DYNAMICS OF PLEURAL FLUID EFFUSION AND CHYLOTHORAX IN THE FETUS AND NEWBORN: ROLE OF THE LYMPHATIC SYSTEM

C. Bellini, Z. Ergaz, F. Boccardo, T. Bellini, C.C. Campisi, E. Bonioli, L.A. Ramenghi

Neonatal Intensive Care Unit (CB,LAR), IRCCS Gaslini Institute, Genoa, Italy; Neonatal Intensive Care Unit (ZE), Department of Neonatology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; Department of Pediatrics (TB, EB), University of Genoa, Genoa, Italy; Department of Surgery - Unit of Lymphatic Surgery (FB), IRCCS S. Martino University Hospital, IST National Institute for Cancer Research University of Genoa, Italy; and Department of Surgery - Unit of Plastic and Reconstructive Surgery (CCC), IRCCS S. Martino University Hospital, IST National Institute for Cancer Research University of Genoa, Italy

ABSTRACT

Pleural fluid effusion particularly chylothorax is a relatively rare occurrence in the newborn, but when it occurs it is often life-threatening. In this article, we describe and illustrate the morphologic features of the visceral and parietal pleura including pleural lymphatics and the physiology and patho-physiology of pleural fluid balance. The role and function of the lymphatic system in controlling the volume and composition of pleural liquid are detailed and a conceptual scheme presented. Finally, the crucial role of inadequate lymphatic drainage (either functional overload from an imbalance in Starling forces or mechanical insufficiency from lymphatic dysplasia) is emphasized.

Keywords: perinatal pleural effusions, congenital chylothorax, newborn, hydrops fetalis, lymphatic dysplasia, lymph dynamics, pathophysiology

Fluid accumulation in the pleural space is commonly defined as pleural effusion. This is a rare event occurring during the neonatal period. Different types of congenital and acquired effusions have been described in the newborn (1,2). Chylothorax, hydrops fetalis, mediastinal extravasation of percutaneously inserted central venous catheter, parapneumonic effusion, and congestive heart failure are the main causes of pleural effusion in the neonate.

Congenital chylothorax (CC) is usually defined as the accumulation of intestinal lymph in the pleural space. Although it is rare indeed, with an estimated prevalence ranging from 1:8,600 to 1:10,000 live births (1-6), CC is the most common cause of congenital pleural effusion during the neonatal period. The aim of this article is to review congenital chylothorax and to provide recent insights on pathophysiology, with particular emphasis on lymphatic involvement.

In general, chylothorax can be classified as traumatic or non-traumatic (7). Traumatic cases can have an iatrogenic or non-iatrogenic (20% of traumatic cases) origin. Thoracic surgery, thoracic duct damage following subclavian vein catheterization, duct blockage due to central venous catheter-related venous

thrombosis, thoracic duct damage following fracture or dislocation of the spine, and childbirth are various conditions reported as causes of traumatic chylothorax of the newborn (8). Various causes of non-traumatic chylothorax, including malignancy, sarcoidosis, retrosternal goiter, amyloidosis, superior vena cava thrombosis, benign tumors, congenital duct abnormalities, lymphangioleiomyomatosis (LAM), and hemangiomatosis are reported (9-11). Many of the listed etiologies are unlikely to be found in the newborn or they are not present during the neonatal period. Lymphatic dysplasia is reportedly a very rare cause of chylothorax. This would be true if we considered both childhood and adult life. However, during the neonatal period and in general early in life, the impact of lymphatic vessel malformations or lymphatic dysplasia syndromes in causing CC may be considered very important.

The first question is: what is the role of the lymphatic system in pleural liquid balance? In this review, we will attempt to answer this question by focusing our attention on the relationship between lymphatic dysplasia and CC, then by evaluating recent insights into the role of lymphatics in the pathophysiology of CC, and more generally, into the role of the lymphatic system in the physiology of the pleural liquid balance.

