The Third Circulation: Radionuclide Lymphoscintigraphy in the Evaluation of Lymphedema*

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Lymphedema—edema that results from chronic lymphatic insufficiency—is a chronic debilitating disease that is frequently misdiagnosed, treated too late, or not treated at all. There are, however, effective therapies for lymphedema that can be implemented, particularly after the disorder is properly diagnosed and characterized with lymphoscintigraphy. On the basis of the lymphoscintigraphic image pattern, it is often possible to determine whether the limb swelling is due to lymphedema and, if so, whether compression garments, massage, or surgery is indicated. Effective use of lymphoscintigraphy to plan therapy requires an understanding of the pathophysiology of lymphedema and the influence of technical factors such as selection of the radiopharmaceutical, imaging times after injection, and patient activity after injection on the images. In addition to reviewing the anatomy and physiology of the lymphatic system, we review physiologic principles of lymphatic imaging with lymphoscintigraphy, discuss different qualitative and quantitative lymphoscintigraphic techniques and their clinical applications, and present clinical cases depicting typical lymphoscintigraphic findings.

Key Words: lymphatic system; radiotracers; lymphatic insufficiency

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CHARACTERISTICS OF LYMPHEDEMA

Lymphedema is a chronic debilitating disease that is frequently misdiagnosed, treated too late, or not treated at all. Lymphedema results from impaired lymphatic transport caused by injury to the lymphatics, infection, or congenital abnormality. Patients often suffer in silence when their primary physician or surgeon suggests that the problem is mild and that little can be done. Fortunately, there are effective therapies for lymphedema that can be implemented, particularly after the disorder is characterized with lymphoscintigraphy.

At the Stanford Lymphedema Center, about 200 new cases of lymphedema are diagnosed each year (from a catchment area of about 500,000 patients). Evidence that the disease is often overlooked by physicians caring for the patient is seen by the fact that about 60% of the patients are self-referred for initial evaluation and treatment, even if they have had lymphedema for years.

Lymphedema is a prevalent disease. Approximately 10 million people have lymphedema secondary to breast and pelvic cancer therapy, recurrent infections, injuries, or vascular surgery. Worldwide, about 90 million people have lymphedema, primarily because of parasitic infection. When chronic venous insufficiency is added as a cause, there may be as many as many as 300 million cases (*1–4*). In our clinic, about 75% of the patients have lymphedema because of malignancy or its therapy, with about half of these related to breast cancer surgery.

Arm Lymphedema

Arm lymphedema is a frequent complication of breast cancer therapy and axillary lymph node dissection, with an estimated frequency of 5%–30%. This incidence is based primarily on studies that use volume and circumference criteria in the first 2–5 y after surgery. Arm volume differences above 100–200 cm^3 or a circumference difference above 2 cm is used as a cutoff point for the diagnosis of lymphedema. All these studies disregard milder forms of lymphedema and miss a significant number of patients with mild lymphedema, especially in the nondominant arm, which could be 200 cm^3 smaller than the dominant arm before surgery. Unfortunately, almost all studies are retro-

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spective and do not include arm measurements before surgery (*5,6*).

One prospective study, by Goltner et al., of 360 women undergoing breast cancer surgery found that arm lymphedema developed after surgery in 42% of women (*7*).

Even clinically "mild" lymphedema may cause a significant disability, especially if it affects the hand. A hand volume increase of 100 cm^3 causes substantial impairment of function, because any work requiring fine movements of the hand, such as typing, writing, or playing piano, are difficult to perform.

A combination of conservative surgery and careful patient selection for nodal radiotherapy may reduce the incidence of postmastectomy lymphedema (*8*), particularly when these therapies are combined with sentinel node biopsy, but their impact on the incidence of postsurgical lymphatic insufficiency has not yet been adequately assessed. Although axillary surgical staging, with or without breast conservation techniques, is characterized as relatively free of significant complications (*9*), a postoperative study of 200 patients suggested that lymphatic complications still occur. Statistically significant changes in ipsilateral arm volume were detected at the mid biceps, antecubital fossa, and mid forearm; furthermore, clinically significant arm edema (arm circumference difference $>$ 2 cm) was detected in 13% of patients at 1 y or more after surgery, whereas 76.5% experienced postoperative sensorineural dysfunction of the medial arm or axilla (*9*).

Axillary lymph node dissection, because it correlates positively with 10-y survival in breast cancer patients (*10*), is still applied to most patients with early breast cancer (*10*). Sentinel node biopsy, however, is gaining clinical acceptance and offers a chance to avoid axillary node dissection in patients with early breast cancer. Sentinel node biopsy will not eliminate the necessity of axillary node dissection in patients with positive sentinel nodes (28%–46% of eligible patients (*11*)) and in patients with advanced breast cancer. One cautionary note about sentinel node biopsy is the limited utility of this procedure in patients with preoperative chemotherapy; up to 33% of patients may have false-negative results (*12*).

The incidence of breast cancer in the United States is projected to increase from 185,000 patients per year to 420,000 per year in the next 20 y (*11*). The higher incidence of breast cancer is likely to increase the incidence of lymphedema despite the developments of breast-conserving surgery and sentinel node biopsy. In addition, the longer survival of breast cancer patients is likely to cause an increased prevalence of arm lymphedema, which may develop many years after surgery.

Leg Lymphedema

Lower-extremity lymphedema resulting from treatment of pelvic cancer also occurs. The reported frequency of secondary leg lymphedema ranges from 10% to 49% (*13–17*). Even "mild" lymphedema of the leg may cause chronic leg discomfort and problems with walking, running, and fitting shoes. Advanced lymphedema of the leg causes severe lifelong disability. Genital lymphedema, frequently secondary to therapy for pelvic cancer, can be devastating for the patient (*18,19*).

In summary, noninfectious lymphedema is a common disease and one can expect an increase in the number of patients rather than a disappearance of this condition over the next decade. Many of these patients suffer because they were not properly diagnosed and treated. Early diagnosis can lead to effective treatment and prevention of secondary effects, including extremity deformity, disuse atrophy, and increased susceptibility to recurrent infections.

Diagnosis

Lymphedema can be surprisingly difficult to diagnose, especially in its early stages. Without a proper diagnosis, therapy is often delayed, allowing secondary fibrosis and lipid deposition to take place. Early treatment often results in rapid clinical improvement and prevents progression to the chronic phase of the disease.

Lymphoscintigraphy offers an objective and reliable approach to diagnose and characterize the severity of lymphedema. The following sections review the anatomy and pathophysiology of the lymphatic system and the technique and interpretation of the lymphoscintigram.

LYMPHATIC ANATOMY, PHYSIOLOGY, AND PATHOLOGY

Components of the Lymphatic System

The lymphatic system is a component of both the circulatory and the immune systems. The lymphatic system consists of a series of conduits (the lymphatic vasculature), lymphoid cells, and organized lymphoid tissues. Lymphoid tissues include the lymph nodes, spleen, thymus, Peyer's patches in the intestine, and lymphoid tissue in the liver, lungs, and parts of the bone marrow (*20*). Lymphatics are found throughout the body, with the exception of the central nervous system, where cerebrospinal fluid fulfills the normal role of lymph. Lymphatic vasculature and lymphoid tissue are prevalent in organs that come into direct contact with the external environment, such as the skin, gastrointestinal tract, and lungs. This distribution is probably a reflection of the protective role of the lymphatics against infectious agents and alien particles. Absorption of fat from the intestine occurs through the lymphatic system, which transports the lipids (chyle) to the liver. The lymphatic system also transports cellular debris, metabolic waste products, and fluid excesses (edema safety factor) from local sites back to the systemic circulation.

