Hemostatic Properties of the Lymph: Relationships with Occlusion and Thrombosis

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Abstract

Lymphatic thrombosis is a rare occurrence, and although its frequency is likely underestimated, its burden remains substantially lower than that of venous or arterial thrombosis. Current evidence suggests that despite measurable levels of fibrinogen, von Willebrand factor and other coagulation factors in the lymph, fibrin generation is substantially inhibited under physiological conditions, essentially making the lymph a hypocoagulable biological fluid. Although factor VIIa-tissue factor-catalyzed activation of factor X is possible in the lymph, fibrin generation is largely counteracted by the unavailability of cell surface anionic phospholipids such as those physiologically present on blood platelets, combined with only low levels of coagulation factors, and the strong inhibitory activity of heparin, antithrombin, and tissue factor pathway inhibitor. Enhanced fibrinolytic activity further contributes to reduce the development and growth of lymph clots. Nevertheless, lymphatic thrombosis is occasionally detected, especially in the thoracic duct, axillary, or inguinal lymphatics. Pathogenetic mechanisms are supported by the release of thromboplastin substances from the injured lymphatic endothelium accompanied by chronic obstruction of lymph flow in the presence of a hypercoagulable milieu, thereby mirroring the Virchow triad that otherwise characterizes venous thrombosis. In theory, any source of lymphatic vessel occlusion, such as internal obliteration, external compression, or increased lymphatic pressure, might predispose to localized lymphatic thrombosis. The leading pathologies that can trigger thrombosis in the lymphatic vessels include cancer (due to external compression, neoplastic obliteration of the lymphatic lumen by metastatic cells, or lymphatic dysfunction after lymph node dissection), infections (especially lymphatic filariasis or sustained by *Chlamydia trachomatis*, *Mycobacterium tuberculosis*, *Treponema pallidum*, or *Streptococcus pyogenes*), congestive heart failure, chronic edema and inflammation of the distal lower limb, complications of central venous catheterization, coronary artery bypass grafting, thoracic outlet syndrome, and amyloidosis.

Keywords
- lymphatic thrombosis
- vessel
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- coagulation

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Historical Premise

Although it is clear that modern immunology had its origin in the mid-17th century, the original discovery of the lymphatic system is still largely debated.[1] Olaus Rudbeck, while dissecting small animals as a young student at Uppsala University, is believed to have been the very first scientist to describe the lymphatic connection between the intestines and the circulating blood, along with the direction of the flow in the lymphatic system.[2] However, as Rudbeck published his own discoveries, the Danish anatomist Thomas Bartholin reported identical observations nearly simultaneously. Nevertheless, Rudbeck charged Bartholin with predating this observation, giving priority of discovery to the latter, with a 752-word letter in Latin.[3] While most studies in the 17th century concerned the anatomical organization of the lymphatic system, those in the following century were mainly planned to elucidate its function. It is still surprising that another dispute emerged a century later, in the late 1750s, between a young Scotch medical graduate (Alexander Monro) and a noted London anatomist (William Hunter), who both advocated the paternity of the discovery of the function of the lymphatic system. Even more interestingly, the priority claims of both scientists were disputed by the earlier findings of an Englishman (Francis Glisson) a full century before.[4]

Biology and Function of the Lymphatic System

Two main components participate to form the lymphoid tissue, namely the interrelated lymphoid tissue and the lymphatic vessels. The former is mainly composed of lymphocytes and other blood cells, which can be structurally organized in primary lymphoid organs such as bone marrow and thymus, secondary lymphoid organs such as lymph nodes, or in less organized lymphoid follicles within the spleen, or representing the mucosa-associated lymphoid tissue in the digestive tract, liver, lung, tonsils, Peyer patches, adenoids, and skin.

The lymphatic system is formed by a complex network of vessels (i.e., the "lymphatic vessels" or "lymphatics"), the essential function of which is to carry a specific fluid called "lymph" unidirectionally from the peripheral tissues to the heart. Basically, the lymphatics can be divided into a peripheral compartment (i.e., from the interstitial space to and within the nearest lymph node) and a central compartment (i.e., efferent lymphatics, cisterna chyli, and thoracic duct).[5] Although the general structure of the lymphatic vessels might resemble that of blood vessels, there are several peculiarities that differentiate the two. Basically, lymphatics are blind-ending vessels with a single, nonfenestrated internal endothelial cell layer, which is tailored to the absorption of fluid and macromolecules, as well as to the transit of cells. Lymphatic vessels are typically characterized by an irregular and wider lumen as compared with blood vessels, their endothelium is attenuated, lies at the surface of an incomplete basement membrane, is not invested by pericytes, and is tightly anchored to the interstitial collagen by filaments. There are also overlapping intercellular junctions, formed by the superimposition of adjacent endothelial cells, which can be opened by increased interstitial fluid pressure to allow the transit of fluid and molecules. These junctions are, however, rapidly closed, thus preventing the retrograde flow of molecules and fluids back into the interstitium, once the fluid entering the lumen is in sufficient amount to reduce the pressure gap.[6]