ESSENTIALS ON MORPHOLOGICAL FEATURES OF THE PLEURA AND PLEURAL LIQUID TURNOVER

Before dealing with the pathophysiology of pleural liquid balance, we must make some preliminary remarks. What is currently known and what is currently hypothesized is mostly based on animal research. Almost nothing is known about the mechanisms regulating pleural fluid filtration or reabsorption in the newborn and even less in the fetus. Thus, what will be described in this article must be treated with caution with regard to the neonatal age.

Structure of the Pleura

The pleura is divided into the visceral pleura, which covers the lung parenchyma and the interlobular fissures, and the parietal pleura, which lines the inside of each hemithorax along the chest wall. These two pleura fuse at the hilum, thus forming the pulmonary ligament. Visceral and parietal pleura both derive from the intraembryonic coelom which is already covered by mesothelial cells by the 7th gestational week. The septum transversum divides the coelom into the pleural and peritoneal cavities and then this process finally divides the two pleural cavities from the pericardial cavity. On light microscopy, the pleura is characterized by 5 different layers (see points A to E in *Fig. 1*, respectively: A) a single layer of mesothelial cells; B) a thin connective tissue layer including a basal lamina; C) a thin superficial elastic layer; D) a loose connective tissue layer containing blood and lymph vessels; and E) a deep fibroelastic layer that is absent in the visceral pleura of small mammals (12). The thickness of the visceral pleura markedly changes among the species and the regions of the lung (13). Most mesothelial cells are flat, like endothelial cells, and the rest are cuboidal, roughly similar