumors.

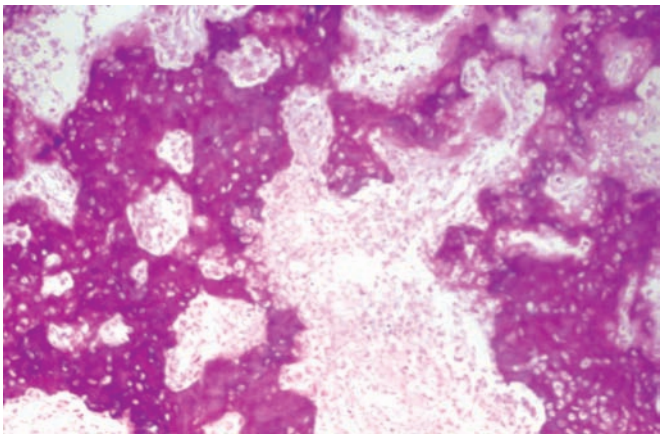


FIGURE 43.46. This mesothelioma showing variable differentiation shows fairly extensive bone formation in the sarcomatoid portion of the mesothelioma.

As mentioned previously, strong expression of CKs by a pleura-based sarcomatoid tumor is a strong indicator of MM. However, CK expression may be depleted in areas of heterologous differentiation, and in this regard the growth pattern of the tumor within the pleura is (again) of considerable value in the differential diagnosis. Confluent pleura-based heterologous sarcomatoid tumors in adults, whether liposarcomatous, chondrosarcomatoid, or osteosarcoma-like,[503] which is radiologically indistinguishable from MM, in our opinion should be designated as pleural MMs, whereas heterologous sarcomatoid tumors arising in relation to chest wall tissue are characteristically localized, without confluence along the pleura itself.

On the other hand, given the distinctive status of epithelioid hemangioendothelioma of the pleura (see later discussion) and pleural angiosarcoma of conventional type,[517] we would not designate those latter two tumors as MMs, because (1) unlike mesotheliomas, angiosarcomas affect the pericardium predominantly, although an origin from other serosal membranes is recorded; (2) serosal involvement as part of an angiosarcoma may constitute part of angiosarcoma of the heart with intramyocardial or intracavitary components, or a multifocal angiosarcoma affecting multiple sites such as the skin, deep soft tissues, liver, and spleen; and (3) so far as we are aware, conventional (nonepithelioid and vasoformative) endothelial differentiation is not part of the documented histologic repertoire of a biphasic or sarcomatoid mesothelioma. (In this context, it is worth recalling that some authors[518,519] use the term *angiosarcoma* interchangeably with *epithelioid hemangioendothelioma* for epithelioid endothelial sarcomas of the pleura.)

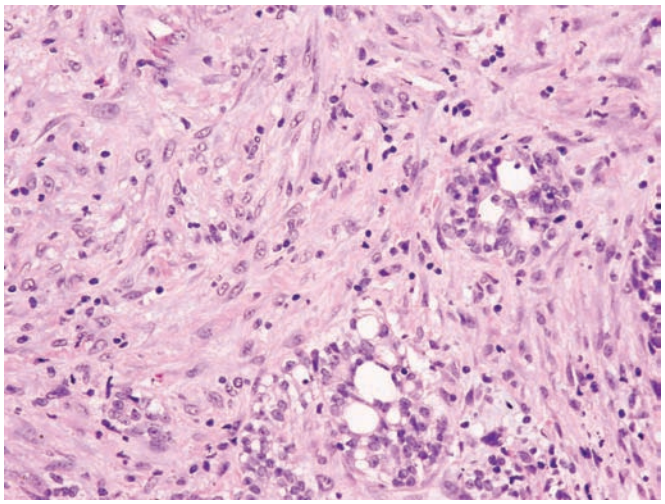


FIGURE 43.47. Biphasic pleural malignant mesothelioma. The epithelial component is represented by circumscribed aggregates of epithelioid cells with rudimentary tubuloacinar structures, seen in the right half of the field illustrated. The spindle-cell stromal tissue shows about the minimal cellularity and atypia required for designation of the stromal component as sarcomatoid (as opposed to a cellular reactive stroma in an epithelial mesothelioma). Note the pleomorphism of some of the stromal cells in the upper left and lower left areas of this field. Compare with Figs. 43.42 to 43.44 in the sarcomatoid section.

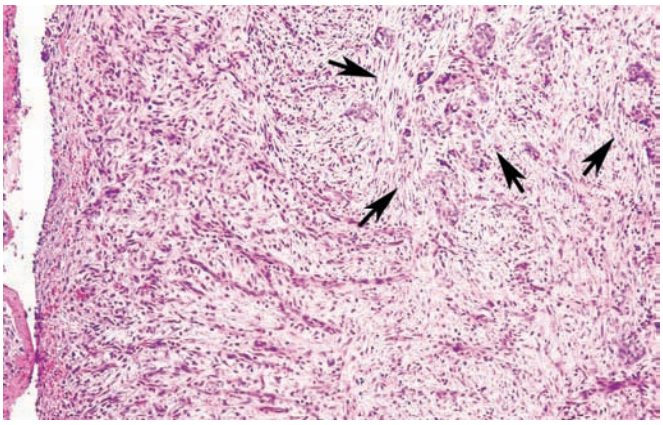


FIGURE 43.48. As opposed to a stromal sarcomatoid component required for diagnosis of a biphasic mesothelioma, this figure depicts an invasive epithelial mesothelioma, with small rounded aggregates of epithelial cells (arrows), surrounded by a prominent reactive stroma. The reactive character of the stroma is indicated by the small parallel blood vessels, orientated almost perpendicular to the free surface of the pleura, and the greater cellularity in the subsurface zone as opposed to the deeper tissues comprising the reactive stroma (a "top heavy" zonal architecture characteristic of a pleural inflammatory process).

Biphasic Malignant Mesothelioma

A mixed (biphasic) epithelial and mesenchymal architecture is perhaps the most distinctive histologic picture encountered with MMs[516]: about 30% of MMs[37,211] within a reported range of 24% to 35%,[37,211,503] But it is worth emphasizing that a mixed histologic pattern can also be encountered, with nonmesothelial tumors affecting the pleural cavities, most notably primary synovial sarcoma of the pleura and secondary spread from a spindle cell carcinoma (carcinosarcoma) of lung, as well as biphasic pulmonary blastoma. Subclassification of MM as biphasic requires that unequivocal epithelial and mesenchymal elements are identifiable, and that each shows malignant features in conventional H&E-stained sections (Fig. 43.47), thereby excluding (1) cellular but not obviously malignant stromal tissue in an epithelial MM (Fig. 43.48); and (2) incorporation of benign alveolar epithelium into a sarcomatoid mesothelioma as it invades into lung parenchyma (staining for thyroid transcription factor-1 [TTF-1] is invaluable in this situation but requires critical evaluation of the histologic distribution of TTF-1–positive epithelial cells, to ensure that a biphasic or sarcomatoid MM is not misdiagnosed as a spindle cell carcinoma of lung).

The appearances of the epithelial component by light microscopy, immunohistochemistry, and electron microscopy are essentially indistinguishable from those of entirely epithelial MMs, and the same considerations apply to the appearances of the sarcomatoid component,[516] which usually resembles either fibrosarcoma or MFH, with heterologous patterns of differentiation on rare occasions (Figs. 43.49 and 43.50). Nonetheless,

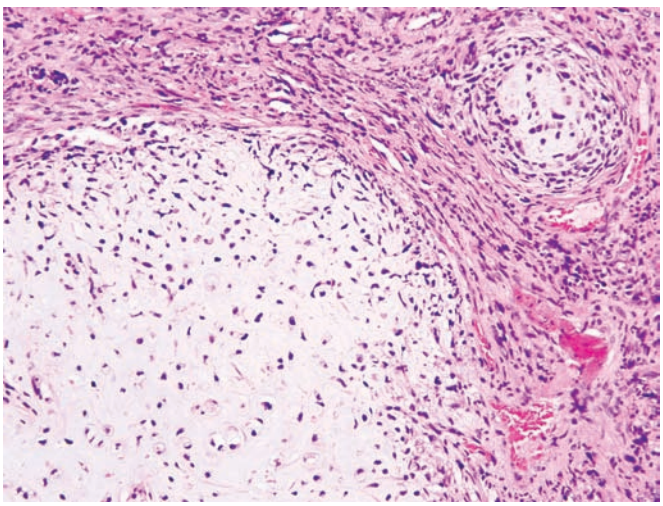


FIGURE 43.49. Heterologous chondroid differentiation in the stromal tissue of a biphasic mesothelioma.

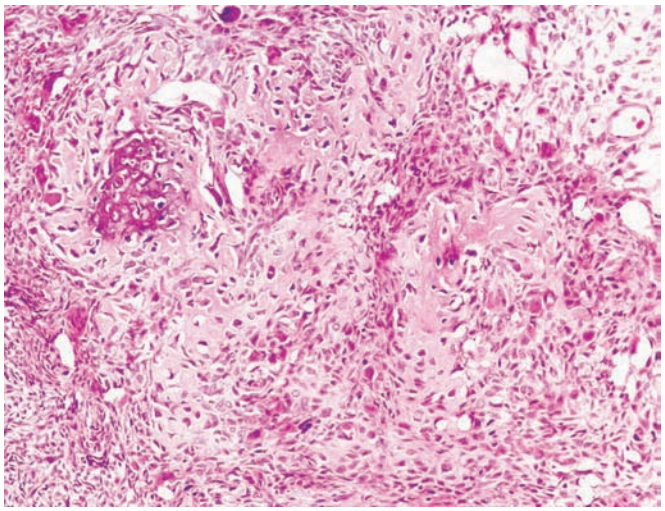


FIGURE 43.50. Heterologous osteoid and bone in the stromal tissue of a biphasic malignant mesothelioma.

the relative proportions of each component in a biphasic MM are highly variable, as are the distribution and the appearances of each component, from one case to another. The tumor may show an intermingling of each of the epithelial and sarcomatoid components, but in other cases and even in different areas of the same tissue sample, the two components may be reasonably discrete (Fig. 43.47), with an abrupt transition from one component to the other.[211,516] As for epithelial and sarcomatoid mesotheliomas, respectively, there may also be considerable histologic variation within each component in a single case, so that tubulopapillary areas and sheets of pleomorphic cells may be encountered within the epithelial component, whereas the sarcomatoid component may vary from cellular and pleomorphic—resembling either fibrosarcoma or MFH—to hypocellular desmoplastic tissue. Heterologous patterns of differentiation within the sarcomatoid tissue include chondroid and osseous differentiation (Figs. 43.49 and 43.50),[211,520] and focal rhabdomyoblastic differentiation was encountered in one biphasic MM in the Australian Mesothelioma Surveillance Program.[211]

At present, the International Mesothelioma Panel recommends arbitrarily that at least 10% of either component should be recognizable in biopsy tissue for MMs to be classified as biphasic. This being so, the proportion of cases classified as biphasic MMs will be dependent in part on the amount of tissue sampled by the biopsy.[516] With limited (for example, core) biopsies, provisional histologic classification of the MM may be modified subsequently by more adequate biopsy tissue or in surgical specimens, or at autopsy.

Distinction of biphasic MM from a biphasic synovial sarcoma (SSa)[521–526] can pose considerable difficulties, especially when the tumor is widely distributed within the pleural cavity (but see Localized Malignant Mesothelioma, below), because there is overlap in the immunoprofile between MM and SSa, for example by way of calretinin expression.[527] Distinguishing features that favor a diagnosis of pleural biphasic SSa include the demonstration of epithelial-type mucosubstances (but see discussion on mucin-positive MMs[505] in Histochemical Features of Pleural Epithelial Mesotheliomas) and the presence of epithelial markers on immunohistochemistry (such as carcinoembryonic antigen [CEA], CD15, or with the antibody Ber-EP4), together with less intense and less extensive cytokeratin expression by the stromal component than is usual in biphasic and sarcomatoid MM. The histologic appearances also differ. In this regard, the bipolar spindle-shaped cells found in SSa typically have an interlacing fascicular pattern, sometimes described as a "school of fish" appearance, in contrast to the fibrosarcomatoid or MFH-like pattern of the stromal component in most biphasic MMs. Synovial sarcoma is also characterized by the t(X;18) translocation,[526,528–531] whereas biphasic mesothelioma is not.

When they spread into the pleura, spindle cell (sarcomatoid) carcinomas of lung (carcinosarcomas)[532] can also pose considerable problems in differential diagnosis, but the radiologic demonstration of an intrapulmonary mass lesion with appearances characteristic of a primary lung cancer can aid considerably in this distinction, together with expression of epithelial-type markers such as CEA, CD15, or Ber-EP4 antigen in the epithelial component of such carcinosarcomas,[532] in the absence of calretinin or CK5/6 expression. Biphasic pulmonary blastomas[533,534] are distinguishable from biphasic MM by their predominantly intrapulmonary localization (although they can spread to the pleura), by their histologic resemblance to fetal lung parenchyma (with an embryonal appearance for the stromal component, which often shows focal chondroid differentiation), and by the resemblance of the epithelial component to endometrial glands (see Figs. 37.18 and 37.19 in Chapter 37). In addition, some pulmonary blastomas show focal expression of CD117.[535]

Transitional Mesothelioma

Transitional mesothelioma refers to a histologic type of mesothelioma that has features transitional between epithelial and sarcomatoid. These were described in 1986.[536] Some mesotheliomas described by Dardick et al.[537] as poorly differentiated mesotheliomas would fit into this category. These mesotheliomas are composed of large, polygonal to plump, occasionally spindle-shaped cells arranged in nests or showing no distinct pattern (Fig. 43.51A,B). These mesotheliomas typically express broad-spectrum keratin (Fig. 43.51C) and vimentin (Fig. 43.51D). These neoplasms usually do not show

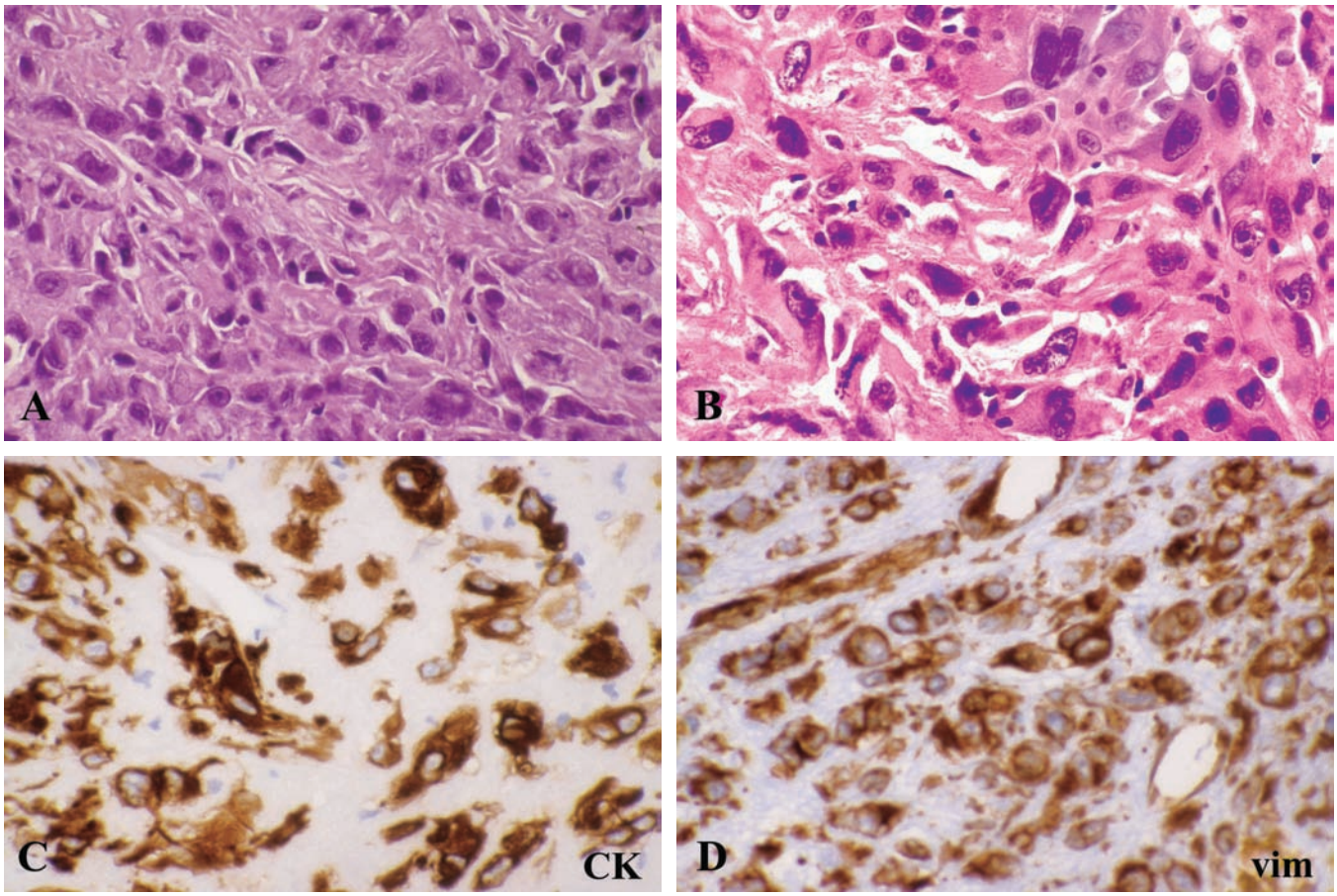


FIGURE 43.51. **(A,B)** Transitional mesotheliomas are composed of epithelioid and spindle cells as seen in these images. **(C)** Transitional mesotheliomas show intense cytoplasmic immunostaining for broad-spectrum (AE1/AE3) keratin. **(D)** As shown in this image, vimentin (vim) is typically expressed in all transitional mesotheliomas.

mesothelial-specific markers and the ultrastructural features are nonspecific.

Pleomorphic Mesothelioma

Pleomorphic mesotheliomas are composed of large, undifferentiated, irregularly shaped cells often having an epithelioid or sarcomatoid morphology. These pleomorphic mesotheliomas (Figs. 43.52 to 43.54) characteristically express broad-spectrum keratin and vimentin, and occasionally other markers of mesothelial differentiation such as CK5/6, calretinin, mesothelin, and epithelial membrane antigen (EMA).

Mesotheliomas Showing Variable Differentiation

In cases where the specimen is large, such as pleural pneumonectomy specimens or autopsy specimens, it is not uncommon to see several different histologic patterns of mesothelial differentiation (Fig. 43.55). This variation can span the entire histologic, immunohistochemical, and ultrastructural expression seen in MM.

Histochemical Features of Pleural Epithelial Mesothelioma

Several standard histochemical tests for the demonstration of carbohydrate/mucopolysaccharide substances are occasionally useful in differentiating epithelial mesotheliomas from other malignant tumors, primarily pulmonary adenocarcinomas and other mucin-producing adenocarcinomas. The two main substances to be considered are mucin and glycogen. *Mucin* is a somewhat vague term and is frequently used synonymously with mucopolysaccharide, glycoprotein, proteoglycan, glycosaminoglycan, mucosubstance, and glycoconjugate. *Glycoconjugate* is the term preferred by some[538]; we prefer *mucosubstance*. The protein portion of a glycoprotein mucosubstance is synthesized in the rough endoplasmic reticulum, and the carbohydrate portion is added in the Golgi apparatus. Mucosubstances can be divided into highly acidic, weakly acidic, or neutral mucosubstances.

Glycogen is observed in the cytoplasm of epithelial mesotheliomas in up to 50% of cases and readily stains

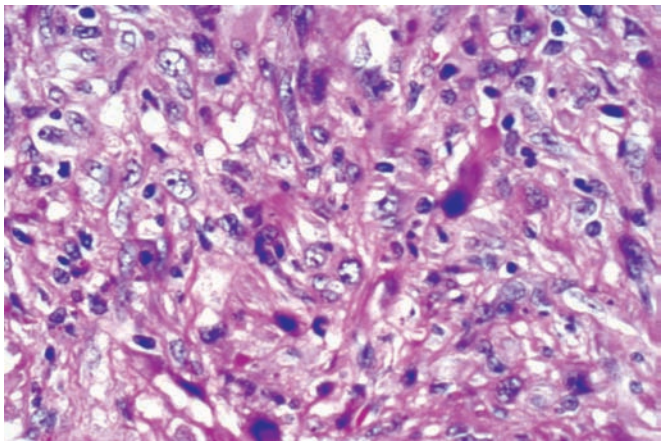


FIGURE 43.52. This pleomorphic mesothelioma is composed of large atypical epithelioid and spindle cells.

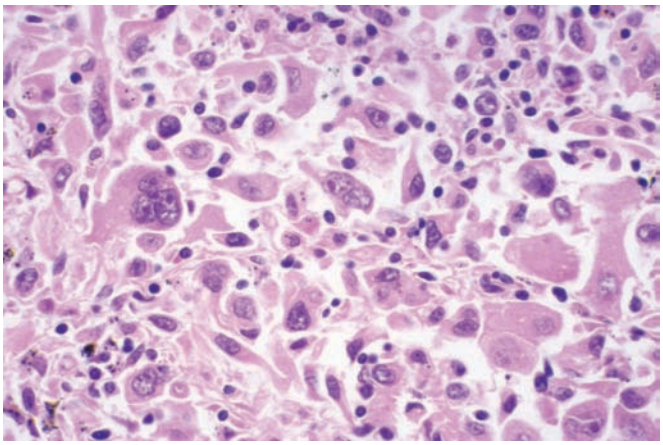


FIGURE 43.54. Most of the neoplastic cells in this pleomorphic epithelial mesothelioma are large epithelioid cells.

with PAS reagent (Fig. 43.56). The glycogen can cause mesotheliomas to have a clear cell morphology (Fig. 43.39) and is usually removed by pretreatment with diastase. This is a nonspecific finding because primary pulmonary carcinomas such as adenocarcinomas frequently contain glycogen, especially those showing degenerative changes. Many so-called clear cell carcinomas of the lung represent neoplasms whose cells contain significant amounts of glycogen, which is removed during processing and causes cytoplasmic clearing. Epithelial mesotheliomas containing significant quantities of glycogen may or may not exhibit a clear cell histologic pattern.

Approximately 20% of epithelial mesotheliomas produce highly acidic mucosubstances, namely, hyaluronic acid and proteoglycan, which can be identified with Alcian blue or colloidal iron stain (Fig. 43.33A). The bluish-staining material is seen within cytoplasmic vacuoles, tubular lumina, or surrounding aggregates of epithelial cells, but is not observed intracellularly. The Alcian blue colloidal iron staining may be removed with hyaluronidase or the intensity of the stain may be decreased (Fig. 43.33B), which is helpful to confirm that the neoplastic cells are producing an acidic mucosubstance consistent with hyaluronic acid or proteoglycan. A note of caution: stromal connective tissue surrounding nests of epithelial mesothelioma cells can be rich in hyaluronic acid and thus misinterpreted as a positive reaction.

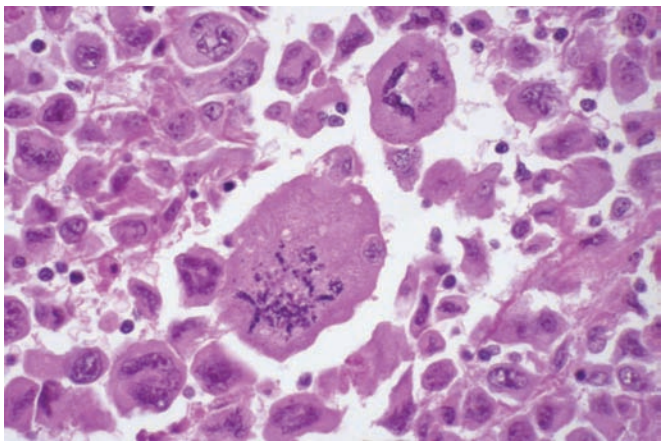


FIGURE 43.53. This epithelioid pleomorphic mesothelioma contains occasional tumor giant cells with abnormal mitoses.

Approximately 65% to 70% of pulmonary adenocarcinomas show intracytoplasmic staining for neutral or weakly acidic mucosubstance that can be identified by PAS-diastase (PAS-D; pretreatment with diastase removes glycogen) or Mayer's mucicarmine. As we reported, most pulmonary adenocarcinomas that show intracytoplasmic staining with PAS-D (Fig. 43.57) or Mayer's mucicarmine (Fig. 43.58) also show Alcian blue/colloidal iron-positive staining at pH 2.5 (Fig. 43.59).[505] The positive-staining glycoprotein material is resistant to pretreatment with hyaluronidase.

Hammar et al.[505] compared the histochemical and immunohistochemical staining reactions of 10 epithelial mesotheliomas (diagnosis documented by ultrastructural examination) that were mucicarmine-positive and compared them with 10 pulmonary adenocarcinomas. The adenocarcinomas were all primary "nodular" lung adenocarcinomas that were mucicarmine-positive. The mucicarmine and PAS-D staining reaction in epithelial mesotheliomas resulted from hyaluronic acid production by these neoplasms. When the tissue sections were pretreated with hyaluronidase, the intensity of staining reactions with mucicarmine and PAS-D usually decreased or disappeared. In some cases, specifically those that showed intracellular droplet-like staining, the staining reaction

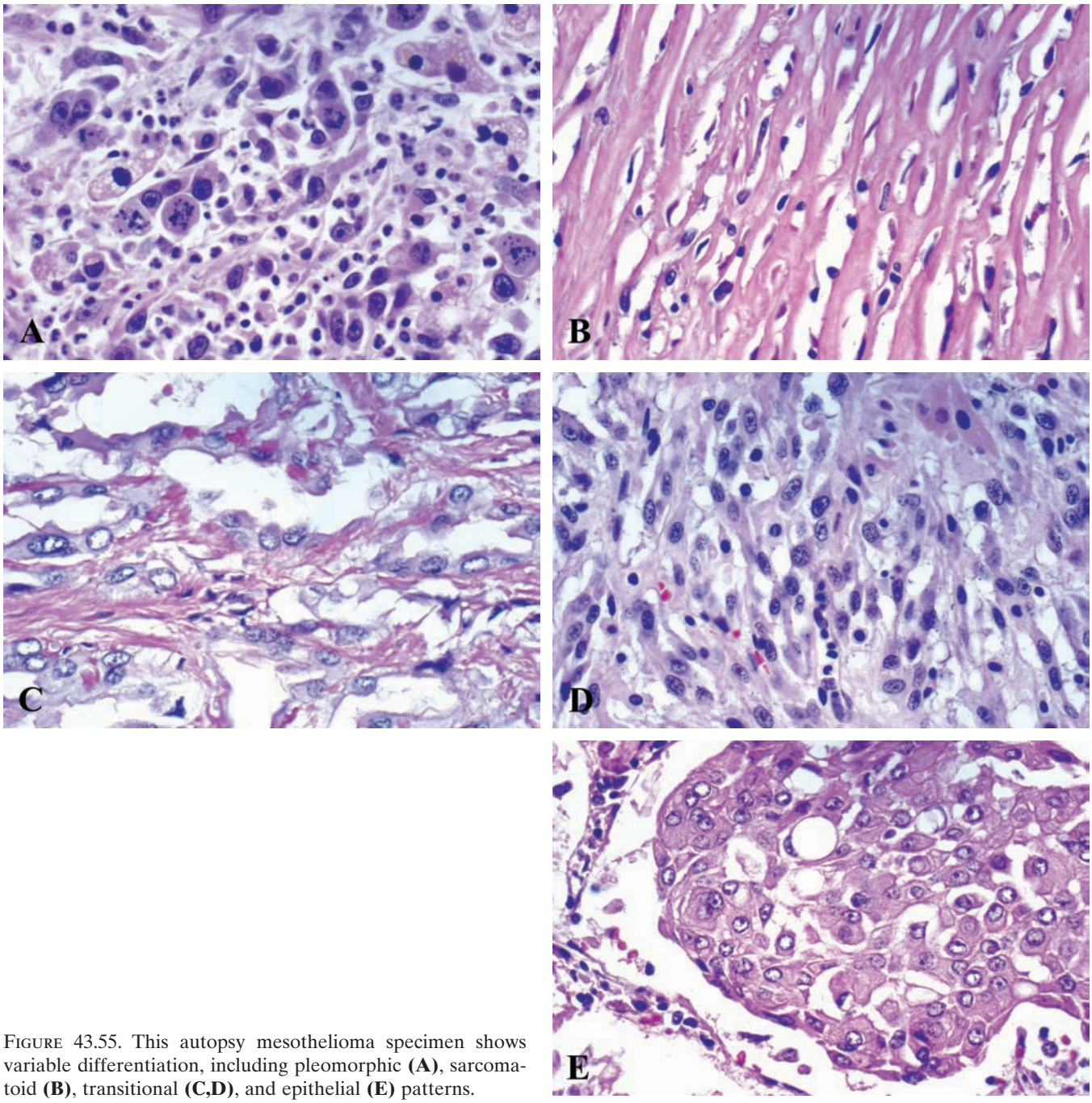


FIGURE 43.55. This autopsy mesothelioma specimen shows variable differentiation, including pleomorphic **(A)**, sarcomatoid **(B)**, transitional **(C,D)**, and epithelial **(E)** patterns.

was not eradicated. All mucin-positive epithelial mesotheliomas we have examined contained crystalloid structures that are described in the section on mucin-positive epithelial mesotheliomas.

Immunohistochemical Features of Pleural Mesothelioma

The cytologic or biopsy diagnosis of MM can be problematic and requires the use of ancillary techniques more frequently than for most other epithelioid tumors and as a routine procedure. As a historical development, supplemental special stains for mucins, including stains for neutral and acidic mucosubstances, notably hyaluronic acid before and after hyaluronidase digestion, have been supplemented in turn, and largely supplanted by, immunohistochemistry.[37] Many adenocarcinomas that commonly spread to the pleura, such as those originating in the breast, may not produce significant amounts of mucin (about 60% to 75% of adenocarcinomas of lung produce

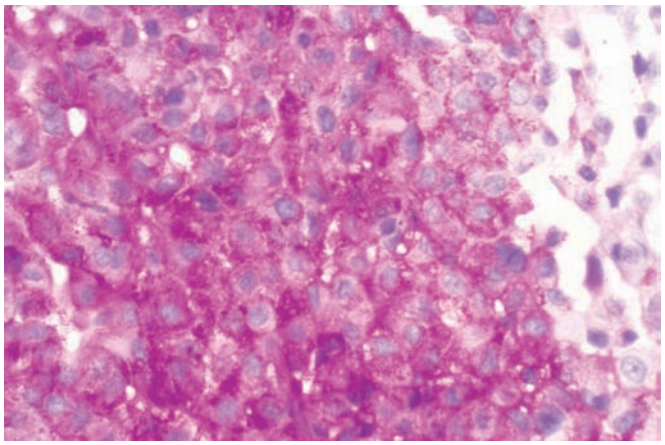


FIGURE 43.56. A significant percentage of epithelial mesotheliomas contain cytoplasmic glycogen that can be shown by a periodic acid-Schiff (PAS) stain.

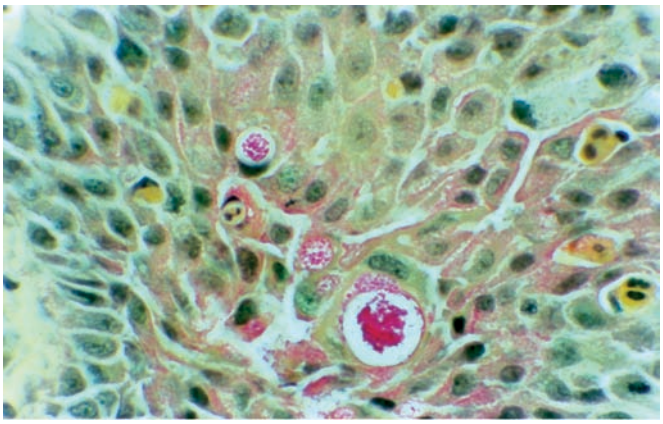


FIGURE 43.58. Intracellular mucicarmine staining is observed in this primary pulmonary adenocarcinoma.

mucin stainable by PAS-D or mucicarmine stains[227,505]). Conversely, mucin-producing mesotheliomas are well described, although rare,[505,539,540] as are PAS-D mucin-like droplets in hyperplastic mesothelial cells, resistant to hyaluronidase pretreatment.[119] Therefore, some authorities[37,541] consider mucin stains to be of limited or little value in diagnosis and to have been largely if not entirely superseded by the immunohistochemical (IHC) techniques available in almost all laboratories in industrialized nations.[542]

Lastly, electron microscopy (EM) can be used when uncertainties remain concerning the diagnosis; EM can be regarded as the "gold standard" for the diagnosis of MMs with an epithelial component,[211,543] but in everyday practice it has been largely replaced in most institutions by IHC investigation (a diminished role aggravated by the closure of many diagnostic EM units). Nonetheless, some authorities[544] argue that EM still plays a role in the independent validation of a diagnosis of mesothelioma, particularly when investigating new antibodies. We continue to find EM useful, often decisively so, including the use of deparaffinized and reprocessed biopsy tissue, when (1) the sample is small (e.g., those that are predominantly cytologic in character, including cell-block preparations); (2) the histologic appearances are atypical; or (3) there are discordant immunohistochemical findings.[213,543] In these circumstances, EM remains an extremely effective ancillary methodology for the diagnosis of epithelial MMs.[213,543,545]

Obviously, the character of the diagnostic problem is dependent on the morphology of the neoplasm. For an epithelioid tumor, the main distinction is between epithelial MM and secondary adenocarcinoma: spread to the pleura is common, with adenocarcinomas arising in

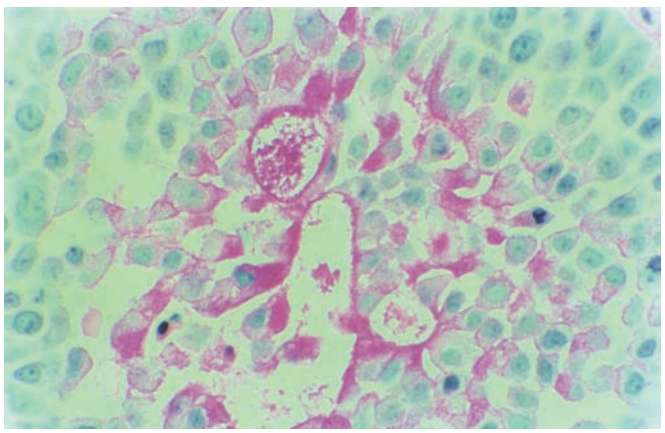


FIGURE 43.57. This pulmonary adenocarcinoma shows intracellular PAS-diastase histochemical staining.

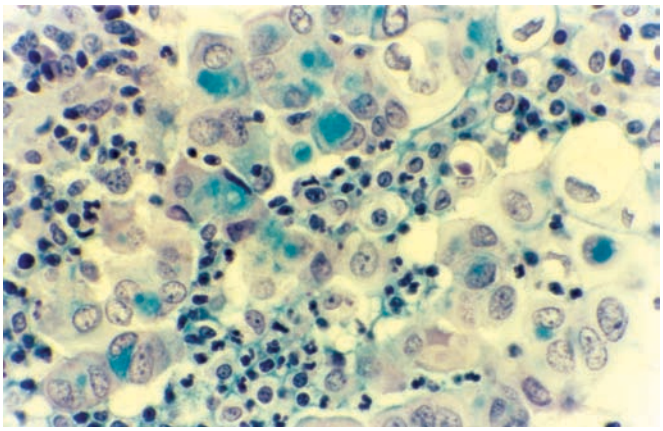


FIGURE 43.59. Alcian blue and colloidal iron are seen in this primary pulmonary adenocarcinoma and are resistant to hyaluronidase predigestion.

various anatomic sites, especially lung and breast, as discussed in a later section of this chapter. For sarcomatoid tumors, the situation is somewhat different, and the differential diagnosis includes solitary fibrous tumor, sarcomas (primary or secondary and including biphasic and monophasic synovial sarcoma), as well as spindle cell carcinoma and other neoplasms where the neoplastic cells can assume a spindle cell morphology (such as renal cell carcinoma and melanoma). Most of the published IHC studies on MM focus on epithelial or biphasic MMs. Immunohistochemistry has a far more restricted role in the diagnosis of sarcomatoid and desmoplastic MMs, but most coexpress broad-spectrum cytokeratins and vimentin.[511,546,547] A further diagnostic difficulty includes the differential diagnosis of (atypical) mesothelial hyperplasia and MM, and ancillary studies may also be of some value for that distinction, although this issue remains the subject of controversy. Finally, some antibodies may also prove useful once a diagnosis of MM has been established, as predictors of prognosis, and this seems to be represent an area of increasing interest.[544–554] In summary, there are three broad indications for immunohistochemistry:

1. The differential diagnosis between MM and other tumors
2. The discrimination between MM and reactive mesothelial hyperplasia
3. The prediction of prognosis

Despite extensive investigations, no definitive mesothelioma-specific antibody has been generated to date (as is the case for most other cancers). Given the protean phenotypic repertoire of MMs, this seems unsurprising. The antibodies currently available can be subdivided into the following broad categories:

1. *Antibodies useful for the positive identification of (epithelioid) mesothelial cells,* and of variable specificity and sensitivity: Although calretinin appears to have high specificity for mesothelial cells, other markers of lesser specificity such as cytokeratin 5/6 are still useful in the differential diagnosis, because some cancers are distinguishable from MM by their histologic appearances or by the expression of some markers and nonexpression of other mesothelial cell markers.

2. *Exclusionary antibodies that are characteristically negative in MMs and that are more frequently and consistently expressed by carcinomas:* Examples include CEA, CD15 (Leu-M1 antigen), and TTF-1 whenever adenocarcinoma of the lung is part of the differential diagnosis. The choice of antibodies in this class can be tailored to the specific circumstances of the case at issue: for example, in a patient with a history of prostatic adenocarcinoma, antibodies against prostate-specific antigen and prostatic acid phosphatase can be added, and in a patient with a background of colorectal cancer, antibodies against CK7 and CK20 can be used in addition to immunostaining for CEA.

3. *Antibodies that can decorate both mesothelial cells and carcinoma cells with reasonable frequency and that have restricted, little, or no discriminatory value in terms of a binary positive or negative result:* An example is immunostaining for EMA. Although positive in MMs and various carcinomas, some such antibodies show differences in the staining pattern between MM and carcinoma (for example, EMA and HBME-1).

4. *Antibodies directed against intermediate filament proteins, most notably cytokeratins (CKs), usually demonstrable in MMs of all histologic types and carcinomas:* Although pan-CK antibodies such as AE1/AE3 have little discriminatory value in general, they assume significance in certain circumstances, such as (a) exclusion of a lymphoma when it is in the differential diagnosis; (b) CK expression by a pleura-based sarcomatoid tumor resembling malignant fibrous histiocytoma or a collagen-rich pleural tumor can provide supportive or confirmatory evidence for a diagnosis of sarcomatoid or desmoplastic mesothelioma; (c) CK5/6 expression by a pleural epithelioid tumor can support a diagnosis of MM with an epithelial component and, substantially less often, a sarcomatoid MM; and (d) as a means to highlight the presence or absence of invasion.

5. *Antibodies that may be of probabilistic value in the discrimination between reactive mesothelial hyperplasia and epithelioid MM.*

6. *Antibodies that may be useful as predictors of prognosis.*

Because no single 100% sensitive and 100% specific antibody has been found, panels of antibodies that include both positive and negative markers are employed. Importantly, those antibodies do not by themselves consistently distinguish between benign and malignant lesions, and application of basic principles of tumor diagnosis is still required. The possible contribution of immunohistochemistry differs according on the diagnostic dilemma at hand.

The reproducibility of immunohistologic diagnosis of MM was examined in the late 1990s by a group of Italian pathologists with an interest in asbestos-related diseases, and they concluded that "the information additionally contributed by IHC did not seem to change the pathologists' diagnoses very much compared with those made by routine H&E [staining]. . . . Careful scrutiny of routinely stained preparations still remains the most rewarding component of the diagnostic pathway."[555] However, all the pathologists involved in that study were experienced in the assessment of asbestos-related disorders, whereas many pathologists do not encounter significant numbers of MM, and it is conceivable that in this particular study

Table 43.14. Markers usually positive in epithelial or biphasic mesothelioma

Positive mesothelial markers	Comment
Calretinin	Currently regarded as the most sensitive and specific marker for mesothelial differentiation
CK5/6	Sensitive and specific for differential diagnosis of epithelial MM versus adenocarcinoma, but not suitable to distinguish ovarian serous and squamous cell carcinoma
WT-1	Good sensitivity and specificity for epithelial mesotheliomas, but possible difficulties with autopsy material; cross-reactivity with renal cell carcinoma is not a problem
D2-40 (Podoplanin)	Similar sensitivities and specificities to calretinin, but less extensively studied
Thrombomodulin	Very variable in literature, but we consider it useful in the distinction of MM from metastatic adenocarcinoma; also avoids misdiagnosis of epithelioid hemangioendothelioma
HBME-1	Variably regarded, but we have found useful if only membrane labeling is considered positive and if dilution is sufficient (1:5000 to 1:15,000)
CD44S	High sensitivity but low specificity
Mesothelin	Some consider it useful (if negative, epithelial MM less likely), but we have found no advantage over calretinin and other positive markers

Table 43.15. Markers usually negative in (epithelial or biphasic) mesothelioma

Markers positive in carcinoma (negative in mesothelioma)	Comments
CEA	Very useful for differential diagnosis of MM and adenocarcinoma but usually negative in renal cell carcinoma and ovarian/peritoneal serous carcinoma
CD15 (Leu-M1)	Well characterized and we consider it a good discriminator; useful in the distinction from renal cell carcinoma (most are positive), but it does not reliably identify squamous cell carcinomas
B72.3	Variable reports, but we (and others) continue its use; sensitivity and specificity of 93% and 80%, respectively (meta-analysis)
Ber-EP4 and MOC31	Both antibodies recognize the same antigen; less reliable than CEA or BG-8, and we have found some labeling of mesotheliomas, but may be useful in certain situations, for example with metastatic breast carcinoma and pleural synovial sarcoma
BG-8	Reliable in distinction of MM and adenocarcinoma, labels 80% of squamous cell carcinomas, but does not label renal cell carcinomas
TTF-1	Useful for differential diagnosis of MM and lung adenocarcinoma; highly specific, but lack of labeling does not exclude lung adenocarcinoma, and squamous cell carcinomas of lung usually do not stain

the experience of the investigators resulted in an underestimation of the role of IHC in the diagnosis of MM. Also, it is unclear how the diagnosis of MM was confirmed, other than by consensus among observers.

We believe that IHC plays an important and often crucial role in the diagnosis of MM and that it routinely contributes to the diagnosis. We have encountered mesotheliomas misdiagnosed as adenocarcinoma histologically and vice versa (pseudomesotheliomatous adenocarcinoma [PMAC]; see later discussion), where the correct final diagnosis was achieved mainly by immunohistochemistry. Although MM and PMAC represent lethal diseases refractory to treatment and with similar mean/median survival times measured in a few months only following diagnosis, we routinely employ carefully considered panels of antibodies, believing the distinction to be important, not least because of the medicolegal implications, but also for strictly scientific reasons.

The considerations presented here are limited to commercially available antibodies that can be used on paraffin-embedded tissues. Apart from the antibodies listed in Tables 43.14 to 43.16, there are many more that have been described in the literature,[556–558] but if they are not commercially available or their use is limited to frozen section material, they are not considered here in detail.

Table 43.16. Other useful markers in the diagnosis of pleural malignant mesothelioma

Antibody	Utility/comment
CK7/CK20	Limited value to ascertain origin of secondary adenocarcinoma; not useful for discrimination between mesothelioma and adenocarcinoma; MM may be CK7+/CK20– or CK7+/CK20+
p63	Useful marker to distinguish MM from squamous cell carcinoma
Gross cystic disease fluid protein (GCDFP)	Limited usefulness to distinguish MM from metastatic breast carcinoma; low sensitivity but high specificity
CD10	Not specific enough to distinguish MM and renal cell carcinoma, because up to 54% of MM are positive
Estrogen receptor (ER)	Useful to distinguish MM from serous carcinoma of ovary or peritoneum and breast carcinoma
Progesterone receptor (PR)	In conjunction with ER, useful to distinguish MM from serous carcinoma of ovary or peritoneum and breast carcinoma
p53	Possibly some limited use in distinguishing reactive mesothelial hyperplasia and MM

There have been numerous studies comparing the usefulness of various panels of antibodies, and different laboratories have recorded different results. For example, some studies have found calretinin to be of little use or "worthless,"[436,559,560] but others have found it to be at least useful[561,562] or even highly sensitive and specific.[511,563,564] Much of the discordance between studies can be explained by the following factors:

1. The use of different materials for assessment (histologic sections of surgical specimens versus autopsy material,[565] versus cell blocks prepared from effusion fluids).
2. The clones of antibodies used: for example, one group that had found immunostaining for calretinin to be "useless" when a Chemicon guinea pig antibody was used, rather than the Zymed or Dako antibodies, remarked that when the Zymed antibody was used, it was the "preferred marker in identifying mesothelial cells in cytological samples, showing the highest sensitivity for mesothelial cells."[566]
3. Methodologic differences, including different dilutions (ranging between 1:50[563] and 1:8000[567] or even more), the use or nonuse of antigen retrieval methods, and, if used, different retrieval methods, incubation temperatures, and times.
4. Variation in what type and intensity of labeling is considered positive in the histologic assessment. For example, in some of the earlier studies on calretinin, cytoplasmic staining was considered positive, leading to the assessment that a high proportion of carcinomas showed positive staining, but when more restrictive criteria were used and nuclear staining was required for a positive result irrespective of cytoplasmic staining, high specificity ensued.[563] In an editorial comment on two successive papers on the IHC assessment of MM versus adenocarcinoma, Ordóñez[568] and Riera et al.,[569] published in the same issue of the same journal in 1997, Wick[570] pointed out that the two papers reached "somewhat divergent conclusions," although both affirmed the value of CEA, TAG-72 (recognized by the B72.3 antibody), and CD15 for the diagnosis of adenocarcinoma, but they differed over the usefulness of Ber-EP4. Ordóñez did not evaluate calretinin, whereas Riera et al. did. They also reached somewhat different conclusions concerning the value of HBME-1 and thrombomodulin. These differences were explicable at least in part by methodology. Among other factors mentioned by Wick, Ordóñez preselected the cases for study on the basis of "strong cytoplasmic staining for keratin"; Riera et al. used epitope retrieval for some probes, whereas Ordóñez did not, except for thrombomodulin; Ordóñez did not set forth specific criteria for a positive result, except that the staining was graded semiquantitatively (1+, corresponding to 1% to 25% of the cells, to 4+ amounting to >76%, and staining of <1% was considered equivocal), whereas Riera et al. considered weak staining of <10% of cells to be a negative result, although intense staining of any number of cells was designated as positive, and their semiquantitative grading system also differed, so that staining of 10% to 25% of cells was assigned to grade 1.

Some such difficulties were highlighted in a published exchange of letters and views on the subject,[571–573] highlighting the differences in approach even among those publishing actively in the field. Finally, despite the large number of studies on this subject, there are only few that attempted to weigh the usefulness of the antibodies in a statistically meaningful manner, for example by using logistic regression or decision tree analysis,[574–577] as opposed to a simple listing of the specificity and sensitivity for each individual antibody.

There are numerous current reviews suggesting various panels of antibodies[547,563,578–581] and meta-analysis has been carried out in an attempt to provide guidance,[547] but the validity of meta-analysis is limited, taking into account the heterogeneity in the methodologic variables in those analyses. The same principle applies to the Web site for Immunoquery,[582] which provides suggested IHC panels for differential diagnosis based on the published literature. Although an immensely useful database, its optimal use requires a critical and discriminatory approach. Finally, studies evaluating the potential use of new antibodies are difficult to interpret. Few provide independent validation of the diagnosis of MM, for example by EM, but if only morphologically unequivocal cases are included, this selective approach may not coincide with the true relative proportion of positive tumors, and thus skew the results. Some recent studies have attempted to overcome this particular problem by using tissue microarrays of both epithelial and sarcomatoid areas of tumors separately, to gain a better understanding of IHC staining profiles of the tumors as a whole.[512] Similarly, some of the studies comparing the immunoprofiles of epithelial MMs versus adenocarcinomas with spread into the pleura either (1) pooled carcinomas arising at different primary sites within the class of *adenocarcinoma*, or (2) used sections of the primary carcinoma rather than the actual pleural deposits.

It is worth mentioning at the outset that no literature review can replace one's own experience and knowledge of the techniques applied in one's own laboratory. In view of the versatility in appearance displayed by MM, it is not surprising that no unique and reproducible immunoprofile has been established that encompasses all types of MM, and that knowledge of immunophenotype of the morphologic subtype of lesion in question, and its differential diagnosis, is necessary to choose the most appropriate studies and for interpretation of the results. One of the most common scenarios that we

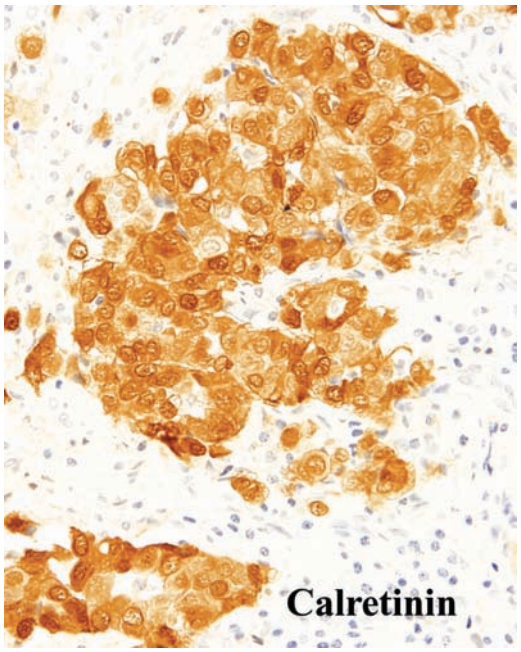


FIGURE 43.60. Pleural epithelial mesothelioma, labeled for calretinin. In addition to the labeling of the cytoplasm, there is convincing decoration of the nuclei of the neoplastic cells. Nuclear labeling of this type or more intense is required for designation of calretinin labeling as positive. If the nuclei are unlabeled, we classify the result as negative.

experience in consultation is a pleural spindle cell lesion with a clinical appearance of mesothelioma but that lacks labeling for the mesothelioma markers—not a surprising finding given the small proportion of sarcomatoid MMs that shows detectable expression of markers such as calretinin, CK5/6, and other mesothelial cell markers. This necessity for familiarity with the strengths of one's own laboratory as well as the specific diagnostic problems with an individual lesion are reflected in the reluctance of both the International Mesothelioma Panel and the Association of Directors of Anatomic and Surgical Pathology (ADASP) to suggest definitive panels of antibodies. Instead, they recommend a panel that includes at least two mesothelial-related antibodies and two antibodies that are commonly negative in mesothelioma, supplemented by immunostaining for cytokeratins in the case of the International Mesothelioma Panel.[37,583] Consequently, the opinions expressed here are largely based on our diagnostic experience with the antibodies suggested, as well as consideration of the current literature.

The discussion in this section focuses on epithelial MMs and the epithelial component of biphasic MMs. The role of immunohistochemistry in the diagnosis of sarcomatoid and desmoplastic MMs is discussed elsewhere in this chapter.

Positive Immunohistochemical Markers for Mesothelial Cells

Calretinin

Calretinin is a 29-kDa calcium-binding protein that belongs to the same family of EF-hand proteins as S-100, and that is thought to play a role in calcium-dependent cell signaling.[584] Typically expressed in the nervous system, it is also found in normal and neoplastic mesothelium.[585–587] There are a number of clones of antibodies available, and we have found the Zymed and the Dako antibodies to be particularly useful. As mentioned above, calretinin has had very variable reports, but we have found this antibody to be highly sensitive, on the order of 98% with a diagnostic accuracy of 95% (unpublished observations). Patchy cytoplasmic staining with this antibody may be observed in some metastatic adenocarcinomas, but if only nuclear staining in tumor cells is considered positive, the diagnostic accuracy of this antibody is high (Fig. 43.60). Calretinin is currently regarded as the most sensitive and specific marker for mesothelioma, and this is reflected by publications that advocate the use of this antibody as a primary antibody in suggested panels.[577,581,588,589] There is some evidence to suggest a complementary role for this antibody if used together with D2-40, particularly in spindle cell lesions.[512]

Cytokeratin 5/6

The use of differential cytokeratin (CK) subtypes, such as CK5/6[590], is of diagnostic value (Fig. 43.61). Initial

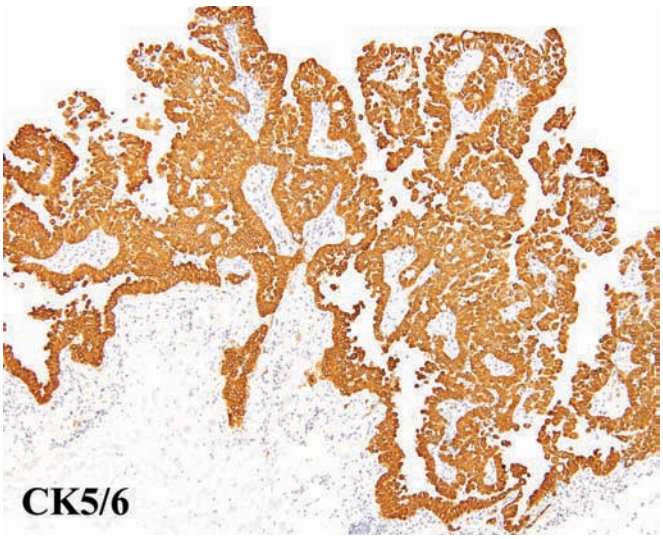


FIGURE 43.61. Exophytic mesothelioma, epithelial in type and in situ in distribution in this micrograph (superficial but undoubted invasion was found in other areas of the same biopsy). Positive labeling of the lesional cells for CK5/6.

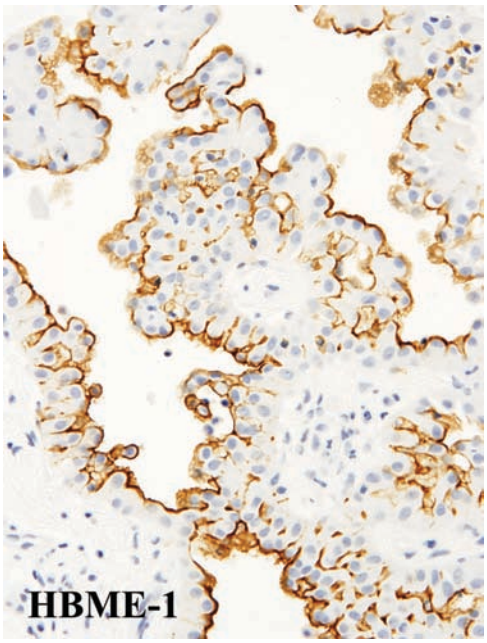


FIGURE 43.62. This tumor shows the positive linear membrane-related labeling characteristic of mesothelial cells and mesotheliomas. Although the diagnostic value of HBME-1 labeling has been questioned, we still find HBME-1 to be one of the most useful markers for epithelial mesothelial cells, provided that the antibody is used at high dilution (1:5000 to 1:15,000 in our laboratories). At higher concentrations, HBME-1 labels a variety of other tumors, although the reaction pattern in such circumstances is often cytoplasmic, rather than the linear pattern shown here.

reports of close to 100% sensitivity and specificity[564] of labeling for CK5/6 for the diagnosis of MM require reevaluation in the light of subsequent data,[547] but nonetheless, we have found this antibody useful for the diagnosis of MM and distinction from adenocarcinoma of lung in particular, although it is not reliable for distinction from ovarian serous or metastatic squamous carcinoma,[564,547,590,591] endometrial adenocarcinomas, and urothelial neoplasms.[592]

HBME-1

HBME-1 is a monoclonal antibody raised from the human mesothelial cell line SPC111. The exact antigen is not known but appears to be associated with microvilli. Reported sensitivities (66% to 100%[436,593]) and specificities (15% to 91%[594,595]) vary widely, but so does the concentration at which this antibody is used: 1:100 and 1:250 and 1:1500 are described,[563,588] and the commercial manufacturer (Dako, Denmark) recommends a dilution of 1:50 to 1:100. However, we have found that high dilutions of this antibody, in the range of 1:5000 to 1:15,000, are required for optimal results.[37,119,507] If used at sufficient dilution, and if only membranous labeling in a distribution similar to that seen with thrombomodulin or EMA is considered positive (Fig. 43.62), we have found a sensitivity of 91% and accuracy of 79% for the positive recognition of epithelial MMs with this antibody (unpublished observations). In a review of published papers, the overall sensitivity and specificity were 85% and 43%, respectively.[547] Unlike Ordóñez,[581] who regards this antibody as "not useful," we continue to find it helpful.

WT1 Protein

This protein is normally expressed by some fetal tissues as well as adult mesothelium and can be detected in up to 93% of epithelial mesotheliomas,[436,527,563,596–598] with overall sensitivity and specificity estimated as 77% and 96%, respectively, in a review of published studies.[547] However, many ovarian tumors also show labeling.[599] Another potential problem with this antibody appears to be that reactivity is significantly reduced or even completely absent in postmortem material compared to surgical specimens, and it is unclear whether this is related to fixation technique or tissue degradation.[436,565] Furthermore, some authors have expressed concern regarding labeling of renal cell carcinoma (RCC) and suggest that RCC should be specifically excluded by radiologic means,[542] but in a comparative study WT1 expression was seen in only 4% of RCCs of clear cell type.[600] We have found nuclear labeling for WT1 to be a very useful marker (Fig. 43.63), particularly in male patients, for the

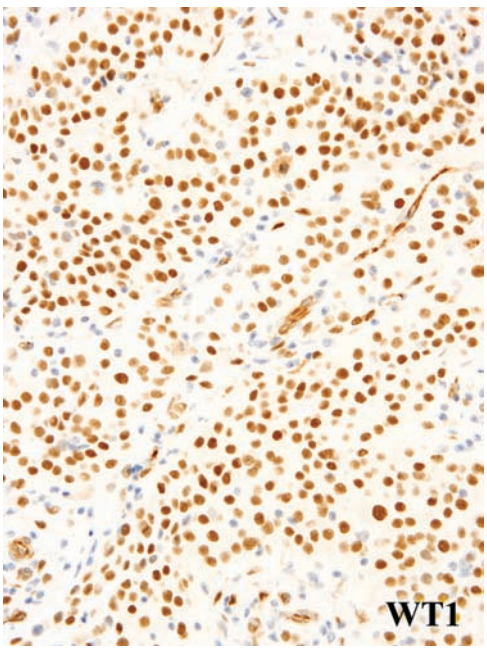


FIGURE 43.63. Epithelial mesothelioma of the pleura, immunolabeled for WT1; the labeling is almost exclusively nuclear in distribution.

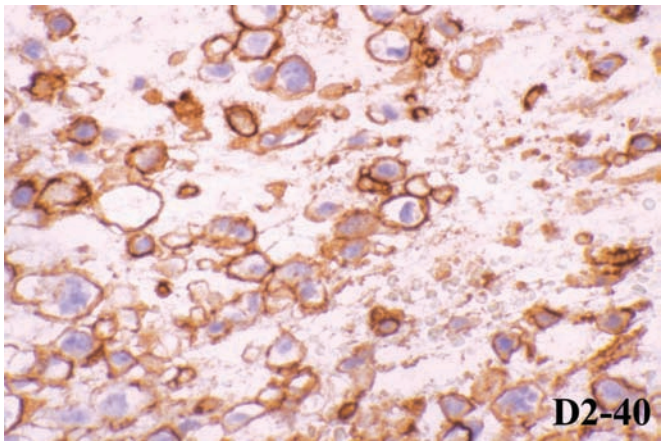


FIGURE 43.64. This epithelial mesothelioma shows cell membrane expression for D2–40.

distinction of MM from lung adenocarcinoma, where there is no possibility of metastatic ovarian carcinoma.

