

## A Cytokeratin- and Calretinin-negative Staining Sarcomatoid Malignant Mesothelioma

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**Abstract.** *Malignant Mesothelioma, or mesothelioma, is a mesothelial-based malignancy that may occur in the pleura, pericardium and peritoneum. Mesothelioma is a very aggressive cancer with limited treatment, and a median survival of about 1 year. At times, the diagnosis of mesothelioma may be problematic. The final diagnosis of mesothelioma relies on histology and often is dependent upon immunohistochemistry. It is generally assumed that mesotheliomas must stain positive for cytokeratin and calretinin and negative staining for these markers would rule out the diagnosis. We encountered a patient with a pleural-based, cytokeratin- and calretinin-negative sarcomatoid malignancy. These negative stainings would rule out the diagnosis of mesothelioma but, after careful consideration of the patient's clinical records, and additional histological and immunohistochemical studies, we conclude that this patient suffered from mesothelioma of the sarcomatoid type.*

Mesothelial cells form the lining of the pericardial, pleural and peritoneal cavities. They are among the most undifferentiated cells of the body (1). Morphologically, mesothelial cells can resemble epithelial or fibroblast cells, but have immunohistochemical characteristics which distinguish them from these cell types. Malignant Mesothelioma (MM) is a malignancy that is derived from mesothelial cells. It usually stains positive for pan-cytokeratin, calretinin, WT-1, and stains negative for numerous epithelial markers such as CEA, LeuM1, BerEp4, B72.3, TTF1, CD31 and CD34. Histologically, MM can exhibit epithelial, fibrous (also called sarcomatoid) and

biphasic morphology. The latter is used to describe MM with both epithelial and sarcomatoid characteristics (2).

The diagnosis of MM can be difficult and is made histologically. The sarcomatoid type of MM is the most difficult to diagnose because it can easily be confused with other types of sarcomas (2). Epithelial MM can occasionally be confused with adenocarcinomas; while sarcomatoid and biphasic MM can be confused with synovial sarcoma, both primary and metastatic sarcoma, and carcinosarcoma (3-8). The current treatment of most cases of MM are of limited effect when considering that even the most novel therapeutic approaches such as pemetrexed, at best, have shown to prolong survival an average of 3 months (9). When correctly treated, other pleural malignancies such as synovial sarcoma may be responsive to chemotherapy and have a much better prognosis. In summary, even though these malignancies may share morphological similarities, they do not share similar treatment options, thus it is very important to correctly diagnose these patients.

We receive pathology samples of patients from different hospitals to offer an opinion on the diagnosis of pleural-based malignancies. Among these referrals, was a patient with a pleural-based sarcomatoid primary malignancy, with negative cytokeratin staining and no definite diagnosis. Cytokeratin-negative staining is thought to rule out the diagnosis of sarcomatoid MM (2) but, after careful review of the pathology, patient's history and history of present illness, it was concluded that the patient did suffer from MM of the sarcomatoid type with a small epithelial component. This case is important because it underscores the belief that immunohistochemistry is an ancillary technique, and should not be the determining factor in the final diagnosis of MM.

### Patient and Methods

*Patient.* The patient was an 80-year-old male with a history of a myocardial infarction at the age of 36, diabetes, a successfully removed basal cell carcinoma with no recurrence, hyperlipidemia, transient ischemic attacks affecting the right side of his body and a history of anemia. He had smoked a pack of cigarettes a day for

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Table I.

Antibody	Immunostaining Results
Vimentin	Positive
PanKeratin AE-1/AE-3 (Ventana and Zymed)	Focally-positive on the epithelial component of the tumor, and negative in the sarcomatoid component of the tumor
both antibodies showed the same results	
CAM 5.2	Focally-positive on the epithelial component of the tumor, and negative in sarcomatoid component of tumor
CK7	Negative
Calretinin	Negative
BerEp4	Negative (very rare focally positive tumor cells)
WT1	Focally-positive in both the epithelial component, and sarcomatoid component
B72.3	Negative
CEA(monoclonal)	Negative
CEA(polyclonal)	Negative
CD15 (LeuM1)	Negative
CD31	Negative on tumor cells, positive on vascular structures
CD34	Negative on tumor cells, positive on vascular structures
TTF1	Negative
EMA	Focally-positive

