FACULTY OF MEDICINE AND HEALTH SCIENCES

THE BIOMECHANICAL PROPERTIES OF THE LYMPHATIC SYSTEM AND THE CHANGES IN LYMPHEDEMA

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ACKNOWLEDGMENTS

I’ve chosen the subject of lymphatics because I was interested to know more about the lymphatic system and lymphedema. From my point of view, the subject of the lymphatic system is not sufficiently covered during my regular courses at university. While lymphatics are important for the functioning of the human body, they remain neglected. Furthermore, I was attracted to the idea of a multi-disciplinary approach to gain insights in the lymphatic system. The more knowledge is gathered, the more we are inclined to specialize and to delve deeply into a subject. A subject as complex as the lymphatics could certainly benefit from different fields of expertise. It is the intention that this thesis can function as a starting point for anyone with either an engineering or medical background who wants to deepen their knowledge on the lymphatic system.

First and foremost, I want to thank my supervisors, Prof. Segers and especially Prof. Debbaut. I would also like to thank my counsellor, Ghazal Adeli Koudehi, for answering my many questions and for giving me very useful feedback. I could not have written my thesis without their guidance and clarification, especially on the subject of biomechanics.

Finally, I want to thank my family and friends for their love and support.

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ABSTRACT

The lymphatic system, which is part of the vascular system, drains fluid from the extracellular spaces, organizes the immune response, and absorbs fat. This thesis gives an overview of the anatomy and physiology of the lymphatic system. Lymphedema is a chronic progressive disease characterized by localized tissue swelling and caused by malfunction of the lymphatic system. This thesis explains the pathophysiology of lymphedema and gives an overview of the state-of-the-art for diagnosis and treatment of lymphedema. Multi-disciplinary biomedical engineering approaches are essential for the progression of our understanding of the lymphatic system. Therefore, this thesis will discuss the biomechanical properties of the lymphatic system and their changes in lymphedema.

A literature review was conducted with several searches in Pubmed and Web of Science. The conclusions of this literature review can be summarized as follows. The type of flow in the lymphatic system can be characterized by a low Reynolds number, a low Womersley number, and the law of Poiseuille. The Poiseuille flow assumption is valid in non-valvular regions of the lymphatic for most of the contraction cycle. Lymph flow velocity and flow rate are discussed. The lymph pump is coordinated by a time delay which results in more effective pumping. The mechanism of valve gating and the valve properties are described. There are four main stresses acting on the lymphatic wall, namely shear stress, circumferential stress, radial stress, and axial stress. Lymphatic vessels show a highly nonlinear pressure-diameter relationship. The lymphatic wall is permeable to macromolecules and the permeability can be regulated by shear stress and specific peptides.

Studies on the altered biomechanics in lymphedema are scarce. The following conclusions can be made based on the limited studies. Inflammatory agents result in increased permeability of the lymphatic wall in an outward direction which results in a decreased efficiency of fluid clearing. The transmural pressure is elevated in lymphedema and increases as lymphedema progresses. The outflow pressure (afterload) is significantly and chronically elevated in edematous limbs. Chronic lymphedema patients have lymphatic contractile dysfunction. The contractility will increase in response to the sustained afterload elevation. However, as lymphedema progresses the vessels will decompensate and the pump function will decline. Lymphatic vessels can remodel to adapt to changes in the mechanical environment, but this will not resolve the pumping failure in the long-term, despite possible temporary beneficial effects in the acute stage of the adaptation.

NEDERLANDSTALIG ABSTRACT

Het lymfevatenstelsel, deel uitmakend van het vaatstelsel, voert vocht af van de extracellulaire ruimtes, organiseert de immuunrespons en absorbeert vet. Deze thesis geeft een overzicht van de anatomie en fysiologie van het lymfevatenstelsel. Lymfoedeem is een chronische en progressieve ziekte gekarakteriseerd door een gelokaliseerde weefselzwelling en veroorzaakt door een stoornis in de werking van het lymfevatenstelsel. Deze thesis beschrijft de pathofysiologie van lymfoedeem en geeft een overzicht van de state-of-the-art op vlak van diagnose en behandeling. Multidisciplinaire biomedische ingenieurstechnieken zijn cruciaal voor de uitbreiding van kennis over het lymfevatenstelsel. Deze thesis zal daarom de biomechanische eigenschappen van het lymfevatenstelsel en hun veranderingen bij lymfoedeem bespreken.

Een literatuuronderzoek werd uitgevoerd met zoekopdrachten in Pubmed en Web of Science. De conclusies van dit literatuuronderzoek kunnen als volgt samengevat worden. De lymfestroom kan getypeerd worden door een laag Reynoldsgetal, een laag Womersleygetal en aan de hand van de wet van Poiseuille. De veronderstelling van Poiseuillestroming is geldig in de regio's op een zekere afstand van een klep en voor het grootste deel van de contractiecyclus. De lymfestroomsnelheid en -debiet worden besproken. De lymfatische pomp wordt gecoördineerd door een vertraging tussen contracties, wat leidt tot effectiever pompen. De klepwerking en de klepeigenschappen worden beschreven. Er zijn vier spanningen die inwerken op de wand van lymfevaten, namelijk de schuifspanning, omtrekspanning, radiale spanning en axiale spanning. Lymfevaten vertonen een niet-lineaire druk-diameter relatie. Lymfevaten zijn permeabel voor macromoleculen en de permeabiliteit wordt gereguleerd door schuifspanning en specifieke eiwitten.

Studies over de verandering van biomechanische eigenschappen in lymfoedeem zijn schaars. De volgende conclusies kunnen gemaakt worden op basis van het beperkt aantal studies. Inflammatie leidt tot een verhoogde permeabiliteit van de wand van lymfevaten wat zorgt voor een minder efficiënte afvoer van vocht. De transmurale druk is gestegen bij lymfoedeem en stijgt verder bij de progressie van lymfoedeem. De afvoerdruk is significant en chronisch gestegen in een oedemateus lidmaat. Patiënten met chronisch lymfoedeem lijden aan contractiele dysfunctie. De contractiliteit zal stijgen als respons op de gestegen afvoerdruk. Bij verdere progressie van lymfoedeem zullen echter de lymfevaten decompenseren en de pompfunctie zal achteruitgaan. Lymfevaten kunnen remodelleren om zich aan te passen aan mechanische veranderingen in hun omgeving, maar dit zal het pompfalen op lange termijn niet kunnen herstellen, ondanks mogelijke tijdelijke verbetering op het acute moment van de remodellering.

INTRODUCTION

The vascular system is composed of the arteries, the veins, and the lymphatic system (1). The importance of the lymphatic system is often disregarded in research and clinical practice and the lymphatic system has been called the forgotten child in the vasculature family (2,3). Despite knowing that the lymphatic system plays an important role in cardiovascular disease, infection, immunity, cancer, and possibly obesity, which are major challenges to healthcare, its role in health and disease is not completely understood (3). The lymphatic system drains fluid from the extracellular spaces, organizes and facilitates the immune response and acts as conduit for immune cells, transporting white blood cells and antigen-presenting cells to lymphoid organs (4–7). In addition, the lymphatic system is tasked with the absorption of fat and maintains the fluid homeostasis and the tissue-fluid balance (5–7).

Lymphedema is a chronic progressive disease characterized by localized tissue swelling. Figure 1 shows three examples of the clinical presentation of lymphedema in which impairment of lymphatic drainage caused the excessive retention of fluid in the interstitium (8,9). Due to its widespread nature, occurring approximately in 1 out of 30 people worldwide, lymphedema is considered a significant problem (2,8). The disease is known to affect primary females and its prevalence increases with age (8,10). Moffatt et al. (11) reported a prevalence of 1,33/1000 which increased to 1/200 in those aged over 65 years. 83% of the lymphedema patients in this study were female (11). If lymphedema is left untreated, it could lead to functional impairment, physical deformity, and a significantly reduced quality of life (1,10). For this reason, early diagnosis and treatment is crucial in preventing its progression (12). However, lymphedema is frequently overlooked by physicians, since they are often unfamiliar with the disease and non-invasive diagnostic tests are insufficiently available (2,10).

The aim of this thesis is to give an overview of the anatomy and physiology of the lymphatic system, pathophysiology of lymphedema, and an overview of the state-of-the-art for diagnosis and treatment of lymphedema. As a result of recent advances through innovations in imaging, tissue engineering, computational modelling, and biomechanics, a multi-disciplinary biomedical engineering approach is deemed necessary for fully understanding the lymphatic system (13). Therefore, the second part of this thesis will discuss the biomechanical properties of the lymphatic system and their changes in lymphedema.

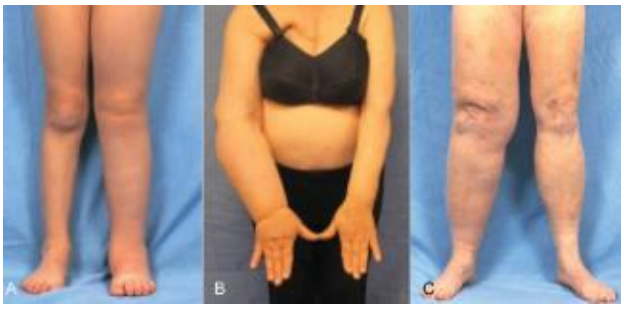

Figure 1: Examples of Lymphedema (14).

METHOD

The introduction and the first two sections are written based on several searches in Pubmed, Web of science, and Google Scholar. The following keywords have been used: “lymphatic system”, “lymphedema”, “lymphatic disease”, “physiology”, “lymph formation”, “pathophysiology”, “pathological”, “inflammation”, ”T cells”, ”T-regulatory cells”, “immune system”, “fibrosis”, “adipose deposition”, “lymphangiogenesis”, “diagnosis”, “lymphoscintigraphy”, “perometry”, “sensitivity”, “CT”, “treatment”, “complex decongestive therapy”, “low level laser therapy”, “VEGF-C hydrogel”, “surgery”, “scaffolds”, “medication”, “sodium selenite”, “targeted drug delivery”, “biomechanical” and “biomechanics”. Several articles were found in the reference lists using the snowball method.

The literature review was conducted based on studies on the biomechanical properties of the lymphatic system. Due to the limited number of studies on this subject, all studies found on the biomechanical properties of the normal lymphatic system or the changes in lymphedema were included. The available literature in the databases Pubmed and Web of Science was reviewed and full text articles were screened when the titles or the abstracts seemed relevant.

The searches in Web of Science with the keywords “lymphatic system biomechanics” and “lymphatic system biomechanical” led to 12 and 13 results, respectively. 5 articles were selected from the first search (4,15–18) and 2 articles were selected from the second search (19,20). The keywords “lymphatic system biomechanics” were also used in Pubmed resulting in 3 articles selected accordingly (13,21,22). 186 results were found in Web of Science using the keywords “lymphatic system physiology” of which 1 article was selected (23). Searching in Web of Science with the keywords “lymphedema shear stress” resulted in 30 articles of which 2 articles were selected (24,25). A search on the author Dixon led to one article and one book (26,27). The snowball method was also applied, 25 articles and one book were selected from the reference lists of the included articles (28–53). The literature search is schematically shown with the help of a PRISMA flow diagram in figure 2.