In the extremities, the lymphatic system consists of a superficial (epifascial) system that collects lymph from the skin and subcutaneous tissue, and a deeper system that drains subfascial structures such as muscle, bone, and deep blood vessels (Fig. 1). The superficial and deep systems of the lower extremities

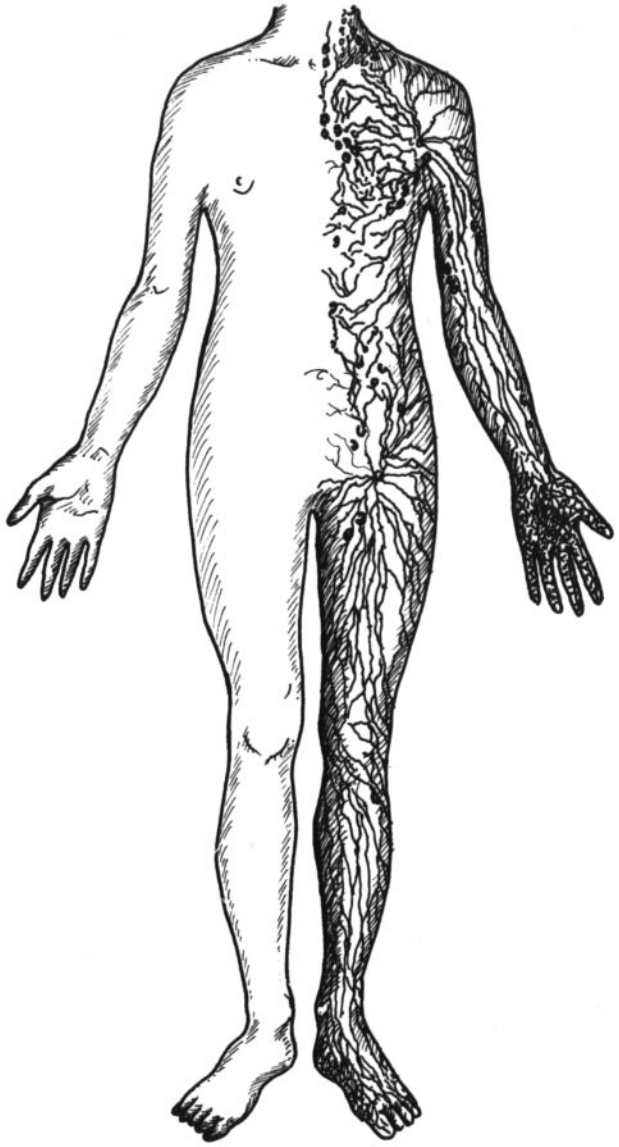

FIGURE 1. Scheme for superficial lymphatic system. Capillary density of skin lymphatic network differs in various parts of body, with higher density in face, soles of feet, and palms of hands than in trunk.

merge within the pelvis, whereas those of the upper extremity merge in the axilla. The 2 drainage systems function in an interdependent fashion such that the deep lymphatic system participates in lymph transport from the skin during lymphatic obstruction (*21*). The superficial and deep systems drain at markedly different rates. In the normal leg, subfascial transport (the deep system) is slower than the epifascial (superficial) system and transports less lymph. Brautigam et al. found median radiotracer uptake in the inguinal area to be 7% when the tracer was administered subfascially versus 13% after subdermal injection (*21*). Mostbeck and Partsch, however, using intramuscular injections of Tc-albumin microcolloid, estimated that deep lymphatic transport is only about 7.7% of superficial lymphatic transport (*22*).

Disorders of the Lymphatic System

Disorders of the lymphatic system cause primary and secondary lymphedema and also include lymphatic malignancies (Table 1).

Regional lymphatic insufficiency causes local lymphedema (Tables 2 and 3). A subclinical form of lymphatic insufficiency can exist when lymphatic transport reserve is diminished. Subclinical lymphatic insufficiency may rapidly progress to clinically apparent edema when the lymphatic system is overloaded. Overload can be caused by local infection (*24–26*), injury (*27–29*), barotrauma (air travel) (*30*), or increased venous pressure (*31–33*).

Primary congenital lymphedema may result from genetic disorders (e.g., missense mutations of vascular endothelial growth factor receptor 3 (*34–36*)). In most cases, however, the etiology remains uncertain. Acquired lymphedema is usually due to filariasis, which is responsible for ~80 million cases worldwide, making secondary lymphedema much more prevalent than primary lymphedema (Table 4). In developed countries, postsurgical lymphedema (due to lymph node dissection; Fig. 2) and postphlebitic syndrome are the most common causes of acquired, regional lymphatic insufficiency.

Regardless of etiology, lymphedema usually presents as slowly progressive extremity edema. Initially, the edema is soft and pitting, but over the course of weeks to months the skin thickens and the swelling becomes hard and nonpitting. Because the cutaneous lymphatics are not functioning, the local immune response is impaired, and recurrent skin infections are common, leading to further insult to the tissue and worsening of edema (*37*,*38*). If lymphedema is untreated it will progress to the point of chronic limb enlargement, with disfiguration of the limb associated with severe functional (*39*) (Fig. 2) and psychologic impairment (*40*). Early diagnosis and therapy to reduce edema are required to minimize the loss of function.

Microanatomy

The lymphatic vasculature consists of initial lymphatics, or lymphatic precollectors, which coalesce into lymphatic ducts, which then drain into the lymph nodes (*41*,*42*). In the skin, the initial lymphatics are present in skin papillae as

TABLE 1
Disorders of Lymphatic System

Description of disorder	Name of disorder
Absence or obstruction of lymphatic vessels	Lymphedema
Inflammation of lymphatic vessels	Lymphangitis
Inflammation of lymph nodes	Lymphadenitis
Obstruction of lymphatic drainage in a specific organ (gastroenteropathy, nephropathy)	Lymphostatic organopathies
Benign neoplasm of lymphatic vessels	Lymphangioma
Malignant neoplasm of lymphatic vessels	Lymphangiosarcoma

TABLE 2
Pathophysiology of Lymphedema

Disorder of lymphatic conduits →	Resulting in . . .
Lymphatic aplasia, hypoplasia, primary valvular insufficiency	Lymphatic hypertension, decreased contractility
	Secondary valvular insufficiency
Primary decreased lymphatic contractility	Lymphostasis with accumulation of lymph, interstitial fluid, proteins, and glycosaminoglycans in skin and subcutaneous tissue
Obliteration or disruption of lymphatic vessels	Stimulation of collagen production by fibroblasts
	Disruption of elastic fibers and activation of keratinocytes, fibroblasts, and adipocytes
	Skin thickening, subcutaneous tissue overgrowth, and fibrosis

blind-end sinuses (*43,44*), which form a superficial subpapillary network of interconnected sinuses (superficial lymphatic plexus). The plexus is formed from single layers of gracile lymphatic endothelial cells (*45,46*). Initial lymphatics range in diameter from 10 to 60 μm, significantly larger than the diameter of arteriovenous capillaries (8 μm) (Fig. 3) (*46,47*). Lymphatic endothelial cells rest on a discontinuous basement membrane that is attached to the surrounding connective tissue by anchoring filaments (Fig. 4) (*48,49*). The basement membrane is composed almost exclusively of type IV collagen. In contrast to the vascular capillary basement membrane, no heparan sulfate, proteoglycan, or fibronectin (*50*) is present in the lymphatic basement membrane. There are no tight junctions between the cells, and interendothelial openings permit extracellular fluid, macromolecules, and cells to drain directly into the lumina of the initial lymphatics through the porous basement membrane (Figs. 4 and 5) (*51–53*). Estimates of the pore size, based on measurements of the intercellular junctional distances, vary from several micrometers to 15–20 nm (*54,55*). Interendothelial junctions form an interdigitated and overlapping structure that can provide a 1-way valve system for fluid movement (*52*). These endothelial clefts can open to dimensions of several micrometers, allowing macromolecules, colloids, cells, and cellular debris to pass unhindered, depending on the degree of distension (*48,51,53,56,57*). Interendothelial junctions open during fluid inflow from the interstitium because of in-plane stretching of the lymphatic endothelium or by edema. In theory, reflux of lymphatic fluid into the interstitium is prevented by reclosure of the endothelial clefts.

