

Diagnosis of pulmonary lymphangiomyomatosis by HMB45 in surgically treated spontaneous pneumothorax

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ABSTRACT: Pulmonary lymphangiomyomatosis (PLAM) is a rare disease with poor prognosis, characterized by an abnormal proliferation of smooth muscle. The patients are females and recurrent pneumothorax is a frequent complication. HMB45 is a monoclonal antibody with specific immunoreactivity for malignant melanoma. Recently, it was reported that some of the smooth muscle cells in PLAM had reactivity for HMB45. The aim of this study was to assess the sensitivity and specificity of HMB45 for the diagnosis of PLAM in cystic pulmonary diseases that cause recurrent pneumothorax.

We compared immunoreactivity of the specimens obtained by open lung biopsy at surgical resection of bullae in 72 patients. The specimens of five females with PLAM, one female with suspected PLAM, 49 patients with primary spontaneous pneumothorax (19 females and 30 males), four with pulmonary eosinophilic granuloma (2 females and 2 males), seven with pulmonary emphysema (7 males), and six with idiopathic pulmonary fibrosis with apical bullous change (2 females and 4 males) were stained with HMB45 and anti-smooth muscle actin.

All PLAM cases had HMB45 positive cells, which also stained with anti-smooth muscle actin. The biopsy specimens of a PLAM suspected case also stained with HMB45. None of the specimens from other diseases reacted with HMB45.

HMB45 appears to provide a highly specific and highly sensitive diagnosis for PLAM in females. It may also be useful in patients with subtle smooth muscle proliferation, where the diagnosis of PLAM is difficult to confirm by conventional histological examination.

Eur Respir J., 1995, 8, 1879-1882.

HMB45 is a monoclonal antibody (MoAb) for melanoma-related antigen [1], and immunoprecipitates a 10 kDa protein from extracts of malignant melanoma [2]. This MoAb is known for its clinical usefulness as a highly specific marker for melanoma [3, 4]. Recently, BONETTI *et al.* [4] tested smooth muscle cells from pulmonary lymphangiomyomatosis (PLAM), chronic infiltrative lung diseases and several pulmonary tumours. Those from PLAM were the only ones to show immunoreactivity for HMB45. There have been no other reports of the sensitivity and specificity of HMB45 in the diagnosis of PLAM among the various pulmonary cystic diseases.

PLAM was first reported in 1955 by ENTERLINE and ROBERTS [5] as a "lymphangiopericytoma". PLAM is characterized by haphazard proliferation of smooth muscle throughout the interstitium of the lung in women, almost always during childbearing age [6]. PLAM patients show isolated abnormalities, usually associated with tuberous sclerosis or renal angiomyolipoma [7, 8]. A definitive diagnosis of PLAM must be made by a lung biopsy specimen; however, when proliferation of smooth muscle cell is faint, diagnosis may be difficult. The most

common presenting symptom of the disease is spontaneous pneumothorax, followed by dyspnoea on exercise. In one series, pneumothorax occurred in 53% of cases at presentation, and 81% during the course of the illness [8]. Radiological and morphological abnormalities of PLAM comprise diffuse cystic lesions resembling honeycombing or emphysema [7, 8].

The aim of this study was to determine the sensitivity of HMB45 in the diagnosis of PLAM and its ability to exclude other cystic pulmonary diseases that cause recurrent pneumothorax: primary spontaneous pneumothorax, pulmonary eosinophilic granuloma, pulmonary emphysema and idiopathic pulmonary fibrosis with apical bullous change.

Materials and methods

Patients

A total of 72 patients including: five with PLAM (5 females); one with suspected PLAM (1 female); 49 with

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Keywords: HMB45
pneumothorax
pulmonary lymphangiomyomatosis
smooth muscle

Received: May 3 1995
Accepted after revision September 7 1995

Table 1. – Immunohistochemical results of HMB45 stain in pulmonary lymphangioleiomyomatosis and other cystic pulmonary diseases

| Diagnosis | n | M/F | Age yrs | HMB45 positive rate |
|----------------------------------|----|------|--------------------|---------------------|
| PLAM* | 5 | 0/5 | 37±9 [#] | 5/5 |
| Case No. 1 | | | 35 | 1/1* |
| Case No. 2 | | | 36 | 2/2* |
| Case No. 3 | | | 40 | 1/2* |
| Case No. 4 | | | 49 | 2/2* |
| Case No. 5 | | | 23 | 4/4* |
| PLAM suspected | 1 | 0/1 | 41 | 1/1 |
| Primary spontaneous pneumothorax | | | | |
| Female | 19 | 0/19 | 31±15 [#] | 0/19 |
| Male | 30 | 30/0 | 42±9 [#] | 0/30 |
| Pulmonary eosinophilic granuloma | 4 | 2/2 | 30±9 [#] | 0/4 |
| Pulmonary emphysema | 7 | 7/0 | 66±6 [#] | 0/7 |
| Idiopathic pulmonary fibrosis | 6 | 4/2 | 64±10 [#] | 0/6 |