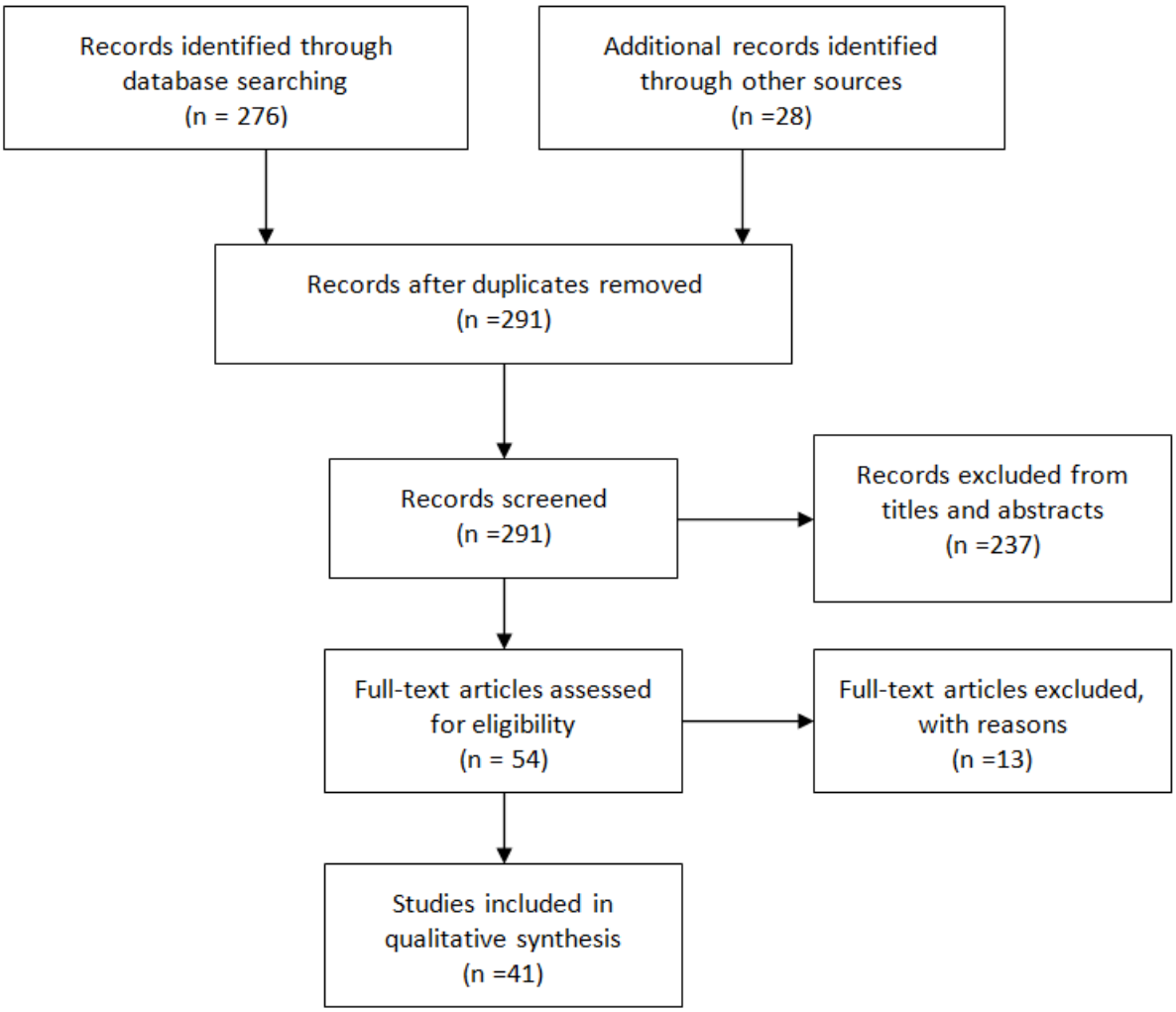

Figure 2: PRISMA flow diagram (54).

RESULTS

This thesis first discusses the anatomy and physiology of the lymphatic system and the pathophysiology, diagnosis, and treatment of lymphedema. These first two sections give essential background information to understand the second part of this thesis (section 3 and 4), namely the biomechanical properties of the lymphatic system and the changes in lymphedema.

1. Lymphatic system

1.1. Anatomy

The lymphatic system consists of a network of lymphatic vessels, lymph nodes, and lymphoid organs including the spleen, thymus, tonsils, and the bone marrow, shown in figure 3 (4,23). The lymphatic vascular system spreads out to every part of the body with the exception of the brain and spinal cord (23). Recently, the Glymphatic system has been identified as being responsible for clearing the brain from solutes and waste products. It is a paravascular pathway that enables the exchange of cerebrospinal fluid with interstitial fluid. The brain meninges contain conventional lymphatic vessels (55). Six main types of lymph conduits are defined: the initial lymphatics (also called lymphatic capillaries or terminal lymphatics), the precollecting vessels, the collecting vessels, lymph nodes, trunks, and ducts (28).

The initial lymphatics drain interstitial fluid and start from blind-ended structures of approximately 10 to 60 µm in diameter (4,23,28). The initial lymphatics have thin walls consisting of a single endothelial cell layer of overlapping oak leaf shaped endothelial cells which are supported by sparse or absent basement membrane (2,7,23,28,56,57). The initial lymphatics are coupled to the extracellular matrix (ECM) using tethering elastic fibres, known as the anchoring filaments, allowing fluid to enter the initial lymphatics through the primary valves (23,28). Initial lymphatics drain into the collecting lymphatics via the precollecting vessels (4,23,28). The precollecting vessels have irregularly spaced valves and its lumen is only partially covered with muscle cells (4). In the meantime, collecting lymphatics have regularly spaced one-way secondary valves and contractile walls (2,23,28). The muscle cells of the contractile wall have both smooth and striated muscle characteristics, which can be considered a new class of muscles which is neither smooth nor cardiac muscle (57,58). The secondary valves subdivide the vessels into short segments: the lymphangions (4,23). A lymphangion serves as a contractile segment that propels lymph towards the next lymphangion (28). Collecting vessels are arranged in a network as a converging tree and move lymph through at least one cluster of lymph nodes (7,28). The collecting vessels can thus be further categorized as prenodal or postnodal. There are hundreds of lymph nodes in the human body of approximately 1 to 10 mm in diameter. The lymph nodes filter and break down bacteria and

viruses and are incubators for white blood cells (28). The postnodal lymphatic vessels drain into larger trunks (7,28).

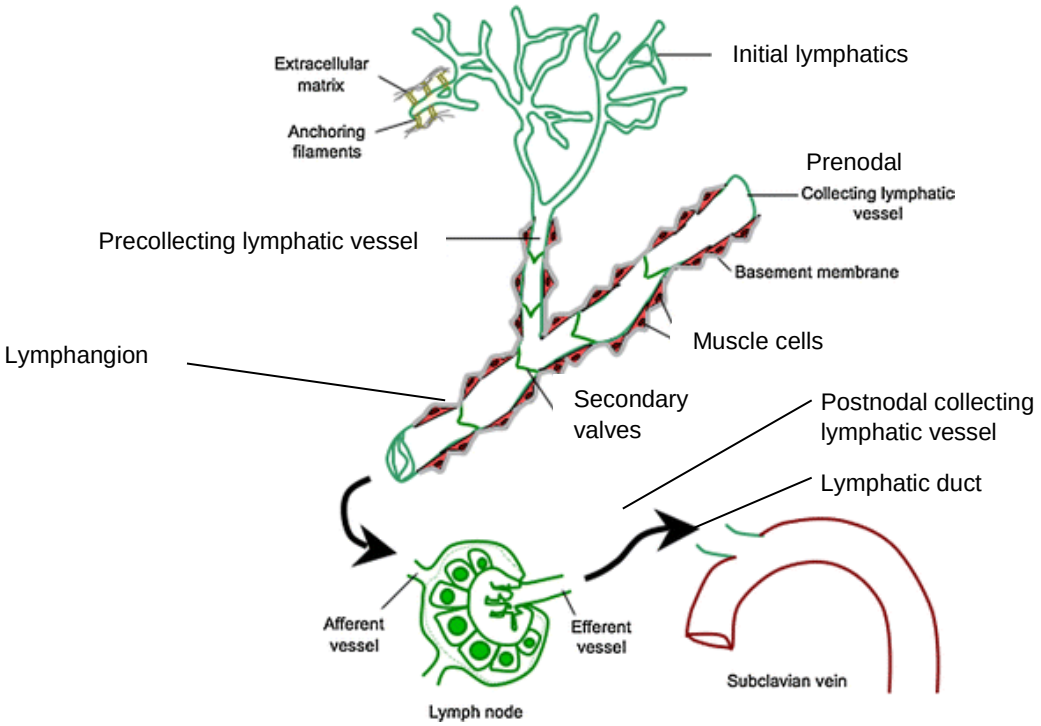

Figure 3: Anatomy of the initial lymphatics, precollecting lymphatic vessel, prenodal and postnodal collecting lymphatic vessel, and lymphatic duct. The secondary valves subdivide the vessel into short segments: the lymphangion. The lymphatic trunks are not depicted. Adapted from (59).

The trunks debouch into the ducts with the exception of the lymphatics of the intestinal, hepatic, and lumbar areas which drain directly into the thoracic duct (28). The ducts return the lymph back to the bloodstream via the subclavian vein (2,28). Figure 4 depicts the relation of the lymphatic system to the cardiovascular system. Lymphatic vessels have thinner walls relative to their diameter when compared with blood vessels, but the multi-layered structure is similar (16).

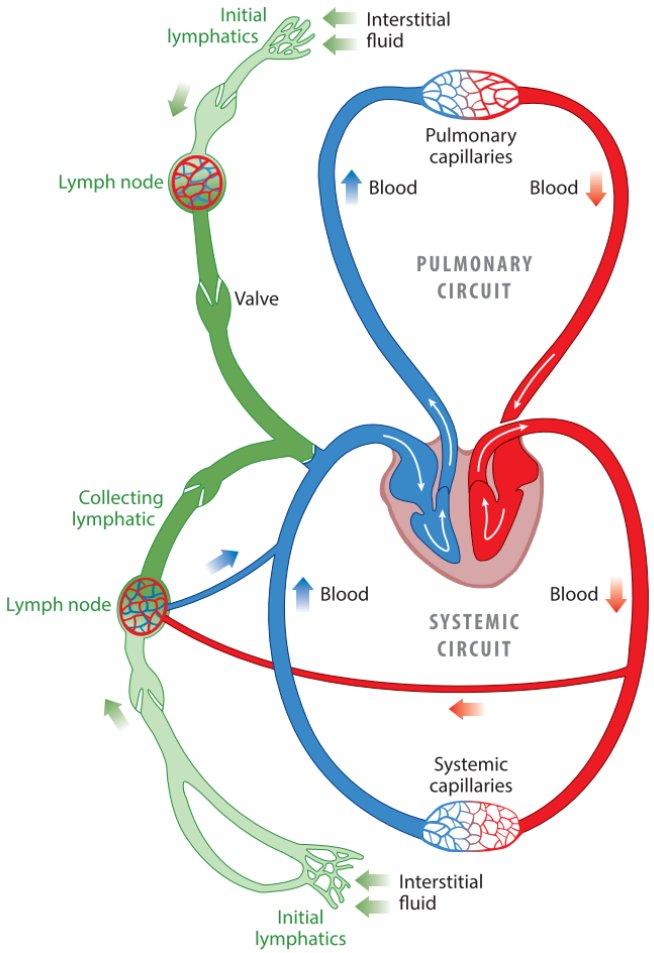

Figure 4: The relation of the lymphatic system to the cardiovascular system (23).

1.2. Physiology

The following section describes how lymph fluid is formed and transported throughout the human body. Fluid is filtered from the capillaries to the interstitium which is conceptualized in the revised Starling principle, after which it is transported from the interstitium into the initial lymphatics (lymph formation). The following lymph propulsion is regulated by stretch, shear, and neural modulators. This section concludes with the important role of the lymphatic valves.