The initial lymphatics are connected in a hexagonal pattern through a set of precollectors, with the deeper lymphatics in the dermis. There, lymph is transported centrally through collecting ducts and, subsequently, to the lymph nodes. The superficial precollectors, like the initial lymphatics, exhibit no detectable vasomotor activity. This observation is consistent with ultrastructural studies that depict a fine endothelial lining without smooth muscle (*53,58,59*). The precollectors coalesce into collecting ducts, which have thick walls (0.50–0.75 mm in diameter) and contain a thin layer of smooth muscle separated from the vessel lumen by a monolayer of endothelial cells (*46,60*). All the collecting lymphatics contain unicuspid or bicuspid valves at regular intervals to prevent backflow of lymph (*41,46,48,61,62*).

Transport of Particles

The interstitial space is similar in all tissues. The interstitial space consists of a fibrous collagen framework that supports a gel phase containing glycosaminoglycans, salts, and plasma-derived proteins (*54,63*). Glycosaminoglycans are polyanionic polysaccharides that are fully charged at physiologic pH. With the exception of hyaluronic acid, they are covalently bound to a protein backbone, thus creating the proteoglycans that are immobilized in the interstitium.

Transport of macromolecules within the interstitium may be physically retarded by the gel structure of the proteoglycans and by electrostatic interactions with charged components of the interstitial architecture (*54,63*). One theory suggests that the negative charge contribution of hyaluronic acid and the proteoglycans provides a net negative charge to

TABLE 3
Lymphangiographic Classification of Primary Lymphedema

Congenital primary lymphedema	Acquired primary lymphedema
Aplasia or hypoplasia of lymphatics	Intraluminal or intramural lymphangio-obstructive edema
Abnormalities of abdominal or thoracic lymph trunks	Distal
Valvular incompetence (associated with megalymphatics and often chylous reflux)	Proximal obliteration, often with distal dilatation
	Combined
	Obstruction of lymph nodes by hilar fibrosis

Modified from Browse et al. (*23*).

TABLE 4
Etiologic Classification of Lymphedema

Primary lymphedema	Secondary lymphedema
Congenital	Parasitic (filariasis)
Familial (Milroy's disease)	Postsurgical (lymph node dissection, after vascular surgery)
Syndrome associated (Turner's, Klippel-Trénaunay, Noonan's, etc.)	Postinfectious
Sporadic	Post-traumatic
Precox	Malignant (secondary to tumor obstruction of nodes)
Familial (Meige's disease)	Lymphedema complicating chronic venous insufficiency
Sporadic	
Tarda	

the interstitium (*64*). An alternative hypothesis suggests that macromolecular diffusion through the interstitium is dictated by molecular size, the presence of diffusional microdomains, and physical and electrostatic interactions with interstitial components (*54*).

Entry of extracellular fluid and protein into the initial lymphatics occurs through interendothelial openings and by vesicular transport through the endothelial cells (*52,65*). Both ways might be equally important in particle transport into the lymphatics. Interendothelial openings may allow cells (macrophages, lymphocytes, erythrocytes) and cellular debris to directly enter lymphatics (*53,66*). Particles can also enter initial lymphatics within macrophages after phagocytosis (*51*). Interstitial fluid pressure in the skin and subcutaneous tissue is slightly negative (-2 to -6 mm H_2O) (*64,67*), whereas lymphatic capillary pressure in skin is positive (*68,69*), thus suggesting that fluid transport into the initial lymphatics occurs against a pressure gradient. Current theory proposes the presence of a suction force that is generated through the contraction of the collecting lymphatics, coupled with the episodic increases in interstitial fluid pressure that are created through tissue movement (*70*). In skeletal muscle, lymphatics are usually paired with arterioles, so that arterial pulsation and muscle contraction contribute to the periodic expansion and compression of initial lymphatics to enhance fluid uptake (Fig. 5) (*61*). Additional mechanisms of particle transport from the interstitium to initial lymphatic include active transendothelial vesicular transport and phagocytosis with subsequent migration of macrophages into the lymphatic vessels (*51,52*). Particle size and surface properties may determine which way is preferred (*71,72*).

Lymph Flow and Lymphatic Contractility

A systemic driving force exists for the basal propulsion of lymph that is independent of the local pressure gradients that promote uptake (*73,74*). Lymph flow in the collectors depends predominantly on lymphatic contraction (*75,76*).

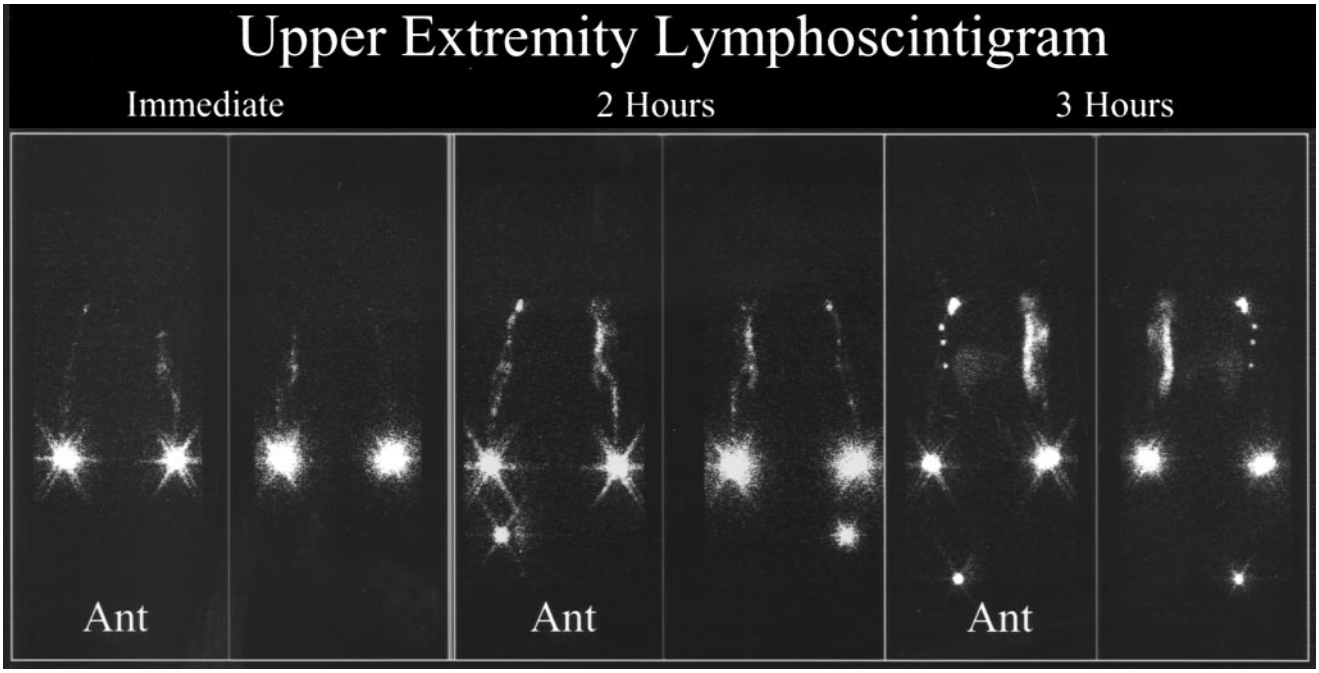

FIGURE 2. Lymphedema of arm in patient after axillary dissection during breast cancer surgery. Ant = anterior.

