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Lymphosome Concept: Anatomical Study of the Lymphatic System

HIROO SUAMI, MD, PhD*
Faculty of Medicine and Health Sciences, Macquarie University, Sydney, New South Wales, Australia

The gross anatomical study of the lymphatic system in humans and animals has been suspended for almost 100 years. This article introduces the author's technique for investigating the lymphatic system using the concept of the lymphosome. In revisiting the anatomical study of the lymphatic system, our updated knowledge can potentially be utilized either to reassure surgeons about their current procedures in the surgical management of cancers and lymphedema or assist them to refine them.
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KEY WORDS: lymphatic system; lymphatic vessel; fluorescence lymphography; cadaver dissection; animal experiment

INTRODUCTION

A better understanding of the anatomy of the lymphatic system is paramount for predicting cancer metastasis pathways and to understand the pathophysiology of lymphoedema. However, the lymphatic system is the least studied field in anatomy. Superficial lymphatic vessels in the upper extremity are much smaller in diameter than cutaneous veins, measuring around 0.3 mm. The lymphatic vessel wall is thin and composed of a single layer of epithelial cells surrounded by a basement membrane, and two to three layers of smooth muscle cells [1]. It is difficult to identify lymphatic vessels during surgery without staining because, unlike blood, lymph fluid is colorless.

Historically, Gasparo Aselli, an Italian anatomist in Pavia, is credited for the discovery of the lymphatic system in 1627 [2]. When he vivisected a well-fed dog, he found white bundle structures in the mesentery which he at first believed to be autonomic nerves. However, upon cutting the vessel and observing the milky liquid leaking from the cut end, he realized that these new structures were responsible for transferring nutrients from the intestine. In 1692, Nuck used mercury in cadaver specimens to visualize the lymphatic system and thereafter investigation of the lymphatic system advanced significantly over the next three centuries [3]. In 1874, Sappey used Nuck's mercury technique and published his findings with superb etching figures of the lymphatic system in humans [4]. He was the first anatomist to recognize that lymphatic drainage of the skin can be divided into territories. His famous engraving figure of the human torso illustrated that lymphatic drainage of the torso can be divided into four skin territories along the frontal and posterior midlines, with the horizontal line at the umbilical level (Fig. 1). The lymphatic vessels in each territory are connected to the ipsilateral axillary or inguinal lymph nodes. His findings formed the basis of our knowledge about the spread of skin cancer and oncologic surgeons still rely on his drawing to determine which regional lymph nodes should be removed to prevent cancer spread. Kubik made further detailed diagrams of lymphatic mapping in humans [5]. For forearm lymphatics, he divided the skin into lymphatic territories according to the position of lymphatic pathways and anatomic regions such as the ulnar, median, and radial bundles.

Nuck's mercury method was terminated early in the of 20th century because of the implications of the toxicity of mercury for scientific research. In 1896, Gerota developed an oil-based color injection technique to demonstrate the lymphatic system in cadaveric specimens, but his mixture could only travel short distances [6]. Therefore, Gerota's method required smaller cadaver materials such as fetuses or small child cadavers. Taking into account this historical background and the limitation of materials, dissection study of the lymphatic system has not been updated for almost 100 years.

In regard to the relationship between the lymphatic system and cancers, Virchow recognized that lymph node metastasis was one of important factors in determining the prognosis of gastric cancer patents [7]. Ewing proposed the anatomical hypothesis of cancer metastasis that postulates that cancers spread along the anatomical course of the lymphatic system [8]. His theory became the basic principle for surgical resection of the regional lymph nodes to prevent cancer spread. In the 1950s, a British surgeon, Kinmonth, developed lymphangiography to demonstrate the lymphatic system radiologically in the clinical setting [9]. This imaging technique consisted of cannulation of a small needle directly into the lymphatic vessel and injection of an oil-based radiocontrast media (lipiodol) by using a syringe pump. Lymphangiography became popular as a standard imaging technique for demonstrating the lymphatic system and expanded our knowledge about anatomy of the lymphatic system. In the examination of upper extremity cases, Kinmonth found that the radiocontrast medium reached one or two lymph nodes and that these lymph nodes were consistently located in the most lateral aspect of the axilla. Lymphangiography was used for diagnostic imaging of lymphedema to identify dermal back flow in lymphedema patients similarly to current diagnostic imaging criteria that uses the more recent indocyanine green (ICG) fluorescence lymphography and lymphoscintigraphy techniques [10].