1.2.1. Revised Starling principle

Interstitial fluid is formed by ultrafiltration (i.e. filtration through a porous membrane) of the capillary microcirculation. The Starling principle states that the ultrafiltration is regulated by the balance between hydrostatic and osmotic pressures (i.e. pressure induced by concentration of macromolecules in the vasculature, attracting fluid) across the blood vessel wall (57). The induced pressure gradient drives the fluid to be filtered at the arterial end of the capillaries and reabsorbed at the venous end (3,57). However, the generally accepted Starling principle was found to be unable to explain the observed fluid-behaviour. In most tissues, the steady-state pressure provides filtration from the capillaries towards the interstitium at both arterial and venous ends since the pressure gradient at the venous end does not cause an absorptive force but rather a slight filtration force

(3,57). Venous blood pressure is higher than all the pressures opposing filtration. Disturbance of the hydrostatic and osmotic pressure balance, e.g. due to haemorrhage, can enable reabsorption for a short period of time, but it will quickly readapt to the state of filtration (3). The exchange of molecules occurs through the glycocalyx, a matrix of glycoproteins and glycosaminoglycans, which acts as a semipermeable membrane (23,57,60). The revised Starling principle distinguishes the subglycocalyx space from the rest of the interstitial fluid, as illustrated in figure 5. The protein concentration of the main part of the interstitial fluid is higher than the protein concentration of the fluid in the subglycocalyx space. The colloid osmotic pressure of the fluid of the subglycocalyx, rather than the colloid osmotic pressure of the interstitial fluid, is considered the driver of the net filtration force. Figure 5 illustrates the differences between the classic and revised Starling principle. According to the revised Starling principle, the filtration force can be expressed by the following equation (60).

$$filtration\ force = (P_c - P_i) - \sigma(\pi_p - \pi_g) \qquad \text{Equation 1}$$

$P_c = local\ capillary\ blood\ pressure\ [Pa], P_i = Interstitial\ fluid\ hydrostatic\ pressure\ [Pa],$
$\sigma = Staverman's\ osmotic\ reflection\ coefficient, \pi_p = colloid\ osmotic\ pressure\ of\ plamsa\ [Pa],$
$\pi_g = colloid\ osmotic\ pressure\ of\ underside\ of\ glycocalyx\ [Pa]$

The semipermeability of the glycocalyx is imperfect and plasma protein leaks into the interstitium. This is represented in the Staverman's osmotic reflection coefficient (60).

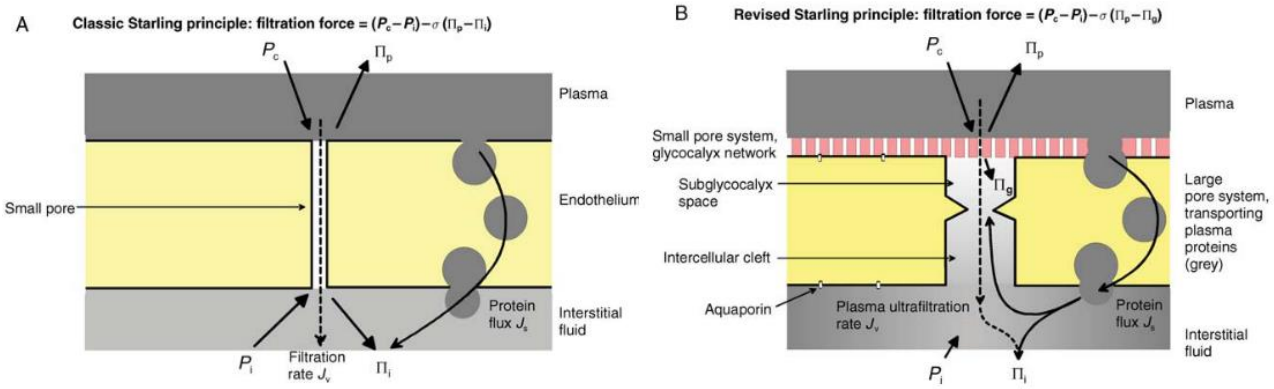

Figure 5: Classic (A) versus revised (B) Starling principle (60).

1.2.2. Lymph formation

The lymph formation is the fluid transport from the interstitium into the initial lymphatics and depends on local effects. The driving forces are the interstitial fluid pressure and strain of the ECM (4,28). The anchoring filaments are sensitive to interstitial stresses and by pulling outward they exert tension on the initial lymphatics to increase its luminal volume (23,28). Fluid enters the initial lymphatic when the pressure in the interstitium is greater than inside the initial lymphatic (56). This mechanism is shown in figure 6. Experimental measurements of pressure showed unexpectedly that the pressure in the initial lymphatics is actually higher than the interstitial pressure. There is

thus a pressure gradient against fluid drainage into the initial lymphatics (12,61). However, there are cyclic changes in both the interstitial and initial lymphatics pressures due to activities in the surrounding tissue and the lymphatics. These changes in pressure will result in a pressure gradient that favours the lymph formation. The activities that have a role in this process are skeletal muscle contraction, heart contraction, gastrointestinal muscle contraction, breathing, intrinsic contractions of the lymphatics, and primary valves (61). The overlapping endothelial cells prevent a backward flow into the interstitium by acting as flap valves when the pressure inside the initial lymphatics becomes greater than the interstitial pressure (23,28,56).

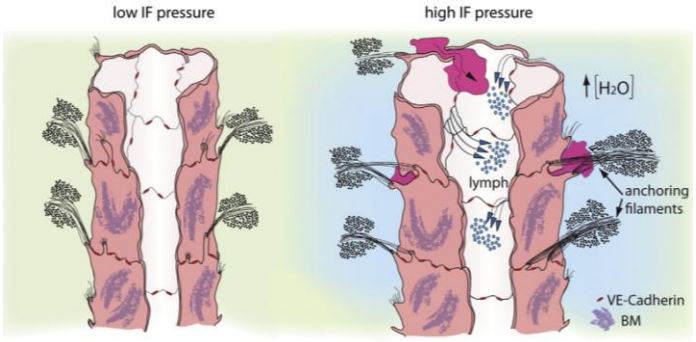

Figure 6: Anchoring filaments exert tension on the overlapping junctions of the lymphatics when interstitial fluid (IF) pressure is increased, which allows the entry of fluid (18).

1.2.3. Lymph composition

The composition of plasma and lymph are both qualitatively and quantitatively different. Lymph has a higher concentration of proteins as a result of ECM processing, tissue growth, tissue remodelling, cellular metabolic activities, cellular catabolic activities, and cell death and contains tissue specific proteins drained from different organs. Eventually, only a small portion of these tissue specific proteins will reach the venous circulation because of phagocytosis by antigen-presenting cells (57). The higher postnodal lymph protein concentration is caused by the absorption of primarily water in the lymph nodes (23). Water, sodium, and urea can also leave the lymph by crossing the lymphatic walls into the surrounding matrix tissue (56). The capillaries lose around eight litres of water a day which results in prenodal lymph. Because of the reabsorption of water, the resulting postnodal lymph flow rate is about four litres a day (23,60). This means that the entire plasma volume, which is considered to be approximately three litres, leaves the circulation once every nine hours (60).

1.2.4. Lymph propulsion

Once the initial lymphatics are filled, lymph has to be pumped against an adverse pressure gradient (4). The average lymph pressure in the more peripheral parts is lower than the final ducts and the lymph pressure in the final ducts is lower than the venous circulation in which they drain. The lymph also needs to be moved against gravitational forces and the hydrostatic pressure gradient. This indicates the need for an active mechanism to allow transportation of lymph (61). The active intrinsic contraction/relaxation cycle of the lymphangions and the passive external compression forces (see

the subsequent section Extrinsic pumping for more details) move the lymph forward (58). At rest, 1/3 of lymph propulsion is caused by extrinsic compression and 2/3 by intrinsic pumping (31). Moderate, tonic form, slow, and long-lasting contractions of the lymphangion are also regulators of lymph transport, besides the strong, fast, and brief contractions of the lymphatic intrinsic pump (61).

Lymphatic tone

The tonic form contractions of the lymphangion play a role in the regulation of lymph transport by influencing the flow resistance. Neural and humoral agents (e.g. α-adrenergic agonists, prostanoids, natriuretic factors, bradykinin, and substance P) regulate lymphatic tone and flow resistance. In addition, local physical factors such as stretch/pressure and shear/flow can also modify lymphatic tone (61).

Intrinsic pumping

Forward lymph propulsion requires rapid and strong contractions coordinated over the length of a lymphangion (31,61). This type of contraction results in a rapid reduction of the lymphatic diameter, of up to 80% of the resting diameter, a decrease in lymphatic compliance, an increase in the local lymph pressure, closure of the upstream valve, opening of the downstream valve, and ejection of lymph to the next downstream lymphangion (13,61). The contractions caused by intrinsic pumping are regulated by lymph pressure/stretch, lymph flow/shear, and neural modulators.

Lymph pressure/stretch mediated regulation of intrinsic pumping

Elevating lymph pressure is an activator of the lymph pump and increases the frequency and amplitude of the contractions. This activation is caused by an increase in the stretch of the lymphatic vessel (13,58,61). This adaptation to increased pressure is rate sensitive, hence lymphatics can compensate for the rapidly varying changes in load (13). Lymphatic vessels of different tissues will reach their maximal intrinsic pumping at different pressures (61). Peripheral lymphatics pump against a greater outflow resistance and can pump at higher pressures acting as strong pumping vessels (13,58,61). Increased preload is defined as increased filling pressure and stretch of the lymphatic during diastolic filling (58). High preload over a certain range increases the frequency and amplitude of the contractions (31,58,61). Contraction frequency is sensitive to pressure, for instance small changes in pressure can double the frequency (31). Further increases in pressure/stretch will eventually decrease the strength of the phasic contractions (61).

Partial outflow obstruction, increased central venous pressure, and gravitational shifts elevate the afterload due to increased outflow pressure. This results in high frequency but low amplitude contractions (31,58). Collecting lymphatics exhibit thus both Starling response

and Anrep effect, similar as described in the cardiovascular system. The Starling response is the increase in cardiac output when the preload is increased. The Anrep effect is the increase of contraction strength of the left ventricle when the aortic pressure is increased (23).

Lymph flow/shear mediated regulation of intrinsic pumping

Shear due to lymph flow causes reduction in contraction frequency and amplitude of the phasic lymphangion contractions, ejection fraction, and fractional pump flow and causes vessel dilatation (7,23,31,61). This is predominantly due to the production of NO (nitric oxide) which acts as vasodilator by inhibiting both the lymphatic tone and the spontaneous contractions (7,61). The primary source of NO is the endothelium, which is the inner cell layer of the lymphatic vessel wall (7). Other endothelial derived relaxing factors such as histamine and prostaglandins are also important in lymphatic physiology (7,31). Lymph flow can also activate the intrinsic lymph pump depending on the pattern and magnitude of the flow. The pump modulation is furthermore dependent on the sensitivity of the lymphatic vessels to shear (61). The shear sensitivity of the vessel is the shear stress threshold at which the vessel will adapt its diameter to the fluid flow (33).

Neural modulation of intrinsic pumping

Sympathetic adrenergic nerve fibres are the principal neural innervation of the lymphatic vessels. A-adrenergic stimulation increases amplitude and frequency whereas β-adrenergic receptor stimulation decreases amplitude and frequency. Muscarinic receptor agonists, serotonin, vasoactive intestinal peptide, calcitonin gene related peptide, and substance P have different effects depending on the species and location of the lymphatic vessel (31).

Extrinsic pumping

The passive external compression forces include systemic forces such as respiration, intestinal peristalsis, passive and active skeletal movements, pulsations of nearby arteries, vasomotion, gravitational forces, variations in central venous pressure, and massage (23,28,58). Extrinsic pumping is especially important in the lymphatic beds of the heart, skeletal muscle, intestinal wall, and the thorax (12,61).

1.2.5. Valve function

The secondary valves, which are semi-regularly spaced, mostly bicuspid and formed by connective tissue overlaid by endothelial cells, enable lymph propulsion by allowing emptying and filling of each lymphangion (4,28,31,57). The synchronization of the valve opening and closure with the vessel contraction is necessary to achieve unidirectional lymph transport and minimize backflow

(31,57,61). The valves are essential during contractions because it enables lymphangions to distend before emptying into the next lymphangion. This distension and thus stretch activates spontaneous contractions (28). On average, there is a potential hydrostatic pressure gradient of -150 cmH_2O (= -110 mmHg) from the feet to the great veins of the neck (61). The valves and lymph nodes interrupt this hydrostatic column of fluid (12,23,58,61). The effective hydrostatic pressure gradient is thus reduced, which promotes central net lymph flow (12,61).