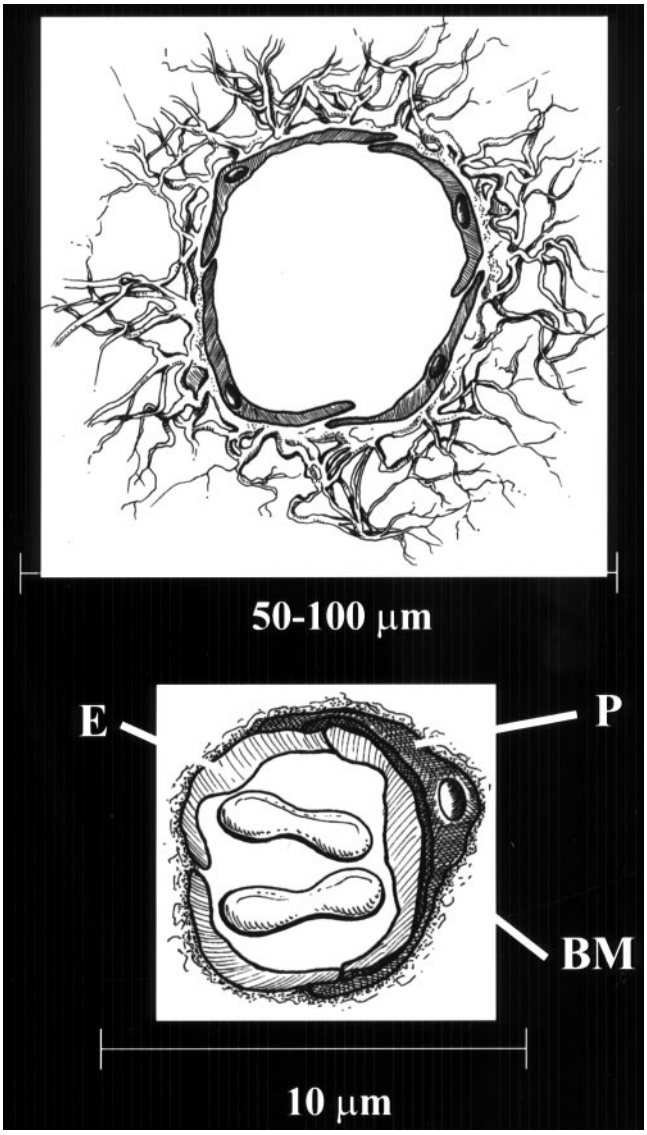

FIGURE 3. Lymphatic capillary (top), in comparison with blood capillary (bottom). Lymphatic capillary has larger diameter, no pericytes (P), and thin and porous basal membrane (BM) and is attached to surrounding tissue with anchoring filaments. Erythrocytes (E) are visible within lumen of blood capillary.

Intrinsic generation of action potentials within the smooth muscle induces the spontaneous contraction of one or more chambers, with the resultant active propulsion of lymph. The rate of lymph transport can be significantly affected by humoral and physical factors that influence the rhythm and amplitude of spontaneous constrictions (*77*,*78*). Activation of β-adrenoceptors has been shown to decrease the frequency and force of spontaneous constrictions in bovine mesenteric lymphatic vessels (*79*). Oxygen free radicals (*80*) and endothelium-derived nitric oxide (*81*) reduce the efficacy of action potential generation of lymphatic smooth muscle pacemaker potentials and, hence, lymphatic phasic constrictions.

Lymph flow and lymphatic contractility increase in response to tissue edema (edema safety factor) (*33*), exercise (*76*), hydrostatic pressure (standing position) (*82*), mechanical stimulation (massage, pneumatic compression) (*83*–*85*), and warm baths (*86*). Interestingly, it has been demonstrated that exposure to cold (ice packs, near 0°C) also stimulates lymphatic flow (*87*).

FACTORS AFFECTING UPTAKE OF COLLOIDS AND PROTEINS

Most radionuclide lymphatic flow studies use particulate materials. The agents studied include ^{99m}Tc-sulfur colloids, ^{99m}Tc-nano- and microaggregated albumin, ^{99m}Tc-antimony sulfide, colloidal gold particles, liposomes, and emulsions administered into the interstitial space of animals and humans (*46*,*88*–*93*). Particles smaller than a few nanometers usually leak into blood capillaries, whereas larger particles (up to about 100 nm) can enter the lymphatic capillaries and be transported to lymph nodes (*46*). However, even large particles were detected in venous blood immediately after subcutaneous injection, probably as a result of direct capillary disruption by the needle (*94*). The optimal colloidal size for lymphoscintigraphy is believed to be approximately 50–70 nm (*91*). Individual estimates vary from 1 to 70 nm (*90*,*92*). Larger particles (>100 nm) are believed to be trapped in the interstitial compartment for a relatively long period (*46*). One study has demonstrated that transport of perfluorocarbon emulsions of 0.08–0.36 μm exhibits an inverse correlation to colloid particle size (*72*). Mechanical massage over the colloid injection site enhances the uptake and weakens this inverse correlation. The same study demonstrated that the particle surface properties may influence the uptake of colloid (*72*). Interestingly, an increase in venous pressure decreased lymph colloid and lymph leukocyte concentration (*72*).

Lymph node uptake of colloids of similar size can vary substantially. Differences in surface characteristics of the colloids may account for these observations (*72*,*76*). Early studies with liposomes have shown that specific surface properties, such as charge, hydrophobicity, and the presence of targeting ligands, can influence both the rate of particle drainage from a subcutaneous injection site and the distribution within the lymphatic system. In rats, for instance, small, negatively charged liposomes localize more effectively in lymph nodes than positively charged vesicles when administered subcutaneously into the dorsal surface of the footpad (*93*,*95*).

Particle uptake by the lymphatic system is temperature dependent. Protein transport across canine lymphatic endothelium is enhanced with increasing temperature (*96*). In addition to temperature, the pH of lymph or interstitial fluid may also alter lymph or particle uptake and transport. The colloid osmotic pressure of body fluids is increased when pH is increased (2.1 mm Hg per pH unit) (*97*). Whether pH differences in interstitial or lymphatic fluid affect particle uptake in vivo, however, remains to be investigated.