ten years, and quit in 1969. After his discharge from the military, he worked as a plumber where he most likely was exposed to asbestos. There was radiological suspicion of pleural plaques because of the presence of pleural densities and this was confirmed histologically. Although pleural plaques are not specific for asbestos exposure, they are often found in patients with above background exposure. In February 2003, the patient developed chest pain on his left side. The pain was accompanied with dyspnea and these symptoms progressively worsened. All of these symptoms were accompanied with increased pleural effusion from the left pleural cavity. The patient subsequently underwent a left thoracoscopy with chest wall pleural biopsy, lung biopsy, and a bronchoscopy to rule out carcinoma of the lung because of his previous tobacco use.

**Immunohistochemistry.** Immunohistochemical staining using the avidin-biotin-peroxidase method was done first at the referral hospital, and then at our laboratory of Surgical Pathology at Loyola University Chicago, IL, USA, according to standard procedures (4). We used the following antibodies: vimentin (Dako), PanKeratin AE-1/AE-3 (Ventana and Zymed), CAM 5.2 (Becton-Dickinson), TTF1 (Dako), WT-1 (Santa Cruz), calretinin (Zymed), B72.3 (Signet), CD34 (Zymed), polyclonal CEA (Dako), monoclonal CEA (Dako), BerEp4 (Dako), CD31 (Ventana) and CK-7 (Ventana) (Table I).

**Results**

**Radiological findings and gross pathology.** The X-ray and CT scan revealed a large dense mass along the left lateral chest wall, measuring approximately 15 cm cranio-caudal and at least 10 cm transversely, and 3 cm in thickness. There was also additional bi-lobed density along the left lateral chest

wall superior to the previously mentioned mass. The tumor caused a rightward shift of the mediastinal structures. There was also evidence of a left pleural effusion, but there was no evidence of a pneumothorax, a right pleural mass, or an effusion on the right side. This evidence suggested the initial diagnosis of a neoplasm of the left pleura, with the possibility of it being a MM.

The next course of action involved the examination of the tumor through thoracoscopy, a pleural biopsy and lung biopsy. Visually, the tumor was described to contain multiple aggregates of grayish-white, rubbery, but pliable tissue measuring in aggregate 6.0 x 4.0 x 0.5 cm, with the largest of the pieces measuring 2.0 x 1.5 x 0.5 cm.

**Histology.** The sections taken from the pleural biopsy showed a poorly-differentiated spindle cell neoplasm with occasional areas having a storiform pattern (Figures 1,2). The tumor cells showed pleomorphic nuclei, a high mitotic count and some multinucleated pleomorphic giant cells. Along with these cells, abundant collagen deposition was also present. Only, one of the five pleural biopsy specimens contained a small epithelial area (less than 1% of the tumor mass) within the sarcomatoid component (Figure 3).

In addition to these histological findings, there were also areas of extensive fibrosis, consistent with pleural plaques. The almost complete absence of tumor cells in these fibrous areas, together with the presence of an inflammatory infiltrate argues that these were indeed pleural plaques rather than areas of desmoplastic reaction. Some biopsy fragments contained large numbers of giant cells. Since the