PLAM: pulmonary lymphangioleiomyomatosis; M: male; F: female. *: HMB45 positive specimens; #: mean±SD.

primary spontaneous pneumothorax (19 females and 30 males); four with pulmonary eosinophilic granuloma (2 females and 2 males); seven with pulmonary emphysema (7 males); and six with idiopathic pulmonary fibrosis with apical bullous change (2 females and 4 males) were enrolled in this study. All patients had been admitted to Sapporo Medical University Hospital or Hokkaido Keiaiikai Minamiichijo Hospital between 1987 and 1994. All specimens were obtained by open lung biopsy for diagnosis and resection of ruptured bullae.

PLAM. Four patients were diagnosed by typical chest radiograms, computed tomographic (CT) scans and open lung biopsy specimens [7, 9]. One patient (case No. 5 in table 1) was not diagnosed as PLAM until her second open lung biopsy for pneumothorax, because only a scant smooth muscle proliferation was observed in the first biopsy specimen. The other patients were diagnosed as PLAM at the first biopsy.

Suspected PLAM. A 41 year old woman suffered from recurrent pneumothorax. She was not a smoker. Her chest CT scan revealed diffuse small lung cysts and the lung specimen obtained by video-assisted thoracoscopic surgery (VATS) showed cystic lesions with sparse smooth muscle cell proliferation. We diagnosed this case as a strongly suspected PLAM and treated her with tamoxifen. She has been followed for 32 months and has had no recurrence of pneumothorax.

Primary spontaneous pneumothorax. Forty nine patients were diagnosed according to physiological, radiological and pathological criteria [10]: no roentgenographic evidence of other pulmonary disease, and the presence of a bulla or a bleb within or immediately close to the visceral pleura. All patients had recurrent spontaneous pneumothorax, and the ruptured cystic lesion was resected by VATS.

Pulmonary eosinophilic granuloma. Four patients had clinical and radiological features compatible with pulmonary eosinophilic granuloma [11]. Pathologically, alveolar septa were widened by an infiltration of histiocytes and eosinophils, and characteristic Birbeck granules were found by electron microscopy.

Pulmonary emphysema. All seven patients were smokers and had centrilobular emphysema. The diagnosis was made according to physiological, radiological and pathological criteria [12]. Although an intercostal drain was inserted for pneumothorax, significant air leak persisted for over 1 week in all cases. Ruptured bullae were resected by VATS.

Idiopathic pulmonary fibrosis. Six patients were diagnosed on the following criteria [13]: predominantly basal and subpleural reticulonodular patterns on the chest radiogram and CT scan; a restrictive pattern of pulmonary function and decreased carbon monoxide transfer factor; hypoxaemia at rest; and elimination of other causes of pulmonary fibrosis.

Immunohistochemical studies

All specimens were fixed in 10% buffered formaldehyde solution for 24 h and embedded in paraffin. Sections from all cases were cut with a microtome at 5 µm and stained with haematoxylin and eosin (H&E). Immunohistochemical studies were performed with the avidin biotin (peroxidase) complex (ABC) method [14]. Primary MoAbs were as follows: smooth muscle actin 1A4 (1:50; Dako Corp., Carpinteria, CA, USA) and HMB45 (1:100; Dako Corp., Carpinteria, CA, USA). Sections were deparaffinized and followed by blocking of the endogenous peroxidase with 3% H₂O₂ for 30 min and washing in phosphate buffered saline (PBS). The sections were incubated with the primary MoAb for 1 h at room temperature in a moist chamber. After washing in PBS the sections were incubated with biotinylated anti-mouse immunoglobulin for 30 min at room temperature. Visualization of the antibody localization was carried out using the avidin biotin immunoperoxidase complex (Vector Laboratories, Burlingame, CA, USA) with 3,3'-diaminobenzidine (DAB) reaction. For specificity controls, the primary antibody was replaced by normal mouse immunoglobulin G (IgG). As a positive control for HMB45, a specimen of malignant melanoma metastatic to the heart was used.

Immunohistochemical slides were interpreted by two clinical pathologists without knowledge of the clinical data.