The endothelium layer is surrounded by smooth muscles, which are arranged circularly around the endothelium. The outermost layer is the adventitia, which consists of fibrous tissue. In analogy with blood vessels, the smaller the caliber of the vessel, the fewer the number of layers, so that the smallest vessels (e.g., lymph capillaries, that are specialized to form the lymph from the interstitial fluid) lack both a muscular layer and adventitia, whereas the largest ones (e.g., lymphatics, that are specialized to transport the lymph) are characterized by a robust and thick wall.

Moreover, at variance with the cardiovascular system, the lymphatic system is "opened," and has no central propulsion from the heart.[6] The movement of the lymph within the vessels is hence facilitated by internal peristalsis of the smooth muscle layer and the external compression due to contraction of surrounding skeletal muscles and pulsation of the near arteries (e.g., the spontaneous and rhythmic contraction of lower limb lymphatics allow the transport of 20 to 250 mL of lymph in a 70-kg man, daily).[7] The lymphatic vessels are also equipped with a two-valve system. The primary system, which is peculiar to initial lymphatics, is located at the endothelium level and cooperates with the secondary (classical) intralymphatic system to permit the unidirectional flow during periodic compression and expansion of lymphatics.[8]

The main function of the lymphatic system is to complement the blood vascular system by regulating tissue fluid balance, facilitating interstitial protein transport, providing essential immunological functions, and transport fat and other components absorbed from the intestine.[6] The lymph generated from the interstitial fluid within the lymph capillary is mainly derived from permeated plasma proteins that enter the interstitium, metabolic products of connective tissue cells, and proteins synthesized and secreted by the lymphatic endothelium. Nevertheless, lymphatic endothelial cells are as heterogeneous as blood vascular endothelial cells.[9]

The leukocyte count in the thoracic duct in healthy men varies from 2 to 20 $\times$ 10^9 and consists almost totally of lymphocytes (i.e., nearly 2 to 10 times the number of lymphocytes that can be found in the peripheral blood), whereas the number of erythrocytes is negligible under physiological conditions, but might increase remarkably in certain disorders characterized by marked capillary damage and extravasation.[10,11] The fluid, the macromolecules, and the cells are transported to the lymph node system and then—through the lymph vessels—to the right and left thoracic ducts, before being reversed into the right or the left subclavian vein, where the lymph is finally mixed with the blood.

Hemostatic Properties of the Lymph

Studies on the hemostatic properties of the lymph are scarce and mostly dated. This field has received poor attention due to a variety of reasons, including objective difficulties in collecting lymph fluid and the paucity of thrombotic and hemorrhagic disorders involving lymphatic vessels. Although the lymph is not completely devoid of hemostatic factors, their relative content is substantially lower than that of the blood and also varies widely according to the composition of the interstitial fluid in the surrounding anatomic regions (e.g., thoracic duct lymph is enriched in several hepatic hemostatic factors), as well as from the molecular weight of the molecule (e.g., due to the dimension of the intercellular junctions, the transit across the endothelial barrier is inversely correlated with the mass of the protein).[12]

One of this first studies designed to investigate the clotting properties of the lymph was performed by Eugene L. Opie, who observed that the coagulation of lymph is slow when compared with that of blood, and attributed this to the scant amount of "thrombokinase" (i.e., activated factor X) present in the lymph, which is further unbalanced by the excessive presence of antithrombin.[13] Nevertheless, thrombosis within lymphatic channels could be triggered by liberation of thrombokinase by the tissues in the wall of the lymphatic (e.g., following necrosis of cells in contact with the lymph stream), or injection of bacteria (*Bacillus pyocyaneus, Staphylococcus pyogenes aureus*) into the blood, or by infection of the tissues in the neighborhood of the lymphatic channel. In 1914, Howell obtained the lymph from anesthetized dogs by placing a cannula into the thoracic duct.[14] In animals starved for 48 hours, whose lymph had little evidence of fat, clotting took place from 10 to 20 minutes after sample collection. Conversely, in the lymph collected immediately from fed animals, whose lymph appeared enriched in chyle fat, clotting was achieved after 30 to 60 minutes in samples collected immediately after feeding, whereas it required 1 to 3 hours in those specimens collected 2 hours after feeding. No evidence of thrombocytes (i.e., platelets) was reported in lymph.