to epithelial cells (12,13). On the luminal side, the flat mesothelial cells are joined by double-stranded tight junction complexes (*Fig. 2*), similar to those of the venular endothelium (12,14). The mesothelial cells have pinocytotic vesicles and are covered by microvilli whose density is generally higher in the visceral than in the parietal pleura, and in the caudal than in the cranial regions. Under physiologic conditions, mesothelial cells secrete numerous glycosaminoglycans, proteoglycans, and phospholipids that constitute a glycocalyx surrounding the cells, thus providing a protective barrier against abrasion and a slippery, non-adhesive surface for intracoelomic movement (15). Water and molecules less than 4 nm can pass passively between the mesothelial cells, also crossing
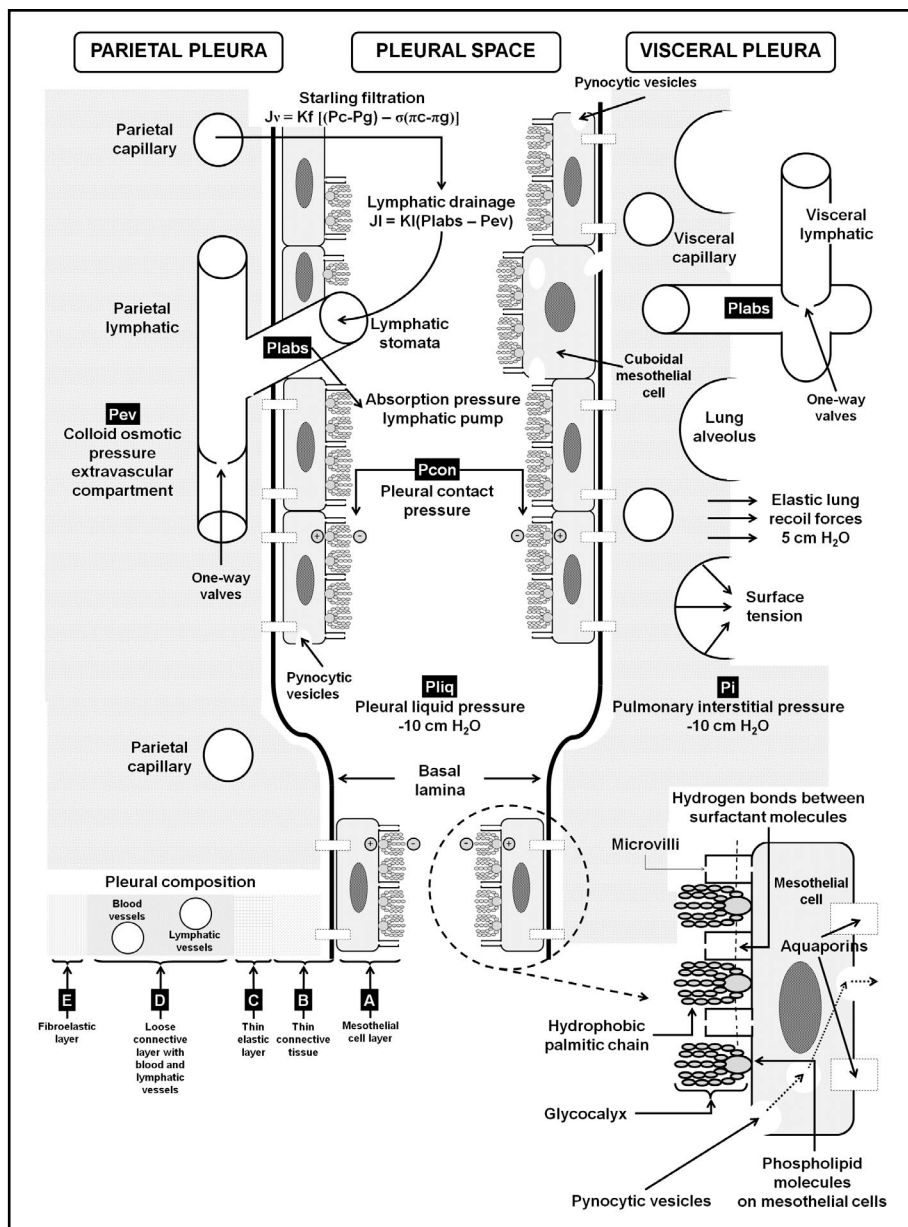
Fig. 1. Schematic diagram of the pathways for normal pleural liquid turnover. Under physiologic conditions, pleural liquid is mainly a filtrate from parietal capillaries in the parietal pleura lining the chest wall. Close control of the volume and composition of the pleural liquid is needed to maintain an efficient balance between liquid entering the pleural space through the parietal pleura down a net filtering pressure gradient and liquid removal by lymphatic drainage through the stomas of the parietal pleura, and by mechanisms involving visceral pleural cellular activity. Arrows from parietal capillary to lymphatic stomata indicate the main flow direction. Physiologic drainage of pleural liquid occurs via lymphatic stomata which are present exclusively in the parietal pleura. The pleural cavity is enclosed between parietal and visceral mesothelium layers; the mesothelium consists of a monolayer of squamous-like flat cells; flat