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*Correspondence to: Hiroo Suami, MD, PhD, Faculty of Medicine and Health Sciences, Bldg. F10A, 2 Technology Place, Macquarie University, Sydney, NSW 2109, Australia.
Fax: +61-2-9812-3600. E-mail: hiroo.suami@mq.edu.au

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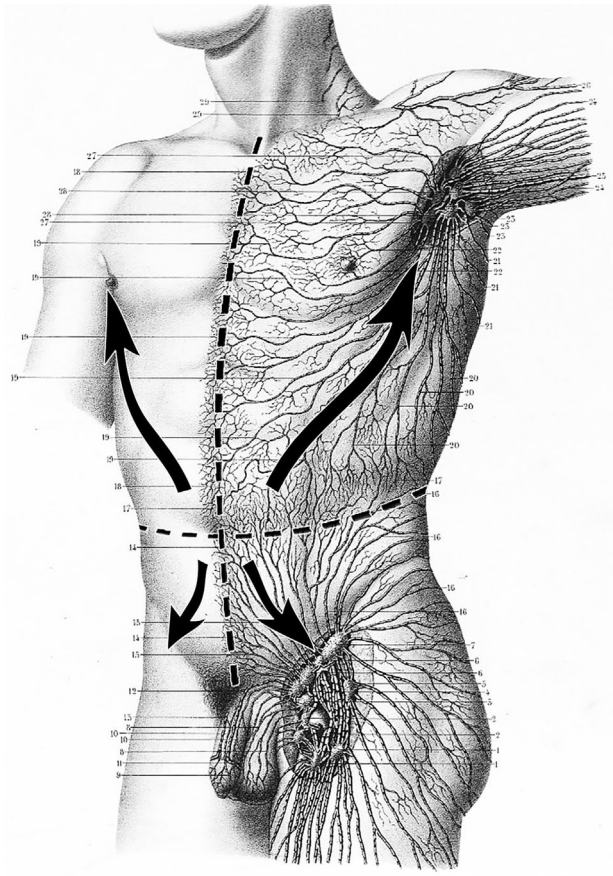

Fig. 1. Suppey's diagram of the front torso illustrates that each lymphatic territory drains to ipsilateral axilla or inguinal lymph nodes (arrows and dotted lines added by author).

Cabanas applied lymphangiography to penile cancers and found a similar lymph drainage pattern to the inguinal lymph node [11]. He coined the term "sentinel lymph node" which describes the first lymph node/s that receive lymphatic drainage from the tumor. He postulated that the presence or absence of cancer cells in the sentinel lymph node would provide useful information to determine prognosis. Morton tested the effectiveness of an intradermal dye injection technique to identify the sentinel node in a ferine model [12]. He then applied the dye injection method in melanoma patients to identify the sentinel lymph node [13]. Oncologic surgeons have been inspired by the advancing knowledge of lymphatic anatomy that has prompted the development of new or refined surgical procedures.

METHODS FOR ANATOMICAL STUDY OF THE LYMPHATIC SYSTEM

This study provides an introduction to our current method for investigating the anatomy of the lymphatic system in animals and humans [14,15]. Identification of the lymphatic vessels in the surrounding fat tissue is the initial crucial step for anatomical investigation, but is also the most difficult. Braithwaite compared the difference in the diffusion of injected dye into the gastric wall between live and postmortem specimens and found that uptake of dye into the lymphatic system was robust in the live specimen but very modest in the postmortem specimen [16]. Thus, identification of the lymphatic system using dye is easier in live specimens, but enormously difficult in dead specimens.