2. Lymphedema

2.1. Classification

2.1.1. Primary lymphedema

Primary lymphedema resulting in underdeveloped or dysfunctional lymphatics is caused by congenital disorders with abnormal development of the lymphatic system. The most common examples are Milroy disease and lymphedema-distichiasis syndrome (23,62). Only affecting approximately 1/100000 children, primary lymphedema is considered a rare disease (14). Further classification can be conducted based on genetics (sporadic or familial) and time of onset (congenital (<1 year), praecox (1-35 year) or tardum (>35 year)) (9,10).

2.1.2. Secondary lymphedema

Secondary lymphedema is caused by the damage to or removal of lymph nodes which often results from surgery, trauma, infection or radiation (23). Approximately 99% of all lymphedema patients suffer from secondary lymphedema (8). A common presentation is breast cancer related upper limb lymphedema. The most frequent cause for secondary lymphedema in tropical regions is a parasitic roundworm that is spread by mosquito bites and results in filariasis. It is estimated that 120 million patients in developing countries suffer from filariasis (23). Direct lymphatic obstruction by the parasites can result in large scale tissue swelling called elephantiasis (23,62).

2.1.3. Staging

The two most common used staging systems for lymphedema are the International Society of Lymphedema (ISL) and the Campisi staging system. Table 1 describes the different stages in relation to these staging systems which are mainly based on clinical findings. It should be noted that imaging and quality of life evaluations are necessary for a more complete assessment (63). Figure 7 gives examples of the clinical presentation of the different stages.

The first phase of lymphedema is an asymptomatic latent phase which is marked by a balance between increased lymph load and decreased outflow capability (stage 0, subclinical stage).

Lymphedema becomes overt when compensatory mechanisms become insufficient (64). The first presentation of overt lymphedema is a puffy pitting edema that can be intermittent (ISL stage I) (14,64). With further disease progression, this pitting edema is reduced as a result of the production of subcutaneous fibroadipose tissue (ISL stage IIb) (14). The maximum swelling of the limb is most often observed within one year after onset, but complications such as cellulitis can increase the swelling (64)

Table 1**:** Staging of lymphedema. Data from (2,14,28).

	International Society of Lymphedema	**Campisi**
Subclinical	Changes only on imaging	
Mild	I accumulation fluid with high protein content	Ia no overt swelling Ib alleviation by limb elevation
Moderate	IIa rarely alleviation by elevation IIb loss of pitting	II Mild persistent swelling III Persistent with recurrent lymphangitis
Severe	III lymphostatic elephantiasis complete loss of pitting, trophic skin changes, infection, and fluid leakage	IV fibrotic changes of the limb, columnlike formation V elephantiasis, limb deformation, lymphatic warts

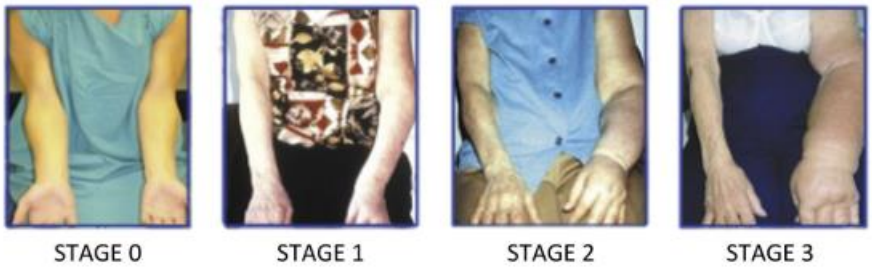

Figure 7: The stages of lymphedema (65).

2.2. Risk factors for the development of lymphedema

The two major risk factors for developing lymphedema are obesity (BMI>30 kg/m^2) and radiation therapy (1,62,63,65,66). In addition, infections, high blood pressure, age, sedentary lifestyle, and underlying genetic makeup are also known to contribute (1,63,65). One out of six patients who undergo solid tumor surgical removal or radiation develop lymphedema (62). The number of nodes resected during oncological surgery and high use of taxane based chemotherapy are also risk factors (63,65,67). Specifically for breast cancer, a larger tumor size, tumor located in the upper

outer quadrant, more advanced tumor stage, and tumor positive nodes are believed to be risk factors for lymphedema development (1,66). Axillary lymph node dissection also increases the risk for lymphedema, especially in combination with radiation therapy (1,68).

2.3. Pathophysiology of lymphedema

This section describes the pathological changes that lymphedema patients undergo in the functioning of their lymphatic system. Interstitial fluid accumulates and this causes inflammation. This inflammation has an influence on the regulation of lymphangiogenesis (i.e. the growth of new lymphatic vessels), fibrosis, and adipose deposition.

2.3.1. Accumulation of interstitial fluid

The cause of lymphedema is the reduction of lymph transport due to lymphatic hypoplasia, absence of lymphatic valves, impairment in intrinsic contractions or surgical, traumatic, inflammatory, and neoplastic disruption of lymph vessels. Lymph stasis leads to accumulation of protein and cellular metabolites in the interstitium (64). This protein accumulation could be the result of the breakdown of damaged initial lymphatics that are overwhelmed by the lymphatic stasis (63). This accumulation increases the colloid osmotic pressure and attracts water due to an increase in filtration force (64). The interstitial fluid, saturated with proteins, results in pitting edema (62). Measurements from the interstitium indicate that the protein concentration in the affected limb is actually lower than in the healthy limb (12,69). This is possibly caused by the increased filtration force driven by the increased colloid osmotic pressure of the interstitium, increased capillary hydrostatic pressure owing to precapillary vasodilatation, and increased vascular endothelial growth factor C (VEGF-C) production (12,69). VEGF-C is a growth factor that plays amongst others an important role in lymphangiogenesis (70). VEGF-C acts in low flow conditions on vasculature and increases the filtration rate instead of acting on the lymphatic contraction or lymphangiogenesis (69).

2.3.2. Chronic inflammation

Lymph stasis results in CD4+ (cluster of differentiation 4) T cell inflammation. T cells are lymphocytes (subtype of white blood cells) and play a role in cell-mediated immunity, in contrast to B cells which produce antibodies. CD4+ T cells are a specific type of T cells and interact with antigen-presenting cells that express class II HLA (human leukocyte antigen) molecules. Antigen-presenting cells process antigen and present fragments of antigen to T cells with the help of HLA molecules on their cell membrane. The interested reader is referred to (71,72).

The CD4+ T cells release cytokines, which are small proteins with a cell signalling function. The CD4+ T cell inflammation in lymphedema is characterized by T helper 2 (Th2) biased mixed Th1/Th2 responses (62,73). Th1 cells and Th2 cells are different phenotypes of T helper cells that

result from differentiation of CD4+ T cells. These cells produce different cytokines and play diverse roles in the immune system. Th2 inflammatory responses result in lymphatic dysfunction by induction of fibrosis, inhibition of collateral vessel formation, and inhibition of the lymphatic pump. The lymphatic contractility and transport is reduced by the increased NO production due to inflammatory cells that upregulate induced nitric oxide synthase (iNOS) (62). T regulatory cells (Tregs) are downregulated in lymphedema patients leading to worsening of lymphedema and increased infiltration of inflammatory cells (74). However, a more recent study by Garcia Nores et al. 2018 (75) shows an increase of Tregs in lymphatic limbs which contributes to immunosuppression in lymphedema (75). Lymphedema is also associated with an increased macrophage infiltration with a favoured M2 differentiation (i.e. macrophages with an immunosuppressive effect). This increased infiltration may be a reaction to the ongoing inflammation in the lymphedematous limb. Macrophages are associated with anti-fibrotic effects, increased VEGF-C production and CD4+ T cell inflammation regulation and Th2 differentiation (76). Inflammation also has an effect on the lymphatic valves. The effectiveness of the primary valves in preventing backflow is reduced by inflammation, which leads to more fluid accumulation (13,29). Inflammatory mediators can accumulate in the tissue due to the leaking initial lymphatics, which can increase the inflammatory reactions (29).

2.3.3. Dysregulated lymphangiogenesis

Lymphangiogenesis plays diverse roles over the course of inflammation. The inflammatory processes may activate early lymphangiogenesis while supressing it at later times (77). Lymphatic fluid stasis regulates the expression of pro-lymphangiogenic cytokines, which promotes lymphangiogenesis (78). CD 4+ T cells (Th1 and Th17) activate macrophages to produce VEGF to promote lymphangiogenesis. VEGF-C generates excessively leaky immature lymphatic vessels (77). Anti-lymphangiogenic cytokines, IL-4 (interleukin 4), IL-13, IFN-γ (interferon gamma), and TGF-β (transforming growth factor beta) are produced by T cell inflammatory reactions in response to lymphatic fluid stasis (62,78,79). These cytokines decrease lymphatic vessel formation by inhibiting endothelial cell proliferation, tubule formation, migration, and function (62).

2.3.4. Fibrosis

Th2 inflammation regulates fibrosis by expression of pro-fibrotic growth factors (IL-4,IL-13, and TGF-β). Functional vessels are replaced by scar tissue, which results in loss of functional initial lymphatics and obstruction of collecting vessels (62). Fibroblasts differentiate into myofibroblasts due to chronic inflammation which leads to collagen deposition and extracellular matrix remodelling (74).

2.3.5. Adipose deposition

Lymphatic damage or insufficient drainage of interstitial fluid cause exposure of leaking lymph or interstitial fluid to adipocytes. This exposure possibly leads to adipocyte hypertrophy because the free fatty acids and metabolites of the fluid are absorbed by the adipocytes. When the lipid storage capacity is at its maximum, hypertrophy of the adipocytes could in turn be followed by hyperplasia (80). Adipose deposition and CD4+ T cell inflammation may increase IL-6 which is a known regulator of adipose homeostasis. This increased IL-6 may limit adipose accumulation and represent a compensatory response to inflammation and lymphatic dysfunction (81).

2.4. Diagnosis

Early diagnosis of lymphedema is important because the more advanced the harder it is to treat the disease (82). The diagnosis of lymphedema can be suspected by assessing the patient's history, clinical examination, and measurements of the limb circumference or volume. In addition, lymphedema can be confirmed by medical imaging techniques such as lymphoscintigraphy, which is considered as the gold standard. Other imaging techniques such as CT and MRI can be used to evaluate the cause of limb swelling. Fluorescence methods have emerged and bioelectric impedance techniques are emerging (1). The use of biomarkers is being studied (65).

2.4.1. Patient history

The onset, axillary or inguinal injury, travel to areas associated with filariasis, family history of primary lymphedema, comorbidities, and symptoms have to be taken into account when assessing the patient's history. Primary lymphedema affects the paediatric population while secondary lymphedema typically begins twelve to eighteen months after injury to lymph vessels (lymphadenectomy/radiation) (14).

2.4.2. Clinical examination

Lymphedema almost always affects an extremity. The distal extremity exhibits lymphedema, including hand or foot. Lymphedema rarely develops in the genital area or other locations (14). A sensitive and specific sign for lymphedema is the Stemmer's sign, which means to be unable to pinch the skin on the dorsum of the hand or foot (14). The toes could become squared and the forefoot puffy (buffalo hump) (82). Figure 8 illustrates both the Stemmer's sign and the buffalo hump. Lymphedema is typically painless, but secondary musculoskeletal pain is possible due to significant swelling of the limb (14).

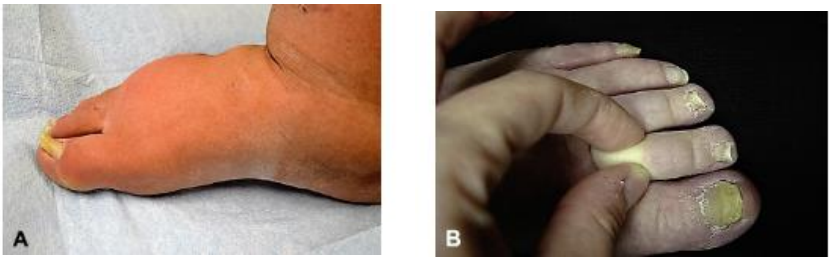

Figure 8: Buffalo hump (A) and the Stemmer's sign (B) (8).