mesothelial cells contain few microtubules, microfilaments, vesicles and vacuoles; cells are connected by tight and adherens junctions, gap junctions, and desmosomes (see Fig. 2). Cells present numerous microvilli, especially on the visceral side. Fewer cuboidal cells are present among flat cells. See text for further details.
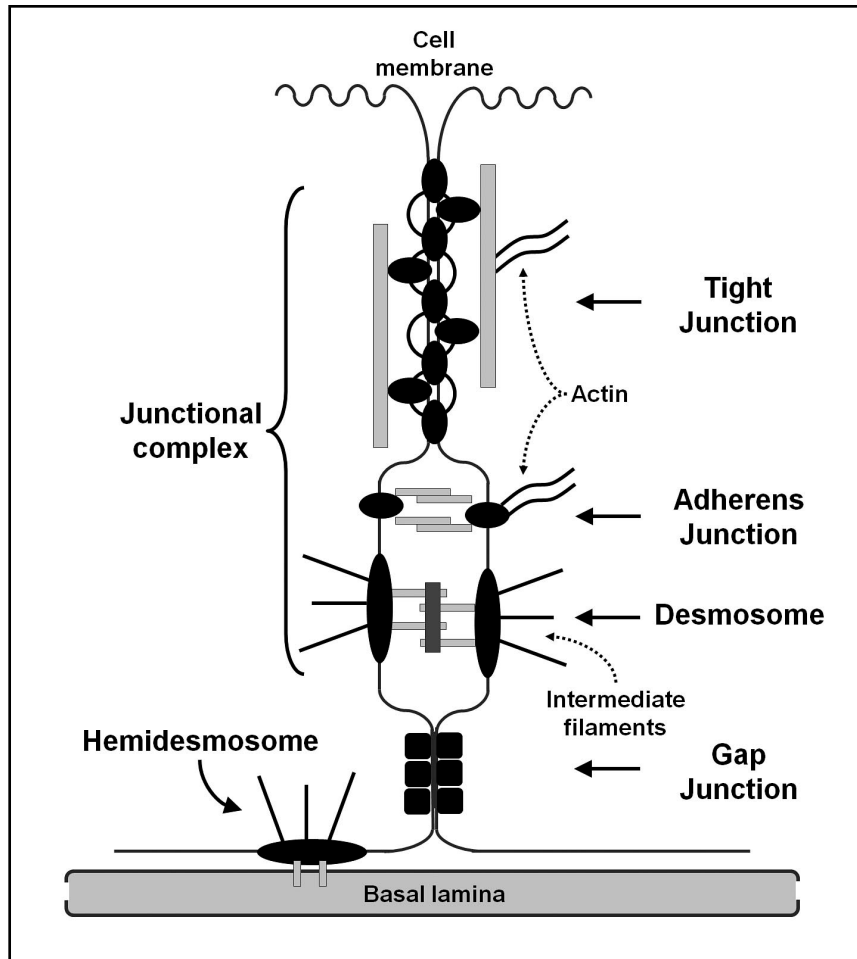
Fig. 2. Schematic diagram of the various cell junctional complexes illustrating the relative locations of tight junction, adherens junction, desmosome, gap junction, and hemidesmosome. Adherens junctions are associated with actin filaments, usually forming an adhesion belt around the cells. Desmosomes form cell-to-cell contacts and are associated with intermediate filaments. Tight junction barriers regulate paracellular movements of solutes down their electro-osmotic gradients. They also act as a fence that maintains differential composition of basolateral and apical membrane domains, limiting the diffusion of lipids and proteins among cellular compartments. Adherens junctions are the building blocks of tissue architecture and can facilitate signaling pathways to govern morphogenesis, tissue homeostasis, and even intercellular communication. Desmosomes and hemidesmosomes are specialized in cell-to-cell and cell-to-extracellular matrix adhesion. They are important in cytoskeletal organization, cell signaling, and tissue patterning. Collectively, these structures form the junctional complex. Behind the junctional complex lie the gap junctions. Gap junctions are clusters of tightly packed channels that allow small molecules (metabolites, second messengers, and ions) to travel between adjoining cells. Cells, in turn, rest on basal lamina, which is composed of extracellular matrix.

