

LYMPHATIC DRAINAGE OF THE PLEURA AND ITS EFFECT ON TUMOR METASTASIS AND SPREAD

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1- Lymphatic Drainage of Pleura; Overview of Basic Anatomy, Recent Studies

The pleura is the serous membrane that covers the lung parenchyma, mediastinum, diaphragm, and rib cage. The pleura is divided into the visceral pleura, which covers the lung parenchyma and inter-lobar fissures, and the parietal pleura that lines the inside of each hemithorax along the chest wall. These pleura fuse at the hilum where they form the pulmonary ligament (1).

Visceral and parietal pleura are both derived from intra-embryonic coelom which is already covered by mesothelial cells by the 7th gestational week. The coelom is divided into the pleural and peritoneal cavities by the septum transversum which arises from the ventral, left and right pleuroperitoneal folds and from the posterior walls. This process ultimately separates the two pleural cavities from the pericardial cavity (1).

Normal pleura exhibits 5 layers via light microscopy; 1. a single layer of mesothelial cells; 2. a thin submesothelial connective tissue layer, including a basal lamina; 3. a thin superficial elastic layer; 4. a loose connective tissue layer; and 5. a deep fibroelastic layer. It is the loose connective tissue layer that serves as the plane of dissection for extrapleural pneumonectomies (1).

Pleural fluid is present between the visceral and parietal pleural layers and is responsible for lubrication. Mesothelial cells line the pleural cavity and are characterized by abundant microvilli and pinocytotic vesicles, with cells at the cranial aspect having less microvilli than those in the caudal portion. Water, and small molecules less than 4 nm,

can pass freely between the mesothelial cells and across the basal lamina, whereas particles of 1000 nm, are engulfed but not transported. Lastly, removal of large particles or cells from the pleural cavity depends on transport via the pleural lymphatics, which cycle pleural fluid at a rate of 0.4 mL/kg/h (2).

The lymphatics within the parietal pleura run along the intercostal spaces and are virtually absent over the ribs. These lymphatic vessels drain ventrally toward nodes along the internal thoracic artery and dorsally toward the internal intercostal lymph nodes near the heads of the ribs. In contrast, the visceral pleura is very rich in lymphatic vessels, with an intercommunicating "network" arranged over the lung surface and penetrating into the lung parenchyma to join the bronchial lymph vessels with drainage to the various hilar nodes. The larger lymphatic vessels in the visceral pleura have one-way valves which also direct flow toward the hilum of the lung (3).

Understanding lymphatic drainage of the lung and pleura is important in the proper assessment and oncologic management of patients with intrathoracic malignancies and there are a few studies investigating the dynamics of pleural lymphatic drainage in vivo. For example, Miura T et al, designed an experimental model utilizing the injection of carbon particles into the pleural cavity of Japanese monkeys. The entrance of carbon particles were identified in the subpleural lymphatic lacunae using stereoscopy and scanning electron microscopy (SEM) studies. These studies defined two populations of mesothelial cells, one rich in microvilli and the other with stomata. The stomata-rich mesothelial cells, present on the parietal pleura, were found to

be rich in carbon particles suggesting that larger particles and cells from the pleural space can migrate out of the pleural space and into the lymphatics via these mesothelial cells. Such a transport system may provide a mechanism for migration of malignant cells to distant organs in patients with positive pleural lavage cytology (4). Broaddus et al. investigated pleural transport by iatrogenically creating a pleural effusion by injecting an autologous protein solution at 10 mL/kg, with a protein level of 1.0 g/dL, into the pleural space of sheep. The artificial effusion was almost completely removed by the lymphatics in a linear manner at a rate of 0.28 mL/kg/hour. The calculated capacity for lymphatic clearance in this study is ~28 times greater than the normal rate of pleural fluid formation (2). The rate of pleural fluid drainage via such lymphatic pathways can be further facilitated by increased NO levels, which can occur in the setting of malignancy (5).

Pleural drainage in humans is less well characterized. The lymphatic relationship of mediastinal lymph nodes and diaphragmatic pleura was studied by Okiemy G et al. Subpleural lymphatics of 30 adult cadavers and 12 fetuses were injected with a modified Gerota's medium, with dissection of the subsequently visualized lymphatic vessels and nodes. The mediastinal lymphatic channels were demonstrated to arise from the mediolateral portion of diaphragmatic pleura in 29 of the 32 cadavers. On the right, lymphatics were observed to ascend within the inferior pulmonary ligament and IVC and then continue further up to the upper mediastinal nodes. On the left lymph vessels were ascending with pulmonary ligament connecting to intertracheobronchial and mediastinal nodes. Involvement of the esophageal nodes was also seen in most of the injections on both sides (6).

Most of the anatomical studies of pleural lymphatics have been done in cadavers or with particles not native to the pleural space, thereby introducing such confounding factors as active and passive migration, lymphatic absorption, and immunogenicity. Furthermore, our current understanding is based on investigations conducted with less advanced imaging techniques. For these reasons, reassessment of pleural physiology, lymphatic drainage patterns and trafficking, etc., using new approaches to in vivo imaging, will not only broaden our understanding of this fundamental process, but will also lead to potentially new treatment paradigms and diagnostic techniques.

