

The Separation of Benign and Malignant Mesothelial Proliferations

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● **Context.**—The separation of benign from malignant mesothelial proliferations is crucial to patient management but is often a difficult problem for the pathologist.

Objective.—To review the pathologic features that allow separation of benign from malignant mesothelioma proliferations, with an emphasis on new findings.

Data Sources.—Literature review and experience of the authors.

Conclusions.—Invasion is still the most reliable indicator of malignancy. The distribution and amount of proliferating mesothelial cells are important in separating benignity from malignancy, and keratin stains can be valuable because they highlight the distribution of mesothelial cells. Hematoxylin-eosin examination remains the gold standard, and the role of immunohistochemistry is extremely controversial; we believe that at present there is no reliable immunohistochemical marker of malignancy in this set-

ting. Mesothelioma *in situ* is a diagnosis that currently cannot be accurately made by any type of histologic examination. Desmoplastic mesotheliomas are characterized by downward growth of keratin-positive spindled cells between S100-positive fat cells; some cases of organizing pleuritis can mimic involvement of fat, but these fatlike spaces are really S100-negative artifacts aligned parallel to the pleural surface. Fluorescence *in situ* hybridization on tissue sections to look for homozygous p16 gene deletions is occasionally useful, but many mesotheliomas do not show homozygous p16 deletions. Equivocal biopsy specimens should be diagnosed as atypical mesothelial hyperplasia and another biopsy requested if the clinicians believe the process is malignant.

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The separation of benign and malignant mesothelial proliferations is crucial to patient management, but for the pathologist this distinction can be exceedingly difficult and even experts in the field frequently cannot come to a consensus on a given case. In 2000, the US-Canadian Mesothelioma Reference Panel published a detailed review on the topic,¹ and noted that in 22% of the cases circulated to the whole panel, there was a disagreement about whether the underlying process was benign or malignant. Similarly, for Group Mesopath (the French equivalent of the US-Canadian Panel), of 97 problem cases circulated to everyone on the panel there was disagreement in 47% on whether the process was benign or malignant. While we still see numerous cases in consultation in which the question asked is whether a clearly malignant tumor is a mesothelioma or not, the issue of whether a mesothelial proliferation is benign or malignant is now the most frequent question in the cases circulated to the whole US-Canadian Panel.

Detailed reviews on benign versus malignant mesothelial proliferations are available in the literature.^{1–3} This article will revisit some of the criteria set out in those publications, to see whether they still are valid or need modification, and will emphasize new and controversial areas.

CLINICAL FEATURES

Clinical features are often very valuable in sorting out benign and malignant processes, but our observation from consultation material is that pathologists frequently are not provided such information and do not ask for it. Patients with abnormal mesothelial proliferations usually have a pleural effusion or ascites, and the fluid is frequently hemorrhagic. The latter is a useful finding because of the limited causes of a hemorrhagic pleural effusion (Table 1), and malignancy always heads the list. However, patients with asbestos exposure can have so-called benign asbestos effusions, which are often hemorrhagic, so care must be taken in interpreting this finding.

More useful is the description of the pleura (peritoneum, hydrocele) on imaging or pleuroscopy (laparoscopy). The story of circumferential pleural thickening involving the mediastinal pleura on computed tomography scan is strongly suggestive of malignancy, and nodular pleural thickening is usually malignant. These findings can be very helpful in deciding whether a suspicious proliferation is really malignant. Similarly, knowledge that the pleuroscopist has seen tumor nodules can save hours of worry in equivocal cases, but this piece of information is frequently not forthcoming unless specifically requested. Conversely, if