In 1941, Brinkhous and Walker first reported that in dogs the average prothrombin concentration is 51% of the plasma concentration in thoracic duct lymph, but decreases to 8% of the plasma concentration in the femoral lymph.[15] Copley and Lalich assessed the bleeding time in 25 men and compared it with the "lymph time" (i.e., measuring the time of clotting of a whitish flow which was observed alone or simultaneously with the red flow after the incision of the thoracic duct), failing to observe any apparent correlation between these measures. Nevertheless, "lymph time" was always much prolonged than the bleeding time.[16]

In a further study, Fantl and Nelson introduced a cannula in the thoracic duct of dogs, which were left without food for 5 to 12 hours.[17] Blood was also obtained from the jugular vein. The clotting times of lymph in silicone-coated tubes spanned over a 16- to 300-minute range whereas considerably shorter times (i.e., 6 to 12 minutes) were recorded when lymph was collected in glass containers, thus suggesting that lymph does not physiologically contain active thromboplastin. It was also observed that the clots did not retract in the lymph specimens, a phenomenon that has been attributed to the lack of thrombocytes in lymph. In most cases, however, the lymph specimens exhibited a longer coagulation time than plasma (e.g., the recalcification time was on average two times longer, whereas the thrombin time was on average prolonged by ~14%). In agreement with previous studies, the activity of prothrombin and the concentration of fibrin were both reduced by ~40% in lymph as compared with plasma. Finally, a test for fibrinolytic activity performed in lymph specimens failed to show any fibrinolytic activity.

Blomstrand et al performed coagulation studies on plasma and thoracic duct lymph collected after introduction of a polyethylene catheter in two patients.[18] The one-stage prothrombin time was in both cases ~20% longer in lymph than in plasma, whereas the recalcification time was 11 to 32% shorter in the lymph. In one subject the prothrombin and factor VII concentration of lymph was almost identical between lymph and plasma, whereas in the other patient it was markedly reduced (i.e., 39% of the plasma value). Reduced concentration of fibrinogen (37 and 46% in the two patients), factor V (18 and 22%), factor VIII (69, only one patient studied), and factor IX (85%, only one patient studied) were consistently observed in lymph as compared with plasma. Although the concentration of antithrombin was found to be identical between lymph and plasma, plasminogen and proactivator activities of the lymph samples were 63 and 36% lower than in plasma. The lymph samples contained no antiplasmin and the so-called "antiactivator activity." Finally, the inhibiting effects of lymph on plasminogen activation by streptokinase and urokinase were 0 to 58% and 15% (only one patient studied) of those recorded in plasma, respectively.

It is also noteworthy that although platelets are absent from the lymph, human thoracic duct lymph contains phospholipid components similar to those present at the platelet surface, although the relative distribution differs. Mayanskii and Minnibaev performed experiments in dogs to compare the coagulant capacity and fibrinolytic activity of blood and lymph.[19] In agreement with previous evidence, the time of recalcification and the thrombin time of the lymph was significantly increased, whereas the concentration of fibrinogen and tolerance of heparin were reduced as compared with blood. The fibrinolytic activity of the lymph was instead remarkably higher than that observed in blood. Kuznik et al also observed that lymph coagulation in dogs might occur much more slowly than in blood, and this was attributed to the low concentration of all coagulation factors and to an increased level of heparin.[20] The fibrinolytic activity of lymph was also found to be higher than that of blood, probably due to the low concentration of inhibitors of the fibrinolytic pathway. Interestingly, it was suggested that the lymphocytes present in the lymph were efficient surrogates of blood platelets during lymph clotting. The hypocoagulability of the lymph was also confirmed in animal models of heterotransfusion shock, whereby hypocoagulation and hyperfibrinolysis observed in blood was accompanied by incoagulability of lymph, ultimately attributed to a sharp increase of heparin.[21]

Müller and Danckworth investigated the presence of coagulation factors in thoracic-duct lymph of dogs.[22] The coagulation activities were found to be substantially lower than those typically measured in the plasma, that is, 30% of factor I, 60% of factor II, 20% of factor V, 50% of factor VII, 30% of factor VIII, 25% of factor IX, 70% of factor X, and 30% of factor XIII. The concentration of antithrombin was also 60% of that of plasma. Finally, platelets were completely lacking in thoracic lymph. Garaev and Mirzabekova created an occlusion of the femoral artery by ligation and investigated the coagulation of blood and lymph.[23] One day after occlusion, hypercoagulation occurred in both blood and lymph. In the latter fluid, the clotting time was reduced by 37 to 49%, whereas the prothrombin time was shortened by ~26%. Heparin tolerance and fibrinogen concentration were instead increased by 47 to 50% and 30 to 39%, respectively.