The author's method was developed primarily on the basis of Kinmonth's lymphangiography technique [9] in the clinical setting, modified for cadaver dissection [17] and downsized for smaller lymphatic vessels less than 0.3 mm [18]. The recently developed procedure of ICG fluorescence lymphography has been used in breast sentinel lymph node biopsy [19], diagnosis of lymphoedema, and lymphoedema surgery [20]. ICG fluorescence lymphography is also a very useful imaging tool for animal experiments and enables enhanced visualization of lymphatic vessels in real time using infrared technology. ICG fluorescence lymphography was found to demonstrate the lymphatic vessels in extremities and the nipple and areolar complex of unfrozen, fresh human cadaveric specimens after intradermal injection of the dye into the finger web space or areola region, respectively [14]. This finding demonstrated that when injected into dead specimens, the ICG is picked up by the lymphatic system in a manner similar to Nuck's mercury injection technique for cadaver dissection. Once the ICG reaches the lumen of the lymphatic vessel, it readily moves proximally with gentle massage, without leaking into the surrounding tissue. If a skinny cadaver is used, the ICG can potentially reach the armpit using extrinsic massage. ICG lymphography thus enables us to locate lymphatic vessels in cadavers and facilitates the consecutive cannulation process. It is worth noting that in our initial efforts, ICG lymphography alone did not easily identify the lymphatic vessels in cadaver torsos. However, when hydrogen peroxide (3%) or a mixture of hydrogen peroxide and acrylic blue dye was injected into the cadaver skin or soft tissue, fine oxygen bubbles were produced and led to an increase in tissue pressure, thus inflating the lymphatic vessels and allowing them to be more easily identified. The blood vessels were also inflated by the bubbles, but the lymphatic vessels were distinguishable from the blood vessels because lymph fluid does not contain any red blood cells and the vessels appear as bead-like formations with thinner walls and extensive valve structures [17].

While ICG lymphography was very helpful in locating lymphatic vessels during cadaver dissection, it was not enough to demonstrate the way in which each lymphatic vessel connected to the corresponding lymph node. Penetration of infra-red rays from the ICG through the soft tissue was limited to 12 mm; therefore, the lymphography could not reveal lymph nodes located in deeper tissue. After identification of the lymphatic vessel in the distal region, each vessel was cannulated with a 30-gauge needle and radiocontrast media or acrylic dye was injected to undertake radiographic recording or dissection, respectively. A micromanipulator was used to hold the small needle steady and cannulate it into the lymphatic vessel, namely, the microinjection technique [21]. If the size of the lymphatic vessel was less than 0.3 mm, a heated and stretched micro glass pipette was selected. The glass needle enabled cannulation of smaller lymphatic vessels up to 80 μm, similar to the size of lymphatic vessels in mice [18]. The needle or glass pipette was connected to a 1 cc syringe via a silicone extension tube and the injectant was pumped manually using the syringe. Multiple injections were required to identify the entire lymphatic pathway until all lymphatic vessels had reached their corresponding lymph node (Fig. 2) [22].

LYMPHOSOME IN ANIMALS

The author has studied the lymphatic system in different animals, including mice [18], rats [21], rabbits [23], canines [24], and swine [15]. The process of identifying the lymphatic vessels in animals was easier than in humans because the subcutaneous fat layers are thinner in animals.

Understanding the anatomical differences of the lymphatic system between animals and humans is crucial when designing animal experiments for lymphatic research. For example, 20–40 lymph nodes can be found located in the human axilla [25], but there are relatively fewer nodes in animals (rat: 1, rabbit: 1, canine: 1–2, and