Results

Lung biopsies from patients with PLAM showed smooth muscle proliferation of benign appearance involving the walls of lymphatic vessels, blood vessels, bronchioles and alveolar septa (fig. 1); and haemosiderin-filled macrophages were often found in the alveoli. The proliferative smooth muscle cells stained with HMB45 (fig. 2) as well as smooth muscle actin. The results of immunostain with

HMB45 in PLAM are summarized in table 1. The PLAM patients had positive results, and 10 out of 11 specimens showed positive HMB45 immunoreactivity. In Case No. 5, the first resected lung specimen did not confirm the diagnosis because the proliferation of smooth muscle cell was not typical for PLAM. However, at her second pneumothorax episode the specimen had typical histological features. Both biopsies contained HMB45 reactive cells. The specimens of the patient with suspected PLAM showed cystic lesions with a faint smooth muscle cell proliferation, and some of the smooth muscle cells of a cystic wall stained with HMB45 MoAb.

A positive immunoreaction with HMB45 was observed only in the cytoplasm of the proliferative smooth muscle cell in case of PLAM and suspected PLAM, and cells with HMB45 positive immunoreaction also had positive reactivity to anti-smooth muscle actin MoAb (fig. 3).

There were no HMB45 positive cells in primary spontaneous pneumothorax, eosinophilic granuloma, pulmonary emphysema, and idiopathic pulmonary fibrosis (table 1). Smooth muscle proliferation with positive reactivity with anti-smooth muscle actin was found in a setting of dense fibrosis in idiopathic pulmonary fibrosis, but there were no cells showing immunoreactivity with HMB45 MoAb.

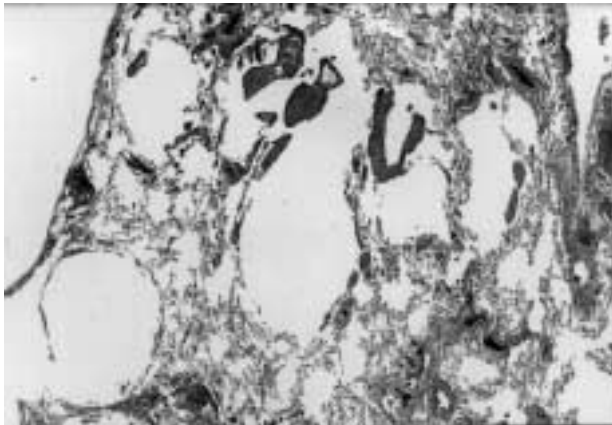


Fig. 1. – Open lung biopsy specimen of pulmonary lymphangioleiomyomatosis (Case No. 4). Marked thickening of parenchymal interstitium with smooth muscle proliferation. (Haematoxylin and eosin stain; internal scale bar=1mm)

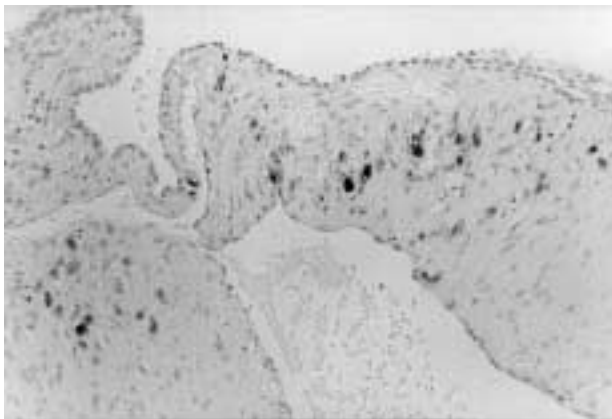


Fig. 2. – Immunostain for HMB45 of pulmonary lymphangioleiomyomatosis (Case No. 4); many proliferative smooth muscle cells showing positive reactivity with HMB45. (Internal scale bar=100 µm).