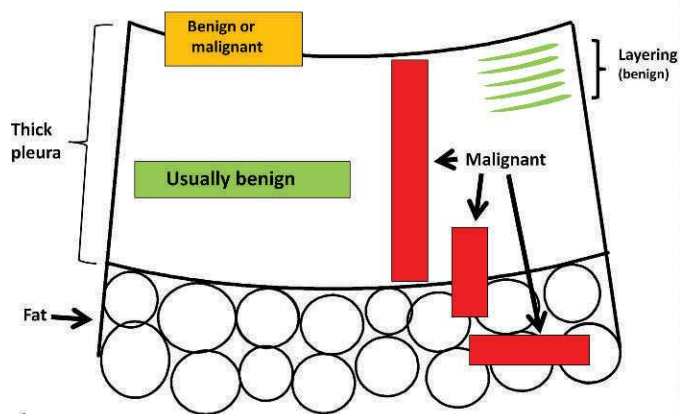
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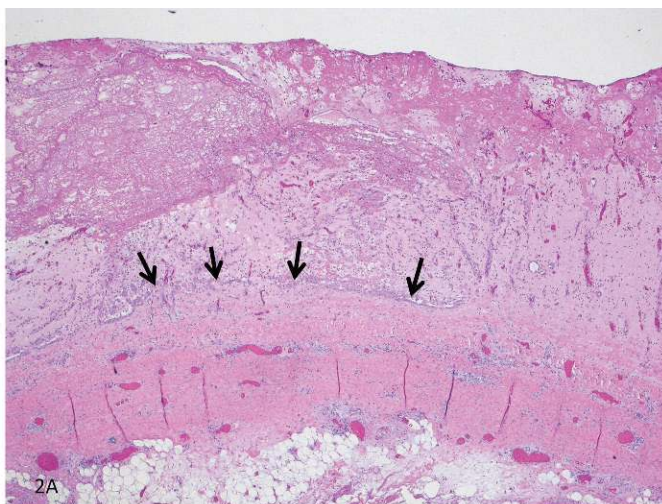
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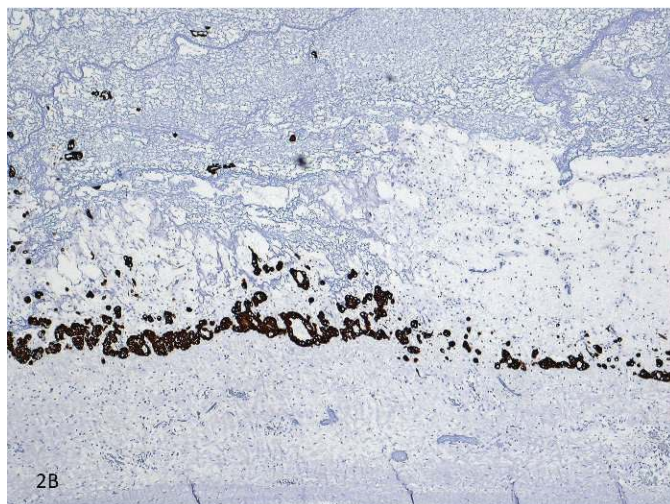
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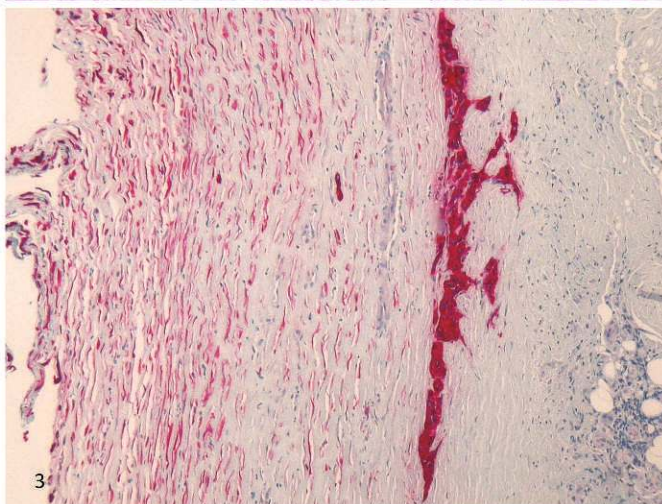
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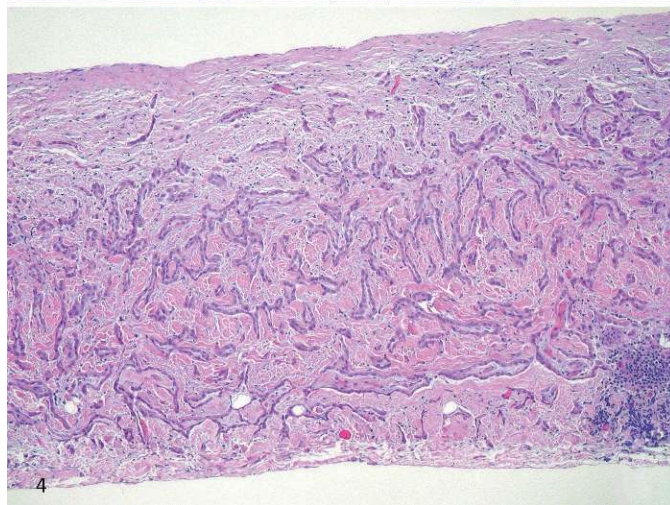
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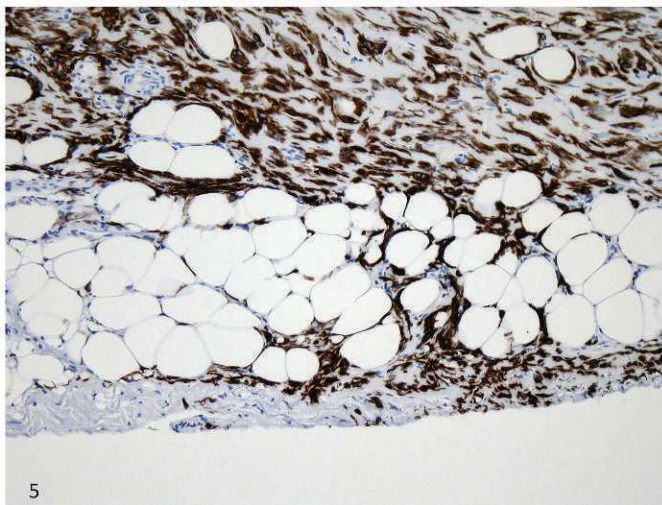
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Figure 1. Schematic diagram showing benign versus malignant processes as a function of the distribution of mesothelial cells in a thickened pleura. This same scheme applies to the pericardium and to hydrocele sacs, and with more difficulty, to peritoneal biopsies where orientation is often problematic. Figure 3 shows an example of layering.

Figure 2. A, Full-thickness view of a markedly thickened pericardium from a young adult with postviral pericarditis and pleuritis. The pericardial space is to the top of the field. There is a linear array of individual mesothelial cells and simple glands (arrows) at the junction of denser fibrous tissue and looser organizing connective tissue/fibrin. B, Pankeratin stain highlights the linear array. This appearance is seen in benign organizing effusions, and the linear array probably represents the original surface line of the pericardium (hematoxylin-eosin, original magnification ×50 [A]; original magnification ×50 [B]).

Figure 3. Mesothelial layering in a hydrocele sac. The lumen is to the left. This type of layering represents repeated effusions with proliferation of surface mesothelial cells and subsequent organization. The darker-staining mesothelial cells to the right probably represent the original lining layer of the sac.

Table 1. Causes of a Hemorrhagic Pleural Effusion

Malignancy
Infection (especially TB)
Pulmonary embolism/infarction
Trauma
Pneumothorax
Benign asbestos effusion

Abbreviation: TB, tuberculosis.

the pleuroscopist says that the pleura looks benign, one should be extremely cautious before diagnosing a mesothelioma. Occasionally, early mesotheliomas can be very “thin” and not show up on gross inspection, but that is not a frequent event.

DISTRIBUTION OF PROLIFERATING MESOTHELIAL CELLS AND MICROSCOPIC “BULK” TUMOR

Figure 1 is a drawing of a thickened pleura with various distributions of proliferating mesothelial cells outlined. A similar scheme can be applied to the pericardium and hydroceles, and sometimes to the peritoneum, but orientation is frequently difficult in the peritoneum and there is much less tendency to end up with a thick fibrous peritoneum than a thick pleura, pericardium, or hydrocele.