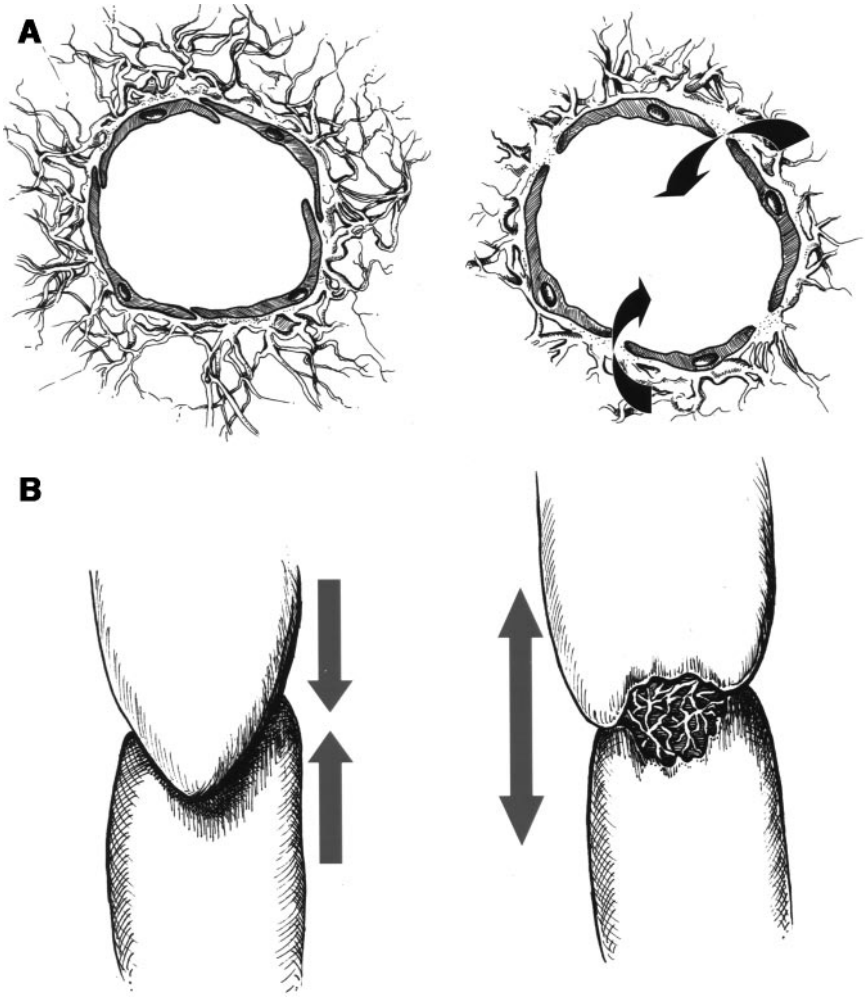

FIGURE 4. Filling mechanism of initial lymphatics: interendothelial clefts. (A) Cross-sectional view shows that stretching of anchoring filaments (tissue edema, massage) pulls apart endothelial cells, allowing interstitial fluid to flow freely into lymphatic capillary. (B) Lymphatic endothelial cells are pulled apart and porous basement membrane is visible, acting as sieve for interstitial fluid entering lymphatic capillary (luminal surface of capillary).

LYMPHOSCINTIGRAPHY

Injection of radiolabeled tracers with subsequent gamma camera monitoring has been used to study the lymphatic system since the 1950s. This minimally invasive procedure simply requires intradermal or subcutaneous injection of the chosen radiolabeled tracer. The method has largely replaced the more invasive and technically difficult technique of lymphangiography (*98*). Specific clinical applications of lymphoscintigraphy are summarized in Table 5.

The protocol for lymphoscintigraphy is not standarized and differs among diagnostic centers. Differences include the choice of radiotracer, the type and site of injection, the use of dynamic and static acquisitions, and the acquisition times themselves.

Methodology

Radiotracers. Deposition of radioactive colloids in regional lymph nodes was first observed by Walker after subcutaneous injection of colloidal gold (^{198}Au) (*99*). Because a significant fraction of the dose remained at the injection site after subcutaneous administration of colloidal ^{198}Au (a tracer with a significant component of β-decay), radiation burden at the injection site limited the administered dose and led to a search for agents with more favorable characteristics. ^{198}Au was replaced by the ^{99m}Tc-labeled tracers. ^{99m}Tc-antimony sulfide colloid, ^{99m}Tc-sulfur colloid, ^{99m}Tc-albumin colloid, and ^{99m}Tc-labeled human serum albumin (HSA) have become the primary agents for clinical use. Unfortunately, neither ^{99m}Tc-antimony sulfide nor ^{99m}Tc-HSA is presently available in the United States. ^{99m}Tc-albumin nanocolloid and ^{99m}Tc-rhenium sulfide colloids are used in Europe (*22,100,101*).

^{99m}Tc-Filtered sulfur colloid (particle size $<$ 100 nm), one of the most commonly used radiotracers for lymphoscintigraphy, is inexpensive, has an excellent safety profile, and has demonstrated clinical value. The agent also has several disadvantages, including minimal absorption from the injection site (typically $<$5% is absorbed) and slow transport from the injection site after subcutaneous administration (intradermal administration is associated with rapid absorption; cutaneous lymphatics are often visualized within 1 min of tracer deposition). Even in the absence of β-radiation, the conversion electrons from ^{99m}Tc result in a dose of 1–5 rad per injection site, for a dose of ~92.5 MBq (depending on the volume administered). The slow transit

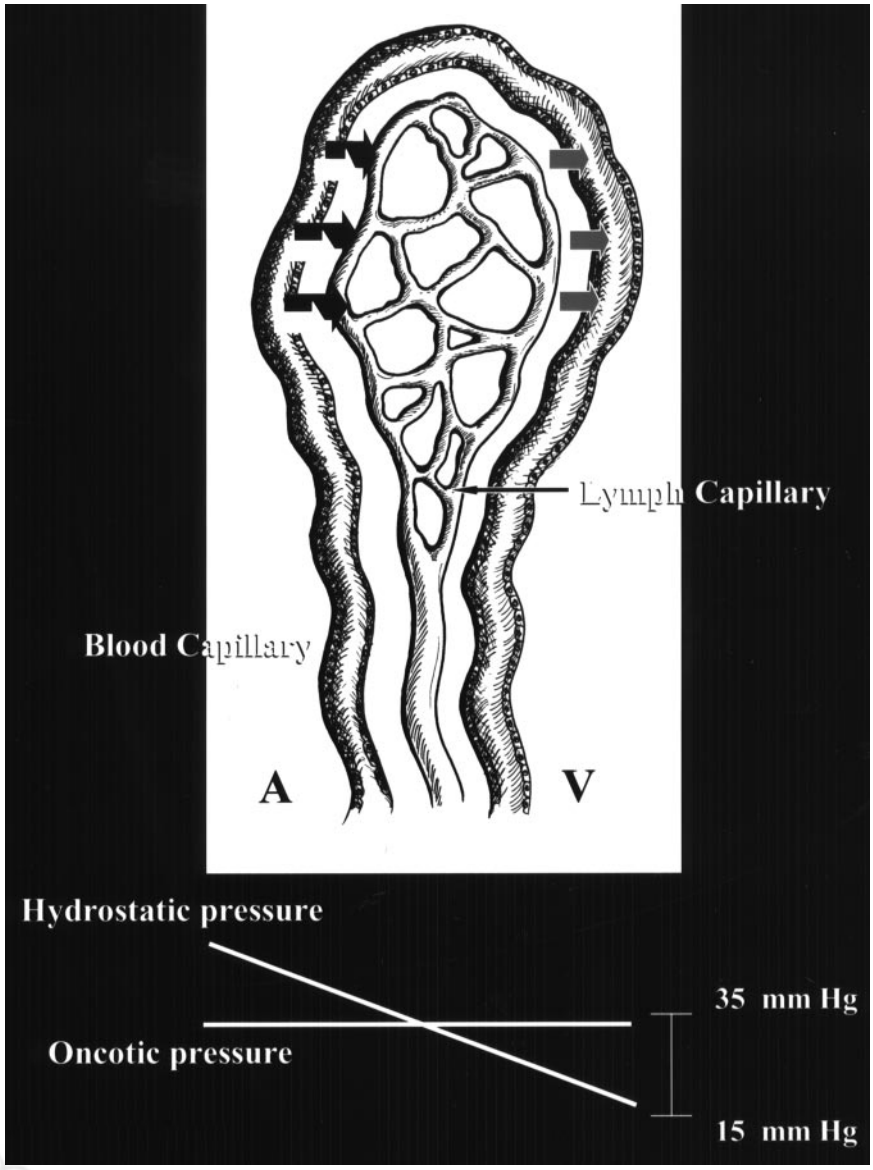

FIGURE 5. Scheme for lymph formation. A = Arterial capillary; V = venous capillary.