Patients also need to be examined for abnormal skin appearance such as bleeding from vesicles, hyperkeratosis, and lymphorrhea (leaking lymph) (14). Dysfunction, disfigurement, lipodermatosclerosis, peau d'orange, nail abnormalities, and rarely lymphangiosarcoma are possible presentations of lymphedema (63,82). Patients with suspected lymphedema require examination for features in the context of syndromic diseases such as lymphedema distichiasis syndrome (14).

2.4.3. Limb circumference

Tape measurements are performed at specific anatomic locations (62). A difference of two centimetres or more relative to the contralateral limb is diagnostic (62,63). This test is easy to perform and commonly used to diagnose and follow-up patient's progression. However, it is less useful for patients having a higher BMI and when there is high inter-rater variability (62). In an effort to minimize this variability, subsequent measurements by the same operator could be performed (63).

2.4.4. Volume measurements

These techniques are superior to limb circumference measurements (62). Volume differences of 200 ml or 10% are diagnostic (62,63,66). Measuring the limb circumference with tape and calculating its volume using the truncated cone formula is a practical and inexpensive alternative (62). This method has a tendency to slightly overestimate volumes in comparison with water displacement (66). There are two techniques to measure the limb volume, namely water displacement and perometry. The sensitivity and specificity of water displacement is high for quantifying overall limb volume but is rarely performed because it is a burdensome technique. Perometry quantifies limb volume with the help of a non-invasive optoelectric device by using infrared light (63). The vertical and horizontal shadow that is cast by the infrared light is used to calculate the circumference of the limb and from that determine the limb volume (83). Perometry may have the same accuracy as water displacement but some studies contradict this assumption (65,83). Perometry is also a quite expensive technique (65).

2.4.5. Lymphoscintigraphy

Lymphoscintigraphy is the most commonly used radiologic technique for diagnosis of lymphedema and is generally considered as the gold standard (62,65). This technique also allows to determine the severity of lymphatic dysfunction (14,84). Lymphoscintigraphy measures the tracer protein (^{99m}Tc-sulfur colloid) uptake by lymph nodes after injection using a gamma camera (14,62). Qualitative lymphoscintigraphy describes the presence and calibre of lymphatic vessels, lymph nodes, collateral networks, and delay in tracer uptake. Quantitative lymphoscintigraphy records the tracer transit time, which is determined by the uptake and clearance time from the injection site and clearance time from the limb (2). Abnormal findings are delayed transit time to the regional lymph nodes, dermal backflow, asymmetric lymph node uptake, and formation of collateral lymphatic channels (10,14,62,63,84). Figure 9B shows an example of an abnormal lymphoscintigram. Dermal backflow, shown in figure 9C, is an indicator of lymph reflux and implies obstruction (10,62). Shortcomings of lymphoscintigraphy are limited visibility of small vessels because of relatively poor spatial resolution and the radiation exposure (2,10,63). The sensitivity for lymphedema is 92-98% and the specificity is 90-100% (10,12,14,84,85). However, a study which compares lymphoscintigraphy with indocyanine green found a lower sensitivity (62%) (10,86).

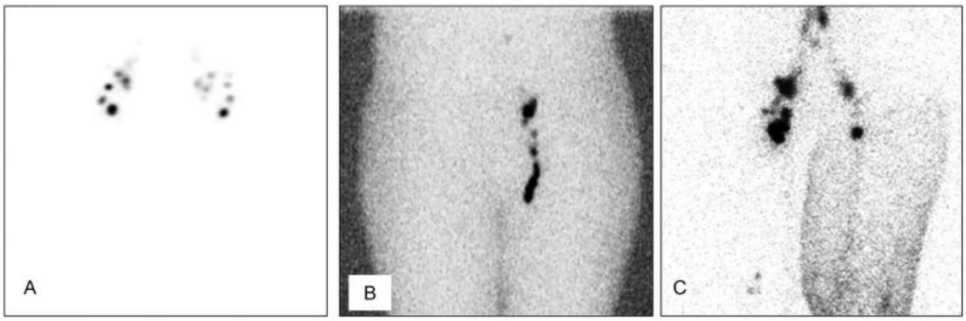

Figure 9: Lymphoscintigrams (A) Normal (B) Abnormal: absent uptake in the right inguinal lymph nodes (C) Abnormal: dermal back flow in the left leg (14).

2.4.6. CT (Computed Tomography) and MRI (Magnetic Resonance Imaging)

The subcutaneous tissue layers, the affected skin, the number and size of lymph nodes can be imaged with CT and MRI. Both techniques are capable of indicating lymphatic abnormalities at different tissue levels and are used to evaluate the cause of limb swelling, for example detection of a tumor that causes lymph obstruction. They can also be used in the evaluation of the presence and severity of lymphedema (2,63,82,86). CT has a reported sensitivity of 33% and a specificity of 100% when using the presence of fibrosis in the subcutaneous fat as diagnostic criterion (86). Fibrosis and fluid depositions around the adipocytes cause the pathognomonic honeycomb distribution of the subcutaneous tissue (2,10,82). The presence of honeycombing on CT has a reported sensitivity of 98,5% and a specificity of 64% (87). MRI can visualize edema in fat tissue, subcutaneous edema, excess subcutaneous water retention, and subcutaneous fat and evaluate the honeycomb

distribution (10,82,86). MRI can also be used to measure circumferences or volumes and detect morphological changes (65). While the non-enhanced MRI techniques cannot detect normal or abnormal lymphatics, dilatation due to lymph stasis can sometimes be observed (2,86). In contrast, MR lymphangiography uses the injection of contrast agents which provides better spatial resolution images than nuclear techniques and shows dilated vessels in greater detail (2,65). Finally, MR lymphangiography does not only provide anatomic information but also captures functional information and visualizes soft tissue changes associated with lymphedema (9). The lymph nodes, however, are best visualized with lymphoscintigraphy (6). The reported sensitivity of MR lymphangiography is 68% (14,84).

2.4.7. Fluorescence methods

Indocyanine green (ICG) near infrared lymphangiography is a near infrared fluorescence (NIRF) method, is nontoxic, and visualizes the initial and superficial collecting lymphatic vessels after intradermal or subcutaneous injection of ICG. A near infrared camera is used after excitation with a laser diode (9,10,62,65). NIRF provides immediate real-time high resolution images of the lymphatic anatomy, contractile lymphatic flow volume, and velocity (9,63). A linear pattern of lymph flow is a normal finding whereas splash, stardust or diffuse patterns are associated with lymphedema (10). Figure 10 shows examples of ICG lymphangiography patterns. Dermal backflow, extended fluorescence in the injection site, and dilated lymph vessels are also flow characteristics of lymphedema (65). Unlike lymphoscintigraphy, NIRF imaging can definitively diagnose early stages of lymphedema (63). In addition, it is helpful for patients with contrast allergies or metal implants, which are contra-indications of MR lymphangiography (9). ICG lymphangiography is further used during lymphatic microsurgery to identify lymphatic vessels (6). A disadvantage of this technique is that morbidly obese patients cannot be properly evaluated because vessels located more than two cm deep in the subcutaneous tissue cannot be observed (10). This technique has a high sensitivity and specificity for diagnosing lymphedema with reports of a sensitivity of 97-100% and a specificity of 78-100% (10,62,63,86,88). There have also been reports of a lower specificity (55%) (14,84).

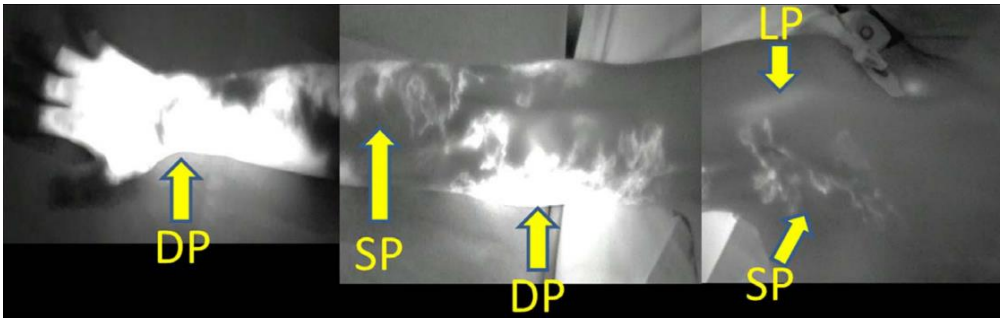

Figure 10: ICG lymphangiography findings (DP) Diffuse pattern (SP) Splash pattern (LP) Linear pattern (86).

Another technique used to investigate initial lymphatics is fluorescence microlymphography which uses fluorescein isothiocyanate labelled dextran (FITC-dextran). FITC-dextran is injected into the

subepidermal layer of the skin, is excited by a visible light with a wavelength of 495 nm, and emits a light of 521 nm (2,10) This technique is also used during lymphatic microsurgery to identify lymphatic vessels (2). Since the drainage of interstitial fluid into deeper collectors of lymphedematous limbs is obstructed, FITC-dextran quickly spreads out in the superficial network. The presence of cutaneous backflow is an extra diagnostic sign (10). This test has a sensitivity of 79-91,4% and a specificity of 78,6-85,7%, depending on several factors such as the cut-off value of normal and pathological dye spread (10,88).

2.4.8. Bioelectric impedance

Bioimpedance can be used for early diagnosis of lymphedema and is believed to be able to identify subtle changes in tissue before detection is possible with tape measurements (62,65,66,83). However, more evidence is needed to validate these claims (83). This method measures the transmission of electrical current to indirectly evaluate the limb volume (63). The more water in the limb, the less electrical impedance/resistance which results in a higher electrical current since interstitial fluid is a conductive medium as opposed to adipose tissue (83). The electrical current flows more rapidly through the lymphedematous areas than normal (62). It should be mentioned that this method cannot diagnose late stage lymphedema since adipose tissue and fibrosis are nonconductive media (83). Postsurgical lymphedema can be more effectively diagnosed by comparison of the postoperative changes to the preoperative data as opposed to comparison to thresholds (65). Two different techniques can be distinguished: single frequency bioimpedance analysis (SFBIA) and multi-frequency bioimpedance analysis (MFBIA) or bioimpedance spectroscopy (BIS). They use different frequencies of current to determine the impedance (83,89). MFBIA is superior to SFBIA in diagnosing lymphedema (83). A study on lymphedema after axillary nodes clearance reports a sensitivity of 73% and a specificity of 84% (90).

2.4.9. Biomarkers

A study found six protein biomarkers that differentiate accurately lymphedema patients with clinical manifestations from healthy controls (1,65). These proteins are linked to lymphangiogenesis, inflammation, fibrosis, and adipocytokine signalling, associated with mechanisms known in disease progression. More research is required to see whether this technique is suitable for detecting early or latent disease (65).

2.5. Treatment

Currently, there is no curative treatment for lymphedema. The aim of the existing treatments is to relieve the symptoms, restore the functionality of the affected limb, and to prevent complications and progression (82). The two most important treatment options are complex decongestive therapy

(CDT) and surgery. In general, surgery is reserved for lymphedema refractory to conservative treatment, however, there is controversy regarding patient selection and timing of surgery. Other treatment options can consist of low level laser therapy and pharmacotherapy. The effectiveness of these therapies still needs to be determined. Stem cell therapy and extracorporeal shock wave therapy are being studied in animal models as possible new treatment options for lymphedema.

2.5.1. Complex decongestive therapy or complete decongestive therapy (CDT)

CDT is aimed to reduce symptoms and prevent lymphedema progression rather than curing it (62). CDT is divided into the intensive phase of volume reduction and afterwards the maintenance phase (91,92). An excess volume reduction of 22% to 73% has been reported in breast cancer related lymphedema (93). Another study reports a mean volume reduction of 30,5% in postsurgical lymphedema patients and a reduction in chronic pain, fear of movement, circumference of the limb, and an improved quality of life (94). The impact of each individual component of CDT on a good outcome is not yet fully understood (95).