Podoplanin/D2-40

D2-40 is a monoclonal antibody that is directed against an M2 protein derived from germ cell tumors and that was found to specifically bind to human podoplanin, making it a useful marker for lymphatic endothelium.[601] It was noted that normal, reactive, and neoplastic mesothelial cells show labeling, and the usefulness of this antibody for the diagnosis of MM has been investigated.[580,602–604] Up to 100% of epithelial MMs investigated showed membrane staining,[604] but there was labeling of other cell types, including metastatic adenocarcinoma. Some authors suggest that membrane staining is specific for D2-40,[604] whereas others found both membrane and cytoplasmic labeling in metastatic carcinoma cells, with membranous labeling being particularly prominent in metastatic ovarian carcinoma.[605] Labeling in sarcomatoid mesotheliomas appears less reliable, with sensitivities of 27%[604] to 58%.[512] Most authors emphasize that only linear membrane staining should be regarded as positive in this context (Fig. 43.64), but since podoplanin expression is found in numerous tissue types,[601] further cross-reactions may be discovered. However, this antibody does show promise in the diagnosis of MM and may be particularly useful in conjunction with calretinin in the diagnosis of pleural spindle cell lesions.[512] However, D2-40 in isolation appears to have no advantage over calretinin.

Thrombomodulin

Thrombomodulin (CD141) is a 75-kDa glycoprotein that is expressed by mesothelium, vascular endothelium, synovium, and placental syncytiotrophoblast.[606–608] In early studies it was found to have very high sensitivity and specificity for MM (92% and 100%, respectively).[606] Many studies have since found thrombomodulin to be useful in the distinction of MM from metastatic adenocarcinoma,[588,609] but in a recent meta-analysis this was not confirmed,[547] with a low sensitivity and specificity of 61% and 80%, respectively. However, we among many others have also found high sensitivity (91%) and acceptable accuracy (79%) (unpublished observations), and we consider thrombomodulin to be a useful marker. It is worth emphasizing that thrombomodulin expression in viable cells is manifested as linear membranous staining (Fig. 43.65), and only membranous staining should be considered positive. In contrast, cytoplasmic staining, which may be seen in degenerate or necrotic tumor, is thought to due to passive uptake of antigen from the serum, and does not represent true binding to the epitope. Also, epithelioid hemangioendotheliomas, angiosarcomas, and squamous carcinomas express thrombomodulin, and this may cause difficulties in the differential diagnosis of pleural spindle cell neoplasms.[511] Positive labeling for thrombomodulin in the absence of detectable labeling for other mesothelial or carcinoma-related markers raises the distinct possibility of an epithelioid hemangioendothelioma,

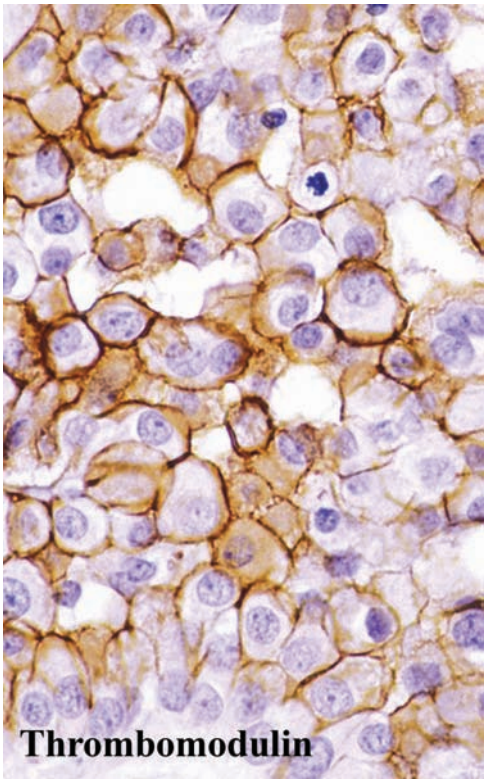


FIGURE 43.65. Pleural epithelial mesothelioma, immunolabeled for thrombomodulin. Characteristically, the labeling is linear and membrane-related, with a "chicken wire" pattern in this area.

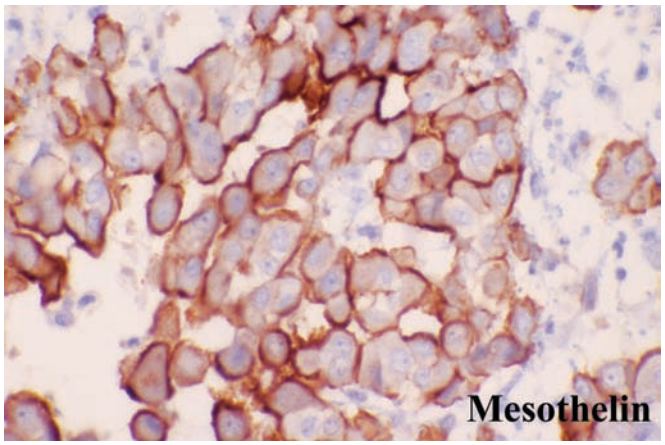


FIGURE 43.66. Fairly intense cell membrane staining for mesothelin is seen in this epithelial mesothelioma.

which can be confirmed by labeling for endothelial markers such as CD31 and von Willebrand factor.

Mesothelin

Mesothelin is a 40-kDa surface glycoprotein that was generated using an ovarian cell line, and it has been reported to be expressed on the surface of normal, reactive, and malignant mesothelial cells (Fig. 43.66).[610] There have been several studies investigating the usefulness of this marker for the diagnosis of mesothelioma.[563,579,580,600,611–614] Some authors describe high sensitivity and specificity for epithelial MMs with no labeling of the metastatic adenocarcinomas investigated,[610] but other investigators found positive labeling in up to 39% of lung adenocarcinomas.[611–613] However, it is worth mentioning that the latter studies utilized a different commercially available clone of antibody. This antibody also shows positive labeling of squamous cell carcinomas of lung, pancreatic carcinomas, and ovarian tumors,[611,615] but no labeling of RCCs where it may play a limited role in the distinction of MM from RCC.[600] In view of the overall low specificity but apparently high sensitivity of this antibody for epithelial mesothelioma in all of the studies published, it has been suggested that a *lack* of labeling could be considered an indication *against* a diagnosis of mesothelioma. In view of the fact that there are now several mesothelioma-related antibodies available that show higher sensitivity and specificity, we consider this antibody to be rather limited in its usefulness.

CD44S

This 85- to 90-kDa transmembrane glycoprotein is expressed by many hematopoietic and lymphoid cells. This protein acts as a receptor for hyaluronic acid as well as facilitating lymphocyte interaction with endothelial cells. After initial encouraging reports describing labeling of up to 92% of mesothelioma cell lines,[616] later studies showed overall disappointing results with fairly high sensitivity (90–100%) but low specificity.[588,609,617]

There are now numerous mesothelial-related markers available, so much so that one might question the need for further markers in this area.[618]

Exclusionary Markers: Characteristically Positive in Adenocarcinomas and Negative in Mesotheliomas

Carcinoembryonic Antigen

Carcinoembryonic antigen is an oncofetal glycoprotein not normally expressed by mesothelial cells but commonly expressed by lung and other adenocarcinomas, most notably colorectal carcinomas. It was the first widely accepted marker to aid in the distinction of MM and adenocarcinoma[619] and remains one of the best of the exclusionary markers.[577,581,589]

In a survey of 598 diffuse MMs comprising 21 separate reports, Henderson et al.[211] found that only 58 (10%) were reactive with antibodies to CEA, whereas 359 of 404 pulmonary adenocarcinomas were CEA positive (89%), and that in those mesotheliomas that are reactive with antibodies to CEA, the staining is usually focal and weak. Polyclonal CEA antibodies (PoAbs) were used in some of the early studies and resulted in some nonspecific staining due to cross-reactions,[211] but an analysis of recent data found a sensitivity of 81% and specificity of 97%.[547]

The significance of immunolabeling for CEA for the diagnosis or exclusion of mesothelioma can be summarized as follows:[119]

1. Intense or extensive cytoplasmic or membrane-accentuated immunoreactivity for CEA is highly characteristic of adenocarcinoma or other carcinomas, and is strong evidence against a diagnosis of mesothelioma.[211]
2. Because CEA is undetectable in 10% to 15% of pulmonary adenocarcinomas and in most serous papillary carcinomas, both ovarian and extraovarian, a negative result on immunolabeling for CEA is not decisive by itself.
3. Numerous CEA polyclonal and monoclonal antibodies are in existence, with different sensitivities and specificities for CEA, so that the results can vary from one study to another. Dejmek and Hjerpe[620] compared patterns of reactivity for CEA in a series of 61 mesotheliomas of different histologic subtypes, using a single PoAb (Dako) and five monoclonal antibodies (MoAbs). Thirteen of the mesotheliomas (21%) were labeled with the CEA PoAb. The staining was focal in 11 cases, and diffuse in two. Four of the five CEA MoAbs were reactive

with variable but smaller proportions of the mesotheliomas (one to seven out of 61 cases). Only the Dako MoAb was unreactive with all mesotheliomas, whereas it decorated 15 of 20 adenocarcinomas.

4. Nonspecific staining with CEA antibodies can be encountered in mesotheliomas and other tumors, including uptake of the antibody in areas of tumor necrosis or in benign alveolar remnants incorporated into mesotheliomas invading lung. False-positive labeling has also been recorded in mesotheliomas with a high content of hyaluronic acid, and this was abolished by pretreatment of sections with hyaluronidase[621]; protracted trypsinization of sections may also lead to nonspecific labeling.[622] Accordingly, interpretation of a positive result for a tumor that resembles MM in all other respects requires some caution. We routinely monitor each immunoreaction with both positive and negative controls and consider only unequivocal labeling of viable tumor remote from any areas of necrosis to be significant.[119]

Cluster of Differentiation 15 (CD15; Clone Leu-M1)

CD15 is a complex cluster of cell surface glycoproteins and glycolipids that share the terminal Lewis^x antigen, a human myelomonocytic antigen. The CD15 antigen is present on more than 95% of mature peripheral blood eosinophils and neutrophils and is present at low density on circulating monocytes. There are over 90 clones of antibodies assigned to CD15 and eight alternate names for CD15. The discussion here is limited to the clone Leu-M1.

CD15 is one of the oldest and best characterized markers for adenocarcinoma and has been used for the distinction from mesothelioma for over 20 years.[623,624] It has established itself in the panels used in most laboratories, although some authors report positivity in up to 32% of MMs.[527,625] In addition, one study using "logic" regression concluded that despite high sensitivity and specificity, some of the newer antibodies such as BG8 and MOC31 are more suitable for the positive identification of adenocarcinoma.[563] We and others have found that MM is only rarely positive for CD15[559,581,626,627] and consider it to represent a useful discriminator. It is also useful in the distinction of MM from RCC, most of which are positive,[600,628] but it does not reliably identify squamous cell carcinomas.[629] Sheibani et al.[623,624] and Battifora[515] found that CD15 was undetectable in all 127 mesotheliomas investigated, whereas it was expressed by 199 out of 268 adenocarcinomas (74%). Wick et al.[627] reported quite decisive results: CD15 was found in all 52 pulmonary adenocarcinomas studied, but none of 51 epithelial mesotheliomas. Battifora has pointed out that pulmonary adenocarcinomas express CD15 more often than adenocarcinomas originating in other sites. He also cautioned that CD15 expression is often focal and that false-negative reactions can be expected with small biopsies.

Blood Group Antigen Lewis^y (BG8 Clone)

BG8 is an antibody that was raised against a lung cancer cell line and was first reported by Jordan et al.[630] to be useful in the distinction of MM and adenocarcinoma. It has since been found to distinguish adenocarcinoma reliably from epithelioid MM.[547,569,631] In a study investigating 12 antibodies and using logic regression, it was found to be one of the three most useful antibodies.[563] This marker also labels 80% of squamous cell carcinomas[632] but does not label RCCs, so that additional antibodies should be included in the panel whenever secondary RCC is suspected.[600]

Antibodies Directed Against Epithelial Cell Adhesion Molecule, Including Ber-EP4 and MOC31

The epithelial cell adhesion molecule (Ep-CAM), which was discovered in the early 1980s, is a type I transmembrane glycoprotein. Expression has been detected at the basolateral membrane of the majority of epithelial tissues, including transitional cell epithelium, but Ep-CAM expression appears to be absent in mature squamous stratified epithelium and in hepatocytes[633–635]; Ep-CAM has also been identified in carcinomas of ovary, colon, breast, kidney, and lung.[634,636] In squamous cell carcinomas, Ep-CAM expression is absent, as detected by the Ber-EP4 antibody.[637]

There are now numerous clones of antibodies commercially available: among those extensively investigated for the distinction between MM and metastatic carcinoma are Ber-EP4 and MOC31, both of which identify the EGF-1–like domain of Ep-CAM.[634] Among the lesser known clones not as extensively studied are HEA125 (also identifying the EGF-1 like domain of Ep-CAM)[638] and AUA1.[634,639,640] Some reports identified high specificity for adenocarcinomas, with no labeling of any of the eight mesotheliomas included in a pilot study using AUA1,[641] but later reports revealed labeling of up to 21% of mesotheliomas,[641] and currently this antibody is not widely recommended for this role.

A meta-analysis of published reports found 80% specificity and 90% sensitivity for Ber-EP4 in the distinction between MM and adenocarcinoma, and 93% sensitivity and specificity for MOC31.[547] An evaluation of 12 antibodies using logic regression included MOC31 in the final three-antibody panel, which reportedly provided 96% sensitivity and specificity for the distinction of MM from adenocarcinoma.[563] Despite these encouraging reports in the literature, in our practice we have removed both Ber-EP4 and MOC31 from our routine mesothelioma proto-

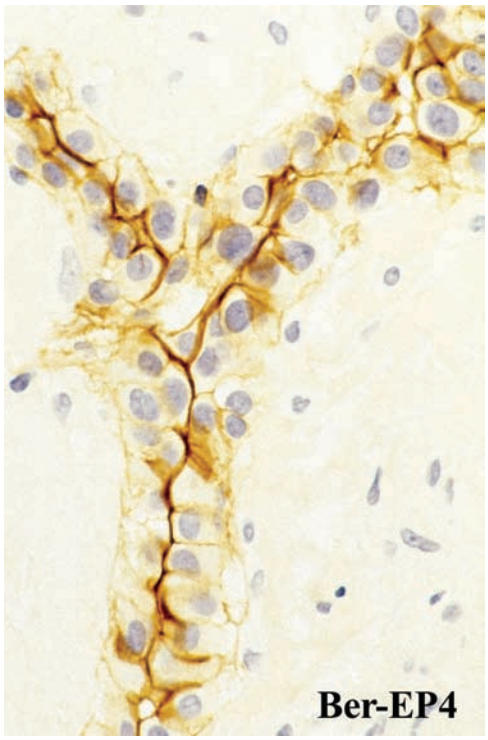


FIGURE 43.67. Pleural malignant mesothelioma. Positive linear immunolabeling with Ber-EP4. No immunohistochemical marker is entirely specific or sensitive for mesothelial cells versus carcinoma cells.

col because in our laboratory each labeled a significant proportion of mesotheliomas (up to about 20–30% with Ber-EP4; Fig. 43.67).

B72.3

The antibody B72.3 identifies the tumor-associated protein TAG-72, a complex glycoprotein expressed in breast carcinoma lines, and has long been used as a positive adenocarcinoma marker, with numerous studies investigating this antibody for the distinction of adenocarcinoma from MM.[627] The published reports have been very variable, with some reporting labeling of more than 40% of MM,[642] and only 50% of adenocarcinomas,[643] in contrast to others that described virtually no labeling of mesotheliomas with this antibody.[579,580] We, like many others, have found acceptable sensitivity and specificity with this antibody, which has been found to be positive in about 85% of lung adenocarcinomas,[625,632,644,645] with overall sensitivity and specificity of 93% and 80%, respectively, as assessed by meta-analysis. In our experience, labeling of epithelial MMs by B72.3 is distinctly uncommon, about three MMs among a few hundred cases tested. In one of the positive cases, the labeling appeared to correlate spatially with prominent lakes of glycogen in the mesothelioma cell cytoplasm as visualized by EM.[119]

E-Cadherin

Cadherins are part of a family of cell adhesion molecules that present as membrane-bound heterodimers. E-cadherins are though to be preferentially expressed by epithelial tissues, in contrast to N-cadherins, which are considered to be preferentially expressed by neural crest tissue. E-cadherin is normally present as a complex with β-catenin, which plays an important role in the *WNT* pathway (the pathway mutated in familial adenomatous polyposis (FAP) and many other malignancies). Some reports see value in using either expression of E-cadherin alone as an adenocarcinoma marker,[646] or assessing differential patterns of expression of E-cadherin (in lung adenocarcinomas) versus expression of N-cadherins (in MM),[560,647] but we, like some others,[579] have found labeling of a significant proportion of mesotheliomas, and a meta-analysis found an overall sensitivity and specificity of 86% and 83%, respectively. With other more reliable markers being available, we have discontinued the routine use of this antibody for this application.

Thyroid Transcription Factor-1

Thyroid transcription factor-1 is a member of the family of homeodomain (HD) transcription factors and is involved in the regulation of genes expressed within the thyroid, lung, and brain, including those that encode thyroglobulin, Clara cell secretory protein, and surfactant proteins.[648] Gene targeting experiments among others have demonstrated that expression of TTF-1 is essential for morphogenesis of the thyroid, lung, and ventral forebrain; TTF-1 knockout mice lack these organs,[648] and suppression of TTF-1 translation inhibits "lung branching morphogenesis."[649] Thyroid transcription factor-1 is expressed at the onset of thyroid differentiation; TTF-1 mRNA is detectable in the endodermal cells of the thyroid rudiment in the rat embryo and precedes the expression of two other known target genes by 5 days.[650] Thyroid transcription factor-1 mRNA and protein are also present at the earliest stages of lung differentiation and are later confined to the bronchial epithelium. In the brain, TTF-1 appears to be restricted to structures of diencephalic origin, including the developing neurohypophysis.[650]

Stahlman et al.[651] studied the IHC localization of TTF-1 in the lungs of 24 human fetuses at 11 to 23 weeks' gestation, three infants without pulmonary pathology at 36 to 42 weeks, and 24 infants aged 2 days to 6.5 months with hyaline membrane disease or bronchopulmonary dysplasia. Thyroid transcription factor-1 was detected in fetal lung epithelial cell nuclei by 11 weeks' gestation. By 17 weeks, labeling was present in scattered nonciliated columnar and cuboidal cells. Throughout gestation, nuclear staining for TTF-1 was prominent in airways that abutted pleural, peribronchial, and perivascular

connective tissue, and was less prominent in centers of lobules. At term, TTF-1 was detected primarily in type II pneumocytes.

In adult normal human lung, TTF-1 expression is restricted to bronchial and alveolar epithelium.[652] Fabbro et al.[652] found TTF-1 expression in seven of 29 cases of non–small-cell lung carcinoma, representing a subset. Curiously, TTF-1 was not expressed in carcinoid tumors, but was "always" expressed in small cell lung carcinomas.[652]

Subsequent studies have shown that TTF-1 is expressed in the nuclei of primary lung (and thyroid follicular) adenocarcinomas and small cell carcinomas, but not in colorectal or breast carcinomas.[653] The specificity and sensitivity of TTF-1 for the diagnosis of adenocarcinomas (and other carcinomas) of lung versus carcinomas of extrapulmonary origin, versus MM, and for the subclassification of lung carcinomas have subsequently been reported in numerous studies.[547,559,579,580,631,654–660] Most such investigations have demonstrated labeling of about 70% to 90% of lung adenocarcinomas for TTF-1,[559,579,655,657–659,661] with a specificity of up to 100%,[547] in comparison to a smaller proportion of large cell carcinomas (~25%[661]) or nonneuroendocrine large cell carcinomas (~50%[659]). Ordóñez[579] found that none of 50 MMs labeled for TTF-1. Our experience is comparable: 4/45 epithelial MMs labeled for TTF-1 (9%) and equivocal labeling at most was found in a further 9%. We consider that definite or strong nuclear labeling in a pleural epithelial tumor represents strong evidence against a diagnosis of epithelial MM (Fig. 43.68) and in favor of an adenocarcinoma of bronchopulmonary origin.

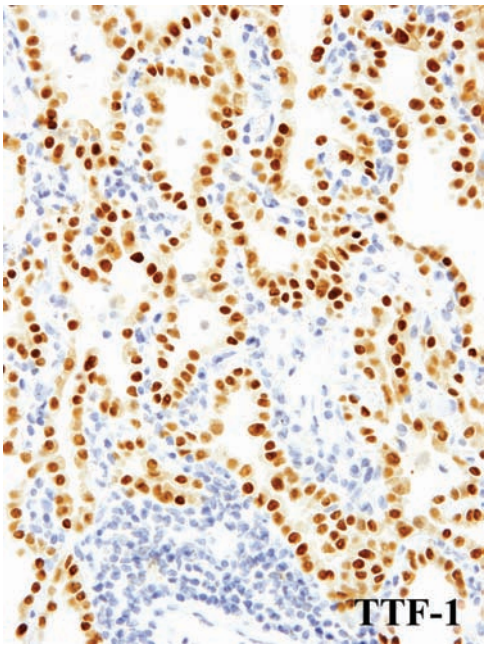


FIGURE 43.68. Strong nuclear staining for thyroid transcription factor-1 (TTF-1), in a peripheral and localized bronchioloalveolar adenocarcinoma (BAC), nonmucinous type, in an 88-year-old man, treated by wedge resection. Nuclear staining for TTF-1 in a pleural tumor is strong evidence against a diagnosis of mesothelioma.

Antibodies that Decorate both Mesothelial Cells and Carcinoma Cells with Reasonable Frequency: Cytokeratins, Epithelial Membrane Antigen, and CA125

Cytokeratins are discussed in a later section.

Epithelial Membrane Antigen

Epithelial membrane antigen (EMA) is a membrane-bound glycosylated phosphoprotein anchored to the apical surface of many epithelia by a transmembrane domain, with the degree of glycosylation varying according to the cell type. It is thought to play a role in the adhesive function of cell-to-cell interaction, including metastasis. Increased expression, aberrant (intracellular) localization, and changes in glycosylation patterns have been associated with carcinomas.

Epithelial membrane antigen is frequently expressed by adenocarcinomas and epithelial MMs alike, but differences in the distribution of staining make this a useful marker. Adenocarcinomas are usually characterized by cytoplasmic staining, whereas epithelial MMs generally show strong, thick, and circumferential membrane-related staining in up to 97% of cases (Fig. 43.69).[588,662–664] Labeling of the atypical cells in this characteristic linear distribution with antibodies based on clone E29 (for example, the Dako antibody) has also been found useful for the distinction between MM and nonmalignant mesothelial proliferations, both in surgical specimens and in cell-block material prepared from effusion fluids.[665–670] This antibody has been extensively studied in effusion fluid cytology[671] and aids in the differentiation of mesothelioma from reactive mesothelial hyperplasia, where labeling is usually undetectable or weak.[402,664,672–678] Although none of the reactive effusions showed staining in this pattern, about 75% of MMs or more in some studies[489,674,678,679] showed this pattern of EMA labeling, resulting in high specificity but low sensitivity. We have found labeling of tumor cells for EMA (E29 clone) to be a useful probabilistic indicator of malignancy, most notably in cells recovered from effusion fluids, but we have encountered numerous tissue biopsies of proven invasive epithelial MMs where there was either no labeling for EMA or where EMA staining was confined to the superficial zone of the tumor tissue, with undetectable staining in the deeper zones.

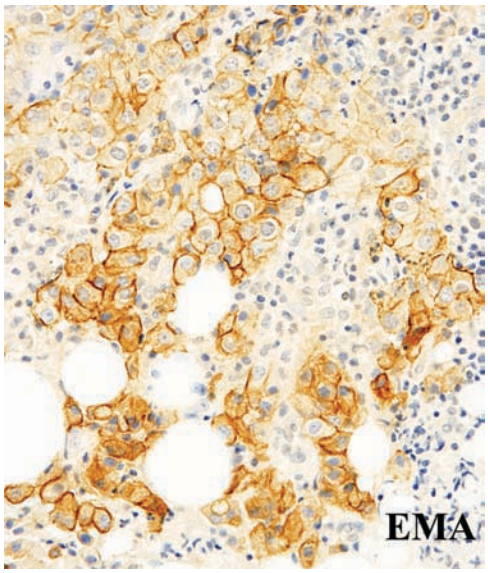


FIGURE 43.69. Pleural malignant mesothelioma, epithelial type. The neoplastic cells, including those invading into subpleural fat, show predominant linear membrane-related labeling for epithelial membrane antigen (EMA), with lesser staining of the tumor cell cytoplasm. Although we have encountered many invasive mesotheliomas that were EMA-negative, as seen in tissue biopsies, it is our experience that the presence of strong thick linear membrane-related labeling for EMA is a probability marker for mesothelioma as opposed to a benign reactive mesothelial hyperplasia, provided that the antibody used is based on the E29 clone (see text). Although not sufficient by itself for diagnosis of mesothelioma as distinct from a reactive mesothelial proliferation, EMA expression in this pattern is an indicator for close follow-up and further investigation of the patient.

CA125

It is well established that immunolabeling of tissue sections for CA125 has no value in the discrimination between MM and adenocarcinomas developing at different anatomic sites, such as those arising in the ovary, lung, and breast.[567,593,680,681] As examples, Bateman et al.[593] found that 15/17 cases of MM labeled for CA125 (88%) in comparison to 7/14 cases of secondary adenocarcinoma in lung and pleura (50%). Attanoos et al.[567] observed positive immunostaining for CA125 in 19/20 ovarian papillary serous adenocarcinomas (95%) and 2/3 primary peritoneal serous adenocarcinomas, in comparison to 8/32 peritoneal MMs (all in females). In a further study from Japan on 90 epithelial MMs and 51 adenocarcinomas of lung, Kushitani et al.[680] found that 85% of the MMs and 80% of the adenocarcinomas were positive for CA125. Finally, in another study based on effusion fluids, Zhu and Michael[681] reported positive staining of all 20 metastatic ovarian carcinomas for CA125, in comparison to 8/13 adenocarcinomas of lung (62%) and 6/13 cases of metastatic breast carcinoma (46%). In all such cases, staining for CA125 is membrane-related.

Therefore, immunostaining of cytology or biopsy samples has essentially no value as a diagnostic discriminator between MM and adenocarcinomas of lung, breast, or ovary. But there is evidence that measurement of serum CA125 levels is a useful and sensitive marker for assessment of the progression of MM and its prognosis, or for the response of MM to treatment. Hedman et al.[682] found that serum CA125 concentrations increased as the disease progressed, whereas stable disease was accompanied by a decrease in CA125 levels. In a study from Turkey on 11 peritoneal MMs, Kebapci et al.[683] found that the mean serum CA125 level was 230 U/mL, within a range of 19 to 1000 U/mL (the normal reference range for this study was 1.2–32 U/mL). In a later study from Italy on 60 cases of peritoneal MM, Baratti et al.[684] recorded a baseline diagnostic sensitivity of 53% for serum CA125 in the MM patients. Forty-six of the patients underwent cytoreductive surgery (CRS) with intraperitoneal hyperthermic perfusion (IPHP): following "adequate" CRS and IPHP, the serum CA125 became negative in 21/22 patients who had elevated baseline levels, but it remained elevated in all nine patients with grossly persistent MM. Elevated CA125 levels developed in all 12 patients who developed progressive disease after CRS and IPHP.

Therefore, there is reasonable evidence that serum CA125 levels represent a sensitive but nonspecific marker for MM, and that serial measurements of the serum levels are a useful means to monitor the progression and prognosis of MM or its response to therapeutic measures, especially when the results are correlated with other serum markers such as soluble mesothelin-related protein (SMRP) and osteopontin (see Serum Osteopontin Levels, below).

Markers of Possible Use for the Distinction of Benign Mesothelial Proliferations Versus MM: EMA, bcl-2, p53, and CD56 (NCAM)

Epithelial Membrane Antigen

The value of IHC staining for EMA in the discrimination between benign versus malignant mesothelial proliferations is discussed above.

Bcl-2

Bcl-2 is a proto-oncogene with a 26-kDa gene product that inhibits apoptosis and therefore promotes survival of individual cells. As discussed earlier, detectable overexpression[478] and direct mutations of *bcl-2* in MM are rare,[391] unlike many other tumors, including follicular lymphoma and even lung carcinoma,[685–687] where overexpression is commonly observed and may be linked to

poorer prognosis. Only a small proportion of MMs has been shown to label with antibodies against *bcl-2*, and none of the "reactive" cases labeled.[665,688] Nonetheless, because only a small percentage of MMs immunolabeled, the IHC detection of *bcl-2* seems to be insufficiently sensitive in isolation to be useful for routine diagnostic work to distinguish MM and reactive pleural lesions,[689,690] although it might find some role as part of a panel, for specific problematic cases.

p53

The tumor suppressor gene *p53* induces cell cycle arrest and is maintained at low levels in normal unstressed cells. Stress may induce increased levels of p53 and result in cell cycle arrest and apoptosis. Because of its short half-life, p53 is rarely detectable in normal cells, but paradoxically, increased levels of p53 are commonly expressed in malignant tumors. This is not due to an increase in functional p53 but rather to mutations that render p53 nonfunctional and resistant to degradation. Such mutations of *p53* are only rarely seen in MM,[410] but the *p53* pathway is affected by numerous mutations.

Studies report the presence of p53 in between 25% and 97% of MMs, whereas p53 was found in between 0% and 82% of reactive mesothelial lesions examined.[689–700] In view of the variability in results, use of this antibody for the distinction of benign from malignant mesothelial lesions seems questionable, but warrants further investigation. A relationship between *p53* expression and prognosis has not been identified.

Neural Cell Adhesion Molecules: CD56

The neural cell adhesion molecules (NCAMs) corresponding to CD56 antigen represent a family of closely related cell surface glycoproteins that are thought to play a role in the development of neural cells and the interactions between them. In a study of 16 MMs that included "all three subtypes" in comparison to normal mesothelial cells and a single specimen of pleural mesothelium, Kettunen et al.[699] found that gene expression for NCAM L1 *(L1CAM)* was upregulated mainly in biphasic MMs in comparison to the reference samples. On IHC analysis of tissue microarrays from 47 MMs (26 epithelial, six biphasic, and 15 sarcomatoid), they also recorded significant *p*-values for *L1CAM* when antigen expression levels for epithelial MM were compared with sarcomatoid MMs.

Lantuéjoul et al.[700] studied 26 cases of epithelial, biphasic, and sarcomatoid MM for NCAM reactivity using the 123C3 antibody in comparison to normal mesothelium and 50 non–small-cell lung carcinomas divided equally between adenocarcinomas and squamous cell carcinomas. Although normal mesothelium was negative, NCAM expression was recorded in 19 of the 26 MMs (73%), including all histologic types. Although this finding raises the possibility that CD56 immunoreactivity might prove useful for the discrimination between benign mesothelial proliferations versus MM, there is too little information on NCAM/CD56 expression in MM and mesothelial hyperplasia to justify inclusion of NCAM/CD56 antibodies (such as that based on clone 1B6) in routine diagnostic protocols until further and more extensive studies become available.

Intermediate Filament Proteins: Cytokeratins (Except CK5/6), Vimentin and Desmin

Cytokeratins

Although CKs are expressed by most MMs (Figs. 43.51C and 43.61) and most carcinomas, so that their simple presence is of no discriminatory value, we consider IHC staining important for the diagnosis of MM and we routinely include a CK antibody in our IHC protocol, for two reasons: to highlight invasion, and for the diagnosis of sarcomatoid mesothelioma.[119] Provided that tissue fixation is prompt and adequate and IHC procedures are carried out correctly, CKs are detectable in most MMs, especially with the use of monoclonal antibodies to a CK cocktail or low molecular weight CKs,[37,119,211,626] and trypsinized sections or other techniques are used for epitope enhancement or retrieval.[119] CK7 is expressed by almost all MMs, and CK20 by about 10%.[37] Within this context, immunostaining for pan-CKs, CK8/18 (Fig. 43.70), or CK7 demonstrates CKs in (1) the overwhelming majority of neoplastic cells in virtually all epithelial mesotheliomas, (2) the epithelial component and usually but not always the sarcomatoid component of biphasic mesothe-

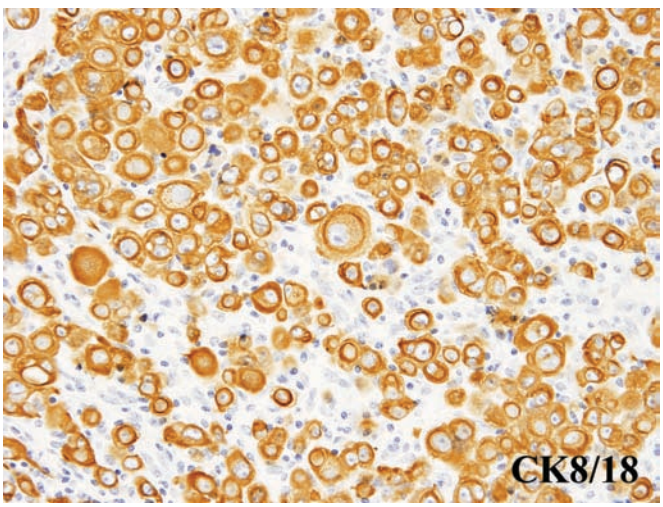


FIGURE 43.70. Pleural malignant mesothelioma, epithelial type, immunolabeled for cytokeratins 8/18 (CAM5.2). There is moderately strong labeling of almost all tumor cells, and some show a perinuclear wreath of intensified labeling.

lioma, and (3) most spindle cells in most but not all sarcomatoid mesotheliomas (see later discussion of sarcomatoid MM in the section Ultrastructural Features of Mesotheliomas). As reviewed by Henderson et al.,[119] CKs were reported in all 137 mesotheliomas comprising nine separate series, in all 94 mesotheliomas in three separate studies that used an antibody against low molecular weight CKs, and in 81 of 94 MMs (86%) with an antibody against high molecular weight CKs. With the use of a broad-spectrum antibody, Mayall et al.[508] identified CKs in 92% and 100% of their epithelial and mixed mesotheliomas, respectively. Lower rates of CK expression in some series seem to be explicable in part by the use of antibodies that recognize stratum corneum keratins, prolonged formalin fixation with loss of immunogenicity, or the use of nontrypsinized sections.[119]

Coexpression of CKs and Vimentin

Vimentin-cytokeratin co-synthesis is characteristic of sarcomatoid, desmoplastic, and transitional MMs and the spindle-cell component of biphasic MM.[119] Mayall et al.[508] identified vimentin in 54% and 74% of epithelial and mixed MMs, respectively, and in 87% of sarcomatoid MMs.

Most sarcomas and other sarcomatoid tumors, and many carcinomas, including metastatic carcinomas and carcinoma cells in effusion fluids,[701] express vimentin so that vimentin by itself is of little or no value in the diagnosis of mesothelioma.[119] Nonetheless, immunolabeling for vimentin in pleura-based tumors is sometimes worthwhile as a check on the immunogenicity of the tissue, and failure to demonstrate vimentin may point to degradation of epitopes, perhaps as a consequence of prolonged fixation.[119]

In addition, disproportionately strong vimentin staining in an epithelioid pleural tumor that shows no or only weak to moderate expression of CKs is an indicator to proceed to immunostaining for CD31 or other markers of endothelial differentiation (epithelioid hemangioendothelioma), especially if the mesothelial cell markers other than thrombomodulin are negative. When investigating a sarcomatoid tumor, it is also worth recalling that sporadic examples of other mesenchymal tumors that express CKs have been also been documented and include malignant fibrous histiocytoma, and smooth muscle cell tumors,[119] but in such instances CK expression is usually weak to moderate at most and is usually confined to a small proportion of the tumor cell population.[119]

Desmin

Desmin is a type III intermediate filament found near the Z-line in sarcomeres. It is only expressed in vertebrates. Scoones and Richman[702] studied desmin and α-smooth muscle actin (α-SMA) in paraffin-embedded biopsy tissue from 10 cases of reactive mesothelial hyperplasia (recurrent pneumothoraces) versus 38 mesotheliomas (27 predominantly epithelioid, four predominantly sarcomatoid, and seven mixed). The reactive hyperplasias expressed desmin and α-SMA more often than mesotheliomas. Similar findings were reported by Attanoos et al.,[689] who found that 85% of reactive mesothelial hyperplasia expressed desmin, but only 10% of mesotheliomas. Mayall et al.[508] detected desmin in 10% of biphasic mesotheliomas, but all of their epithelial or sarcomatoid tumors were negative.

Other Markers

Increased nuclear labeling for the transcription factor β-*catenin*, which is normally found in complex with the cell surface glycoprotein E-cadherin, may be useful in the distinction of reactive and malignant mesothelial proliferations and shows some promise in effusion fluids,[703] but further studies are necessary to further assess its utility in this context.

Labeling for the *X-linked inhibitor of apoptosis proteins* (XIAPs) also shows some promise in distinguishing benign from reactive pleural effusions, although this can be positive in mesotheliomas as well as some (but not all) metastatic adenocarcinomas, colonic adenocarcinomas being a notable exception.[704]

P glycoprotein (also known as p170) plays a role in cell membrane transport, and expression has been associated with resistance to chemotherapy.[689,705,706] Normal mesothelium has not been found to express this protein, but expression has been found in a high proportion of MMs, with no demonstrated effect on patient survival.[705] The overall sensitivity of this antibody for malignancy is relatively low at 52%; however, if labeling is present specificity is high, at about 92%.

GLUT-1[689] is part of a family of transmembranous glucose transporters, which facilitate the entry of glucose into cells. It is largely undetectable by immunohistochemistry in normal epithelial tissues and benign tumors, but is expressed in a variety of malignancies. In a study on pleural effusion fluids, GLUT-1 was expressed in 72% (28 of 39) of cases of malignant effusions: 100% from the ovary, 91% from the lung, 67% from the gastrointestinal tract, and 12% from the breast, but none (0/25) of the benign effusions expressed GLUT-1.[707] Thus, the expression of GLUT-1 appears to be a potentially useful marker of malignant transformation, but additional investigations are required to assess this marker further.

In rare instances, unusual substances have been demonstrated immunohistochemically in mesotheliomas. Okamoto et al.[708] reported two neoplasms consistent with primary pleural mesotheliomas that contained anaplastic tumor giant cells that demonstrated human chorionic gonadotropin on immunohistochemistry.

TABLE 43.17. Markers potentially useful in the distinction of reactive and malignant mesothelial proliferations

Antibody	Utility/comment
EMA (clone E29)	Strong, diffuse, linear labeling supports diagnosis of malignancy
p53	Sensitive but not very specific; labeling may support diagnosis of malignancy
Bcl-2	Specific but not very sensitive; labeling may support diagnosis of malignancy
Desmin	Positive in reactive lesions (and in some MMs with sarcomatoid features)

McAuley et al.[709] evaluated a patient with MM who had hypercalcemia and an elevated serum concentration of parathyroid-like hormone. They also evaluated nine epithelial mesotheliomas for parathyroid-like peptide and found abundant immunopositive cells in eight of nine cases. They also observed parathyroid-like peptide immunoreactivity in normal and reactive epithelial mesothelial cells.

Markers Related to Prognosis

A high proliferative index as assessed by *Mib-1* labeling has been found to be associated with a poorer prognosis in MM.[550] However, because there appears to be correlation between Mib-1 labeling index and the subtype of MM, the possibility that this represents poor survival associated with tumor type cannot be excluded.[549,550] Also, a mitotic activity index, assessed by direct count of mitotic figures, was not found to be an independent prognostic factor.[470]

Expression of the proliferation-associated antigen *p27*, which blocks progression of the cell cycle to mitosis, was also found to be related to prognosis, with lower expression being predictive of poorer survival,[554] but interestingly, and somewhat surprisingly, this was not linked to mitotic indices, so that the mechanism of action for p27 in this context appears uncertain.

Apart from being used as indicator of malignancy, labeling for XIAPs has also been suggested to predict poorer response to apoptosis-inducing chemotherapy regimens. Development and testing of XIAP-blocking drugs is underway, but further studies are needed before the value of this investigation can be assessed.[704]

Unsurprisingly, increased expression of *vascular endothelial growth factor* (VEGF), which may be triggered by tumor necrosis and which is an established growth factor for MM, has also been identified to predict a poorer outcome.[470]

The value of serial serum estimations of CA125 as a marker for progression of MM and hence prognosis, or its response to treatment, was discussed earlier in this section on immunohistochemistry.

Currently, it appears that although a number of markers are under investigation, no clinically or therapeutically useful marker has emerged.

Recommended Panel

The various antibodies/markers for MM diagnosis discussed in the preceding text (and some others) are summarized in Tables 43.14 to 43.18.[436,527,547,552,559,563,564,569,577,579–581,588–591,593–598,600,602–604,606,609–613,617,623–632,642–645,655,658,689–692,710–719] As also indicated in that discussion, we consider that an optimal approach to the IHC evaluation of possible or suspected mesothelioma entails each laboratory establishing its own protocol from proven cases of MM and non-MM lesions, and validating its methodology for each immunoreaction. Like the International Mesothelioma Panel,[37] we believe a reasonable and systematic first-line protocol would include the following:

- Immunostaining for CKs, for example, pan-CKs, CK8/18, or CK7
- Epithelial membrane antigen (EMA)
- *At least two mesothelial cell markers,* from a panel that would include calretinin as the most useful and specific marker at present, and one of the following: CK5/6, HBME-1, WT-1, podoplanin/D2-40, or perhaps thrombomodulin (the last also useful as an endothelial marker) (Table 43.18)
- *At least two carcinoma-related markers:* CEA, CD15 (Leu-M1 antigen), B72.3, BG-8, and TTF-1[648–656,661] (now standard in many protocols)

TABLE 43.18. Summary of immunoreactivity of malignant mesotheliomas with an epithelial component versus adenocarcinoma of lung

Tumor	CKs	CK5/6	CALR	HBME-1	WT1	TM	D2-40	MT	EMA	CEA	CD15	B72.3	BerEP4 MOC31	TTF-1
Malignant mesothelioma	+	+	+	+*	+	+	+	+	+*	0	0	0	0/+	0
Lung adenocarcinoma	+	0	0	0/+**	0	±	0	±	+**	+	+	+/0	+	+

CK, cytokeratins (AE1/AE3, CK8/18, CK7); CALR, calretinin; WT1, Wilms' tumor-1 antigen; TM, thrombomodulin; D2-40, podoplanin antibody; MT, mesothelin; EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; CD15, Leu-M1 antigen; TTF-1, thyroid transcription factor-1; +, usually positive; 0, usually negative; ±, may be positive or negative; *, linear, membrane-related; **, cytoplasmic.

In the event of discordant or equivocal findings, other members of each group can be added, or one can proceed to EM (for example, when there is one major discordant immunoreaction such as positive labeling for CEA, or two discordant reactions with antibodies of lesser specificity, such as Ber-EP4 or MOC31).

If the IHC protocol shows that the lesion is a carcinoma, the following labels can then be used according to the specific circumstances of the case:

- The CK7/CK20 profile[655,710]
- Others depending on the clinical background (e.g., prostate-specific antigen and prostatic acid phosphatase, especially if there is a suspicion or a past history of prostate cancer; CD99 and bcl-2 for biphasic tumors where synovial sarcoma enters the differential diagnosis; CD10, erythropoietin and RCC antigen if there is a suspicion of secondary RCC[711]; CD31, CD34, factor VIII–related antigen whenever epithelioid hemangioendothelioma enters into the differential diagnosis; and S-100 protein, HMB-45, and melan A if there is a suspicion of secondary melanoma (CK-negative tumor)

For pleura-based sarcomatoid tumors, the following simplified protocol can be used:

- Pan-CKs or low molecular weight CKs, ± vimentin
- ±CK5/6, calretinin (negative in about 50% of sarcomatoid mesotheliomas or more)
- CD34, bcl-2, CD99 (if the differential diagnosis includes solitary fibrous tumor)
- Ber-EP4, other carcinoma-related markers, bcl-2, CD99 (if the differential diagnosis includes synovial sarcoma)
- Others depending on the clinical background

Ultrastructural Features of Mesotheliomas

Several reports in the literature have illustrated the ultrastructural features of mesotheliomas.[79,720,721] Similarly, the ultrastructural features of primary lung neoplasms have been described extensively. In our experience, epithelial mesotheliomas have ultrastructural features that can be used to differentiate them from pulmonary adenocarcinomas and other primary lung carcinomas. The converse is also true: pulmonary adenocarcinomas and other primary lung carcinomas have electron microscopic features that can be used to differentiate them from epithelial mesotheliomas. This does not mean that every epithelial mesothelioma or every primary pulmonary carcinoma looks identical by electron microscopy, but there are enough ultrastructural differences to allow their separation.

Ultrastructurally, well and moderately well differentiated epithelial mesotheliomas are formed by cuboidal, polygonal, columnar, and round cells that are often connected to each other by well-formed desmosomes and junctional complexes (Fig. 43.71). Tumor cell nuclei are round, occasionally indented, and have medium-sized nucleoli. Their cytoplasm contains numerous mitochondria, short profiles of rough endoplasmic reticulum, and numerous intermediate filaments that are often aggregated into tonofilaments, which insert into large desmosomes connecting the cells together (Fig. 43.72). The most conspicuous ultrastructural feature of neoplastic epithelial mesothelial cells is the presence of numerous long, slender, sinuous microvilli that arise from the cell membrane (Fig. 43.73). These are often referred to as bushy microvilli. The neoplastic mesothelial cells are characteristically separated from the fibrovascular tissue by a well-defined basal lamina that is often infolded and is associated with micropinocytotic vesicles in the cell membrane of the adjacent mesothelial cells (Fig. 43.74). Epithelioid mesotheliomas composed of round cells have ultrastructural features similar to those of tubulopapillary mesotheliomas. They have long cell-surface microvilli, numerous cytoplasmic intermediate filaments, including tonofilaments, and aggregates of cytoplasmic glycogen (Fig. 43.75). Some mesotheliomas show microvillus-matrix interaction in which the microvilli of an

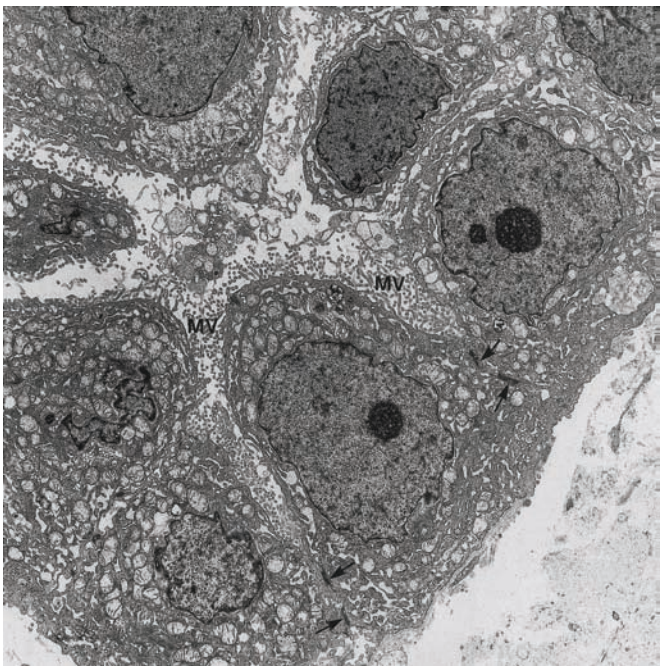


FIGURE 43.71. Electron micrograph shows representative region of tubulopapillary mesothelioma. Tumor cells are similar in size and shape and are connected to each other by well-formed desmosomes (arrows). Round nuclei located near center of cell have medium-sized nucleoli. Cytoplasm contains numerous mitochondria and other organelles. Note microvilli (MV) arising from cell surface.

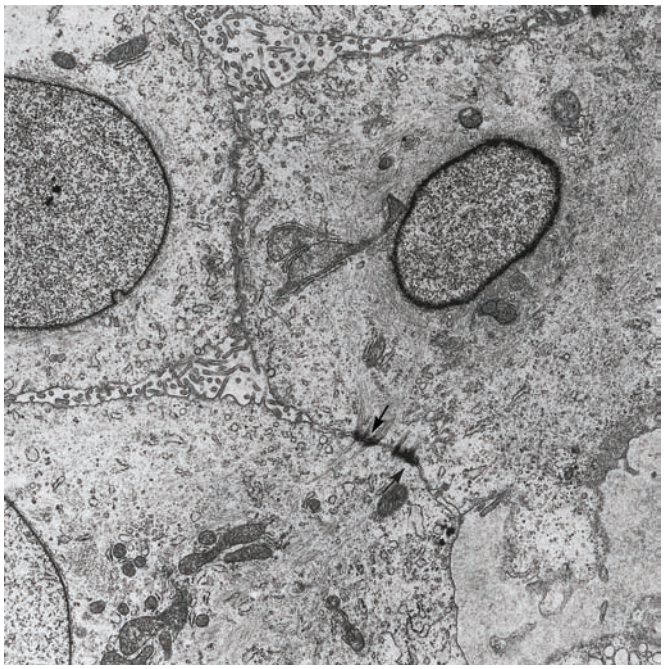


FIGURE 43.72. Mesothelial cells are usually connected by large desmosomes into which intermediate filaments insert (arrows).

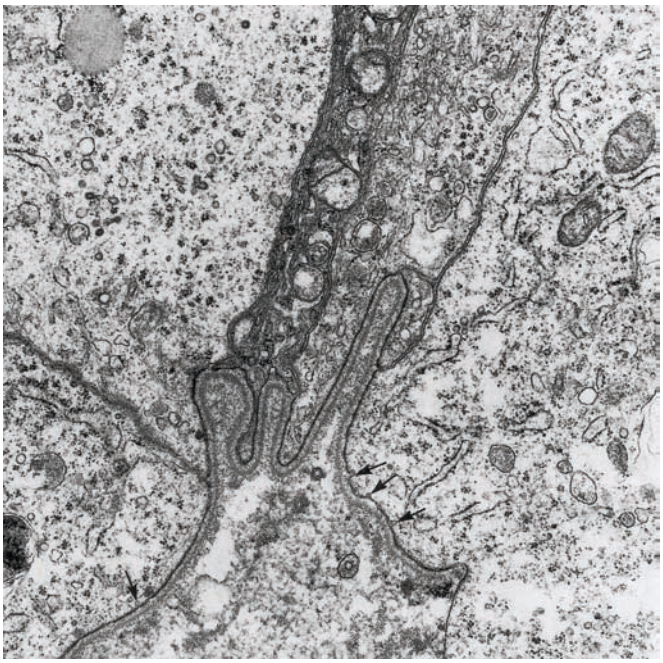


FIGURE 43.74. Portions of several mesothelioma cells show invagination of their cytoplasm and investment by basal lamina. Note micropinocytotic vesicles in cell membrane of tumor cells (arrows).

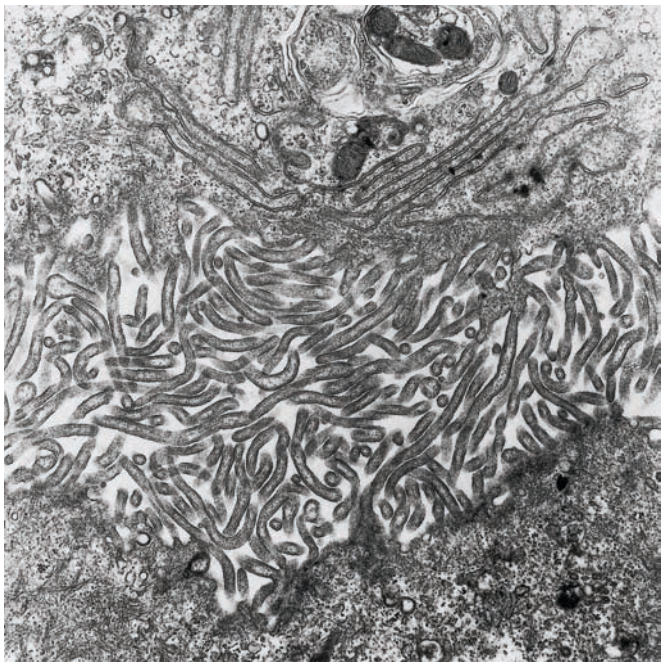


FIGURE 43.73. Most characteristic feature of epithelial mesothelioma cells is long sinuous microvilli. These have length-to-width ratios averaging 10 to 15, significantly greater than the length-to-width ratio of microvilli of pulmonary adenocarcinomas, and are not covered by a fuzzy glycocalyx.

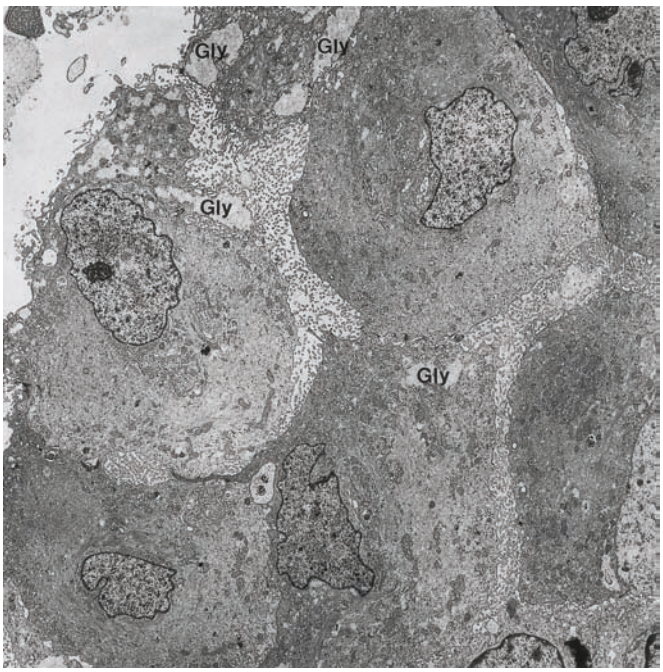


FIGURE 43.75. Ultrastructural appearance of epithelioid mesothelioma. Cells are round with abundant intracellular intermediate filaments and aggregates of glycogen (Gly).

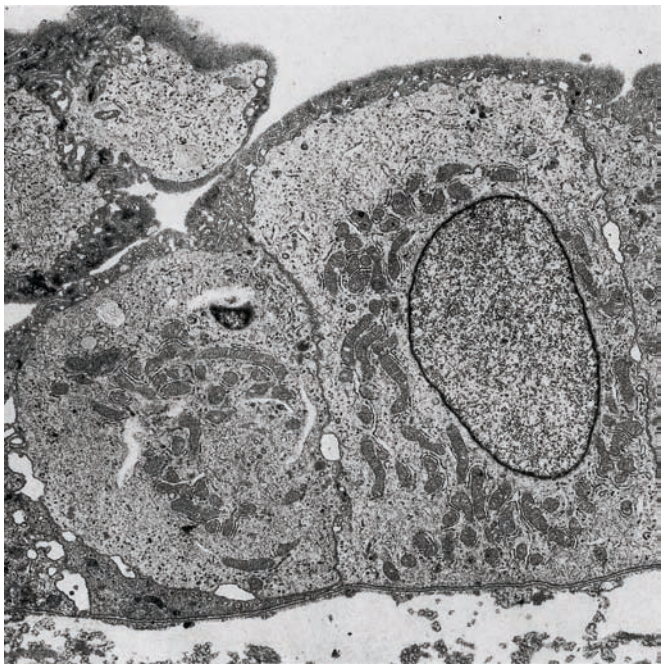


FIGURE 43.76. Some epithelial mesotheliomas produce hyaluronic acid. This is not seen within cytoplasm of tumor cells but appears as medium electron-dense material on cell surface in which microvilli are "embedded."

epithelial mesothelioma directly penetrate into adjacent collagen fibrils.

Approximately 20% of epithelial mesotheliomas produce a mucosubstance, hyaluronic acid and proteoglycan, that can be identified ultrastructurally as a medium electron-dense material associated with the cell microvilli (Fig. 43.76). This material is often seen in intracellular neolumina and often crystallizes (Fig. 43.77). Hyaluronic acid may form scroll-like crystalline structures (Fig. 43.78). Mesotheliomas that show the crystalloid material typically are "mucin-positive," showing intracellular PAS-D, mucicarmine, hyaluronidase-resistant and Alcian blue/colloidal iron-hyaluronidase–resistant material.

Sarcomatoid MMs have variable ultrastructural features. The tumor cells may resemble fibroblasts (Fig. 43.79), containing short profiles of distended rough endoplasmic reticulum, a prominent Golgi apparatus, and occasionally inspissated electron-dense material in the cisterna of the rough endoplasmic reticulum. Other sarcomatoid MMs show more variability in size and shape (Fig. 43.80) and occasionally show epithelial differentiation in the form of well-formed intercellular junctions (Fig. 43.81), basal lamina formation (Fig. 43.82), and tonofilaments (Fig. 43.83). They may even show a few microvilli arising from the cell surface (Fig. 43.80). Some sarcomatoid mesotheliomas have an ultrastructural appearance resembling myofibroblasts, containing peripherally located actin filaments and centrally located short profiles of rough endoplasmic reticulum[722] (Fig. 43.84). Desmoplastic MMs have variable ultrastructural features, being composed of cells that resemble fibroblasts or myofibroblasts.

Transitional mesotheliomas are composed of cells with electron microscopic features of both epithelial and mesenchymal cells. The tumor cells are frequently connected to each other by relatively well-formed intercellular junctions, have aggregated mitochondria, and have cytoplasmic intermediate filaments that may represent vimentin (Fig. 43.85). In some tumor cells, thin actin filaments are observed in association with the cell membrane. The tumor cells typically do not show the long, sinuous microvilli observed in better differentiated epithelial mesotheliomas.

The epithelial component of biphasic mesotheliomas shows the ultrastructural features of epithelial mesotheliomas with long bushy cell-surface microvilli and abundant intracellular tonofilaments and other organelles. The sarcomatoid portion is composed of cells with electron microscopic characteristics of sarcomatoid MMs. In transition zones, the tumor cells may have an ultrastructural appearance transitional between cells expressing epithelial features and other cells expressing sarcomatoid features.

Most of the controversy concerning the ultrastructural features of mesotheliomas has centered around epithelial

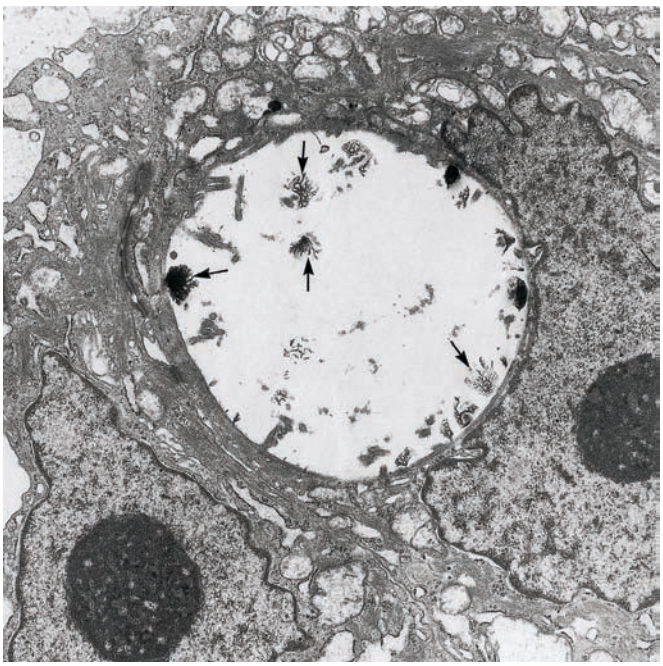


FIGURE 43.77. Intracellular lumen in mesothelioma cell shows crystallized mucosubstance (arrows) that has a fern-like appearance.

