A Pleuro-Peritoneal Communication through the Diaphragm affected with Lymphangiomyomatosis

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Abstract

A 30-year-old Japanese woman with lymphangiomyomatosis (LAM) developed a left chylothorax and chylous ascites. A pleuro-peritoneal communication was confirmed by a scintigram with ^{99m}Tc-labeled macroaggregated-albumin injected into the peritoneal cavity. Video-assisted thoracic surgery revealed a protruding papillary lesion on the left diaphragm. Chyle was oozing into the pleural cavity through this lesion. Histopathological analyses demonstrated that the protrusion was a diaphragmatic LAM lesion and that LAM-associated lymphangiogenesis enabled communication between the pleural and peritoneal cavities through lymphatic vessels. This case demonstrated a new mechanism for chylous pleural effusion in LAM and illustrates the significance of LAM-associated lymphangiogenesis.

Key words: lymphangiomyomatosis, chylothorax, chylous ascites, pleuro-peritoneal communication, lymphangiogenesis

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Introduction

Lymphangiomyomatosis (LAM) is an uncommon disease that occurs exclusively in women of reproductive ages. This condition is characterized by the infiltration of abnormal smooth muscle-like cells (LAM cells) and the cystic destruction of the lung (1). Clinical manifestations of LAM include progressive dyspnea on exertion, recurrent pneumothoraces, hemoptysis, and cough. Furthermore, extrapulmonary abnormalities, including angiomyolipomas or lymphangiomyomas of the axial lymphatics, are present in some patients (2-5).

Chylothorax is a well-known complication of LAM (2-4, 6-8). It usually results from the obstruction or disruption of the thoracic duct or its tributaries (7, 9-11). We herein report a patient who experienced a left chylothorax

and chylous ascites at the time of the diagnosis of LAM. The patient underwent video-assisted thoracic surgery and a protruding papillary LAM lesion on the left diaphragm was confirmed to be the conduit of chyle leaking from the peritoneal space into the pleural cavity. The present case indicates a new mechanism for the development of LAM-associated chylothorax and illustrates the significance of LAM-associated lymphangiogenesis in the pathophysiology of LAM.

The histopathological findings of the diaphragm and cytological findings of the ascites of this patient have been in part reported in a previous study as the registered case number of LNF79 (12). However, we consider that a description of this patient's clinical characteristics is valuable in order to elucidate a new mechanism for the simultaneous occurrence of chylous pleural effusion and ascites in LAM patients.

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Table 1. Laboratory Findings of Chylous Pleural Effusion and Ascites

	Total Protein (g/dL)	LDH (IU/mL)	Specific gravity	Cell number (/μL)	Fibrin	T-Cho (mg/dL)	TG (mg/dL)	VEGF-D (pg/mL)
Pleural effusion	5.4	361	1.019	14,124	+	80	1391	7,008
Ascites	5.8	261	1.020	15,598	+	84	1226	7,129

Figure 1. Radiological findings on admission. A: A chest radiograph showed an abnormal density at the left costophrenic angle, which indicated the presence of a pleural effusion. B: Computed tomography (CT) of the chest revealed the left pleural effusion and multiple thin-walled cysts in the both lung fields. C: Contrast-enhanced CT of the abdomen and the pelvis revealed a moderate amount of ascites and cystic tumors suggestive of lymphangioleiomyomas near the right iliac vein. D: Appearance of the pleural effusion: prior (left) and after (right) low-speed centrifugation.

Case Report

A 30-year-old woman was admitted to our hospital for an evaluation of a left pleural effusion that had been detected during an annual health checkup at her workplace in May 2003. She worked as a clerk in a hospital and had experienced exertional dyspnea for several months prior to the admission. The physical examination suggested that she had ascites in addition to a pleural effusion and these findings were confirmed by computed tomography (CT) of the chest and abdomen. Because the chest CT also revealed multiple thin-walled cysts scattered through both lung fields, the patient was suspected to be affected with LAM and was referred to our hospital.