Volume reduction phase of CDT

Most volume reduction findings take place in the first ten days of CDT therapy and the entire volume reduction phase usually takes between four to six weeks (82,92). The volume reduction is achieved by wrapping the affected area with short-stretch bandages and manual lymph drainage (MLD). This way, a rapid volume reduction can be achieved with patients with a significant pitting edema (91). The bandages, kept on as long as possible, apply a high pressure during activity and a low, even pressure during rest (91,95,96). The lymphatics are manually pumped by compression between the muscle and the bandage (96). Adherence is essential in maintaining a stable volume reduction (62,91,92). Contra-indications of the use of bandages are arterial insufficiency, acute cellulitis, and uncontrolled congestive heart failure (91). Manual lymph drainage (MLD) increases the lymphatic fluid transport rate, develops new routes for lymphatic drainage, breaks up fibrosis, and redirects the lymph towards healthy lymph nodes (91,96). The activity of macrophages is increased to remove protein deposits. The indications of MLD are significant pitting edema with fibrosis or sclerosis (91). Four basic hand strokes of MLD consist of intermittent gentle pressure applied on the skin in a directional manner to move lymph fluid away from damaged lymphatics to other drainage pathways (62,95). MLD causes the initial lymphatics to stretch open which allows fluid to enter the system. MLD also stimulates the lymph flow by influencing the contractility of collecting lymphatics which generates a suction effect pulling the lymph towards the lymph nodes (62,96). Contra-indications of MLD are untreated neoplasia, decompensated right-sided heart failure, deep venous thrombosis (DVT), and cellulitis (91).

Maintenance phase of CDT

Lifelong compliance is necessary to control the edema and prevent progression. Compression garments are worn during the day while the use of short-stretch bandages can be recommended during the night (91).

Adjunctive treatments

Intermittent pneumatic compression (IPC) stimulates the flow of lymph to the functional lymphatics (91). The device consists of pneumatic cuffs that inflate and deflate to simulate the natural pump of lymphatic contractions and promote drainage (62). IPC should be used in both the intensive reduction phase and the maintenance phase. The pump is effective at removing the fluid, but MLD is still necessary for breaking up the protein deposits (91). IPC extracts water but leaves proteins in the interstitial space which may worsen the lymphedema by retracting the water. When IPC is applied at high pressures, it may cause damage to the lymphatic structures (82). These two possible adverse effects make the use of IPC controversial, but recent meta-analysis suggests that IPC may be effective for volume reduction (62,82). Contra-indications of IPC are infection, DVT, malignancy, and anticoagulant therapy (91). Lymphedema exercises can increase the return rate to the venous system three to four folds (91,96). Patients wear short-stretch bandages (in the intensive reduction phase) or compression garments (in the maintenance phase) during the exercises to facilitate lymph transport (91). Exercises also improve the range of movement and quality of life (82). Another important aspect of CDT is patient education on skin care and nail management to reduce the risk of cellulitis (91). Lymphedema is associated with an increased risk of soft tissue infection, doubling the risk of cellulitis, because the excess fluid and protein accumulation promotes microbial growth (14,64,82). Cellulitis is a potential life-threatening complication, is more severe and progresses quickly in lymphedema patients, can accelerate fibrosis and sclerosis of lymphatic vessels, and recurring infections progressively damage the initial lymphatics (64,82,91). Avoiding dry skin and maintaining the correct pH is achieved by daily skin cleaning and moisturizing (91,96). Supple skin is less prone to chapping and cracking which decreases the risk of entering bacteria (95). In addition, patients need to be trained in daily inspection of their limbs for redness, tenderness, and warmth development (91,96). Furthermore, they need to avoid injury to the affected limbs and treat injuries immediately (91).

Limitations of CDT

This conservative treatment often leads to the improvement of lymphedema. However, the effects are temporary and lifelong compliance is necessary. Patients need to invest a significant amount of time and effort in maintaining their lymphedema. Each component of

CDT has his own contra-indications which means that not every lymphedema patient can undergo CDT treatment.

2.5.2. Surgery

Two types of surgery for lymphedema exist: physiologic and ablative operations. Physiologic interventions increase lymphatic drainage through bypasses (LVA) or through induction of lymphangiogenesis (VLNT), while ablative procedures debulk lymphedema areas in an effort to reduce morbidity (9). Controversy regarding patient selection criteria, type and timing of surgery, and postoperative evaluation of outcome exists (97). Significant pitting edema and responsiveness to conservative therapy are indications for physiologic operations. Lymphedema surgery is individualized based on the clinical stage of lymphedema, anatomical considerations, and the preference of the patient (9).

Physiologic operations

Lymphaticovenous anastomoses (LVA) or lymphovenous bypass (LVB) is a technique that consists of bypassing the diseased lymphatics and restoring adequate lymphatic drainage by redirecting excess lymphatic fluid directly into the venous circulation (9,63,98). The anastomosis is created using microsurgical techniques between small lymphatics and subdermal venules, less than one centimetre in diameter (9,63). Figure 11A illustrates this technique. The indication of this procedure is failed conservative therapy and ISL stage II (or Campisi stage Ib, II and early III) disease with partial obstruction (63,98). LVA is most effective in early, reversible lymphedema (62,63,98). Patients with secondary edema tend to respond better and upper extremity lymphedema improves more than lower extremity (98). The contra-indication of LVA is complete occlusion of the lymphatics because functional lymphatics are necessary to create the anastomosis (63). 70% of the patients show objective volume improvement at 6 months and 12 months post-op (99). Volume reductions of 35% and 42% at 12 months have also been reported (9,68). LVA improved the quality of life in the majority of the patients and decreased the time, resources, and efforts needed to maintain lymphedema (68). The symptoms improved in 50 to 100% of the patients and the frequency of cellulitis dropped by almost 50% (99–101). Studies objectifying the late patency of the anastomosis and reporting long term outcome are still required (68,99). LVA surgery is missing guidelines on optimal approach, surgical technique, and outcome measurement (100).

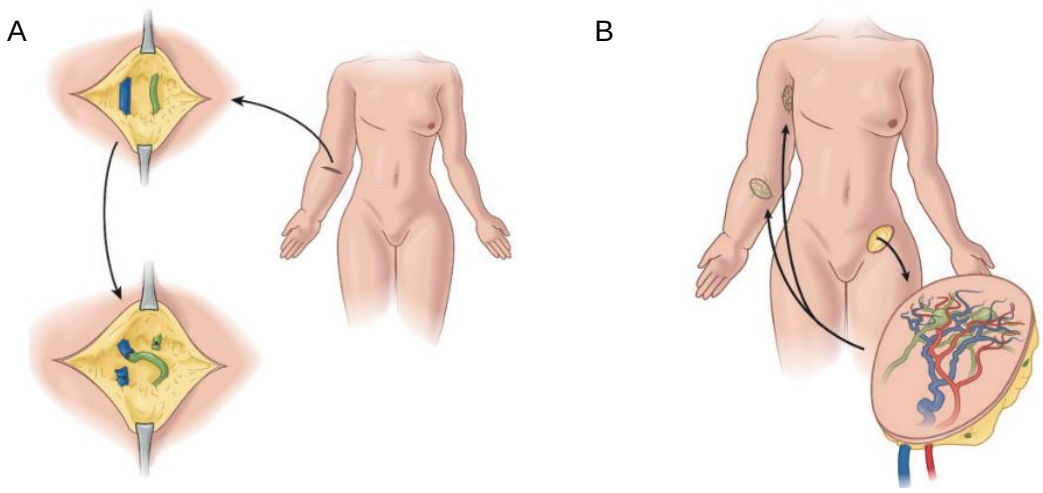

Figure 11: Illustration of physiologic operations. A: Lymphaticovenous anastomosis (LVA) between a venule (blue) and a lymphatic vessel (green). B: VLNT of inguinal lymph nodes. Lymph nodes can be transplanted orthotopically (axilla) or heterotopically (forearm) (62)

Vascularized lymph node transfer (VLNT) promotes lymphatic drainage in situations where lymph nodes are dysfunctional (lymphadenectomy and radiation therapy) (9). VLNT relocates a soft tissue flap containing healthy lymphatic vessels and nodes from an unaffected donor site to the lymphedematous area (62,63). Diverse donor sites are available including cervical area, omentum, supraclavicular, axilla, lateral thoracic area, and most commonly the groin (9,62). Lymph nodes can be transplanted orthotopically (region of lymph node dissection) or heterotopically (distal region of affected limb), which is shown in figure 11B (62). The transplantation of lymph nodes is believed to promote lymphangiogenesis through the production of VEGF-C (9,63). VLNT in combination with the exogenous administration of VEGF-C is being studied (74). Clinical use of VEGF-C in cancer related lymphedema has been limited due to the risk of tumor recurrence since these are the same growth factors that regulate the tumor microenvironment (62,74). The indications are Campisi stage II to V lymphedema, failed conservative management, absolute occlusion of lymphatic vessels, fibrosis preventing LVA, and chronic infections. The results for volume reduction and improved quality of life are similar to the ones obtained using LVA (63). Reductions in extremity circumference and volume of 30 to 60% are reported (9). In addition, VLNT patients are more likely to be able to discontinue wearing compression garments and show greater subjective improvement (102). In general, VLNT is considered a higher risk operation and is associated with higher complication rates (63). The most feared complication of VLNT is iatrogenic lymphedema at the donor sites (9,63). Three possible strategies are known to lower the risk of iatrogenic lymphedema in the context of VLNT. The first strategy consists of harvesting only the lymph nodes whose absence will not cause lymphedema. This can be achieved by using reverse lymphatic mapping during the harvest of lymph nodes in the donor site to differentiate between expendable lymph nodes and

lymph nodes that need to be preserved because they drain the extremities. A gamma probe is used during surgery to identify these critical lymph nodes after injection of technetium in the hand or foot (9). The nodes that can be harvested are identified by injection of indocyanine green in the lateral chest or lower abdomen (103). A second strategy is based on using a different source of lymph nodes, namely vascularized omentum lymphatic transplant (VOLT). The omentum contains a rich supply of lymphatic vessels and VOLT allows two lymph node transplants in the same limb (104). The third and final strategy is the creation of artificial lymph nodes instead of harvesting them and the development of artificial lymph node scaffolds (105). In addition, the use of nanofibrillar collagen scaffolds in combination with VLNT and VEGF-C is currently being studied (74).

Ablative operations

Ablative operations are best suited in case of more advanced stages of lymphedema with swelling caused by increased adiposity. These patients have minimal to no improvement with conservative therapy and have minimal to absent pitting edema (9). The Charles procedure is the first described technique for surgical excision or radical debulking for severe lymphedema (9,63). Longitudinal skin incisions are made to remove the skin and subcutaneous tissue up to the deep fascia. Afterwards, the defects are covered with split-thickness skin grafts (9,62). Indications of this technique are advanced fibrosclerotic lymphedema not responsive to other procedures, recurrent cellulitis, severe disfigurement or dysfunction, and the inability to exclude sarcoma. Superficial skin lymphatic vessels are removed or further damaged and this technique is associated with morbidity and the risk of skin graft failure (63). Suction-assisted protein lipectomy (SAPL) is a technique that consists of removing fat and fibrosis by suction. Since it removes only the fibrotic adipose tissue and not the fluid, it is necessary to drain the fluid using conservative methods. Indications of SAPL are moderate to severe disease and failing of conservative management (63). The most favourable candidates are patients with advanced lymphedema who do not have significant pitting edema but have significant adipose deposition (>600 ml volume difference) and are not candidates for physiologic operations (LVA, VLNT) (9,62). An average excess volume reduction of 97%, symptomatic relief, and decreased risk of cellulitis have been described. SAPL is a safe technique and has only minor and self-limiting complications (62). Contra-indications of SAPL are active cancer, infection, and wounds (63). It should be noted that this procedure does not treat the underlying cause of lymphedema. As a result, the fibrofatty tissue deposition recurs without further action within three months (62). Compression therapy, therapist treatment, VLNT or LVA must be applied to maintain the effects of SAPL (9,63).