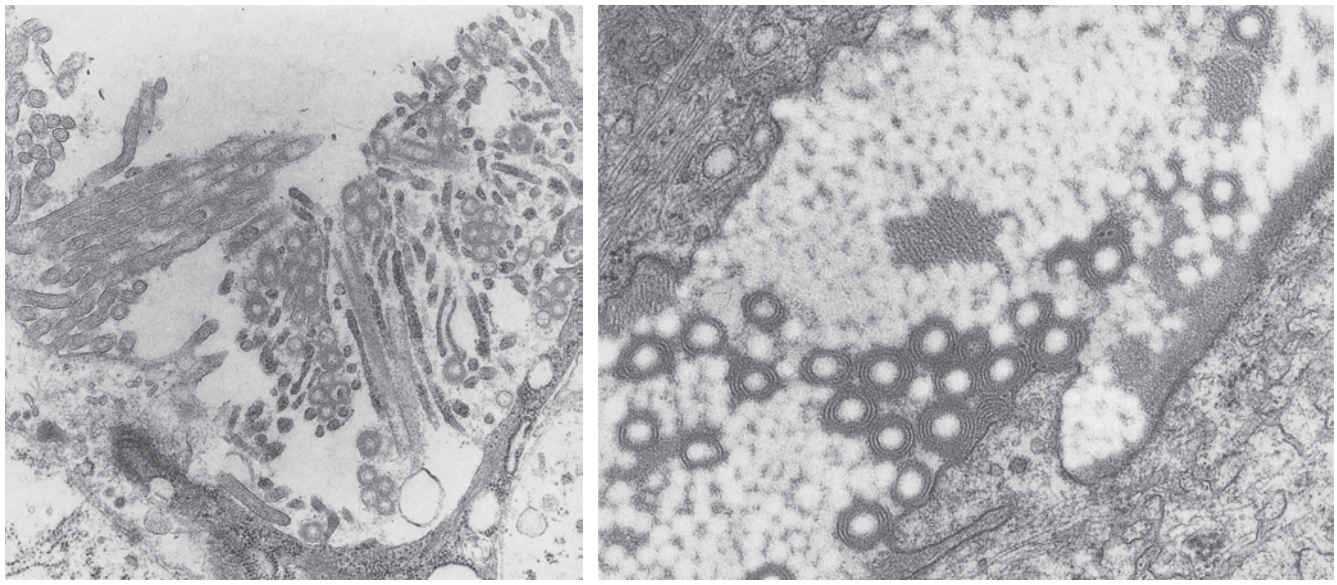


FIGURE 43.78. **(A)** In this hyaluronic acid–producing epithelial mesothelioma, hyaluronic acid crystallized to form hollow tubular structures with a scroll-like appearance on cross section. **(B)** In cross section, the hyaluronic acid crystals have a scroll-like morphology and resemble hollow chrysotile fibrils.

mesotheliomas, specifically with respect to whether they can be differentiated ultrastructurally from pulmonary adenocarcinomas or other types of adenocarcinomas. Warhol et al.[723] and Warhol and Corson[724] studied quantitatively the difference between the microvilli of epithelial mesotheliomas and pulmonary and breast adenocarcinomas. They found the mean length-to-diameter ratio of epithelial mesothelioma microvilli was 15.7, whereas pulmonary adenocarcinoma microvilli had a length-to-diameter ratio of 8.7. Burns et al.[725] found

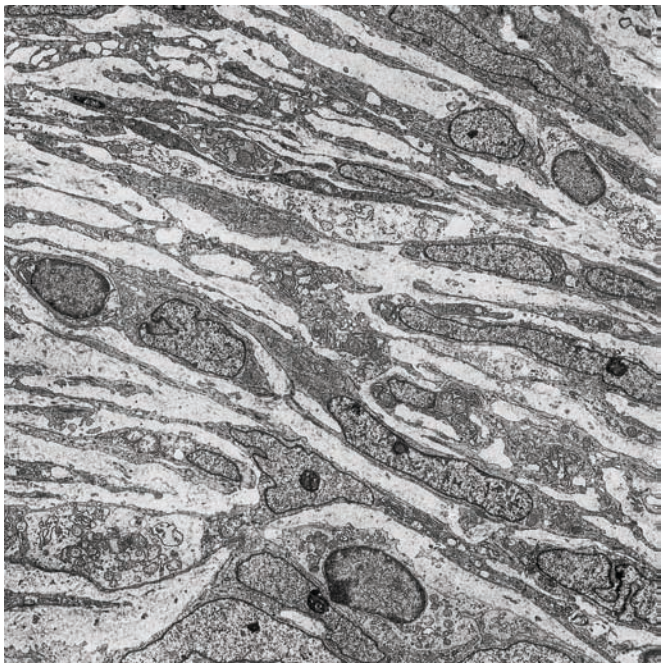


FIGURE 43.79. Sarcomatoid mesothelioma composed of spindle cells that resemble fibroblasts.

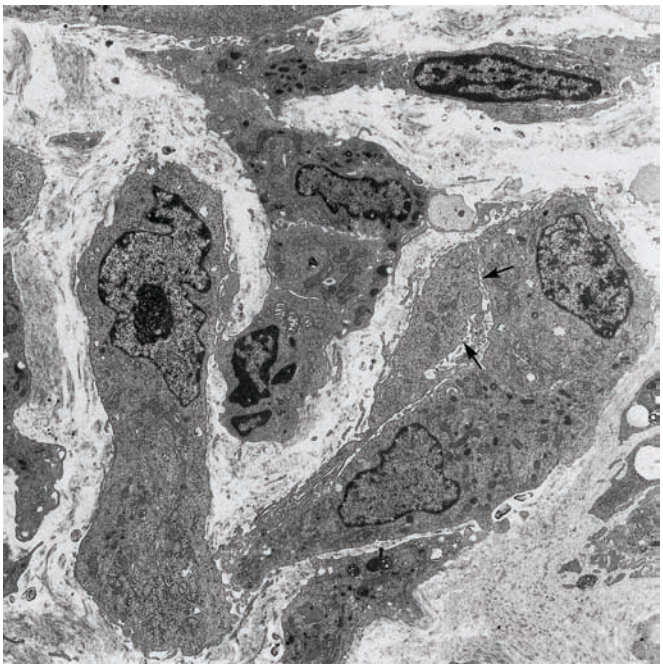


FIGURE 43.80. Sarcomatoid mesothelioma composed mostly of spindle-shaped cells with large nuclei. An occasional cell shows a few cell-surface microvilli (arrows).

FIGURE 43.81. Some neoplastic cells in this sarcomatoid mesothelioma are connected to each other by well-formed desmosomes.

similar results with a mean length-to-diameter ratio of 11.44 for seven epithelial mesotheliomas and 5.39 for three pulmonary adenocarcinomas. Warhol and colleagues also found epithelial mesotheliomas had more cytoplasmic tonofilaments than pulmonary adenocarcinomas. Hammar et al.[79,669] have emphasized the overall difference in the pattern of the microvilli of epithelial mesotheliomas and pulmonary adenocarcinomas. As shown, the microvilli of epithelial mesotheliomas are numerous, long, and sinuous, whereas the microvilli of pulmonary adenocarcinomas are frequently short, straight, and covered by a fuzzy glycocalyx. We do not

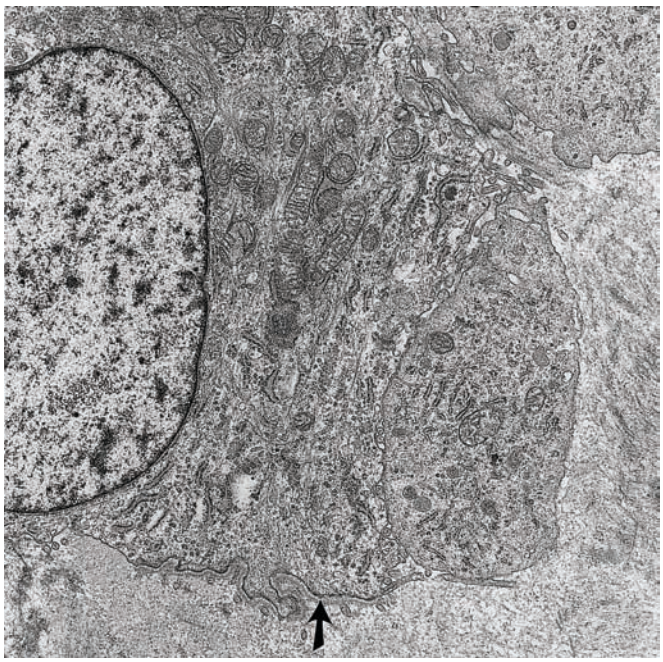


FIGURE 43.82. Some neoplastic cells forming this sarcomatoid mesothelioma are surrounded by basal lamina (arrow).

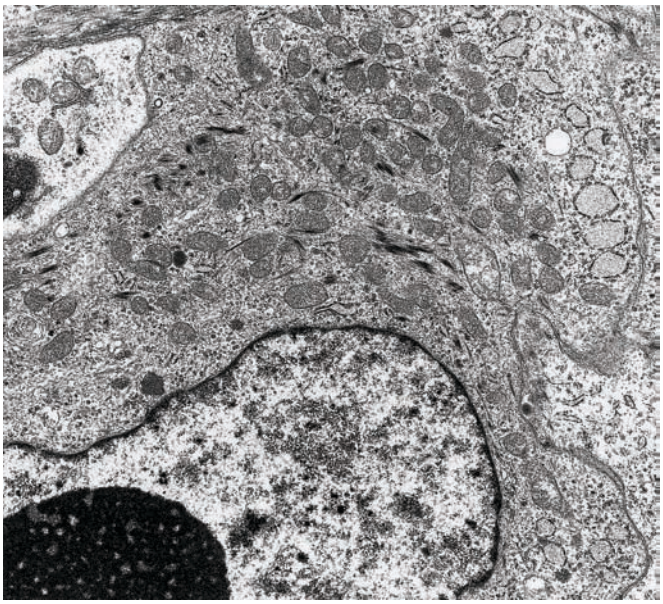


FIGURE 43.83. In this sarcomatoid mesothelioma, many neoplastic cells contain aggregates of intermediate filaments in their cytoplasm consistent with tonofilaments.

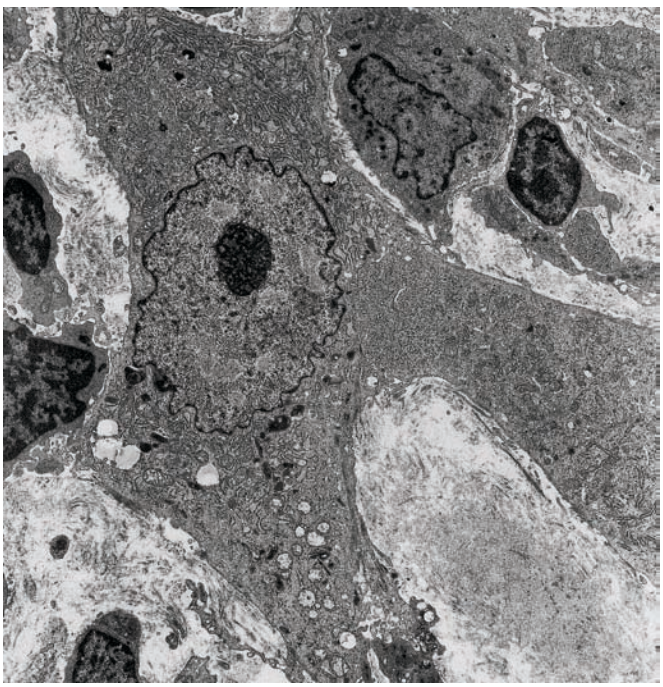


FIGURE 43.84. Some cells of this sarcomatoid mesothelioma have ultrastructural features of myofibroblasts, with peripherally located thin filaments consistent with actin filaments and abundant short profiles of rough endoplasmic reticulum.

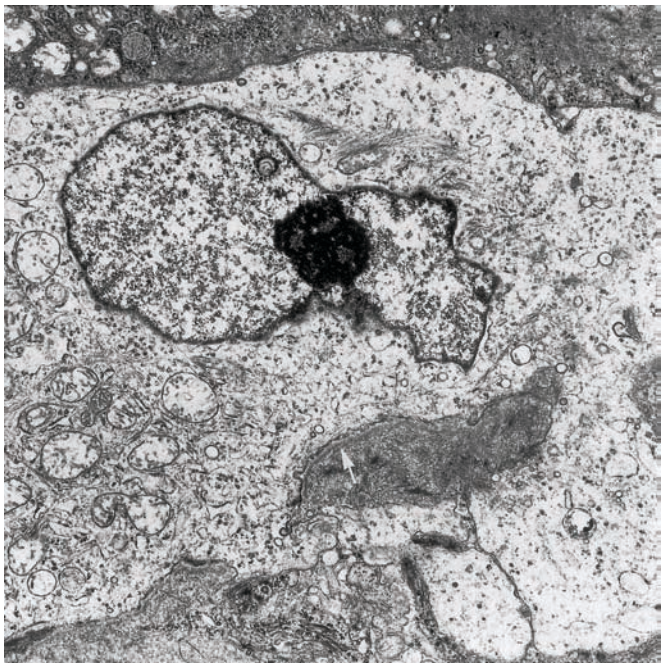


FIGURE 43.85. Transitional mesothelioma composed of large polygonal cells with large nuclei and relatively nonspecialized cytoplasm. A few intermediate filaments in cell cytoplasm resemble tonofilaments. Note focal basal lamina (arrow).

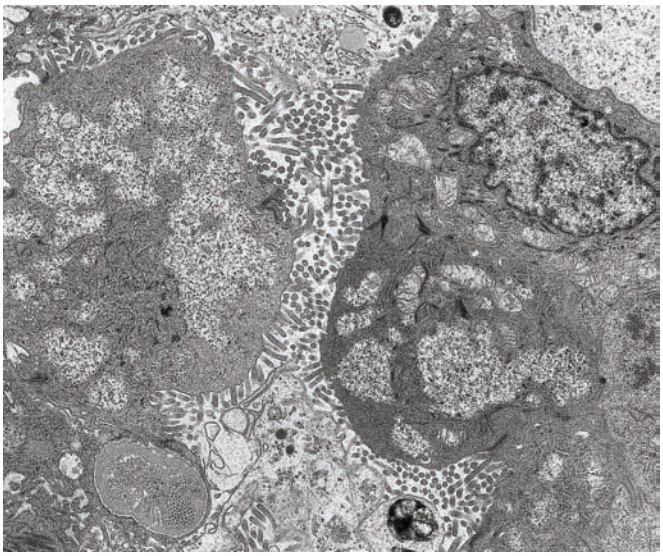


FIGURE 43.86. Peripheral pulmonary adenocarcinoma had long slender microvilli resembling those seen in epithelial mesothelioma.

believe it is necessary to determine the length-to-width ratio of microvilli to tell the difference between epithelial mesothelioma and pulmonary adenocarcinoma. Determining the length-width ratio is difficult because the long, thin, sinuous microvilli are usually not in the same plane of section, and the entire length cannot be measured. Rare pulmonary adenocarcinomas exist that have relatively long microvilli and at first glance may resemble an epithelial mesothelioma (Fig. 43.86), but on closer inspection are covered by fuzzy microvilli (Fig. 43.87), a finding incompatible with an epithelial mesothelioma.

There are other ultrastructural differences between epithelial MMs and pulmonary adenocarcinomas. The cells forming epithelial mesotheliomas and pulmonary adenocarcinomas are connected to each other by intercellular junctions. Where the tumor cells form glands, they are attached by junctional complexes and elsewhere are connected predominantly by desmosomes. As a general rule, the desmosomes connecting mesothelioma tumor cells are larger than those connecting pulmonary adenocarcinoma cells. This observation has been confirmed by a semiquantitative study.[726]

As stated and shown previously, about 20% of epithelial mesotheliomas produce hyaluronic acid, which can be identified ultrastructurally as a medium-electron-dense material in which the cell microvilli appear embedded. Hyaluronic acid–producing mesotheliomas do not contain mucosubstance granules in their cytoplasm, which is in contrast to the 60% to 75% of pulmonary adenocarcinomas that are mucus-producing and contain cytoplasmic mucous granules of variable size and density that are often associated with a prominent terminal web. Pulmonary adenocarcinomas of Clara cell or type II pneumocyte origin frequently contain cytoplasmic multivesicular bodies and lamellar bodies. These structures are infre-

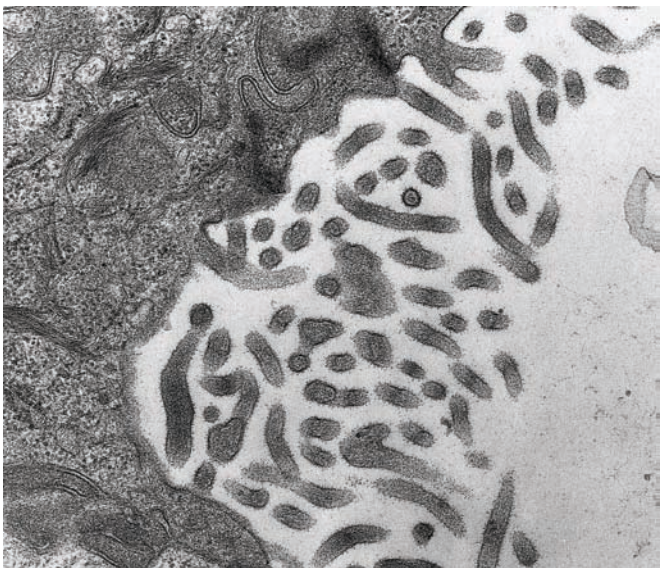


FIGURE 43.87. At greater magnification, long microvilli of neoplastic cells were covered by fuzzy glycocalyx, a finding not seen in epithelial mesothelioma.

Table 43.19. Comparison of the ultrastructural features of epithelial mesothelioma and pulmonary adenocarcinoma

Ultrastructural features	Epithelial mesothelioma	Pulmonary adenocarcinoma
Microvilli	Long, sinuous, smooth	Short, usually straight; covered by fuzzy glycocalyx
Intercellular junctions	Junctional complexes; large desmosomes	Junctional complexes; small desmosomes
Mucosubstance production	No mucosubstance granules in cytoplasm; mucosubstance on cell surface; crystallization	Mucous granules in cytoplasm; glycocalyceal bodies; mucus in gland lumen
Cytoplasmic intermediate filaments	Abundant; often in a perinuclear distribution; tonofilaments frequent	Common; often distributed throughout cytoplasm; tonofilaments variable
Cytoplasmic inclusions	Infrequent; some lysosomes	Frequent; multivesicular bodies and lamellar bodies frequent in bronchioloalveolar cell carcinoma

quently seen in epithelial mesotheliomas. A comparison of some of the ultrastructural features of epithelial mesotheliomas and pulmonary adenocarcinomas is shown in Table 43.19.

Cytogenetic and Molecular Features in Mesothelial Cell Proliferations

Cytogenetic abnormalities are commonly found in MMs. Tiainen et al.[727] performed successful cytogenetic analyses on cells obtained from solid tumors and from pleural effusions in 34 of 38 patients with MM. Clonal chromosomal abnormalities were detected in 25 patients, the majority being complex and heterogeneous with no chromosome abnormality specific to mesothelioma. Nine patients had normal karyotypes or nonclonal chromosomal abnormalities. Translocations and deletions involving a breakpoint at 1p11-p22 were the most common structural abnormality. The number of copies of chromosome 7 short arms was inversely correlated with survival, and a high concentration of asbestos fibers in the lung tissue was associated with partial or total loss of chromosomes 1 and 4, and a breakpoint at 1p11-p22.

Hagemeijer et al.[728] evaluated 40 confirmed cases of MM, in 90% of cases using malignant cells in pleural fluid. A normal karyotype was found in nine cases, and complex karyotypic abnormalities were identified in 30 cases. The chromosomal changes were all complex and heterogeneous, with no consistent specific abnormality found. Two main patterns of nonrandom abnormalities were found: (1) loss of chromosomes 4 and 22, 9p and 30p in the most abnormal cases, corresponding to a hypodiploid and for hypotetraploid modal chromosome number; and (2) gain of chromosomes 7, 5, and 20 with deletion or rearrangement of 3p.

Hicks[729] recently reviewed the biologic, cytogenetic, and molecular factors in mesotheliomas and mesothelial cell proliferations. As Hicks pointed out, these types of studies have been performed in an attempt to identify specific, nonrandom alterations that may be useful in diagnosing mesotheliomas and mesothelial cell proliferations. Hicks and others have reported that karyotyping mesotheliomas has not provided any specific diagnostic abnormalities. The changes one sees are listed in Tables 1 and 2 of Hicks's review article (see also the discussion of chromosomal abnormalities in Molecular Events in the Development of Mesothelioma III, above.)

Davidson et al.[730] reported on chemokine receptors expressed on malignant or benign mesothelial cells. They concluded that chemokine receptors were widely expressed on leukocytes in MM and reactive mesothelial effusions, but were rarely found on normal cells of mesothelial origin. The findings were stated to argue against an autocrine chemokine pathway in MM. An increased monocyte infiltration and higher expression of chemokine receptors in these cells in MM effusions could possibly have tumor-promoting rather than inhibiting effects.

Jaurand[731] reported on asbestos, chromosomal deletions, and tumor suppressor gene alterations in human MM, and found the most frequent alterations were on chromosome losses involving chromosomes 1, 3, and 9 (most often p arm), and chromosomes 6, 13, 14, 15, and 22 (most often q arm). Chromosomal gains were reported on chromosome 5 and 7 (most often on the p arm).

Janne[732] developed two proteomic methods to identify potential therapeutic targets. The first had to do with a pan-receptor tyrosine kinase and the second had to do with activators of the PI3K/Akt pathway.

Christensen et al.[733] reported on asbestos burden and epigenetic silencing in pleural MM and found that asbestos induced a pronounced epigenetic silencing of tumor suppressor genes in a fashion directly related to measurable lung function burden. They stated that this novel tumorigenic mechanism of action for asbestos had not been previously described and could help understand the role of asbestos in the development of MM, as well as the clinical course (see Molecular Pathogenesis and Pathology of Malignant Mesothelioma).

Rihn[734] evaluated oxidative stress gene modulation in pleural mesotheliomas as assessed by microarray and found dozens of overexpressed genes in mesothelioma that promoted local invasion; protected cells against oxidative stress; and counteracted the anticancer therapies. Rihn concluded the portrait of normal and cancerous

TABLE 43.20. DNA indices and proliferative rates of malignant mesotheliomas versus reactive mesothelial cells and other nonmesothelial malignant neoplasms

	Malignant mesothelioma					Reactive mesothelial cells					Non-mesothelioma malignant neoplasms				
		DNA index		S phase			DNA index		S phase			DNA index		S phase	
Study	No. of cases/ specimens	Diploid	Aneuploid	≤6%	>6%	No. of cases/ specimens	Diploid	Aneuploid	≤6%	>6%	No. of cases/ specimens	Diploid	Aneuploid	≤6%	>6%
Croonen et al.[736]	13[a]	10	3[b]	ND	ND	45[a]	40	5[c]	ND	ND	29[a]	7[d]	22	ND	ND
Hafiz et al.[737]	18[e]	30.5±[f]	7.2[f]	ND	ND	14[e]	15.2[f]	±2.9[f]	ND	ND	—				
Frierson et al.[738]	19[g]	9	10[i]	ND	ND	28[h]	28	0	ND	ND					
Burmer et al.[739]	46[j]	30[k]	15[l]	23[m]	22[m]	—	—	—	—	—	31[n]	4[o]	27[o]	15[p]	15[p]
Dazzi et al.[740]	70[q]	3[r]	34[r]	19[s]	36[s]	—	—	—	—						
Tierney et al.[741]	25[t]	u	U	ND	ND	11[t]	u	u	ND	ND	20[t]	—	20	ND	ND
El-Naggar et al.[742]	23[v]	18	6[w]	x	x				—	—	41[y]	5[z]	36[z]	[aa]	[aa]
Esteban and Sheibani[743]	45[bb]	30	5	cc	5[cc]	—	—	—	—	—	41[dd]	10	31	[ee]	[ee]

[a]Malignant cells in pleural/peritoneal fluids.
[b]Autopsy diagnosis was lung adenocarcinoma in two cases.
[c]In two of 54 cases there was an associated malignancy, but no evidence of malignancy on follow-up.
[d]In one case a primary tumor was not identified, and there was no evidence of recurrence or metastases.
[e]Cells in pleural or peritoneal fluid.
[f]Mean DNA content of 50 mesothelial cells in arbitrary absorbance units as determined by analysis of Feulgen-stained cells. The DNA content of mesothelial cells was compared to the DNA content of lymphocytes.
[g]Deparaffinized malignant epithelial mesothelioma.
[h]Reactive cells in pleural or peritoneal effusions.
[i]None of the malignant epithelial mesotheliomas had multiple aneuploid peaks.
[j]Of the 46 mesotheliomas, 30 were epithelial, five were sarcomatous, and 11 were biphasic.
[k]Diploid or near-diploid.
[l]All but two of the aneuploid mesotheliomas exhibited a single aneuploid peak. No significant difference between percentage of aneuploid mesotheliomas according to histologic type.
[m]Only "fresh" tissue specimens were analyzed and one case could not be evaluated. Average S phase for diploid mesotheliomas was 5.0% (17 cases) and 8.7% for aneuploid mesotheliomas (10 cases). No significant correlation between S phase and histologic subtype.
[n]Seven primary pulmonary adenocarcinomas; six primary poorly differentiated squamous carcinomas; three primary poorly differentiated carcinomas, not otherwise specified; four primary lung sarcomas; three metastatic sarcomas; four metastatic breast carcinomas; and four metastatic renal cell carcinomas.
[o]Nonmesothelioma malignant tumors that were diploid included one primary pulmonary adenocarcinoma, one metastatic renal cell carcinoma, one primary pulmonary sarcoma, and one metastatic sarcoma.
[p]One case could not be analyzed.
[q]168 paraffin-embedded tissue specimens from 70 patients with malignant pleural mesothelioma, 31 epithelial mesotheliomas, 21 sarcomatoid mesotheliomas, and 18 biphasic mesotheliomas.
[r]37 cases diploid or near-diploid, 34 cases aneuploid or multi-aneuploid.
[s]Phase % could be calculated in 55 cases. Range was 0.8–16.1%, median S phase was 6%.
[t]Feulgen stained nuclei of 100 tumor cells/reactive cells were measured using a DNA image analyzer. Lymphocyte nuclei were used as controls. Aneuploidy determined by measuring 5*c* exceeding rate (5*c*ER), which was defined as the percentage of aneuploid cells having a DNA content >5*c* where diploid = 2*c*. Previous studies suggested a 5*c*ER or greater than 0.1 was malignant. In this study, a 5*c*ER of 1 was used as a cutoff for malignancy.
[u]Information not given. Using the cutoff of 1 for 5*c*ER (see footnote t), 14 "mesothelial" cases were classified as benign, and 22 as malignant, which equated to a false-negative rate of 57% and a false-positive rate of 23%. All of the nonmesothelial tumors had a 5*c*ER >1, which indicated they were aneuploid.
[v]All cases were of pleural origin and had epithelial histology. Tumor tissue from multiple blocks were analyzed.
[w]The mesotheliomas that were aneuploid exhibited a "solid" growth pattern.
[x]S + G_2M of 18 diploid mesotheliomas was 5.83 ± 2.62 SD. S + G_2M of five aneuploid mesotheliomas was 5.0 ± 1.23 SD.
[z]Of the 36 aneuploid pulmonary adenocarcinomas, 31 were well to moderately differentiated, and five were poorly differentiated. Of the five diploid pulmonary adenocarcinomas, four were well to moderately differentiated, and one was poorly differentiated.
[aa]SG_2M of 5 diploid pulmonary adenocarcinomas was 12 ± 7.48 SD. SG_2M for 36 aneuploid pulmonary adenocarcinomas was 16.42 ± 10.21 SD.
[bb]Thirty-one epithelial mesotheliomas, six sarcomatoid mesotheliomas, eight biphasic mesotheliomas. Five of the 45 mesotheliomas could not be analyzed because the histograms obtained were uninterpretable; five other cases of mesothelioma were excluded because the coefficients of variation were >9.
[cc]Not all cases could be analyzed; in most aneuploid mesotheliomas the S phase could not be determined. Five (17) of the diploid mesotheliomas had an S phase >10%.
[dd]All cases were pulmonary adenocarcinomas.
[ee]Nine of the diploid adenocarcinomas had an S phase >10%.
ND, not done.

pleura achieved at the mRNA level seemed meaningful for the understanding of asbestos-mediated carcinogenesis, and for mesothelioma stratification and management. Rihn stated mesothelioma markers described in the study should improve the accuracy of mesothelioma diagnosis and therapy.

Bahnassy et al.[735] evaluated the role of $p14^{ARF}$, $p16^{INK4A}$, and their related genes in MM and concluded that pleural MM is a complex disease characterized by multiple genetic aberrations in the cell cycle regulatory genes. The authors identified regulatory genes that seemed to play a role in the pathogenesis of mesothelioma and also other pathways that were involved in the progression and survival of mesothelioma.

DNA Analysis and Proliferative Index in Malignant Mesothelioma

DNA concentrations or proliferative rates have been evaluated in reactive mesothelial cell proliferations and in MMs[736–743] (Table 43.20). Croonen et al.[736] concluded that mesotheliomas were usually DNA-euploid, whereas most adenocarcinomas were aneuploid. Hafiz et al.,[737] using cytophotometry to evaluate the DNA content of cells in Feulgen-stained sections of effusion specimens, found the mean DNA content of malignant mesothelial cells (30.5 ± 7.2) was significantly higher than the mean DNA content of reactive mesothelial cells (15.2 ± 2.9). Frierson et al.[738] determined that 53% of epithelial mesotheliomas were aneuploid, but considered that the finding of DNA aneuploid cells in an effusion specimen supported the diagnosis of MM. Burmer et al.[739] found most MMs to be DNA diploid with low to intermediate proliferative rates, whereas 85% of primary lung carcinomas were DNA aneuploid and had high proliferative rates.

In the study of Dazzi et al.,[740] 38.6% of mesotheliomas were diploid and 61.4% were aneuploid, and a higher percentage of epithelial mesotheliomas were diploid. The authors found no significant difference in survival in the patients whose mesotheliomas were aneuploid versus diploid. Patients whose tumor showed an S-phase percentage greater than the median of 6% had a significantly shorter survival than those whose tumors had a lower S-phase percentage. Tierney et al.[741] determined DNA cellular concentrations using DNA image analysis of Feulgen-stained tissue sections. These authors concluded that mesothelial lesions appeared to have a wide range of ploidy values regardless of their biologic behavior, and that ploidy could not be used as a reliable diagnostic index in diagnosing primary mesothelial tumors.

El-Naggar et al.[742] analyzed epithelial mesotheliomas by flow cytometry and compared them with pulmonary adenocarcinomas, and found that 80% of pulmonary adenocarcinomas and 100% of pleural mesotheliomas showed a homogeneous DNA ploidy; 78% of epithelial mesotheliomas were diploid, whereas 88% of pulmonary adenocarcinomas were aneuploid. The proliferative fraction (S-phase percentage) of aneuploid adenocarcinomas was significantly greater than aneuploid epithelial MMs, leading the authors to conclude that the DNA indices of epithelial mesotheliomas were significantly different from pulmonary adenocarcinomas. Esteban and Sheibani,[743] in their flow cytometric analysis, found that 14% of mesotheliomas were aneuploid; in contrast, 75% of pulmonary adenocarcinomas were aneuploid. These authors recommended that ploidy analysis should be used in diagnostically difficult cases of possible mesothelioma.

More recently, Cakir et al.[744] evaluated cell proliferation rate and telomerase activity in the differential diagnosis between benign and malignant mesothelial cell proliferations. By means of immunohistochemical analysis for Ki-67 and human telomerase reverse transcriptase (hTERT), the mean value of Ki-67 proliferation index in MMs was found to be significantly higher than that of benign mesothelial lesions. Ki-67 immunohistochemistry was reported to have a sensitivity of 74%, a specificity of 86%, and a positive predictive value of 94% in detecting MM. The hTERT immunohistochemistry detected MM with a sensitivity and specificity of 68%. The authors suggested that immunohistochemistry profiling for Ki-67 and hTERT was useful in differentiating malignant and benign mesothelial lesions in routine formalin-fixed, paraffin-embedded material.

Rare/Unusual Mesotheliomas or Mesothelial Proliferations

Benign Mesothelial Inclusions in Lymph Nodes

Although regional lymph node metastases can occur with pleural MMs—to axillary, cervical, bronchial, mediastinal, and retroperitoneal lymph nodes—as part of late-stage disease (for example, as an autopsy finding[211]) or even as a presenting manifestation.[745–748] Such metastatic deposits require distinction from benign mesothelial inclusions within subpleural, bronchial, or mediastinal lymph nodes,[119,749,750] related to chronic inflammatory processes affecting the pleura (and occasionally other serosal membranes). In some cases, the reactive mesothelial cell inclusions are confined to or concentrated within the subcapsular sinuses (Fig. 43.88), but deeper extension into lymph node tissue is also recorded and no clear criteria for histologic discrimination between benign inclusions of this type and metastatic MMs have been delineated. Accordingly, the International Mesothelioma

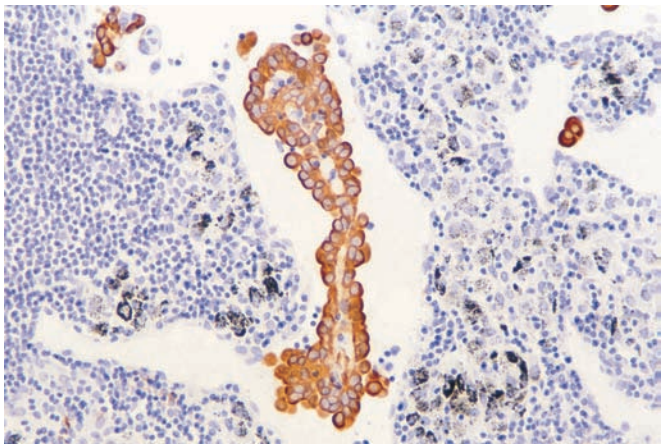


FIGURE 43.88. One of multiple benign mesothelial inclusions with a papillary architecture found in bronchial lymph nodes in an elderly woman who had undergone pneumonectomy for treatment of a non–small-cell carcinoma of lung. The mesothelial cells are concentrated within sinusoids. Their identity as mesothelial inclusions was established in this case by immunohistochemistry and by electron microscopic examination of deparaffinized tissue.

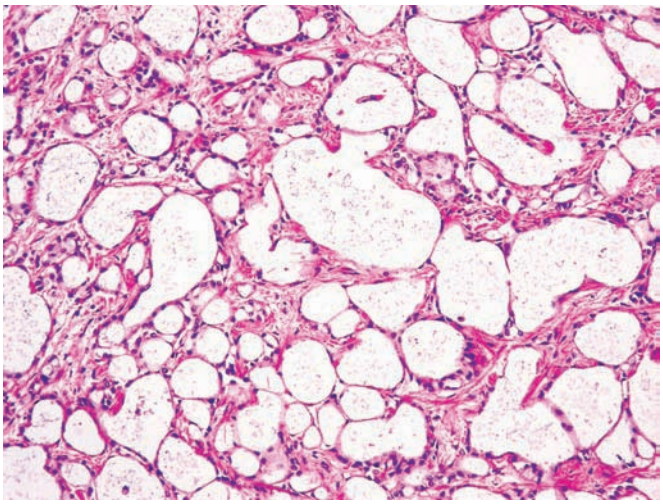


FIGURE 43.89. Pleural adenomatoid tumor discovered as an incidental autopsy finding in an elderly man. The tumor consists entirely of microcystic spaces lined by attenuated cells, with a sparse intervening fibrocollagenous stroma.

Panel[37] recommends that a diagnosis of metastatic mesothelioma within lymph nodes should be supported by one or both of the following criteria: (1) a diagnostic biopsy of the corresponding serosal membrane, or (2) radiologic evidence supportive of an underlying pleural mesothelioma (such as diffuse pleural thickening with encasement of the lung, accompanied by evidence of nodularity).

Adenomatoid Tumor of the Pleura

Characteristically, adenomatoid tumors represent benign mesothelial tumors that develop in relation to the reproductive tract of either males (testis/epididymis[751]) or females (uterus[752]). In these locations they are often clinically silent lesions, although they can produce clinically detectable localized mass lesions (especially in relation to the testis/epididymis).

On gross examination, adenomatoid tumor is a nonencapsulated and usually poorly delineated firm, pale yellow mass. Histologic examination reveals unencapsulated lesions that comprise multiple microcystic spaces and complex tubules, embedded within a fibrous stroma and lined by flattened epithelial-type cells that express immunohistochemical markers of mesothelial differentiation. The differential diagnosis includes lymphangioma, and it is important to emphasize that the antibody D2-40 labels both lymphatic endothelium and mesothelial cells.[632,753]

Pleural adenomatoid tumors[37,754,755] (Fig. 43.89) are exceedingly rare and typically represent small and clinically silent lesions.[37] The major differential diagnosis for pleural adenomatoid tumor is that of a conventional MM with focal or extensive microcystic change producing an adenomatoid appearance (Fig. 43.90; also see Figs. 43.27 and 43.28). Accordingly, the following criteria for the diagnosis of pleural adenomatoid tumor are suggested[37]:

- The tumor typically is a lesion found incidentally either in surgery (thoracoscopy or thoracotomy) carried out for other reasons[755] or at autopsy (Fig. 43.89); that is, there should be no clinical manifestations such as a

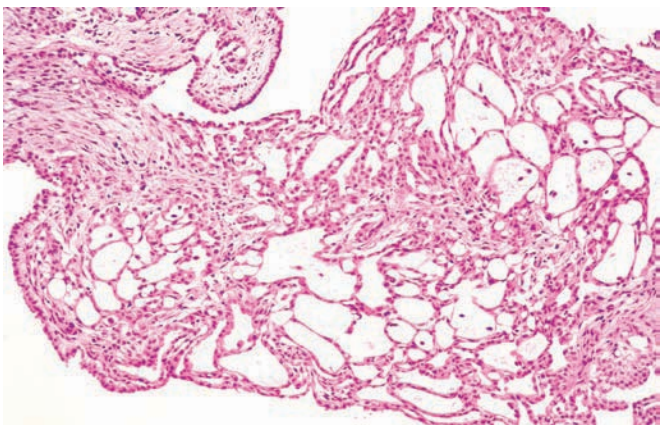


FIGURE 43.90. Pleural malignant mesothelioma with focal microcystic (adenomatoid) features, in a woman in her 30s, who had sustained childhood environmental exposure to crocidolite at Wittenoom in Western Australia. In its advanced clinical stage, this mesothelioma showed prominent transdiaphragmatic spread into the peritoneum, with intractable ascites.

pleural effusion directly attributable to the adenomatoid tumor.
- The tumor is a small localized lesion; the International Mesothelioma Panel has suggested that it should be less than 5 mm in greatest dimension,[37] but occasionally it may be larger (Fig. 43.89).
- The histologic appearance is of a benign adenomatoid tumor throughout. It is recommended that the entire lesion be embedded and sectioned, with no areas characteristic of conventional MM of epithelial type.[37] Tumors that show areas of conventional MM with either focal or extensive adenomatoid features should be designated as a conventional MM showing microcystic change (Fig. 43.90).
- The phenotype should conform to a mesothelial lesion on either immunohistochemistry or electron microscopy, or both.
- The differential diagnosis in the pleura also includes an epithelioid hemangioendothelioma, from which adenomatoid tumors are distinguishable by absence of labeling for markers of endothelial differentiation (CD31, CD34, and factor VIII–related antigen[37]). In addition, although some epithelioid hemangioendotheliomas show weak to moderate expression of cytokeratins, most do not, whereas adenomatoid tumors characteristically show moderate to strong cytokeratin expression, like other mesothelial lesions.

Well-Differentiated Papillary Mesothelioma

Well-differentiated papillary mesothelioma (WDPM) is well recognized in the peritoneum, usually in middle-aged women.[756–767] The median age in the series of 22 cases reported by Daya and McCaughey[759] was 40 years (range, 25–69 years), and 18 of the 22 patients were women. A WDPM may represent either solitary and localized lesions or multifocal tumors, and it generally measures about 5 to 20 mm in diameter. Some authors consider the localized lesions to be benign and amenable to cure by local resection,[757,764,768] whereas others designate WDPMs as tumors of borderline or attenuated malignant potential,[758,763] with an indolent natural history[765] even when they are multifocal.[762] Even so, one of 14 cases so diagnosed by Butnor et al.[767] pursued an aggressive clinical course. Rare examples of WDPM have also been encountered in the pericardium,[769] the tunica vaginalis testis,[767,768] and the pleura.[766,767,770]

Butnor et al.[767] reported 14 cases of WDPM, seven of which affected the pleura, six in the peritoneum, and one in the tunica vaginalis. Eleven of the patients were men and three were women (presumably reflecting a selected group of patients, as expected for a tertiary referral center), with an average age of 58 years (range, 32–82 years). Six of the patients had a history of asbestos exposure. Of nine cases with complete follow-up, six had clinically indolent disease, but one case pursued an aggressive course. The authors concluded that WDPM represents "a rare variant of mesothelioma with a variable clinical prognosis . . . etiologically related to asbestos exposure in some cases" (whereas peritoneal WDPMs affecting young to middle-aged women are typically not associated with a background of asbestos exposure).

Subsequently, Galateau-Sallé et al.[770] reported a series of 24 cases that were classified as WDPM affecting the pleura, in 11 men and 13 women, with a mean age of 60 years (range, 31–79 years). The cases were selected on the basis of a "relatively uniform spreading of papillary formations with very limited or no invasion." In 10 cases, invasion was present at the time of diagnosis "but was strictly limited to the submesothelial layers," with no extension into lung parenchyma or subpleural adipose tissue. However, the histologic appearances of the tumors "in 2 cases at the time of progression of the disease was like . . . [that] of conventional epithelioid mesothelioma." Twenty-two of the cases presented with pleural effusion, hemorrhagic in some, and accompanied by pneumothoraces in two patients, and only one was an incidental finding. Nine cases had radiologic evidence of "thin focal pleural thickening" and the oldest patient showed contraction of the affected hemithorax. The findings at thoracoscopy for six patients were those of multiple small (millimeter-sized) nodules over the parietal or visceral pleura, producing a "velvety" appearance. With progression of disease, pleural nodularity developed, sometimes with encasement of the lung, and in one case there was dissemination into the peritoneum. Eleven of the patients had a history of asbestos exposure, occupational in character except for two patients with household contact (domestic exposure). Among 11 patients with follow-up data for a minimum of 24 months, the average survival was 74 months (range, 36–180 months) with a 10-year survival rate of almost 31%, in comparison to an average survival of about 10 months for 1248 paired patients with conventional MM.

We have encountered occasional cases of pleural mesothelioma where areas histologically indistinguishable from WDPM coexisted with other areas characteristic of conventional MM of epithelial type.

Here is our approach to these lesions:

- The diagnosis of *apparently benign, well-differentiated papillary mesothelioma* should be restricted to such solitary and localized terms when they are discovered as an incidental finding at thoracoscopy, thoracotomy, at autopsy, with no clinical symptoms or an effusion directly attributable to the lesion itself, and when the lesions comprise papillary to club-shaped processes with a core of fibrovascular tissue covered by a layer of bland mesothelial cells, with no evidence of invasion (Fig. 43.91). Benign WDPMs so diagnosed do

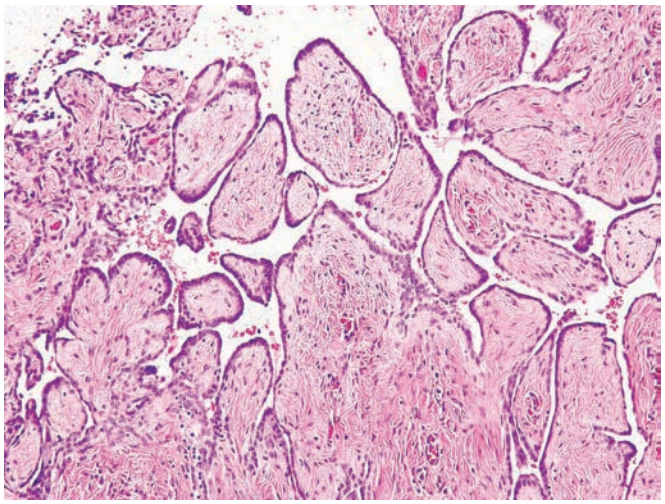


FIGURE 43.91. Well-differentiated papillary mesothelioma (WDPM) of the peritoneum, discovered incidentally at laparotomy carried out for other reasons; this lesion comprised this pattern of tissue entirely and was noninvasive in character.

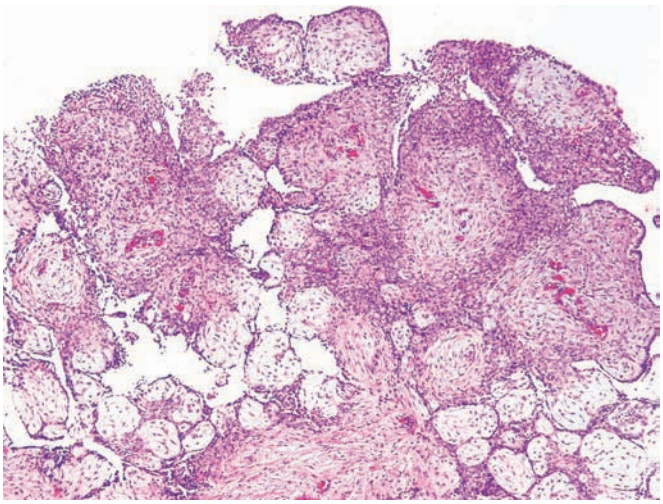


FIGURE 43.92. Pleural mesothelioma with areas of WDPM. Multilayering of the mesothelium covering some of the WDPM formations.

not require radical surgery or chemotherapy,[762] but instead should simply be observed by way of clinical follow-up.

- Multifocal pleural tumors with features of WDPM (Figs. 43.92 and 43.93), when associated with pleural effusion or pleural thickening, frequently pursue a progressive clinical course even when they are only minimally and superficially invasive, with significant morbidity and mortality[770]; however, evidence indicates that the WDPM appearances are associated with a more indolent course than pleural MM, with longer survivals in most cases.
- When areas of WDPM are admixed with other areas of invasive mesothelioma, where the appearances would allow a diagnosis of conventional MM in the absence of the WDPM-like foci, we diagnose such lesions as a pleural MM with WDPM-like areas. The WDPM-like tissue may point to a more indolent clinical course than ordinary pleural MMs. In this regard, the natural history of WDPM in terms of survival times seems to be related directly to the proportion of the WDPM-like tissue, and inversely to the extent of the lesion(s) and their invasiveness.

Noninvasive Atypical Mesothelial Proliferations: The Concept of Mesothelioma In Situ and Discrimination Between Early-Stage Mesothelioma and Reactive Mesothelial Hyperplasia

In the 1980s Bolen et al.[536,771] proposed a multipotential subserosal fibroblastoid cell as the stem cell for mesothelial healing and regeneration, and as the progenitor cell for mesothelioma development, and proposed that an origin of mesothelioma from such subserosal cells could account for the bidirectional differentiation characteristic of biphasic mesotheliomas. As an alternative model,

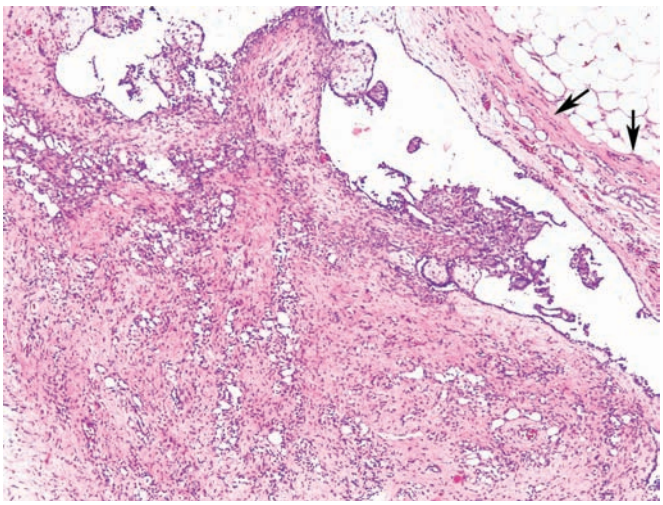


FIGURE 43.93. Pleural mesothelioma with WDPM-like areas. (Same case as in Fig. 43.92.) The apparent invasion in the lower part of this field is explicable in part by an en face appearance resulting from a tangential plane of section, but not entirely so, taking into account the extent of the epithelial-type tumor within the submesothelial fibrous tissue. En profile infiltration into the pleural fibrous tissue is also evident, where there is no suggestion of an oblique plane of section (arrows), and there were multiple other areas of undoubted invasion into the pleural fibrous layer.

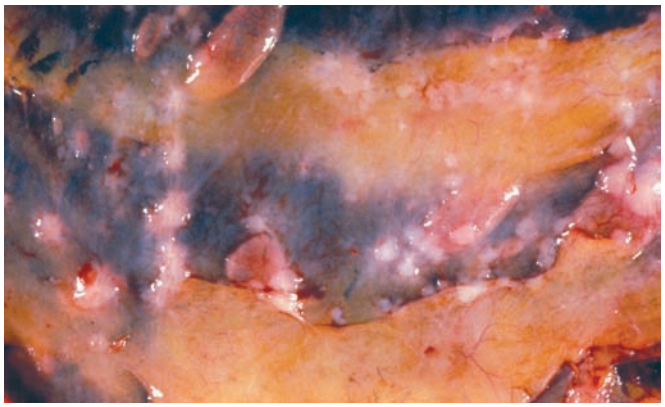


FIGURE 43.94. Pleurectomy specimen from a patient who presented with a massive pleural effusion. No distinctive abnormality was seen at thoracoscopy, but multiple random biopsies revealed an extensive atypical mesothelial proliferation, in situ in most areas of the biopsies, but with small foci of invasion. A pleurectomy was subsequently carried out, and in the surgical specimen, small foci of white invasive tumor were found, some of which extended into subpleural adipose tissue. The entire pleurectomy specimen was examined as a series of Swiss roll sections, and the areas devoid of invasive mesothelioma were seen to show extensive in situ mesothelial atypia.

Whitaker et al.[489] advanced the concept of mesothelioma in situ, based in part on experimental models of mesothelial healing following injury that did not disrupt the submesothelial basal lamina,[492,493,495,] and on their observation of a number of cases of apparently early-stage MM of epithelial type, where mesothelial atypia appeared to be predominantly in situ, in the absence of any radiologic or gross anatomic evidence of pleural thickening or nodularity. This being so, Whitaker's group[489,679] suggested that mesothelioma in situ could be defined as the replacement of benign surface mesothelium by mesothelial cells with markers of malignancy. The problem was to define an acceptable and consistently reproducible marker of neoplastic change. Accordingly, they described 22 cases of pleural disease characterized by atypical and predominantly in situ mesothelial proliferation.[489] The cases had presented in conventional fashion with a pleural effusion with either no identifiable pleural tumor or only tiny nodules at thoracoscopy (Fig. 43.94), and the diagnosis in a number of cases was established by existing cytologic criteria. Whitaker et al.[489] suggested that the markers for MM in situ in pleural biopsies included the following[119,490]:

- *Absence of background inflammation* as a potential drive for reactive mesothelial hyperplasia (to which one could add the clinical absence of any underlying cause or association for pleural inflammation and reactive mesothelial proliferation).
- *An abnormal architecture of the mesothelium at the surface of the affected pleural tissue.* The architectural abnormalities included noninvasive, linear, papillary and tubulopapillary patterns, sometimes with a complex exophytic architecture (Fig. 43.95). Whitaker's group[679] emphasized that a prominent papillary pattern of mesothelial proliferation in pleural biopsies is a disturbing feature, not usually seen with reactive mesothelial hyperplasias (although this observation does not apply to mesothelial proliferations affecting the peritoneum and in relation to the omentum in particular).
- Substantial cytologic atypia (Fig. 43.95B), but Whitaker's group also considered that other cases might occur where there is substantially less cytologic atypia, so that such cases would be diagnosable (if at all) only by ancillary techniques. Among these techniques they included strong linear labeling for EMA or areas occupied by silver labeling of nucleolar organizing regions (AgNORs), in excess of the areas found in proven benign reactive mesothelial proliferations.
- In relation to labeling for EMA, Whitaker et al.[489] found that 17 of 22 cases showed thick linear labeling

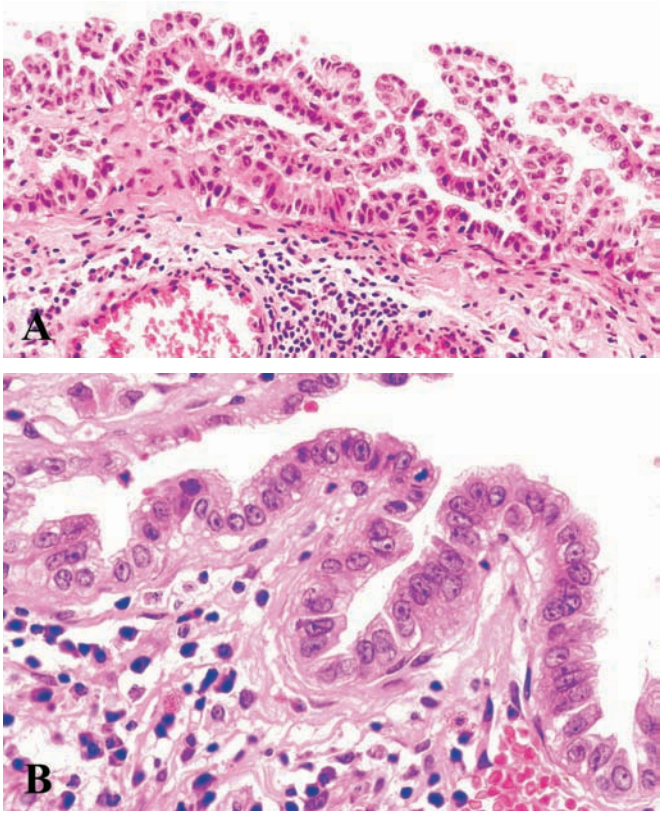


FIGURE 43.95. **(A)** Atypical mesothelial proliferation seen in a pleural biopsy, with an exophytic papillary architecture at the surface. **(B)** Same pleural biopsy illustrating the mesothelial atypia at higher magnification.

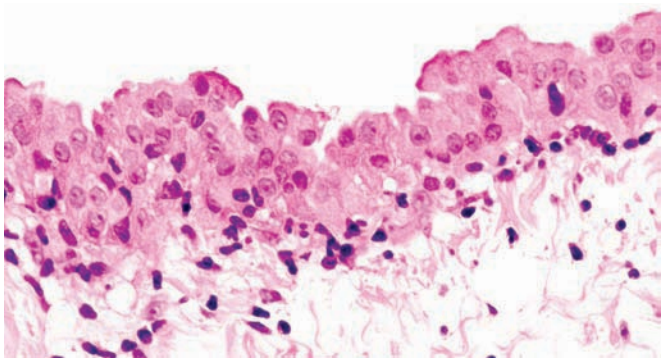


FIGURE 43.96. Example of benign reactive mesothelial proliferation in a pleural biopsy taken from a patient with proven lung cancer. Compare with Figure 43.95B.

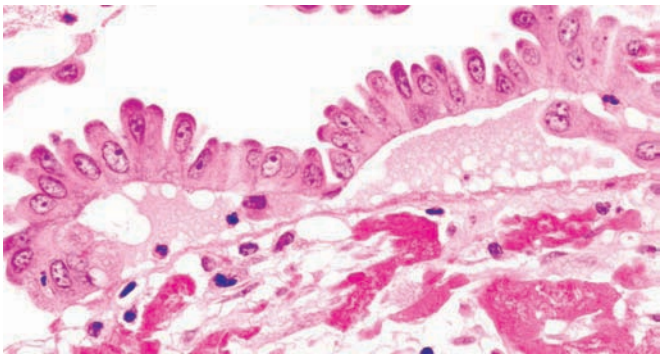


FIGURE 43.98. Prominent reactive mesothelial atypia in a case of organizing fibrinous pericarditis. Fibrinous exudate is evident in the lower half of this field.

of the mesothelial cells for EMA, whereas proven benign reactive mesothelial proliferations usually showed no significant labeling or only patchy weak labeling, as studied in the same laboratory.[119] (On the other hand, we emphasize that in tissue biopsies, a substantial proportion of cases of invasive mesothelioma may show no detectable immunohistochemical labeling for EMA.) Saad et al.[671] investigated EMA expression in 20 cases of reactive mesothelial proliferation (RMP) versus 20 cases of MM, using antibodies based on the Mc5 and E29 clones. For the Mc5 clone, there was positive staining in 14/20 cases of MM (70%) and 12/20 cases of RMP (60%); for the E29 clone, the corresponding results were 15/20 for MM (75%) and 0/20 for RMP. For the E29 clone, the sensitivity and specificity for MM were 75% and 100%, respectively. The authors concluded that EMA antibodies based on the E29 clone are a reliable discriminator between RMP and MM. Simon et al.[402] also commented on this pattern of EMA labeling as a discriminator between benign RMPs and areas of seemingly in situ MM.

Nonetheless, because it is known that there is overlap in the degree of cytologic atypia between benign reactive mesothelial proliferations (Figs. 43.96 to 43.98) versus mesothelioma[37,490,503,513] (Fig. 43.95), Whitaker et al.[489] and Henderson et al.[119] emphasized that the only consistently reliable marker for mesothelioma as opposed to RMP is the presence of acceptable neoplastic invasion in the same biopsy, or a different biopsy taken at a different time, or at autopsy (Figs. 43.99 to 43.103). Accordingly, Henderson et al. commented in 1997:

> We caution against rash or premature diagnosis of mesothelioma in situ from conventional light microscopy examination of

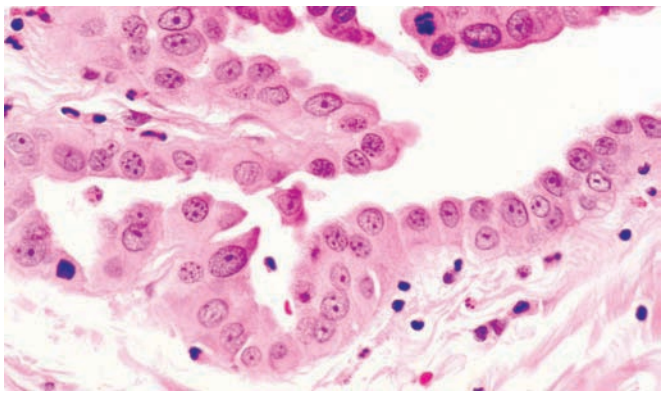


FIGURE 43.97. Extreme reactive mesothelial atypia as seen in the visceral and parietal layers of the pleura, in an apical wedge resection specimen of lung and in the pleura, from a man in his 20s, with a history or recurrent pneumothoraces on the same side.

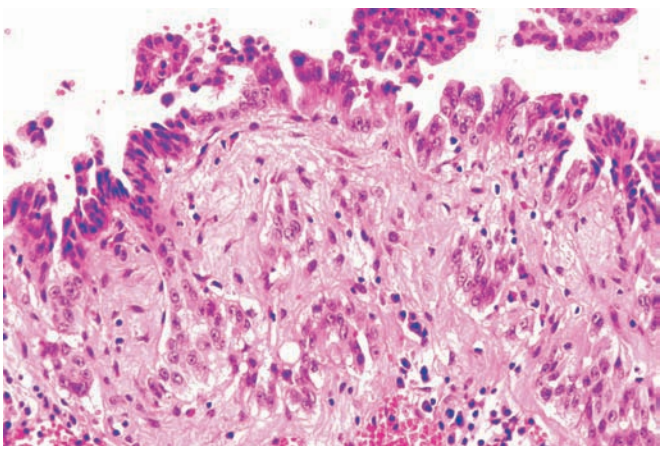


FIGURE 43.99. Different area of the same biopsy shown in Figure 43.95. Foci of infiltration into the submesothelial fibrous tissue are evident, so that the findings overall were interpreted as those of an exophytic-papillary mesothelioma in situ with multiple foci of superficially invasive mesothelioma.

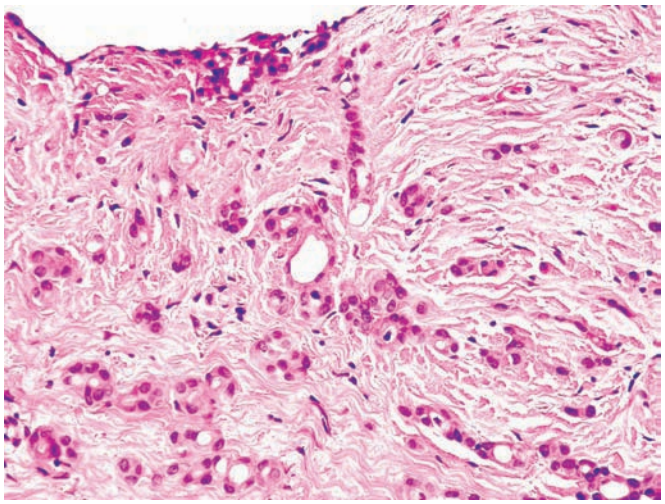


FIGURE 43.100. Early-stage invasive mesothelioma of epithelial type, with infiltration of the submesothelial fibrous tissue, in a pattern that is inconsistent with benign mesothelial entrapment as part of a fibroinflammatory process. In addition, this biopsy showed no evidence of exudative inflammation. There is only minor cytological atypia.

biopsy tissue, taking into account that there is overlap in the cytologic abnormalities that occur in reactive mesothelial hyperplasias versus mesothelioma. However, [findings suggestive of a component of mesothelioma in situ] (especially in conjunction with effusion fluid cytology) may delineate "at risk" patients with "early" stage disease who require further investigation and follow-up. Because of the minimal and perhaps predominantly in situ tumor burden, the mesotheliomas may also be amenable to new modalities of therapy, and some of our "in situ" patients have had prolonged survivals.