the basal lamina. Larger molecules, particles, and cells are cleared and then transported from the pleural cavity via the pleural lymphatics. The blood supply to the visceral pleura comes from the pulmonary circulation in species with thin pleura but from the bronchial (systemic) circulation in the other species, like humans. In all species, however, the blood from the visceral pleura is drained by the pulmonary veins (16). The endothelial
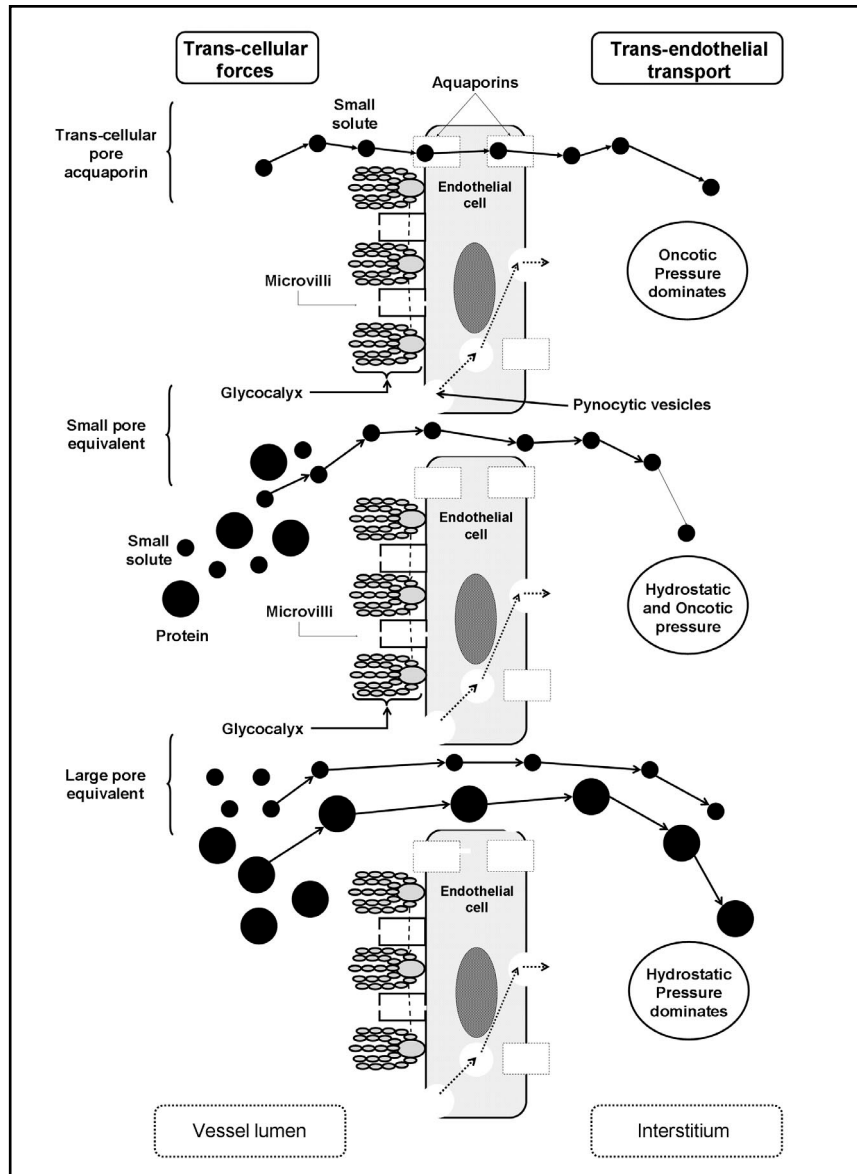
Fig. 3. Schematic diagram of fluid transport across the microvessel endothelial barrier. Fluid moves through paracellular pathways according to Starling forces (see text and Fig. 1). The microvessel endothelial barrier is portrayed as four main points across the endothelium. Trans-aquaporin transport (A), intercellular gap (cell junctional complex) lined by a dense glycocalyx layer (B), intercellular gap (cell junctional complex) with a less dense glycocalyx layer (C), and transport through cells via vesicles (D,1) or transendothelial channels (D,2). Transcellular fluid transport forces are listed on the right. Partially modified from (17).