Limitations of surgical treatment of lymphedema

Surgical interventions are invasive and associated with the risk of complications but remain important since less invasive techniques such as SAPL are insufficient and require further CDT treatment. At this point, the long term outcome of the surgical techniques and guidelines on surgical approach are not clear and no standardized surgical techniques of which effectiveness has been proven on short and long term exist.

Surgery and prevention of lymphedema

Surgery is an important risk factor in the development in lymphedema. Several methods to reduce the risk of iatrogenic lymphedema exist. Sentinel lymph node biopsy reduces the risk of lymphedema compared to axillary lymph node dissection (1,66). Breast reconstruction is also associated with a decrease in risk (1). Lymphatic microsurgical preventing healing approach (LYMPHA) is a preventive LVA technique that consists of an anastomosis of a disturbed arm lymphatic with an axillary vein during axillary lymph node dissection. Only 12,5% of breast cancer patients who underwent axillary lymph node dissection with LYMPHA developed transient lymphedema compared to 50% of patients without LYMPHA in a lymphedema surveillance study (98). However, the impact on cancer treatment and metastasis is not yet clear (68).

2.5.3. Low level laser therapy (LLLT) = photobiomodulation (PBM)

This non-invasive phototherapy uses low-power lasers with wavelengths between 650 and 1000 nm to stimulate tissue changes via non-thermal mechanisms. It is a safe technique that can reduce pain and swelling and can be a useful adjunctive in secondary lymphedema treatment. It has anti-inflammatory and anti-fibrotic effects and has been used to promote lymph vessel regeneration and lymphatic motility (62,106). Different energy generating components of cells absorb light which leads to increased levels of ATP (adenosine triphosphate). This sets off a cascade of secondary reactions which result in the release of growth factors, increased metabolism, increased NO production, cell proliferation, and stimulation of lymphatic vessels. Data suggests that LLLT is more effective than no treatment at all, but is not proven to be beneficial compared to other conventional treatments (106).

2.5.4. Mesenchymal stem cell therapy

The use of bone marrow or adipose-derived stem cells may provide a therapeutic benefit since they have the ability to differentiate into lymphatic cells in vitro culture and increase the drainage of interstitial fluid when administrated in vivo (62,74). Combination treatment with VLNT could improve the outcome of VLNT (74). The results of co-administration of VEGF-C hydrogel and human

adipose-derived stem cells in a mouse model suggest a favourable effect on lymphangiogenesis (70).

2.5.5. Extracorporeal shock wave therapy (ESWT)

ESWT has been demonstrated to stimulate lymphangiogenesis and ameliorate secondary lymphedema in a lymphedematous rabbit model (107). Extracorporeal shock waves are low energy shock waves and induce cavitation (collapse of bubbles) in a localized area. This cavitation results in an increase in cell permeability, gene expression, and cellular metabolism (82,107,108). ESWT may also have anti-inflammatory, vasodilatory, and neo-angiogenic effects. The effectiveness and safety of this method still needs to be determined (82). VEGF-C hydrogel and ESWT showed to be synergistic in a mouse model of lymphedema and was able to alleviate the symptoms and stimulate lymphangiogenesis (108).

2.5.6. Pharmacotherapy

A large amount of lymphedema patients has a selenium deficit and their selenium status declines with the progression of lymphedema. The administration of sodium selenite can result in reducing lymphedema volume, increasing the effectiveness of CDT, and reducing the infection risk in cancer related lymphedema patients (109). Sodium selenite could be a possible cost-effective, nontoxic, and anti-inflammatory drug in the treatment of lymphedema (66,109). Treatment and prevention with neutralizing antibodies to block IL-4 and IL-13 and to inhibit Th2 differentiation have shown to be effective in preclinical models (62). Inhibition of TGF-β using monoclonal antibodies possibly results in the decrease of fibrosis and inflammatory cell infiltration, together with an improvement of vessel function (79). An advantage of the inhibition of Th2 cytokines over the administration of VEGF-C or other pro-lymphangiogenic cytokines is the avoidance of increased risk of tumor growth (62). Benzopyrones (coumarin) stimulate the macrophages and increase proteolysis and protein catabolism. The effectiveness of benzopyrones in reducing lymphedema is still under discussion (82). Atrovastatins is considered another possible treatment method. Statins suppress Th1 and Th17 differentiation, modulate fibrosis and adipocyte deposition, and inhibit the Th1/Th17/macrophage interaction in the early lymphangiogenesis process during the development of lymphedema. Further studies are essential to evaluate statins as a possible prophylactic drug (77). Diuretics are sometimes proposed by health professionals as a treatment option for lymphedema (11). However, diuretics have not been proven useful and are believed to potentially worsen lymphedema (9,82,91). Diuretics are often used to treat other types of edema. This emphasises the importance of correct and timely diagnosis of lymphedema.

3. Biomechanics of the normal lymphatic system

The physiology of the lymphatics is dependent on the biomechanical function. This section gives an overview of the basic mechanical principles applied to the lymphatic system to understand the biomechanical function (26). This thesis section starts with a discussion of the lymph flow. This includes a characterization of the type of flow, the flow velocity, and the flow rate. Next, the pump coordination and the lymphatic valves are discussed. The four main lymphatic wall stresses are explained as well as the compliance of the lymphatic vessel and the modulus of elasticity. This section concludes with the discussion of permeability.

3.1. Lymph flow

Time-varying active contractions of the lymphatics and their consequent diameter changes result in lymph flow. The flow is also influenced by passive external compression forces due to systemic forces such as respiration, skeletal muscle movement, and pulsations of nearby arteries and the flow is limited by segmental viscous and valvular resistance (35). The mechanism of lymph propulsion is explained in detail in section 1.2.4. In order to describe this behaviour, the type of flow has to be defined with the help of the Reynolds number, the Womersley number, and the law of Poiseuille. Next, the velocity of the flow is discussed. Furthermore, the flow rate and pump coordination is explained. This section concludes with the mechanism of valve gating and the valve properties.

3.1.1. Type of flow

In fluid dynamics, the flow is described with the help of parameters such as the Reynolds number and the Womersley number, and the law of Poiseuille. The Reynolds number (Re) is dimensionless and characterizes the flow by reflecting the ratio of inertial to viscous forces (18,42). The Reynolds number can be expressed by the following equation (42).

$$Re = \frac{\rho v D}{\mu}$$

Equation 2

$\rho = lymph\ density\ [kg/m^3]$, $v = mean\ velocity\ of\ lymph\ [m/s]$, $D = vessel\ inner\ diameter\ [m]$, $\mu = dynamic\ viscosity\ of\ lymph\ [Pa \cdot s]$

The flow is considered laminar (i.e. dominant viscous effects) when the Reynolds number is below the critical value of 2200. If the Reynolds number exceeds this critical number, the flow may become turbulent. A turbulent flow implies that the fluid follows a highly irregular/chaotic flow pattern, opposed to a laminar flow characterized by a highly organized flow with the fluid flowing along fluid layers (~laminae) (42). Studies concluded that the Reynolds number in the lymphatic system is typically less than one, with reports of up to five in close presence of valves (4,18). The flow in lymphatic vessels smaller than 100 µm is always viscous and in the thoracic duct always laminar (23).

Another number that can be used to characterize flow is the Womersley number. The Womersley parameter (α) is expressed by the following equation and shows the relative importance of inertial effects over viscous effects during pulsatile flow (42).

$$\alpha^2 = \frac{{r_i}^2 \omega \rho}{\mu}$$ Equation 3

$\omega = angular\ frequency = 2\pi f$, $f = frequency\ [Hz]$, $r_i = vessel\ inner\ radius\ [m]$

Viscous effects dominate when the Womersley number is low (α<3) and the flow profile becomes parabolic as in Poiseuille flow (42). The Womersley number in the lymphatic system is typically less than one which indicates the dominant viscous effects (18).

A third way to characterize flow is the law of Poiseuille. This law represents the relation between lymph flow and pressure drop in a stiff vessel. Lymph as Newtonian fluid (i.e. constant viscosity), laminar steady flow, and a velocity of zero at the wall are assumed. The law of Poiseuille is expressed with the following formula (42).

$$Q = \frac{\Delta P \pi r_i^4}{8 \mu l}$$ Equation 4

$Q = lymph\ flow\ [m^3/s]$, $\Delta P = pressure\ drop\ [Pa]$, $l = vessel\ length\ [m]$

Poiseuille is an idealized interpretation of flow which can be used to simplify the modelling of flow. The assumption of Poiseuille law as a model for flow seems reasonable as the Womersley number and the Reynolds number are low in the lymphatic system (23). There are two conditions namely that fluid velocity is greater than the wall velocity and velocity is measured far enough from the lymphatic valves (13). Poiseuille flow assumes rigidity, but studies have shown that lymphatic vessels are dynamic and have a significant wall motion with reports of a ratio of radial to axial velocities greater than one, for example during flow reversal or rapid dilatation. This may mean that the condition that states that the fluid velocity needs to be greater than the wall velocity is not fulfilled and that the Poiseuille assumption is not always appropriate (48). Dixon et al. 2006 (49) measured with a high-speed video camera system both the lymph velocity and wall velocity in a rat mesenteric collecting vessel. They concluded that these conditions are fulfilled for most of the contraction cycle except when the lymph velocity is near zero during flow reversal (49). In section 3.2.1, another study will be described that gives further arguments for the validity of the Poiseuille flow assumption.

3.1.2. Flow velocity

Poiseuille flow can be used as an approximation for the velocity distribution in the lymphatic vessel, as the previous section has indicated the validity of the Poiseuille assumption (13). The velocity has a parabolic distribution and can be expressed as a function of the radius (42).

$$v_r = \frac{\Delta P(r_i^2 - r^2)}{4\mu l}$$

Equation 5

$\Delta P = pressure\ drop\ [Pa]$, $l = vessel\ length\ [m]$, $\mu = dynamic\ viscosity\ of\ lymph\ [Pa\ \cdot s]$, $r_i = vessel\ inner\ radius\ [m]$

The velocity at the wall ($r = r_i$) is zero and the velocity at the axis ($r = 0$) is maximal. The average velocity can be expressed as the following equation with the help of equation 4 (42).

$$v_{mean} = \frac{v_{max}}{2} = \frac{Q}{\pi r_i^2}$$

Equation 6

$Q = lymph\ flow\ [m^3/s]$

The lymph flow velocity has been measured in initial lymphatic vessels in human skin (10 µm/s at the end of the filling period and 0,51 mm/s during filling) (28,46). The average lymph velocity measured in a rat mesenteric prenodal collecting lymphatic vessel is 0,87±0,18 mm/s. Dixon et al. 2006 (49) suggests that the vessel contraction and the velocity pattern are 180° out of phase with one another which is shown in figure 12 (49).

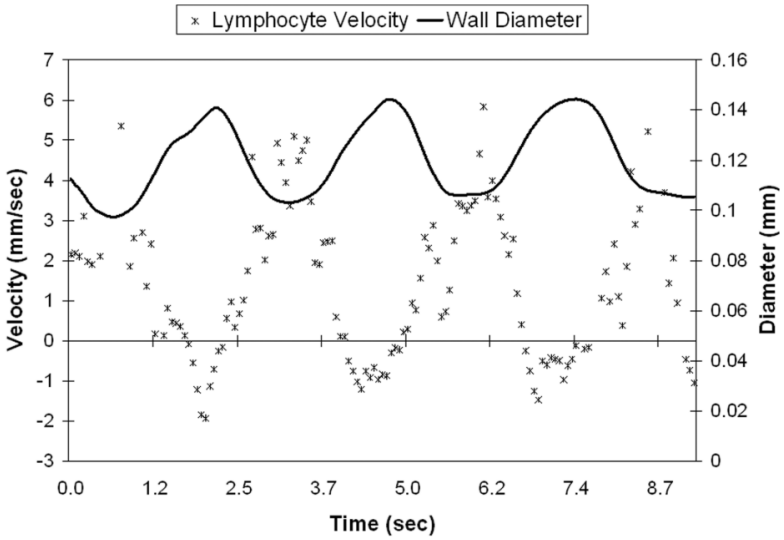

Figure 12: Relationship between fluid velocity and wall diameter (49).