Epithelial mesothelial proliferations that are confined to the surface can be benign or malignant; proliferations that reach from the free surface to the fat of a *considerably thickened* pleura are usually malignant, and those that invade the fat are always malignant. Interestingly, lines of mesothelial cells or simple glands arrayed parallel to the pleural (pericardial, hydrocele) surface and located deep in a thickened pleura (Figures 1; 2, A and B; and 3) are usually benign; they typically represent the original surface of the pleura (pericardium, hydrocele), which has now been buried by organization of an overlying effusion. A more florid example of the same process is layered lines of mesothelial cells aligned parallel to the pleural surface (Figures 1 and 3); these represent repeated cycles of organization, followed by growth of a new mesothelial layer, followed by further surface organization.

The number of proliferating mesothelial cells is also a helpful but much more subjective measure. This is not an issue when a 1-cm biopsy specimen is completely filled by mesothelial cells, but the cutoff from “obvious” tumor to “suspicious” is not sharp, and is particularly problematic in small biopsy specimens where separating invasion from en face cuts may not be possible. The problem is not dissimilar to that encountered in fluid cytology, where the number of atypical groups or individual cells plays a role in diagnosis. Figure 4 shows a mesothelial proliferation that runs from one end of a medium power field to the other and from the pleural surface to the junction with fat. This process is malignant by distribution of mesothelial cells (pleural surface to chest wall of a greatly thickened pleura, and

linear extent along the direction of the pleura) and number of mesothelial cells.

INVASION OF THE STROMA VERSUS ENTRAPMENT AND EN FACE CUTS

What the preceding paragraphs are really discussing is invasion of the stroma, and invasion of the stroma continues to be by far the most reliable criterion for separating benign from malignant mesothelial proliferations.^{1–3} Fat is the stroma most frequently encountered and the finding of mesothelial cells in fat makes the proliferation malignant unless there is an extraordinarily good reason to believe otherwise. The same comment applies to invasion of muscle or invasion of lung or another organ.

All active mesothelial proliferations, whether benign or malignant, are pankeratin positive, and pankeratin stains are extremely helpful in showing the distribution of mesothelial cells. They are particularly valuable for detecting subtle invasion of fat by a few cells that may not be readily apparent on hematoxylin-eosin (H&E) staining (Figure 5); however, care should be taken that the “invading” cells are really in the tissue because occasionally artifactual “carry” can mimic invasion (Figure 6).

A mesothelial proliferation extending through the whole thickness of a greatly thickened pleura is really a form of stromal invasion (Figure 4). Another variant is the formation of expansile nodules of stroma (Figure 7); these can be found within both epithelial mesotheliomas and desmoplastic mesotheliomas (DMMs). They may contain relatively few mesothelial cells, but benign processes do not make stromal nodules. However, one confounder is the formation of nodules of fat surrounded by mesothelial cells in the peritoneal cavity; these are common in inflammatory process, but they are not stromal nodules and are not malignant unless the mesothelial cells invade the fat.

Elastic stains are not usually performed in this setting, but we have seen some equivocal cases in which mesothelial cells transgress the elastic layer of the visceral and parietal pleura. This might be an indicator of malignancy, although interpretation is not simple because there can also be elastic duplication in reactive processes. This issue needs further study, but we suggest that if there is transgression of the elastica in a case that is suspicious for mesothelioma, deeper sections be performed or another biopsy proposed.

An important issue in this setting is whether one is dealing with invasion or entrapment. Entrapment of mesothelial cells is common and can occur with any type of inflammatory reaction. The inflammation in turn appears to drive mesothelial cell proliferations and these can be cytologically quite atypical (see below); a good rule of thumb is to be exceedingly cautious in diagnosing a mesothelioma in the midst of an active inflammatory process.

Reactive lymphoid proliferations are commonly seen in the pleura in conjunction with all types of mesothelial

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the hydrocele sac. Note the sharp circumscription (lack of invasion) of the process, another sign that one is dealing with a benign process (pankeratin, original magnification ×100).

Figure 4. Malignant mesothelioma in a greatly thickened pleura. The lumen is to the top. The extent of the proliferation, full thickness top to bottom and from left to right, indicates that this is a malignant process (hematoxylin-eosin, original magnification ×75).

Figure 5. Invasion of chest wall fat demonstrated on pankeratin staining. Invasion of fat by mesothelial cells is always a sign of malignancy unless there is some extraordinarily good reason to believe otherwise. Keratin stains are useful for picking up subtle invasion that may be hard to detect with hematoxylin-eosin (original magnification ×200).