Le et al measured the concentration of a variety of hemostatic factors in rabbit limb lymph, and compared it with that present in plasma,[24] observing that the relative content of lymph was 28% for fibrinogen, 27% for factor X, 26% for prothrombin, 17% for factor VII, and 8% for both factor V and VIII. The concentration of lymph von Willebrand factor (VWF) antigen, which is most probably produced by lymphatic endothelial cells, was also very low (i.e., 5%, range 2 to 10%) as compared with plasma, and mainly consisted in lower molecular weight multimers. The concentration of the natural inhibitors was also markedly reduced as compared with plasma (i.e., 40% for tissue factor pathway inhibitor [TFPI], and 38% for antithrombin), whereas fibrin degradation products (FDPs) were virtually unmeasurable. Interestingly, lymph fibrinogen was however clottable.

More recently, Miller et al collected peripheral afferent lymph by cannulation of a collecting vessel in healthy men.[25] Both activity and concentration of most clotting factors were confirmed to be dramatically reduced in lymph as compared with plasma. In particular, the percentages in lymph as compared with plasma were 5% activity and 26% antigen for fibrinogen, 47% antigen and 50% activity for prothrombin, 21% antigen and 4% activity for factor V, 29% activity and 13% antigen for factor VII, 4% activity for factor VIII, 13% activity and 9% antigen for factor IX, 25% activity and 28% antigen for factor X, 49% antigen for factor XII, and 12% antigen for VWF. As regards the natural inhibitors, the percentages in lymph as compared with plasma were 43% activity and 4% antigen for TFPI, 65% antigen for antithrombin, 24% antigen for protein C, 11% antigen for protein S, 55% for α-2 antiplasmin antigen, and 9% for α-2 macroglobulin. The plasma D-dimer assay averaged 187 fibrin equivalents/mL, whereas the lymph concentration exceeded 1250 fibrin equivalents/mL in 90% of subjects (i.e., more than fivefold higher in lymph than in plasma). Interestingly, the ratio between plasma and lymph for activated factor VII and for the TFPI-Xa complex was remarkably high, being 2.21 and 1.45, respectively.

Interesting results emerged from a recent proteomics analysis of normal ovine lymph by protein chip technology, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS), two-dimensional polyacrylamide gel electrophoresis (2-DPAGE), and mass spectrometry (MS). Overall, SELDI-TOF-MS analysis showed three exclusive protein peaks only contained in lymph, and five exclusive protein peaks only contained in plasma. As regards the hemostatic factors, the lymph contained peaks attributable to fibrinogen α- and β-chains, fibrinogen γA-chain, α2-macroglobulin, plasminogen, prothrombin, α2-antiplasmin, and antithrombin.[26] A further study focused on the proteomic evaluation of postshock mesenteric lymph, revealed the presence of protein released from tissue injury, depletion of protective protease inhibitors, increased lipid carriers as well as depletion of coagulation factors.[27]

As the results of previous studies that have assessed the fibrinolytic properties of the lymph were rather contradictory, Laschinger et al investigated whether lymphatic endothelial cells in culture might be capable of producing plasminogen activators and their inhibitors.[28] Analysis by reverse fibrin autography from lymphatic endothelial cells revealed the presence of three bands with molecular weight of 110, 60, and 50 kDa, which were identified with tissue plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) complex, tPA and PAI-1, respectively. Treatment with human tumor necrosis factor-α (TNF-α) resulted in a three- to sevenfold increase in the amount of PAI-1, and in a fourfold increase of the amount of tPA-PAI-1 complexes, whereas tPA production was decreased by 80% from the baseline. No urokinase type plasminogen activator (uPA) activity could be, however, identified in both control and TNF-α treated cells.

Leak et al also investigated the role of the lymphatic endothelium on the regulation of endogenous fibrinolytic activity,[29] by studying in particular the effects of various agonists on the production of tPA and PAI-1 by the lymphatic endothelium. Fibrin autography demonstrated that a plasminogen-dependent fibrinolytic activity occurs at molecular weight of 110, 65, and 55 kDa bands, which have been identified with the tPA-PAI-1 complex, tPA and uPA, respectively. The analysis of the lymph also showed a lytic activity for a 110 kDa band, which is consistent with the generation of complexes between tPA and PAI-1. The use of various agonists to stimulate lymphatic endothelium also triggered a remarkable increase of both tPA and PAI-1 mRNAs. Overall, these findings suggest that both tPA and PAI-1 are effectively produced and released by the lymphatic endothelium, and that fibrinolytic activity is present in the lymph and might play a crucial role in the regulation of endogenous fibrinolytic activity within the lymphatic vascular lumen for the maintenance of lymph fluidity.