requires prolonged times for imaging. The unpredictable nature of the absorption and transit makes reliable calculation of tracer disappearance rates difficult. ^{99m}Tc-sulfur colloid also requires an acidic pH to remain stable; such a pH often causes the patient to experience burning at the injection site. (To minimize discomfort at the time of injection, some investigators use a cutaneous cream containing a eutectic mixture of local anesthetics or add some lidocaine to the injection. Even when the tracer is administered without these aids, the discomfort is usually minimal and disappears within a few minutes of injection.) The large particle size of ^{99m}Tc-sulfur colloid (30–1,000 nm) (*102*) contributes to the minimal absorption and slow transit. To circumvent this problem, filtered sulfur colloid was advo-

TABLE 5
Clinical Applications of Lymphoscintigraphy

General application	Specifics
Differential diagnosis	Distinguish lymphatic from venous edema, myxedema, lipedema, or other etiology
Assessment	Assess pathways of lymphatic drainage
Identification	Identify sentinel nodes in patients with melanoma, breast, or genitourinary cancer
	Identify patients at high risk for development of lymphedema after axillary lymph node dissection
Quantitation	Quantify lymphatic flow

cated for lymphoscintigraphy (*102*). Use of a 0.1-μm filter yielded sulfur colloid with a stable particle size of <50 nm. The properties of this filtered colloid are similar to those of antimony trisulfide colloid. Albumin microcolloid has a reproducible colloid size distribution (95% is <80 nm) and ease of labeling. Its rapid clearance from the injection site makes it suitable for quantitative studies, and injections are painless. Thus, ^{99m}Tc-albumin microcolloid may be more suitable for quantitative studies than is ^{99m}Tc-sulfur colloid. Colloidal radiotracers and their particle size are summarized in Table 6.

Noncolloidal tracers reported in the literature include ^{99m}Tc-HSA (*108,109*), ^{99m}Tc-labeled dextran (*110*), and ^{99m}Tc-labeled human immunoglobulin (*111*). The more rapid absorption of ^{99m}Tc-HSA allows shorter study times, provides better visualization of lymphatic trunks, and may be preferable for quantitative analyses (*108,109,112,113*). Although the noncolloidal tracers clear from the injection site, they clear by a dual mechanism, with both resorption into capillaries and transport through lymphatics. As a result, use of these agents requires different criteria for interpretation than does use of colloidal tracers.

Subcutaneous, Intradermal, and Subfascial Injection. Both subcutaneous and intradermal injections are used in routine studies of superficial lymphatics of the extremity. Weissleder and Weissleder prefer subcutaneous injections of ^{99m}Tc-HSA microcolloid, arguing that intradermal injections lead to significant uptake of radiotracer by blood vessels (*98*). According to Mostbeck and Partsch, who compared subdermal and intradermal injections of ^{99m}Tc-albumin microcolloid, subcutaneous injections produced more reliable results (*22,114*). In patients with primary lymphedema of the entire lower extremity, slow uptake was seen after intradermal injection, whereas in distal and secondary lymphedema, uptake in nodes was nearly normal. Subcutaneous injections, in contrast, suggested lymphatic obstruction. Opinions differ about which injection technique is best. Subcutaneous tracer injection is recommended by many investigators (*98,100,115*), but intradermal injection is preferred by others (*82,112,116–118*). Intradermal administration of noncolloidal agents (^{99m}Tc-HSA) is associated with rapid lymphatic transport, facilitating rapid evaluation and better quantification of lymphatic flow (*82*). Intradermal injection of colloidal tracers or other noncolloidal agents may not be as efficacious as HSA. However, comparison of intradermal and subdermal injections with ^{99m}Tc-HSA reveals better tracer kinetics after intradermal injection and slow or no transport after subcutaneous injections (*119*). Available data suggest that the optimal route of injection may vary depending on the tracer used, with subcutaneous injection being optimal for the colloidal agents (*22,114*).

Subfascial injection of radiotracers is used for investigations of the deep lymphatic system of the extremities. Injection can be intramuscular (*22*), subfascial in the lateral retromalleolar region (*120*), or in aponeurotic sites of the soles or palms (G. Mariani, written communication).

Two-compartment lymphoscintigraphy (epifascial + subfascial) may be preferable for the differentiation of various mechanisms of extremity edema (*21,101,114,120*). The injection sites are prepared by swabbing the area with either an iodine solution (especially in patients with frank lymphedema) or alcohol. The 9.25 MBq per injection in a 0.05- to 0.1-mL dose is administered using a 26-gauge needle for each of 4 injection sites (the web space between the first and second and the second and third digits of the hands or feet). Generally, both limbs receive injection (typically to use one side as a control for patients with unilateral lymphedema).

Imaging. Images should be recorded with a dual-detector instrument, using high-resolution parallel-hole collimators, in the whole-body scanning mode. Images should be recorded with a 20% window centered on the 140-keV photopeak of ^{99m}Tc, using a scan speed of 10 cm/min, into a dedicated computer. The data should be displayed with the upper level set to display the small fraction of tracer that emigrates from the injection site to the nodes (this setting usually causes substantial blooming of the image near the injection site but optimizes the likelihood of seeing the nodes). A transmission scan should also be recorded to

TABLE 6
Colloidal Radiotracers and Their Particle Size

Agent	Particle size	Reference
^{198}Au-Colloid	5 nm; 9–15 nm	Strand (*92*), Kazem (*103*)
^{99m}Tc-Rhenium colloid (TCK-1)	10–40 nm; 50–500 nm	Nagai (*104*), Bergqvist (*90*)
^{99m}Tc-Rhenium colloid (TCK-17)	50–200 nm; 45 nm; 3–15 nm	Bergqvist (*90*), Nagai (*105*)
^{99m}Tc-Antimony sulfur colloid	2–15 nm; 40 nm	Warbick (*106*), Bergqvist (*90*)
^{99m}Tc-Sulfur colloid	100–1,000 nm	Davis (*107*)
^{99m}Tc-Filtered sulfur colloid	38 nm (mean)	Hung (*102*)
^{99m}Tc-Stannous sulfur colloid	20–60 nm	Kleinhans (*124*)
^{99m}Tc-Albumin microcolloid	<80 nm	Manufacturer
^{99m}Tc-Microaggregated albumin	10 nm	Bergqvist (*90*)

Modified from Bergqvist et al. (*90*).

permit anatomic localization of the areas visualized. The transmission source is typically placed on the posterior detector, and the camera windows are reset to image both the cobalt-sheet source activity for the transmission data and the ^{99m}Tc activity for the emission data, using the anterior detector to record the data. Typically, the body scan data are recorded within about 10 min of injection, at 1–2 h, and finally at 4–6 h after tracer administration.