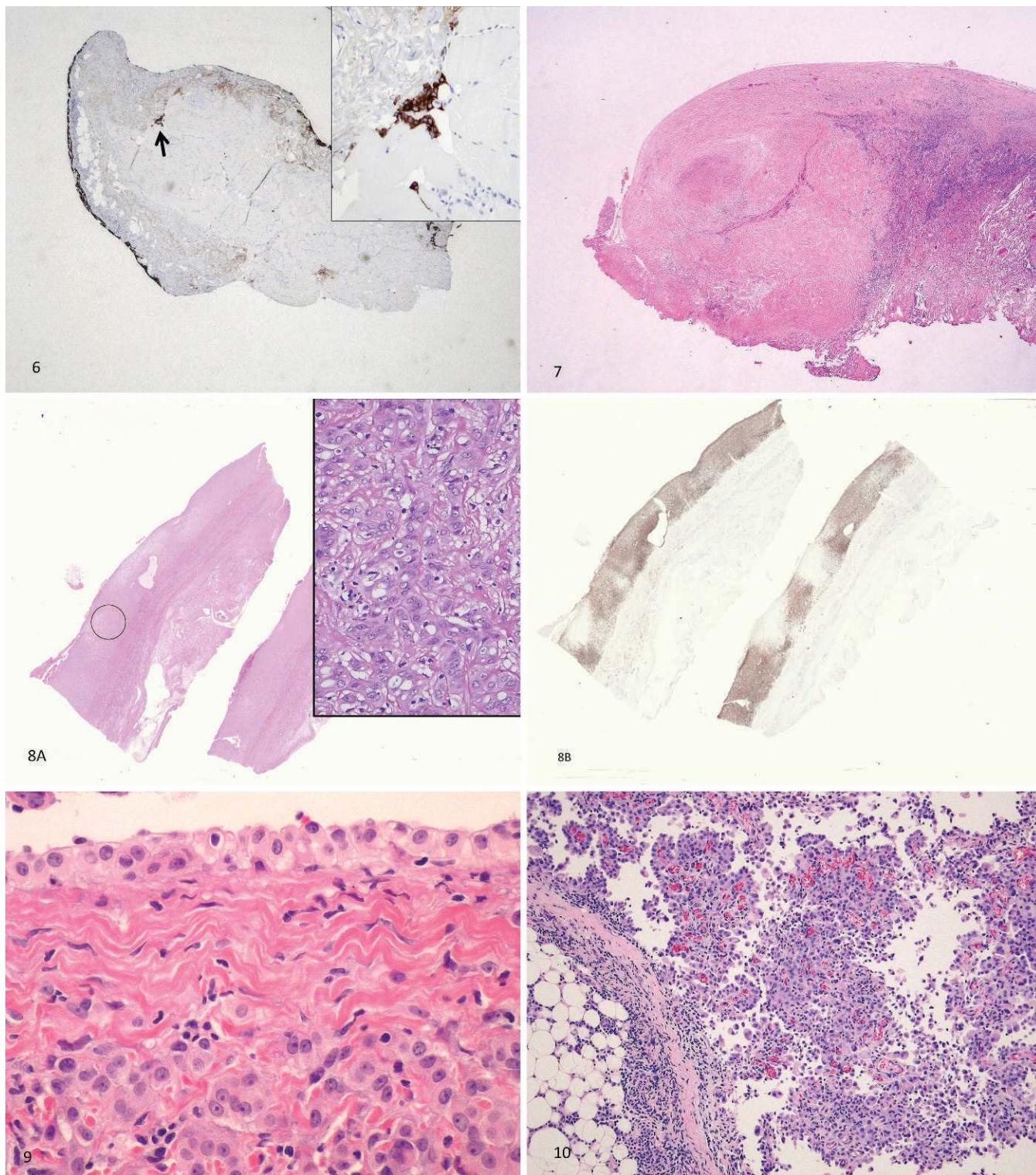


Figure 6. Spurious invasion ("carry"). Keratin stain of this pleural biopsy shows that the mesothelial cells are nicely confined to the surface and there is no fat invasion. However, there is an apparently positive isolated focus deep in the tissue (arrow); on higher power (inset) this is clearly carry (original magnifications $\times 20$ and $\times 200$ [inset]).

Figure 7. Nodular stromal expansion in a desmoplastic mesothelioma. Nodule stromal expansions are a sign of malignancy and can be seen with both epithelial and desmoplastic mesotheliomas (hematoxylin-eosin, original magnification $\times 20$).

Figure 8. A, Low-power view of a greatly thickened hydrocele sac. The area in the circle is shown at higher power in the inset, and by pattern and cytologic appearance raises the question of mesothelioma. B, Pankeratin stain demonstrates sharp circumscription, indicating that the process is benign (hematoxylin-eosin, original magnifications $\times 20$ [A] and $\times 200$ [A, inset]; original magnification $\times 20$ [B]).

Figure 9. Invasive mesothelioma below and identical cells forming a line on the pleural surface, that is, they constitute mesothelioma in situ. Note

Table 2. Causes of Necrosis in Mesothelial Proliferations

Malignant tumor
Empyema
Mycobacterial and fungal infections
Talc pleurodesis

proliferations, but a dense lymphocytic infiltrate with entrapment of mesothelial cells should raise the question of low-grade lymphoma or chronic lymphocytic leukemia, and appropriate immunochemical and molecular workup may be indicated.

The linear arrays and layered arrays described above (Figures 2 and 3) are a form of entrapment in which the inflammatory process is usually no longer evident. A helpful hint in circumstances in which there are proliferating mesothelial cells but no inflammation is the distribution of mesothelial cells, and this is particularly well shown with keratin stains. Benign processes tend to be sharply circumscribed, with a few glands evident beneath the pleural surface, or with a sharp line beyond which no mesothelial cells are found (Figures 2; and 8, A and B), whereas mesotheliomas are always invasive.

In small biopsy specimens, it may not be possible to tell whether one is dealing with invasion, entrapment, or merely in face cuts of the surface. Because of the treatment and prognosis associated with a diagnosis of mesothelioma, we suggest that worrisome proliferations that are not unequivocally malignant simply be called *atypical mesothelial hyperplasia* or *atypical mesothelial proliferation*, with a comment that another biopsy may be appropriate if the specimen is clinically suspicious.

NECROSIS

Necrosis is usually an indicator of malignancy in mesothelial proliferations. However, it can occasionally be seen in bacterial empyemas (where the necrotic tissue typically is made up of inflammatory cells with relatively few mesothelial cells), tuberculous and fungal infections in the pleura, and as a reaction to talc poudrage (Table 2). Talc can also induce cytologically worrisome mesothelial reactions, so considerable caution should be exercised in diagnosing (de novo) a mesothelioma after talc instillation.

MESOTHELIOMA IN SITU

There is every reason to believe that mesotheliomas must start with an in situ phase, and identification of mesothelioma in situ (MIS) would in theory allow for curative therapy. Processes that have been proposed as MIS include a single layer of atypical mesothelial cells along the pleural surface,⁴ and more complicated heaped-up collections of mesothelial cells along the pleural surface, either as solid sheets or papillary structures.

Occasionally, one can find single layers of cells on the surface and identical cells invading the underlying tissue (Figure 9), and the surface phase thus qualifies as MIS,

Table 3. Desmoplastic Mesothelioma Versus Organizing Pleuritis

Organizing Pleuritis	Desmoplastic Mesothelioma
Cellularity greatest under effusion and decreases away from effusion (zonation)	No zonation
No stromal invasion (but fibrous tissue may develop in fat along with small vessels)	Stromal invasion into fat, muscle, lung
Cells immediately under effusion often very atypical	Atypia often hard to discern
Capillaries perpendicular to surface	Capillaries inconspicuous
Usually no necrosis	Bland necrosis
No sarcomatous foci	Sarcomatous foci
No nodular stromal expansions	Nodular stromal expansions

although whether this is the original mesothelioma in situ, or simply an area where tumor cells have accessed the pleural surface and grown along it, is not resolvable. These surface proliferations are often remarkably bland (Figure 9), such that cytologic atypia cannot be counted on. Complex papillary proliferations (Figure 10) on the surface are very worrisome, and some patients, by follow-up, turn out to have or develop mesotheliomas, but others with similar patterns do not (Figure 10).