2- Significance of Pleural Invasion; Relationship With Pleural Drainage

Differences in the lymphatic drainage of pleura vs. lung parenchyma are of more than academic interest and may be an important factor to take into account before tailo-

ring overall therapy. The study by Rahman et al focused on this relationship investigating patterns of lymphatic spread in patients with malignant pleural mesothelioma. Positive mediastinal lymph nodes were commonly noted despite the absence of disease in hilar nodes in patients with pleural invasion, whereas positive hilar lymph nodes were more associated with the invasion of lung parenchyma (7). They also noted unusual sites of spread such as the internal mammary nodes and paraesophageal nodes as predicted by the above study by Okiemy.

Pleural invasion by a parenchymal lung cancer is a bad prognostic factor and is associated with higher incidence of pN2 disease in patients. Manac'h et al studied more than 1,200 patients with visceral pleural invasion (VPI) and related this to prognosis. The presence of VPI was associated with tumor size >3 cm, adenocarcinoma histology, positive N2 disease, and a worse overall prognosis. The impact of VPI was an independent contributor to bad prognosis and its impact was found to be additive to the effect of nodal status (8). Similarly, Mizuno et al reported that in the documented absence of pleural invasion, prognosis is similar between IA and IB disease and that lymphatic and vessel invasion have less impact on prognosis than VPI, suggesting that pleural lymphatic drainage is a more efficient pathway for metastasis (9).

The prognostic significance of visceral pleura involvement and the subsequent increase in positive nodal status has prompted discussion of changes in the lung cancer staging system. The current TNM lung cancer staging system focuses on the T descriptors on tumor size alone. Several studies have proposed the incorporation of other prognostic factors, including pleural invasion. Shimizu et al reported that tumor size >3 cm and extension to the pleural elastic layer or surface resulted in similar survival to T3 whereas tumors <3cm exhibited survival similar to T2 tumors(10). The clinical importance of malignant pleural disease is reflected in the reclassification of T4 tumors with malignant pleural cytology as M1a based on the survival obtained from a pool of over 60,000 patients (11).

To date, most studies examining the clinical significance of pleural invasion have been descriptive in nature. Few have characterized the lymphatic drainage of the pleura in such a fashion as to be used therapeutically. Future innovative studies are sorely needed to allow us to gather more accurate information of the unique lymphatic pathways draining a specific malignancy and thus help identify those nodes at risk for metastatic spread.

3- Advances in Pleural Lymph Node Mapping

Evidence that VPI and subsequent lymphatic spread are significant prognostic factors has lead to several attempts

at lymphatic mapping of the pleural space with subsequent identification of sentinel nodes. Liu et al injected activated charcoal, latex and PLGA-rhodamine particles for the purpose of lymph node mapping of the pleural space in healthy rats, rats with orthotopic lung cancer or following pneumonectomy. Lymphatic mapping was superior when particles between 0.7 and 2 micrometers in diameter were used (12). They also noted that particle distribution in nodes with actual metastatic spread was peripheral in location, as compared to the uniform distribution noted in non-affected lymph nodes.

Real time lymphatic trafficking has been performed in vivo in rats and pigs by Parungo et al using near infrared (NIR) fluorophores (14). Due to low background and high NIR signal that is visible in intact tissue, these studies documented lymphatic drainage from the pleural space to superior mediastinal, intercostal, paraesophageal and extrathoracic lymphatics. Analysis at different time points demonstrated, in real time, travel of the labeled particle through different caliber lymphatic channels. Drainage of pleural lymphatics to such distant nodes as the superior mediastinal nodes could explain why positive cytology from a pleural effusion is associated with survival more akin to metastatic disease than local extension. Communication between abdominal lymph nodes and the pleural space was also demonstrated. Considering that nodal metastases is a major prognostic factor, additional studies to identify nodes at risk of metastases are extremely important. A second study using NIR fluorescence was performed by Parungo et al to demonstrate sentinel lymph node groups draining the pleural cavity (13). Quantum dots (QD), which are composed of an inner core of semiconductor nanocrystal and an outer coat of solubilizing organic material, emit NIR fluorescence while migrating through lymphatic channels in real time. Given their size of 20nm, they are filtered by the first nodes encountered and thus accumulate in the sentinel nodes and can be imaged in vivo to identify the location of the sentinel node intra-operatively. Superior mediastinal nodes were found to be the primary nodes for QD accumulation following pleural injection. This pattern of drainage also can be evident following contralateral injection, demonstrating the variability of pleural drainage and suggesting the potential importance of lymph node mapping in accurate staging for patients with pleural involvement (14). Since lymph node basins are not as directly accessible in thoracic surgery as in patients with breast cancer and melanoma patients, adaptation of NIR imaging systems for intraoperative diagnosis and planning holds great promise and is the focus of a recently funded clinical trial in patients with early stage lung cancer.

4- Discussion

Clinical observations and findings from studies of normal pleural anatomy have shown significant individual variability in lymphatic drainage pattern with ramifications in malignant disease progression and prognosis. Given the clinical implications of pleural invasion and subsequent lymphatic spread on prognosis, it is likely that markers of pleural involvement will be important to include in future staging systems. However, such progress is limited by the current inability to identify pleural involvement and lymphatic spread until late in the disease process or histologically at the time of surgical exploration. For the first time, new advances in imaging and lymphatic mapping now offer the potential to better stage patients with malignant pleural disease with the goal of developing more effective, individualized treatment strategies for these patients.

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