barrier is portrayed as three points of transfer across the endothelium: transcellular, water-only aquaporin; intercellular gap lined by a dense glycocalyx (equivalent to the "small pore" model); intercellular gap with a less dense glycocalyx that allows macromolecules to pass through (equivalent to the "large pore" model) (17) (*Fig. 3*).

Origin and Structure of Pleural Lymphatics

In general, the origin of the lymphatic vessels may vary in different tissues. In the connective tissue, there are variously formed fissures or clefts filled with fluid. They freely communicate with one another and lead into the beginnings of the network of lymphatic capillaries. The lymph capillaries run midway between the blood capillaries, and are made up of a single layer of nucleated endothelial cells. Lymphatics may originate by encircling the blood vessels in the shape of channels. If lymph vessels originate on the surface of serous membranes, as in the case of the pleura, they are intimately connected with the lymphatic system, thus inordinately and randomly forming lymph spaces (16,18).

The organization of the lymphatics differs between the visceral and parietal pleura. In the visceral pleura the lymphatic network is widely represented, penetrating into the lung parenchyma to join the bronchial lymph vessels. The lymphatics of the parietal pleura run along the intercostal spaces and are virtually absent over the ribs (16,18,19).

In most parts of the endothelial surface of the serous cavities are a number of so-called stomata, or small apertures surrounded by a few cells which differ from ordinary endothelial cells in many respects. In particular, they present a unidirectional valve system which exerts a sort of control in the passage of the fluid from the serous cavity into the lymph vessels. These stomata are found at the commencement of the dense network of lymph capillaries which lie in the subserous tissue. The two main differences between parietal and visceral pleura are that only the parietal pleura presents stomata, i.e., 2-12 μm lymphatic openings situated between mesothelial cells (16,18,19), and that the parietal pleura tends to be uniform in thickness among various species, unlike the visceral pleura whose thickness varies. Humans present intermediate visceral pleural thickness (16,18,19). This architecture has

important consequences on pleural fluid formation and removal.

ENDOTHELIAL CELL PLASTICITY

Pleural Space and Pleural Liquid

Understanding of the fluid mechanics of the pleural space has undergone continuous evolution. The hydrostatic equilibrium of pleural liquid and the surface forces due to the opposing elastic recoils of the lung and chest wall are fundamental principles. An important function of the liquid in the pleural space is to lubricate the pleural surfaces. It is of the utmost importance that the thickness of the lubricating layer is maintained uniform; this feature is obtained by the recirculation of pleural liquid driven by gravity, ventilatory and cardiogenic motions. Liquid enters the pleural space through the parietal pleura down a net filtering pressure gradient (19,20). Fluid movements between vascular and interstitial spaces are regulated by the filtration of fluid across the capillary wall as described by the Starling equation [$J_f = K_f * (P_c - P_g) - \sigma(\pi_c - \pi_g)$] (*Fig. 1*) which describes the role of hydrostatic and oncotic forces (the so-called "*Starling forces*") in the movement of fluid across capillary membranes. In *Fig. 1*, K_f represents the capillary filtration coefficient; ΔP ($P_c - P_g$), the transcapillary hydrostatic pressure gradient derived from the difference between the capillary hydrostatic pressure (P_c) and interstitial hydrostatic pressure (P_g); $\Delta\pi$ ($\pi_c - \pi_g$), the oncotic pressure gradient derived from the difference between the capillary oncotic pressure (π_c) and interstitial oncotic pressure (π_g); σ, the reflection coefficient; J_f, the net fluid movement between compartments per unit endothelial area; π_g, the oncotic pressure within the glycocalyx. A high K_f indicates a highly water permeable capillary bed and a low K_f reflects low capillary permeability.

Controversies exist on the magnitude of filtration rate K_f. The difficulties in estimating J_f are that the involved parameters are

not precisely established, and there are wide variations among different species (20-22).

Vesicular transport provides an additional contribution in the endothelium (20-22). Permeability to protein is relevant in determining transpleural colloid osmotic pressure (23-27). The transfer of liquid and solutes across cell layers is often described as occurring through aqueous channels ("pores"). The pore theory of permeability (*Fig. 2*), which was formulated for microvascular endothelium (23), provides a reference model in this regard. In the model, small solutes, like inulin, spread through "small pores" with a radius of approx 4-5 nm, represented by clefts in the intercellular junctions. Macromolecules, which are too large to diffuse through these "pores," use a population of "large pores" with a radius of approximately 20-30 nm, less numerous than small "pores" (1:4), which could either be real openings or ones created by the transient fusion of vesicles through the cell (21,22). Vesicular transcytosis transport contributes to the transfer of all molecules and is essential to transfer large macromolecules. In addition to these pathways, water also flows through aquaporin water-exclusive channels of the cell membrane (21,22). Aquaporins have been to be expressed in the placenta, fetal membranes and tissues and organs of the developing fetus (28,29). The concept of rigid intercellular gaps as pathways of solute transport has changed with the discovery of the glycocalyx (29,30). Glycocalyx is an extracellular coating of anionic polysaccharides, and this finding led to a revision of the classic Starling equation (*Fig. 1*). Abnormal glycocalyx causes increased capillary transendothelial permeability. This "pore" model, with its refinement of glycocalyx and aquaporin features, should be considered a functional simplification useful for understanding a very likely for more complex pathway (22,29).