The consensus at present is that there are no reliable rules for separating MIS from reactive processes on routine staining, and at this point there are no immunostains or molecular techniques that will solve the problem. We strongly advise against diagnosing MIS and suggest that such cases be signed out as atypical mesothelial hyperplasia.

ORGANIZING PLEURITIS VERSUS DESMOPLASTIC MESOTHELIOMA

The preceding discussion has largely focused on epithelial mesothelial proliferations. Spindle cell mesothelial proliferations fall into 3 categories: organizing pleuritis (OP, also called *fibrous pleurisy* or *fibrosing pleurisy*), DMMs/sarcomatous mesotheliomas, and atypical mesothelial proliferations (atypical mesothelial hyperplasia), used for lesions worrisome for, but not diagnostic of, DMM or sarcomatous mesotheliomas.

Sarcomatous mesotheliomas are usually easy to diagnose, but DMMs are often problematic. The histologic features of DMMs have been reviewed elsewhere^{1,3,5} and are summarized in Table 3. Desmoplastic mesotheliomas are paucicellular processes that at low power look like scars or organizing pleuritis (Figure 11, A). Desmoplastic mesotheliomas may form expansile stromal nodules (Figure 7), a phenomenon that is not seen in OP.

At higher power, DMMs characteristically show a short storiform pattern ("patternless pattern"; Figure 11, B), but this is not specific and can be seen in OP, nor is it present in every case of DMM. As is true of epithelial proliferations, invasion of the stroma (usually fat [Figure 11, C], muscle, or lung) is by far the most reliable indicator of malignancy.

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the remarkable blandness of the surface process. The diagnosis of mesothelioma in situ, absent invasive tumor, is unreliable and should not be made (hematoxylin-eosin, original magnification ×200).

Figure 10. Complex surface mesothelial proliferation. Complex surface proliferations cannot be reliably separated into benign and malignant on histologic grounds. This example was benign on follow-up (hematoxylin-eosin, original magnification ×75).

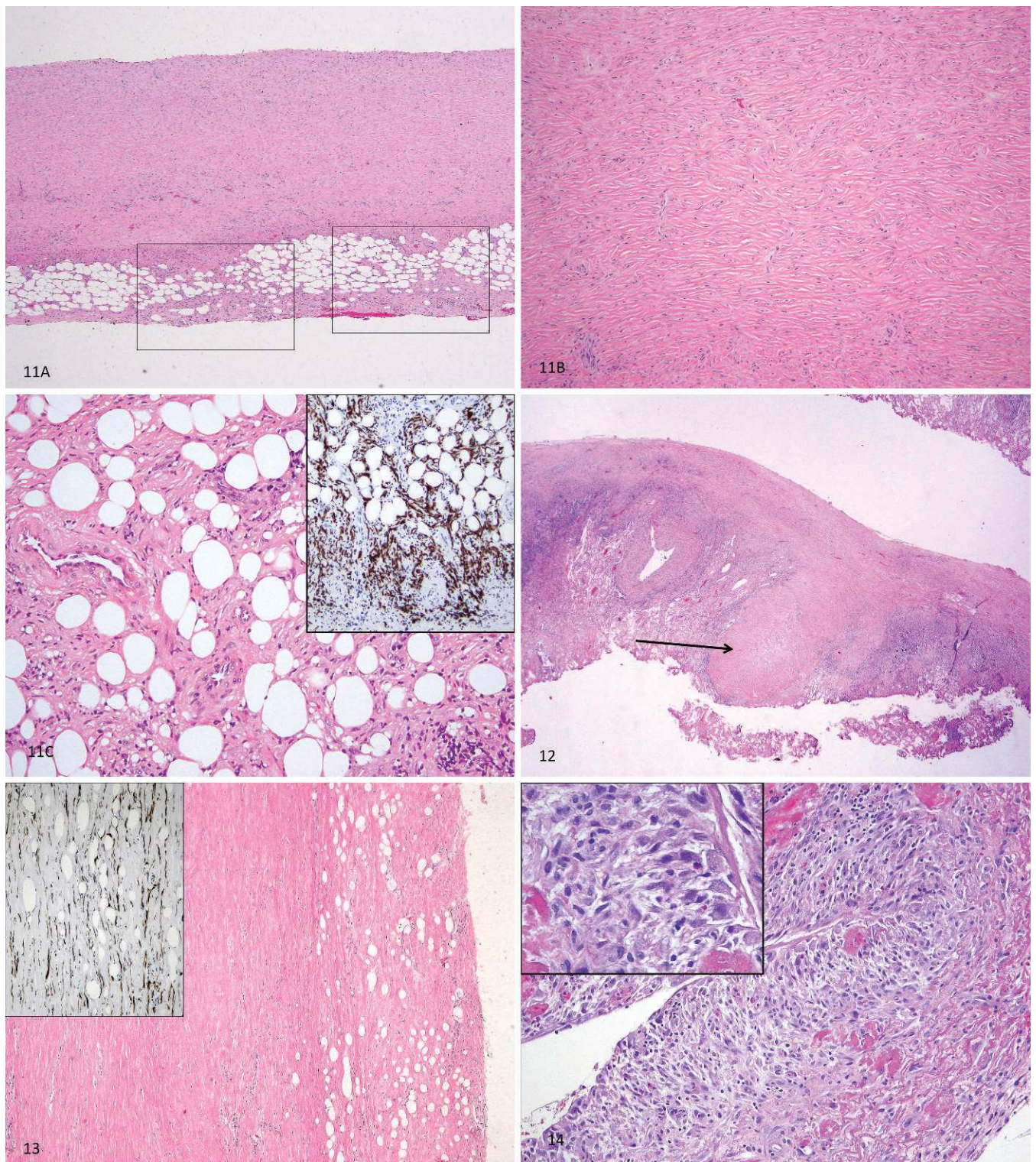


Figure 11. A, A thin desmoplastic mesothelioma. At low power this appears to be organizing pleuritis, but the areas of cellular processes involving the fat (boxes) are worrisome and need to be carefully examined. B, Higher-power view of the fibrotic portion, showing a short storiform pattern with ropey collagen. This finding is typical of desmoplastic mesotheliomas but is sometimes seen in organizing pleuritis. C, High-power view of another area of fat invasion. Keratin-positive spindle cells (inset) course down through the fat, indicating that this is a desmoplastic mesothelioma (hematoxylin-eosin, original magnifications $\times 50$ [A] and $\times 200$ [B and C]; original magnification $\times 100$ [C, inset]).