Henderson et al.[119,490] also emphasized that in all of their cases,[489,679] biopsy or autopsy examination did

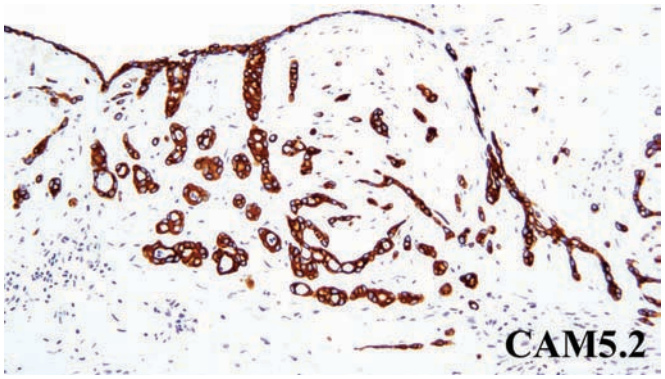


FIGURE 43.101. Same biopsy as illustrated in Figure 43.100, immunostained for low molecular weight cytokeratin (CAM5.2), illustrating the pattern of infiltration into the pleural fibrous layer.

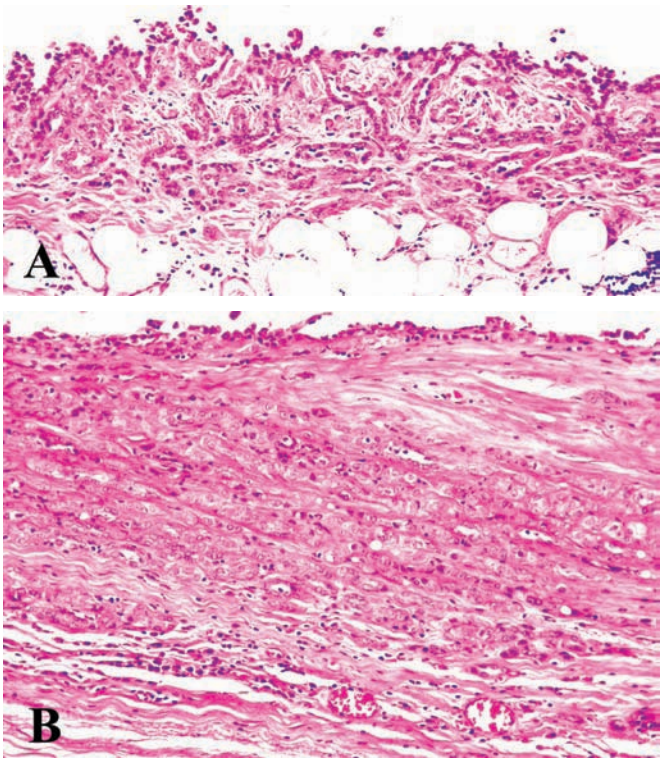


FIGURE 43.102. **(A,B)** Early-stage invasive malignant mesothelioma of epithelial type. Both of these figures are from the same case. The parietal pleural biopsy showed multiple foci of infiltration into the fibrous layer of the pleura, in the absence of exudative inflammation, with only equivocal and focal extension of a few mesothelial cells into the subjacent fat. The pattern of infiltration into the fibrous layer, with near-filling by linear and compressed tubular aggregates of mesothelial cells **(B)** is inconsistent with benign entrapment.

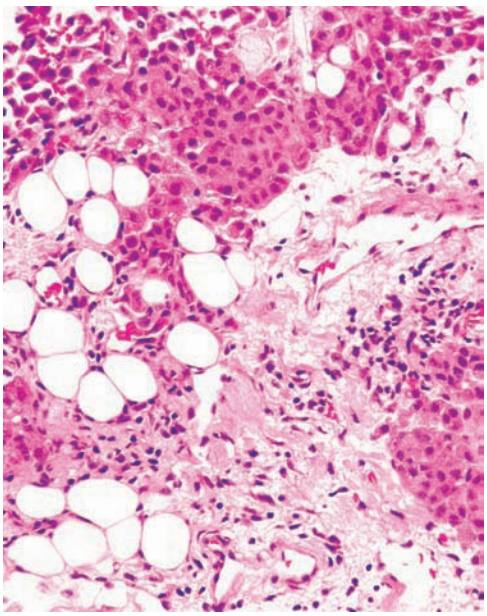


FIGURE 43.103. Area of invasion into subpleural adipose tissue, as a marker of malignancy for this mesothelial proliferation. The proliferation was noninvasive elsewhere in the biopsy.

confirm the development of invasive mesothelioma, but one patient was still alive at the time of writing[119] (with only a short period of follow-up). (Subsequently, Churg et al.[503] commented that in one instance in which Whitaker et al.[489] and Henderson et al.[119,679] "made a diagnosis of mesothelioma in situ without the presence of invasive tumor, the lesion appeared to have been benign on follow-up.")

Churg et al.[503] suggest that the term *mesothelioma in situ* not be used, and instead noninvasive atypical mesothelial proliferations should be designated as either "atypical mesothelial hyperplasia" or "atypical mesothelial proliferation" (the latter being favored by the International Mesothelioma Panel[37]). We have no argument with the term *atypical mesothelial proliferation* for entirely noninvasive atypical mesothelial lesions, but we would discourage use of the term *atypical mesothelial hyperplasia*, because by definition the word *hyperplasia* indicates a benign process, whereas the reactive versus neoplastic status for such lesions is indeterminate.

As a further point, we would emphasize that complex exophytic mesothelial proliferations, such as illustrated by Churg et al.[503] in their Fig. 4.23A,B, are not patterns usually or typically encountered with benign inflammation-induced mesothelial proliferations. Such appearances (Fig. 43.95A) raise a suspicion of MM where the invasive component (if present) has not been sampled by the biopsy. Such lesions should not be dismissed as benign; they are an indicator for close follow-up and further cytologic or biopsy investigation, as indicated by Churg et al. In other words, noninvasive atypical mesothelial proliferation in biopsy tissue does not correspond to a treatable disorder, but instead is a requirement for follow-up or further investigation.

It is sometimes stated that there is no proof that in situ mesothelial atypia in association with areas of invasive mesothelioma represents the same lesion.[503] However, Simon et al.[402] did report a single case of mesothelioma in situ in association with focal early-stage invasive mesothelioma. They investigated the lesion by laser microdissection and comparative genomic hybridization and found similar chromosomal alterations in both the areas of in situ mesothelial atypia and in the foci of early invasive mesothelioma. Accordingly, in the areas of mesothelioma in situ they recorded losses at 3p, 5q, 6q, 8p, 9p, 15q, 22q, and Y, with a gain on 7q; in the area of early invasive mesothelioma there were losses at 3p, 5pq, 6q, 8p, 9p, 15q, and 22q with no gain. In contrast, the advanced mesothelioma showed losses at 1p, 4pq, 6q, 9p, 13q, 14q, and 22q, with gains at 1q, 7pq, and 15q.

We still consider mesothelioma in situ to be a useful concept concerning the development of MM. In addition, by refocusing attention on the mesothelium itself as the target for neoplastic transformation, this concept points to the potential for diagnosis of noninvasive mesotheliomas, with the promise of more effective therapy in the future. We continue to believe that the term *mesothelioma in situ* represents a valid retrospective diagnosis in cases where at least early-stage invasive mesothelioma has been demonstrated.

As discussed above and illustrated in Figures 43.95B to 43.98, there can be substantial overlap in the degree of cytologic atypia encountered in proven atypical reactive mesothelial hyperplasias, versus proven invasive MMs of epithelial type. Although thick linear membrane-related labeling for EMA (using antibodies based upon the E29 clone) may sway the probability index toward a diagnosis of early-stage mesothelioma, this finding cannot be considered decisive or definitive, and at present there is no universally accepted immunohistologic or molecular marker for consistent discrimination between reactive mesothelial hyperplasia versus MM. This being so, histologic assessment of invasion is crucial to the diagnosis of MM and its discrimination from an atypical reactive mesothelial proliferation, in everyday diagnostic practice. We have found the following guidelines and caveats to be useful in the approach to differential diagnosis of mesothelial lesions where the discrimination between mesothelioma and hyperplasia is problematic:

- It is useful to correlate the histologic appearances with the findings on pleural effusion fluid cytology and with any abnormalities revealed by imaging studies, such as chest radiographs or CT scans. In this regard, the radiologic investigations can be regarded as a surrogate for gross anatomic findings. For example, radiologic demonstration of a confluent and nodular pleural lesion with encasement of the lung and contraction of the affected hemithorax together with an effusion (in which a florid atypical papillary mesothelial proliferation was found) can effectively substitute for the histologic detection of invasion, at a high order of confidence.
- Neoplastic invasion of subpleural adipose tissue (Fig. 43.103) or deeper structures by epithelioid cells that show a mesothelial phenotype on immunohistochemistry or by spindle-shaped fibroblastoid cells that express cytokeratins represents a decisive indicator of malignancy, for either epithelial mesothelioma or sarcomatoid mesothelioma respectively.
- Even so, mesothelioma remains diagnosable even when there is no infiltration into subpleural tissues such as fat, provided that the pattern of invasion within more superficial tissues, namely the pleural fibrous layer, is characteristic or diagnostic of neoplastic invasion (Figs. 43.99 to 43.102) as opposed to artifact or benign entrapment of mesothelial cells as part of an organizing fibroinflammatory process (see below).

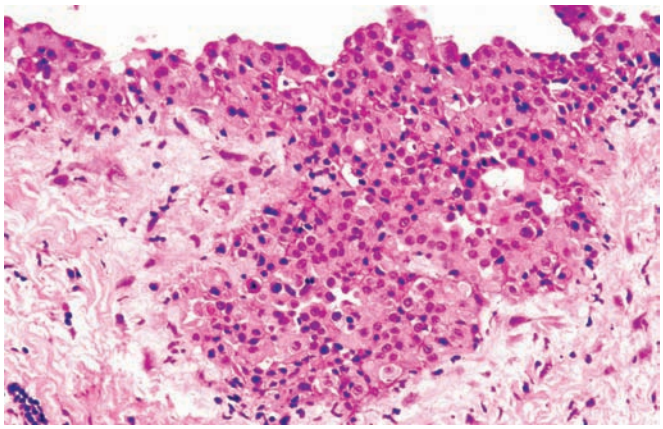


FIGURE 43.104. Pseudo-invasion in a case of benign reactive mesothelial hyperplasia, resulting from folding of the pleural membrane. (Same case as in Fig. 43.96.)

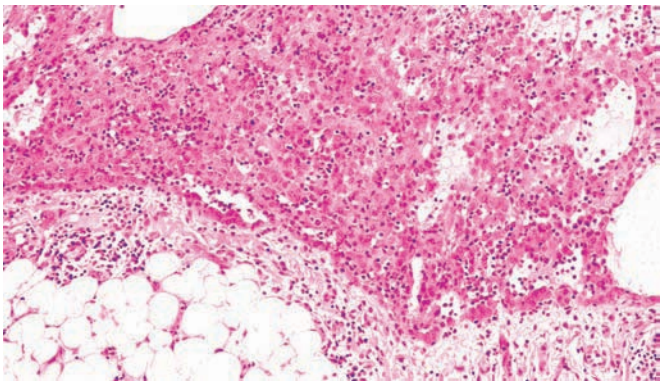


FIGURE 43.105. Prominent benign reactive mesothelial hyperplasia in a patient with proven tuberculous pleuritis. It is evident that the mesothelial proliferation is entirely noninvasive in character, and admixed with numerous inflammatory cells.

- We take great care to ensure that pleural biopsies are orientated correctly when subject to histologic sectioning, so that the tissue is embedded on edge, resulting in *en profile* as opposed to *en face* sections, because the latter can create problems concerning interpretation over what is, and what is not, acceptable evidence of invasion. When sufficient pleural membrane is available, we find it useful to prepare a *Swiss roll* from the biopsy and to fix the pleural tissue after the Swiss roll has been prepared, then taking a series of transverse sections, to facilitate correct orientation of the pleural membrane.
- It is necessary to discriminate between pseudo-invasion, for example, resulting from an *en face* plane of section through the biopsy or from folding of the pleural membrane (Fig. 43.104) versus genuine neoplastic infiltration of the submesothelial tissue. When there is doubt over whether the process represents pseudo-invasion versus genuine neoplastic invasion, we dismiss the appearances as inconclusive.
- Although most inflammation-driven reactive mesothelial hyperplasias are noninvasive (Fig. 43.105), some organizing serosal inflammatory reactions, especially in the pericardium in our experience, can result in the burying of hyperplastic mesothelial cells within the organizing and proliferative fibrous tissue, so that this well-recognized pattern of benign mesothelial entrapment requires distinction from genuine invasion (cf. Figs. 43.99 to 43.102 with Figs. 43.106 and 43.107). The presence of a florid fibrinous or neutrophilic inflammatory reaction should alert one to the likelihood of benign entrapment, but the authors have encountered cases of proven invasive mesothelioma with prominent associated inflammatory exudate. In such organizing inflammatory processes, the entrapment appears to be the result of burying of the site where the mesothelium is normally located, by a layer of inflammatory exudate that extends across the surface of the membrane, with subsequent organization. This process of entrapment of mesothelium is sometimes designated as mesothelial sequestration, but in many instances the lowermost level of the entrapped mesothelium seems actually to be situated at its original level. Instead, it is the surface of the pleura that has moved inward, into the lumen of

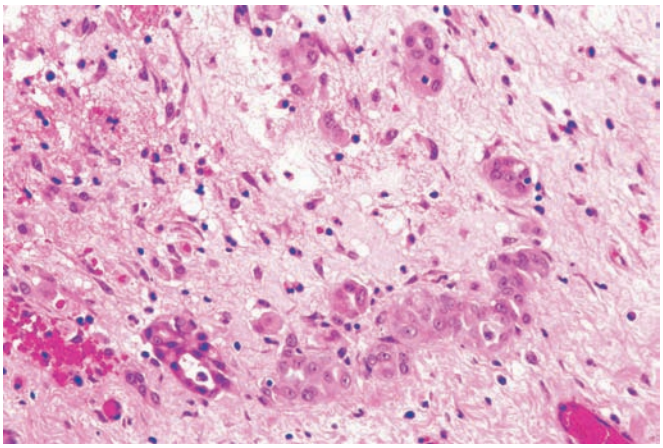


FIGURE 43.106. Benign mesothelial entrapment in a case of constrictive pericarditis, in a young man. Islands and tubules formed by mesothelial cells are evident within the fibrous tissue. There was prominent fibrinous inflammatory exudate near the surface of the pericardium (near the top left hand corner). In addition, the proliferative mesothelial cells in this biopsy showed scattered intracytoplasmic mucin-like droplets, stainable by the PAS-diastase stain. Follow-up for a period of over 5 years was entirely benign.

the pleura (or pericardium), a process that we sometimes liken to the shrinking of the Aral Sea and that we designate as the *Aral Sea effect.* In other words, ships marooned by the shrinkage of the Aral Sea have not moved into the surrounding desert, but rather the shoreline has moved away from the ships. In this regard, we find immunohistochemical staining for cytokeratins (or calretinin) to be of value, because it delineates a clear boundary between the zone of the proliferative and entrapped mesothelial cells, versus the deeper tissues, as shown in Figure 43.107.

- Therefore, neoplastic invasion remains the linchpin for diagnosis of early-stage mesotheliomas of epithelial type. When there is any doubt over whether genuine invasion is present or not, we prefer to err on the side of underdiagnosis of mesothelioma as opposed to inappropriate overdiagnosis. We base this approach on the principle that if the lesion is mesothelioma, it will declare itself as such soon enough, whereas inappropriate overdiagnosis of mesothelioma can lead to erroneous cytotoxic chemotherapy or even radical surgery, together with the anguish that a diagnosis of mesothelioma usually entails.
- Even when invasion cannot be found in a biopsy sample, there are several findings in combination that are suspicious of mesothelioma, although nondiagnostic by themselves. They include the extent of the mesothelial proliferation, the presence of a complex exophytic or papillary architecture at the surface of the pleura (in the absence of exudative inflammation), prominent cytologic atypia, focal necrosis within sheets of proliferative mesothelial cells in the pleura (Fig. 43.108), prominent intracytoplasmic vacuoles devoid of mucin-like content, and strong thick linear labeling for EMA (using antibodies based on the E29 clone). The presence of two or three or more such features is an indication for clinical follow-up or further investigation, to clarify the hyperplastic versus neoplastic properties of the mesothelial proliferation.

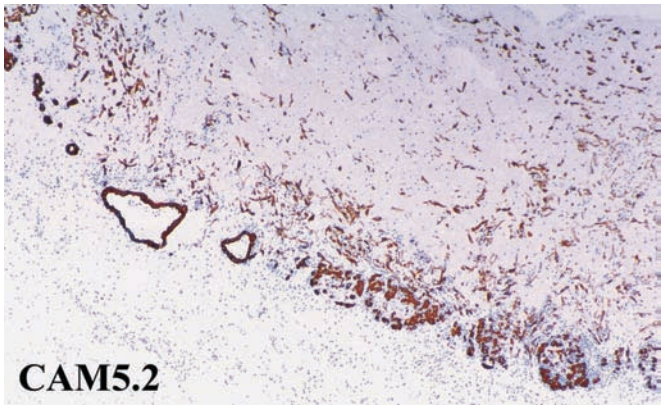


FIGURE 43.107. Same biopsy as illustrated in Figure 43.106, immunostained for low molecular weight cytokeratin (CAM5.2). Note the reasonably clear demarcation or boundary zone between the entrapped mesothelial cells and the deeper tissues, the appearances being unlike those of neoplastic invasion by a malignant mesothelioma, where the deep boundary of the lesion is less sharply demarcated and is infiltrative in character.

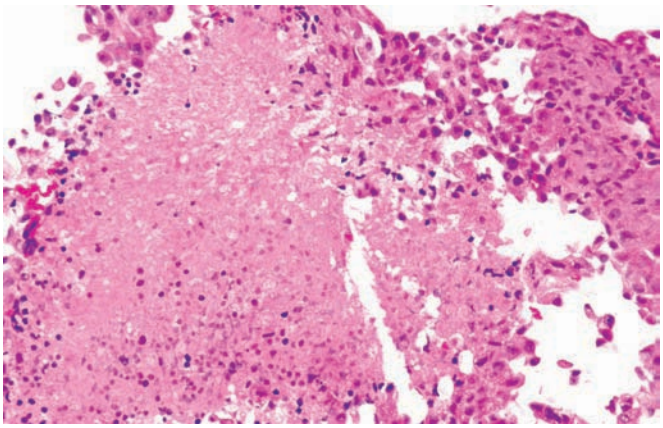


FIGURE 43.108. Focal tumor necrosis in an atypical mesothelial proliferation, as one indicator of malignant mesothelioma. Neoplastic invasion into subpleural adipose tissue was found in a different area of the same biopsy.

Small Cell Mesothelioma

In 1992, Mayall and Gibbs[772] drew attention to a small cell variant of MM, likely to be confused with small cell carcinoma of lung. In this regard, it is also worth emphasizing that Falconieri et al.[773] reported four cases of small cell carcinoma of lung with spread into the pleura, simulating pleural MM.

It is notable that most of the cases reported by Mayall and Gibbs[772] represented autopsy cases, with the potential for the small cell features being explicable at least in part by postmortem artifact. Krismann et al.[774] have expressed doubt about the existence of small cell mesothelioma, because the German Mesothelioma Registry, which contained more than 6000 mesothelioma cases as of 2004, did not contain a single example of small cell mesothelioma. Nonetheless, we have encountered extremely rare cases of mesothelioma with a small cell pattern (fewer than even lymphohistiocytoid mesothelioma). The following findings aid distinction of this form of mesothelioma from small cell carcinoma infiltrating pleura:

- In the cases of small cell mesothelioma the we have encountered, the tumor showed a transition from the

small cell areas to other regions where the appearances were more characteristic of epithelioid mesothelioma.
- The nucleocytoplasmic features of small-cell mesothelioma differ subtly from those of small cell carcinoma (Figs. 43.30 and 43.31), so that the mesothelioma cells often possess greater amounts of cytoplasm, or alternatively, the nuclei are more open and vesicular in pattern with finely divided chromatin, in comparison to the "salt and pepper" nuclear chromatin pattern characteristic of small cell carcinomas, with nuclear molding.
- Immunohistochemical studies on these mesotheliomas reveal features characteristic of mesothelial differentiation, with no evidence of neuroendocrine differentiation as shown, for example, by immunostaining for synaptophysin or chromogranin.

Nonetheless, we have encountered extremely rare cases of mesothelioma where there was some focal evidence of neuroendocrine differentiation, but such cases appear not to have been described in any detail in the literature.

Deciduoid Mesothelioma

In 1994, Nascimento et al.[775] described three cases of peritoneal mesothelioma in young females, where the tumor cells possessed abundant eosinophilic cytoplasm and showed a resemblance to decidual cells, and such cases had no identifiable prior exposure to asbestos.[776] Reports of other cases of "deciduoid" mesothelioma followed.[776–780]

It is now recognized that deciduoid mesotheliomas (Fig. 43.25)[126,778,779,781–785] are confined neither to the peritoneum nor to young women, and they can arise in the pleura and in men.[786] Their natural history is akin to other epithelial mesotheliomas, although a few patients have had long survivals,[779] whereas the tumors comprising the original report[775] pursued an aggressive clinical course. Mesotheliomas that consist only of deciduoid tissue are rare, but it is not uncommon in biopsy tissue to see a transition from more usual patterns of epithelial mesothelioma to foci of deciduoid tissue. We do not consider deciduoid mesothelioma to represent a distinctive subtype, and instead we refer simply to these mesotheliomas as epithelioid mesotheliomas with focal deciduoid features. The immunophenotype of such "deciduoid" mesotheliomas is essentially the same as for other MMs of epithelioid type.[786]

Mucin-Positive Epithelial Mesotheliomas

Up to about 5% of epithelial mesotheliomas show focal staining with Mayer's mucicarmine, PAS-diastase, and Alcian blue/colloidal iron with hyaluronidase. We refer to these mesotheliomas as mucin-positive mesotheliomas.[505]

Ernst and Atkinson[787] reported seven of 18 epithelial mesotheliomas to be mucicarmine positive. They attributed the positive staining reaction to hyaluronic acid. The review article on MM by the U.S.–Canadian Mesothelioma Panel[788] illustrated a case of mucicarmine-positive mesothelioma and indicated this finding did not exclude the diagnosis of mesothelioma. Some mucin-positive epithelial mesotheliomas show staining of the cell membrane with mucin stains and are sensitive to hyaluronidase pre-digestion (Fig. 43.109). Others show intracellular droplet staining with Mayer's mucicarmine (Fig. 43.110), PAS-diastase (Fig. 43.111), and Alcian blue with and without hyaluronidase (Fig. 43.112). In our experience, these mucin-positive epithelial mesotheliomas are the ones that show crystalloid structures ultrastructurally (see Ultrastructural Features of Mesotheliomas, above).

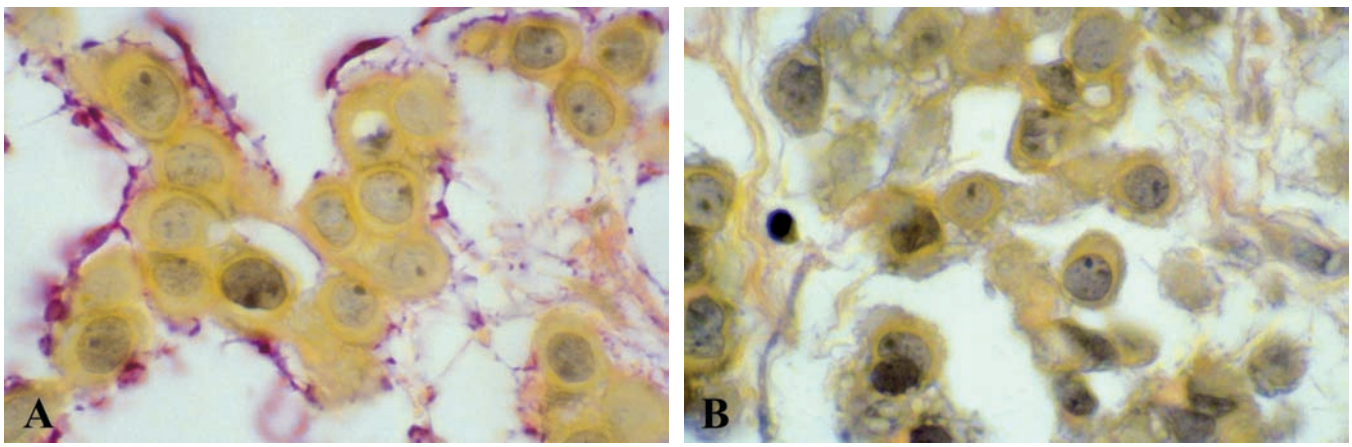


FIGURE 43.109. **(A)** This epithelial mesothelioma shows cell membrane staining for mucicarmine. **(B)** When pretreated with hyaluronidase, the mucicarmine staining does not occur, suggesting the mucicarmine staining is caused by hyaluronic acid.

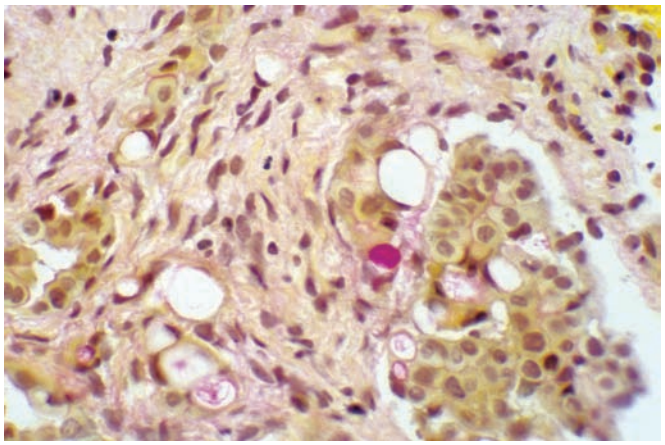


FIGURE 43.110. This epithelial mesothelioma shows intracellular droplet-like staining for mucicarmine that is resistant to hyaluronidase pre-digestion.

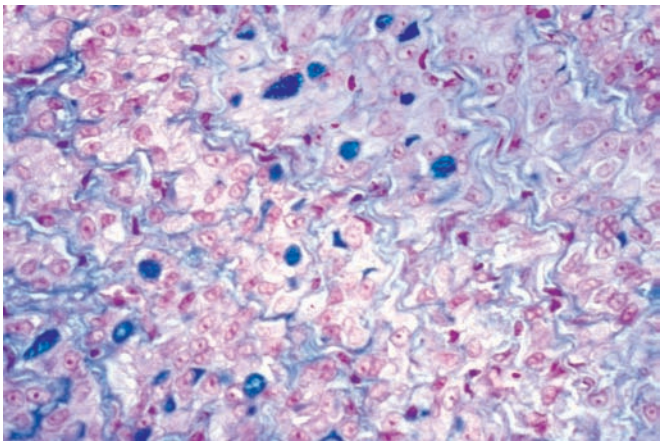


FIGURE 43.112. Alcian blue droplet-like staining is seen in this epithelial mesothelioma and is resistant to hyaluronidase pretreatment.

Benjamin and Ritchie[789] examined the staining results for glycogen and mucosubstance of 30 diffuse epithelial mesotheliomas. Tissue was fixed in formalin and processed using standard techniques. Tissue sections were stained with the WHO stain for mucin, PAS reagent with and without diastase, Hale's colloidal iron stain with and without hyaluronidase, potassium hydroxide–PAS technique, and Alcian blue at pH 1.0 and 2.5. They found that seven of the 30 mesotheliomas failed to stain by any method tested, and concluded the staining reactions of epithelial mesotheliomas with mucopolysaccharide stains were too inconsistent to be of much value in diagnosing epithelial mesotheliomas.

MacDougall et al.[539] reported a case of epithelial MM, the diagnosis documented by electron microscopy and immunohistochemistry, which was mucicarmine and PAS-D positive.

Gaucher Cell–Like Mesotheliomas

Gaucher cell–like mesotheliomas are one of the rarest, if not *the* rarest, epithelioid type of mesothelioma. These mesotheliomas are composed of large cells that are mostly round and contain intracytoplasmic inclusions and resemble Gaucher cells (Figs. 43.113 and 43.114). Ultrastructurally, these cells show some very unique crystalloid structures within the cisternae of the rough endoplasmic reticulum[211] (Fig. 43.115). We have seen this neoplastic pattern only in mesotheliomas and not in any other type of tumor.

Multicystic Mesothelioma

Multicystic mesotheliomas are well recognized in the peritoneal cavity,[790–807] mainly in women and less often in

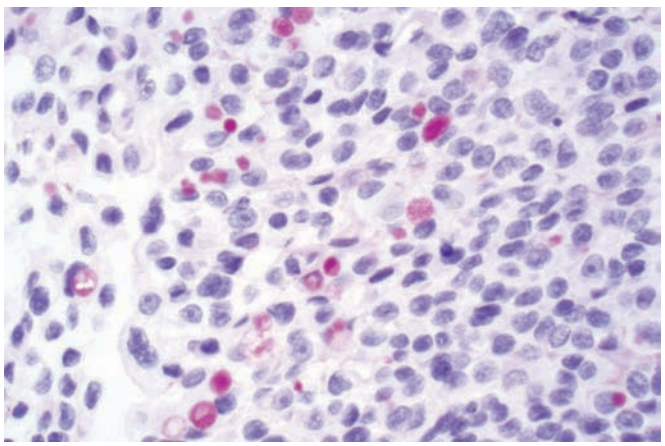


FIGURE 43.111. Intracellular PAS and PAS-diastase droplet-like staining is observed in this epithelial mesothelioma.

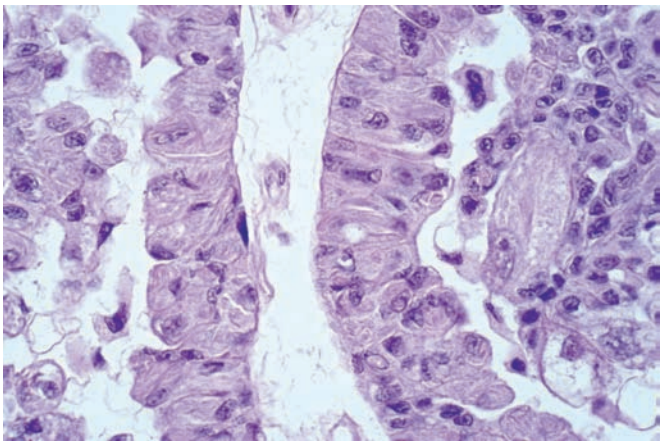


FIGURE 43.113. This epithelial mesothelioma is composed, in part, of numerous large cells with intracytoplasmic inclusions that resemble those seen in Gaucher cells.

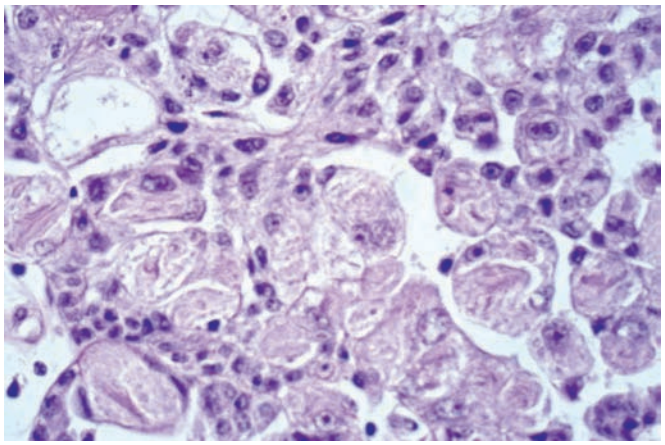


FIGURE 43.114. Gaucher-like cells with intracytoplasmic inclusions are a prominent component of this epithelial mesothelioma.

men.[808–813] In the peritoneal cavity, some lesions of this type appear to represent benign postinflammatory cystic lesions (for which an association with peritoneal inflammatory disorders, endometriosis, and antecedent surgical procedures has been recorded),[801,813–816] whereas other peritoneal multicystic mesotheliomas appear to represent indolent neoplasms of intermediate or low-grade malignant potential,[817] occasionally forming massive lesions that can recur locally[812,818,819] and require repeated surgical removal, although spread beyond the peritoneal cavity appears not to have been recorded.

We have encountered one case of a cystic mesothelioma of the peritoneum found during appendectomy in a man, with repeated local recurrences and with transition to a conventional malignant-appearing epithelial mesothelioma in late recurrences of the lesion. Gonzalez-Moreno et al.[820] also described malignant transformation of a peritoneal cystic mesothelioma in a 36-year-old woman.

Multicystic mesotheliomas most often affect young adults to middle-aged premenopausal women and they are found most often in the pelvic region, often localized to the pouch of Douglas. The patients may present with abdominal pain or abdominal swelling or a detectable mass lesion.

Characteristically, the cystic nature of this form of mesothelioma is evident on naked-eye inspection, and the cysts are lined by a single layer of flattened cells that express a mesothelial phenotype on immunohistochemistry, with fibrous tissue in the septa separating the individual cystic locules.

Multicystic mesotheliomas localized to the pleura are exceedingly rare. We know of only one report in the literature,[821] in a 37-year-old woman with a history of childhood exposure to asbestos (the size of the cystic locules was not specified). A single case of pleural cystic mesothelioma was also encountered in the Australian Mesothelioma Surveillance Program, in a young woman (the case being misdiagnosed initially as a cystic lymphangioma).[822] Again, the size of the cystic locules in that case is unknown, but the lesion did recur.

Multicystic mesotheliomas have no proven relationship to asbestos, and it seems likely that any association[821] is coincidental rather than causal. Given the extreme rarity of pleural multicystic mesothelioma, the following criteria are suggested for its diagnosis:

- The cystic character of the lesion should be evident on gross examination (either at thoracoscopy or thoracotomy, or on examination of a resected specimen).
- Throughout the entire lesion, the histologic appearances should be indistinguishable from those of a cystic mesothelioma of the peritoneum, with a requirement for it to be embedded in its entirety and sectioned.
- In particular, there should be no areas characteristic of conventional MM of epithelial type. Tumors showing areas of conventional mesothelioma we believe should be designated as MM with focal microcystic change. Nonetheless, adenomatoid areas are well recognized in conventional multicystic mesotheliomas.
- The mesothelial phenotype of the cells lining the cysts should be confirmed on immunohistochemistry or electron microscopy or both. In this regard, we reemphasize that cystic lymphangioma represents one differential diagnosis for these lesions, and that the antibody D2-40 labels both lymphatic endothelium and mesothelial cells,[632,753] as well as other cell types[605]; labeling of the

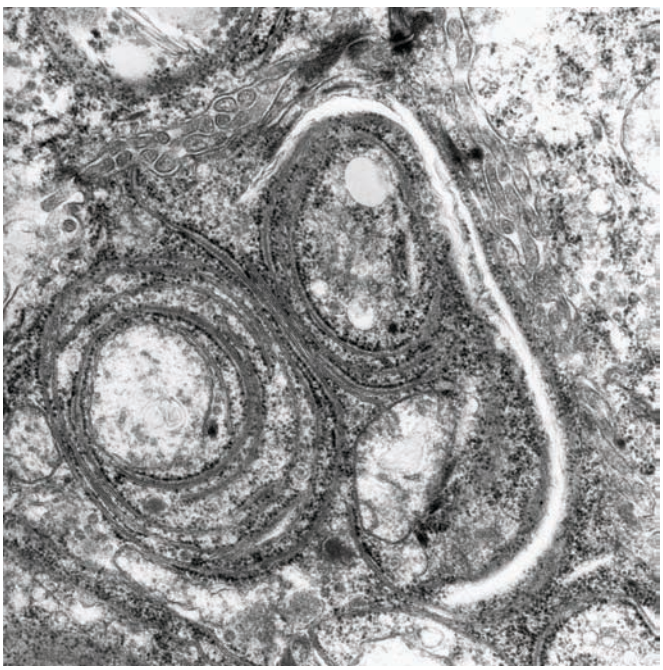


FIGURE 43.115. The cells with the inclusions show parallel arrays of membrane-like material within the cisterna.

relevant sections for cytokeratins or mesothelial markers such as calretinin facilitates the distinction.

Simple postinflammatory mesothelial cysts seen in the peritoneum do not seem to occur in relation to the pleura.

We have also seen several cases of peritoneal cystic mesothelioma where the patients had been informed that they had a (malignant) mesothelioma, and other cases where the patients were subjected to aggressive combination chemotherapy. Because of the distinct risk of clinical overreaction to these lesions, we prefer to designate most such lesions as peritoneal mesothelial inclusion cysts. If the term *cystic mesothelioma* is used in pathology reports, we consider it imperative to include a comment on the character of these lesions and their distinction from conventional MM.

Desmoplastic Sarcomatoid Mesothelioma of the Pleura and Its Distinction from Benign Fibrous Pleuritis

The first description of desmoplastic MM (DesMM) is usually attributed to Kannerstein and Churg[823] in 1980, and these lesions were further documented in 1982 by Cantin et al.,[824] but McCaughey[497] had emphasized the diagnostic problems imposed by "large amounts of hyaline collagen" in mesotheliomas as early as 1965. Much earlier, in their 1920 report of a case of pleural mesothelioma, Du Bray and Rosson[825] commented that much of the tumor showed a "marked desmoplastic reaction with the tumor cells scattered rather diffusely throughout the fibrous tissue," with few mitotic figures in those areas where the desmoplastic reaction was prominent. In 1998, Mangano et al.[826] reported a series of 31 DesMMs and proposed criteria for their diagnosis.

DesMMs are usually pleural in localization, although we have encountered uncommon cases of desmoplastic MM in the peritoneum. Of the 27 cases reported by Cantin et al.,[824] 26 were pleural in localization and only one was peritoneal; 19 represented sarcomatoid MMs, as opposed to six biphasic and two epithelial MMs.

About 2% to 10% of mesotheliomas can be described as desmoplastic,[37,119,507,823,827] and they are arbitrarily so designated when 50% or more of the tumor in an adequate biopsy represents hypocellular fibrous tissue[37] (when the proportion of paucicellular desmoplastic tissue is <50%, the authors simply designate the tumors as a sarcomatoid or other MM with desmoplastic features).

Characteristically, DesMMs comprise interweaving bundles of hyalinized fibrocollagenous tissue with variable numbers of intervening tumor cells, and the gross morphology is that of firm rubbery fibrous tissue that may even be described as "woody" in consistency.

Desmoplastic sarcomatoid MM is perhaps the most deceptive pattern of mesothelioma encountered in surgical pathology practice, and it stimulates greater diversity of diagnostic opinion and disagreement among expert mesothelioma panels than any other histologic type of mesothelioma,[788] because of its liability to misdiagnosis as either inflammatory pleural fibrosis[826] or parietal pleural fibrous plaque.

In our experience, accurate diagnosis of desmoplastic MM is often impossible with closed and core biopsies of pleura, and surgical biopsy is required for confident diagnosis, such as thoracoscopy-guided biopsies. Because of the bland appearance of the MM in many cases, assessment of invasion is often the most valuable pointer to the diagnosis.[119,503,822] This being so, it is important for the biopsy to include not only the pleura but also subpleural tissues for the assessment of invasion; the confidence index for a diagnosis of desmoplastic MM can be correlated directly with the extent of the biopsy and its depth. Even so, it is our experience that some cases of DesMM continue to be misdiagnosed histologically as benign fibrous pleuritis. As recorded by Mangano et al.[826] and in our experience,[119,822] several major features aid in the diagnosis of these deceptive lesions:

- *The architecture of the lesion and the presence or absence of "bland" necrosis*. Unlike the paucicellular laminated architecture of benign pleural fibrous plaques, DesMMs are usually characterized by interweaving areas of fibrocollagenous tissue, with a branched, whorled, micronodular, or storiform pattern, different from the architecture characteristic of benign pleural fibrous plaques or the more orderly stratified (zonal) pattern of benign fibrous pleuritis (cf. Figs. 43.116 and 43.117 with 43.118 to 43.120).

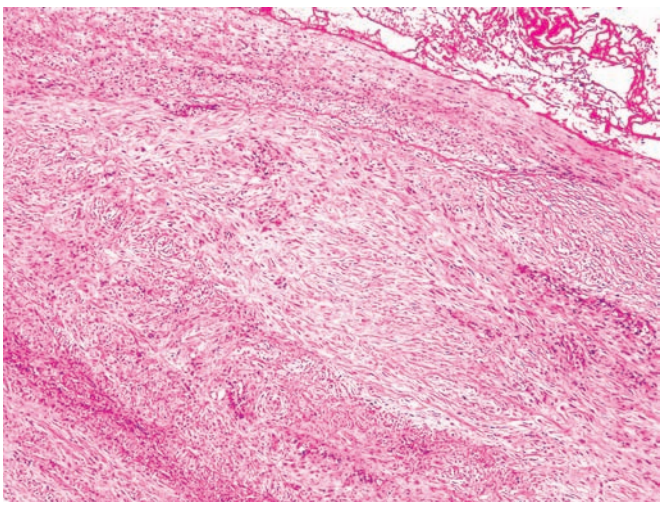


FIGURE 43.116. Desmoplastic sarcomatoid mesothelioma of pleura. At low magnification, the disordered architecture of the collagen-rich hypocellular tumor tissue is evident, especially in the lower left of this field.

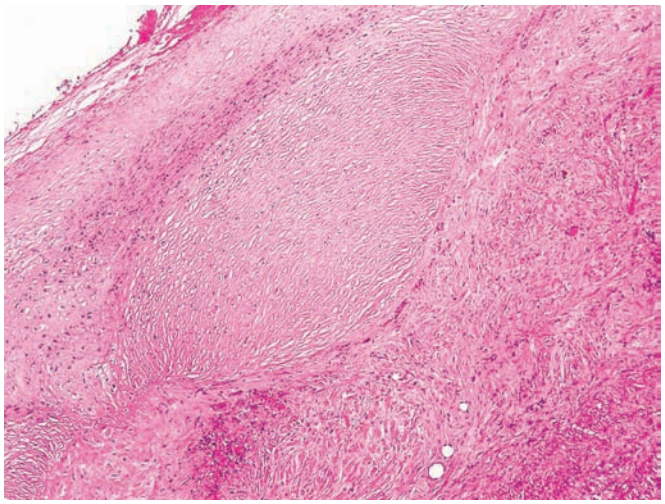


FIGURE 43.117. Pleural desmoplastic sarcomatoid mesothelioma. In addition to a disordered architecture of the desmoplastic tissue, this lesion shows a focal micronodular pattern, located near the center of the field. The desmoplastic tissue also shows greater cellularity in the deeper zone of the tumor (lower right field) than in the subsurface zone, a reversal of the zonation characteristic of a fibroinflammatory process affecting the pleura.

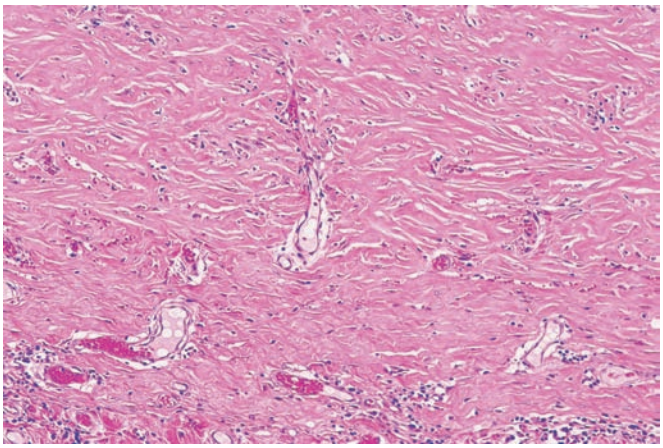


FIGURE 43.119. Benign fibrous pleuritis. This micrograph was taken close to the interface between the pleural fibrous tissue and subpleural adipose tissue and shows a reasonably orderly to laminated architecture, with no augmentation of cellularity in this zone. Small thin-walled blood vessels are evident within the fibrocollagenous tissue, one of which (center) extends almost vertically toward the pleural surface.

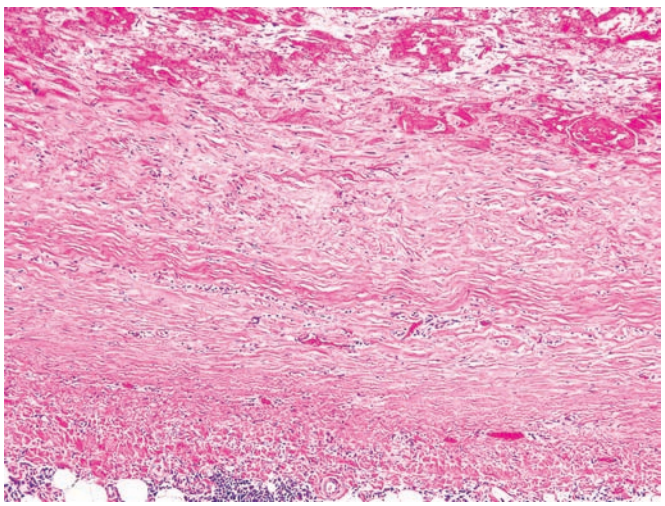


FIGURE 43.118. Benign fibrous pleuritis. Fibrinous exudate is evident near the upper zone of this field and the fibrous tissue shows no increase in cellularity, for example, near the interface between the pleural fibrous tissue and the subpleural fat, where there is a focal lymphocytic infiltrate, a feature often seen with benign fibrous pleuritis and also with pleural malignant mesotheliomas on occasions. The appearances of the fibrous pleuritis in this case are nonspecific, but the patient had a background of occupational exposure to asbestos with no clinical evidence of any alternative cause for pleuritis, so that the appearances were considered consistent with benign asbestos pleuritis with pleural fibrosis. Compare with Figures 43.116 and 43.117.

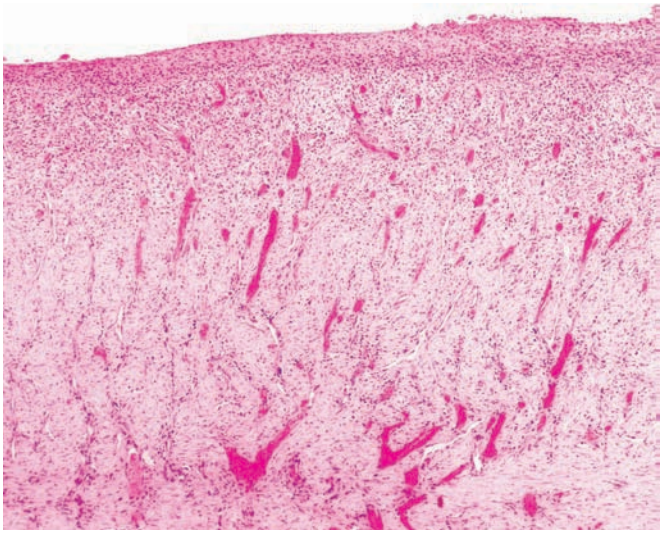


FIGURE 43.120. Benign fibrous pleuritis. In comparison to the desmoplastic mesothelioma illustrated in Figures 43.116 and 43.117, this benign inflammatory process shows a "top heavy" zonal pattern in terms of cellularity, whereby the most cellular tissue is located in the subsurface zone, with diminishing cellularity and increasing collagen deposition in the deeper zones of the thickened fibrous layer. In addition, there are multiple small and congested blood vessels that extend through most of the fibrous tissue illustrated, near-perpendicular to the free surface of the pleura and roughly parallel to each other. The overall architecture and zonation are characteristic of a benign fibroinflammatory process.

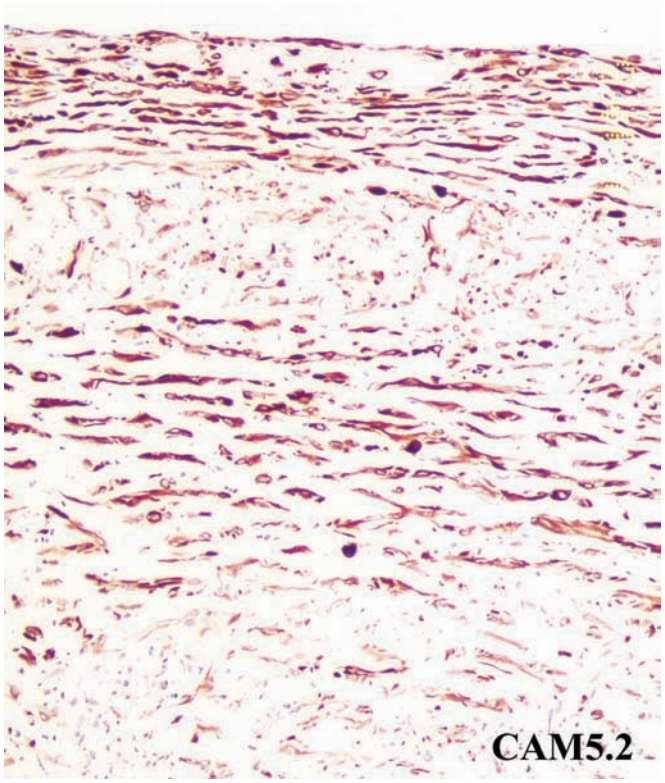


FIGURE 43.121. Benign fibrous pleuritis. The fibroblastoid cells in cases of benign fibrous pleuritis usually show positive expression of cytokeratins (CKs), illustrated here by staining for CK8/18 (CAM5.2). Again, the pattern of CK expression conforms to the zonal pattern of a benign fibroinflammatory disorder, whereby the most cellular tissue is located near the free surface, with diminishing cellularity toward the lower zone of this field. In addition, the fibroblastoid cells in this area are disposed with their long axes parallel to the surface of the pleura and roughly parallel to each other. This orderly pattern of zonation and cellularity is characteristically not seen in cases of desmoplastic mesothelioma.

The zonal architecture of the lesion is also of importance for diagnosis.[828] As mentioned in a previous discussion and shown in Figures 43.120 and 43.121, the most cellular and atypical tissue in benign fibrous pleuritis is characteristically located at or near the surface of the pleura, with gradually diminishing cellularity and increasing collagen deposition in the deeper zones of the fibrous tissue ("top heavy" cellularity[507]). In contrast, the most cellular and atypical tissue in DesMM is usually found near the deep boundary of the lesion (Fig. 43.117); in other words, DesMMs are characterized by reversal of the zonation typical of organizing pleural inflammation.

The architecture of the microvasculature within the fibrous tissue may be of diagnostic significance. In some instances, small blood vessels within benign fibrous pleuritis are arranged roughly in parallel and perpendicular or nearly so to the free surface of the pleura (Fig. 43.120), and they traverse almost the full thickness of the fibrotic tissue,[37,507] whereas this orderly and near-perpendicular vascular architecture is typically not seen in cases of DesMM.[507] Even so, two caveats are worth emphasis concerning this finding: (1) blood vessels with this pattern are not always or consistently evident in benign fibrous pleuritis, and (2) we have encountered rare cases of proven epithelial MM accompanied by a prominent fibroproliferative stromal reaction where there were parallel and near-perpendicular blood vessels of this type (Fig. 43.48 in biphasic mesothelioma section). Therefore, it seems that only the presence (not absence) of these blood vessels is of significance, and that they indicate that the fibrous tissue is benign in those areas where they are located.

When a disordered, storiform, or micronodular architecture is seen in combination with foci of so-called bland necrosis—defined as such by absence of a boundary inflammatory reaction (Fig. 43.122) and perhaps resulting from compression, invasion, or neoplastic outpacing of the stromal microvasculature—these two findings in combination can allow a diagnosis of desmoplastic sarcomatoid MM at a high order of confidence, even in the absence of overtly sarcomatoid tissue or in the absence of invasion (for example, when the biopsy is too superficial in character for this assessment).[826] Even so, laminated fibrocollagenous tissue that is essentially indistinguishable from pleural fibrous plaque tissue can be encountered in desmoplastic mesotheliomas,[822] and in such cases it is arguable as to whether such areas represent benign plaque tissue overgrown by the desmoplastic mesothelioma or whether the laminated paucicellular fibrocollagenous tissue is an integral part of the mesothelioma (as we consider it sometimes to be).

- *The cellularity and cytomorphology of the fibrocollagenous tissue*. Areas of overtly sarcomatoid tissue—defined as such by cellularity, cytologic atypia, and mitotic figures that are excessive for a benign fibrocollagenous lesion of the pleura such as benign fibrous pleuritis—are important markers of DesMM.[37,119,503,822,826] It is our impression that the most cellular and atypical tissue is sometimes found at the mediastinal aspect of the pleura, and in one of our cases a definitive diagnosis of DesMM could not be made on a surgical biopsy from the lateral parietal pleura, because of absence of overtly sarcomatoid tissue or invasion, but the diagnosis was suspected from the collagen pattern; because of this and the operative appearances, a further biopsy was taken from the mediastinal pleura and this revealed obvious sarcomatoid tissue.[822]
- *Clear evidence of invasion of chest wall structures or lung*. Invasion of subpleural adipose tissue (or even deeper chest wall structures) is one of the most impor-

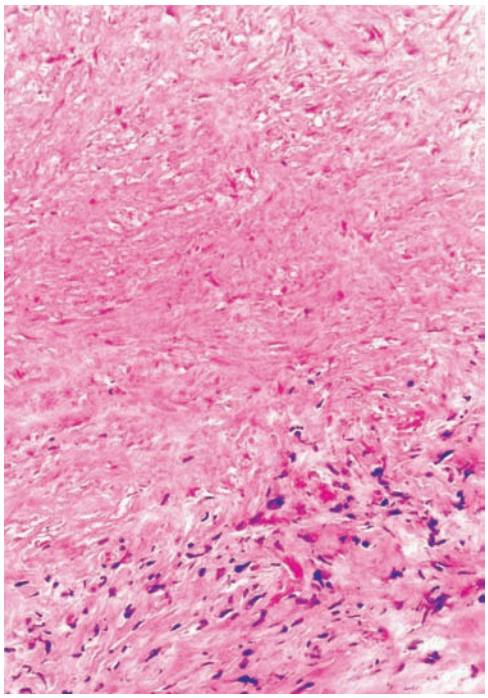


FIGURE 43.122. Desmoplastic sarcomatoid mesothelioma of pleura. An area of "bland" necrosis is illustrated, characterized by absence of an inflammatory reaction at the interface between the necrotic zone and the adjacent apparently viable desmoplastic sarcomatoid tissue.

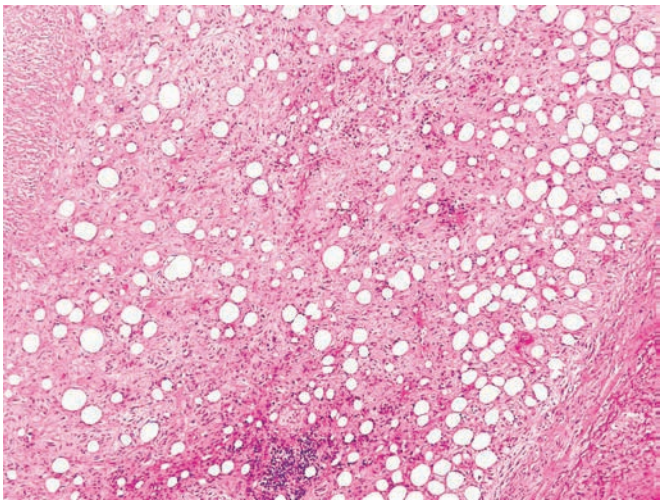


FIGURE 43.123. Desmoplastic sarcomatoid mesothelioma of pleura. The hypocellular tumor tissue shows a characteristic pattern of infiltration into the subpleural adipose tissue, whereby the neoplastic cells insinuate between individual adipocytes, splaying them apart and incorporating them into the advancing edge of the tumor, a pattern that we sometimes describe as Swiss cheese invasion.

tant markers for a diagnosis of desmoplastic sarcomatoid MM, and perhaps the most decisive. In particular, the demonstration of infiltration into subpleural tissue or deeper structures or into lung parenchyma by cytokeratin-positive spindle-shaped cells is perhaps the clearest indicator of sarcomatoid DesMM in surgical biopsy tissue (Figs. 43.123 to 43.127).[37,119,503,822,826] In this regard, it is our experience and that of others[503,515,829] that the great majority of sarcomatoid DesMMs show intense and widespread expression of cytokeratins (CKs), and the demonstration of invasion of subpleural tissues by CK-positive spindle cells represents a decisive indicator of MM[119] (Fig. 43.125). It is emphasized that the presence of CK-positive fibroblastoid cells is not of diagnostic importance by itself, because benign fibroinflammatory disorders of the pleura are usually characterized by CK expression by the reactive fibroblastoid cells (Fig. 43.121)[37]: instead, immunostaining for CKs is of value in this situation to facilitate assessment of invasion as a marker of malignancy (Fig. 43.125). In contrast, in our experience[119,822] and that of others,[37,515,829] infiltration of CK-positive fiboblastoid cells into subpleural adipose or other tissues is almost never seen with benign fibroinflammatory disorders (exceptions include rare examples of a biopsy of an antecedent biopsy site or needle track, with displaced mesothelial cells restricted to the zone of the wound).

Invasion into subpleural adipose tissue by the fibrocollagenous tissue comprising DesMM is often characterized by an insinuative pattern of invasion whereby the tumor cells extend between individual adipocytes, splaying them apart and incorporating them into the poorly delineated deep margin of the DesMM (Figs.

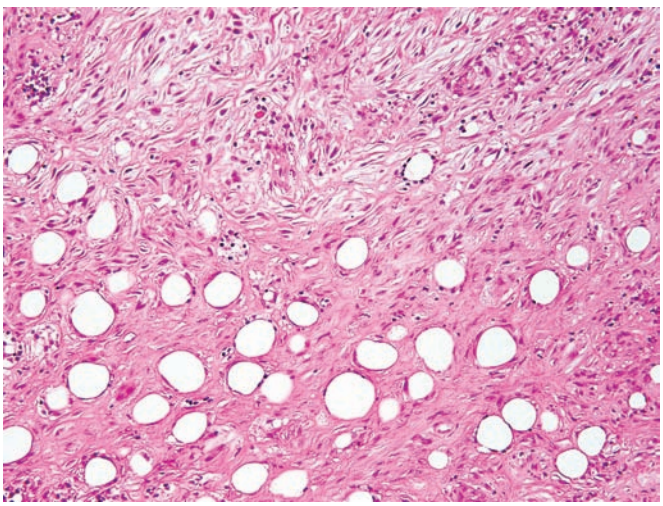


FIGURE 43.124. Desmoplastic sarcomatoid mesothelioma of pleura. This field illustrates the invasion at higher magnification, with splaying apart of the adipocytes by the hypocellular fibroblastoid tumor tissue.

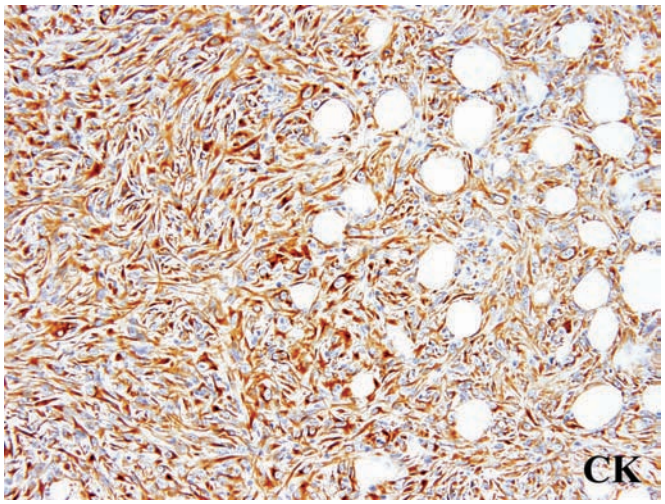


FIGURE 43.125. Pleural desmoplastic sarcomatoid mesothelioma. The demonstration of CK-positive fibroblastoid cells infiltrating into adipose tissue with separation of individual adipocytes is virtually diagnostic of malignancy in this context.