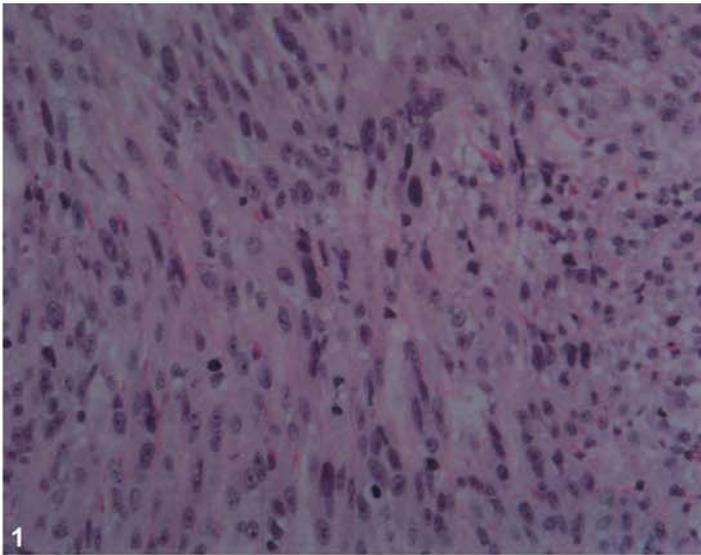


Figure 1. *Representative histology. Over 99% of the tumor in each of the 5 tumor blocks submitted for diagnosis showed a non characteristic sarcomatoid morphology. H/E stained. 200x.*

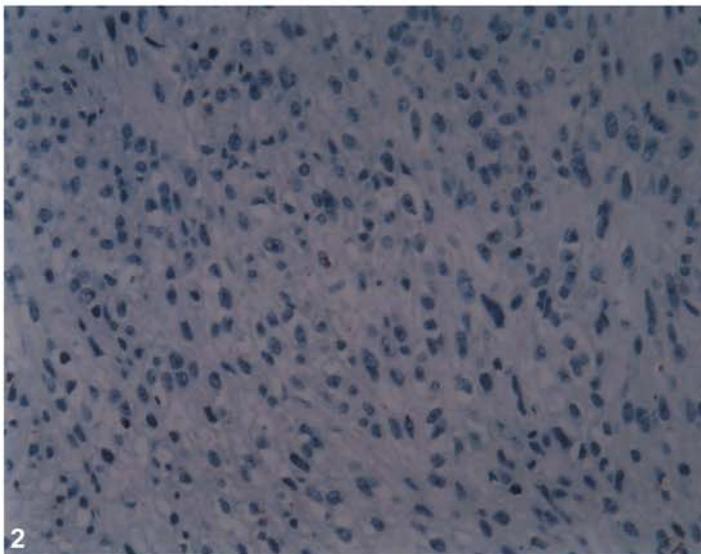


Figure 2. *Pankeratin-negative staining. 200x.*

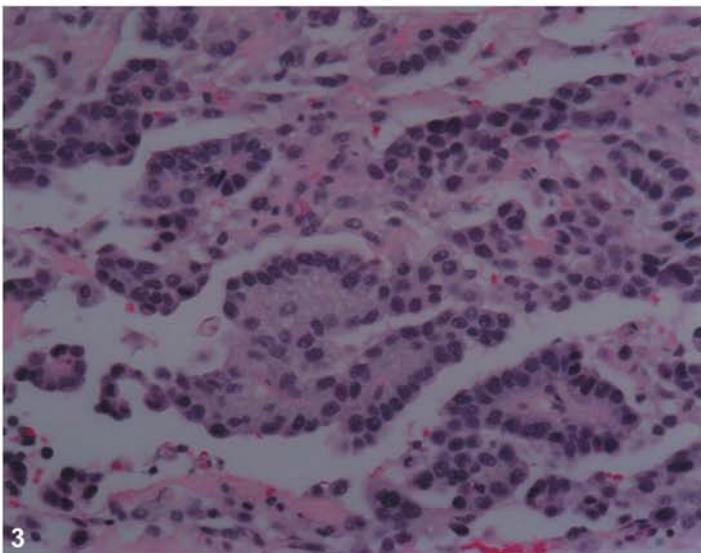


Figure 3. *Epithelial component. One of the 5 tissue blocks contained a rare area of epithelial differentiation shown here. H/E stained. 200x*