Figure 12. Another thin desmoplastic mesothelioma. The visceral pleura is slightly thickened by tumor but could be easily passed off as benign; however, the markedly thickened and fibrotic interlobular septum (arrow) represents invasion of the lung and indicates that this is a desmoplastic mesothelioma (hematoxylin-eosin, original magnification $\times 20$).

Figure 13. The fake fat phenomenon in organizing pleuritis. An array of spaces, which at first glance look like fat, is aligned along the chest wall side (to the right) of a fibrotic pleura, parallel to the pleural surface. Keratin-positive mesothelial cells (inset) pass between these spaces, mimicking

Desmoplastic mesotheliomas often show so-called bland necrosis, an area of stroma or fat that loses nuclei without any inflammatory reaction. Desmoplastic mesotheliomas may have overtly sarcomatous foci. Occasionally, DMMs present with distant metastases.

Desmoplastic mesotheliomas typically produce a markedly thickened pleura; however, occasionally, DMMs are thin (Figures 11 and 12) and can be all too easily passed off as OP. If the case is suspicious, evidence of a spindle cell process growing into fat or into lung should be looked for (Figures 11 and 12), and if a fairly large biopsy sample has been taken, the whole specimen should be processed for histology because only a few fields may be diagnostic. "Fibrous" expansions of pulmonary interlobular septa that connect up to a thickened pleura are suggestive of DMM (Figure 12) because very few processes in the lung cause fibrosis of the interlobular septa. Desmoplastic mesotheliomas can also produce patterns that look like bronchiolitis obliterans organizing pneumonia when they invade lung.

Keratin stains are very helpful in diagnosing DMMs because they typically show spindled cells running downward (ie, away from the pleural surface) into fat (Figure 11, A and C). Care should be taken to ensure that what appears to be fat really is fat. Old paucicellular OP may show deep, fatlike spaces running parallel to the pleural surface, with keratin-positive cells between the "fat" cells (Figure 13). In a recent report,⁶ we have shown that this phenomenon, which we call *fake fat*, is really a biopsy/tissue processing artifact and the spaces are not fat but rather, traction artifacts in a fibrotic stroma; by contrast, true DMMs are both more cellular and have *downward* growth of spindle cells between fat cells (Figure 11, A and C) rather than growth parallel to the pleural surface. S100 stains can be helpful because true fat is S100 positive and artifactual spaces are not.

Table 3 contrasts DMM and OP. As opposed to DMM, OP usually shows zonation, that is, it is more cellular immediately under the effusion and progressively less cellular and more fibrotic away from the effusion (Figure 14). Fibrin appears to provoke atypical and sometimes bizarre mesothelial reactions, and if there is an active effusion with fibrin present, the surface cells in OP can be remarkably pleomorphic (Figure 14), but such processes still show zonation. Desmoplastic mesotheliomas and sarcomatous mesotheliomas do not produce zonation.

Organizing pleuritis can also extend into (real) chest wall fat (Figure 15, A and B). In general, OP in fat is virtually acellular or shows only a few capillaries and inflammatory cells, as opposed to the much more cellular spindle cell proliferations of DMM. Occasionally, it can be difficult on H&E to be sure what process is occurring within fat and here keratin stains are again helpful (Figure 15, B) because in OP, keratin-positive cells are not present in fat.

ROLE OF IMMUNOHISTOCHEMISTRY

One attempt to resolve the problem of benignity versus malignancy has involved immunohistochemical staining,

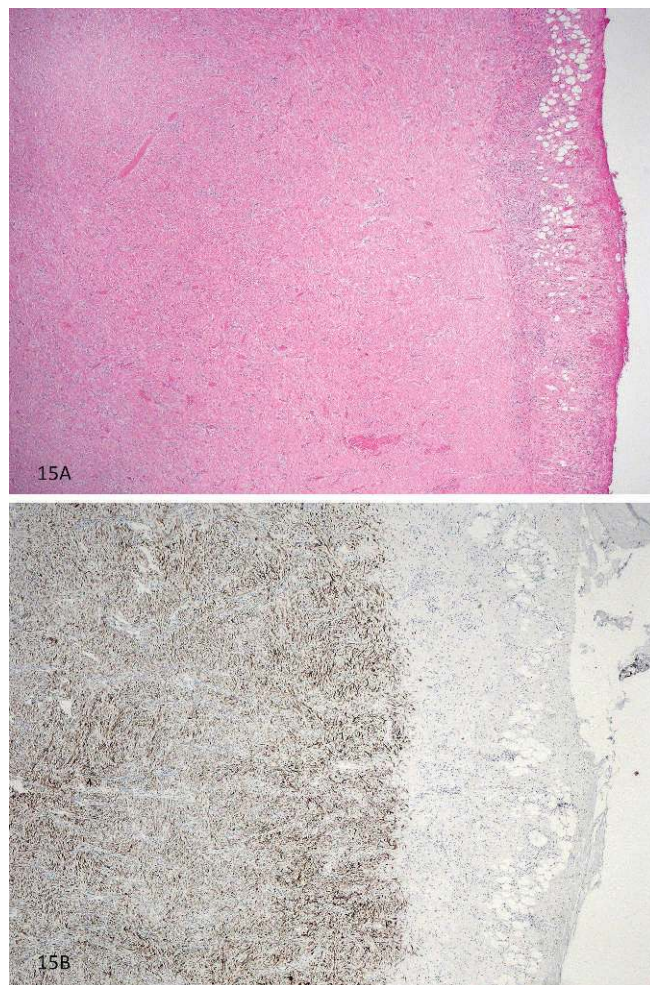


Figure 15. Organizing pleuritis post empyema. A, At first glance this process is worrisome for a desmoplastic mesothelioma because there appears to be a cellular process in the chest wall fat. B, Pankeratin stain shows sharp circumscription of the proliferating mesothelial cells with no keratin-positive cells in the fat, supporting a benign diagnosis (hematoxylin-eosin, original magnification $\times 20$ [A]; original magnification $\times 20$ [B]).

but the role of immunohistochemistry in this situation is very controversial. A variety of markers have been reported to be of use in this setting (Table 4). Among these, p53^{7,8} and epithelial membrane antigen⁹ are claimed to be indicators of malignancy, while desmin is claimed to mark benign mesothelial cells.⁹ King et al¹⁰ have provided a summary of the literature to 2006 and found that the reported specificity of desmin is 83%; of epithelial membrane antigen, 89%; and of p53, 91%; they nonetheless concluded that H&E stains were more reliable than immunohistochemical stains.