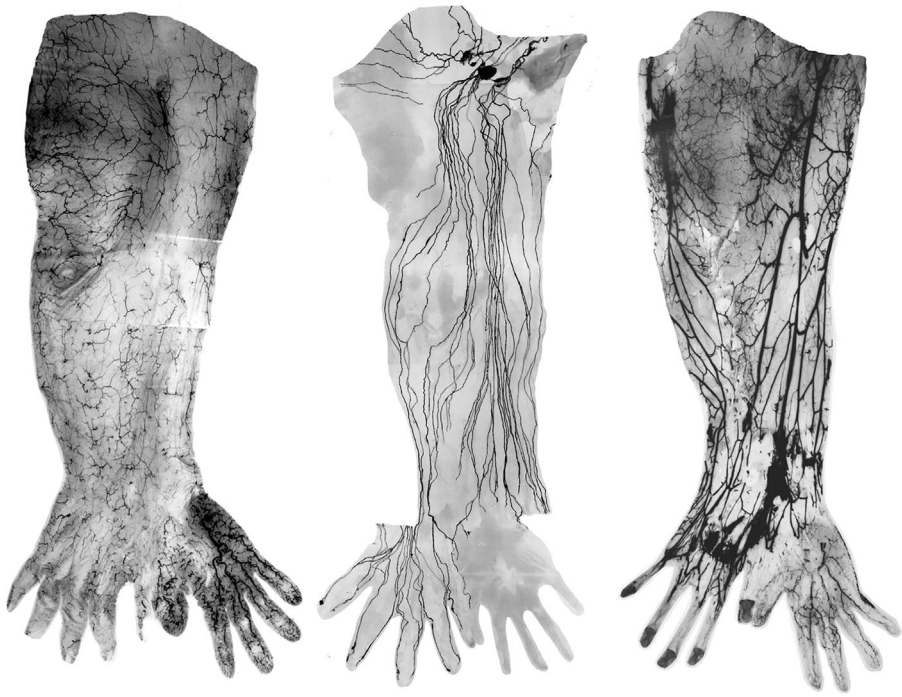

Fig. 2. Radiographs of the skin and fat tissue of the upper extremity with the arterial (left), lymphatic (center), and venous (right) injections from three different cadaver specimens. (Reproduced from Ref. [22]).

swine: 0) [15,21,23,24]. The number of lymphatic vessels is very small in rodents and rats have only three to four lymphatic vessels in the hind limb. In contrast, the size of lymphatic vessel was less varied between animals and humans relative to body size. The diameter of the lymphatic vessel in the rat hind limb is around 0.2 mm, while in humans, it is between 0.2 mm and 2.2 mm [26].

Lymphatic mapping in animals varied between species but was specific between specimens within species. For example, the dominant lymphatic pathway in a canine forelimb drained to the superficial cervical node instead of the axillary nodes as is the case in humans [24]. The lymphatic drainage pathways in both the upper torso and forelimb in the swine connected to the superficial cervical node, possibly due to the absence of a superficial axillary node in this species [15]. The combination of ICG fluorescence lymphography and the microinjection technique enabled us to determine the connections between lymphatic vessels and their corresponding lymph node. Each group of regional lymph nodes was color-coded to match the color of the lymphatic vessels that drained into them [14]. The superficial lymphatic vessels rarely crossed each other, so it was possible to divide the skin into lymphatic territories (Fig. 3). The author coined the term "lymphosomes" to demarcate the lymphatic territories determined in this manner. Lymphosomes have proved to be instrumental in providing crucial anatomical information to map which area of the skin drains into which corresponding regional lymph nodes [15,23,24].

LYMPHOSOMES IN HUMANS

In human cadaver dissection, the same method as described above can be used to identify and track lymphatic vessels to the point where they connect to their corresponding lymph nodes. Previously, lymphatic vessels were described as running alongside their corresponding veins, but this description has been found to be inaccurate. Some lymphatic vessels accompany cutaneous veins, such as the cephalic, basilic, great and short saphenous veins, but other lymphatic vessels bear no close relationship with cutaneous veins. In normal conditions, the superficial lymphatic vessels were independent from the deep lymphatic vessels that are located below the deep fascia. While some lymphatic vessels accompanying cutaneous veins ran gradually from the superficial region to the deep region, there were no vertical connections like those found in the venous system. The cutaneous vein branches anastomosed each other frequently, appearing as a honeycomb-like network structure (Fig. 2) [22]. However, in the case of lymphatics, the superficial vessels diverged and merged on their way to the lymph node without any of the interconnections observed in cutaneous veins. Similarly to lymphosomes in animals, these anatomical characteristics of the superficial lymphatic vessels in humans allowed us to color-code each lymphatic vessel with the color of its corresponding lymph node, thus permitting the successful demarcation of the human skin into lymphosomes (Fig. 4).