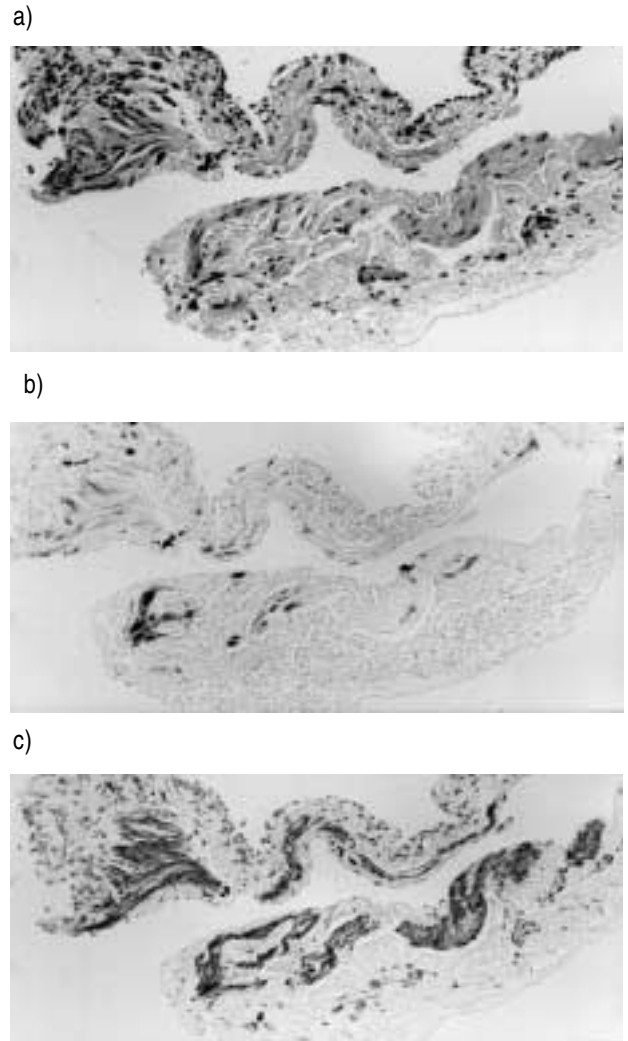


Fig. 3. – Immunostain of serial sections of pulmonary lymphangioleiomyomatosis. a) Haematoxylin and eosin stain; b) HMB45; c) smooth muscle actin. Some of the proliferative smooth muscle cells show positive reactivity with HMB45 in the cytoplasm, and almost all HMB45 positive cells also have a positive reaction for smooth muscle actin. (Internal scale bars=100 µm).

Discussion

We present a high degree of specificity of HMB45 in the pathological diagnosis of PLAM in contrast to other cystic pulmonary diseases that cause recurrent pneumothorax especially in females. We also found that HMB45 staining was diagnostic in lung cyst specimens with subtle smooth muscle proliferation that suggested PLAM. It was suggested that this MoAb was useful in diagnosis in the early phase of PLAM.

BONETTI *et al.* [4] reported that HMB45 positive cells were present only in PLAM, and not in other lung disorders with smooth muscle proliferation and neurogenic tumour. They examined samples from patients with focal muscle hyperplasia, bronchiolitis obliterans organizing pneumonia, diffuse alveolar damage after irradiation, leiomyosarcoma metastatic to the lung, haemangioendothelioma, sclerosing haemangioma, paraganglioma, granular cell tumour, spindle cell carcinoma, Langerhans' cell granulomatosis, Wegener's granulomatosis and sarcoidosis.

Hoon *et al.* [15] reported that smooth muscle tumour (leiomyoma and leiomyosarcoma) were negative for HMB45. In this study, idiopathic pulmonary fibrosis showed the proliferation of smooth muscle cells with positive smooth muscle actin reactivity that might mimic PLAM. Those samples were, however, never positive for the present MoAb. From these results, this peculiar immunoreactivity appears to be quite useful for confirmative diagnosis of PLAM, especially in patients with spontaneous pneumothorax associated with bullous change and other symptoms typical of PLAM.

Our results (table 1) also show a high degree of sensitivity for HMB45 MoAb tested in PLAM. The HMB45 negative specimen of Case No. 3 was small and demonstrated no smooth muscle proliferation in H&E stain. This negative result may be due to an inadequate volume of the specimen to evaluate the disease. BONETTI *et al.* [4] reported that small transbronchial lung biopsy specimens allowed the evaluation, though it was difficult when the sample had only slight smooth muscle cell proliferation.

HMB45 binds to antigen present in the cytoplasm of neoplastic melanocytic cells [2, 3], melanocytes in foetal skin at a gestational age of 50 days [16], and foetal and neonatal retinal pigment epithelium [17]. This distribution suggests that this MoAb binds cytoplasmic oncofoetal glycogen associated with immature melanosomes, and changes in immunoreactivity with maturation or malignant transformation may be a function of post-translational modification [17]. In an electron microscopic study, the derivation of the PLAM proliferating cells from smooth muscle was questioned [18], and it was suggested that the origin might be the pulmonary interstitial cell or pericyte [5]. BONETTI *et al.* [4] reported that the morphological heterogeneity of the PLAM lesions was reflected in the HMB45 immunoreactivity, and most cells with epithelioid features were strongly positive with HMB45, whilst only rare spindle cells were positive. In our study, many spindle cells were positive with HMB45 in contrast to the results of BONETTI *et al.* [4].