On admission, a physical examination revealed the following: height, 157.5 cm; body weight, 45 kg; body temperature, 36.2°C; blood pressure, 88/54 mmHg; pulse rate, 72/min; and respiration rate, 18/min. Her respiratory sounds were slightly diminished in the left lower lung field. The abdomen was slightly distended, which indicated the presence of ascites. No cutaneous or neurological abnormalities, suggestive of tuberous sclerosis complex (TSC) were observed. There was no family history of TSC, although she had a

past history of ulcerative colitis at 25 years of age. Blood tests showed a white blood cell count of 4,100/μL with 13.8% lymphocytes, hemoglobin of 12.1 d/dL, and platelet count of 22.8×10⁴/μL, total protein level of 7.3 g/dL, albumin level of 3.8 g/dL, total cholesterol level of 130 mg/dL, and triglyceride level of 85 mg/dL. An arterial blood gas analysis under the room air conditions showed a moderate degree of hypoxemia (PaO₂ 66.7 Torr) at rest. The chest X-ray on admission (Fig. 1A) revealed a left pleural effusion with mild to moderate retention, and the chest CT revealed a moderate accumulation of the left pleural effusion with the multiple thin-walled cysts in the both lung fields (Fig. 1B). These findings were consistent with LAM. The pelvic CT demonstrated ascites and cystic tumorous lesions near the right iliac vein, which was suggestive of lymphangioleiomyoma (Fig. 1C); however, there was no evidence of any renal angiomyolipoma.

Thoracentesis yielded milky-red fluid. Low-speed centrifugation separated the milky turbid fluid from the pellet of red blood cells (Fig. 1D). The fluid was confirmed to be chyle due to the fact that it had a total cholesterol level of 80 mg/dL and a triglyceride level of 1,391 mg/dL (Table 1). The ascites obtained by abdominocentesis also consisted of bloody chyle, which possessed an appearance and laboratory

Figure 2. Scintigraphic examinations suggested the communication between the peritoneal and thoracic cavities as well as the obstruction of lymphatic flow. A: ^{99}Tc -labeled macroaggregated albumin was injected into the peritoneal cavity with the patient in the supine position. The radioactive signal was detected in accord with the left pleural cavity only 5 minutes after the injection, thus suggesting the existence of a pleuro-peritoneal communication. B: The lymphoscintigraphy using ^{99}Tc -labeled human serum albumin (HAS) demonstrated the blockade of lymphatic flow at the pelvic level. The left-sided lymphatic flow was blocked at a lower level than the contralateral flow, in accordance with the existence of the cystic tumor (Fig. 1C). The thoracic duct and axial lymphatics, which should normally be depicted, were not detected. This figure represents the scintigraphy at 20 minutes after the subcutaneous injection of the ^{99}Tc -HAS into the bilateral dorsum pedis. The scan was performed from the anterior side of the patient.

findings similar to the pleural effusion (Table 1). Therefore, we postulated that there was a communication between the peritoneal cavity and left pleural cavity. The concentrations of VEGF-D in the pleural effusion and ascites were higher (7,008 and 7,129 pg/mL, respectively) than that in serum (2,861 pg/mL). In order to confirm the pleuro-peritoneal communication, we injected ^{99}Tc -labeled macroaggregated-albumin into the peritoneal cavity of the patient. Radioactive signals were detected in the left pleural cavity only 5 minutes after the injection. The peritoneal cavity was visualized more intensely than the left pleural cavity (Fig. 2A). Lymphoscintigraphy, a scan following subcutaneous injection of ^{99}Tc -labeled human serum albumin (HSA) into the dorsum pedis, revealed a lack of depiction of the thoracic duct and axial lymphatics at the level of the pelvis, suggesting an extensive congestion or obstruction of lymphatic flow (Fig. 2B).

Based on the characteristic clinical features, including the fact that the patient was a woman of reproductive age, the presence of multiple thin-walled cysts on the chest CT, and the chylous pleural effusion and ascites, we suspected that the patient was affected with LAM. In order to confirm the

diagnosis and to evaluate the lesion on the diaphragm, video-assisted thoracic surgery (VATS) was performed on the 10th hospital day. Macroscopic observation via thoracoscopy revealed milky-red colored chyle and a protruding papillary lesion on the dome of the left diaphragm (Fig. 3A). It was located in the muscular portion near the boundary with the tendinous portion of the left diaphragm. No obvious fenestra was observed on the diaphragm and the chyle appeared to be oozing into the left pleural cavity from the diaphragmatic papillary lesion. This lesion was resected at the base using an automatic stapling device under VATS for the histopathological analyses. An area of erosion was detected on the top of the lesion (Fig. 3A, inset), which was identified as the conduit for chyle to leak from the peritoneal to the pleural cavity. The S8 region of the left lung was also biopsied for the histopathological diagnosis. Microscopic examinations disclosed the proliferation of LAM cells in the lung specimen (Fig. 3B). The protruding papillary lesion was identified as a diaphragmatic LAM lesion where LAM cells had proliferated and destroyed the original diaphragmatic structure including the tendon (Figs. 3C, 3D). Immunohistochemical examination clearly demonstrated that