Stress Lymphoscintigraphy. Lymphoscintigraphy can be performed after an intervention designed to augment lymphatic flow—such as changes in temperature, physical exertion, or administration of a pharmacologic agent. Although stress lymphoscintigraphy is recommended by most authors for its enhanced sensitivity and for its utility in the quantitation of lymphatic flow (*114,121*), this approach is not universally used (*100,119*). In the lower extremities, stress maneuvers include walking (*122*), standing (*82*), limb massage (*116,123*), standardized treadmill exercise (*22*), and bicycle exercise (*101*). In the upper extremities, repetitive squeezing of a rubber ball, use of a hand-grip exercise device (*124*), or massage (*116*) have been proposed. Massage, exercise, and standing enhance radiotracer transport from the injection site (*52,82,123*).

Lymphoscintigraphy is usually performed after injection into the web space of the upper or lower extremities, followed by imaging for 30–60 min. Thereafter, the patient performs the stress activity (walking, massage, or squeezing a ball) for ~20 min, and then imaging is repeated. A marked change in the appearance of nodes or clearance of the tracer identifies a response to the intervention.

Quantitative Lymphoscintigraphy. Quantitation of lymphatic flow may be a more sensitive approach to the diagnosis of lymphatic impairment (Table 7) (*98*). Quantitation of the regional lymph node accumulation of radiotracer is preferred (*22,114*). Clearance from the injection site may not allow discrimination between healthy patients and patients with lymphedema (*114*). Quantitation of disappearance rates from the injection site is preferred by investigators using labeled HSA (*127*).

Weissleder and Weissleder compared quantitative and qualitative lymphoscintigraphy in 308 extremities with different grades of lymphedema and found that qualitative interpretation confirmed the diagnosis of lymphedema in 70% of extremities, whereas quantitative analysis detected abnormal lymphatic function in all 308 examined limbs. All cases missed by qualitative interpretation were mild, grade I, lymphedema (*98*).

Clinical Applications of Extremity Lymphoscintigraphy

Differential Diagnosis of Extremity Edema. Lymphoscintigraphy offers objective evidence to distinguish lymphatic pathology from nonlymphatic causes of extremity edema (*100,115,127–130*). Criteria for lymphatic dysfunction include delay, asymmetric or absent visualization of regional lymph nodes, and the presence of "dermal backflow." Additional findings include asymmetric visualization of lymphatic channels, collateral lymphatic channels, interrupted vascular structures, and lymph nodes of the deep lymphatic system (i.e., popliteal lymph nodes after web space injection in the lower extremities) (Figs. 6 and 7) (*100*). Quantitative analysis may increase the sensitivity and specificity of lymphoscintigraphy in the diagnosis of lymphatic disorders (*98*).

Scoring systems have also been suggested to enhance diagnostic differentiation in borderline cases (*115,124,131*). Baulieu et al. proposed factor analysis of lymphoscintigraphic data to increase specificity and provide an objective evaluation of the efficacy of therapy (*132*).

Assessment of the Results of Therapeutic Interventions in Lymphedema. Qualitative and quantitative lymphoscintigraphy has been widely used in the assessment of therapeutic interventions for lymphedema, ranging from microsurgery (*128,133–135*) and liposuction (*136*) to manual lymphatic massage (*137–140*), pneumatic compression (*86,132*), hyperthermia (*141*), and pharmacologic interventions (*142–144*).

Slavin et al. evaluated lymphatic regeneration after free-tissue transfer with lymphoscintigraphy (*145*). These investigations used both quantitative and qualitative lymphoscintigraphy.

TABLE 7
Quantitative Lymphoscintigraphy in Lymphedema

Radiotracer	Route	ROI	Stress	Measurement	Reference
^{99m}Tc-HSA	sc	IS	Walking	Clearance rate	Kataoka (*122*)
^{99m}Tc-Albumin microcolloid	sc (im)	LN	Treadmill	LN uptake, depth correction	Mostbeck (*22*)
^{99m}Tc-Albumin microcolloid	sc	LN, IS, lymph vessels	Bicycle	LN uptake	Brautigam (*21*)
^{99m}Tc-Albumin microcolloid	sc	IS, LN	Passive exercise	Clearance rate, LN uptake	Weissleder (*98*)
^{99m}Tc-Rhenium colloid	sc	IS	None	Clearance rate, LN uptake	Pecking (*100,142*)
^{99m}Tc-Sulphur colloid	sc	IS, LN	None	Clearance rate, LN uptake	Carena (*125*)
^{99m}Tc-HIG	ic	IS	None	Clearance rate	Svensson (*111*)
^{99m}Tc-HSA	ic	IS	None	Clearance rate, LN uptake	Nawaz (*126*)

HSA = human serum albumin; sc = subcutaneous; IS = injection site; im = intramuscular; LN = regional lymph nodes; ic = intracutaneous; HIG = human immunoglobulin.