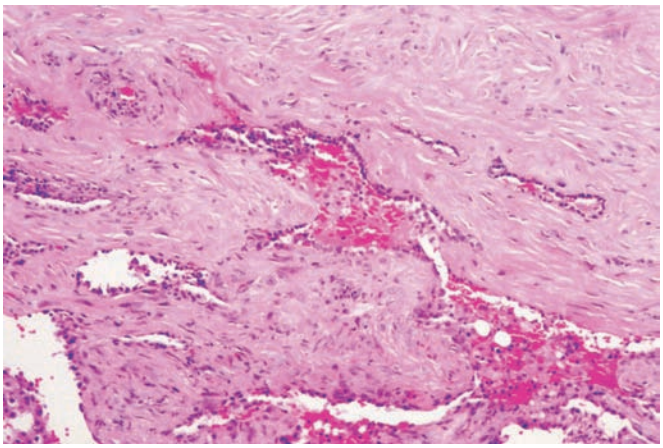


FIGURE 43.127. Desmoplastic sarcomatoid mesothelioma of pleura, infiltrating into the interstitium of the peripheral lung parenchyma, with incorporation into the tumor of remnants of alveolar spaces lined by alveolar epithelium. In other instances, the desmoplastic tissue can erupt into alveolar spaces, producing mimicry of organizing pneumonia, or even the architectural pattern of an epithelioid hemangioendothelioma of lung.

43.123 to 43.125). We often refer to this pattern of infiltration as *Swiss cheese* invasion. Although characteristic of DesMM, it is by no means diagnostic and can be found with other tumors, including non-Hodgkin's lymphomas.

When DesMM invades into lung parenchyma, it can infiltrate along the interstitium and interlobular septa, incorporating remnants of alveoli into the invasive margin of the mesothelioma (Fig. 43.127). The mesotheliomatous tissue may also burst into alveolar spaces, to mimic the histology of organizing pneumonia or even epithelioid hemangioendothelioma of lung.[37,503]

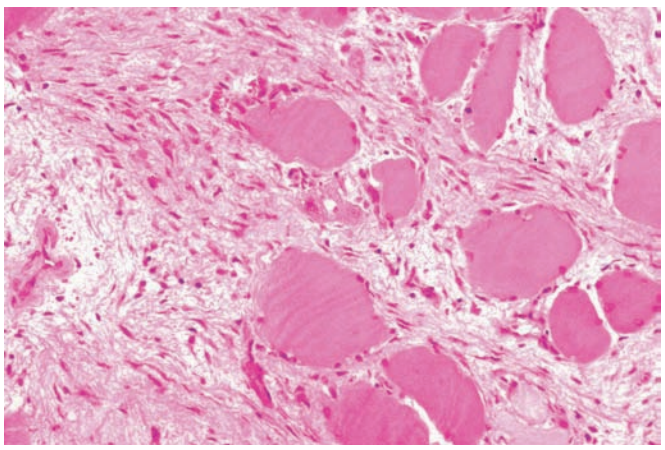


FIGURE 43.126. Desmoplastic mesothelioma of pleura. This micrograph depicts insinuative invasion of the hypocellular fibroblastoid tumor tissue into chest wall skeletal muscle, with separation of individual myocytes. The desmoplastic tumor tissue in this case extended almost to the perichondrium of a rib, where there was a CK-negative periosteal reaction with subperiosteal new (woven) bone formation.

- *Rarely in surgical pathology, the identification of metastatic DesMM*. Wilson et al.[827] found evidence of metastatic spread in 14 of 16 cases of DesMM that came to autopsy. The contralateral lung was the site affected most commonly (75%), and on rare occasions an intrapulmonary metastatic deposit of DesMM may be found in biopsy tissue.[503] Other sites of metastasis recorded by Wilson et al. included liver, thyroid, kidney, adrenal gland, myocardium, and bone.

  DesMM appears to have a propensity to metastasize to bone,[37,503,830] with the potential for misdiagnosis as a primary bone tumor if the antecedent medical history is unknown to the pathologist or if the metastatic deposit(s) represent the presenting manifestation of the DesMM. We have encountered several such cases (Figs. 43.128 and 43.129). In most cases, the bone metastasis presented as a pathologic fracture after diagnosis of the pleural DesMM, but one referral case presented as a fractured neck of femur in an elderly woman who had been diagnosed a short time beforehand with benign fibrous pleuritis. As in the other cases, the bone deposit was characterized by strong CK expression by the desmoplastic tissue, and was followed by reexamination of the original pleural biopsy and a revised diagnosis of pleural DesMM. The bone in such skeletal deposits is distinguishable from osseous differentiation within a DesMM[503] by (1) knowledge of the site whence the biopsy was taken; and (2) the presence of well-

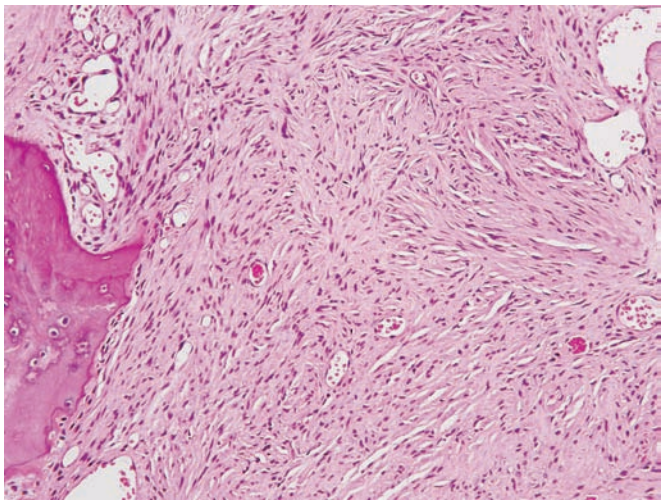


FIGURE 43.128. Metastatic deposit of desmoplastic sarcomatoid mesothelioma in bone, depicting the hypocellular tumor tissue.

developed trabeculae of lamellar bone, in addition to any woven bone related to a pathologic fracture.

Finally, it is worth emphasizing that although desmoplastic MMs lack many of the cytologic indicators of malignancy, these lesions represent a highly lethal form of pleural MM, with a mean survival of approximately 6 months following diagnosis,[824,827] in comparison to about 8 to 12 months following diagnosis of other forms of pleural MM.

Lymphohistiocytoid Mesothelioma

In 1988, Henderson et al.[831] described three cases of pleural MM with a striking lymphomatoid appearance in biopsy tissue, which they designated as lymphohistiocytoid mesothelioma (LHM). They considered this type of mesothelioma to represent a variant of predominantly sarcomatoid mesothelioma where the neoplastic cells were histiocytoid in appearance but were obscured by a prominent infiltrate of lymphocytes, accompanied by plasma cells and in one case eosinophils, imparting a histologic resemblance to either Hodgkin's or non-Hodgkin's lymphoma (Figs. 43.130 and 43.131); all three cases had been misdiagnosed at some stage as lymphoma.

The three cases represented 0.8% of all cases of pathologically proven mesotheliomas across Australia as accessioned in the Australian Mesothelioma Register as part of the Australian Mesothelioma Surveillance Program. Subsequently, additional cases have been reported by Khalidi et al.[832] and by Yao et al.[833] The cases reported by Yao et al. represented 3.3% of accessions, probably reflecting a referral bias for cases submitted to a reference center for ultrastructural pathology in the U.S. Galateau-Sallé et al.[37] reported a series of 22 cases reported by the MesoPath Group in France in 2003, representing less than 2% of their cases.

Of 12 cases of LHM described in detail in the literature,[831–835] 11 were pleural in location, and one was peritoneal (we have seen an additional case of peritoneal LHM (Fig. 43.131). The ages of the patients ranged from 31 to 74 years, with a mean of 59 years approximately, with a male-to-female ratio of 2:1.

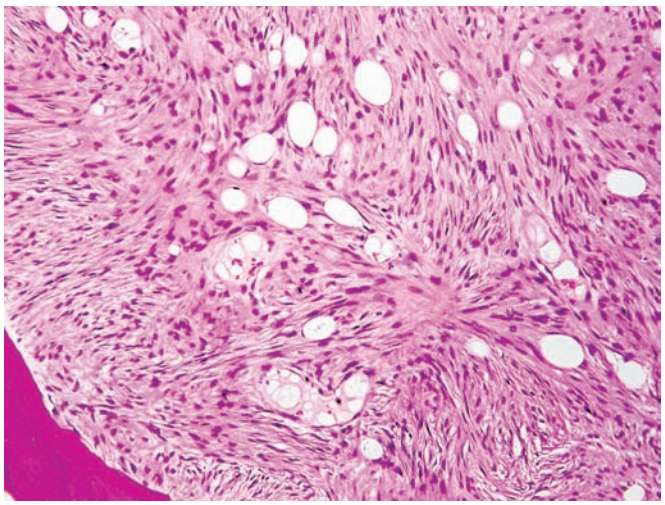


FIGURE 43.129. Metastatic deposit of desmoplastic sarcomatoid mesothelioma in bone. This was the most cellular area of the tissue in this biopsy specimen, showing a focal storiform architecture. The bone trabecula at the upper right of the field was predominantly lamellar in character.

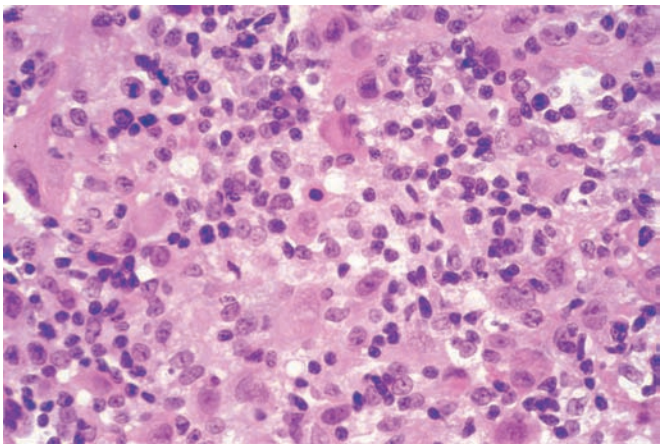


FIGURE 43.130. Pleural malignant mesothelioma, lymphohistiocytoid type. The tissue comprises an admixture of histiocytoid cells, with moderate amounts of pale eosinophilic cytoplasm, with numerous interspersed lymphocytes. (Case 3 from Henderson et al.[831])

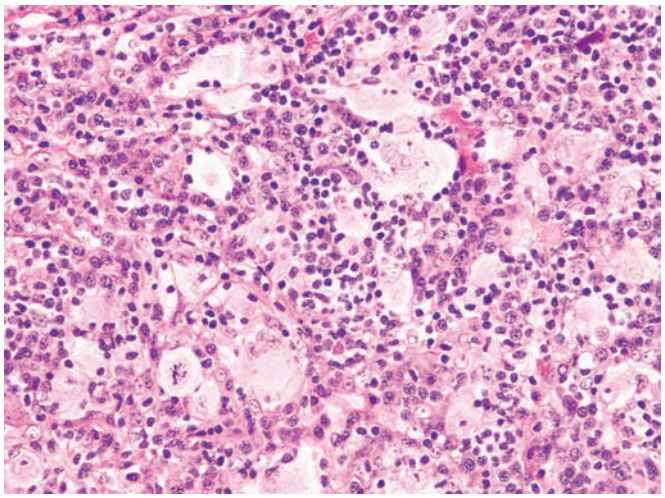


FIGURE 43.131. Peritoneal malignant mesothelioma of lymphohistiocytoid type. Among the background lymphocytes and plasma cells, there are larger pale neoplastic cells with multilobated nuclei, and one mitotic figure is evident. The large pale cells showed strong immunostaining for low molecular weight cytokeratins.

All three cases originally described by Henderson et al.[831] had a background of occupational exposure to asbestos, but no such history was recorded in the three cases reported by Khalidi et al.[832] and details of any asbestos exposure were unknown for three of the cases reported by Yao et al.,[833] whereas one of their cases had no history of exposure. There was a history of minor exposure to asbestos in the single case reported by Dorfmüller et al.[834] in 2004.

There was no evidence that the lymphohistiocytoid appearances of the cases conferred any major survival advantage. Three of the cases reported by Khalidi at al.[832] were alive with disease at 2, 3, and 72 months postdiagnosis, whereas the survival range for other cases averaged about 7 months, within a range of 2 to 20 months.

The differential diagnosis for LHM includes both Hodgkin's and non-Hodgkin's malignant lymphoma as well as lymphomatoid granulomatosis, primary or secondary thymoma affecting the pleura, inflammatory pseudotumor (inflammatory myofibroblastic tumor), and sarcomatoid carcinoma with a prominent stromal inflammatory reaction.[831–833,836]

Several findings facilitate the diagnosis of LHM:

- The presence of a confluent pleura-based (or, even more rarely, a peritoneal) lesion with an anatomic distribution indistinguishable from mesothelioma on imaging studies or at operation (Fig. 43.132).
- A lymphoma-like appearance on light microscopy, with scattered dispersed or indistinctly clustered atypical large histiocytoid cells (Figs. 43.130 and 43.131).
- Areas of transition to conventional spindle-cell sarcomatoid tissue, or even small foci of epithelial mesothelioma.
- Cytokeratin and vimentin expression by the large histiocytoid cells (Fig. 43.133) and, occasionally, expression of mesothelial markers such as calretinin or CK5/6 on immunohistochemistry, whereas the same large cells are devoid of lymphoid markers[503] such as CD45, CD3, or CD20.
- Evidence in some instances of mesothelial differentiation on electron microscopy, such as elongated serpentine microvilli devoid of a glycocalyx. Henderson et al.[831] found evidence of mesothelial differentiation in terms of elongated serpentine microvilli in two out of their three cases, and three of the four cases reported by Yao et al.[833] also showed ultrastructural evidence of mesothelial differentiation, whereas no electron microscopy findings were recorded in three cases described by Khalidi at al.[832]

Four further facets of LHM are worth emphasis:

- This variant of mesothelioma does not simply represent prominent lymphocytic infiltration in an epithelial mesothelioma.[837] Henderson et al.[831] considered LHM to be a variant of predominantly sarcomatoid mesothelioma, where there was an intimate admixture and intermingling of the background histiocytoid tumor

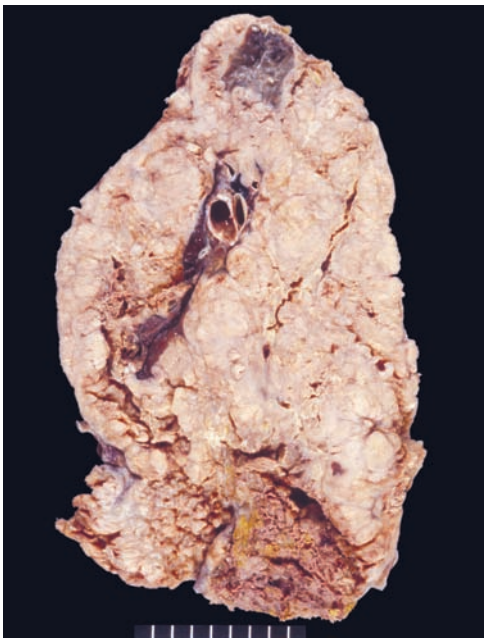


FIGURE 43.132. Pleural lymphohistiocytoid mesothelioma, gross appearances at autopsy. On histologic examination of the autopsy tissues, the lymphohistiocytoid features were depleted, and the tissue comprised mainly spindle-cell sarcomatoid tissue. (Case 3 from Henderson et al.[831])

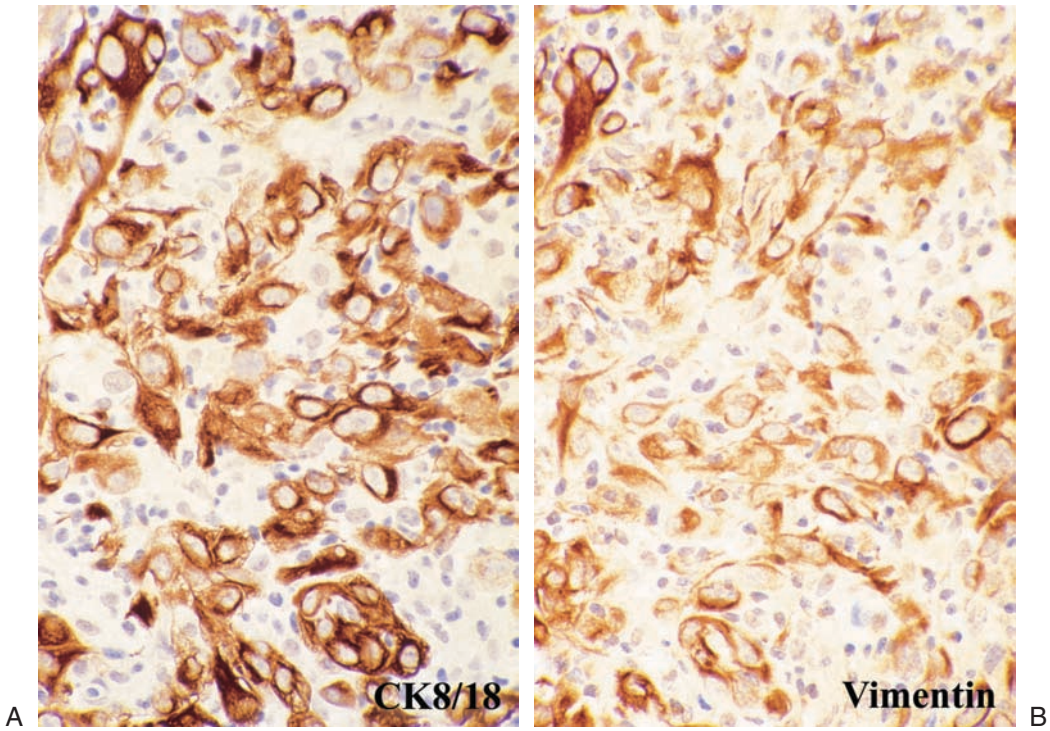


FIGURE 43.133. **(A)** Pleural lymphohistiocytoid mesothelioma. The neoplastic cells show obvious expression of low molecular weight cytokeratins. (Case 3 from Henderson et al.[831]) **(B)** Coexpression of vimentin by the neoplastic cells.

cells with tumor-infiltrating lymphocytes, plasma cells, and in some areas, eosinophils.

- Focal lymphohistiocytoid features occur in otherwise conventional sarcomatoid mesothelioma, so that it is suggested—by analogy with desmoplastic mesothelioma—that at least 50% of the tissue in an adequate biopsy should be lymphohistiocytoid in appearance for a diagnosis of LHM.[37] When the proportion falls below 50%, we simply designate such cases as sarcomatoid mesotheliomas with focal lymphohistiocytoid features.
- The lymphohistiocytoid appearances presumably reflect an immunologic response on the part of the host to the mesothelioma itself. Henderson et al.[822] described the immunohistochemical findings in the tumor-infiltrating lymphocytes in 24 biopsies and autopsy tissue from 22 cases of mesothelioma (epithelial, biphasic, and sarcomatoid in type, including LHMs), and they found T-lymphocyte predominance in about 60% of cases, approximately equal representation of T and B cells in 20%, and B-lymphocyte predominance in the remaining 20%. In their cases, Khalidi et al.[832] found a predominance of T lymphocytes, but with the additional presence of B cells. Yao et al.[833] also recorded a predominance of T lymphocytes in all four cases, but with a minor component of CD20-positive B cells, accompanied by occasional eosinophils.
- The lymphohistiocytoid appearances may reflect a transient phase in the development of some sarcomatoid MMs. One of the three cases originally reported by Henderson et al.[831] had the histologic appearances of a conventional sarcomatoid mesothelioma at autopsy, suggesting depletion of immune-effector cells in the later stages of the mesothelioma. Robinson et al.[835] reported a single case of LHM in a woman who survived for 20 months. In contrast to the initial biopsy, no significant lymphoid infiltrate was detected at autopsy in her mesothelioma.

Pleomorphic Mesothelioma

Many epithelioid mesotheliomas show only low-grade cytologic atypia with minor nuclear pleomorphism and relatively little nuclear hyperchromasia, in comparison to the carcinomas from which they require distinction. Equally, although sarcomatoid MMs closely resemble equivalent soft tissue sarcomas, most notably fibrosarcoma and malignant fibrous histiocytoma (MFH), they may show only low-grade cytologic atypia and pleomorphism, especially desmoplastic mesotheliomas. However, rare mesotheliomas can show extreme cellularity, nuclear atypia, hyperchromasia, and pleomorphism, producing a close histologic resemblance to either an undifferentiated large cell carcinoma of lung or to the pleomorphic variant of MFH (Figs. 43.40, 43.41, and 43.134, respectively).

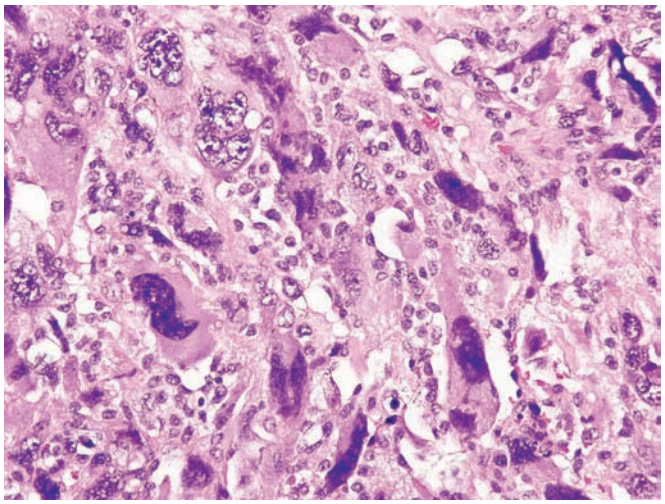


FIGURE 43.134. Pleomorphic predominantly sarcomatoid mesothelioma showing extreme nuclear atypia, pleomorphism, and hyperchromasia, with the presence of multinucleated tumor cells.

Accordingly, we believe that pleural tumors showing extreme pleomorphism should not be dismissed as large cell carcinoma or secondary sarcoma and that when they are pleura-based and have an anatomic distribution consistent with mesothelioma, they should be investigated accordingly.

The diagnosis of pleomorphic mesothelioma, whether epithelial or sarcomatoid, can be based on the following findings:

- A pleura-based tumor with an anatomic distribution that conforms to a diagnosis of mesothelioma, as revealed by imaging studies.
- A transition from the pleomorphic areas to other regions where the appearances are more characteristic of either epithelial or sarcomatoid MM.
- An immunohistochemical profile that conforms to a diagnosis of mesothelioma of either epithelial or sarcomatoid type, as opposed to secondary carcinoma or even secondary sarcoma (for example, when the neoplastic tissue shows strong positive labeling for cytokeratins throughout).
- Occasionally, ultrastructural evidence of mesothelial differentiation.

Localized Malignant Mesothelioma

In 1992, Henderson et al.[211] briefly referred to two cases of localized pleural MM, and further cases were subsequently described by Crotty et al.[838] and Allen et al.,[839] among others.[840–844] Localized pleural MM has been reported in men and women with about equal frequency, within an age range from the 40s to the 70s, although we have encountered localized and even polypoidal pleural malignant MMs in young adults of ages 20 to 30. Typically, these tumors represent localized sessile or pedunculated lesions, ranging in size from 100 mm to a few centimeters (Fig. 43.135).

As the term implies, localized pleural MMs represent circumscribed tumors with histologic, immunohistochemical, and ultrastructural features essentially identical to their diffuse malignant counterparts, and they include epithelial, biphasic, and sarcomatoid lesions. Again, the immunohistochemical profile of these lesions corresponds to that of ordinary confluent epithelial, biphasic, or sarcomatoid MMs.

Churg et al.[503] commented that localized MMs tend not to spread over the pleura, unlike conventional pleural MMs, and that they can be resected successfully in some cases, apparently with no recurrence of the tumor. However, other localized MMs can recur following surgery, and metastasize. In one of the first reports of such localized tumors, Crotty et al.[838] recorded six cases treated by surgical resection, of which three had a disease-free survival for an extended period following excision, but the other three patients sustained local recurrence of the their disease and died within 2 years of initial resection. In one case mentioned by Henderson et al.[211]—a cytokeratin-positive sarcomatoid MM histologically resembling a malignant fibrous histiocytoma, located in an interlobar fissure and treated initially by surgical resection (bilobectomy)—the gross appearances of the recurrent tumor at autopsy were characteristic of mesothelioma.

When dealing with limited biopsy tissue, recognition of localized as opposed to diffuse MM requires information beyond that obtainable from the histologic sections alone. Some diffuse MMs can present with a dominant mass lesion, accompanied by other smaller tumor nodules, so that evidence of the purely localized character of the MM is needed for diagnosis of localized MM,[503] necessitating

FIGURE 43.135. This localized pleural mesothelioma arose in the pleura and invaded lung parenchyma. It was diagnosed radiographically as a solitary pulmonary nodule.

integration of the histologic findings with organ-imaging studies or the gross appearances at operation.

It is sometimes claimed that the relationship between localized pleural MM and prior asbestos exposure is less well established than for diffuse pleural MMs. This may be so, perhaps explicable by the unusual gross and radiologic findings in such cases, so that an exhaustive exposure history may not be sought, and by the paucity of such localized cases reported to date; however, we have encountered such cases where there has been a clear history of antecedent asbestos exposure (including one case with childhood exposure). Therefore, on the basis of the prevailing evidence at this time, we believe that there is no compelling evidence to consider the relationship of such localized MMs to asbestos to be essentially different than for diffuse MMs.

Approach to Diagnosis/Differential Diagnosis

Our approach to the diagnosis of pleural neoplasms is to accurately classify a neoplasm according to its cytologic, histologic, immunohistochemical, and ultrastructural features. All types of specimens are potentially useful in making a specific diagnosis. In general, with respect to biopsy specimens, the larger the specimen, the more useful and potentially less difficult it is to make a specific diagnosis. Cytologic evaluation is also a potentially useful technique as described below.

The Cytology of Malignant Mesothelioma

The cytology specimens used for the investigation of a lesion suspicious of MM include effusion fluids and, less commonly, fine-needle aspiration biopsies (FNABs). As noted by some authors,[845] the difficulties that beset interpretation of effusion cytology specimens have "kept researchers and publishers in business over the last 20 years." Unfortunately, those difficulties can also lead to confusion among clinicians who may be uncertain over the interpretation of the cytopathology reports and assessment of the confidence index for a diagnosis. There are two main difficulties in pleural effusion cytology: (1) the distinction between MM and metastatic malignancy, and (2) the distinction between a reactive pleural effusion from MM. Nowadays, it is the second that is more problematic.

Numerous diagnostic criteria and ancillary investigations, such as immunohistochemical studies, electron microscopy,[846] flow cytometry,[847] atomic force microscopy,[848] and many more, have been proposed. Some techniques initially appeared to show promise in research laboratories, but that early promise was either not borne out in more extensive routine diagnostic testing, or the techniques were impractical for everyday diagnosis. There is currently no consensus concerning the optimal approach for difficult cytology specimens. There are several excellent textbooks and recent reviews on this subject[845,849–854] and it is not our aim to duplicate those comprehensive accounts. Rather, we highlight some of the problem areas and offer our approach to them (see also Mesothelioma in Chapter 45).

The main issues of importance in the cytologic diagnosis of pleural MM, as we see them, are as follows:

1. Some pathologists require the presence of invasion in a tissue specimen for a definitive diagnosis of MM, and consequently argue that a definitive diagnosis cannot be made from a cytology specimen alone.[854] Even when a combination of clinical and cytologic criteria is used, there is no consensus about the confidence index for a cytodiagnosis. Some authors believe that even distinction of MM in situ and invasive MM is possible in skilled hands.[849] However, the literature and, in particular, the criteria proposed for the diagnosis of MM in situ indicate that this specific diagnosis is almost impossible on cytology. Henderson et al.[490] recommended that invasive MM should be identified elsewhere in the same biopsy, a follow-up biopsy, or at autopsy as a requirement for the diagnosis of MM in situ. In our practice, we consider a biopsy-proven diagnosis to be optimal, but in many cases a confident diagnosis of mesothelioma can be reached from careful correlation of the cytologic findings with clinical-radiologic information, whereby the radiologic demonstration of a confluent pleura-based lesion with nodularity or other evidence of invasion can substitute for gross or histologic evidence of invasion. In particular, we require an atypical pleural mesothelial proliferation *plus* classic radiologic findings for a clear diagnosis of MM. Correlation with clinical and radiologic information can also avoid false-positive diagnosis.

These considerations also highlight the importance of clinicopathologic correlation in general; for example, if an FNAB is performed, the exact location of the biopsy (pleura-based lesion versus an intraparenchymal lung lesion impinging on pleura) must be recorded.

2. Different processing procedures can result in different appearances on the slide. It is important to be thoroughly familiar with the procedures employed in one's own laboratory.

3. Not all types of MM are equally amenable to diagnosis from effusion fluid cytology; MMs with an epithelial component (i.e., epithelial mesotheliomas and biphasic mesotheliomas) are far more likely to shed atypical and identifiable mesothelial cells into effusion fluids than sarcomatoid MMs, for which effusion fluids usually show low cellularity and a low frequency of atypical cells. It is our experience that desmoplastic sarcomatoid mesothelioma

is never diagnosable in practice on the basis of either effusion fluid cytology or FNAB.

4. Assessment of pleural effusion cytology (like assessment of biopsy tissue) is critically dependent on the adequacy of the specimen, the quality of specimen preparation, and the experience of the pathologist providing the service. The reported sensitivity and specificity of cytology on the diagnosis of MM varies greatly. In a 1989 review of 30 years of publications, sensitivity varied between 0% and 93%.[678] In later publications, sensitivity remained variable, between 32%[855] and 76%,[856] although the main problem appeared to be the adequacy of the specimen, rather than its assessment. Practicing cytopathologists seem now well aware of the problems in making the diagnosis, and we are not aware of any recent reports of false-positive diagnoses of MM based on cytologic specimens alone.

5. A dedicated service where the entire effusion fluid is received by the pathology laboratory and can be used for microscopy and ancillary studies is likely to give the greatest diagnostic yield. This is highlighted by Whitaker et al.[850] who, on reviewing slides for a published study that claimed low sensitivity of effusion fluid cytology for diagnosis of MM,[855] found that "poor samples were the cause of poor results." The use of immunohistochemical studies on cell-block sections can increase the sensitivity and specificity of cytologic assessment; in other words, a cell block is an essential adjunct to cytologic diagnosis.

There is no doubt that the interpretation of pleural effusion cytology is fraught with difficulty. We agree with Whitaker et al.[850] that "the cytological diagnosis of MMs can be a relatively straightforward exercise though it is often a challenge and occasionally, especially in desmoplastic cases, impossible."

The first step, the distinction of malignant cells, whether mesothelial or metastatic, from benign reactive mesothelial cells, can be problematic. Attention to cytologic detail and additional features in the specimen, such as background inflammation as well as relevant clinical-radiologic details, may all assist in cytodiagnosis. However, it is our approach to err on the side of underdiagnosis when there is uncertainty, on the basis that if the process is malignant (whether MM or secondary cancer), it will declare itself as such soon enough (the prognosis for any kind of pleural malignancy is poor and usually measured in months, with little available in terms of effective treatment options at the moment; see discussion of malignancy-associated pleural effusions in the section Secondary Malignant Neoplasms Affecting the Pleura).

Rapport with Clinician

Effective communication between the cytopathologist and the clinician can aid significantly in the assessment of specimens, and relevant radiologic information should be communicated. It is unfortunate that the current guidelines issued by the British Thoracic Society Pleural Disease Group state that "20 mL of pleural fluid is adequate for cytological examination," and although some of the recommendations regarding biochemical examination have been questioned, this statement seems not to have been challenged, despite the recommendation from some cytologists that the effusion fluid should be submitted in its entirety for optimal results.[850,857,858] No less unfortunate is the statement from the European Respiratory Society (ERS) on the management of malignant pleural effusions[859] that "monoclonal antibodies . . . cannot be relied on for diagnosis" and instead the ERS recommends that "identification of . . . aneuploidy by flow cytometry may add to routine cytology by detecting false negatives."

Macroscopic Appearance of Specimen and the Use of Tumor Markers

Useful information can be gained from observation of the volume, color, clarity, and viscosity of the effusion fluid. A massive effusion is more likely to be due to a malignant process than a small one, and exudative effusions are more likely to be malignant than transudates (see later discussion on malignancy-associated pleural effusions). Highly cellular fluids (as is typical of malignant effusions) may show a thick whitish layer at the bottom of the container if they have been allowed to stand for some time. High viscosity due to high levels of hyaluronic acid is characteristic of MM.[860–862] This finding is particularly useful when quantitative assessment of hyaluronic acid concentration is used in combination with cytologic criteria.[863] Sometimes the hyaluronic acid can be seen on the slides as flocculent background material.[851] Measurement of mesothelin levels in effusion fluid may also contribute to diagnosis[864] (see later discussion in this chapter). Other tumor markers have not been found to be particularly helpful, with the possible exception of CEA, which may be increased in malignant effusion related to secondary neoplasms but was not found to be elevated in any of the cases of MM investigated.[865]

Specimen Preparation

Cytology slides may be prepared as direct smears made from the pellet after centrifugation of the specimen, as smears of the clotted specimen, as direct cytospins of the whole effusion fluid, or as cytospin preparations after Ficol Hypaque gradient centrifugation. Finally, some laboratories also use the Thin-Prep technique originally developed for cervical smears. Each of these techniques has certain characteristics and advantages, but these technical variations may lead to variation in the appearances of the specimen. No significant advantage has been iden-

tified in the use of Thin-Prep preparations over cytospin slides,[866,867] in regard to background and the preservation of cytologic detail. Whenever sufficient material is available, a cell block should be prepared. Immunohistochemical studies are most reliable when performed on sections of cell blocks, as compared to cytospins or direct smears, with the least background staining (apparently due to the reduced proteinaceous background and the reduction of three-dimensional clusters of cells that may trap antibody, resulting in false-positive results[868]). Cell-block sections also allow for the best morphologic interpretation, approximating the results seen in surgical specimens except for invasion, and are the most economical of the techniques tested.

Not only is it important to have comprehensive knowledge of the preferred techniques used in one's own laboratory, but cytologists also need to be aware of these different types of specimen preparation when reviewing slides from other laboratories.

Specimen Adequacy

There is no quantitative rule for the minimal number of mesothelial cells on a slide to assess a specimen as adequate, but in general one can argue that the more cells the better. Our experience suggests that a reasonable assessment is generally possible on samples of 50 mL at least.

Although there is no doubt that specimens are best received fresh, it appears that storage at 4°C for up to 14 days does not significantly compromise assessment of effusion fluid specimens.[869] In particular, apart from increased numbers of cytoplasmic blebs and cytoplasmic vacuolation, morphologic detail remained sufficiently preserved for diagnosis, and immunocytochemistry performed on cell block material did not reveal significant loss of antigenicity. Even though the number of specimens examined was relatively small, the results nonetheless suggested that examination can be attempted with a good chance of obtaining a diagnosis on those specimens that reach the laboratory after considerable delay.

General Aspects of Specimen Assessment

The main differential diagnoses encompass a MM, an atypical but reactive mesothelial proliferation, and secondary neoplasia. The cytologic features that suggest mesothelial differentiation do not by themselves definitively distinguish between benign and malignant mesothelial processes, but a combination of features may be used to make the distinction. Ancillary techniques including immunocytochemistry may also be used, but some are somewhat controversial. In contrast, it is widely accepted that a distinction between a mesothelial process and a metastatic malignancy can usually be made with certainty using an appropriate immunocytochemical panel, discussed below.

Features Indicative of Mesothelial Differentiation, and Discrimination Between Benign Mesothelial Hyperplasia and Malignant Mesothelioma

Normal mesothelial cells may contain one or more round or oval nuclei with one or more nucleoli. There is uniform staining of nuclei and cytoplasm, and most nuclei are located centrally or slightly eccentrically within cells, but only rarely does the nucleus abut the cell border. The cells tend to form flat sheets, with obvious fenestrations between cells (Fig. 43.136), related to the presence of long microvilli between apposed cell membranes.[829] Single cells have finely microvillous (fuzzy) borders, again corresponding to the characteristic elongated and serpentine microvilli. Small three-dimensional balls may be present but usually comprise less than 20 cells. A central collagenous core may be noted. The background may contain erythrocytes, leukocytes, and necrotic debris.

Denser cytoplasm may be seen in reactive mesothelial cells, and larger three-dimensional cell balls containing

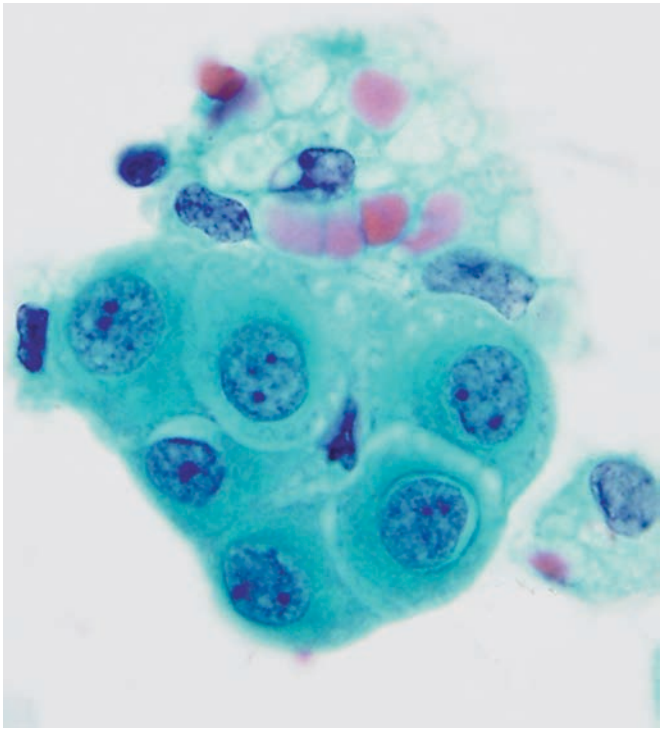


FIGURE 43.136. Atypical mesothelial cells in pleural effusion, thought to represent an atypical mesothelial hyperplasia. No biopsy was taken, but the patient was alive and well 4 years later. The cells are from a cytospin preparation, stained by the Papanicolaou (Pap) technique and show marked cytologic atypia with obvious fenestrations between cells.

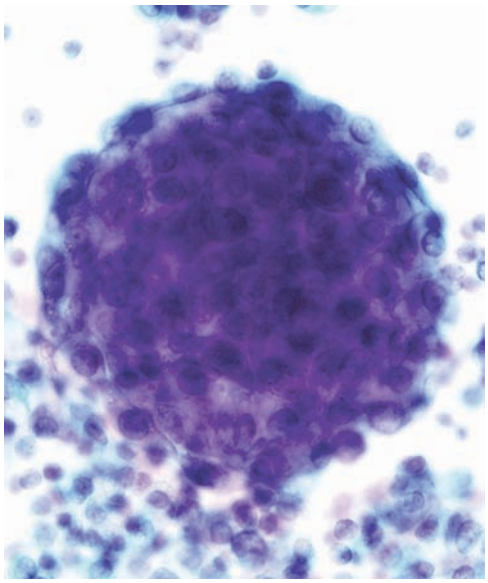


FIGURE 43.137. This Pap-stained cytospin preparation shows a large three-dimensional morule. There is some nuclear pleomorphism and some prominence of nucleoli. The nuclei remain central within most of the cells. This specimen of pleural effusion fluid came from a patient with biopsy-proven invasive MM of epithelial type.

between 20 and 50 cells may become apparent, but numerous tridimensional morules (Fig. 43.137) are more characteristic of MM than a benign mesothelial proliferation. Papillary structures may be obvious (Fig. 43.138), but in pleural effusion fluid numerous papillary formations with prominent collagen cores or abundant basement membrane material (Fig. 43.139) are a feature of MM rather than reactive effusions.[849,870] The background may contain erythrocytes, leukocytes, and cellular debris. Squamous-like cells may also sometimes be seen in pleural effusions (Fig. 43.139) and are thought to be a feature associated with degenerative mesothelial cells, but they do not equate to malignancy. However, such squamoid cells are more common in mesothelioma; if prominent, this finding can cause confusion with metastatic squamous cell carcinoma.[871]

Mesothelial cells may show large single or multiple small cytoplasmic vacuoles. Multiple small vacuoles may represent lipid vacuoles, others are usually considered to be degenerative in nature, and larger glycogen-filled vacuoles may also be seen. Occasionally large single vacuoles may be present, which may mimic the mucin-filled vacuoles seen in adenocarcinoma (Fig. 43.140). These are now thought to contain hyaluronic acid. However, adenocarcinoma cells may also contain different types of vacuoles, and all of these findings can be misleading. We routinely stain spare slides or cell-block sections with PAS and PAS-diastase stains.

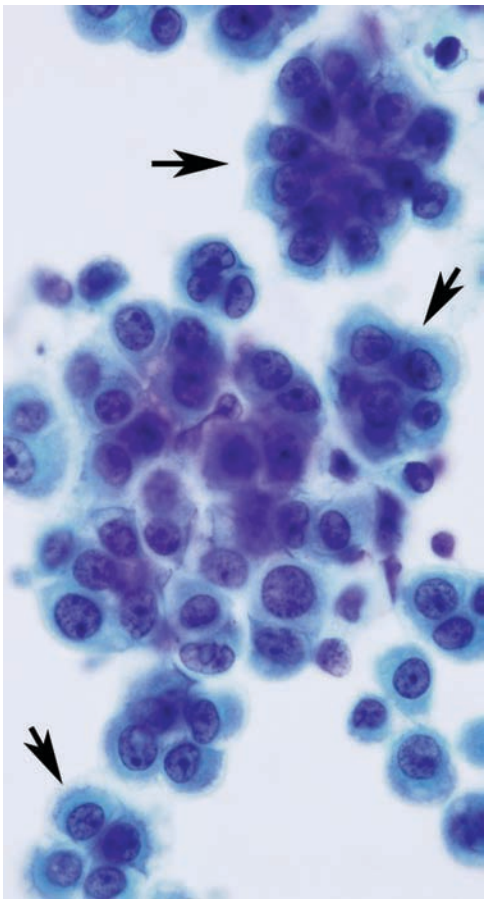


FIGURE 43.138. Atypical mesothelial proliferation. (Same case as in Figure 43.136.) This Pap-stained cytospin demonstrates papilla formation in a pleural effusion fluid (arrows).

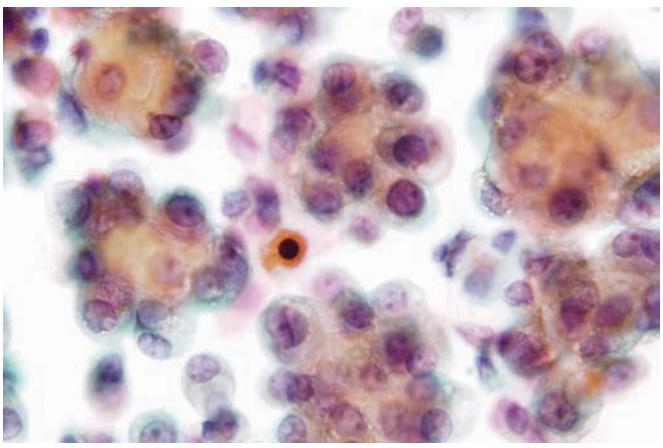


FIGURE 43.139. Mesothelial cells in pleural effusion, from a patient with biopsy-proven invasive MM of epithelial type, stained by the Pap technique. Thus micrograph depicts several papillary clusters of atypical mesothelial cells. The core in some of the papillae shows glassy orange staining, which correlated with the presence of PAS-positive basal laminal material. A small rounded squamoid cell with a pyknotic nucleus and intensely orangeophilic cytoplasm is also evident.

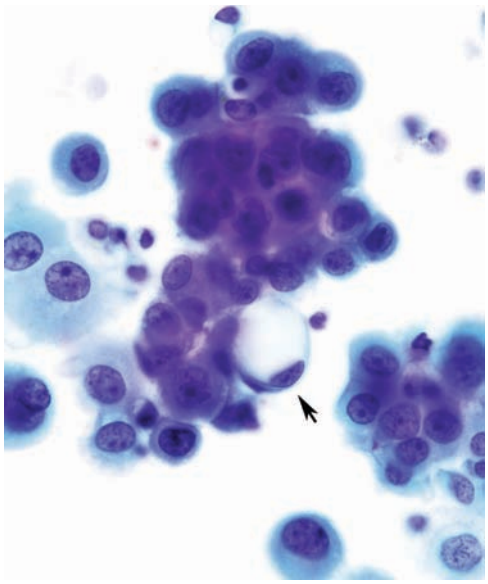


FIGURE 43.140. This Pap-stained cytospin preparation depicts a large intracytoplasmic vacuole in a mesothelial cell, with displacement of the nucleus to the periphery of the cell (arrow). For cells like this, the main differential diagnosis is adenocarcinoma. This patient had a biopsy-proven invasive MM, epithelial type.

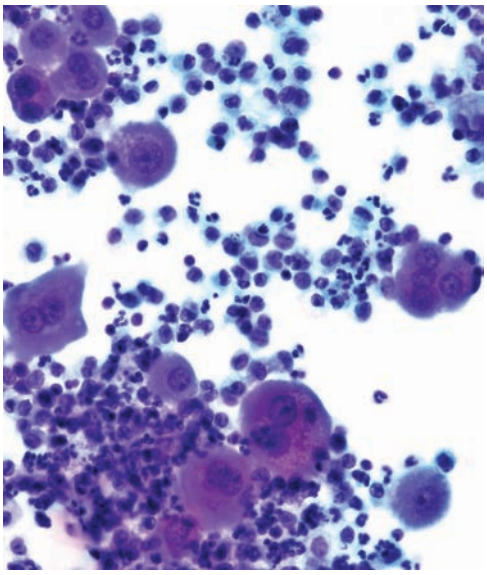


FIGURE 43.141. Multinucleation of cells may be seen in reactive processes as well as in MM. However, the presence of numerous multinucleated cells in virtually every high-power microscopic field examined supports a diagnosis of MM. This pleural fluid was taken from a patient with biopsy-proven invasive MM of epithelial type.

The cellularity of a specimen is important. In general, malignant effusions show greater cellularity than benign "reactive" effusions, but the cellularity seen on the slide may depend in part on the preparatory method used, and cellularity alone is insufficient for a diagnosis of malignancy. With increased cellularity, papillary structures can often be found in the effusion fluid; if found in significant numbers, they should suggest a malignant diagnosis (cf. Figs. 43.138 and 43.139). As mentioned previously, cytologic atypia alone is insufficient for a diagnosis of malignancy; MMs often do not show marked cytologic atypia and characteristically maintain a stable nuclear to cytoplasmic ratio, although a high nuclear-to-cytoplasmic ratio may occasionally be seen, and fenestrations may be evident between apposed cells (Fig. 43.136). Conversely, hyperplastic mesothelial cells can show substantial cytologic atypia, as well as nucleoli.

Although multinucleated mesothelial cells may be seen in reactive processes, the presence of numerous multinucleated cells—with multiple multinucleated cells in any given high-power microscopic field—has been found to correspond to MM (Fig. 43.141). The presence of cell-cell engulfment[872] or "cannibalism" in a pincer-like arrangement is also commonly seen in MM (Fig. 43.142) and may support a diagnosis of malignancy versus a reactive process. The presence of necrotic debris is a strong indicator of a malignant process.

The cytologic features that may aid in the differential diagnoses between a malignant pleural mesothelioma and a benign reactive mesothelial hyperplasia are summarized in Table 43.21, but a host of ancillary techniques has also been employed and these are discussed below.

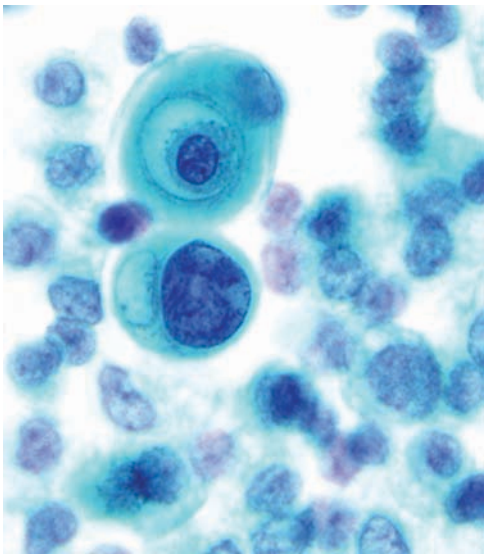


FIGURE 43.142. Cell-cell engulfment or "cannibalism" is considered to be a general feature of malignancy, but is common in MM. Shown here is the typical pincer-like cell-in-cell arrangement in a biopsy-proven case of invasive MM. The adjacent cell shows a high nuclear-to-cytoplasmic ratio.

TABLE 43.21. Summary of cytologic discriminants among reactive mesothelial hyperplasia, epithelial malignant mesothelioma, and secondary adenocarcinoma

Feature	Reactive mesothelial hyperplasia/mesotheliosis	Mesothelioma (epithelial, biphasic)	Metastatic adenocarcinoma
Low-power	Moderate to high cellularity	Cellular	Cellular
Cell population	• Single epithelioid cell population • ± Inflammatory cells	• Single epithelioid cell population	• Classically dual epithelioid cell population, but may be single malignant population
Cell disposition	• Single cells • Small 2D clusters/sheets and clumps (<20 cells)	• Single cells • Large 3D morules, (>50 cells) • Scalloped and complex outline of clusters • Papillary structures • Pseudoacini with collagen core	• Large clusters (>12 cells) smooth "cannonball" outline • Acini with peripheral nuclei • Cells in single-file row
Cytologic features	• Enlarged cells • Enlarged central nucleus	• Enlarged cells, N/C ratio same or less • Range of cell sizes • Many multinucleated cells, "cell-in-cell" • Giant mesothelial cells • Squamous-like cells	• Enlarged cells • Atypical and bizarre cells
Differentiating features	• Fenestrations between the cells in clusters/sheets • Central nuclei • Bi-tonal staining cytoplasm (dense orange around nucleus to green-blue at periphery in Pap stain, denser centrally in DQ) • Peripheral fringe • Cytoplasmic vacuoles may be present (no or only minimal PAS-diastase staining) • Atypia usually only moderate		• Mucin vacuoles indenting nucleus, PAS-diastase positive • Nuclei peripheral • Nuclei may be very atypical, often with coarse chromatin
IHC	• Calretinin (nuclear) • CK5/6 • WT1 (nuclear) • HBME-1 (linear membrane) • Thrombomodulin (linear membrane) • EMA: strong circumferential linear labeling more common in MM than reactive (clone E29), cytoplasmic labeling in adenocarcinoma		• CEA • B72.3, • CD15 • BG8 • Site specific markers, e.g., TTF-1, gross cystic disease fluid protein (GCDFP)
EM	Long slender serpentine microvilli; no glycocalyx		Short stubby microvilli; antennular glycocalyx

DQ, Diff Quick; EM, electron microscopy; IHC, immunohistochemistry; 2D, two-dimensional; 3D, three-dimensional.

When assessing pleural effusions, we emphasize that mesotheliomas are histologically diverse tumors, and consequently the cytologic features and presentation can also be diverse. For example, the effusions in biopsy-proven cases of sarcomatoid mesotheliomas are often paucicellular with minimal cytologic atypia, and hence not diagnostic of malignancy. In summary, we concur with Whitaker,[849] who stated, "There is no single or set of morphological criteria that are entirely specific for mesothelioma, yet there are common patterns that often permit us to confidently assert the diagnosis."

Ancillary Techniques used to Distinguish MM and Reactive Mesothelial Hyperplasia

Immunocytochemistry can be applied to Thin-Prep preparations and direct smears,[873] but we, like most others, prefer cell-block sections.[866,868] Labeling of cells in the typical linear distribution with antibodies against EMA[667] (clone E29) has also been found to be useful for the distinction between MM and reactive effusions,[662–664] because only MM showed strong and widespread (>10% of cells) membranous staining.[671] Although none of the reactive effusions showed staining in this pattern, only 75% of MMs tested showed this pattern of EMA labeling, with high specificity but low sensitivity. However, at the moment, this is the only ancillary technique available in most routine diagnostic laboratories to aid in the distinction between mesothelial hyperplasia and MM,[576] with the E29 clone being commercially available (Dako); we have found this to be a useful indicator of malignancy (Fig. 43.143).

In addition, immunohistochemical labeling for the one of the inhibitors of apoptosis proteins (IAPs), the X-linked-IAP (XIAP), has been found to be of value in distinguishing benign from malignant effusions, irrespec-

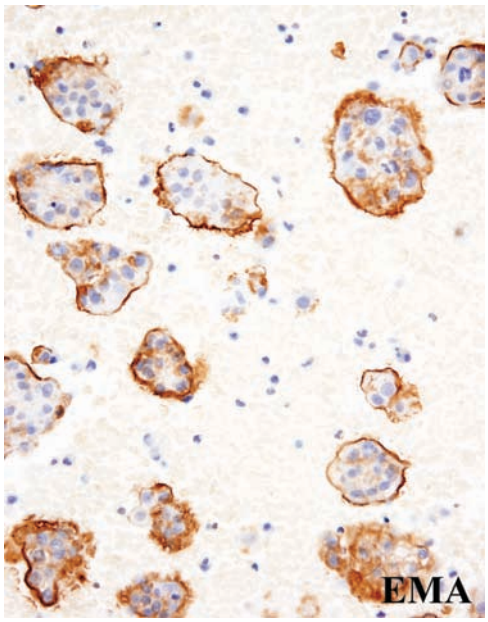


FIGURE 43.143. Immunocytochemical labeling of a cell-block section for EMA (clone E29) in a biopsy-proven case of MM reveals strong, circumferential membrane labeling of the cells and cell clusters, supporting a diagnosis of malignancy.

tive of the type of malignancy.[704] In the one study published to date, 80% of mesotheliomas showed positive labeling of mesothelial cells, whereas in reactive effusions positive labeling was limited to histiocytes in a minority of specimens (6%). This technique may prove to be useful, particularly in the distinction of reactive mesothelial hyperplasia and MM, but further validation of the results is required before routine use can be advocated, and identification of the type of malignant cells by other means (cytomorphology or immunohistochemistry) would still be necessary.

The use of immunohistochemistry for the distinction between MM and metastatic carcinoma is discussed in detail below.

Flow cytometry has been used to distinguish reactive and malignant mesothelial cell populations in pleural effusion fluids, based on the fact that malignant cells commonly show aneuploidy. Although high specificity has been reported in research laboratories, this approach appears at present to be too insensitive for routine diagnostic use.[874–876]

Image cytometry on de-stained Papanicolaou (Pap)-stained slides, which were then re-stained with the Feulgen stain, also assesses ploidy, and was found to be particularly helpful in the distinction of reactive mesothelial proliferations (all diploid) from MM (most aneuploid), but this technique is less useful for the distinction of MM from secondary adenocarcinoma, because both are mostly aneuploid.[876] Other studies found ploidy studies in isolation to be less useful, but suggested that prognostic information may be gained from ploidy studies on histologically confirmed MMs.[874,877]

Additional techniques that have been investigated include *silver nucleolar organizer region (AgNOR) staining*, which resulted in 95% sensitivity in small closed biopsies when combined with linear EMA labeling.[679] AgNOR testing appears to be fairly specific for malignant effusions and possibly more sensitive than ploidy studies by either fluorescence in-situ hybridization (FISH) or flow cytometry, but because of the high demands on either staff time or image analysis equipment, this technique has also not entered into routine diagnostic practice.[679,878,879]

Distinction Between Secondary Neoplasms Affecting the Pleura and Malignant Mesothelioma

The distinction between a malignant mesothelial process and a metastatic malignancy makes use of cytomorphology and ancillary techniques. On microscopic examination, the most obvious and important feature is the presence of a dual cell population (Fig. 43.144), although, rarely, a single population of metastatic malignant cells may be present and may mimic MM. The most common distinction is between MM and adenocarcinoma, with lung (for males) and breast (for females) being the most common primary sites (see Secondary Malignant Neoplasms Affecting the Pleura, below). However, other

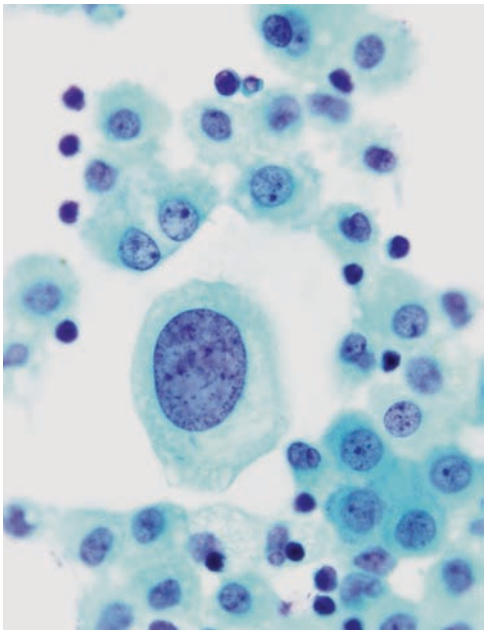


FIGURE 43.144. Pleural effusion fluid from a patient with documented disseminated breast carcinoma, showing a dual population of malignant cells. The large cell illustrated shows a high nuclear-to-cytoplasmic ratio.

types of malignancies, such as lymphoma or squamous cell carcinoma (SCC) may also enter the differential diagnosis. The exclusion of SCC is particularly important if the squamous-like cells sometimes seen in mesothelial proliferations are numerous. Lack of immunohistochemical labeling for calretinin in SCC is particularly useful in this situation.[637]

The effusions of metastatic adenocarcinoma are often very cellular, and may contain cell aggregates, but unlike the morules seen in MM, the cell aggregates in metastatic adenocarcinoma often have smooth contours. They may also show obvious acinar arrangements, with columnar cells featuring eccentric nuclei. Malignant mesothelial cells may be enlarged and even "giant," but classically the nuclear to cytoplasmic ratio is retained and frankly bizarre cells indicate a diagnosis of carcinoma.

Ancillary Techniques for the Distinction of Malignant Mesothelioma from Metastatic Neoplasms

Once a diagnosis of malignancy has been reached, and a distinction between MM and, for example, adenocarcinoma is required, ancillary techniques are particularly useful, and can be employed successfully by routine diagnostic laboratories. For example, mucin stains may distinguish intracytoplasmic mucin droplets of adenocarcinoma from prominent vacuoles in mesothelial cells. However, occasional mucin-producing mesotheliomas have been described.[505,539]

Unlike the distinction between reactive mesothelial processes and MM, a clear distinction between metastatic carcinoma and MM can be made with confidence in most cases, using appropriate immunocytochemical protocols. Different laboratories have found different panels of antibodies useful, and there are numerous current reviews suggesting various panels of antibodies for effusion fluids.[566,658,666,676,880–884] There are many more studies focusing on histologic sections[547,563,578–580] and one would expect similar staining results for cell-block sections, although such findings require verification. Meta-analysis has been attempted on the studies of surgical specimens, in an a effort to provide guidance,[547] but we are not aware of any such attempt for the panels of antibodies used in cytologic preparations.

In everyday diagnostic practice, we employ a standardized immunocytochemical protocol that includes mesothelial cell markers, markers that react with both mesothelial cells and other epithelial cells, and carcinoma-related markers. A suggested panel includes CAM5.2 or AE1/AE3 and EMA as general epithelial markers; CK5/6, calretinin, HBME-1, WT1, and thrombomodulin as mesothelial cell markers; and CEA, CD15, B72.3, and BG8 as carcinoma-related markers. Like many others, we have found calretinin to be particularly useful.[566] The marker mesothelin has been found to be less sensitive and specific than calretinin.[614] In contrast, D2-40 was reported to show some promise in cytologic specimens by some authors,[714] but other publications suggest low specificity.[605] We have not used this antibody extensively in this setting. Because the most common secondary malignancy in the pleura is a metastasis of pulmonary carcinoma, we routinely include TTF-1 in our panel. Other site-specific markers such as gross cystic disease fluid protein (GCDFP) may also be included. If the distinction is between SCC and MM, labeling for high-molecular weight cytokeratins in the absence of labeling for calretinin has been found to be of value.[632,637]

Should this protocol yield inconclusive findings, and depending on the cytomorphology of the cell population in the individual case, as well as the clinical background and past medical history, additional markers can be added (for example, further mesothelial, carcinoma-related markers and markers for endothelial and melanocyte differentiation and even lymphoid markers).[37,213] It also has been recommended that each laboratory should establish its own protocol that best meets its requirements and that yields consistent results, with high specificity and a high predictive value overall, keeping in mind that it is unlikely that a unique and reproducible immunoprofile will ever be established for a morphologically protean tumor such as MM.