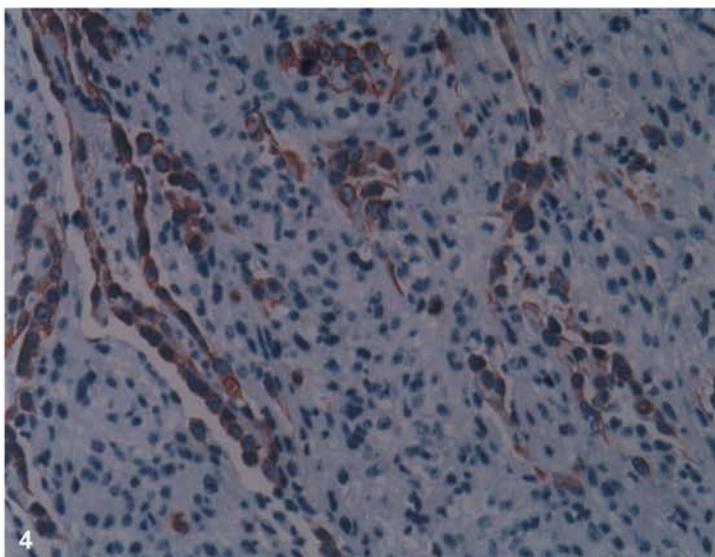


Figure 4. *Pankeratin* staining, positive on the epithelial component. 200x

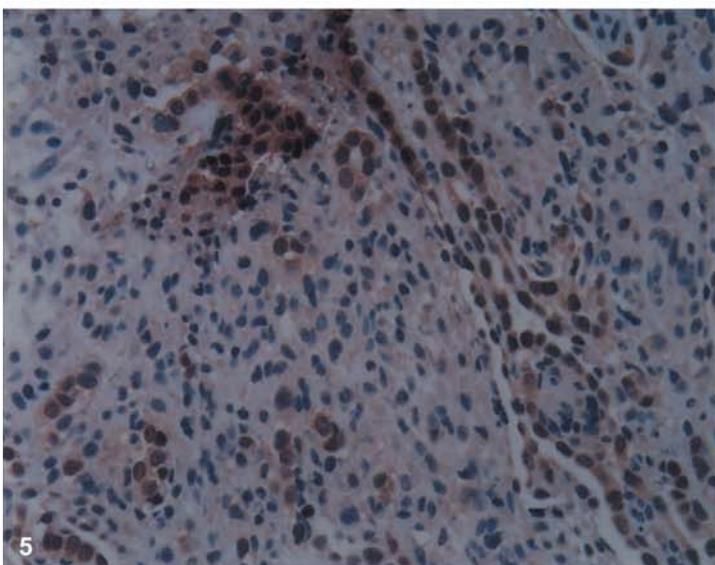


Figure 5. *WT-1* staining, positive in the epithelial component and positive in sporadic cells in the sarcomatoid component. 200x

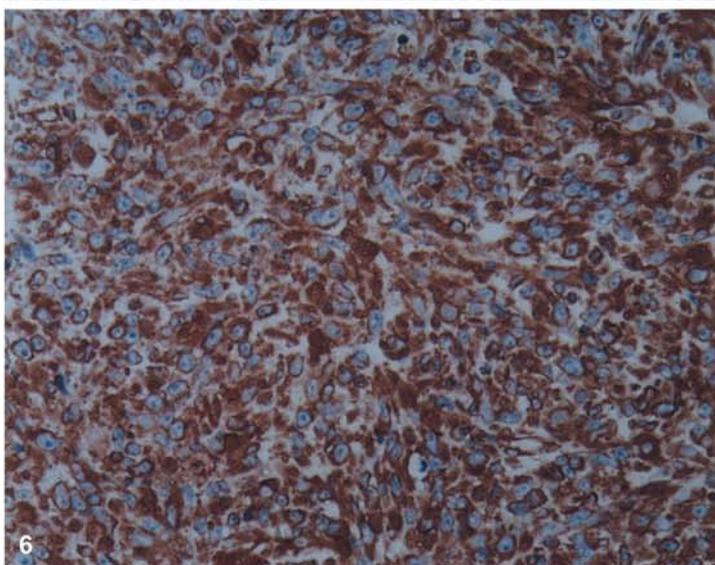


Figure 6. *Vimentin*-positive staining. 200x

patient did not receive any treatment prior to biopsy, the large number of giant cells was consistent with a poorly-differentiated tumor, and somewhat less characteristic of MM. The differential diagnosis was between MM and a carcinosarcoma, a synovial sarcoma, a vascular tumor or a malignant fibrous tumor. The morphology was not consistent with a synovial sarcoma or vascular tumor, but was consistent with a carcinosarcoma or a poorly-differentiated sarcomatoid MM.