At first glance these numbers sound quite good and it is certainly true that, if a marker provides 90% specificity for

desmoplastic mesothelioma. However, desmoplastic mesotheliomas always invade down, as in Figure 11. These spaces are traction or cutting artifacts and some contain pale-staining ground substance (hematoxylin-eosin, original magnification $\times 50$; original magnification $\times 50$ [inset]).

Figure 14. Organizing pleuritis with marked cytologic atypia of the surface cells. Note that the process still shows zonation with loss of the proliferating surface cells and increasing collagen as one moves away from the pleural surface. Inset, Higher-power view of the atypical surface cells (hematoxylin-eosin, original magnifications $\times 100$ and $\times 200$ [inset]).

Table 4. Immunohistochemical Markers Claimed in the Literature to Separate Benign From Malignant Mesothelial Proliferations

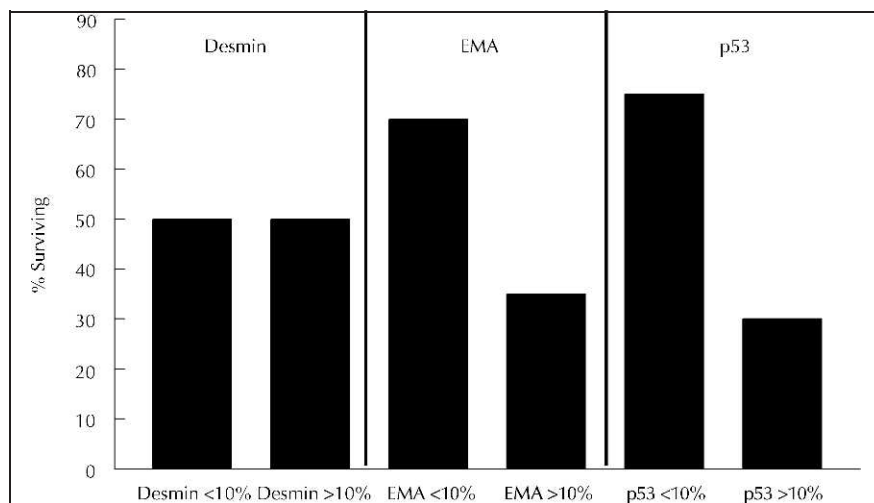
Marker	Proposed Significance
Pankeratin	Seen in both benign and malignant mesothelial processes
EMA	Claimed to be marker of malignancy
p53	Claimed to be marker of malignancy
Desmin	Claimed to be marker of benign mesothelial cells
GLUT-1	Claimed to be marker of malignancy
X-linked inhibitor of apoptosis	Claimed to be marker of malignancy
IMP-3	Claimed to be marker of malignancy

Abbreviations: EMA, epithelial membrane antigen; GLUT-1, glucose transporter-1.

one malignant tumor versus another in the pleura, we would all be satisfied with it. In the setting of benign versus malignant process, however, a 10% error is probably not acceptable because the issue involves telling the patient that he or she will die (and embarking on a variety of unpleasant and largely ineffective therapies) versus telling the patient that the process is at least not an overt cause of worry.

As well, our own experience indicates that if one uses these markers to predict outcome, as opposed to retrospectively selecting benign and malignant cases, the specificity is nowhere near as high as 90%. Figure 16 shows data from Group Mesopath in which specimens from a set of mesothelioma and atypical mesothelial hyperplasia cases were stained and the patients followed up for 5 years after biopsy. Using the frequently cited cutoff of 10% cell staining as a positive result, desmin provided no predictive value at all; for cases with positive staining, half of the patients were alive at 5 years and half had died. For epithelial membrane antigen, survival at 5 years was about 70% for cases with less than 10% staining versus 35% for cases with more than 10% staining, a statistically significant difference, but not one that is really of use in treating patients. For p53, corresponding numbers were 75% and 30%, again a statistical difference with little practical application. Our conclusion is that these markers have no diagnostic utility in an individual case for separating benign from malignant mesothelial proliferations.

Figure 16. Percentage survival in a group of 55 patients followed up for 5 years, separated according to whether the biopsy specimen showed greater or less than 10% staining for desmin, epithelial membrane antigen (EMA), and p53. None of these markers provides a diagnostically useful separation of benign from malignant process.



Positive staining for glucose transporter 1 (GLUT-1),¹¹ X-linked inhibitor of apoptosis,¹² and IMP-3¹³ have also been proposed as indicators of malignancy in mesothelial proliferations, but most of the literature on these markers relates to effusion cytology specimens. Kato et al¹¹ reported that, in histologic sections, 40 of 40 mesotheliomas and 0 of 40 reactive mesothelial proliferations stained for GLUT-1; while Monaco et al¹⁴ found positive staining in 27 of 41 mesotheliomas (66%), 5 of 70 benign mesothelial proliferations (7%), and 1 of 14 cases of atypical hyperplasia (7%). Wu et al¹² observed staining for X-linked inhibitor of apoptosis in 0 of 9 normal samples of normal mesothelium, 1 of 13 hyperplasia cases (8%), and 25 of 31 mesotheliomas (81%). More recently, Shi et al¹³ reported that 0 of 64 reactive mesothelial proliferations stained for IMP-3, whereas 33 of 45 mesotheliomas (73%) did, as did 2 cases of atypical hyperplasia that later turned out to be mesotheliomas.