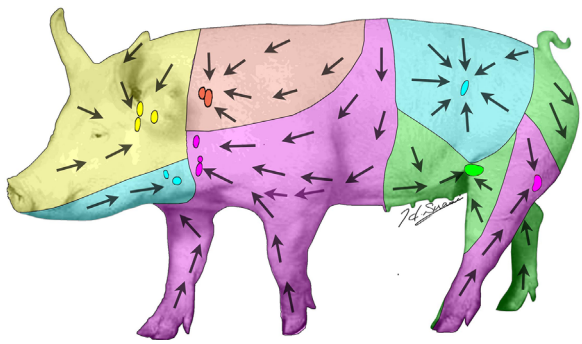

Fig. 3. The skin of the swine was successfully divided into lymphosomes after we mapped the whole lymphatic system by using ICG fluorescence lymphography and the microinjection technique.

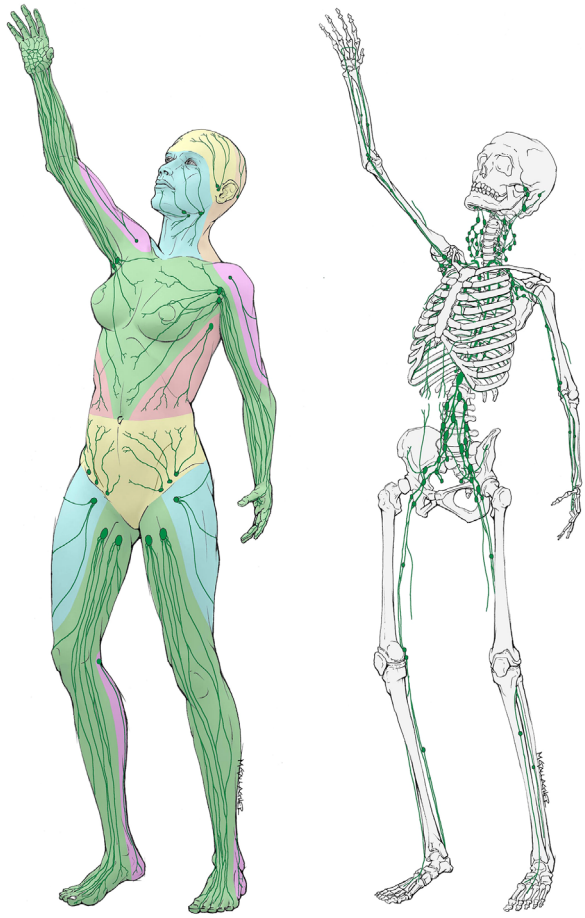

Fig. 4. Lymphosomes in the human: superficial lymphatic system (left) and deep lymphatic system (right).

Kinmonth identified the dominant lymph node in the upper extremity with lymphangiography [27]. The dominant lymph nodes in the limbs were also identified in our dissection studies. The dominant lymph node/s in the upper extremity was/were located in the most lateral aspect of the axilla and correspond to level I lymph nodes using the breast cancer classification [22]. The dominant lymph nodes in the lower extremity were identified as being located at the bottom of the inguinal triangle next to the great saphenous vein [14]. The small number of dominant lymph nodes were connected to the majority of lymphatic vessels in the extremities and covered a large area. These lymph nodes also acted as sentinel lymph node in the extremities. However, there were collateral lymphatic pathways in the extremities which skipped the dominant lymph nodes and connected to other lymph nodes. The lymphatic vessels accompanying the cephalic vein in the upper extremity skipped the axillary lymph nodes and connected to the subclavicular lymph nodes via the deltopectral node located in the front shoulder [28]. The lymphatic vessels originating in the heel region accompanied the short saphenous vein in the lower extremity, skipping the superficial inguinal lymph node and connecting to the deep inguinal lymph node via the popliteal lymph node in the knee fossa [29]. Knowledge of these collateral lymphatic pathways is of great importance for skin cancer management, because the conventional targets of lymph node dissection, such as axillary or inguinal dissection, may miss metastasized lymph nodes corresponding to these pathways.