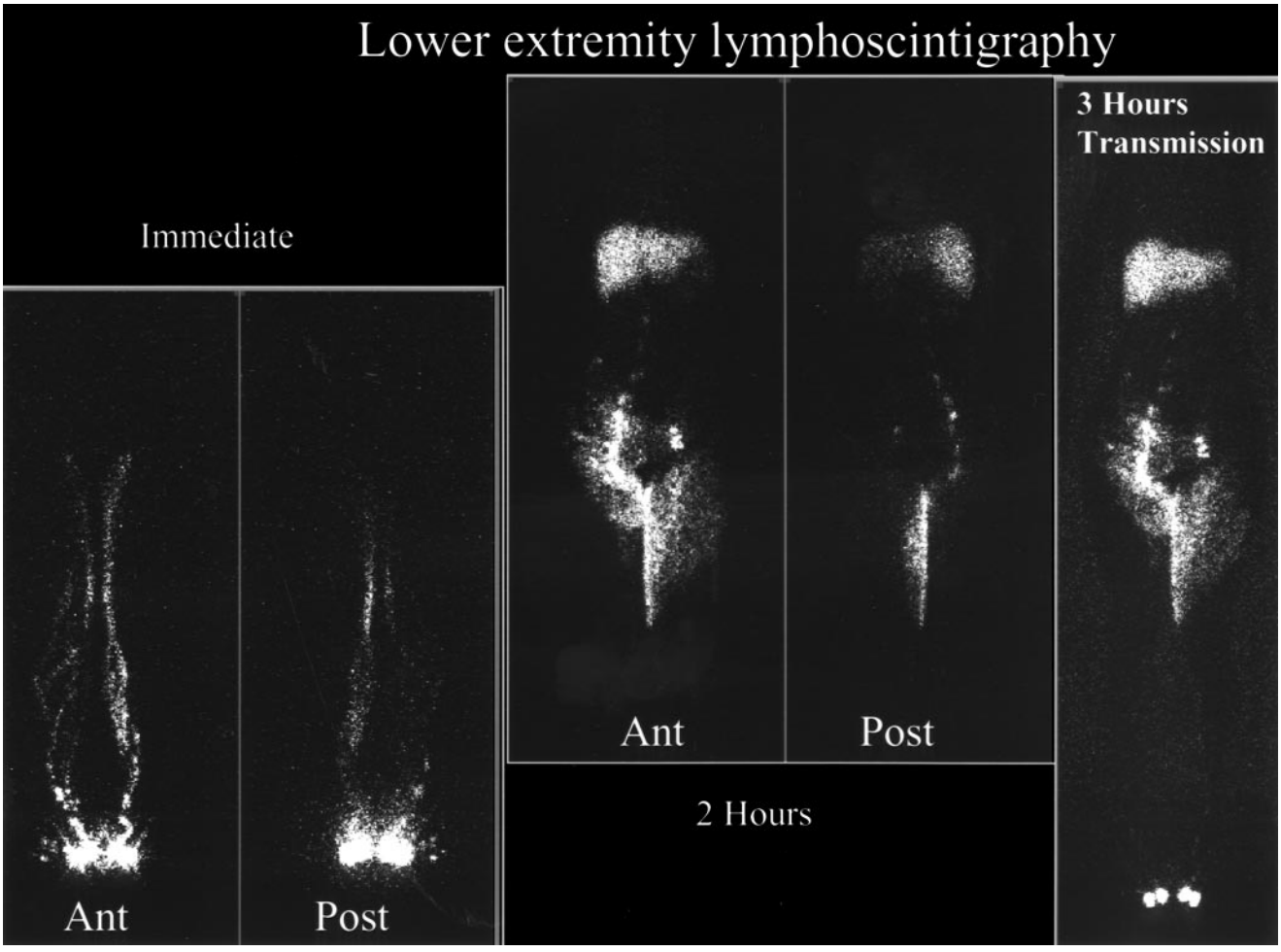

FIGURE 6. Lower-extremity lymphoscintigram from patient with history of lymphadenitis in right groin because of herpes zoster (shingles) affecting her right buttock and inguinal area. Shown are immediate images in anterior and posterior views (left), late images (about 3 h after injection) in anterior and posterior views (middle), and superimposition of anterior emission scan on transmission scan (right). Inguinal node visualization on right and dermal backflow on medial aspect of upper thigh are minimal, suggesting lymphatic obstruction of superficial system. Ant = anterior; Post = posterior.

Prediction of the Outcome of Lymphedema Therapy. In a recent study of 19 women undergoing therapy for postmastectomy lymphedema, Szuba et al. found that the degree of impairment of lymphatic function before the treatment correlated with the outcome of manual lymphatic therapy (*131*).

Assessment of the Risk of Development of Lymphedema. When postoperative lymphoscintigraphy in patients undergoing axillary dissection during breast cancer surgery demonstrates disruption of lymphatic drainage, the risk that arm lymphedema will develop increases (*146*). Pecking et al., in their study of 60 women treated with surgical axillary lymph node dissection and radiation therapy, demonstrated that an abnormal lymphoscintigram 6 mo after radiation therapy can predict the development of arm lymphedema (*147*). Baulieu et al. analyzed 32 lymphoscintigrams from patients with tibial fractures treated surgically (*29*). Lack of visualization of inguinal lymph nodes predicted late postoperative leg edema.

These studies suggest that postoperative lymphoscintigraphy can identify patients with a high risk of development of extremity lymphedema. Early identification of these patients will allow specific implementation of preventive strategies to minimize the risk of lymphedema. However, more studies are necessary to establish clinical guidelines for the performance of lymphoscintigraphy in patients undergoing therapeutic lymph node excision or radiation therapy.

CONCLUSION

Lymphatic flow and sites of lymph drainage can readily be evaluated with lymphoscintigraphy. Lymphatic imaging can play a pivotal role in defining the etiology of extremity swelling and in predicting the success of common therapies.

From a technical perspective, better radiopharmaceuticals are needed. Agents that clear from the injection site and localize in the nodes could markedly enhance the value of the procedure. Although radiolabeled albumin has been

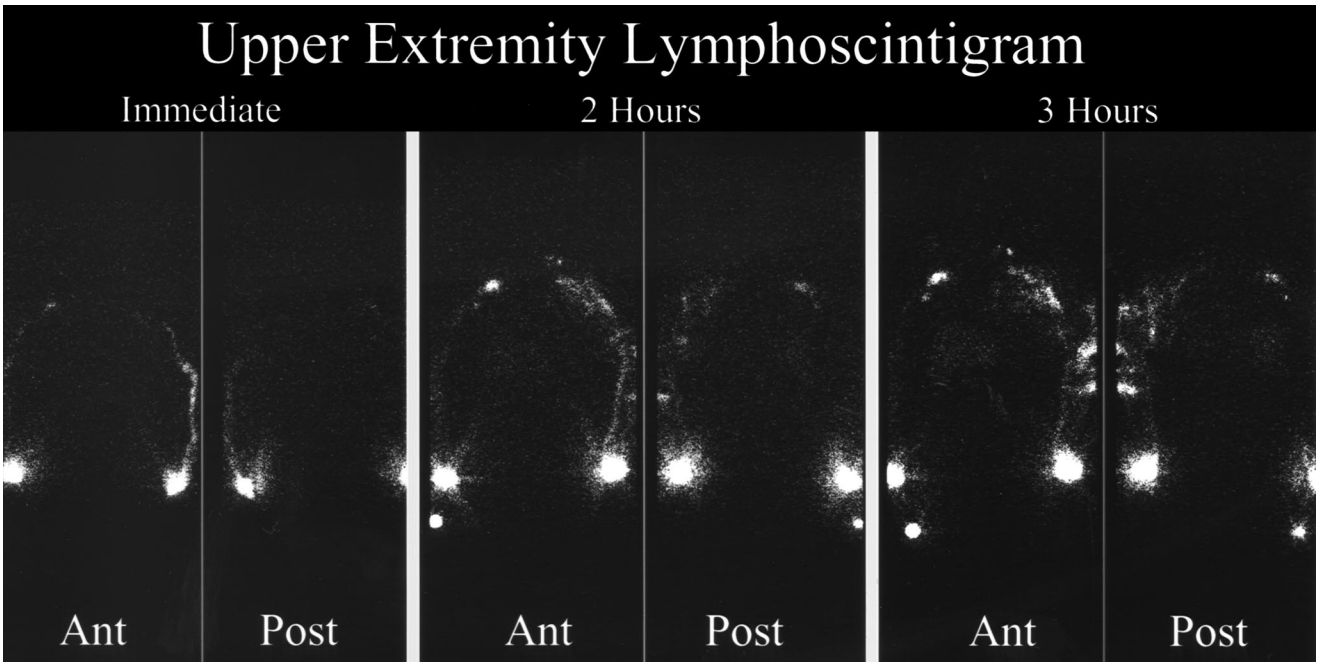

FIGURE 7. Upper-extremity lymphoscintigram from patient who had left-sided pacemaker. Several months after pacemaker was placed, patient noticed swelling of left arm. Shown are immediate images in anterior and posterior views (left), images 2 h after injection in anterior and posterior views (middle), and images 3 h after injection in anterior and posterior views (right). Axillary nodal visualization and appearance of dermal backflow in upper portion of left arm and in area of pacemaker implantation are weak, confirming that cause of extremity edema is lymphatic obstruction, possibly related to surgical intervention. Ant = anterior; Post = posterior.

used for many years, it does not remain in the nodes and requires continuous imaging to define the lymphatic channels. An alternative, ^{99m}Tc-annexin V, has been tested in experimental studies in rabbits. This agent was selected because the protein is small, enhancing its clearance from the injection site, but it also concentrates in lymph nodes because lymphocytes undergo apoptosis (a target for annexin V) in the nodes. Such an agent will shorten the procedure and enhance the ability to obtain quantitative data in patients with partial lymphatic obstruction.

Because many institutions are establishing lymphedema centers, lymphatic imaging will become more prevalent. As this occurs, it will be important to develop standardized procedures and radiopharmaceuticals to perform these examinations and standardized criteria to interpret the results. Imaging at rest and after stress allows the procedure to provide more useful data than do rest studies alone. As a result, application of physical stress should be considered part of the routine approach to assess lymphatics. Until a new radiopharmaceutical is approved, filtered ^{99m}Tc-sulfur colloid should become the standard, since it is available throughout the world.

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