As regards lymphoid germinal centers in human lymph nodes, Yamakawa et al identified 19 blood coagulation factors and fibrinolysis factors by immunohistochemistry.[30] Most of these factors (i.e., high-molecular-weight kininogen, kallikrein, factors XII, X, V, II, XIIIa, XIIIs, plasminogen, tPA, and PAI-1) were detected within lymphoid germinal centers. Although VWF, factor I, protein C, tetranectin, antithrombin, type 2-plasminogen activator inhibitor, and α2-plasmin inhibitor were almost entirely lacking in germinal centers, they were however detectable in vascular wall and lumen. Similar results were obtained by Kudo et al, who localized 15 blood coagulation factors in the germinal centers of human Peyer patches.[31] Although factor VIII, α-thrombin, and fibrinogen

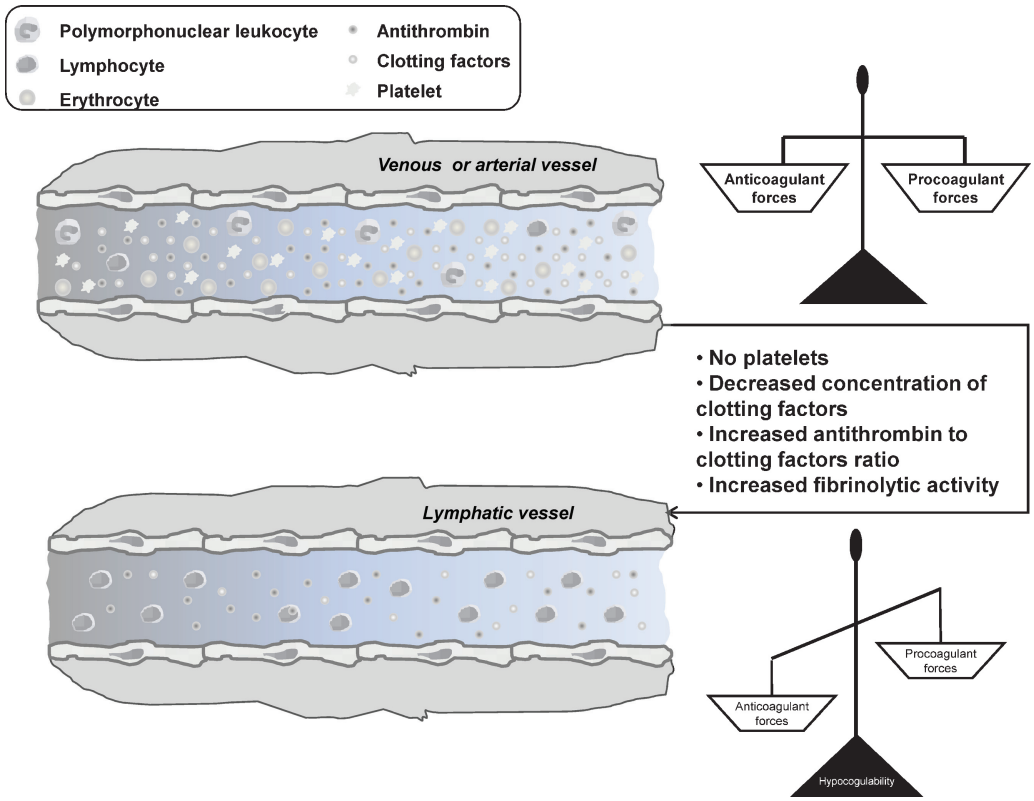

Figure 1 Difference of hemostatic properties between blood and lymph.

were hardly evident in the germinal centers, the majority of clotting factors including kallikrein, high-molecular-weight kininogen, factors XII, X, IX, VII, V, XIIIa, and XIIIb, prothrombin, antithrombin, and inactive α-thrombin were instead detectable.

Taken together, this evidence suggests that despite a measurable concentration of fibrinogen, fibrin generation is however inhibited under physiological conditions in the lymph. In particular, although a factor VIIa-tissue factor-catalyzed extravascular activation of factor X might be theoretically possible in the lymph, fibrin generation would be however limited by the unavailability of cell surface anionic phospholipids (i.e., those physiologically present on platelets in blood) which would therefore support only minimal activation, insufficient levels of factor VIII and V, as well as by the strong inhibitory activity of heparin, antithrombin, and TFPI (►**Fig. 1**). In agreement with this hypothesis, the unusual observation of remarkably increased concentrations of D-dimer in the lymph in the study of Miller et al has not been attributed to degradation of polymerized fibrin, rather to the cross-linking of intact fibrinogen molecules in the interstitial fluid by transglutaminases, which are then degraded by plasmin or other enzymes with release of dimeric D-domains that are also measured as D-dimer with certain immunoassays.