Ancillary Techniques to Increase the Detection of Malignant cells in Effusion Fluids

To decrease the rate of false-positive fluids, the use of ancillary techniques has been suggested. However, immunocytology has not yielded convincing results in this regard. In one study there were only three of 26 cases of false-positive serous effusions where malignant cells could be detected using a panel of markers,[885] and similar results were seen in earlier studies.[639] This low cost-benefit ratio for such expensive and labor-intensive techniques has been considered as the main reason for the continued use of conventional cytology as first-line investigation.

Fine-Needle Aspiration in the Diagnosis of Malignant Mesothelioma

Fine-needle aspiration (FNA) has been performed with success on a range of pleural lesions, including MM, solitary fibrous tumors, synovial sarcoma, and unusual lesions such as myelolipoma.[886–892] The technique has also been found useful in the identification of local recurrence of MM.[893] In addition, primary diagnosis of MM based on an aspirate obtained from a supraclavicular lymph node has also been described.[894] The diagnostic considerations are similar to those associated with the assessment of

pleural effusion fluid, in that clinicopathologic correlation is required. As for effusion fluids, a correct diagnosis of epithelial or biphasic MM may be possible based on the cellular findings in a sufficiently cellular specimen, but the diagnosis of sarcomatoid and, in particular, desmoplastic MM can be challenging or impossible. Ancillary techniques, in particular, immunohistochemistry and EM, may be extremely useful in reaching the correct diagnosis.

In addition, FNA has been employed in the diagnosis of metastatic MM, but there are only few reports available.[895–898] It appears that the cytomorphologic features of the metastatic tumors vary greatly, as might be expected in view of the morphologic variability of MM, and immunohistochemical techniques and clinical information, including knowledge of previous malignancy, play a major role in the diagnosis of these tumors.

Finally, percutaneous cutting needle biopsy under radiologic guidance, yielding a thin core of tissue, may be employed if insufficient material is sampled by FNA. This technique has a reported sensitivity of 86%, with 100% specificity,[899] and we have found this to be occasionally helpful in fibrous or desmoplastic lesions. The different techniques and lines of investigations available for diagnosis should be regarded as complementary.

Secondary Malignant Neoplasms Affecting the Pleura

Secondary neoplasms represent the most common pattern of malignancy affecting the pleura, and it has been estimated that malignant disease accounts for about 25% of all pleural effusions[900,901]—ranking after effusions related to congestive cardiac failure in the elderly and as a complication of pneumonia (parapneumonic effusion)[902]—and amounting to about 75% of exudative pleural effusions.[903] According to Matthay et al.,[902] among 1868 pleural effusions reported by different groups, 785 (42%) were linked to cancer, with a large increase in the percentage of malignancy-associated pleural effusions from the third and fourth decades with a further proportional rise in the seventh decade, followed by a fall in the eighth.

Because of its frequency and anatomic proximity to the pleura, carcinoma of the lung represents the most frequent cancer associated with malignant pleural disease—about 35% to 45% of pleural effusions related to cancer[900,902]—and it has been estimated that about 7% to 15% of lung cancer patients develop pleural effusion during the course of their disease.[903] Metastatic breast cancer accounts for about 25% of malignant pleural effusions,[900,902] followed by malignant lymphoma, including both Hodgkin's and non-Hodgkin's malignant lymphomas (about 10%).[900,902,903] In one series of cases,[904] women were almost twice as likely to develop metastasis to the pleura than men, related to the high frequency of pleural metastasis from breast cancer. These three categories of cancer account for about 75% of all malignancy-associated pleural effusions.[900] Metastatic carcinomas of ovarian or gastric origin, malignant melanoma, and sarcomas account for only a small percentage of cancer-associated pleural disease (about 5%).[900,902,903] In about 5% to 15% of cases with malignancy-associated pleural effusion, the primary site is unknown,[900,902,903] but it can often be identified using a panel of immunocytochemical markers.[905]

Adenocarcinoma represents the most frequent histologic type of lung cancer to result in a malignant pleural effusion—presumably because adenocarcinomas comprise a greater proportion of peripheral cancers than the other histologic types[903]—followed by squamous, small cell, and large cell undifferentiated carcinomas. As expected, adenocarcinomas represent the histologic type for cancers of breast, ovary, and stomach metastatic to the pleura.[902]

In some cases, malignancy-associated pleural effusions do not involve direct infiltration of the pleura by the cancer, and Sahn[903] designates such effusions as "paramalignant." As Sahn has emphasized, the lymphatic system of the parietal pleura, which joins the intercostal trunk vessels that drain predominantly toward the mediastinal lymph nodes, is the only pathway for clearance of fluid from the pleural cavities. Obstruction of this pathway at any point (for example, by mediastinal lymph node metastases) can result in a pleural effusion. Alternatively, a paramalignant effusion can result from obstructive pneumonitis as a consequence of lung cancer, or even from venous obstruction (for example, as part the superior vena cava syndrome).[902] In some instances, notably those resulting from lymphatic or venous obstruction, the effusion represents a transudate as opposed to a exudate. In contrast, effusions resulting directly from neoplastic infiltration of the pleura are characteristically exudative. Other causes of paramalignant pleural effusion include pulmonary embolism and low serum protein levels, or the effects of radiation or chemotherapy.[902] Depending on the anatomic site of the primary tumor, infiltration of the pleura can result from direct invasion of the visceral pleura by an underlying lung cancer or, alternatively, infiltration into the subpleural lymphatic plexus or from invasion of small branches of the pulmonary artery, with embolism of tumor cells to the periphery of the lung where they can then invade the visceral pleura. In the case of malignant pleural effusions resulting from subdiaphragmatic tumors, it has been suggested that the pleural involvement represents tertiary spread from hepatic metastases.[902,903]

Malignancy-associated pleural effusions need not be bilateral. Patients with lung cancer usually develop unilateral pleural effusion on the same side as the primary carcinoma, but occasionally the effusion is bilateral; an

effusion restricted to the contralateral side is rare.[902] In contrast, with patients with breast cancer and subdiaphragmatic neoplasms (for example, stomach or ovary), there is no such predilection for the ipsilateral side.[902] It has been estimated that 50% of patients with disseminated breast cancer develop a pleural effusion during the course of their disease, on the same side as the original breast cancer in 60% of the patients, on the contralateral side in 25%, and bilaterally in about 15%.[902] In general, the interval between the diagnosis of the primary breast cancer and the subsequent development of an associated pleural effusion is about 2 years, but it can be as long as 20 years or more.[902]

The size of the pleural effusion in metastatic malignancy varies greatly. In about 75% of patients the effusion is moderate to large, within the range of about 500 to 2000 mL; in about 10% the effusions are massive (with complete opacification of the affected hemithorax); and in a further 10%, approximately, the effusions are small (less than 500 mL).[903] About 70% of patients with a massive effusion have an underlying cancer as the basis for the effusion.[903] Matthay et al.[902] referred to one series of 46 patients with massive pleural effusions from all causes: 31 (67%) had malignant pleural effusions, 27 as a consequence of metastatic carcinoma and one patient had a MM.

From an analysis of 500 documented cases of pleural effusion as a consequence of metastatic malignancy, Matthay et al.[902] found that the diagnostic yield from cytologic examination of pleural effusion fluid was 66%, versus 46% from pleural biopsy. Matthay et al. commented that pleural fluid cytologic examination is more sensitive for the diagnosis of metastatic cancer than pleural biopsy, and although cytology and biopsies are complementary to each other, pleural biopsy added little to cytologic examination. Matthay et al. commented further that the lower yield from pleural biopsy may represent operator technique or sampling error, the latter known to be a problem in that metastatic deposits can be widely scattered over the pleural membrane. They suggested that diagnostic yield can be increased by repeat cytology examinations and pleural biopsy. If a diagnosis is not obtained following repeat cytology examination and biopsy, thoracoscopy can be considered, and when multiple biopsies are taken at thoracoscopy, the diagnostic yield rises to about 80% to 97%.[902] Vargas and Teixeira[900] commented that pleural biopsies in cases of malignant pleural effusion establish the diagnosis in about 40% to 75%, but the combination of cytologic evaluation of the effusion fluid and a needle biopsy allows a diagnosis in about 80%. Medford and Maskell[906] commented that "blind" pleural biopsy increased the diagnostic yield over cytologic examination of effusion fluid by only 7% to 27%, and that at least four samples from one site are required to optimize the diagnostic return. These authors also set forth their perception that "blind" pleural biopsy no longer has a role in the investigation of malignant pleural disease and that it should be replaced by guided biopsies under imaging control.

In general, pleural metastatic deposits are a marker of advanced disease,[906] and survival of patients with pleural deposits from cancer of the lung, stomach, or ovary is usually measured in only a few months following diagnosis of the malignant pleural effusion.[902]

Although it is emphasized that lung and breast cancer and malignant lymphomas account for about 75% of malignancy-associated pleural effusions, almost any cancer with the capacity for metastasis to the lungs in particular also has the capacity for metastasis or spread to the pleura. Such unusual metastases can range from renal cell carcinomas to ependymomas arising in the central nervous system, among many others.

Pseudomesotheliomatous Tumors Affecting the Pleura Including Pseudomesotheliomatous Adenocarcinoma of Lung

By definition, pseudomesotheliomatous neoplasms affecting the pleura are characterized by diffuse infiltration of the pleura in a pattern essentially identical to, and indistinguishable from, pleural MM on gross examination or on radiologic studies, including CT scans.[907] In this regard, the neoplasm characteristically takes the form of multiple nodules, plaques, or a confluent rind of tumor, with an associated pleural effusion in many instances and with frequent obliteration of the pleural cavity in the later stages of the disease, sometimes with invasion into the chest wall, diaphragm, and pericardium, as seen at autopsy.

Most pseudomesotheliomatous neoplasms affecting the pleura are thought to originate from the lung,[908–923] but pseudomesotheliomatous metastases from carcinomas arising in other sites are well recorded, including the kidney,[918,924–926] thyroid gland,[497] larynx,[927] stomach,[918] and cutaneous malignant melanoma as well as various sarcomas, including malignant phyllodes tumor.[928]

In addition, with pseudomesotheliomatous carcinomas (PMCs) of the lung, adenocarcinoma is the most frequent histologic type, but other cell types can produce pseudomesotheliomatous spread, including SCC, small cell carcinoma,[773] large cell undifferentiated carcinoma, and carcinosarcoma.[532]

Pseudomesotheliomatous carcinomas of the lung were first described by Babolini and Blasi[929] in 1956, to emphasize that the symptoms in these patients were related predominantly to involvement of the pleura with recurrent exudative effusion, often accompanied by chest pain and dyspnea. Of five cases reported by Babolini and Blasi, two appear to have represented small cell carcinoma and the other three were adenocarcinomas. About

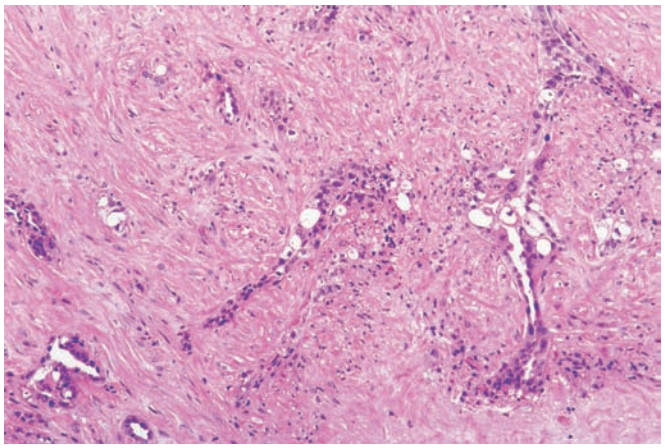


FIGURE 43.145. Pseudomesotheliomatous adenocarcinoma. Pleural biopsy from a 77-year-old man with a right pleural effusion. At thoracoscopy, the appearances were considered suggestive of a malignant mesothelioma. The neoplastic acini are embedded in a prominent fibrous stroma ("tubulo-desmoplastic adenocarcinoma").

20 years later, Harwood et al.[908] reported six cases of primary lung cancer with mimicry of mesothelioma in terms of the distribution of the carcinoma within the pleura, and they introduced the term *pseudomesotheliomatous carcinoma*. In two of their six cases there were small intraparenchymal nodules in the underlying lung parenchyma and all tumors were adenocarcinomas, with bronchioloalveolar features in five. The patients were all men of ages 50 to 76 years, and they had symptoms of dyspnea on exertion, chest pain, and weight loss. Koss et al.[916] also reported an underlying adenocarcinoma in the lung in seven out of 14 autopsy cases. Nonetheless, in some instances, pleural pseudomesotheliomatous adenocarcinomas show no evidence of an underlying intraparenchymal tumor, probably explicable by overgrowth of a small peripheral primary lung cancer by the predominant pleural extension.

In their review, Koss et al.[916] reviewed 15 previously published pseudomesotheliomatous adenocarcinomas of lung and added a further 15 examples from the files of the Armed Forces Institute of Pathology (AFIP) in Washington. Ninety percent of the patients were men with a median age of 61 years, and 17% had possible to definite occupational exposure to asbestos; one patient had proven asbestosis. The prognosis for pseudomesotheliomatous adenocarcinoma was similar to that of mesothelioma: the mean survival time in this series[916] was 4.7 months and the longest survival was 25 months.

Although PMCs are defined entirely by the gross anatomic distribution of the neoplasm (or on radiologic examination as a surrogate for gross examination), the acinar structures in pseudomesotheliomatous adenocarcinoma may or may not resemble an epithelial mesothelioma; that is, these tumors may comprise simplified or isolated glands in a fibrotic stroma, with appearances characteristic of adenocarcinoma; however, in some instances they can show a complex branching and anastomosing architecture producing a histologic resemblance to epithelial mesothelioma (Figs. 43.145 and 43.146). The acini, tubules, and nests of tumor cells in PMC are characteristically surrounded by thickened and fibrotic stromal tissue (Fig. 43.145), heightening the resemblance to mesothelioma (an appearance that Hammar and Dodson[907] have described as "tubulo-desmoplastic adenocarcinoma").

In the series reported by Koss et al.,[916] the main feature used for the diagnosis of pseudomesotheliomatous adenocarcinoma was the presence of PAS-diastase–positive mucin in gland lumina or as intracytoplasmic droplets (but "all of the AFIP surgical specimens . . . were selected on the basis of mucin-positivity within tumor cells"). The distinction between mesothelioma and pseudomesotheliomatous adenocarcinoma is usually straightforward on immunohistochemical staining, and the distinction is facilitated by use of a panel of mesothelial cell markers and generic carcinoma-related antibodies (Figs. 43.147 and 43.148), together with immunostaining for TTF-1.

A further issue that awaits clarification is whether a causal relationship between these tumors and asbestos exposure differs from other bronchopulmonary carcinomas. In our experience, a high proportion of pseudomesotheliomatous adenocarcinomas appear to have a background of occupational exposure to asbestos, but it is unclear whether this seemingly high proportion is

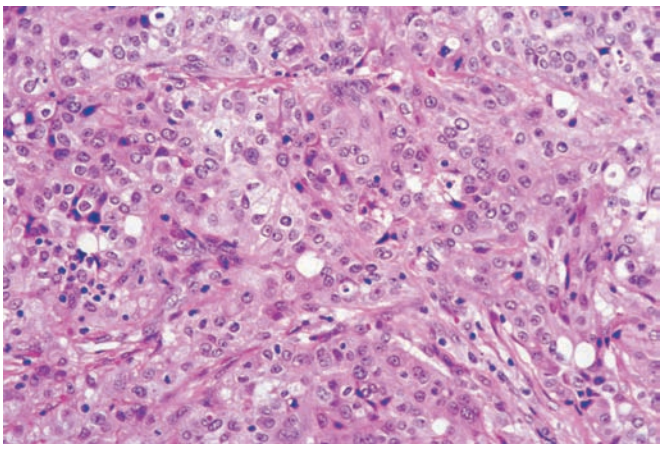


FIGURE 43.146. Pseudomesotheliomatous adenocarcinoma of pleura. This carcinoma is more cellular than the tumor illustrated in Figure 43.145, with a paucity of stromal tissue. The nuclei of the neoplastic cells are nonhyperchromatic and they show only moderate cytologic atypia. The appearances are similar to those seen in some epithelial mesotheliomas.

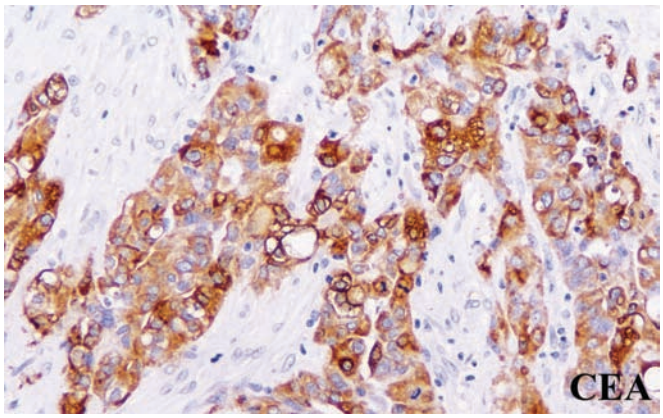


FIGURE 43.147. Pseudomesotheliomatous adenocarcinoma. (Same case as in Fig. 43.145.) The tumor shows positive staining for carcinoembryonic antigen (CEA).

explicable by (1) patterns of referral of cases for which mesothelioma is the differential diagnosis, or (2) whether the clinical and radiologic mimicry of mesotheliomas by these tumors stimulates a more detailed history concerning asbestos exposure than would be the case for conventional lung cancers (see also Chapter 27).

Spindle Cell Carcinoma and Carcinosarcoma of Lung

Although spindle cell (sarcomatoid) carcinomas of lung usually form localized intraparenchymal mass lesions, they can invade the pleura, with the potential for histologic mimicry of biphasic mesothelioma. In this regard, Mayall and Gibbs[532] reported two carcinosarcomas that presented as pleural tumors, with encasement of the lung in a pseudomesotheliomatous fashion in one patient. No site of origin within the lung could be identified for either tumor. These authors suggested that the following findings in such tumors militate against a diagnosis of mesothelioma: (1) neutral mucin production; (2) expression of CEA; (3) squamous differentiation, although squamous differentiation can occur rarely in MMs of epithelial type; or (4) evidence of neuroendocrine differentiation.

Serosal-Surface Serous Papillary Tumors

Because serous papillary adenocarcinomas arise predominantly from the ovaries or the peritoneal mesothelium itself, mimicry of *pleural* mesothelioma is exceptional, but it can constitute a significant diagnostic problem, especially because a high proportion of serous papillary carcinomas show no evidence of CEA expression on immunohistochemistry.[119,930–933] Even so, three patients with an underlying serous papillary adenocarcinoma of the peritoneum encountered by the authors[119] (Figs. 43.149 and 43.150) presented with unilateral pleural effusion, apparently related to spread from the underlying peritoneal tumor (in at least one of these cases, the primary peritoneal lesion was demonstrable only on CT imaging). The diagnosis in most instances can be made on detailed immunohistochemical studies, for example, including labeling with antibodies such as Ber-EP4 (Fig. 43.150), B72.3,[119,930,932,934] and BG8. In two cases in our files the diagnosis was established primarily by electron microscopy, which demonstrated short blunt microvilli with an antennular glycocalyx characteristic of carcinoma in one case, and by the presence of elongated branched microvilli in another case, where the microvilli lacked the sinuous and serpentine architecture characteristic of mesothelial microvilli.[119] The resemblance of such serous

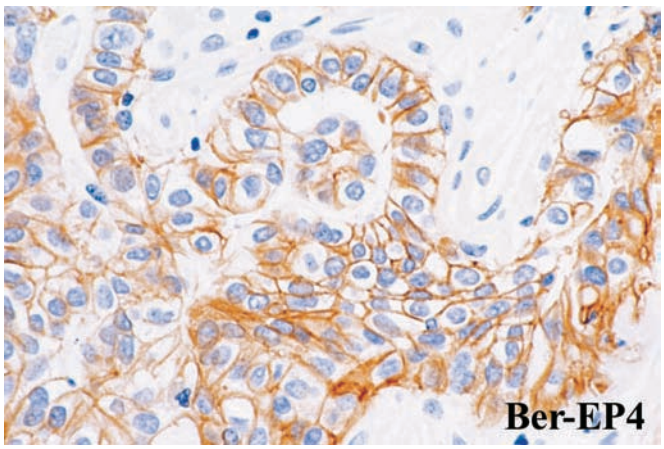


FIGURE 43.148. Pseudomesotheliomatous adenocarcinoma. Positive linear labeling of the neoplastic cells with Ber-EP4.

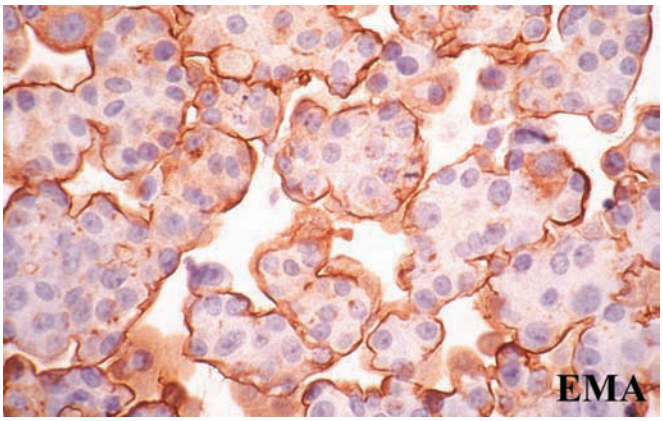


FIGURE 43.149. Pleural metastasis of a serous papillary adenocarcinoma of the peritoneum (cytology cell block section). Linear membrane-related staining for epithelial membrane antigen (EMA), essentially indistinguishable from labeling that characterizes epithelial mesotheliomas.

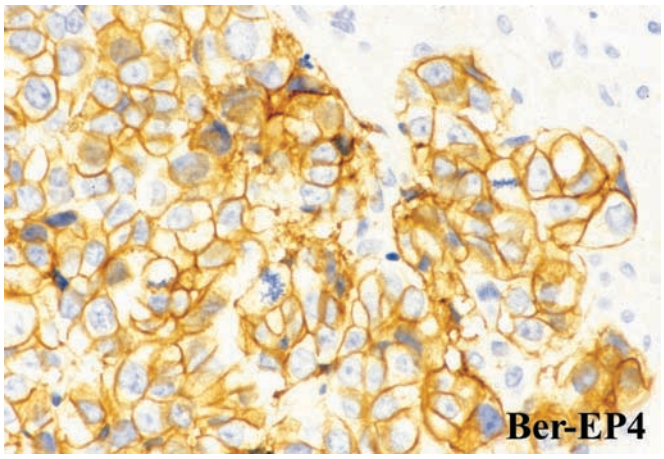


FIGURE 43.150. Pleural metastasis of a peritoneal serous papillary adenocarcinoma. (Same case as in Fig. 43.149.) positive linear labeling of the neoplastic cells with Ber-EP4 in a "chicken-wire" pattern.

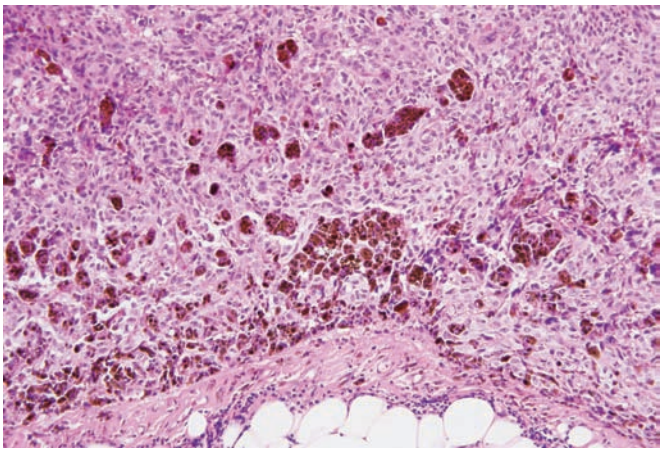


FIGURE 43.151. Pleural metastasis of malignant melanoma of unknown primary site, in an 83-year-old man with a recurrent blood-stained pleural effusion, thought on clinical grounds to be suspicious of mesothelioma. As illustrated, the melanoma showed confluent spread over the pleura. Plentiful melanin pigment is evident, mostly concentrated in stromal macrophages.

papillary carcinomas to mesothelioma is further enhanced by the pattern of EMA staining in some cases, with linear membrane-related labeling in some instances (Fig. 43.149).

Other Tumors that can Invade or Spread to the Pleura

We have also encountered cases of renal cell carcinoma and amelanotic malignant melanoma metastatic to the pleura[662] (Figs. 43.151 and 43.152), with mimicry of mesothelioma on rare occasions, and renal cell carcinomas with a spindle cell sarcomatoid pattern represent a potentially difficult differential diagnostic problem. In such instances, labeling of the tumor for renal cell carcinoma–related markers such as CD10 and renal cell carcinoma antigen may facilitate the diagnosis,[711] but whenever renal cell carcinoma enters into the differential diagnosis, we routinely recommend exclusion of an underlying renal tumor by noninvasive imaging procedures such as ultrasound or CT scanning.

Metastatic melanoma is distinguishable from mesothelioma by the absence or paucity of CK expression in most instances, and by positive labeling for S-100 proteins and other melanoma-related markers such as HMB-45[119,662] (Fig. 43.152) and melan-A.

We have also encountered rare cases of sarcoma metastatic to the pleura, with clinical and even histologic mimicry of mesothelioma on rare occasions, including one case of metastatic sclerosing epithelioid fibrosarcoma.[935,936] Such cases highlight the importance of comprehensive clinical data, including a history of any other neoplasm with the capacity for metastasis to the pleura, to avoid misdiagnosis of secondary sarcomas and other cancers as mesothelioma. At the same time, because mesotheliomas are most often encountered in patients over 55 years of age, many of our patients with proven pleural MM have had a history of antecedent cancer (for example, carcinoma of the prostate). When dealing with cases of this type it is crucial to compare the pleural lesion

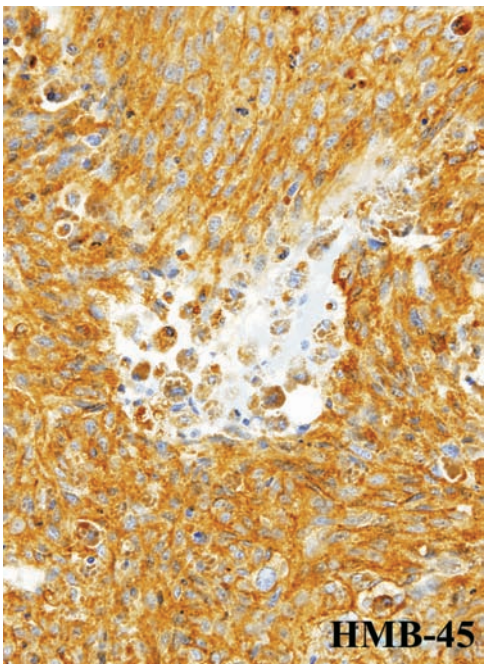


FIGURE 43.152. Positive staining of tumor cells with HMB-45. (Same case as in Fig. 43.151.) Immunostaining for cytokeratins was negative.

with any tissue available from the antecedent tumor whenever possible and to adjust the immunohistochemical protocol to encompass not only mesothelial cell and generic carcinoma markers but also more specific markers for the relevant carcinomas and other tumors (for example, TTF-1, prostate-specific antigen, prostatic acid phosphatase, and so forth).

Thymoma Affecting the Pleura

The literature contains several reports of thymoma affecting the pleura, either as spread into the pleura from an anterior mediastinal thymoma,[937] or as primary pleural thymomas.[836,938–941] Moran et al.[940] documented eight cases of thymoma that presented as pleural tumors requiring distinction from mesothelioma (most notably the lymphohistiocytoid variety). Six of their patients had diffuse pleural thickening, with encasement of the lung in four cases, and the tumor in one patient was obscured by a massive unilateral effusion. All of the cases comprising this series lacked radiographic evidence of a mediastinal tumor, but there was some uncertainty as to whether the thymomas were ectopic within the pleura or whether they represented spread from an underlying thymic tumor. More recently, the concept of primary pleural thymoma has become established,[836] but such pleural thymomas are distinctly rare and only about 25 to 30 cases have been reported in the literature to date.[836,938–941] They can present as localized masses or with diffuse pleural thickening.

The main histologic feature distinguishing lymphocyte-rich thymoma from lymphohistiocytoid mesothelioma is subdivision of the thymoma by bands of fibrocollagenous tissue, producing a lobulated architecture, and by a double cell population comprising epithelial cells and small lymphocytes only, the lymphocytes showing an immunohistochemical pattern of immature thymic lymphocytes. In other cases, the epithelial component predominates, with nesting, spindle-cell, and trabecular patterns, together with perivascular microcystic spaces.

Attanoos et al.[836,942] reported eight cases of pleural thymic epithelial tumors, four in males and four in females, with an age range of 19 to 75 years (median, 56 years). Three tumors occurred in the left hemithorax and four in the right, and the laterality was unknown in one case. In seven of the eight cases, the tumors were multinodular, with pleural thickening and partial encasement of the ipsilateral lung. In seven cases, low-magnification histologic examination showed a strikingly lobulated architecture, with fibrous septa subdividing cellular epithelial islands of tumor cells. In each case, there was a variable lymphoid cell population and one case had an extensively cystic appearance. The cases comprised WHO type A (medullary) thymic epithelial tumors, WHO type B1 (predominantly cortical) tumors, and WHO type B2 (cortical) tumors.[943] The differential diagnosis for the type A tumors included solitary fibrous tumor, monophasic synovial sarcoma, angiosarcoma, and sarcomatoid mesothelioma, whereas the differential diagnosis for the type B1 tumors included lymphohistiocytoid MM, metastatic lymphoepithelial carcinoma, and non-Hodgkin's lymphoma. The differential diagnosis for the type B2 tumors included epithelioid mesothelioma, secondary carcinoma, and secondary melanoma.

Attanoos et al.[836,942] also emphasized that thymic epithelial tumors can show variable expression of cytokeratin 5/6 and thrombomodulin, but nuclear expression of calretinin was not found in their cases. These authors also commented that CD20 expression in a cytokeratin-positive epithelial neoplasm and the presence of an immature lymphocyte population (demonstrable by immunostaining for CD1a, CD2, CD99, and terminal deoxynucleotidyl transferase [TdT]) indicates a thymic epithelial neoplasm, whereas nuclear expression of calretinin "favors MM."

Other Neoplasms Arising in the Pleura

Spindle Cell Neoplasms

Synovial Sarcoma of the Pleura

Both biphasic and monophasic synovial sarcomas (SSas) affecting somatic soft tissues and other sites have been extensively documented in the literature,[944–950] comprising up to about an estimated 5% to 14% of all sarcomas,[526,951] and characterized by a distinctive t(X;18) chromosomal translocation and the production of the resultant alternative fusion genes, *SYT-SSX1* or *SYT-SSX2*.[528–530] Most commonly, SSa affects the soft tissues of the extremities near—but only exceptionally in continuity with—large joints, and they have been described in most anatomic sites, including the head and neck region, the hypopharynx, abdominal wall, central nervous system, and prostate, among others.[951] They are now well recognized also as primary intrathoracic neoplasms in the mediastinum,[526,952,953] heart and pericardium,[954–957] lung,[526,958,959] and pleura[521–526,951,960–966] where the histologic appearances can potentially lead to confusion with either biphasic or sarcomatoid mesothelioma or carcinosarcoma (spindle cell carcinoma) of pulmonary or other origin, or biphasic pulmonary blastoma.

It is worth emphasizing that the term *synovial sarcoma* is quite inappropriate for these neoplasms, which have no phenotypic relationship to either synovial A or B cells (histiocytoid and fibroblastoid cells, respectively).[951,967–970] Instead, the epithelioid component of biphasic SSa shows clear evidence of epithelial differentiation as demonstrated by immunohistochemical studies and by electron microscopy (the term *carcinosarcoma* might be more correct for soft tissue SSas,[969] but *synovial sarcoma* is now standard, and terms such as *carcinosarcoma* for pleuro-

pulmonary tumors would only invite confusion with carcinosarcoma of lung). For example, Ordóñez et al.[948] described the pathologic findings in 39 primary SSas of which 15 were biphasic and 24 monophasic, as well as 19 cases of metastatic SSa. The epithelial or spindle cells in each biphasic tumor, whether primary or metastatic, showed reactivity for cytokeratins and EMA, but only six primary tumors (five biphasic and one monophasic) showed detectable expression of CEA, which was confined to the epithelial component of the biphasic tumors. Of the monophasic SSas, 15 primary (63%) and four metastatic (25%) cases showed reactivity for cytokeratin, whereas seven primary and two metastatic SSas (29% and 13%, respectively) showed detectable expression of EMA. The same authors found that EM could facilitate the diagnosis when markers of epithelial differentiation were not expressed on immunohistochemical staining, and EM aided in differentiating monophasic SSas from other sarcomas with histologic similarities. (See also later discussion of the study reported by Miettinen et al.[527] concerning the immunohistochemical repertoire of biphasic, monophasic and poorly differentiated SSas, in comparison to mesothelioma.)

In 1989, Witkin et al.[952] reported four cases of primary mediastinal biphasic SSa, with a fifth case mentioned as an addendum to their report, and they also referred to another case, in a 5-year-old boy who had a localized pleural tumor with a histologic resemblance to SSa. Although the SSas described by Witkin et al. were frequently adherent to the pericardium or pleura, none appeared actually to arise from the mesothelial surface at either site.

Subsequently, Gaertner et al.[521] recorded five cases of pleural biphasic SSa. The average age of their patients was 25 years (significantly younger than the mean age of mesothelioma patients), and the tumors presented as a localized mass lesion, often surrounded by a pseudocapsule (Fig. 43.153).[119] Jawahar et al.[522] reported a further case of pleural biphasic SSa, and in the same year Kashima et al.[971] reported a case of peritoneal biphasic SSa that showed the characteristic t(X;18) translocation; in the following year, Langner et al.[955] described a pericardial SSa in a patient with occupational exposure to asbestos, thought initially to represent a pericardial mesothelioma.

Nicholson et al.[523] described three cases of pleural SSa, in a 28-year-old man and two 42-year-old men, with no known background of exposure to asbestos. Two of the tumors were monophasic in character and one was biphasic. All three tumors showed focal expression of either cytokeratins or EMA in the spindle-cell tissue, and they also showed positive staining for bcl-2 protein and CD99. Bégueret et al.[526] also reported a series of 40 t(X;18) cases of primary intrathoracic SSa, at least 19 of which represented lung tumors, whereas six affected the pleura. The others were designated as pleuropulmonary or they affected mediastinal structures, sometimes in apparent continuity with the pericardium or lung. In this series, only one SSa was biphasic. The remaining 39 were classified as monophasic (24 cases) or poorly differentiated SSas (15 cases). Aubry et al.[960] reported five cases of primary monophasic SSa of the pleura, confirmed by identification of the SYT-SSX fusion transcript. In the following year, Praet et al.[972] reported four cases of pleural SSa, three of which were monophasic. Molecular analysis revealed SYT-SSX transcripts in three of the four cases, with results pending for the remaining case.

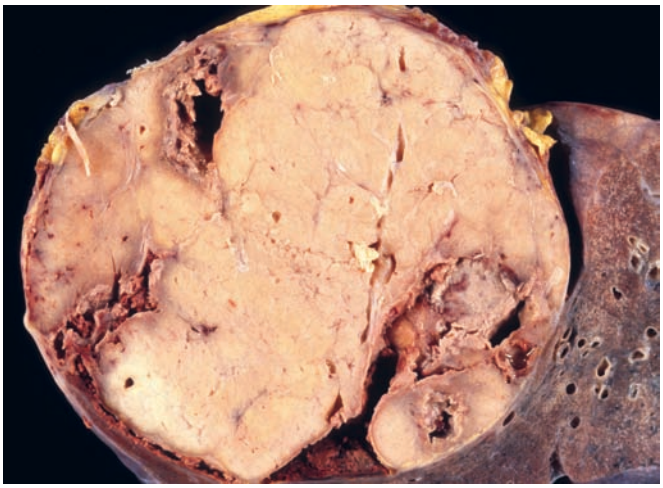


FIGURE 43.153. Gross appearances of a pleuropulmonary synovial sarcoma. Surgical resection specimen of upper lobe from an elderly woman. The tumor is well demarcated, and it indented the adjoining upper lobe. Yellow mediastinal fat is attached to the outer aspect of the tumor, in the upper part of this field. The tumor tissue itself is tan in color, with areas of necrosis and cystic degeneration. Other examples of pleural synovial sarcoma may take the form of pedunculated tumors or multinodular to confluent tumors that can mimic mesothelioma in their gross appearances.

Powers and Carbone[951] summarized the findings in 23 cases of primary SSa of the pleura reported in the literature.[419,521–523,961–965] The patients' ages ranged from 9 to 77 years (mean, 35.5 years, significantly less than the mean ages recorded for patients with pleural MM). There were 14 males and 9 females (M/F ratio = 1.56:1). Twelve of the SSas were monophasic, whereas 10 were biphasic, and the histologic type was unspecified for the remaining case.

In one of the largest studies reported to date, Miettinen et al.[527] described the immunohistochemical findings in 103 *extrapleural* SSas that included 41 biphasic tumors, 44 monophasic sarcomas, and 18 poorly differentiated SSas, in comparison to 23 epithelial and seven sarcomatoid mesotheliomas. They found that most biphasic SSas

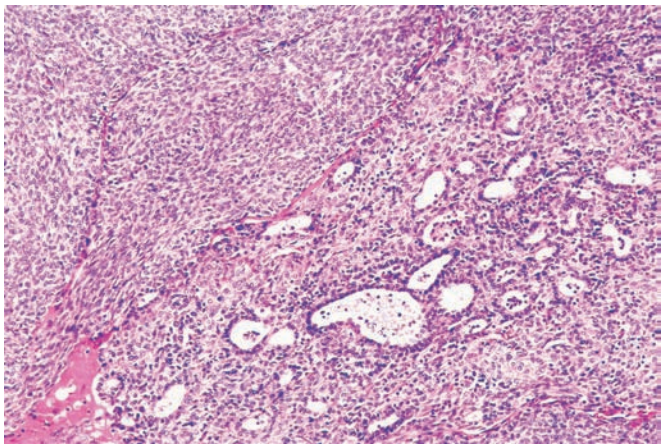


FIGURE 43.154. Pleuropulmonary synovial sarcoma, biphasic in type. The stromal component is illustrated in the upper left of this field, and the glandular component in the remainder of the field.

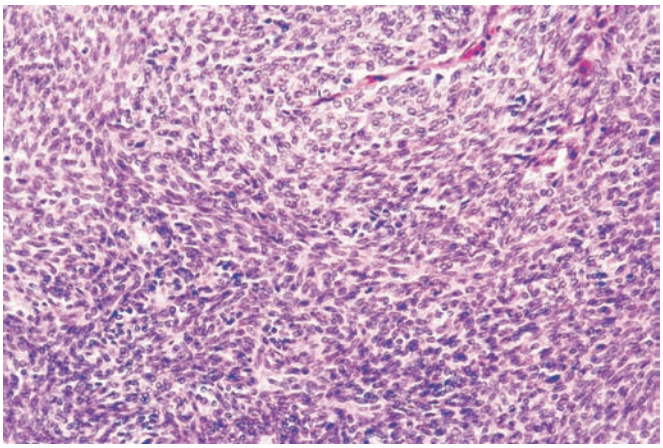


FIGURE 43.155. Stromal component of a pleuropulmonary synovial sarcoma. The stromal tissue is more cellular than the sarcomatoid tissue usually encountered in biphasic and sarcomatoid mesotheliomas, and typically the tumor cells tend to form curving poorly delineated fascicles as opposed to the storiform architecture often encountered in sarcomatoid and biphasic mesotheliomas.

(29/41; 71%) showed focal to extensive calretinin positivity, more often in the spindle-cell tissue (24/41 cases; 59%) than in the epithelial cells (14/41 cases; 34%), but only five of those cases showed calretinin positivity in ≥10% of the epithelial component; all of the biphasic SSas also stained with HBME-1. The monophasic and poorly differentiated SSas showed foci of calretinin positivity in 52% and 56% of cases, respectively. In comparison, all 23 epithelial mesotheliomas showed extensive calretinin positivity, and variable focal positive calretinin staining was seen in seven sarcomatoid mesotheliomas. They also found that two of 15 malignant peripheral nerve sheath tumors showed focal calretinin positivity, whereas there was no evidence of calretinin expression in epithelioid sarcomas, leiomyosarcomas, gastrointestinal stromal tumors (GISTs), or angiosarcomas. The biphasic SSas differed from mesothelioma by their more common Ber-EP4 positivity (90%), whereas focal Ber-EP4 staining was found in 13% of epithelial mesotheliomas. Expression of CD15 was rare in both mesotheliomas and SSas. Expression of Wilms' tumor antigen-1 (WT1) was not detected in any of the cases of SSa but was found in 12 out of 17 epithelial mesotheliomas. Miettinen et al. found that cytokeratins were present in the epithelial cells of both biphasic SSas and mesotheliomas (CK7 and CK19), but the expression was focal in both the monophasic and poorly differentiated SSas.

The findings of the International Mesothelioma Panel[37] and ours are useful for discrimination between biphasic/monophasic SSa and pleural MM:

- Typically, pleural SSas occur at a younger age (mean, 25–35 years) than pleural MM (mean, 65 years), although we have encountered some cases of SSa in the elderly.
- In terms of gross morphology, pleural SSa usually takes the form of a circumscribed mass lesion (Fig. 43.153), ranging from a few millimeters to 250 mm in diameter,[951] sometimes surrounded by a fibrous pseudocapsule and often accompanied by focal cystic degeneration[951] (Fig. 43.153), although diffuse pleural SSas can occur, mimicking MM in their anatomic distribution.
- There are significant histologic differences between either biphasic or monophasic SSa and biphasic/sarcomatoid MM (Figs. 43.154 to 43.156). The spindle-cell

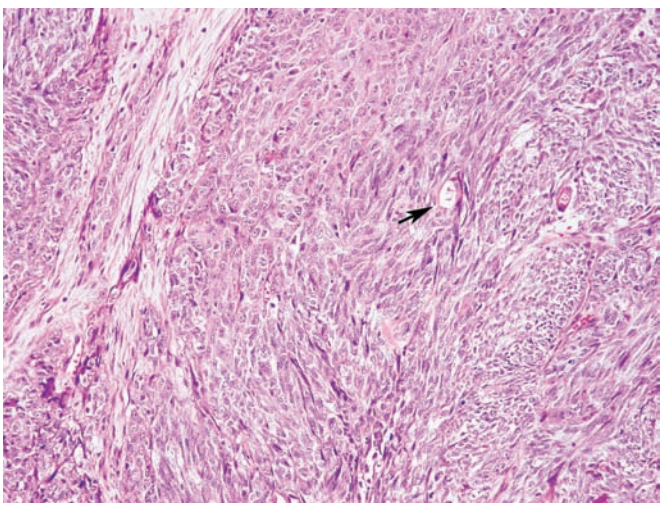


FIGURE 43.156. Localized pleural synovial sarcoma resected in a 67-year-old man. The fascicular architecture of the spindle-cell tissue is more obvious than in Figure 43.155. A rudimentary glandular structure can be seen (arrow).

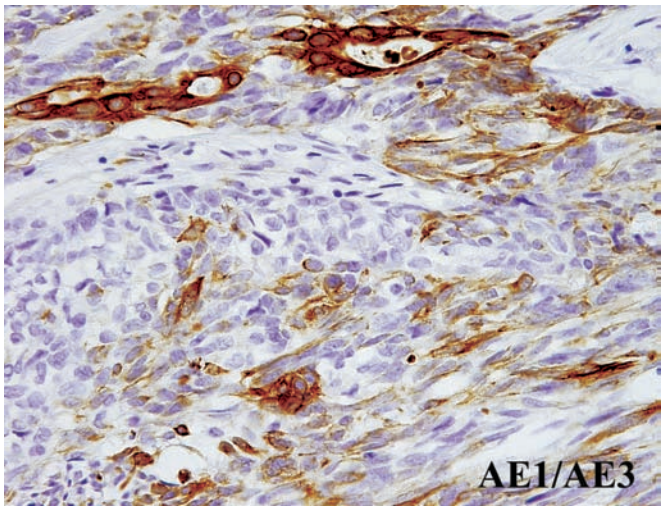


FIGURE 43.157. Biphasic synovial sarcoma of pleura, immunostained for pan-cytokeratins (AE1/AE3). Both the glandular component and the spindle-cell stromal tissue show expression of CKs, but labeling is more intense in the glandular tissue.

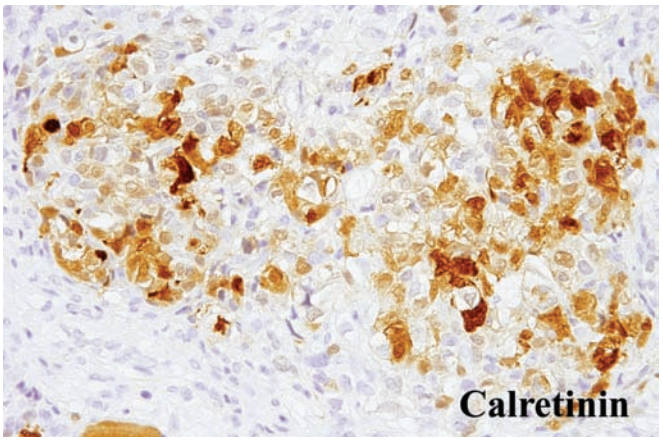


FIGURE 43.158. Pleural synovial sarcoma, biphasic type, showing focal staining for calretinin in both the cytoplasmic and nuclei of the tumor cells. (Same case as in Fig. 43.157.)

tissue in SSas is usually more cellular than the sarcomatoid component of mesotheliomas, and the cell size is smaller (Fig. 43.155). In addition, the spindle cells in SSa typically form interweaving fascicles (Figs. 43.155 and 43.156)—a "school of fish" pattern, a hemangiopericytic pattern, and foci of hyaline fibrosis (and even calcification[951]) are common in SSa but are not characteristic of mesothelioma. Frequent stromal mast cells are a characteristic finding in SSa, but not pleural mesothelioma.

- The glandular component of SSas (when present) frequently shows evidence of neutral mucin, whereas this finding typically does not occur in biphasic mesotheliomas, although mucin-positive mesotheliomas are well described.
- Powers and Carbone[951] considered that focal CK expression together with labeling for bcl-2, CD56, and CD99 in the context of undetectable staining for calretinin and WT1 suggests a diagnosis of SSa as opposed to pleural mesothelioma. In addition, expression of CKs by the stromal component of SSas is usually less intense and less extensive than in most cases of biphasic or sarcomatoid mesotheliomas (Fig. 43.157), and two cases of pleural monophasic SSa reported by Praet et al.[972] showed no detectable CK expression (the diagnosis in both was confirmed by detection of *SYT-SSX* transcripts).
- As indicated above, there is some overlap in calretinin expression between SSa and mesothelioma (Fig. 43.158), whereas nuclear staining for WT1 is frequent in mesothelioma but not in SSa.
- Both biphasic SSa and biphasic mesothelioma typically show positive staining for EMA, but whereas EMA expression in mesothelioma is typically linear and membrane-related in distribution, both membranous and cytoplasmic staining is found in biphasic SSa. Furthermore, expression of the epithelial markers, most notably Ber-EP4, CEA, or CD15 (Fig. 43.159) is not uncommon in biphasic SSa, but is substantially less frequent in mesotheliomas.
- By electron microscopy, the microvilli found on the epithelial cells of SSas are short and blunt,[946,947] and may even show structures resembling glycocalyceal bodies,[119] whereas the microvilli in mesothelioma are characteristically elongated, serpentine, and intertwining, with no evidence of a glycocalyx.

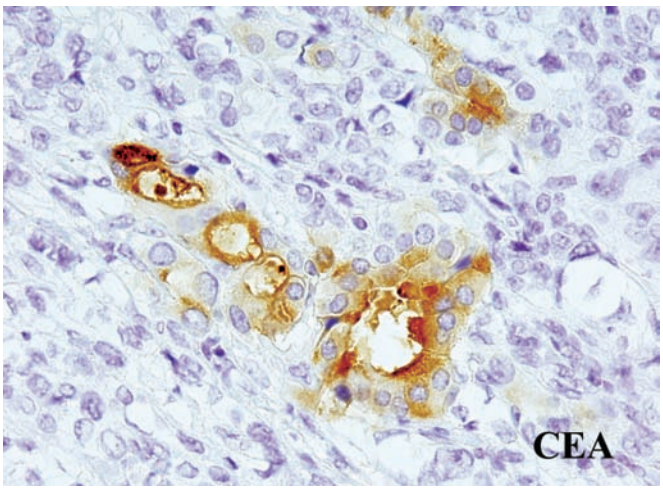


FIGURE 43.159. Biphasic synovial sarcoma of pleura, showing focal staining for carcinoembryonic antigen in the glandular component. (Same case as in Figs. 43.157 and 43.158.)

- Finally, the t(X;18) chromosomal translocation and expression of the resultant chimeric gene *SYT-SSX1* or *SYT-SXX2* are virtually diagnostic of SSa—both biphasic and monophasic—but are absent in mesotheliomas. Identification of this characteristic translocation is of particular value for the discrimination between poorly differentiated SSa and mesothelioma.[526]

Also, a diagnosis of primary pleuropulmonary SSa requires exclusion of a history of an antecedent SSa of somatic soft tissues or other anatomic sites, to exclude SSa metastatic to lung or pleura.[973]

In rare cases there appears to be some as yet unreported and unexplained linkage between pleural SSa and mesothelioma. We have encountered one case of a surgically resected pleural SSa that was followed about 1 year later by recurrent tumor in the same hemithorax, but the pathologic features of the recurrence were classical of mesothelioma and not SSa. In another referred case, biopsy of a confluent pleural tumor revealed features classical of epithelial mesothelioma, but the thoracic surgeon also identified a small and apparently separate polypoidal tumor in the same hemithorax, and biopsy of this lesion yielded findings characteristic of SSa.

The prognosis for pleural SSa, at least the localized tumors, appears to be somewhat more favorable than for patients with diffuse MM. About half of the 14 cases in the literature as tabulated by Aubry et al.[960] were alive without evidence of disease at 4 to 13 months postresection, and one patient was alive with disease at 8 years. However, diffuse pleural SSas and poorly differentiated SSas appear to represent highly aggressive lesions. The distinction of pleural SSa from pleural MM is also important, for two additional reasons:[951]

1. SSas may be responsive to ifosfamide-based chemotherapy, which is not the case for pleural MM.
2. Pleural SSas have no proven or consistent causal relationship to prior asbestos exposure, unlike the majority of pleural MMs.

Solitary Fibrous Tumors of Pleura

Solitary fibrous tumors (SFTs) are uncommon localized spindle-cell fibroblastoid neoplasms that usually occur in relation to the pleura, where they are thought to arise from submesothelial mesenchyme.[974–976] First described in 1931 by Klemperer and Rabin,[8] SFTs have been reported under a variety of different names, including *submesothelial fibroma*.[503] *Localized fibrous tumor* is arguably the best descriptor because these tumors are not always solitary, but *solitary fibrous tumor* is the preferred nomenclature at present.[503] The former designation *fibrous mesothelioma* is to be avoided, because the spindle cells comprising these lesions show no evidence of a mesothelial phenotype, and the term *fibrous mesothelioma* invites confusion with conventional mesothelial tumors.

Intrathoracic SFTs most often arise in relation to the visceral pleura (~80% of pleural SFTs[503])—where they frequently represent pedunculated lesions (Figs. 43.160 to 43.162)—or the parietal pleura,[974] but they can also arise within the mediastinum or as intraparenchymal lung tumors[977] (see Chapter 39 for complete discussion of intrapulmonary SFT), and in relation to the pericardium[978] and diaphragm.[979] Within the thorax, they can vary greatly in size (Figs. 43.160 and 43.161), ranging from 13 to 330 mm in greatest diameter in one series of cases.[980] Extrathoracic SFTs have been recorded with increasing frequency in a variety of sites,[981,982] such as the orbit,[983–986] nasal cavity,[981,987] paranasal sinuses[987] and nasopharynx,[988] soft tissues of the extremities,[981,989] retroperitoneum,[990] kidney, urinary bladder,[981,991] seminal vesicle and prostate,[981] spermatic cord, vagina,[992] parotid gland,[993] thyroid,[994] liver,[995] pancreas, omentum/mesentery,[996] and meninges.[985]

Solitary fibrous tumors have been recorded in patients of ages 5 to 87 years, but they are rare in patients under the age of 10 years, and the peak incidence is between the fourth and sixth decades of life. One review of 55 patients with pleural SFTs recorded an age range of 18 to 80 years, with a mean of 55 years.[997] A smaller series of 14 intrathoracic SFTs recorded an older age range of 44 to 73 years, with a mean of 60 years.[980] Both intrathoracic and extrathoracic SFTs have been recorded rarely during childhood, for example, in an 8-year-old boy (intrapulmonary)[998] and an 11-year-old girl (parotid gland).[993] In

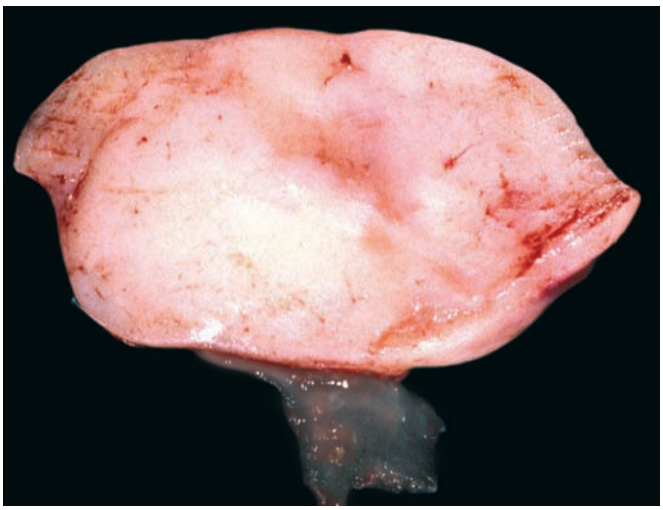


FIGURE 43.160. Small solitary fibrous tumor (SFT) from the visceral pleura. This lesion was pedunculated, and a portion of the pedicle can be seen in the lower center of this field, extending to the foot of the photograph.

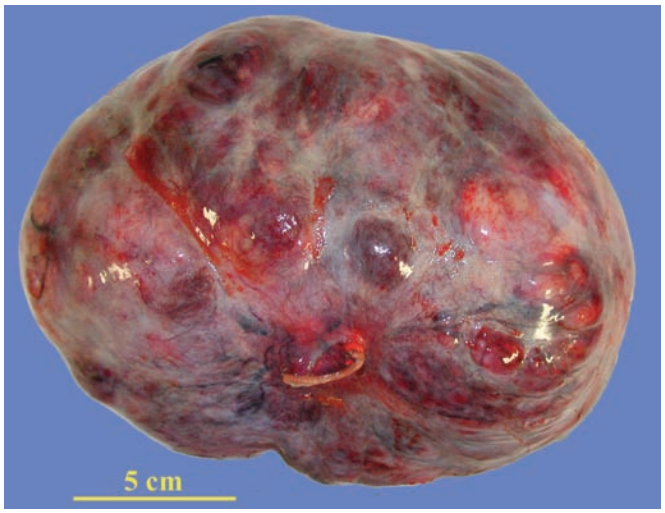


FIGURE 43.161. Pleural SFT. This lesion was resected from a 45-year-old woman, and required the use of obstetrics forceps to "deliver" the SFT through the thoracotomy incision. The lesion has a smooth if slightly bosselated surface, with areas of congested and hemorrhagic tumor tissue alternating with paler areas. The pedicle for this pedunculated tumor is shown near the lower center of this field. The scale at the foot of the photograph is in centimeters.

several series of intrathoracic SFTs, the tumors occurred more often in females than males, but one larger study had a male predominance (32 of 55 cases).[997] In a series of 27 consecutive intrathoracic SFTs from the files of one of the authors (D.W.H.), there were 13 male patients and 14 females, with an average age of 64 years; the tumors ranged in size from 16 to 224 mm (mean, 75 mm), as recorded for 16 cases.

Most commonly, SFTs are discovered incidentally on routine chest x-rays or CT scans in asymptomatic patients,[503,999] and the radiologic appearances may give some inkling of the diagnosis (for example, a smooth localized pleura-based tumor[503]), but definitive diagnosis requires histologic examination of either a biopsy or surgical resection specimen. When present, symptoms can be related to the size of the tumor and to compression of—or intrusion into—surrounding tissues.[999] In such circumstances, symptoms related to intrathoracic SFTs include systemic symptoms such as fatigue, fever, night sweats, and weight loss, whereas symptoms related to the intrathoracic location include cough, dyspnea, chest pain, digital clubbing, hypertrophic osteoarthropathy, and, less commonly, hypoglycemia related to production of insulin-like growth factor[1000] (Doege-Potter syndrome[503]). In one review of 79 cases of SFT[1001]—54 intrathoracic and 25 extrathoracic—89% of the intrathoracic lesions were asymptomatic, whereas 83% of the extrathoracic SFTs were associated with symptoms, which varied according to the range of sites in which the tumors arose.

The histologic appearances characteristically vary from one area to another within a single tumor and from one SFT to another, and they can range from the "patternless pattern" of Stout to "herringbone," cellular, short storiform, diffuse sclerosing, myxoid and hemangiopericytic or angiofibromatoid areas, and areas with neural-type palisading, and, in some instances SSa-like areas (Figs. 43.163 to 43.168).[503,974,976] The bipolar spindle-shaped cells resemble fibroblasts, and they often show a distinctive localization along and parallel to stromal collagen bundles (Fig. 43.164). Multinucleated giant cells occur in some cases, and calcification or ossification may be present (Fig. 43.169). Other changes include cystic degeneration, necrosis, and hemorrhage (Fig. 43.162). Varying degrees of nuclear atypia and pleomorphism, and mitotic activity can be found, and the mitotic index in particular appears to be a probability marker for a diagnosis of malignant SFT (see following discussion). Entrapped mesothelium may be present (or entrapped alveolar epithelium in the case of intrapulmonary SFTs)[503] (Figs. 43.170 and 43.171).

The differential diagnosis includes a variety of other spindle-cell fibroblastoid tumors that can arise in relation to the pleura, chest wall, mediastinum, and other sites where both intrathoracic and extrathoracic SFTs have

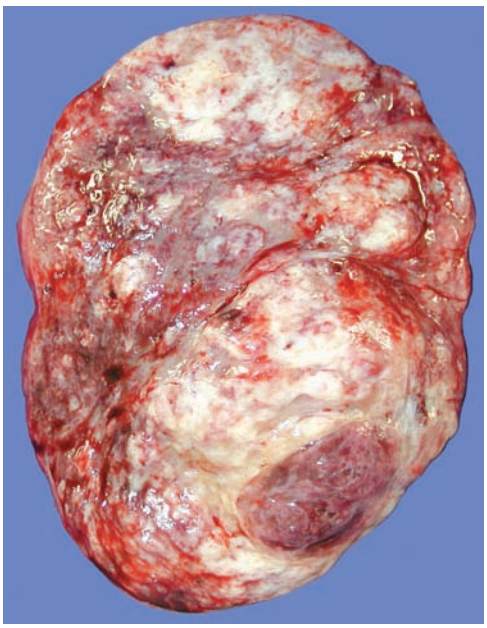


FIGURE 43.162. Pleural SFT. (Same case as in Fig. 43.161.) Areas of pale white tumor tissue alternate with hemorrhagic zones. Areas of necrosis were evident in this tumor, histologically resembling ischemic necrosis, so that the areas of hemorrhagic necrosis were thought probably to be related to partial torsion of the SFT around its pedicle. There were no histologic markers of malignancy, and the tumor tissue was uniform in appearance with only rare mitotic figures.

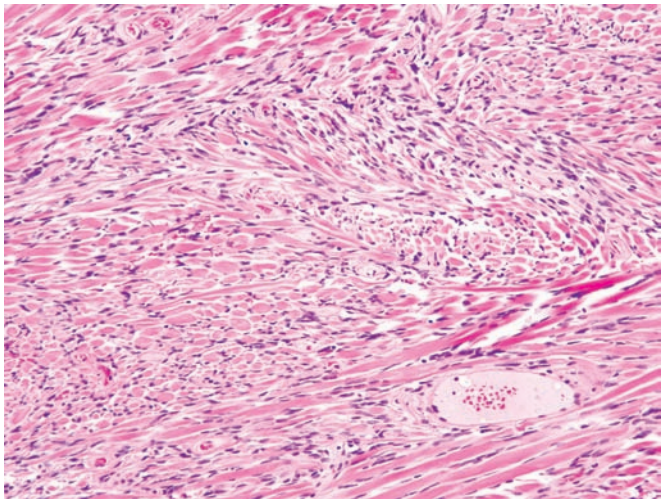


FIGURE 43.163. Pleural SFT. This field depicts intertwining fascicles of collagen bundles with intervening fibroblastoid cells, the appearances being characteristic of an SFT.