Figure 3. Histopathological findings of the LAM lesions in the lung and the diaphragm. A: Thoracoscopy demonstrated a papillary protrusion (arrow) from the dome of the left diaphragm. The lesion was resected under the video-assisted thoracic surgery (VATS). The inset shows a formalin-fixed specimen and the arrowheads indicate an erosion on the top of the protruding lesion (the bar=1 cm), which was considered to be the channel for the leakage of chyle from the peritoneal cavity to the pleural cavity. B: Histopathological findings of the lung (Hematoxylin and Eosin staining, with the original magnification $\times 20$). Spindle-shaped LAM cells proliferated in nodules in the respiratory bronchiole region. Each LAM nodule had slit-like or dilated spaces, which were considered to be the LAM-associated lymphatic vessels. C-E: Histopathological findings of the papillary protrusion on the left diaphragm. Hematoxylin and Eosin staining (C and D) and double immunostaining (E) with VEGFR-3 (red) and cytokeratin (brown) of the diaphragmatic lesion are presented (the original magnification C, $\times 200$; D and E, $\times 400$). Histologically, the resected protruding lesion was identified to be a part of the diaphragm itself that was abnormally thickened and drawn (C and D). The upper and the lower portions of the figures correspond with the pleural and the peritoneal sides, respectively. Proliferating LAM cells (arrowheads) were identified on the pleural side of the diaphragm (C and D). Note that the original structure of the diaphragm, including striated muscle or tendon, was not observed. Immunohistochemical examination (E) clearly demonstrated that the surface of the LAM nodules was not covered with KL1 (cytokeratin)-positive mesothelial cells (brown) and was directly exposed to the pleural cavity (arrowheads). LAM cells were confirmed by HMB45 staining (brown) (the inset in E, the original magnification $\times 400$). The extensive LAM-associated lymphangiogenesis (red) and the proliferating reactive mesothelial cells (brown) in the diaphragm were demonstrated with anti-VEGFR-3 immunostaining. F: LAM cell clusters (LCCs) isolated from the ascites (smear, Diff-Quik stainTM, the original magnification $\times 150$). Three clearly demarcated and globular-shaped LCCs, which were surrounded by mesothelial cells and lymphocytes, were observed.

the thoracic surface of the resected diaphragmatic lesion, especially the top of the protrusive lesion, was not covered with KL1 (cytokeratin)-positive mesothelial cells; furthermore, proliferating LAM cells were directly exposed to the thoracic cavity (Fig. 3E). Antibody against VEGFR-3, a marker of lymphatic endothelial cells, revealed extensive LAM-associated lymphangiogenesis in the LAM-affected diaphragm (Fig. 3E). The abundant LAM-associated lymphatic vessels formed a lymphatic network throughout the diaphragm, thus suggesting that the source of the communication between the pleural and peritoneal cavity was through LAM-associated lymphatic vessels. Numerous LAM cell clusters (LCCs), a globular-shaped cluster of LAM cells enveloped by lymphatic endothelial cells (12), were identified in fluid samples from the ascites (Fig. 3F) and the pleural effusion (not shown). Immediately after the operation, the left pleural effusion disappeared. However, thereafter the patient felt a stronger degree of abdominal distension than before the operation since her abdominal circumference increased approximately 3-5 cm after the operation. Low-fat diet and as-needed use of diuretics was initiated, but she needed an occasional abdominocentesis to relieve abdominal distension. She was discharged on the 15th post-operative day and thereafter the administration of gonadotropin-releasing analogue (subcutaneous injection of leuporelin acetate 1.88 mg, every 4 weeks) was initiated for the treatment of LAM. She remained free of pleural effusion while she had massive but a tolerable amount of ascites without abdominocentesis. A small amount of left pleural effusion reappeared approximately one year later.

Discussion

Although chylothorax is one of the major complications of LAM, the incidence of chylous ascites is less common. Moreover, the simultaneous occurrence of chylothorax and chylous ascites is quite rare. Chylothorax has been reported in less than 14% of LAM patients at presentation and its incidence seems to increase to 22-39% of patients during the course of disease (2-4, 6-8). In a retrospective study, Ryu et al reported that eight out of 79 patients (10.1%) with LAM experienced a chylothorax (10). However, none of these eight patients had chylous ascites during their clinical course, with the exception of one patient who had been diagnosed with chylous ascites 12 years before the onset of the chylothorax (10). Hayashida et al reviewed a total of 173 Japanese LAM patients, including the present patient and found that 21 (12%) patients had a chylothorax and 11 out of 165 patients (7%) who received abdominal CT or ultrasonography had ascites (2). Regarding this series of patients, only 3 patients (including this patient) simultaneously experienced chylothorax and ascites at the same time (personal communication).