In addition to malignant melanoma and PLAM, renal angiomyolipomas [15], clear cell tumours of the lung [19], and adrenal pheochromocytoma [17] have been reported to contain HMB45. PEA *et al.* [19] found that HMB45 stained the majority of the neoplastic cells in clear cell tumour of the lung, but only 5% of the lesions in renal angiomyolipoma. UNGER *et al.* [20] reported that 4 of 12 adrenal pheochromocytomas were reactive with HMB45, but the proportion of reactive tumour cells was 50% in one case and 5% in the other three. Since the results of BONETTI *et al.* [4] and our study showed that all PLAM patients had HMB45 positive cells, this MoAb appears to be of great value for enabling a quick, simple and positive diagnosis of PLAM, if a lung biopsy specimen is available.

References

- Gown AM, Vogel AM, Hoak D, Gough F, McNutt MA. Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocytes. *Am J Pathol* 1986; 123: 195–203.
- Esclamado RM, Gown AM, Vogel AM. Unique proteins defined by monoclonal antibodies specific for melanoma: some potential clinical applications. *Am J Surg* 1986; 152: 376–385.
- Leong AS-Y, Milios J. An assessment of a melanoma-specific antibody (HMB45) and other immunohistochemical markers of malignant melanoma in paraffin-embedded tissues. *Surg Pathol* 1989; 2: 137–145.
- Bonetti F, Chiopera PL, Pea M, *et al.* Transbronchial biopsy in lymphangiomyomatosis of the lung: HMB45 for diagnosis. *Am J Surg Pathol* 1993; 17: 1092–1102.
- Enterline HT, Roberts B. Lymphangiopericytoma: case report of a previously undescribed tumor type. *Cancer* 1955; 8: 582–587.
- Colby TV, Carrington CB. Infiltration lung disease. In: Thurlbeck WM, ed. *Pathology of the Lung*. New York, Thieme, 1988; pp. 425–517.
- Fraser RG, Pare JAP, Paré PD, Fraser RS, Genereux GP. In: *Diagnosis of Disease of the Chest*. 3rd edn. Philadelphia, W.B. Saunders, 1991; pp. 2672–2677.
- Taylor JR, Ryu J, Colby TV, Raffin TA. Lymphangiomyomatosis: clinical course in 32 patients. *N Engl J Med* 1990; 323: 1254–1260.
- Kitaichi M, Nishimura K, Itoh H, Izumi T. Pulmonary lymphangioliomyomatosis: a study of 46 patients including a clinicopathologic study of prognostic factors. *Am J Respir Crit Care Med* 1995; 151: 527–533.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 134: 225–244.
- Friedman PJ, Liebow AA, Sokoloff J. Eosinophilic granuloma of lung: clinical aspects of primary pulmonary histiocytosis in the adult. *Medicine* 1981; 60: 385–396.
- Snider GL, Kleinerman J, Thurlbeck WM, Bengali ZH. The definition of emphysema. Report of a National Heart, Lung and Blood Institute, Division of Lung Diseases Workshop. *Am Rev Respir Dis* 1985; 132: 182–185.
- Crystal RG, Fulmer JD, Roberts WC. Idiopathic pulmonary fibrosis: clinical, histologic, radiologic, cytologic and biochemical aspects. *Ann Intern Med* 1976; 85: 769–788.
- Hsu SM, Raine L, Fanger H. Use of avidin biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 1981; 29: 577–580.
- Hoon V, Thung SN, Kaneko M, Unger PD. HMB-45 reactivity in renal angiomyolipoma and lymphangioliomyomatosis. *Arch Pathol Lab Med* 1994; 18: 732–734.
- Holbrook KA, Underwood RA, Vogel AM, Gown AM, Kimball H. The appearance, density and distribution of melanocytes in human embryonic and fetal skin revealed by antimelanoma monoclonal antibody, HMB-45. *Anat Embryol* 1989; 180: 443–455.
- Kapur RP, Bigler SA, Skelly M, Gown AM. Antimelanoma monoclonal antibody HMB45 identifies an oncofetal glycoconjugate associated with immature melanosomes. *J Histochem Cytochem* 1992; 40: 207–212.
- Kane P, Lane BP, Cordice JWV. Ultrastructure of the proliferating cells in pulmonary lymphangiomyomatosis. *Arch Pathol Lab Med* 1978; 102: 618–622.
- Pea M, Bonetti F, Zamboni G, Martignoni G, Fiore-Donati L. Clear cell tumor and angiomyolipoma. *Am J Surg Pathol* 1991; 15: 199–201.
- Unger PD, Hoffman K, Thung SN, Pertsemliades D, Wolfe D, Kaneko M. HMB45 reactivity in adrenal pheochromocytomas. *Arch Pathol Lab Med* 1992; 116: 151–153.