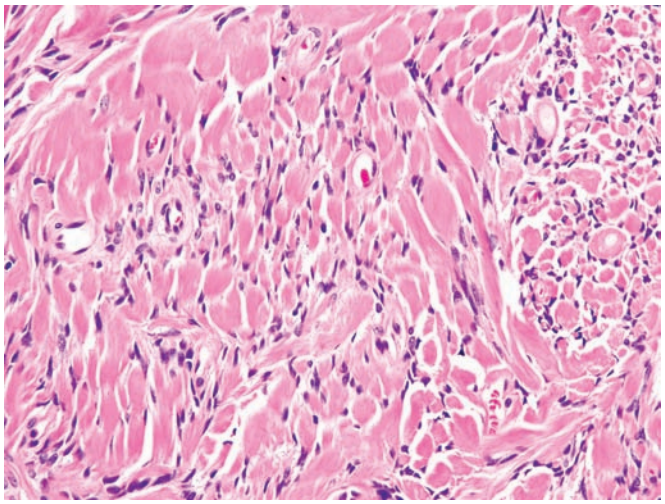


FIGURE 43.164. Pleural SFT. (Same case as in Fig. 43.163.) At higher magnification the collagen bundles and their intervening fibroblastoid cells are seen.

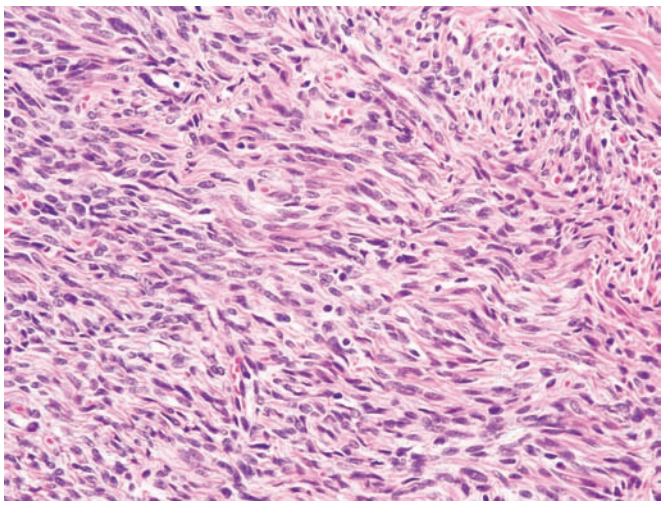


FIGURE 43.165. Pleural SFT. Area of cellular fibroblastoid tissue. This lesion showed no detectable expression of cytokeratins, but staining for CD34 was positive. The tumor cell nuclei are reasonably uniform, and mitotic figures were extremely rate in this case.

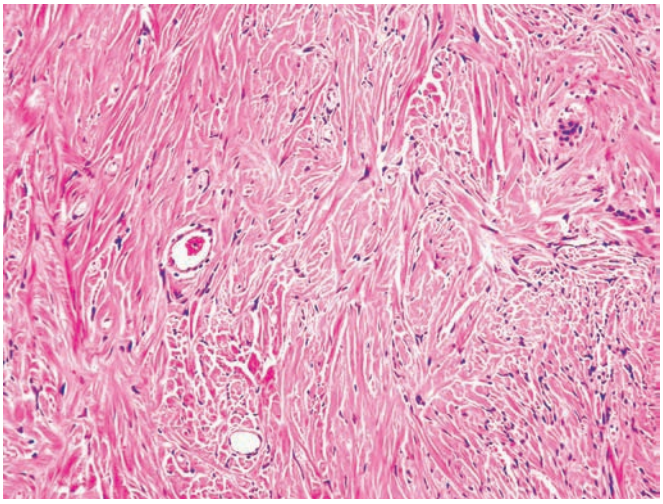


FIGURE 43.166. Pleural SFT. Sclerotic area.

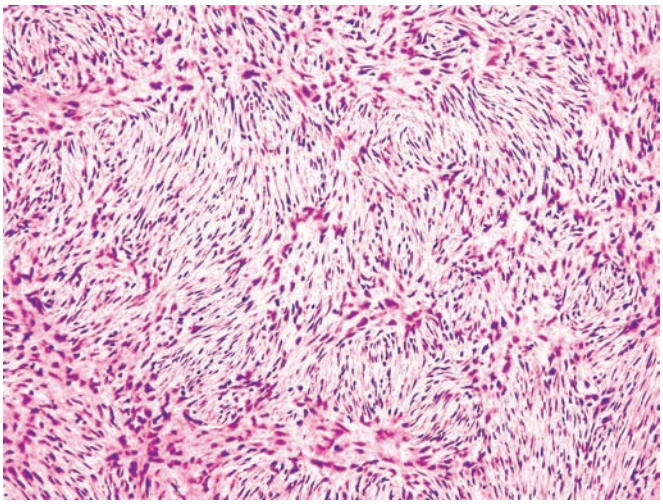


FIGURE 43.167. Pleural SFT that was considered to be malignant on the basis of invasion and areas of cytologically malignant tissue. The tumor has a prominent storiform architecture in this region.

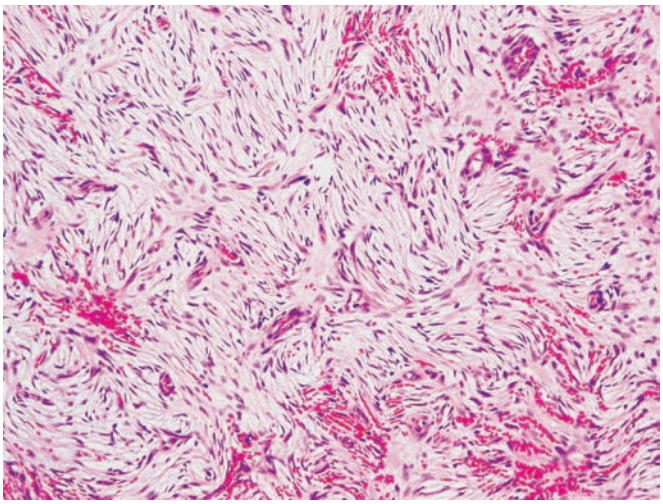


FIGURE 43.168. Pleural SFT, assessed as malignant on the basis of invasion and cytologic indicators of malignancy. An area of myxoid storiform tissue is shown.

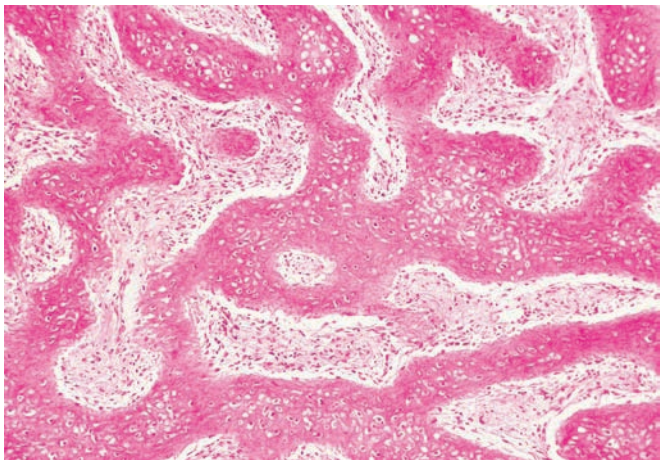


FIGURE 43.169. Area of bone formation in a solitary fibrous tumor of pleura that was malignant in terms of invasion but showed no cytologic markers of malignancy, with no identifiable mitotic figures.

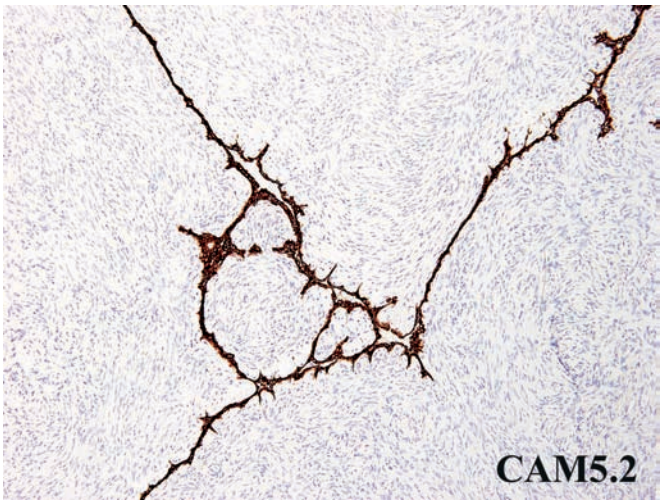


FIGURE 43.171. Malignant SFT of pleura, showing the multilobated pattern of tumor growth, with inclusion of linear formations of cytokeratin-positive mesothelium (CAM5.2).

been recorded, including extraintestinal gastrointestinal stromal tumors (EGISTs). In the case of pleural tumors, the major differential diagnoses include sarcomatoid and desmoplastic mesothelioma (which can occur as a localized tumor on occasions), fibroblastoid tumors arising in relation to the chest wall or ribs and including pleural desmoid tumors, monophasic SSa, schwannoma, inflammatory myofibroblastic tumor (inflammatory pseudotumor), calcifying fibrous (pseudo)tumor,[999] and perhaps a spindle cell carcinoma of lung with invasion of the pleura.

In most instances, the gross and histologic findings can discriminate between the differential diagnoses at a reasonable order of confidence, but immunohistochemical studies are crucial if there is doubt. Characteristically, the fibroblastoid cells comprising benign SFTs are devoid of CK expression (Fig. 43.171) in contrast to most sarcomatoid mesotheliomas, whether localized or not, and instead the cells show positive immunohistochemical staining for vimentin and CD34[503] (within a range of about 66% to 95%; Fig. 43.172), and less consistently for bcl-2[1002] and CD99.[971,985,1003,1004] However, in some malignant SFTs, the tumor may show depletion of CD34 expression either throughout the tumor or over extensive areas.[1005] In addition, malignant SFTs may show a lobulated growth pattern, with incorporation of linear arrays of hyperplastic mesothelial cells into the tumor, but the background fibroblastoid cells are still devoid of CK expression. The

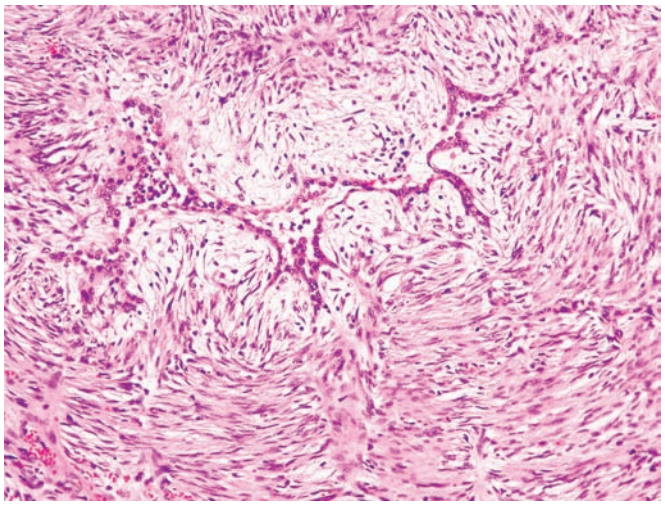


FIGURE 43.170. Malignant SFT of pleura, showing an area of incorporated mesothelium thought to have been enclosed by a multinodular pattern of tumor growth.

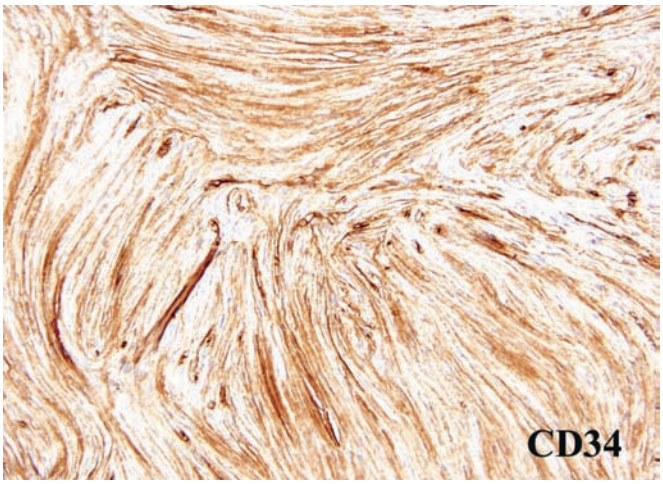


FIGURE 43.172. Pleural SFT. Expression of CD34.

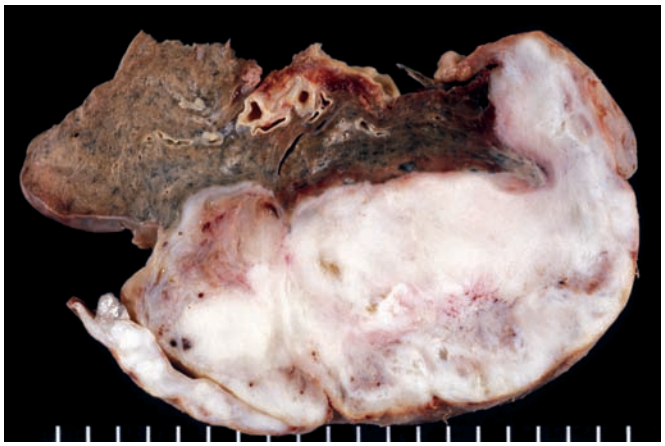


FIGURE 43.173. Malignant SFT of pleura (surgical resection specimen). The lesion forms a massive sessile tumor attached to the visceral pleura and lung, which measured almost 19 cm in vertical dimension (scale is in centimeters). The tumor tissue is pale and white, but there are no obvious areas of necrosis. Nonetheless, the lesion showed invasion into lung parenchyma, and there was also invasion along the parietal pleura. This tumor also showed focal osseous metaplasia (Fig. 43.169). The tumor tissue comprised uniform-appearing fibroblastoid cells throughout, with no mitotic figures identifiable on a protracted search of the sections; nonetheless, this lesion recurred rapidly within the same hemithorax, with a fatal outcome 10 months after the original presentation.

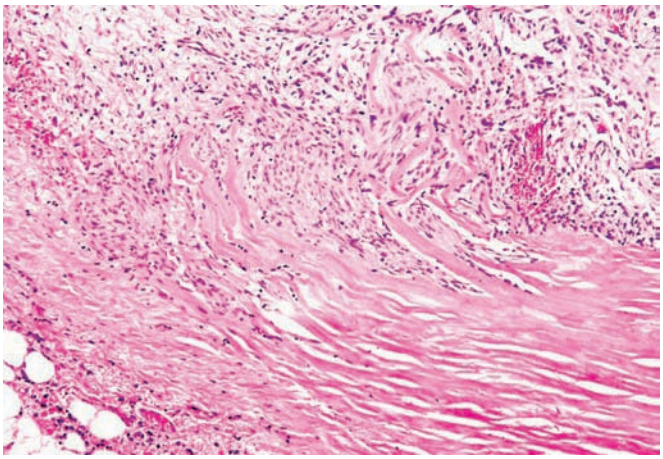


FIGURE 43.174. Malignant SFT of pleura. This patient had a background of occupational exposure to asbestos, with the presence of pleural plaques. Nonetheless, this lesion had histologic features and an immunoprofile characteristic of solitary fibrous tumor. In this field, the tumor is seen invading into pleural plaque, dissecting along and between the collagenous laminae making up the plaque.

EGISTs affecting the thorax (e.g., the mediastinum) can be excluded by the absence of staining for CD117 (c-kit); however, Miettinen et al.[1006] found that about 47% to 100% of GISTs showed positive staining for CD34, and others[1007] have reported positive staining of SFTs for CD117. Schwannomas can be excluded by labeling for S-100 proteins and other markers of schwannian differentiation.

Discrimination between benign and malignant SFTs can be problematic and is analogous in many ways to the problems of assessing the malignant potential of GISTs. In their series of 223 SFTs, England et al.[974] commented that there appeared to be no clearly defined histologic discriminators between benign and malignant tumors. As indicators of malignancy they invoked high cellularity, nuclear atypia, pleomorphism, and more than four mitotic figures per 10 high-power fields (HPFs), among others. At the same time, about 45% of the cases so designated as malignant appeared to have been cured by surgical resection, suggesting either that such tumors have a favorable prognosis or, alternatively, that the histologic indicators of malignancy were not consistently reliable.

Therefore, by extension of the criteria put forward by others,[503,842,974,976] it appears that the major discriminators, in perhaps the following order of rank, favor assessment of an SFT as malignant as opposed to benign, in biopsy tissue or a surgical resection specimen (Figs. 43.173 to 43.176):

- Invasion of adjacent structures (pleura, chest wall, lung (Figs. 43.173 and 43.174)
- Areas of overtly sarcomatous tissue within an SFT, and not resembling SFT (Fig. 43.176)
- Areas of tumor necrosis (as opposed to ischemic-type necrosis possibly related to partial torsion of the lesion; Fig. 43.175)
- More than four mitotic figures per 10 HPFs

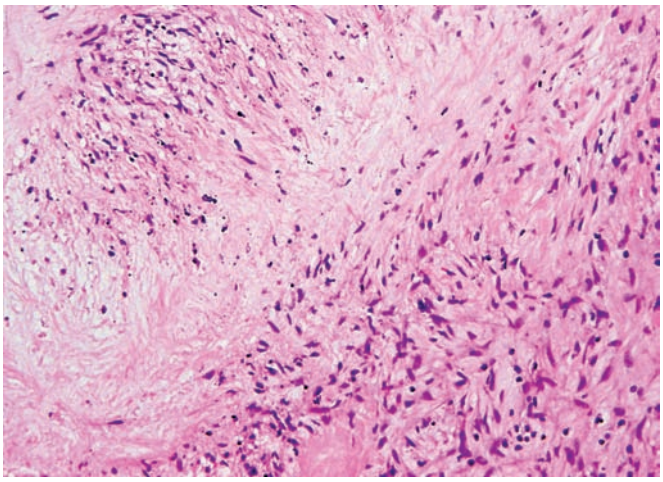


FIGURE 43.175. Malignant SFT of the pleura, showing an area of necrosis with a minor associated inflammatory infiltrate, accompanied by nuclear karyorrhexis.

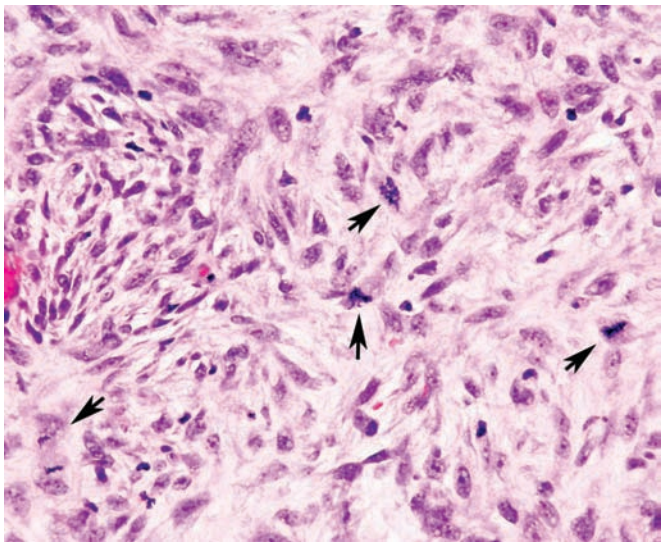


FIGURE 43.176. Malignant SFT of pleura. At least four mitotic figures are evident in this single high-power field (*arrows*). The tumor cells also show moderate nuclear atypia and pleomorphism.

- High cellularity, with prominent nuclear atypia and pleomorphism
- Occurrence on the parietal pleura
- Sessile tumor (Fig. 43.173)
- Large tumor size (>10 cm; Fig. 43.173)
- Associated pleural effusion
- Local tumor recurrence following surgical resection (although otherwise benign SFTs can recur locally as multiple tumor nodules following incomplete resection)

With the exception of invasion (or metastasis), most of these markers may be regarded as probability indicators, and some are probably linked (nonindependent) variables. Assessment of the benign versus malignant status of an SFT is arguably best based on a combination of findings. Depending on the above combination of findings, we report SFTs as benign (no histologic evidence of malignancy), SFTs with features of malignancy (e.g., SFT with invasion), and SFTs of uncertain malignant potential. Accordingly, it seems that a small pedunculated tumor arising from the visceral pleura is likely to have a "benign" course following apparently complete surgical resection, irrespective of the cellularity and cytologic atypia seen focally within such a lesion. On the other hand, a large sessile tumor arising on parietal pleura, with areas of tumor necrosis and obvious invasion of the pleura is likely to pursue a "malignant" course, irrespective of the degree of nuclear pleomorphism and atypia. For example, we have encountered a case of a massive malignant SFT that arose as a sessile lesion in relation to the parietal pleura, with invasion of the pleura, chest wall, and lung, and which recurred with a fatal outcome within 10 months of incomplete surgical resection, although exhaustive histologic sampling of the tumor revealed no evidence of excessive cellularity, nuclear atypia, or pleomorphism, and no mitoses could be found (Fig. 43.173).

In the literature, benign SFTs appear to predominate within the thorax. In one study of 36 cases, only two recurred locally.[1008] Another series of 55 cases[997] revealed features of malignancy in four, but only one case showed aggressive behavior, with local recurrence. In another study, four of 14 cases were assessed as malignant[980]; the malignant tumors were larger in diameter (>20 cm) and were soft and fleshy, and they showed high mitotic activity, with an average of about seven mitoses per 10 HPF. In a series of 92 extrathoracic SFTs reported by Vallat-Decouvelaere et al.,[1009] 10 recurred or had atypical histologic features (11%), with tumor relapse in eight cases and the development of metastases in five (in lung, liver, and bone). These authors concluded, "Nuclear atypia, hypercellularity, greater than 4 mitoses/10 HPFs, and necrosis . . . [occur] in up to 10% extrathoracic SFTs, and are associated with, but are not themselves predictive of, aggressive clinical behavior."

Wherever possible, management of SFT is by surgical resection.[503] It has been observed that incompletely resected pleural SFTs can recur locally, sometimes as multiple tumor nodules, even where there are no other indicators of malignancy. Therefore, we recommend that local resection of pleural SFTs should include a tumor-free margin of about 10 mm around the base of the pedicle or base of the tumor, whenever feasible.

Calcifying Fibrous (Pseudo-)Tumor of the Pleura

Calcifying fibrous tumor (CFT) typically affects the subcutaneous and deeper soft tissues of the limbs, trunk, and neck of children, adolescents, and young adults,[503] but cases have been reported in relation to the pleura,[1010–1013] chest wall,[1014] mediastinum,[1015,1016] peritoneum,[1017] and mesentery.[1018] Pinkard et al.[1011] described three cases of pleural CFT in young adults, ages 23, 28, and 34 years. Typically, pleural CFTs are located in the inferior chest region, and they may represent either solitary mass lesions or multifocal tumor-like lesions, measuring about 30 to 120 mm in greatest diameter.[503,1010] One case with multiple pleural lesions has been recorded in a 29-year-old woman who had no symptoms referable to the tumor.[1013]

On histologic examination, CFT comprises paucicellular fibrocollagenous tissue without a laminar (plaque-like) architecture, accompanied by a sparse lymphoplasmacytic infiltrate and with variable numbers of rounded calcified bodies of variable size, resembling psammoma bodies (Fig. 43.177). Points of distinction of CFT from either desmoplastic MM or solitary fibrous

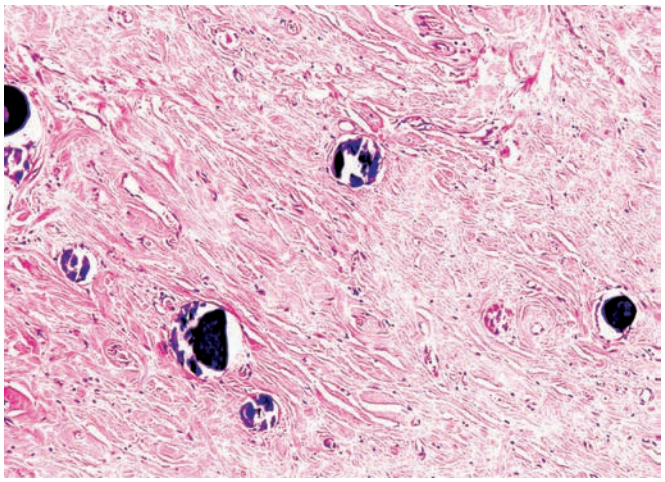


FIGURE 43.177. This calcifying fibrous (pseudo)tumor comprises paucicellular fibrocollagenous tissue with several rounded calcified bodies, some of which are partly shattered as a consequence of cutting the histologic section. (Courtesy of Dr. Goran Elmberger, Stockholm, Sweden.)

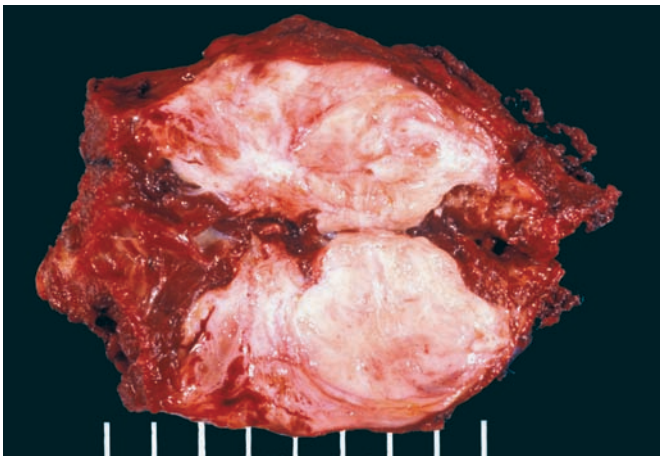


FIGURE 43.178. Desmoid tumor of the chest wall, impinging upon the parietal pleura. The specimen has been bivalved, with pleura at the top of the specimen as depicted, and at the bottom. The lesion is reasonably well localized, although obviously unencapsulated. It was clearly invasive on microscopy, and it had a firm rubbery white (slightly bosselated) cut surface. Scale is in centimeters.

tumor include negative reactions for both cytokeratins and CD34, whereas the fibroblastoid cells show positive staining for vimentin.[503] Pleural plaques are distinguishable by their paucicellular, laminated, and (frequently) hyalinized appearance; in addition, the pattern of psammoma-like calcification in CFTs differs from the finely punctate to sheet-like calcification seen in plaques.

A relationship to inflammatory myofibroblastic tumor has been debated,[1019–1021] but the pathogenesis of CFT remains obscure. These lesions are entirely benign in character and are usually treated successfully by surgical extirpation, but local recurrence has been recorded.[1022]

We have observed one case that occurred in a diffuse pleural distribution and was misdiagnosed as a desmoplastic mesothelioma.

Desmoid Tumors of the Pleura

Desmoid tumors in the region of the thorax, including the shoulder girdle region and chest wall, are well recognized in the literature; some chest wall lesions can impinge upon the parietal pleura (Fig. 43.178), but primary desmoid tumors of the pleura and lung are extremely rare. Pleural desmoid tumors carry the potential for misdiagnosis as an SFT in particular, as well as benign neurogenic tumors and even localized sarcomatoid mesotheliomas with desmoplastic features.

Wilson et al.[1023] reported four cases of pleural desmoid tumors, in two men and two women of ages 16 to 66 years (mean, 44 years). Three of the patients presented with chest pain and one had dyspnea. Three of the tumors affected the parietal pleura and one was located in the visceral pleura. The mean tumor size was 125 mm, and all showed a bosselated firm white cut surface (Fig. 43.178). The histologic appearances were essentially identical to those of desmoid tumors in extrapleural sites (Figs. 43.179 and 43.180). As with desmoid tumors in other locations, the lesions invariably showed invasive features, with

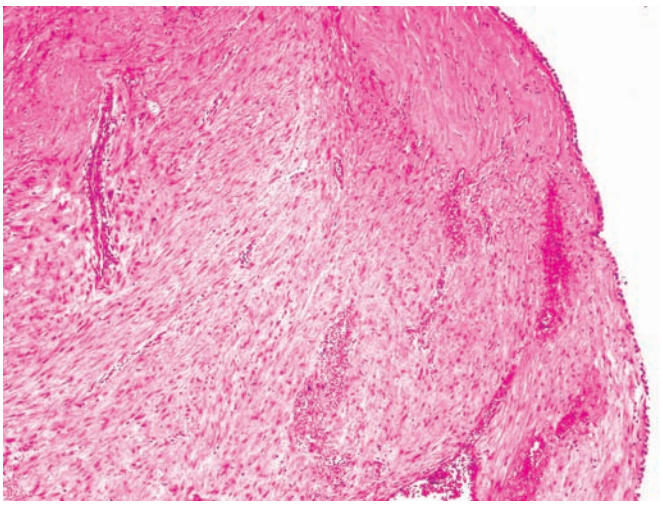


FIGURE 43.179. Pleural desmoid tumor from a 69-year-old man. This lesion was located near the apex of the pleura, with invasion of the thoracic inlet, so that complete surgical resection was impossible. A layer of cuboidalized mesothelium can be seen at the surface of the pleura, and the submesothelial tissues are expanded by a hypocellular collagen-producing spindle-cell lesion with histologic appearances and a pattern of invasion elsewhere that were characteristic of a desmoid tumor.

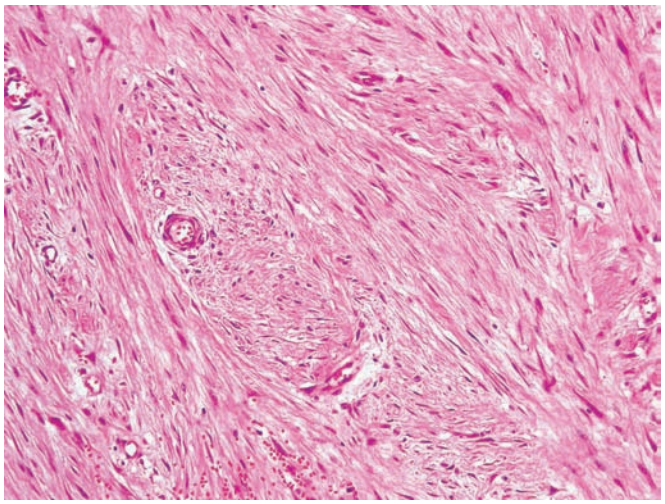


FIGURE 43.180. Detail of desmoid tumor of the pleura. The tumor comprises reasonably uniform spindle-shaped fibroblastoid cells separated by a collagenous matrix, with reasonably prominent blood vessels.

extension into fat or skeletal muscle. Wilson et al. found that the tumor cells showed immunoreactivity for vimentin, smooth muscle and muscle-specific actin, and desmin in three out of the four cases, and the lesions showed no evidence of S-100 protein immunoreactivity. The patients were treated by surgical resection, either complete or incomplete, and one case where resection was incomplete was managed further by radiation therapy and then complete surgical resection. Follow-up revealed stable residual disease at 12 months after treatment in one patient, and two of the patients had no evidence of residual disease at 12 and 96 months.

Subsequently, Andino et al.[1024] studied β-catenin expression and cyclin D-1 in a series of four thoracic desmoid tumors—one representing a pleural desmoid tumor, one intrapulmonary in location, and two affecting the pleura-chest wall—in comparison to five benign and six malignant SFTs of pleura. Diffuse, moderate to strong nuclear staining for β-catenin was found in all of the desmoid tumors, four out of five benign SFTs, and two of six malignant SFTs. Nuclear and cytoplasmic cyclin D-1 staining was seen in all groups. These authors also found that the distinction between desmoid tumors and SFTs was best made from CD34 expression (0/4 desmoid tumors versus 8/11 SFTs) and smooth muscle actin (found in all four desmoid tumors but in none of the 11 solitary fibrous tumors). Lack of S-100 protein expression also distinguishes pleural desmoid tumors from neurogenic lesions, and the distinction from a localized sarcomatoid mesothelioma with desmoplastic features can be made on the distinctive histologic appearances of desmoid tumors and the absence of cytokeratin expression (although, as mentioned elsewhere, cytokeratin-negative sarcomatoid mesotheliomas are well recognized).

Benign and Malignant Nerve Sheath Tumors

Neoplasms that have histologic and immunohistochemical features of nerve sheath tumors have been reported primary in the pleural cavity.[1025,1026] The benign growths typically show morphologic features of Verocay bodies with Antoni A and B areas, as well as hyaline vascular changes. They have features similar to nerve sheath tumors seen elsewhere. When malignant, these cells frequently do not show the typical benign features of nerve sheath tumors. Immunohistochemical staining with neural markers such as S-100 protein is helpful in confirming a neurogenic origin of these neoplasms.

Inflammatory Myofibroblastic Tumors

Inflammatory pseudotumors, also referred to as *plasma cell granulomas* and *inflammatory myofibroblastic tumors*, may occasionally involve the lung and rarely involve the pleura.[1027] These tumors have the histologic features of those neoplasms involving the lung and occurring elsewhere, typically made up of a proliferation of spindle cells with varying numbers of inflammatory cells, usually with an excess number of plasma cells. There has been a significant debate whether these tumors are true neoplasms or are reactive changes.[1028] (See Chapter 39).

Epithelioid Hemangioendothelioma and Angiosarcoma of the Pleura

Epithelioid hemangioendothelioma (EHE) is a distinctive malignant angioformative neoplasm in which the neoplastic endothelial cells are epithelioid and sometimes bland in appearance,[1029] often arranged as solid sheets or in a linear fashion, embedded in a hyaline or myxohyaline stroma.[237] These epithelioid endothelial neoplasms have been described in soft tissue,[1029] bone, liver, and lung; in the lung they were designated as intravascular bronchioloalveolar tumors (IVBATs) before their endothelial character was recognized.[533] The epithelioid appearances of the neoplastic cells stand in contrast to the angioformative and even papillary patterns of conventional angiosarcomas; EHEs in soft tissues are often considered to represent neoplasms intermediate in malignancy between conventional aggressive angiosarcomas and benign hemangiomas, but they have the potential for local recurrence and metastatic spread. The anatomic site where these tumors arise correlates with mortality, so that the mortality rate for EHEs of bone or liver is about double the mortality rate for those that arise within soft tissues.[503]

In 1993, Battifora[1030] recorded mimicry of mesothelioma by pleural EHE, and his report was followed in 1996 by the study carried out by Lin et al.[1031] on 14 cases of

malignant vascular tumors of serous membranes producing mimicry of mesothelioma. The EHEs (epithelioid angiosarcomas) diffusely involved pleural, peritoneal, or pericardial cavities, producing a clinical picture that closely simulated mesothelioma. The patients ranged in age from 34 to 85 years at the time of diagnosis, with a mean age of 52 years. The patients included two women and one man with peritoneal EHE, eight men with pleural EHEs, and three men with pericardial tumors.

The histologic appearances took the form of a diffuse sheet-like and clustered pattern of tumor cells with variable degrees of vascular differentiation, and a tubulopapillary growth pattern was encountered in four cases. Nine cases showed varying numbers of spindle-shaped cells producing a focal biphasic architecture, heightening the resemblance to mesothelioma.

The initial diagnoses made on those cases included mesothelioma, secondary adenocarcinoma, and leiomyosarcoma. On immunohistochemical analysis, they were characterized by extensive strong vimentin staining (14/14 cases) in the face of weak (4/14) to moderate (2/14) immunostaining for CKs. The tumor cells expressed at least two of the four endothelial markers employed in the study (CD31, CD34, von Willebrand factor [factor VIII–related antigen; factor VIII–RAG], and *Ulex europaeus* agglutinin-1). Markers for mesothelial, epithelial, myoid, and neuronal differentiation were all negative. These serosal EHEs pursued a highly aggressive course; 12 of the patients presented with disseminated disease and most died within months of the initial presentation.

Subsequently, additional cases have been reported by Attanoos et al.,[1032] Crotty et al.,[1033] Zhang et al.,[519] Sporn et al.,[1034] and Al-Shraim et al.[1035] Zhang et al. found a total of 26 cases in the literature, to which they added five; 22 cases came from Western nations and nine from Japan. The patients were 22 to 79 years of age, with an average of 57 years, and with a male-to-female ratio of 9:1. A history of exposure to radiation or asbestos was noted in a few Western cases. The most common presentation took the form of pleural thickening accompanied by effusion, producing radiological mimicry of MM.

All three cases of pleural EHE reported by Attanoos et al.[1036] had a background of occupational exposure to asbestos, but ferruginous bodies were found in histologic sections from only one of the cases, and only in this patient was the asbestos fiber burden raised in comparison to the range of fiber counts for a nonexposed "background" population. The latent period between asbestos exposure and the diagnosis of the EHEs ranged from 18 to 60 years. These authors reported that no definitive conclusion concerning a relationship between asbestos and pleural EHE could be drawn from this small series of three cases, "but further investigation [was] warranted."

The six patients (five men and one woman) reported by Sporn et al.[1034] ranged in age from 55 to 80 years. All six presented with pleural thickening with or without an accompanying pleural effusion, and for the five for whom follow-up was available, all had died at periods ranging from 3 to 14 months. Oliveira and Carvalho[1037] reported a pleural EHE in a woman who survived for 29 months after diagnosis.

Not only do pleural EHEs essentially mimic mesothelioma in their presentation and the anatomic distribution of the pleural tumor as revealed, for example, by radiologic imaging studies, but the epithelioid appearances of the neoplastic cells can produce a pattern in H&E-stained sections that is virtually indistinguishable from mesothelioma. The neoplastic cells can closely resemble epithelioid cells in an MM, being disposed as sheets or as irregular clusters as shown in Figures 43.181 and 43.182. In some areas, abortive vascular differentiation may be found, and in many cases the neoplastic cells possess empty-appearing intracytoplasmic vacuoles that appear on electron microscopy examination to represent rudimentary vascular lumina (Figs. 43.181 and 43.183). The stroma of these tumors can vary from myxoid (Figs. 43.181 and 43.182) to hyaline, and there may be a spindle-cell sarcomatoid pattern producing mimicry of biphasic mesothelioma.

In 1984 three cases of angiosarcoma of serosal surfaces were described by McCaughey et al.[517] In general, the angiosarcomas are more pleomorphic and less epithelioid than the EHEs (Fig. 43.184).

Clues to the correct diagnosis of EHE include the following:

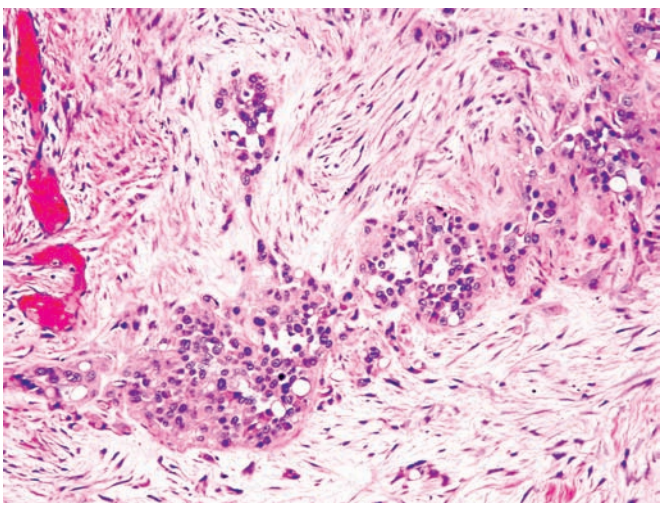


FIGURE 43.181. Pleural epithelioid hemangioendothelioma (EHE) in a middle-aged woman who presented with a unilateral pleural effusion. The tumor comprises an irregular ramifying collection of epithelioid cells embedded in a myxoid fibroproliferative matrix. Vacuoles are discernible in some of the neoplastic cells.

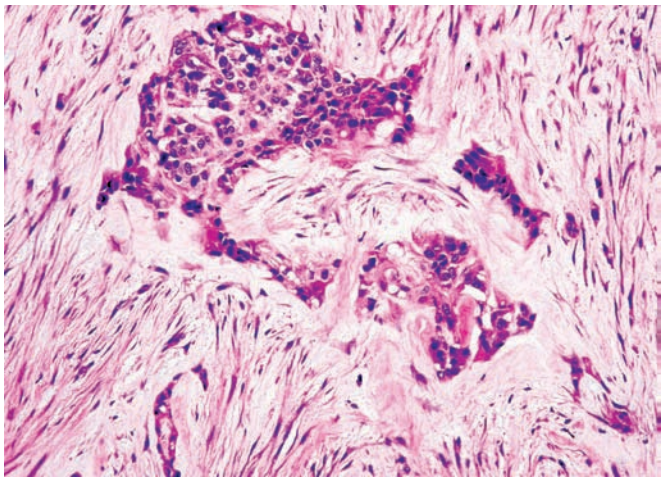


FIGURE 43.182. Pleural EHE. (Same case as in Fig. 43.181.) Collection of epithelioid tumor cells, surrounded by abundant myxoid matrix.

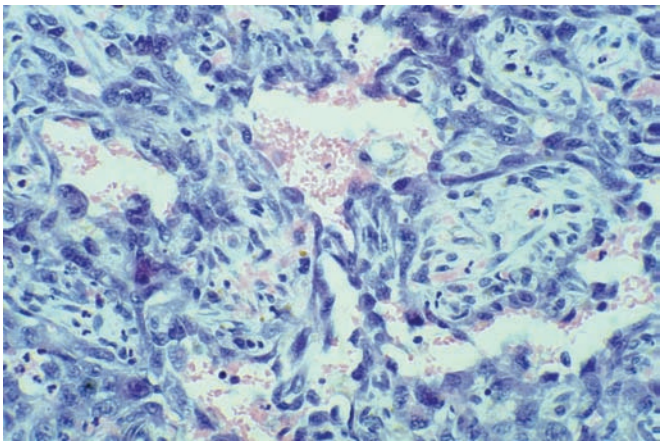


FIGURE 43.184. Histologically, angiosarcomas of the pleura are formed by pleomorphic cells showing vascular spaces

- Negative to weak or only moderate immunostaining for CKs, in comparison to disproportionately prominent reactivity for vimentin
- Absence of staining for mesothelial cell markers such as calretinin or with HBME-1 or for carcinoma-related markers
- Positive immunostaining for endothelial markers such as CD31, CD34 (Fig. 43.185) or factor VIII–RAG

For these reasons, we always include an endothelial marker as part of our immunohistochemical workup on cases of suspected mesothelioma, and we have encountered only two cases of proven mesothelioma that showed positive reactivity of the epithelioid cells for CD31.

On electron microscopy, these tumors show distinct features of endothelial differentiation, including the formation of rudimentary vascular structures, a surrounding basal lamina, and in some instances the presence of tubulated Weibel-Palade bodies in the cytoplasm (Figs. 43.186 and 43.187).

Desmoplastic Round Cell Tumors

Most desmoplastic round cell tumors occur in the pelvic cavity in young adults; rare cases have been reported in the pleura and thorax.[1038–1041]

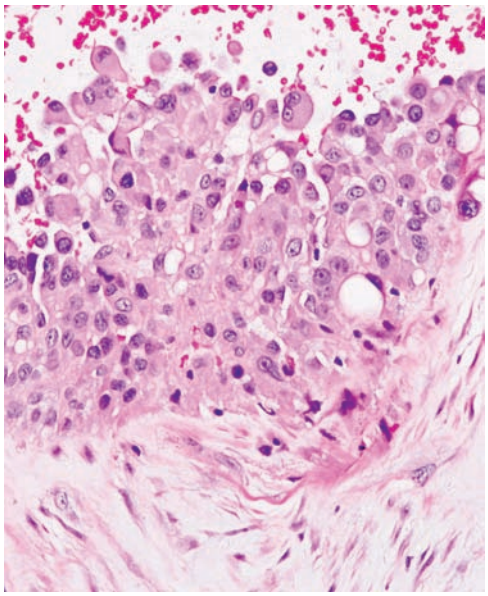


FIGURE 43.183. Pleural EHE. The epithelioid cells are depicted in greater detail, showing nuclear atypia and lucent intracytoplasmic vacuoles.

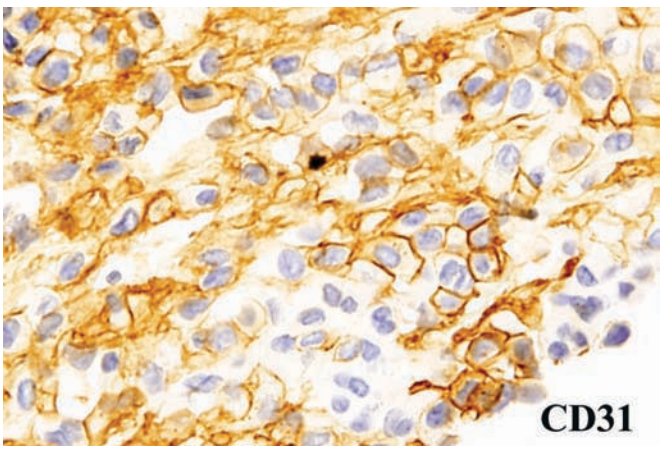


FIGURE 43.185. Pleural EHE. (Same case as in the preceding figures.) The immunoreactivity is seen on labeling for CD31. Identical labeling was seen for CD34. This case showed no detectable cytokeratin expression.

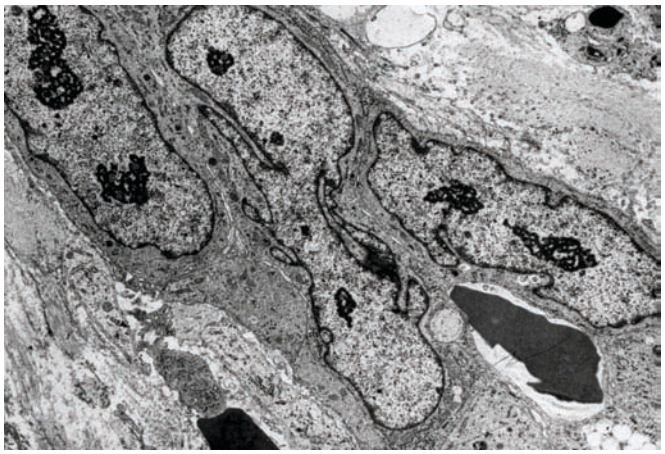


FIGURE 43.186. This electron micrograph shows elongated cells forming primitive vascular structures.

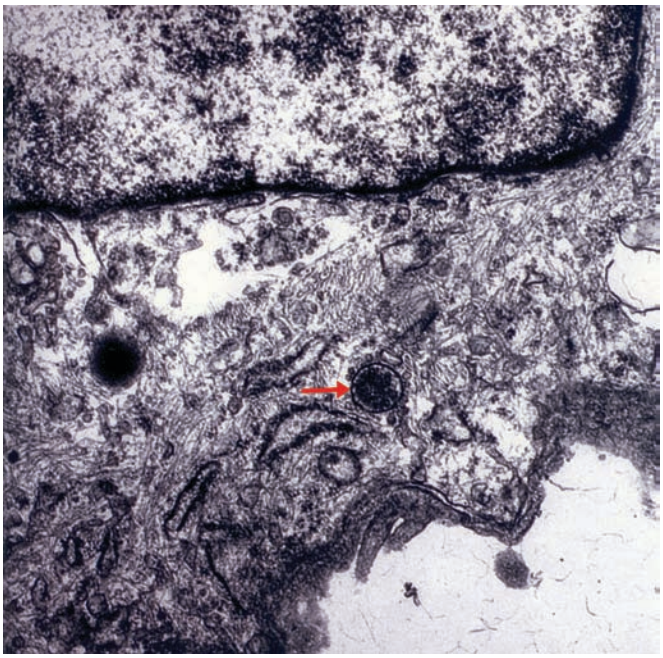


FIGURE 43.187. The structure (arrow) in the cytoplasm of the cell shown here is referred to as a Weibel-Palade body and is pathognomonic of an endothelial cell.

These neoplasms have the same morphology in the pleura as they do in the abdominal cavity, typically consisting of nests of small round cells with hyperchromatic nuclei and a dense fibrous or cellular spindle stroma (Fig. 43.188). The cytoplasm typically contains dot-like structures that correspond to intermediate filaments when examined ultrastructurally (Fig. 43.189). These neoplasms typically show immunostaining for cytokeratin and desmin, with the desmin being in a dot-like configuration (Fig. 43.188B) corresponding to the intermediate filaments seen ultrastructurally. In addition, these neoplasms typically show nuclear staining for WT1. Desmoplastic round cell tumors also characteristically show the translocation t(11;22)(p13;q12) by molecular analysis.[1042]

Primitive Neuroectodermal Tumor

Primitive neuroectodermal tumors (PNETs) are part of the spectrum of small round cell neoplasms that also includes Ewing's sarcoma. These tumors are also referred to as Askin tumors and are composed of sheets of small round cells with hyperchromatic nuclei that show areas of necrosis (Fig. 43.190).[1042] Rosette structures are common and cystic spaces are occasionally seen. The neoplastic cells have a high nuclear-cytoplasmic ratio, and the nuclei typically have vesicular, finely granular chromatin. Glycogen is frequently present in the neoplastic cells and can be demonstrated with a PAS stain or by ultrastructural examination. By immunohistochemistry, the neoplastic cells express CD99 and are usually negative

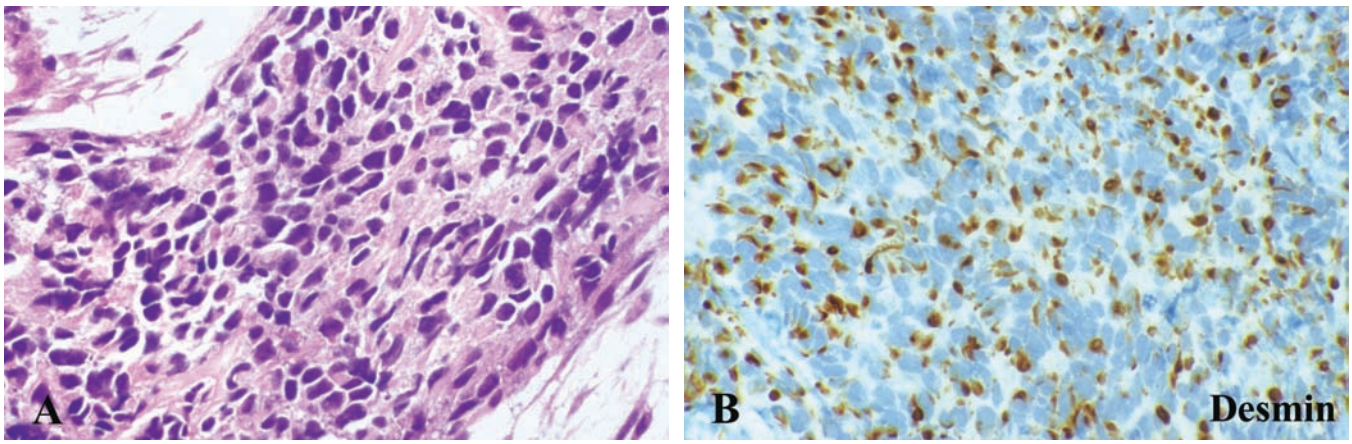


FIGURE 43.188. **(A)** This pleural tumor is composed of small round cells surrounded by cellular fibrous stroma. **(B)** Immunostain for desmin is positive in a dot-like pattern.

for keratin, although focal keratin positivity as well as chromogranin and synaptophysin immunostaining have been observed.[1043,1044] Histologically, these tumors can be confused with small cell mesotheliomas. Molecular analysis typically shows the characteristic translocation, t(11;22)(q24;q12), although this translocation is not specific (see Figs. 36.99 to 36.101 in Chapter 36, and Chapter 42).

Pleuropulmonary Blastoma

Pleuropulmonary blastomas are rare neoplasms that occur in the lung and pleura, predominantly in early childhood.[156,1045] Pleuropulmonary blastomas often have a hamartomatous appearance and frequently are associated with a family history. This neoplasm is different from the pulmonary blastoma that characteristically occurs in an adult. Pleuropulmonary blastoma is composed of primitive cells underneath an epithelium with a cambium layer-like appearance as seen in sarcoma botryoides. Rhabdomyoblasts may be found among the small cells. Occasional anaplastic sarcomatous elements, including embryonal rhabdomyosarcoma, fibrosarcoma, chondrosarcoma, and undifferentiated sarcoma (see Figs. 42.7 to 42.9 in Chapter 42) are observed.

Pleural Lymphomas

Primary pleural lymphomas are rare. The two lymphomas that are mentioned most frequently as involving the pleura are *primary effusion lymphoma (PEL)* and *pyothorax-associated lymphoma*.[1046,1047] Primary effusion lymphomas are composed of large B lymphoid cells (Fig. 43.191) and typically present as pleural effusions without detectable tumor masses elsewhere in the body. Primary effusion lymphomas are associated with human herpesvirus 8 and Kaposi's sarcoma, and typically occur in individuals with acquired immune deficiency syndrome (AIDS).[1046–1048] (See Chapter 32).

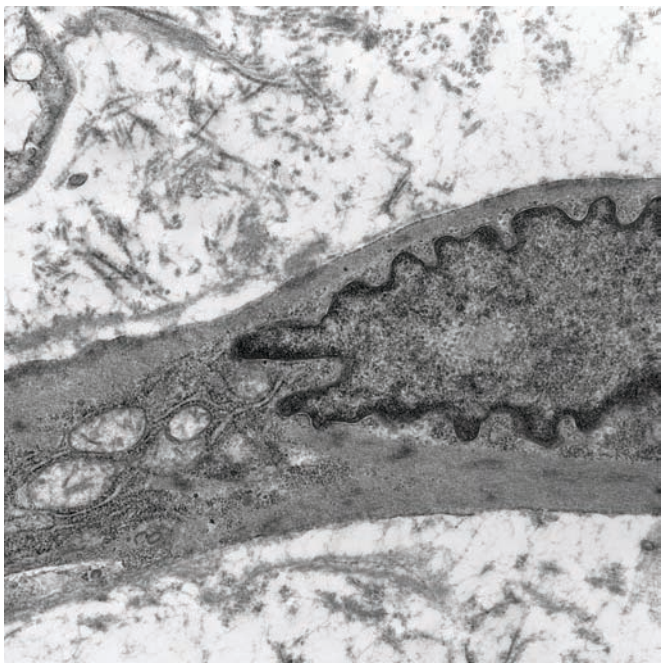


FIGURE 43.189. Ultrastructurally, the cells are round to spindle shaped and show intracytoplasmic intermediate filaments.

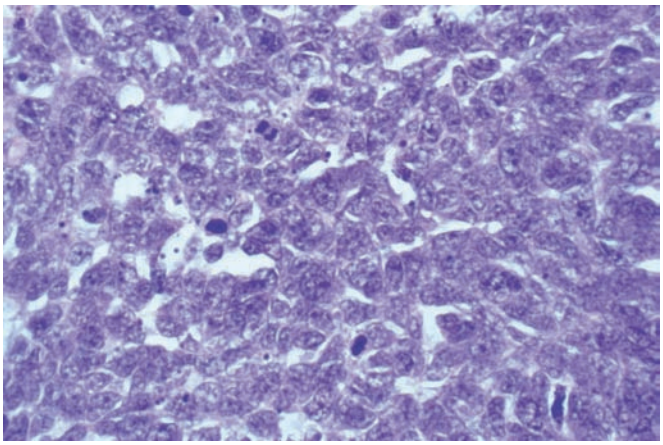


FIGURE 43.190. This small cell tumor involving the pleura has the histologic and immunohistochemical features of a primitive neuroectodermal tumor (PNET).

Pyothorax-associated lymphoma typically occurs in persons with a chronic pyothorax, often decades after the initial injury.[1049–1051] Pyothorax-associated lymphomas were first described in Japan and the largest series is from that country. Clinically, persons with pyothorax-associated lymphomas present with effusion, chest pain, weight loss, and dyspnea. Males are typically more frequently affected than females. Patients with pyothorax-associated lymphoma do not have a history of HIV infection or immunosuppression. The potential causes of pyothorax include tuberculosis and other inflammatory/infectious conditions. The pathogenesis is thought to be due to chronic antigen stimulation analogous to mucosa-associated lymphoid tissue (MALT) lymphomas of the stomach. Pyothorax-associated lymphomas typically are large (usually ≥10 cm) and are associated with pleural fibrosis. They often invade adjacent structures. Pyothorax-associated lymphomas are composed of large B lymphocytes with a smaller number of lymphoplasmacytoid cells. At the time of autopsy, over half the patients have disease limited to the thoracic region and the other half show extrathoracic extension. The neoplastic cells typically show expression of CD45, CD20, CD79, and occasionally CD138. The lymphoid cells are typically negative for CD3.

Diffuse large B-cell lymphomas have been reported to show pleuropulmonary involvement and typically are

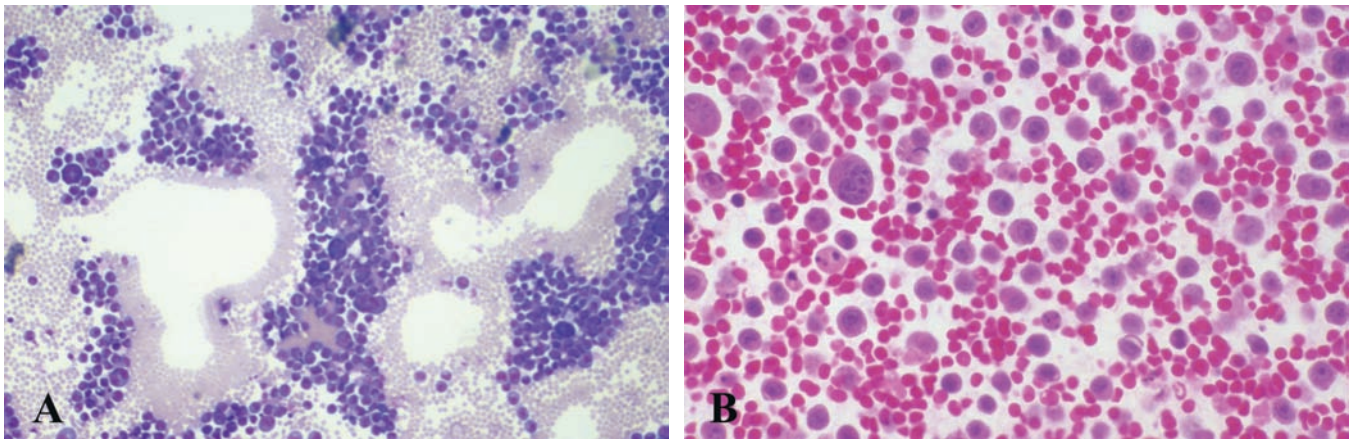


FIGURE 43.191. **(A,B)** The patient whose pleural fluid was evaluated was HIV positive. All cells in the fluid were CD20 positive and were diagnosed by flow cytometry as a large B cell lymphoma. There was no evidence of lymphoma elsewhere in the patient's body.

composed of cells that have immunoblastic features with plasmacytoid differentiation. These cells show frequent mitoses and have a high proliferative rate as demonstrated by MIB-1 evaluation. The cells also show immunostaining for CD45, CD79, and CD20 (see Chapter 32).

Primary sclerosing mediastinal B-cell lymphomas typically occur in young females and can show pleuropulmonary involvement. These lymphomas are thought to arise from perithymic B lymphocytes and typically show immunostaining for CD45, CD20, and CD30. They are CD15 negative.

Multiple myeloma has also been identified as primarily involving the pleura.[1052–1054]

The most recent report on lymphomas involving the pleura is by Vega et al.,[1055] who reviewed the clinicopathologic features of 34 patients with lymphoma involving the pleura proven by biopsy and classified these lymphomas according to the WHO classification. Nine (26.5%) patients had pleural involvement as the only site of disease, whereas 22 (64.7%) had other sites of involvement. Eighteen (56.3%) of 32 patients with adequate clinical data had a history of lymphoma, including three patients with pleural involvement as the only site of disease. According to the WHO classification, 17 (50%) were diffuse large B-cell lymphomas; five (14.7%) were follicular lymphomas, including a case with areas of diffuse large B-cell lymphoma; two (5.9%) were small lymphocytic lymphoma/chronic lymphocytic leukemia; two (5.9%) were precursor T-cell lymphoblastic lymphoma/leukemia; one (2.9%) was mantle cell lymphoma; one (2.9%) was posttransplant lymphoproliferative disorder; and one (2.9%) was a classical Hodgkin's lymphoma. The other five cases were B-cell lymphomas that could not be further classified. The authors concluded that most patients with lymphoma involving the pleura had simultaneous evidence of systemic involvement. The most frequent type was a diffuse large B-cell lymphoma followed by follicular lymphoma.