This patient is the first reported case in which a protruding papillary LAM lesion on the left diaphragm was confirmed to be the channel through which chyle leaked from

the peritoneal space to the pleural cavity. The putative mechanisms of chylothorax formation in LAM include the following: 1) the leakage of chyle from the thoracic duct or its tributaries as the result of the proximal lymphatic obstruction or direct involvement by LAM, 2) general oozing from pleural lymphatics or collateral vessels, and 3) transdiaphragmatic flow of chylous ascites (10). Porous diaphragm syndrome, an anatomical defect of the diaphragm through which a substance passes from the peritoneal cavity to the pleural space, is a possible explanation of the transdiaphragmatic flow of chyle (13). However, the defect may favour the right side of the diaphragm in this syndrome, and the condition is known to be associated with catamenial pneumothorax (14), hydrothorax complicating peritoneal dialysis (15, 16) or Meigs's syndrome (13, 17). We were unable to detect a fenestra on the left diaphragm of the patient under thoracoscopy. We instead directly observed the leakage of chyle through the papillary LAM lesion. Furthermore, the pleural effusion disappeared immediately after the surgical resection of the lesion. Consequently, the transdiaphragmatic flow of chyle from the abdominal cavity through the diaphragmatic lesion was considered to be the etiology of the chylothorax in this patient. Morimoto et al reported that chylorrhea from swollen vessels on the right diaphragm was observed with thoracoscopy in a Japanese LAM patient who had a right chylothorax (18); however, there was no evidence of ascites although an abdominal CT scan revealed iso-density masses in the retroperitoneum that were compatible with lymphangiomyomas. Unfortunately, it was unclear in that patient whether the lesion was affected with LAM and LAM-associated lymphangiogenesis since the histopathological evaluation of the swollen vessels on the diaphragm was not conducted.

The human diaphragm has naturally extensive lymphatic networks consisting of stomata, lymphatic vessels, and lacunae (19-22). Two layers of lymphatic vessels are present in the muscular portion of the peritoneal diaphragm while one layer is present in the tendinous portion (20, 22). The existence of pleural stomata remains controversial. Li observed round or oval stomata on the diaphragmatic pleura using scanning electron microscopy (23) while Shinohara et al did not (22). Because LCCs travel in the lymphatic stream, a diaphragmatic LAM lesion may develop when LCCs anchor at a specific point inside the lymphatic network in the diaphragm. Alternatively, LCCs in ascites may be implanted onto the diaphragmatic peritoneum through lymphatic stomata, thus causing LAM lesions in the diaphragm. To date, pathological findings of the diaphragmatic LAM lesion are not commonly documented, with the exception of our previous report including the present patient and five autopsy cases (12). In that study, we examined the diaphragms from six patients with LAM, including two patients with a history of chylous pleural effusion, two patients with a history of chylous pleural effusion and ascites, and two patients without a history of chylous effusion. Diaphragmatic LAM lesions were identified in three out of the four patients with a

history of chylous effusion; however, these lesions were not detected in two autopsy cases without a history of chylous effusion (12). These results suggested the possible pathogenic relationship between chylous effusion and diaphragmatic LAM lesions. Since one patient (LYTK59) (12) had a diaphragmatic LAM lesion only on the pleural side of the left diaphragm, it is plausible that pleural stomata may exist in some patients or develop due to some disease-related condition.

Consistent with the extensive lymphatic involvement in LAM, a lymphangiogenic growth factor, VEGF-D, is expressed by LAM cells and is increased in the serum of LAM patients (24). In this patient, the VEGF-D serum level rose to 2,861 pg/mL; moreover, the VEGF-D levels in pleural effusion and ascites were much higher than that in the serum. The appearance of pleural effusion and ascites in this patient was bloody chyle (Figs. 1D, 3A), suggesting that the chyle was not simply oozing from the normal lymphatic stream or leaking due to the proximal obstruction of lymphatic vessels. Kopfstein et al used an elaborate transgenic mouse model to demonstrate that VEGF-D induced lymphangiogenesis with blood-lymphatic vessel shunts (25). Accordingly, LAM-associated lymphangiogenesis seems to play a central role in LAM-associated chyle leakage, especially

in this patient. A surgical ligation of the thoracic duct is often performed in order to control refractory chylous effusion (3, 10). However, thoracic duct ligation may fail to control chylothorax or its effect lasted transiently and chylothorax recurred (26). Together with the observation that the prevalence of chylous effusion increased during the course of the disease, these findings may indicate that, not only the lymphatic obstruction or direct involvement by LAM, but also the occurrence of an abnormal communication between the pleural or peritoneal space and the lymphatic circulation through LAM-associated lymphangiogenesis thus play a role in the development of chylothorax and ascites in LAM patients.

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