At this point, the literature on these 3 markers is too scanty to recommend them for general use, and to us, overall, immunohistochemical staining has yet to provide a marker that reliably separates benign from malignant mesothelial proliferations.

p16 FLUORESCENCE IN SITU HYBRIDIZATION

p16 (CDKN2A) is a gene involved in cell cycle regulation, and some proportion of mesotheliomas lose p16. Illei et al¹⁵ were the first to suggest that homozygous loss of p16 gene, detected by fluorescence in situ hybridization, could be used to separate benign from malignant mesothelial cells in cytology preparations, and Chiosea et al¹⁶ reported that this approach could also be applied to histologic sections.

In our experience the technique works on carefully selected cases (Figure 17, A and B), but has a number of limitations. First, because of section thickness, in some cells, 1 or both copies of any gene will not be in the plane of section; therefore, control values have to be generated for the range of artifactual versus real p16 gene loss (we use >20% of nuclei lacking both copies of p16 as a cutoff value). Second, picking out individual cells of interest (eg, a line of single mesothelial cells) by fluorescence microscopy can be very difficult, so that p16 fluorescence in situ hybridization works best on nodules of mesothelial cells, a feature that may not be present in a case of atypical hyperplasia. But

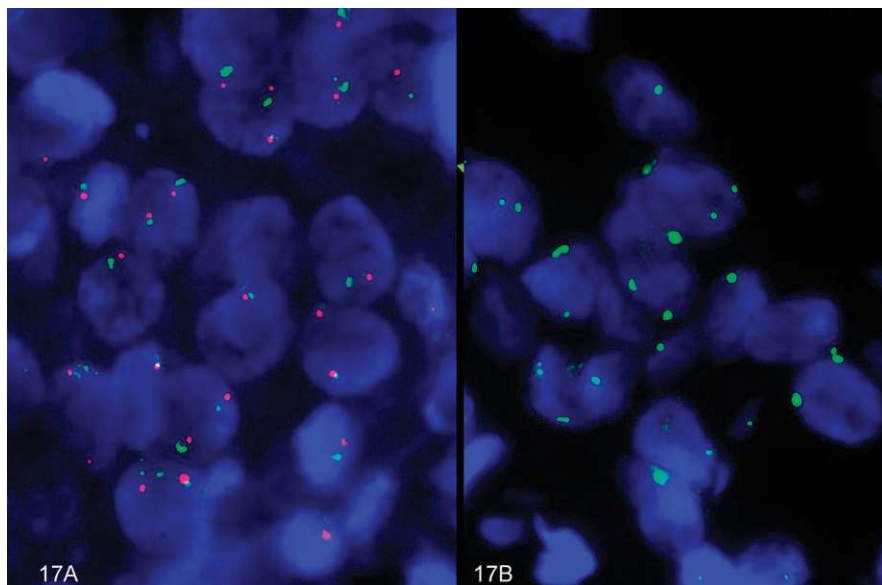


Figure 17. Fluorescence in situ hybridization images of p16 gene. A, A benign mesothelial proliferation shows 2 green signals (chromosome 9 centromere) and 2 red signals (p16) in each cell. B, A mesothelioma in which all cells in the field have lost the red p16 signals, indicating homozygous deletion of p16, a marker of malignancy (original magnifications $\times 1000$ [A and B]).

most important, only a proportion of mesotheliomas show homozygous p16 loss: Monaco et al¹³ reported that p16 gene was lost in 70% of pleural and 51% of peritoneal mesotheliomas, so that the presence of p16 does not rule out a malignant process.

SIGNIFICANCE OF A DIAGNOSIS OF ATYPICAL MESOTHELIAL HYPERPLASIA

There is very little in the literature on what a diagnosis of atypical mesothelial hyperplasia means in terms of patient outcome. We have data on survival from Group Mesopath in 67 cases that were labeled *atypical mesothelial hyperplasia* and 640 that were labeled *outright mesothelioma*. As shown in Figure 18, the 3-year survival associated with the atypical hyperplasia cases was about 60% versus approximately 15% for the mesothelioma cases. What this says is that our ability to predict malignancy is in one sense quite good because cases with definite histologic features of malignancy behave as mesotheliomas. However, if overt features of malignancy

are not present, and we make a diagnosis of atypical mesothelial hyperplasia, then we are basically flipping a coin in terms of predicting outcome. The implication is that better markers of malignancy are needed.

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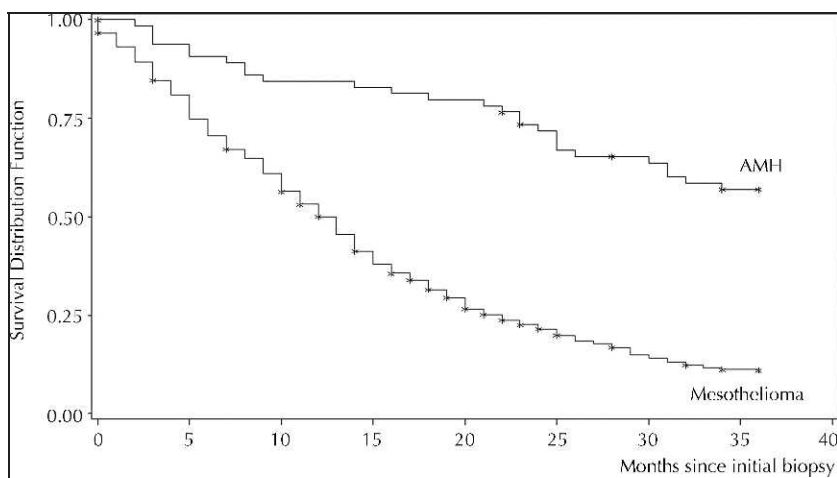


Figure 18. Survival for 67 patients with a diagnosis of atypical mesothelial hyperplasia (AMH) on biopsy, compared to 640 patients with a diagnosis of mesothelioma. A histologic diagnosis of mesothelioma is an accurate predictor of a poor prognosis, whereas the atypical mesothelial hyperplasia group clearly represents a mixture of benign and malignant processes. Survival curves are significantly different ($P < .001$).

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