The lymphatic vessels in the upper torso originated from the anterior and posterior midlines and lumber horizontal line and they converged toward the axilla, as illustrated by Sappey (Fig. 1) [4]. This arrangement of lymphatic vessels did not differ between males and females. A dissection study by Bartels proved that this arrangement of lymphatic vessels was already developed in the infant [30]. Most current anatomical atlases tend to illustrate breast lymphatic drainage radiating from the areolar region [25,31]. However, these diagrams fail to demonstrate that there are lymphatic vessels in the torso that drain the breast. Some of the lymphatic vessels in the torso passed through the breast paranchyma and connected to the group of lymph nodes located in the upper part of the axilla [32,33]. Lymphsomes in the front torso were divided into two subgroups of regional lymph nodes, those which connected to the lymphatic vessels passing through the breast (color-coded green) and those which did not (color-coded orange) (Fig. 4).

FUTURE ANATOMICAL STUDIES FOR LYMPHATICS

It has been possible to refine oncologic surgery to prevent cancer spread by taking lymphatic anatomy into consideration to predict cancer metastatic sites. Historically, semi-integumentectomy for malignant melanoma [34] and radical mastectomy for breast cancer [35] aggressively removed all possible tissue, including the lymphatic system, by performing en-bloc resection from the primary tumor site to the corresponding regional lymph nodes. These procedures were later modified and primary resection and lymph node dissection became standard. The introduction of the sentinel lymph node biopsy concept for melanoma [13] and breast cancer [36] provided a personalized surgical option to avoid redundant dissection.

Histological study of the lymphatic system has significantly progressed with development of immnohistochemical staining methods for microscopic analysis [37,38]. However, gross anatomical study of the lymphatic system has been hampered for the past century due to the lack of a suitable dissection technique to replace Nuck's and Gerota's methods. The importance of the author's method of combining ICG fluorescence lymphography with the microinjection technique as highlighted in this paper and development of the lymphosome concept is significant for further investigation and better understanding of the lymphatic system. The lymphosome concept will not only provide a normal anatomical template for interpreting lymphoscintigraphy to locate the sentinel lymph node or assess lymphoedema, but also has the potential to inform further refinement of surgical oncology techniques. For example, precise mapping of breast and upper extremity lymphatic drainage would help to improve the axillary reverse mapping technique [39] for breast cancer and contribute to preserving arm lymphatic drainage without compromising breast cancer treatment, thus prophylactically preventing postoperative lymphedema.

Despite the efforts of Baum [40,41], a German veterinarian, and Kampmeier [42] who undertook extensive study of lymphatic anatomy in different species, lymphatic mapping in animals has not been sufficiently investigated. Lymphoedema is a debilitating life-long problem for cancer survivors, but we still do not fully understand the etiology of the disease. Animal experiments must play an important role in investigating the pathophysiology of lymphoedema and test different modalities as pre-clinical studies. Better understanding of animal anatomy is crucial for comparing the pathological and normal condition. I hope that the lymphosome concept will assist lymphatic research in animal experiments.

SUMMARY

Gross anatomical study of the lymphatic system in humans and animals has been suspended for over 100 years, due to lack of a suitable dissection method. In contrast, oncologic surgery continues to advance

with new imaging techniques for detecting metastasized lymph nodes in the clinical setting. Now that the anatomical study of the lymphatic system has been revisited, the improvements in knowledge can be applied to reassure surgeons about their current procedures in the surgical management of cancers and lymphedema or assist them to refine them, including sentinel node biopsy and axillary reverse mapping, and in so doing promote better understanding of the etiology of lymphedema. The author hopes our new investigative technique and the lymphosome concept will make an invaluable contribution to the field of oncological research.

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