Pathogenesis of Lymphatics Occlusion and Thrombosis

According to the data discussed in the previous paragraph, the onset of thrombosis such as that typically occurring in venous[32–36] and arterial vessels[35–37] is less likely, although still possible, in the lymphatic system (►**Table 1**). Lymphatic thrombosis occasionally occurs in the thoracic duct, axillary lymphatics, or inguinal lymphatics, especially in the uterine tract during puerperal fever. Nearly 100 years ago, Opie postulated that thrombokinase released by the injured lymphatic endothelium would provide a favorable condition to thrombosis within lymphatic channels, when accompanied by chronic obstruction to lymph flow and a hypercoagulable milieu.[13] This would thereby mirror the typical elements of the Virchow triad that also characterize venous thrombosis, with the only difference that occlusion of the vessel generally anticipates the onset of thrombosis in the lymphatic system.[32] The essential contribution of endothelial cell injury has also been demonstrated in the pathogenesis of lymphatic thrombosis associated with *Brugia malayi*.[38]

An elegant study performed by Fader and Ewert, who examined by scanning electron microscopy the lymph thrombi in cats experimentally infected with this pathogen, revealed the presence of a multitude of morphological forms of thrombi.[39] It was also observed that thrombi undergo a maturation process characterized by three subsequent phases. In phase I the thrombus mainly consists of erythrocytes encased in fibrin, in phase II the thrombi are then characterized by the appearance of phagocytic cells and fibroblasts on their surface whereas, and in the third phase, the thrombi surface only consists of fibroblasts and endothelial cells originated from the vessel wall. Lymphatic thrombosis is less clinically severe than arterial or venous thrombosis,

Table 1 Leading Pathologies Associated with the Onset of Lymphatic Thrombosis

Cancers
Infections • Lymphatic filariasis (*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*) • *Chlamydia trachomatis* • *Mycobacterium tuberculosis* • *Treponema pallidum* • *Streptococcus pyogenes*
Congestive heart failure
Chronic edema and inflammation of the distal lower limb
Complications of central venous catheterization and coronary artery bypass grafting
Thoracic outlet syndrome
Amyloidosis

and the abrupted part of the thrombi is usually retained within the regional lymph nodes.

Lymphedema

Lymphedema is typically sustained by a lymphatic dysfunction caused by mechanical obstruction or destruction of the lymphatic wall, and causes an abnormal accumulation of interstitial fluid containing high-molecular-weight proteins. As a consequence, edema develops. Chronic stasis of lymphatic vessels also predisposes to substantial inflammatory and architectural modifications, frequently characterized with an increase in the number of fibroblasts, adipocytes, and keratinocytes in the edematous tissues.[40] In protracted lymph stasis, lymphatic segments still contract as they are stretched by high lymph volume, but the gradually worsening lymphatic valve insufficiency along with the ineffectiveness of the contractions become progressively ineffective in unidirectionally propelling the lymph.[7]

Lymphedema can essentially be classified as primary or secondary. The former entails three major forms according to the depending on age at presentation (i.e., congenital lymphedema, lymphedema praecox, and lymphedema tarda). Congenital lymphedema accounts for 10 to 25% of all primary cases, occurs more frequently in the lower extremity, is often bilateral and is characterized by an anaplastic pattern without subcutaneous lymphatic trunks but with normal dermal plexus. Lymphedema praecox is the most frequent form (65 to 80% of all primary lymphedema cases). Nearly 70% of cases are unilateral, with a net prevalence of the left lower extremity. The histological analysis is consistent with a hypoplastic pattern, with a substantial reduction of caliber and number of lymphatic vessels. Lymphedema tarda (also known as Meige disease), is the less frequent form, accounting for less than 10% of cases. The histological pattern is characterized by hyperplasy, with tortuous lymphatics increased both in caliber and number, frequently accompanied by incompetent valves.[40]