Role and Function of Lymphatics

Strict control of the volume and composition of the pleural liquid is necessary to ensure efficient mechanical coupling between the lung and chest wall. Liquid removal is carried out by an absorptive pressure gradient through the visceral pleura, by lymphatic drainage through the stomas of the parietal pleura, and by cellular mecha-nisms. Mesothelial cells are metabolically active and possess the cellular features that are needed for the active transport of solutes, including vesicular transport of protein; microvilli, pinocytic vesicles, and electrolyte transport are topical mesothelial mechanisms (21,22).

The mesothelium presents a less perme-able barrier than previously believed and is similar to the microvascular endothelium. The parietal pleura is the only membrane which is involved in lymphatic drainage of pleural liquid. Direct lymphatic drainage through the stomas of the parietal pleura is crucial for removing particles and cells and is important for removing protein from the pleural space but should not be the main effector of fluid removal. When pleural liquid volume increases, an imbalance occurs in the forces involved in turnover, thus favoring fluid removal. In case of a primary abnor-mality of one or more of the mechanisms of pleural liquid turnover, a pleural effusion ensues. The factors responsible for pleural effusion may be subdivided into three main categories: those changing transpleural pressure balance, those impairing lymphatic drainage, and those producing increases in mesothelial and capillary endothelial permeability. Except for the first case, pleural fluid protein concentration increases above normal: this feature underlies the classifi-cation of pleural effusions into transudative and exudative.

The Lymphatic "Vacuum Cleaner"

Lymphatic drainage of the interstitial fluid is crucial. It is defined by the equation $Jl = Kl(Plabs - Pev)$ (*Fig. 1*), where Kl is the conductance of the initial lymphatics, and $Plabs$ and Pev are the absorption pressure of

the lymphatic pump and the pressure in the extravascular compartment, respectively. Plabs has been estimated to reach approximately -10 to -15cmH$_2$O, thus, supporting the concept of the lymphatics as a sort of vacuum cleaner (21,22). The lymphatic conductance Kl is a coefficient proportional to the extension of the lymphatic network. The Plabs - Pev difference has a negative value, thus indicating that lymph flow provides an outflow from the extravascular compartment. Note that Pev is sometimes also referred to as Pliq or as Pi, pressure in the pleural space and pressure in lung interstitial space, respectively, as shown in *Fig. 1.*

Although there may be some pressure differences among various body compartments, in general it can be affirmed that Starling dependent filtration is balanced by lymphatic drainage. Thus, under steady state condition, Jv + Jl is 0 (zero).

The relationship between volume and pressure of the extravascular fluid (Pev) is unequivocally determined by the extravascular compartment compliance of either the pleural or the lung interstitial space. Pleural space compliance (Pliq) is calculated to be -10 cm H$_2$O, and increases by 1 cm H$_2$O for each 10-fold increase in pleural liquid volume.

The rigid control mechanism resides in the ability of lymphatics to increase the draining flow in response to an increase in filtration rate. Considering the equation Jl = Kl(Plabs - Pev), whenever filtration increases, volume accumulates, thus making Pev less negative and increasing the driving pressure for lymphatics (Plabs - Pev). The modulating function of the lymphatics is cardinal since an increase in function by 20 times is possible due to the fact that the Plabs - Pev difference is minimal under steady state conditions (20,21,31).