**Immunohistochemical studies.** Most of the tumor (99% of the tumor cells) consisted of a sarcomatoid component (Figure 1), which stained negative for pankeratin (Figure 2) which, by itself, almost rules out the diagnosis of MM. However, we noticed that in 1/5th of the tissue blocks, there was a small area in which the tumor cells had biphasic epithelial differentiation. The epithelial component (Figure 3) of the tumor was focally-positive for pankeratin AE-1/AE-3 (from both Ventana and Zymed), WT-1 (Figures 4 and 5), and that some of these cells were also BerEp4-positive. The same cells stained negative for TTF1, B72.3 and CEA. These findings identify these cells as mesothelial cells and ruled out a lung carcinosarcoma. The fact that rare cells stained positive for BerEp4 is consistent with MM (1, 2), while in a carcinosarcoma, adenocarcinoma, or synovial sarcoma a larger number of cells would be expected to stain positive for BerEp4, (1, 2, 4). A very unusual finding was that the tumor cells of both the sarcomatoid component and scattered epithelial components were negative for calretinin. Calretinin stains the vast majority of epithelial MM and can also stain sarcomatoid MM (10, 11). The overall highly aggressive morphology of this tumor may explain this finding, because the tumor cells may have lost keratin and calretinin production in the process of de-differentiation. The negative staining for CD31 and CD34 ruled out a vascular tumor or a malignant fibrous tumor. All tumor cells stained positive for vimentin, confirming the immunoreactivity of the tumor cells (Figure 6). These immunohistochemical and histological findings, along with the clinical findings and the age of the patient, support the diagnosis of MM of the sarcomatoid type.

## Discussion

MM usually occurs in males (8:1 male to female ratio) between 70 and 80 years old, as was this patient. The onset is, as in this case, usually experienced as chest pain or dyspnea due to fluid build up and the growth of the tumor in the pleural cavity. Being employed as a plumber for some time, the patient was at risk for asbestos exposure and the presence of pleural plaques support the hypothesis that he was indeed exposed to asbestos.

Immunostaining for intermediate filaments such as cytokeratin has a pivotal role in the diagnosis of MM.

Cytokeratin has been shown by many groups to be a reliable marker for MM. Ordonez reported 40 out of 40 and Clover showed 23 out of 23 epithelial MM staining positive for cytokeratin, and many other groups have had the same results and have shown cytokeratin to be the most reliable marker for MM (2, 12-15). Our experience over the years has shown that 100% of epithelial and sarcomatoid MMs stain positively for cytokeratin, and we considered cytokeratin staining a must for the diagnosis of MM.

Similarly, many other groups have reported that all of their MMs stained positive for pankeratin, and that most MMs stained positive for calretinin. Calretinin is often positive in sarcomatoid MM, but a negative staining is not infrequent (11). However, a double-negative keratin and calretinin sarcomatoid tumor, almost by definition, is not considered a MM. This case has forced us to reconsider our belief that a cytokeratin- and calretinin-negative sarcomatoid malignancy of the pleura is not a MM. Although they are very rare, sarcomatoid, cytokeratin-negative MM does exist and this is the first one we have seen. The fact that the tumor cells were negative for pankeratin and calretinin would have most likely caused us to misdiagnose this tumor if we had not identified a small biphasic epithelial component in 1/5th of the tumor tissue blocks. Immunostaining of this small (less than 1% of tumor cells) biphasic component allowed us to identify this malignancy as a MM.

Our finding underscores the importance of studying multiple biopsy samples to correctly diagnose MM, and the fact that immunohistochemistry may, on occasion, be misleading. Thus, at times, the importance of performing multiple immunohistochemical tests on multiple samples cannot be overemphasized for a correct diagnosis of MM.

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