At variance with primary lymphedema, the secondary cases are due to an identifiable cause, which variably compromise the function of lymphatic vessels. The lymphatic obstruction causes accumulation of interstitial fluid, massive dilatation of the remaining outflow tracts, and valvular incompetence, which reverse flow from subcutaneous tissues into the derma. Protracted lymphedema is associated with fibrosis of the vessels and might trigger the formation of fibrinoid, obliterant thrombi within the lumen. The most frequent cause of secondary lymphedema is represented by injury or dissection of regional lymph nodes caused by surgery, radiation, infection (e.g., filariasis), tumor invasion or compression, vein stripping, peripheral vascular surgery, lipectomy, burns, insect bites, and cellulitis commonly associated with diabetes.[40] It has also been demonstrated that there is a high prevalence (i.e., up to 25%) of protein-losing enteropathy (PLE) in patients who have undergone surgery for complex congenital heart disease, and that physical lymphatic obstruction plays an important, and previously unrecognized, role in the development of PLE in these patients.[41]

Treatment options of lymphedema are very limited and usually entail physiotherapeutic interventions that reduce the volume of edema, but can only ensure a limited relief, and does not enable freedom from the occurrence of irreversible fibrosis.[40]

Lymphatic Thrombosis

Theoretically, any known cause of lymphatic vessels occlusion due to internal obliteration, external compression or increased lymphatic pressure (i.e., poor venous function, trauma, shock, or cardiac disease) might predispose to localized lymphatic thrombosis (►**Fig. 2**). Venous thrombosis is a well-recognized complication of cancer.[42] The "passive" contribution of the lymphatic system to the metastatic dissemination of cancers is well recognized, as most metastasis initially spread through the lymphatic vessels and the relative degree of lymph node involvement in cancer patients is still considered among the leading criteria for staging and defining the prognosis. Although the main mechanism of metastatization involves passive dissemination of cancer cells through the lymphatic vessels, emerging evidence has been provided that enlarged lymphatic vessels and lymphangiogenesis (i.e., newly formed

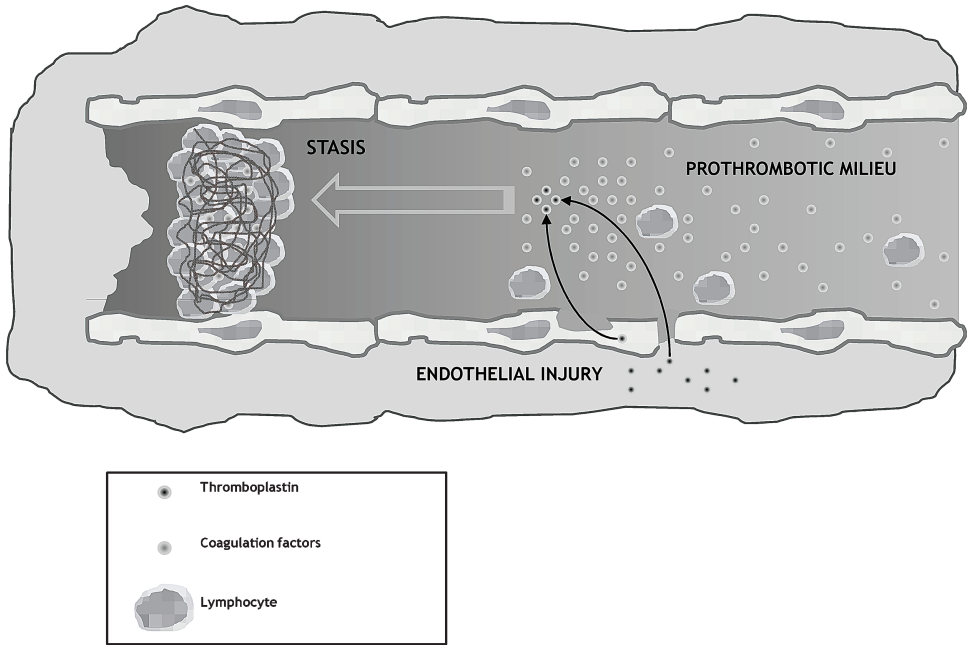

Figure 2 Pathogenesis of lymphatic thrombosis.

lymphatic vessels), along with angiogenesis in the peritumoral areas of several cancers might also play an essential role in cancer spread.[6]

Different mechanisms might contribute to the onset of lymphatic thrombosis or occlusion in cancer patients, including external compression from the tumor mass, neoplastic obliteration of the lymphatic lumen by metastatic cells, or lymphatic dysfunction after lymph node removal. Surgical (sentinel) lymph node dissection in patients with malignancies, especially those with breast cancer, is probably the most common cause of lymphatic thrombosis, as reported in several studies.[43–45] The pathogenesis involves lymphovenous damage, hypercoagulation of the lymph, superficial venous and lymphatic stasis as well as disorders and injuries of tissues as a result of the disruption of superficial lymphatics and vessels during dissection.[46]