The lymphatic conductance (Kl) is more than 10 times higher than the filtration coefficient of the parietal pleura (Kf). The presence of two membranes in series, such as the capillary endothelium and the parietal

mesothelium, creates a substantially high-resistance system for water and plasma proteins. The greater thickness of the visceral pleura essentially excludes it from pleural fluid turnover (20,21,31).

CLINICAL EFFECTS

The next question is: how can impaired lymphatic function lead to congenital chylothorax?

As reported above, the key factors that may be responsible for pleural effusion can be subdivided into three main categories: those changing transpleural pressure balance, those impairing lymphatic drainage, and those producing increases in mesothelial and capillary endothelial permeability. All these mechanisms can be involved in congenital chylous effusion. Very often they are tightly linked to each other.

In the fetus or newborn it is difficult to establish the exact role and contribution of lymph flow as related to the active water reabsorption and the Starling dependent absorption through the visceral pleura which supplies pleural fluid. Nonetheless, it should be noted that lymphatics can act as an efficient negative feedback system that provides efficient control of the pleural fluid dynamics setting a rather sub-atmospheric pressure due to their powerful draining action, and increased draining flow capacity in response to increased fluid filtration. Pleural lymphatics constitute a high-rate powerful mechanism which is able to guarantee the physiological steady-state condition. This cardinal equilibrium is lost when inflow rate exceeds lymphatics maximum draining capacity (32-34) or when a condition of lymphatic dysplasia leads to an impairment of lymphatic function and effectiveness.

The cardinal control mechanism is the ability of lymphatics to increase the drainage flow in response to an increase in filtration rate.

Considering the equation Jl = Kl(Plabs - Pev) (*Fig. 1*) reported above, Pev becomes

less negative as the volume accumulates, thus increasing the driving pressure for lymphatics, i.e., Plabs - Pev. In steady state conditions, the Plabs - Pev difference is approximately zero, with Pev very close to Plabs, although it can increase by 20 times. The equation Jl = Kl(Plabs - Pev) has been processed (33), and the results showed that $\Delta Jl / \Delta Pev = Kl$. In this equation, the ratio $\Delta Jl / \Delta Pev$ means the lymphatic capacity to increase for unit increase in liquid pressure. Thus, in other words, if the lymphatics are able to increase their flow, the ratio stays very close or equal to lymphatic conductance Kl. The higher the ratio, the smaller the increase in volume of the pleural or lung interstitial fluid. The lack of adaptation of lymphatics to increased flow inevitably leads to fluid accumulation (20,21).

In the case of congenital lymphatic dysplasia affecting the lung and the pleura, it is intuitive that the impaired capacity of lymphatics leads to a type of blockage of both interstitial tissue and pleural space drainage. The condition of lymphatic dysplasia is generally permanent, and although the degree of impairment may vary at various ages (35), one can expect that the lymphatic draining capacity will never reach normal function. The cases of insufficient draining capacity due to causes not directly affecting the lymphatics system structure, but affecting the function, is different. Nonimmune hydrops fetalis due to heart failure, for example, may lead to an increase in central venous pressure that strongly influences lymphatic flow or, as reported above, the $\Delta Jl / \Delta Pev$ ratio (5).

In conclusion, the efficacy of lymphatic function is the cornerstone of pleural fluid balance. Each prenatal or perinatal condition that can negatively influence the pleural and pulmonary lymphatic vessel function, including both primary or secondary causes, results in a lack of control of pleural fluid volume and a possibly difficult recovery from pleural effusion, thus leading to congenital chylothorax and not uncommonly, subsequent mortality.

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Carlo Bellini, MD, PhD
Neonatal Intensive Care Unit
Department of Intensive Care
IRCCS Gaslini
University of Genoa
Largo G. Gaslini, 5
16147 Genoa, Italy
Tel: 0039-56361
Email: carlobellini@ospedale-gaslini.ge.it