We have recently seen a mantle zone lymphoma proven by flow cytometry and immunohistochemistry primarily involving the pleura and associated with an epithelial mesothelioma.

Leukemic Involvement of the Pleura

The incidence of leukemic involvement of the pleura is difficult to determine. Relatively few cases have been reported. Bourantas et al.[1056] reported pleural effusion in four patients with chronic myelomonocytic leukemia. Two of four patients presented with pleural effusion as the initial symptom of the disease, whereas the other two developed pleural effusions during the course of the disease. In only one patient was the pleural effusion found to be due to leukemic infiltration. In the other three patients, it was considered a reactive phenomenon.

Schmitt-Graff et al.[1057] reported identification of focal leukemic infiltrates as the initial manifestation of acute myeloid leukemia. Eight patients had myelodysplastic syndrome, and over a 2-year period developed acute myelogenous leukemia. Focal leukemic infiltrates were localized in the skin, oral mucosa, lymph nodes, gastrointestinal tract, pleura, and retroperitoneum. These myelosarcomas were usually regarded as putative malignant lymphomas until further evaluation by immunohistochemistry or flow cytometry. By immunohistochemistry, the neoplastic cells reacted with an antibody against lysozyme, myeloperoxidase, CD68, CD43, CD56, CD117, and CD34. The authors stated that although bone marrow findings were inconclusive, a straightforward diagnosis

was reached by considering the possibility of a myelosarcoma and performing the appropriate immunohistochemical/flow cytometric analyses.

Screening for Mesothelioma: Serum Levels of Soluble Mesothelin-Related Proteins and Osteopontin

Soluble Mesothelin-Related Proteins

A potentially significant recent development for the investigation of MM has been the retrospective demonstration[1058–1065] of elevated serum mesothelin-related protein (SMRP) levels in patients with mesothelioma; similar findings have also been reported in relation to osteopontin levels as a marker for MM.[1066,1067] Even so, these approaches are still at an investigational stage of development. The positive predictive value (PPV)[1068]* for an elevated blood level of SMRP or osteopontin (or both together) has yet to be established, as a precondition for the introduction of these tests into routine clinical practice for the screening or clinical investigation of individual patients for the prospective diagnosis of MM.

Mesothelin is a cell-surface glycoprotein present on normal mesothelial cells and is expressed in several cancers,[563,611,612] including mesotheliomas with an epithelioid component,[563,611,612,680] ovarian adenocarcinomas in particular,[563,864,1069,1070] squamous and large cell carcinomas and adenocarcinomas of lung,[583,680,1071] pancreatic adenocarcinomas,[615,1072] and some gastrointestinal cancers.[1069] The precursor protein product of the mesothelin gene occurs as a 69- to 71-kDa polypeptide with a glycosyl-phosphatidyl-inositol linkage that anchors it to the cell membrane.[1069,1073] This anchored precursor protein can be cleaved by a furin-like protease to yield a 31-kDa soluble protein called megakaryocyte potentiating factor (MPF)[1069,1073] and a 40-kDa cell membrane-bound protein called mesothelin. There is some evidence that mesothelin may be implicated in cell-cell adhesion,[1069] but knowledge of its normal biologic function is incomplete, and mice with a knockout of the mesothelin gene(s) have no obvious phenotypic abnormality.[1070] Although mesothelin is attached to the cell membrane, it can be shed like other cell membrane proteins, and some investigators, including Robinson's group,[1058–1062] have described a 42- to 44-kDa protein called soluble mesothelin/MPF-related (SMR) protein detectable in sera from patients with pleural MM and also ovarian carcinoma.

Antibodies to cell membrane-bound mesothelin were first prepared by inoculating BALB/c mice with the human ovarian carcinoma cell line OVCAR-3, generating the monoclonal antibody K-1,[615,1070] and K-1 has been used for some years for assessment of cancers by immunostaining of histologic sections.[611,612] However, we abandoned the use of antibodies against mesothelin for the immunohistochemical investigation of suspected MM because of its cross-reactivity with other cancers,[611,615,1070] and it appeared to have no particular advantage over other antibodies raised against mesothelial cells. Of course, detection of mesothelin by immunohistochemical analysis of histologic sections is an exercise different from quantitative estimates of blood SMRP levels.

The mechanisms whereby mesothelin is released from cell membranes are unclear as yet, but the release of SMRP might be due to an abnormal splicing event that unbinds or cleaves it from the cell surface.[1064] Robinson's group[1058,1062] detected SMRP using the OV569 monoclonal antibody, but Hassan et al.[1063] appear to have used a different approach to the generation of a mouse anti-mesothelin monoclonal antibody, making it difficult to compare their results with those of both Robinson's group[1058–1062] and Scherpereel et al.[1064] (the OV569 antibody appears to be the basis for the commercially-marketed Mesomark™ Fujirebio Diagnostics, Inc. Malvern, PA test[1065]). Testing for serum SMRP levels is determined by an enzyme-linked immunosorbent assay (ELISA) test using two monoclonal antibodies (e.g., OV569 and 4H3),[1062] which bind to different SMRP epitopes. Shiomi et al.[1074] found that the renal cell carcinoma gene *ERC*, which is expressed in a renal carcinoma model in Eker rats, which carry a mutation in the Tsc2 gene,[1073] is a homologue of the human mesothelin gene, and these investigators[1074] developed an ELISA system for the detection of mesothelin in the sera of mesothelioma patients, using specific antibodies prepared in the same laboratory against the 31-kDa N-terminal fragment of *ERC*.

Robinson et al.[1058] reported elevated blood SMRP levels in 37 of 44 patients previously diagnosed with MM (sensitivity = 84%) in contrast to one of 22 lung cancers (histologic types not specified) and seven of 40 asbestos-exposed control patients (three of whom developed MM 15 to 19 months after the SMRP sample had been taken). Robinson et al. reported their results in terms of the optical absorbance at 420 nm; in a more recent (2006) publication from the same laboratory, Creaney et al.[1062] reported the results as nanomoles (nM), with a mean value of 15.33 ± 20.48 nM in the mesothelioma group, in

*PPV is defined lucidly by Gigerenzer[1068] as "the proportion of p among all those who test positive who actually do have the disease (or condition); i.e. the true positives divided by the total number who test positive"; validity is the extent to which a test measures what it is intended to measure; reliability is the extent to which a test produces the same result when it is carried out at different times and by others using the same methodology. High reliability is necessary but does not guarantee high validity, and vice versa; both are required for a high PPV, among other factors. The sensitivity of a test can be defined as the proportion of patients with the disease in question who return a positive test for that disease.

comparison to a level of 0.925 ± 0.831 nM for healthy controls. Hassan et al.[1063] recorded elevated serum mesothelin levels in 40 of 56 mesothelioma patients (71%) and in 14 of 21 patients with ovarian cancer (67%); their results were expressed as nanograms per liter (ng/L).

Nonetheless, although a sensitivity of 84% and a claimed specificity of 100% as recorded by Robinson et al.[1058] may seem impressive at first sight, these figures do not necessarily translate to a PPV of the same order.

Beyer et al.[1065] investigated SMRP levels in the serum of 409 apparently healthy individuals, 177 patients with nonmalignant disorders, and 500 cancer patients who included 88 with pleural mesothelioma. The 99th percentile level for the reference group was 1.5 nM/L, whereas the mean level for the 88 mesothelioma patients was 7.5 nM/L (95% CI, 2.8–12.1). The SMRP levels were increased in 52% of the MM patients and 5% of asbestos-exposed individuals.

In another series, Scherpereel et al.[1064] reported blood SMRP levels in 74 mesothelioma patients, 35 patients with carcinomas metastatic to the pleura, and 28 cases of benign pleural lesions associated with asbestos exposure (BPLAE). They found that the serum SMRP levels were significantly higher for epithelioid MMs than for biphasic or sarcomatoid MMs. They also found that the median value for patients with pleural MM was 2.05 ± 2.5 nM/L, in comparison to a level of 1.02 ± 1.79 nM/L for the metastatic carcinoma group—there is significant overlap between these two values in term of the standard deviations (SDs)—and in BPLAE cases the level was 0.55 ± 0.59 nM/L.

In 2007, Creaney et al.[864] also reported mesothelin levels in effusion fluids from 52 patients with pleural MM, in comparison to 56 patients with malignancies other than mesothelioma and 84 with benign pleural effusions. Creaney et al. found significantly greater concentrations of mesothelin in pleural fluid from the MM patients than in the other two groups, with a specificity of 98% and a sensitivity of 67% for the mesothelioma group in comparison to those with nonneoplastic effusions. In seven of 10 cases, the mesothelin levels were elevated before the diagnosis of MM was made (by 0.75–10 months); four of eight such cases had elevated mesothelin concentrations in the effusion fluid but not in the serum. The highest mesothelin levels were found in peritoneal fluid in patients with ovarian carcinoma (exponentiated mean of log transformed data = 73.7 ± 0.77 nM); there were significant differences in the corresponding mean mesothelin values in pleural effusion fluid for epithelial (46.9 ± 1.1 nM), biphasic (30.1 ± 0.8), and sarcomatoid (4.5 ± 1.38) MMs, and for the cases designated "cytology only" the mesothelin level in pleural fluid was 39.2 ± 0.96 nM. For the pleural sarcomatoid MMs, the mesothelin concentrations did not differ significantly from patients with nonmalignant effusions. The median survival for MM patients with high concentrations of mesothelin in effusion fluid was 14 months, versus 8 months for those with low mesothelin levels, probably reflecting MMs with an epithelial component as opposed to sarcomatoid mesotheliomas.

Therefore, we draw the following conclusion:

1. Although blood SMRP levels are elevated in most cases of mesothelioma, nonmesothelial cancers can also be associated with significantly elevated serum SMRP concentrations, including lung and, in particular, ovarian cancers.[1063,1075]
2. Epithelial mesotheliomas are associated with higher mesothelin levels in serum and effusion fluid than biphasic or sarcomatoid mesotheliomas.
3. The diagnosis of MM remains an essentially pathologic exercise that employs routine light microscopy of cytology and biopsy specimens and autopsy tissue on occasion, together with mucin histochemistry, immunohistochemistry, and, in some cases, transmission electron microscopy.
4. At present, investigation of serum SMRP levels cannot replace cytologic or biopsy diagnosis of MM, except perhaps in extraordinary circumstances (e.g., an elderly patient whose poor physical condition precludes biopsy procedures or for whom past biopsies have been nondiagnostic, but who has high serum SMRP levels, such as levels >15 nM/L).
5. At present, it seems difficult or impossible to compare the SMRP results obtained by different laboratories, because of methodologic differences.
6. High serum SMRP levels (for example >7.5 or >15 nm/L) probably have a greater predictive value as a marker of MM, whereas levels in the range of ~2.0 nM/L are more problematic, and the PPVs for different blood levels of SMRP have yet to be evaluated.
7. Use of serum SMRP levels as a screening test for patients at high risk of MM should be approached with awareness of the limitations of the test and its potential ethical ramifications: (a) any test will produce occasional false-positive results, with a requirement to investigate further, and such further investigations for mesothelioma are necessarily invasive, with the potential for resultant morbidity; (b) a false-positive result can generate unnecessary anguish in the patient and family concerning a cancer well known to be highly aggressive; and (c) screening procedures are most cogently justifiable when there is an effective intervention or treatment for the disorder so detected, but there is no consistently curative or definitive treatment for mesothelioma at the present time.
8. High mesothelin levels in effusion fluid may prove useful as an adjunct to cytodiagnosis of such fluids when ovarian carcinoma is not an issue.
9. Apart from a role as a screening procedure or as an adjunct to pathologic diagnosis, assays of serum SMRP

levels may find a role as an indicator of prognosis (with the exception of sarcomatoid MMs) and as a means to assess the progress of the disease or its response to treatment.

Serum Osteopontin Levels

The significance of serum osteopontin (OPN) levels as a marker for mesothelioma is more problematic and open to greater doubt than testing for serum SMRP concentrations. OPN is an acidic glycoprotein normally synthesized by osteoblasts and—like the angiopoietin-1 (ANG-1) also produced by osteoblasts—OPN acts as a "constraining factor"[1076] on hemopoietic stem cell proliferation in the bone marrow. Although elevated blood OPN levels have been recorded in patients with mesothelioma,[1066] elevated levels have also been recorded in a variety of other disorders that include carcinomas of the head and neck region[1077,1078] and cervix,[1077] as well as ovarian,[1079] gastric,[1080] and hepatocellular carcinomas[1081]; elevated levels have also been found in patients with inflammatory bowel disease.[1082] Therefore, it appears that serum OPN levels have poor specificity for a diagnosis of mesothelioma, but serum OPN assays may find a role in assessment of the extent and prognosis of mesothelioma and its response to treatment.

Chemical Analysis of Pleural Fluid and Pleural Neoplasms for Hyaluronic Acid

The concentration of hyaluronic acid in pleural fluid and pleural neoplasms has been evaluated to determine if it is helpful in making a diagnosis of mesothelioma. The results have been variable. Friman et al.[1083] found an increased concentration of hyaluronic acid in pleural fluid in three cases of mesothelioma. Arai et al.[1084] reported a hyaluronic acid concentration of 7 μg/mL in a case of diffuse mesothelioma, 14 ± 8.6 μg/mL in four cases of tuberculous pleurisy, and 9.43 ± 5.13 μg/mL in seven cases of cancerous pleurisy. Other investigators[1085–1087] found similar variable results of hyaluronic acid concentration in pleural fluid. An anecdotal case report also noted increased pleural fluid hyaluronic acid in a patient with mesothelioma.[1088]

In 1988, Pettersson et al.[1089] reported their evaluation of hyaluronic acid concentration in pleural fluid from 85 patients with pleural effusions, including 15 with MM, 32 with other types of neoplasms, 31 with nonmalignant inflammatory disease, and seven with congestive heart failure. Eleven of 15 (73%) patients with MM and seven of 31 (23%) with nonmalignant inflammatory conditions had pleural fluid hyaluronic acid concentrations greater than 100 mg/L, whereas all 32 patients with other types of cancers and the seven patients with congestive heart failure had hyaluronic acid concentrations less than 100 mg/L. The authors also evaluated the usefulness of pleural fluid CEA concentrations in differentiating MM from other types of cancer. Four of 15 (27%) patients with MM and 12 of 32 (38%) patients with other malignant neoplasms had CEA concentrations greater than 10 μg/L. The authors concluded that, in pleural effusions associated with a malignant tumor, a high hyaluronic acid concentration and low CEA concentration in the pleural fluid suggested the diagnosis of MM as opposed to other malignant neoplasms. Using a cutoff of 100 μg/mL, Atagi et al.[860] also found that pleural fluid hyaluronic acid levels were higher in patients with mesothelioma versus metastatic carcinoma, and that the combination of elevated hyaluronic acid and low CEA levels possibly supported the diagnosis of mesothelioma.

In a somewhat similar study, Hillerdal et al.[1090] determined the hyaluronic acid concentration in serum and pleural fluid in 78 consecutive patients with pleural effusions. In three of nine (33%) patients with MM and five of 42 (12%) patients with metastatic malignant neoplasms, pleural fluid hyaluronic acid concentration was greater than 100 mg/L. In addition, in two of 11 (18%) patients with cardiac disease, three of four (75%) patients with viral infection, one patient with a postinfectious effusion, and two of two (100%) patients with benign asbestos-induced effusion had pleural fluid hyaluronic acid concentrations greater than 100 mg/L. The serum hyaluronic acid concentrations were lower than those found in the pleural fluid, and there was no correlation between pleural fluid hyaluronic acid concentrations and serum hyaluronic acid levels. In contrast to the conclusion of Pettersson et al.,[1089] Hillerdal et al. concluded that a high concentration of hyaluronic acid in pleural fluid was not specific for MM and could be found in other malignant conditions and in benign diseases. They also concluded that a low pleural fluid hyaluronic acid concentration did not exclude the diagnosis of MM. Soderblom et al.[1091] also concluded that elevated hyaluronic acid levels could be found not only in mesothelioma but also in patients with benign pleural effusions, especially those with rheumatoid arthritis. They speculated that hyaluronic acid was related to proinflammatory cytokines.

In tissue specimens, Arai and colleagues[1084] found at least 0.10 mg of hyaluronic acid per gram of dry tissue in four cases of mesothelioma, but only 0.02 to 0.03 mg of hyaluronic acid per gram of dry tissue in two cases of carcinomatous pleural tissue and in pleural tissue from two patients with asbestosis. Chiu et al.[1092] isolated glycosaminoglycans from 21 mesotheliomas, 34 primary lung carcinomas, 12 carcinomas from other sites, and four soft tissue sarcomas. Hyaluronic acid was identified qualitatively in 20 of 21 mesotheliomas, approximately half of the lung carcinomas, and all of the soft tissue sarcomas. Quantitatively, hyaluronic acid constituted 45% of the

total glycosaminoglycan in mesotheliomas and 28% of the total in carcinomas of the lung. The mean value of hyaluronic acid in mesotheliomas was significantly higher (0.74 mg/g) than lung adenocarcinomas (0.08 mg/g), but was not significantly higher than in soft tissue sarcomas (2.01 mg/g) or ovarian serous carcinomas (0.92 mg/g). They concluded that a hyaluronic concentration of greater than 0.4 mg/g dry tissue extract supported the diagnosis of mesothelioma when the alternative diagnosis was primary pulmonary adenocarcinoma.

Nakano et al.[1093] also studied glycosaminoglycan concentration in five pleural mesotheliomas and contrasted it to that seen in one pulmonary adenocarcinoma. The average total amount of glycosaminoglycan was 7.9 times higher in the mesotheliomas than in the pulmonary adenocarcinoma, and hyaluronic acid and chondroitin sulfate were the main types of glycosaminoglycans found. They also found an increase in hyaluronic acid and chondroitin sulfate in pleural fluid from two patients with mesothelioma. Iozzo[1094] reviewed the subject of proteoglycans and their role in neoplasia in 1985, having previously reported[1095] that tissue extracts of mesotheliomas contain large amounts of chondroitin sulfate.

Afify et al.[1096] evaluated archival paraffin-embedded cell blocks of serous fluids from 28 cases of reactive mesothelial cells, 14 cases of MM, 20 cases of metastatic ovarian carcinomas, 17 cases of metastatic breast carcinomas, 12 cases of metastatic lung adenocarcinoma. and 12 cases of metastatic gastrointestinal adenocarcinoma by means of immunohistochemical staining for hyaluronic acid using a biotinylated hyaluronic acid binding protein (HABP) and CD44S. All MMs and 93% (26 of 28) of benign mesothelial cells were positive for intracytoplasmic hyaluronic acid versus none of the adenocarcinomas. CD44S was expressed in 100% of mesothelial hyperplasia cases and 86% (12 of 14) of MMs, 70% (14 of 20) ovarian carcinomas, 29% (five of 17) of breast carcinomas, 25% (three of 12) of gastrointestinal adenocarcinomas, and 8% (one of 12) of lung adenocarcinomas. The authors concluded immunostaining for hyaluronic acid was a reliable marker that could distinguish between cells of mesothelial origin (reactive mesothelial cells and MM) and adenocarcinoma. The authors also concluded that immunostaining for CD44S could be useful with other stains in the differential diagnosis of adenocarcinoma and mesothelioma.

Thylen et al.[1097] in a multivariate analysis confirmed that an elevated concentration of hyaluronan in pleural fluid was an independent predictor of longer survival in older patients and in patients receiving therapy for mesothelioma.

In summary, most mesotheliomas show reactivity for hyaluronic acid and manifest elevated concentrations of hyaluronic acid in pleural fluid, but the findings are neither specific nor sensitive enough to be used in a diagnostic setting.

Clinicopathologic Correlations

Patients with pleural mesothelioma usually present with nonspecific signs and symptoms consisting of chest pain, dyspnea on exertion, cough, weight loss, and a unilateral pleural effusion. Physical examination is usually nonspecific, but characteristically reveals dullness to percussion on the involved side and distant breath sounds by auscultation.

Approximately 10% to 20% of patients diagnosed with mesothelioma have "B" symptoms consisting of fever, night sweats, weight loss, and anorexia. About 20% to 30% of patients have anemia, typically a microcytic anemia. About 20% to 30% develop thrombocytosis, thought to be mediated by interleukin-6. We have seen four cases of individuals who have presented with spontaneous thrombosis of the subclavian vein with elevated platelet counts, the highest being over 1 million platelets per microliter, and other cases where a diagnosis of mesothelioma has been followed by thrombotic or thromboembolic complications related to thrombocytosis, such as cerebral infarction.

It is currently thought that the systemic manifestations of MM, including fever, cachexia, and thrombocytosis, may be related to the production of interleukin-6 by malignant cells.[1098]

Spread and Staging of Malignant Mesothelioma

The clinical course of MM is usually dominated by the primary tumor and its locoregional spread. Accordingly, pleural mesotheliomas typically compress and invade lung, mediastinum, and chest wall structures. On occasion, the neoplasm and its associated effusion may be so massive that it constitutes a tension effusion with tumor, with displacement of mediastinal structures to the contralateral side.[1099] Because mesothelioma can produce contraction of the affected hemithorax (Fig. 43.192), it can also displace mediastinal structures toward itself. Invasion of the mediastinum and pericardium may be complicated by the development of hemopericardium with tamponade, or by encasement of the great vessels or esophagus, sometimes with the development of dysphagia. Invasion of the chest wall is frequent,[211,190] especially along needle tracks, biopsy sites, or drainage wounds (Fig. 43.193),[211,503,716] with extension through the chest wall into the subcutaneous plane, sometimes complicated by ulceration.

Local invasion into lung parenchyma is also common, and when this occurs, unusual patterns of infiltration can develop, including a desquamative interstitial pneumonia (DIP)-like appearance whereby the invasive epithelial mesothelioma is accompanied by innumerable alveolar macrophages[1100] (Fig. 43.194); lepidic spread along preexisting alveolar walls can also occur, producing histologic

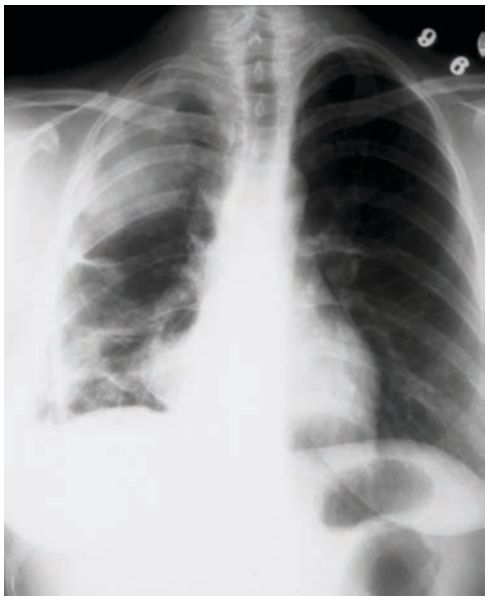


FIGURE 43.192. Right-sided pleural malignant mesothelioma in a young woman. Chest radiograph following aspiration of a massive pleural effusion. This was the first "environmental" mesothelioma from Wittenoom. The patient was 28 years old at the time of her presentation in 1975, with an abrupt onset of right pleuritic chest pain in the middle of the night. She had given birth to her third child a few weeks earlier and at first her pleural effusion was thought to be explicable by pulmonary thromboembolism. A pleural biopsy revealed a biphasic malignant mesothelioma with heterologous osseous differentiation (see Fig. 43.50). The patient had lived at Wittenoom for the first 12 years of her life, where her father was a miner (and subsequently developed asbestosis). Mine tailings were used to top-dress the lawn in the backyard of the family residence, and the patient frequently played in the tailings, looking for "fool's gold." She died from her mesothelioma about 6 months after presentation.

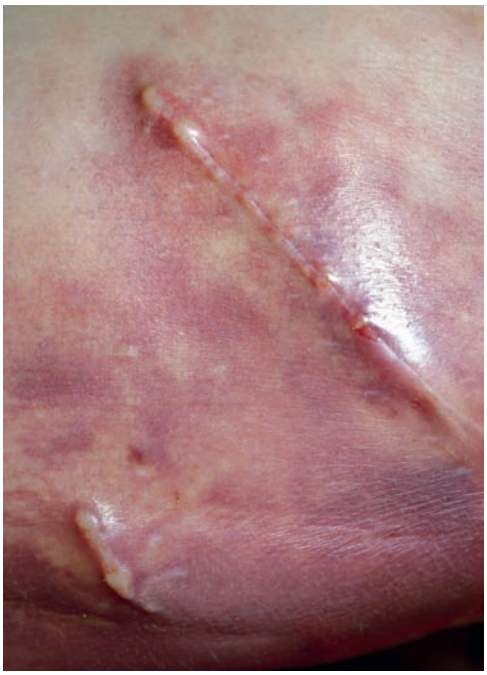


FIGURE 43.193. Pleural malignant mesothelioma with direct invasion into a thoracotomy scar and extension into the skin, which displays postmortem lividity. A similar pattern of chest wall invasion is also evident through the nearby drainage site. (Figure 4-5 from Churg A, Cagle PT, Roggli VL. Tumors of the Serosal Membranes, AFIP Atlas of Tumor Pathology, Fourth Series, American Registry of Pathology, Washington, DC 2006.)

mimicry of a bronchioloalveolar carcinoma.[1100,1101] When sarcomatoid and desmoplastic MMs invade into lung, they can infiltrate into and along the interstitium, and they can also erupt into alveolar spaces, producing histologic mimicry of either organizing pneumonia[37] or an intrapulmonary epithelioid hemangioendothelioma.[1100] Spread to the contralateral pleura or lung is also common in late-stage disease.[211]

Extension of mesothelioma through the diaphragm ("gravitational spread") can lead to seeding of the mesothelioma into the peritoneal cavity, complicated by the development of ascites[211] (Fig. 43.195). For right-sided pleural mesotheliomas, extension through the diaphragm may be accompanied by direct invasion into the liver. In some cases, ascites as a consequence of transdiaphragmatic spread dominates the clinical picture, and identification of the mesothelioma by cytologic examination of ascitic fluid or biopsy tissue from the abdomen can lead to misdiagnosis of the mesothelioma as a primary peritoneal lesion. Therefore, before diagnosis of a mesothelioma as a primary mesothelioma of the peritoneum (or pericardium or tunica vaginalis testis), we routinely recommend exclusion of an underlying pleural mesothelioma on the basis of the clinical and radiologic findings,

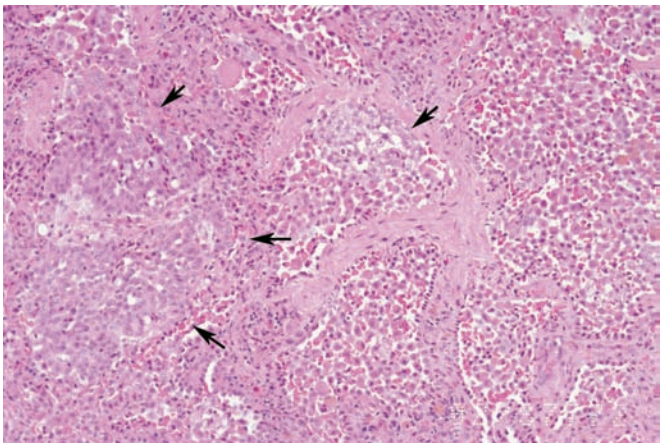


FIGURE 43.194. Invasion into lung parenchyma by a pleural malignant mesothelioma of epithelial type. The mesothelioma (arrows) extends into alveolar spaces where it blends with numerous alveolar macrophages, creating a histologic resemblance to desquamative interstitial pneumonia.

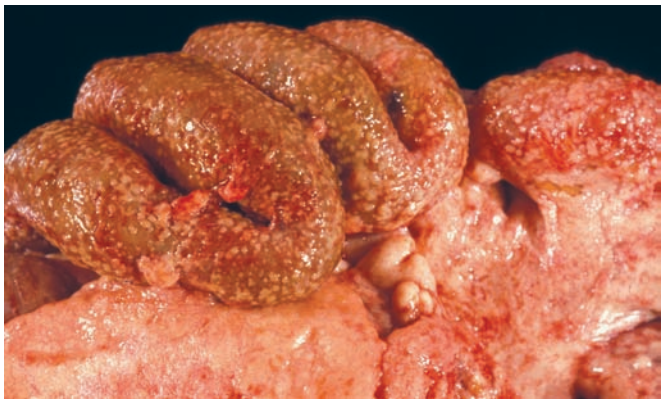


FIGURE 43.195. Transdiaphragmatic extension into the peritoneal cavity from a biopsy-proven pleural malignant mesothelioma. Apart from symptoms referable to the primary pleural tumor, the patient suffered from intractable ascites during the final few months of his life, as a consequence of this pattern of spread. Innumerable serosal nodular deposits of tumor are evident over the loops of small intestine, accompanied by a mesenteric "cake" of metastatic mesothelioma.

taking into account the fact that about 90% of all mesotheliomas arise in the pleura.[211] For example, a diagnosis of MM in one of our cases was established on an omental biopsy taken at an exploratory laparotomy for ascites. The primary pleural tumor was recognized in retrospect from abnormalities in the chest radiographs—contraction of one hemithorax plus a pleural effusion on the same side—which antedated the abdominal manifestations.[211] In another case, the diagnosis of mesothelioma was established from a resected vermiform appendix, in a patient suspected on radiologic grounds to have a pleural mesothelioma.[211]

In contrast to spread of mesothelioma from the pleura to the peritoneum, the reverse direction of spread is distinctly uncommon and seems to have occurred in only two cases accessioned into the Australian Mesothelioma Surveillance Program, as assessed from the clinical findings and the distribution of tumor at autopsy.[211]

Local invasion into and along lymphatic channels is often encountered (especially in pleuropneumonectomy specimens), accompanied in some instances by metastatic deposits in regional and more distant lymph nodes. Sussman and Rosai[746] documented lymph node metastasis as the initial manifestation in six cases of MM. This pattern of spread seems to occur more frequently with peritoneal mesotheliomas than pleural tumors, and four of the five peritoneal tumors had lymph node metastases above the diaphragm, in cervical and mediastinal lymph nodes.[746] Invasion along peribronchial lymphatic channels can also occur, producing cuffs of neoplastic tissue surrounding bronchial walls.[1102] Lymphangitic spread has also been recorded as a presenting manifestation,[1103] as has miliary spread.[1104] In addition, spread into the mediastinal and hilar region can be accompanied by retrograde infiltration along bronchi—sometimes within bronchial lymphatic vessels—with eruption of the tumor into the bronchial lumen, accounting for rare cases where mesothelioma is sampled by endoscopic bronchial biopsies (Fig. 43.196).

Autopsy studies have also shown that hematogenous metastases from mesothelioma often develop in sites such as lung, liver, adrenal glands, bone marrow, brain, and even kidney.[211] In this regard, such hematogenous spread can be encountered in three main circumstances:

1. *At autopsy:* In general, distant metastatic deposits from mesothelioma remain silent during life, so clinical evidence of extrathoracic spread is uncommon (about 10%).[190]

2. *Clinically apparent metastases in cases with antecedent biopsy-proven MM:* Such metastases include cerebral[1105–1111] and cutaneous[1112,1113] metastases. Brain metastases in three cases of mesothelioma in our files produced prominent clinical manifestations. In fact, MM has the capacity to metastasize to virtually any anatomic

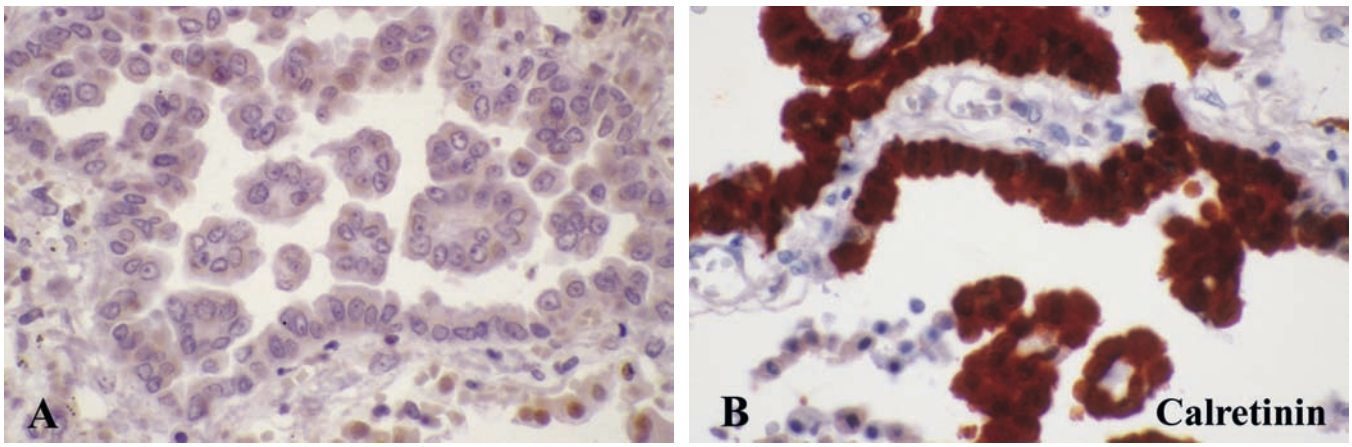


FIGURE 43.196. This transbronchial biopsy shows involvement by an epithelial neoplasm **(A)** that shows nuclear and cytoplasmic immunostaining for calretinin **(B)** and no immunostaining for CEA or TTF-1.

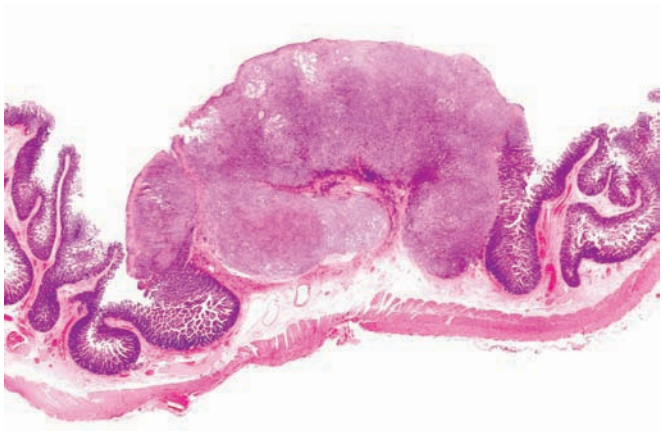


FIGURE 43.197. Metastasis of pleural malignant mesothelioma to small intestine. The patient was a 51–year-old man with a history of antecedent minor exposure to asbestos and who was found to have a pleural mass lesion. Fine-needle aspiration cytology and a core biopsy from the affected pleura yielded a diagnosis of highly probable to near-definite mesothelioma of epithelial type. About 2 years later, he developed intestinal obstruction and was found at laparotomy to have tumor nodules in the small intestine. Pathologic examination of the resected segment of small bowel revealed mucosal/submucosal deposits of metastatic mesothelioma, as shown in this figure. There is no evidence of extension into the muscularis externa and the serosa is unaffected.

site. Unusual other sites where metastases have been recorded on rare occasions include the orbit,[1114] tongue,[1115] intestine (Fig. 43.197), thyroid,[898,1116] and prostate,[1116] among others. As mentioned elsewhere in this chapter, desmoplastic mesotheliomas appear to have a propensity for metastasis to bone,[37,503,830] where they can be confused with primary fibroblastoid bone tumors.

3. *Rarely, metastases as the presenting manifestation of an underlying and hitherto undetected MM.*

Brenner et al.[85] reviewed 123 patients with pleural mesothelioma and found that the tumor was apparently confined to the thorax in all but nine at the time of diagnosis, but spread to the peritoneum or distant sites developed later in 33 of the remaining 114 patients (29%). "Distant" metastases were also recorded in 12 of 16 autopsy cases of pleural mesothelioma reported by Adams and Unni,[500] whereas Whitaker[495] recorded them in 45% of cases and Roberts[1117] in 47%. Huncharek and Muscat[1118] detected lymph node deposits in 19 of 42 autopsy cases (45%), whereas "distant" metastases were found in 32 cases (76%). Hulks et al.[1119] found autopsy evidence of metastatic disease in lymph nodes and distant sites on either side of the diaphragm, in 32 of 40 pleural MM patients from Western Glasgow (80%). In the last two series,[1118,1119] the histologic type of the mesothelioma did not appear to influence either the frequency of metastases or their distribution. In a later autopsy study of 22 cases of mesothelioma, King et al.[1120] found metastases in multiple sites that included omentum, stomach, intestine, mesentery, adrenal glands, ovary, pancreas, kidneys, liver, spleen, and vertebrae. Henderson et al.[211] recorded similar observations (Table 43.22), as did Hammar[289] in a tabular analysis of 11 different autopsy studies,[83,500,1117–1119,1121–1126] across which 58% of the cases had metastatic disease. Malignant mesothelioma has also been reported in other more unusual metastatic sites such as scalp, fingers, tonsil, and gluteal muscle.

TABLE 43.22. Spread of pleural malignant mesothelioma as found in 143 autopsy cases

Anatomic pattern of spread	Number of cases	Percentage
Direct/intrathoracic spread		
Contralateral pleura/lung	73	51
Pericardium	74	52
Myocardium/endocardium	17	12
Mediastinal/brachial great vessels	17	12
Esophagus	9	6
Transdiaphragmatic spread into peritoneal cavity	63	44
Lymph nodes: cervical, mediastinal, hilar, retroperitoneal	67	47
Distant metastases		
Axial bone marrow: sternum, ribs, vertebrae	23	16
Liver	36	25
Spleen	6	4
Kidney	19	13
Adrenal gland	20	14
Pancreas	4	3
Central nervous system: meninges, brain, spinal cord	5	4
Skin and subcutis	5	4
Other (muscle, thyroid, cecum)	11	8
Total with distant metastases	69	48

Source: Modified from Henderson DW, Shilkin KB, Whitaker D, Attwood HD, Constance TJ, Steele RH, Leppard PJ, The pathology of malignant mesothelioma, including immunohistology and ultrastructure. In: Henderson DW, Shilkin KB, Langlois SLeP, Whitaker D, eds. Malignant mesothelioma, pp. 69–139. Copyright 1992 by Hemisphere. Reproduced with permission of Informa Healthcare Books via Copyright Clearance Center.

TABLE 43.23. Staging of mesothelioma

DEFINITION OF TNM

IMIG Staging System for Diffuse Malignant Pleural Mesothelioma

Primary Tumor (T)

TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
T1 Tumor involves ipsilateral parietal pleura, with or without focal involvement of visceral pleura
T1a Tumor involves ipsilateral parietal (mediastinal, diaphragmatic) pleura. No involvement of the visceral pleura
T1b Tumor involves ipsilateral parietal (mediastinal, diaphragmatic) pleura, with focal involvement of the visceral pleura
T2 Tumor involves any of the ipsilateral pleural surfaces with at least one of the following:
—confluent visceral pleural tumor (including fissure)
—invasion of diaphragmatic muscle
—invasion of lung parenchyma
T3* Tumor involves any of the ipsilateral pleural surfaces, with at least one of the following:
—invasion of the endothoracic fascia
—invasion into mediastinal fat
—solitary focus of tumor invading the soft tissues of the chest wall
—non-transmural involvement of the pericardium
T4** Tumor involves any of the ipsilateral pleural surfaces, with at least one of the following:
—diffuse or multifocal invasion of soft tissues of the chest wall
—any involvement of rib
—invasion through the diaphragm to the peritoneum
—invasion of any mediastinal organ(s)
—direct extension to the contralateral pleura
—invasion into the spine
—extension to the internal surface of the pericardium
—percardial effusion with positive cytology
—invasion of the myocardium
—invasion of the brachial plexus

*T3 describes locally advanced but potentially resectable tumor
**T4 describes locally advanced, technically unresectable tumor

Regional Lymph Nodes (N)

NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastases
N1 Metastases in the ipsilateral bronchopulmonary and/or hilar lymph node(s)
N2 Metastases in the subcarinal lymph node(s) and/or the ipsilateral internal mammary or mediastinal lymph node(s)
N3 Metastases in the contralateral mediastinal, internal mammary, or hilar lymph node(s) and/or the ipsilateral or contralateral supraclavicular or scalene lymph node(s)

Distant Metastasis (M)

MX Distant metastases cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

STAGE GROUPING			
Stage I	T1	N0	M0
Stage IA	T1a	N0	M0
Stage IB	T1b	N0	M0
Stage II	T2	N0	M0
Stage III	T1, T2	N1	M0
	T1, T2	N2	M0
	T3	N0, N1, N2	M0
Stage IV	T4	Any N	M0
	Any T	N3	M0
	Any T	Any N	M1

Source: Used with the permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois. The original source for this material is the AJCC Cancer Staging Manual, Sixth Edition (2002) published by Springer Science and Business Media, LCC, www.springer.com.

Staging of Pleural Malignant Mesothelioma

The Butchart staging system[1127] for pleural MM has now been superseded by the tumor, node, metastases (TNM) staging system as developed by the International Mesothelioma Interest Group (IMIG)[37,1128] and as essentially set forth in the Cancer Staging Handbook from the American Joint Committee on Cancer (AJCC) (Table 43.23).[1116]

As previously stated, malignant pleural mesotheliomas not infrequently show diffuse spread to lung parenchyma when evaluated at autopsy. Radiographic diffuse metastases to lung parenchyma by pleural mesothelioma may show no abnormalities or a diffuse reticulonodular or variably nodular pattern. Ohishi et al.[1129] reported identifying mesothelial metastases by transbronchial biopsy. We have seen several cases of this phenomenon (Fig. 43.196).

Prognosis of Malignant Mesothelioma

Chailleux et al.[1130] evaluated 167 cases of pleural MM diagnosed between 1955 and 1985 in the St. Nazaire region of France; 135 mesotheliomas were epithelial, 25 biphasic, and seven sarcomatous; 131 (78%) were related to occupational exposure to asbestos. Eighty-eight patients were treated, including 14 by pleurectomy, 25 by partial pleurectomy, four by pleuropneumonectomy, 42 with chemotherapy (consisting of cisplatin alone, cisplastin, adriamycin and bleomycin, cyclophosphamide alone, and other combinations), 20 with talc pleurodesis and 1 with radiation plus chemotherapy. Survival from first symptoms was 54% at 1 year and 22% at 2 years with a median of 11 months. Survival from pathologic diagnosis was 39% at 1 year and 14% at 2 years with a median of 10 months. No patient was alive 4 years after diagnosis. Patients treated by chemotherapy, surgery, or talc poudrage had a longer survival, but there was no indication that one form of therapy was superior to another. One woman treated with cisplatin had a 15-month complete remission; no partial remissions were observed with chemotherapy. The histologic type of mesothelioma and a history of asbestos exposure had no predictive survival value. Patients younger than 60 years of age when the mesothelioma was diagnosed lived longer than those 60 years or older.

Antman et al.[1131] evaluated 180 patients with MM identified between 1965 and 1985, of which 136 were pleural, 37 peritoneal, five pericardial, and two testicular in origin. The median survival for those patients with pleural mesothelioma was 14 to 15 months. There was a significantly increased survival for those patients with a performance status between 0 and 1 (median, 31–32 months) versus those with a performance status >1 (median survival, 7 months), for those with epithelial histology (median survival, 17 months) versus sarcomatous histology (median survival, 7 months), for those with an absence of chest pain (median survival, 24 months) versus those with chest pain (median survival, 16 months), and those with an interval >6 months from the onset of symptoms (median survival, 16 months) versus those with an interval of ≤6 months from the onset of symptoms (median survival, 13 months), and possibly a better survival for those patients treated with chemotherapy or pleuropneumonectomy.

Alberts et al.[1132] evaluated survival rates and prognostic factors in 262 patients diagnosed between 1965 and 1985 with pleural MM who were treated with chemotherapy only, radiotherapy only, radiotherapy and chemotherapy, or with decortication combined with chemotherapy and radiotherapy. The median survival for all patients from the time of diagnosis was 9.6 months, which was the same for all treatment groups. In a univariate analysis, favorable prognostic factors included good performance status, duration of symptoms >6 months at the time of diagnosis, early stage of disease, white race, and female gender. In a multivariate analysis, good performance status, white race, duration of symptoms, and stage of disease were significant favorable prognostic factors. The authors found that the stepwise addition of treatment modalities did not increase survival.

Ruffie et al.[1126] performed a retrospective study of 332 patients diagnosed with pleural MM between 1965 and 1984. The median survival was 9 months. Using univariate analysis, three factors were found to have a significant effect on survival: (1) disease stage: stage 1, median survival 16.6 months, versus stage IV, 1.4 months; (2) weight loss: no weight loss, median survival, 10.5 months, versus weight loss, median survival, 4.8 months; and (3) histologic type: epithelial or mixed median survival of 9.9 and 9.2 months, respectively, versus sarcomatous median survival of 5.2 months. The authors found there were no drastic differences in survival among groups of patients subjected to different therapeutic measures. Radical surgery and radiotherapy were found to be ineffective; there was a low response rate to chemotherapeutic agents.

Harvey et al.[1133] also performed a retrospective analysis on 94 patients with pleural MM treated at one institution between 1965 and 1988. Group I patients ($n = 76$) received supportive care only, including pleurodesis as needed. Group II patients ($n = 9$) were managed with debulking procedures including decortication and pleurectomy. Group III patients ($n = 7$) were treated by extrapleural pneumonectomy. Median survival in group I patients was 231 days. Four patients in group I survived more than 2 years, and one patient who was treated with chemotherapy and tangential field external beam irradiation survived more than 5 years. Group II patients had a median survival of 360 days, and none were alive at the end of 2 years. Four of seven group III patients expired within 6 months after treatment, although one patient died 7 years after therapy and one 36-year-old man was alive 8 years after diagnosis. The authors concluded that selected patients (seven young patients) benefit from radical surgery and that debulking may also extend survival.

Ribak and Selikoff[1134] studied the clinical course of 457 consecutive fatal cases of pleural and peritoneal MM that occurred among 17,800 asbestos insulation workers observed prospectively from January 1967 to January 1987. In the pleural mesotheliomas, mean survival time was 11.4 months and median survival time 10 months. The mean survival time in peritoneal mesothelioma was 7.4 months. The median survival time from diagnosis to death for patients with pleural mesotheliomas was 5 months and for peritoneal mesothelioma 2 months. The authors found no differences for survival time between various treatment modalities or between treated and untreated patients. The authors concluded that survival time in MM was short, most patients die within 1 year from the onset of symptoms, and no effective therapy for MM was available.

Tammilehto[1135] prospectively studied 98 patients with histologically proven MM, 93 pleural and five peritoneal, diagnosed between 1981 and 1990. Treatment consisted of surgery ($n = 15$); surgery and chemotherapy ($n = 11$); surgery and radiotherapy ($n = 14$); surgery, chemotherapy, and radiotherapy ($n = 28$); chemotherapy ($n = 3$); chemotherapy and radiotherapy ($n = 9$); radiotherapy ($n = 8$); and no treatment ($n = 10$). The median survival for all 98 patients calculated from the date of histologic diagnosis was 9 months with a range of 0 to 81 months. Eighteen patients were alive 2 years after diagnosis and two patients 5 years after diagnosis. By univariate analysis, good prognostic factors included age ≤65 years (11 months median survival versus 6 months median survival for those >65), female gender (13 months median survival versus 8 months for males), epithelial histology (median survival 14 months versus 2 to 5 months for sarcomatous histology), performance status WHO ≤1 (13 months median survival versus 3 months for WHO >1), stage I to IIA (11.5 months versus 5 months for stage IIB, III, and IV), and a diagnostic delay of more than 6 months from first symptom to histologic diagnosis (14.5 months median survival versus 8 months for diagnostic delay of ≤6 months). Low S-phase fraction was associated with a better survival (16 months median survival) than a high S-phase fraction (median survival of 8 months), although DNA ploidy had no effect. Lung tissue fiber content of $<10^6$ fibers per gram of dry lung tissue was associated with a median survival of 26 months whereas a concentration $\geq 10^6$ fibers per gram of dry lung tissue showed a median survival of 13 months. Factors by multivariate analysis

that were prognostically favorable included good performance status (WHO diagnostic delay of more than 6 months, epithelial histology, and clinical stage I or IIA). Although the patients who were treated with surgery, chemotherapy, or irradiation appeared to survive longer, this apparent increased survival was not significant when other factors were considered.

Sridhar et al.[1136] evaluated survival rates and prognostic factors in 49 patients with MM diagnosed between 1977 and 1991. The male-to-female ratio for patients with mesothelioma was 4:1, and the patients ranged in age between 36 and 77 years with a mean and median of 58 years. Asbestos exposure was identified in 75% of patients in whom a history was available. Most patients presented with Butchart stage 1 to 2 disease. Thirty-three patients were treated with a variety of combinations of chemotherapeutic agents, 14 were treated by various surgical modalities, and 10 patients received some type of radiation therapy. The median time from first symptom to diagnosis was 3 months. The median survival for pleural mesotheliomas was 13 months, and 15 months for peritoneal mesotheliomas from the onset of first symptom. Survival was longer in patients with earlier stage disease, a good performance status, a longer duration of symptoms, an absence of pain, and those who were treated with combined surgery and chemotherapy.

Pistolesi and Rusthoven[113] reviewed pleural MM, including current management and new therapeutic options. They stated that the stage of the disease was but one of the known variables that might influence survival. Two prognostic scoring systems were stated to have been developed for evaluating pleural MM on data collected from patients entered into large cooperative trials. Multivariate Cox analysis of a variety of variables (performance status, chest pain, dyspnea, platelet count greater than 400,000 per microliter, weight loss, serum lactate dehydrogenase level greater than 500 IU/L, pleural involvement, low hemoglobin level, high white blood cell count, and age greater than 75) demonstrated that pleural involvement, lactate dehydrogenase greater than 500 IU/L, poor performance status, chest pain, platelet count greater than 400,000 per microliter, nonepithelial histology, and age greater than 75 were independent predictors of reduced survival. Performance status was stated to have produced the most significant prognostic split. Six distinct prognostic subgroups were identified, with survival times ranging from 1.4 to 13.9 months. The best survival time was in patients less than 49 years of age with a performance status of 0 and a hemoglobin of 14.6 g/dL. The worst survival time was in patients with a performance status of 1 or 2 and a white blood cell count of greater than 15,600 per microliter. See Box 43.9 for a summary of prognostic factors.

Curran et al., of the European Organization for Research and Treatment of Cancer (EORTC)[1137] evaluated 13 factors via Cox proportional hazard regression model. Poor prognosis was stated to have been associated with a poor performance status, a high white blood cell count, a probable/possible histologic diagnosis of mesothelioma, male gender, and sarcomatoid histologic subtype. The EORTC classified patients into two prognostic groups: a good prognostic group (1-year survival of 40% having two or fewer poor prognostic factors) and a poor prognostic group (1-year survival of 12% having three or more poor prognostic factors).

Box 43.9. Prognostic Factors
(Expected Survival: 1.4 to 13.9 Months)

Independent predictors of reduced survival
(3 or more of the following)
Age 75 or older
Performance status 1 or 2
Nonepithelial histology or sarcomatoid subtype
Pleural involvement
Chest pain
Platelet count greater than 400,000 per microliter
WBC greater than 15,600 per microliter
Lactate dehydrogenase greater than 500 IU/L
Independent predictors of increased survival
Age 49 or younger
Performance status of 0
Hemoglobin of 14.6 g/dL

Among treatment modalities, radiation was stated to have been shown to have palliative benefit in reducing pain and symptoms of dyspnea. Surgical pleurodesis was stated to have reduced symptoms associated with recurrent or persistent pleural effusions. Chemotherapy was stated to have demonstrated palliative benefits in overall quality of life. Pistolesi and Rusthoven[113] concluded that treatment of pleural MM with more than palliative intent remained inadequate at all stages of presentation. Surgery, as a single modality, was stated to have failed to improve survival. Chemotherapy was stated to have generally failed to significantly impact survival.

Pistolesi and Rusthoven[113] discussed three procedures that are used in surgical management of pleural MM, including thoracoscopy with pleurodesis, pleurectomy/decortication, and extrapleural pneumonectomy. Thoracoscopy was stated to be useful not only for obtaining tissue for a diagnosis, but also for palliating recurrent symptomatic pleural effusions. Talc was stated to be the least expensive and could be administered via thoracoscope or instilled as a slurry through a chest tube. The authors stated that although often attempted with curative intent, neither extrapleural pneumonectomy nor pleurectomy/decortication appeared to offer a significant improvement in survival. The authors cited the Brigham and Women's Hospital Tri-Modality therapy. Those who survived surgery achieved a 2-year and 5-year survival rate of 38% and 15%, respectively.

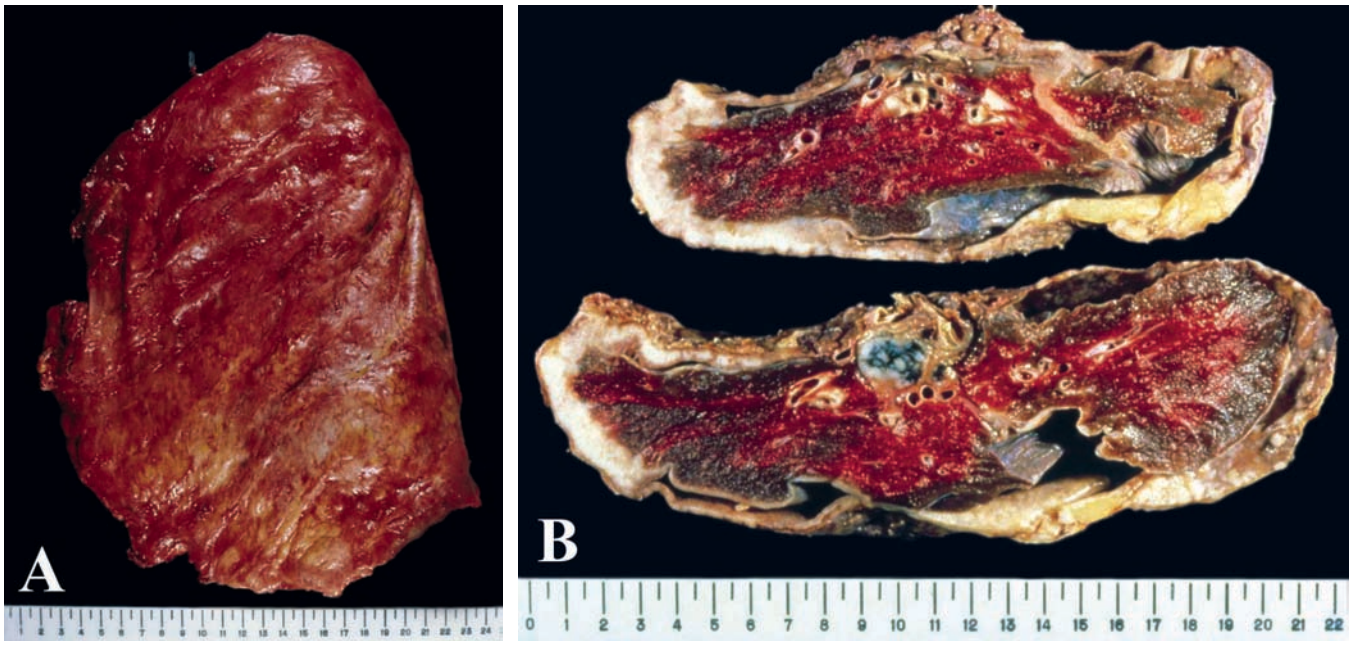


FIGURE 43.198. **(A)** Pleuropneumonectomy specimen resected from a patient with stage 1 epithelial mesothelioma. **(B)** Two cross-sectioned portions of lung and pleura are shown. Note the lack of complete encasement of the lung. Also note the whitish tissue within the hilar lymph node; this represents metastatic mesothelioma.

Reviews of radiation therapy were stated by Pistolesi and Rusthoven[113] to show no suggestion of a clear survival benefit for extensive radiation therapy. They stated that a report from the Joint Center for Radiation Therapy in Boston suggested a minimum effective dose of 40 Gy in order to achieve palliation.

With respect to chemotherapy, Pistolesi and Rusthoven[113] stated that most single agents that have been tested in malignant pleural mesothelioma have had response rates less than 20%, and survival benefit for single-agent chemotherapy has not been suggested in a single cohort study. A common combination of chemotherapy agents used at the present time is pemetrexed (Alimta®) and cisplatin. A response rate of about 42% has been reported.[113] Pistolesi and Rusthoven also discussed novel therapies for the treatment of mesothelioma. At this point in time, it is difficult to know whether these will be of any significance.

A more recent study from the Sugarbaker International Mesothelioma Group[1138] found a 5-year survival rate of 55% of those patients with anatomic stage 1 disease and epithelial histology. A typical pleuropneumonectomy specimen in shown in Figure 43.198. Note the extent of tumor and the rind of tumor that encases the lung. Also note that, in areas, the visceral and parietal pleura are not fused. Also note that in this case there is metastatic tumor in a hilar lymph node.

Takagi et al.[1139] reported on the surgical approach to diffuse pleural MM in Japan. They evaluated 189 surgical cases of diffuse MM between 1987 and 1996. The patients ranged between 18 and 80 years old and 154 were males, 33 were females and 2 were unspecified; 104 patients had an epithelial histology, 29 had a sarcomatous histology, and 46 had a biphasic histology. Pleuropneumonectomy was performed on 116 cases (61%) and limited resection was performed in 73 cases (39%). The goal of radical pleuropneumonectomy was stated to be radical resection of the tumor, which often required resection of adjacent structures. The tumor was stated to have been completely removed macroscopically in 84 cases (72%) of the 116 patients who underwent pleuropneumonectomy. Among those who had an epithelial mesothelioma that was completely removed by pleuropneumonectomy, the tumor recurred postoperatively in 43% of patients. Perioperative adjuvant therapy was performed in 83 of 116 patients who underwent pleuropneumonectomy. The 2-year and 5-year survival rates of those who underwent pleuropneumonectomy was 29.7% and 9.1%, respectively. The perioperative mortality was 6%.

Pass et al.[1140] analyzed the impact of preoperative and post-resection solid tumor volumes on the outcomes in 47 of 48 consecutive patients undergoing resection for pleural MM who were treated prospectively and randomized to photodynamic therapy or no photodynamic therapy. Forty-eight patients with pleural MM had cytoreductive debulking to 5 mm or less residual tumor by extrapleural pneumonectomy (n = 25) or pleurectomy/decortication (n = 23). Three-dimensional CT reconstructions of preresection and post-resection solid tumor were prospectively performed and the disease was staged postoperatively according to the new IMIG/AJCC staging. Median survival for all patients was 14.4 months (extrapleural

pneumonectomy 11 months; pleurectomy/decortication 22 months). Median survival for preoperative volume less than 100 cc was 22 months versus 11 months if 100 cc or greater. Median survival for postoperative volume less than 9 cc was 25 months versus 9 months if there were 9 cc or greater. Tumor volumes associated with negative nodes were stated to be significantly smaller than those with positive nodes. The authors concluded that pre-resection tumor volume was representative of T status in pleural MM and could predict overall progression-free survival as well as postoperative stage. Large volumes were associated with nodal spread and post-resection residual tumor burden could predict outcome.

Edwards et al.[1141] evaluated the significance of tumor necrosis in cases of MM. They reviewed 171 routine formalin-fixed, paraffin-embedded, H&E-stained tumor sections by two independent observers. Angiogenesis was stated to have been assessed by microvessel count (MVC) using CD34 immunostained sections. Tumor necrosis correlated with survival by Kaplan-Meier and log rank analysis. Stepwise multivariate Cox models were used to compare tumor necrosis with angiogenesis and establish prognostic factors and prognostic scoring systems. Tumor necrosis was stated to have been identified in 39 cases (22.8%) and correlated with low hemoglobin level, thrombocytosis, and high microvessel counts, and was a poor prognostic factor in univariate analysis. Patients with tumor necrosis had a median survival of 5.3 months versus 8.3 months in cases without necrosis. Independent indicators of poor prognosis in multivariate analysis were nonepithelioid cell type, poor performance status, and increasing microvessel counts, but not tumor necrosis. Tumor necrosis contributed independently to prognosis according to the EORTC and to the Cancer and Leukemia Group B prognostic groups. Tumor necrosis correlated with angiogenesis and was stated to be a poor prognostic factor in MM.

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