The most common clinical sign of lymphatic thrombosis in breast cancer patients is the appearance of cording, that is, tender cord-like structures either on the chest wall, in the axilla, or down the inner aspect of the arm.[47] In the lower limb the prevalence of lymphedema varies according to the type of primary cancer and the extent of the resection.[48] The metastatic obliteration of lymphatic drainage is typically observed in breast cancer patients and, less frequently, in those with other tumors such as Hodgkin disease, non-Hodgkin lymphoma, malignant melanoma, Kaposi sarcoma, and seminoma.[48]

Lymphatic filariasis is reported to be the most prevalent cause of secondary lymphedema worldwide (especially in sub-Saharan Africa, India, Southeast Asia, parts of South America, the Caribbean, and the South Pacific), even though it only develops in a minority of the over 120 million filariasis patients. The causative agents are the filarial nematodes *Wuchereria bancrofti*, *B. malayi*, *and B. timori*.[49] The pathogenesis of lymphatic filariasis is the presence of adult worms in the lymphatic system. Lymphatic vessel dilation is the early event following antigenic stimulation, which takes place while the adult worm is still alive. The death of the worm, either spontaneous or as a consequence of the therapy, then triggers an inflammatory reaction which leads to the development of granulomas characterized by macrophages, plasma cells, eosinophils and neutrophils. Obliteration of the lymphatic lumen by thrombi and the accompanying inflammatory response triggered by the nematode infection is a major factor in the pathogenesis of lymph stasis in this disorder.[39] Additional fungal, bacterial and viral infections such as *Chlamydia trachomatis*, *Mycobacterium tuberculosis*, *Treponema pallidum*, or *Streptococcus pyogenes* may cause cellulitis or progressive lymphatic destruction, which sporadically predispose to thrombosis of lymph vessels.[50]

Another important pathology that might be associated with development of clot in the lymph is congestive heart failure, which determines a gradual increase in venous pressure that is then followed by a rise in capillary pressure and filtration, as well as extravascular fluid accumulation. In heart failure patients, reabsorption of interstitial fluid is progressively abolished. As venous congestion grows, edema accumulates at a rate which would only be limited by compliance of the interstitial space and lymphatic flow. The possibility to increase the flow of lymph under high pressure to balance extravascular fluid accumulation is however impeded, especially in the thoracic duct, due to the increased local resistance at the thoracic duct-jugular vein junction. Accordingly, stasis of lymph and development of clots in the thoracic duct has been described in patients with congestive heart failure.[51,52]

Lymph clot might also develop in patients with chronic venous failure, chronic edema and inflammation of the distal lower limb. Olszewski investigated 153 patients with

protracted lymph stasis of the lower limb due to lymphatic damage from soft tissue bacterial inflammation of mechanical trauma of soft tissues and bones.[7] Newly formed lymph nodes could be detected in 10 and 25% of patients with postinflammatory and posttraumatic lymph stasis, respectively. Histological evaluation revealed various architectures, including lymph node structure without differentiation into cortical and medullary areas, follicle-like structure within dilated lymph vessels as well as lymph clots in the dilated lymphatic vessel, mainly composed of organized lymphoid structures containing also lymphocytes and dendritic cells. Recurrent cellulitis may also obliterate lymphatic vessels, especially in the limbs.[48]

Additional sporadic cases of lymphatic thrombosis have been reported in the current literature. Spontaneous lymphatic thrombus formation was described by Chan et al in protein C/factor XI double-deficient mice.[53] Moskalik et al showed that irradiation with neodymium laser, which is successfully applied to the treatment of slightly elevated skin melanoma, caused necrosis of melanoma, epidermis, and dermis. In particular, foci of laser destruction were characterized by the presence of stasis, thrombosis, and coagulation of blood and lymphatic vessels.[54]

Some additional cases have been then described, whose common denominator was the injury of thoracic duct or its tributaries or thrombosis in the confluence of the jugular and left subclavian veins, which would thereby obstruct the drainage of the thoracic duct, generate lymph stasis, and trigger lymph clotting. These include complications of central venous catheterization,[55] coronary artery bypass grafting,[56] and thoracic outlet syndrome.[57] Finally, intrinsic amyloid obstruction of the lymphatics has been occasionally reported in patients with amyloidosis.[58]

Conclusions

Lymphatic thrombosis is a rare occurrence. Although its frequency is much lower than that of venous or arterial thrombosis, its real prevalence is likely to be largely underestimated. For this reason, as well as for objective difficulties in collecting lymph for coagulation studies and the uncertain therapeutic approach, lymphatic thrombosis has been somehow overlooked in the recent literature. Further experimental and clinical studies are indeed required to understand the pathogenetic mechanisms of this challenging pathology, as well as its real prevalence and optimal therapeutic strategy.

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