3.1.3. Flow rate

Transmural pressure is an important modulator of lymph flow. Jamalian et al. 2016 (30) developed a lumped parameter model of a branching network of lymphangions, shown in figure 13A, to investigate the pumping capacity of the network under increasing transmural pressure. This study suggests the possibility of an optimum transmural pressure that results in peak lymph flow (30). A parameter sensitivity analysis of a lumped parameter model of a chain of lymphangions suggested

that the transmural pressure that results in a maximal mean flow rate is close to zero. Further investigation followed with the superposition of transmural pressure-diameter loops on the passive pressure-diameter curves. The flattest point of the pressure-diameter curve occurs close to a transmural pressure of zero, as seen in figure 13B. This means that the maximum mean flow rate is generated when the vessel is most compliant (50). The compliance of lymphatic vessels is further explained in section 3.3.

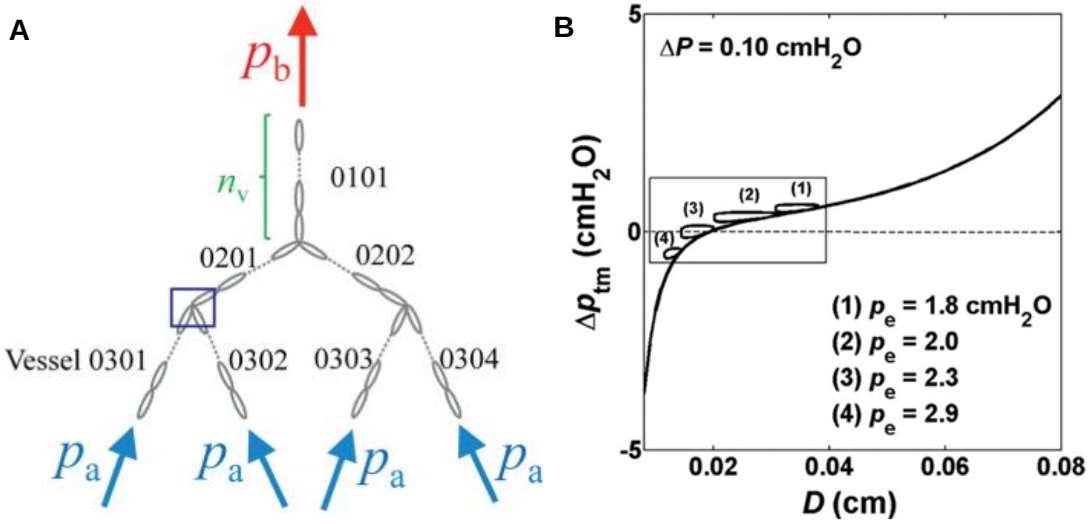

Figure 13: A: Schematic presentation of a lumped parameter model of a branching network of lymphangions. P_a= inlet pressure, p_b= outlet pressure, n_v= number of lymphangions per vessel (30). B:Transmural pressure Δp_{tm}-diameter loops superimposed on the passive pressure-diameter curve (50).

Not only the transmural pressure is an important modulator of flow rate but also the contraction frequency. A parameter sensitivity analysis concluded that the output is highly sensitive to the contraction frequency. The average flow rate increases with higher contraction frequency and the sensitivity is the highest at the physiological low frequencies. The average flow rate doubled with physiological increases of 0,2 to 0,4 Hz in contraction frequency (50). The average flow rate calculated in rat mesenteric prenodal collecting vessels was 13,95±5,27 µl/h (49). The flow rate in the initial lymphatics depends on the pressure gradient between the interstitial pressure and the initial lymphatic pressure. A continuum model of primary lymphatic valves has shown that a relatively small pressure gradient (10-100 dyn/cm^2 =1-10 Pa) across the endothelium is necessary to fill the initial lymphatics. The flow rate at a pressure gradient of 10 dyn/cm^2 is estimated to be 10^{-14} cm^3/sec per µm length of cell junction (36).

3.1.4. Pump coordination

The lymph pump efficiency is influenced by coordination of spontaneous, active contractions. Studies on rat mesenteric lymphatic vessels have shown that the contractions of adjacent lymphangions are coordinated (i.e. a small time delay between contractions and the same contraction frequency) more than 80% of the time (30). A lumped parameter model of a chain of

lymphangions indicated that pumping was more effective when there was a time delay between contractions (i.e. sequential contraction) (30,35). The coordination of contractions between adjacent lymphangions is suggested to be affected by the transmission of mechanical and electrical signals. Lymphatic pumping is not only coordinated by the presence of a time delay between contractions of adjacent lymphangions but also by a time delay between subsequent contractions of one lymphangion (30). This period of muscle relaxation before the next contraction is the refractory period. A parameter sensitivity analysis showed that when the refractory period increases, the maximum power decreases, but the pumping efficiency increases. Contracting less than the maximum rate (i.e. no refractory period) results in a more efficient metabolic energy use (50). A computational model of a branching lymphatic network showed that the flow rate is optimized if the refractory period is 1,5 s (30). A lumped parameter multi-lymphangion model showed that pumping is most efficient if the refractory period is long and the lymphangion starts to contract when the contraction of the adjacent upstream lymphangion is peaking (53).

3.1.5. Lymphatic valves

Lymphatic valves must allow forward flow and prevent backward flow to provide a net pumping effect (15). The mechanism of valve gating, valve geometry, and valve properties are discussed next.

Valve gating

Valve gating is supposed to be passive, which is deduced from assumptions based on the funnel-like shape and the small size of the valves. The low Reynolds number for lymph flow predicts that transvalvular pressure gradients and viscous forces control valve gating (40). The valves have an open bias with a predisposition to be open when the transvalvular pressure gradient is zero. In lymphatic vessels with a small adverse pressure gradient, this property results in a lower resistance to flow (31). A study on rat mesenteric vessels measured the pressure gradients that open and close valves. This study concluded that the transvalvular pressure gradient necessary for valve closure depends on vessel distension. This pressure gradient varied >20 fold as the vessel distended. Valve gating is thus different comparing low and high intraluminal pressure. At low intraluminal pressure, the vessel has a small diameter and thus the transvalvular pressure gradient necessary to close the valve is relatively small (40). The bias of the valve to remain open increases as the vessel distends (23). An indirect effect of muscle tone on valve gating has been suggested. Active muscle contraction can facilitate valve opening, possibly via the indirect effect of collagen fibres instead of muscle cells in the valves, but the influence of active contraction on valve gating remains controversial. Furthermore, the residual tone between contractions leads to a smaller diameter which makes valve closure under a smaller pressure gradient possible (40).

As the pressure gradient moves lymph forward, the valve leaflets remain open, which allows relatively unobstructed flow. With the pressure gradient decreasing and eventually reversing, the flow slows down, stops, and starts moving in the other direction. The valve remains open during a short period of time which allows backflow. The pressure drop then forces the valve to close which increases the backflow resistance. Ballard et al. 2018 (15) developed a computational model to study the valve behaviour in which the valves showed a delayed response to changes of flow condition. The gap distance is the distance between the tips of the top and bottom leaflets of the valves. There is a hysteresis of the gap distance between increasing and decreasing pressure gradient as shown in figure 14. Opening of the valve lags the pressure gradient when the pressure gradient increases to open a closed valve. As seen in figure 14, the valve is not at its unstressed state (the gap distance is lower than the dotted line) when the pressure gradient is zero and the flow direction changes to forward flow. Valves are not maximally opened when the maximum positive pressure gradient is reached, additional time is necessary to be fully opened. Valve closing lags the pressure gradient which causes a larger gap distance than when the valve was opening from the closed state. The delayed valve response is dependent on the valve properties which is explained in the subsequent section valve geometry and properties (15).

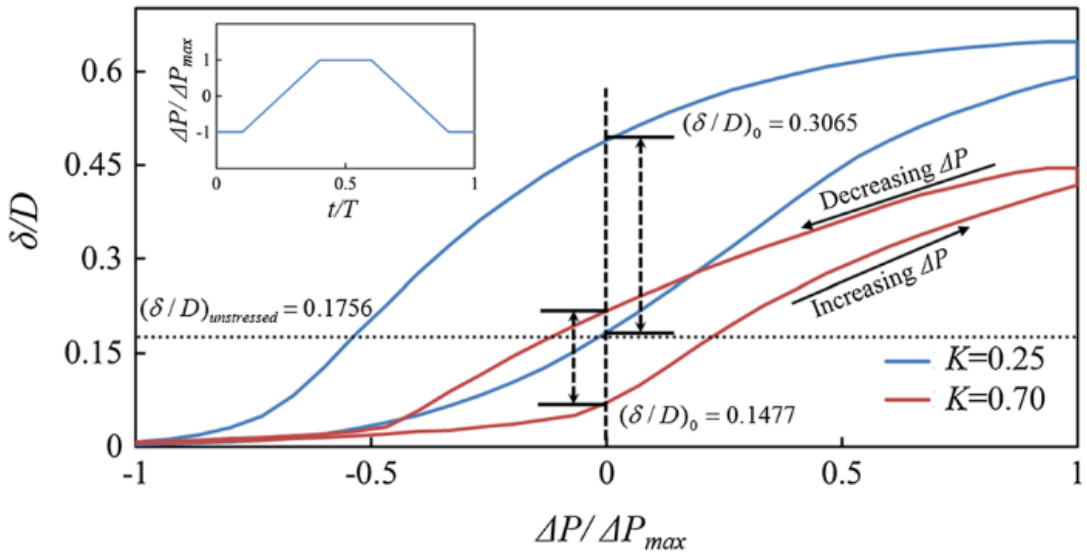

Figure 14: Normalized valve gap distance on the y-axis and normalized pressure gradient on the x-axis. The dotted line represents the gap distance at the unstressed state. K is the bending stiffness parameter. K=0,25 represents more flexible valves than K=0,70. δ =gap distance D= vessel diameter at base of the valve. $(\delta/D)_{unstressed}$= normalized gap distance at unstressed state. ΔP=pressure gradient. ΔP_{max}= maximum pressure gradient (15).

Valve geometry and properties

The valve geometry is probably optimized to both allow forward flow and prevent backward flow. The aspect ratio A of the lymphatic valves is the length of the valve divided by the valve width (i.e. vessel diameter at the base of the valve). The valve aspect ratio in sheep and rat lymphatic vessels has a range between 1,15 and 3. The aspect ratio can be different

depending on species and lymphatic vessel location. Simulations of lymphatic vessels containing valves of different lengths show that a larger aspect ratio (i.e. long valves) leads to increased flow resistance. However, shorter valves cannot fully occlude the vessel which allows backward flow. Valve stiffness also plays a role. Simulations have shown that stiffer valves are less effective in preventing backflow because they are not easily closed by pressure drops. More flexible valves are better suited to allow forward flow and prevent backward flow. The effect of valve stiffness depends on the valve geometry, as shorter valves must deflect more to close fully and thus is flexibility more necessary compared to longer valves. There is a significant effect of the valve stiffness on the hysteresis during the pumping cycle which is also shown in figure 14. Flexible valves have a larger gap opening, except with full closure of the valve with a maximum negative pressure gradient. Flexible valves thus need to travel greater distances during opening and closing than stiff valves which leads to greater valve velocities and hydrodynamic forces (15).

3.2. Lymphatic wall stresses

There are four main stresses acting on the lymphatic wall. First, the wall shear stress τ_{wall}, induced by the lymph flow component parallel to and in contact with the lymphatic wall. Second is the wall tension σ_{hoop}, which is the circumferential stress (i.e. hoop stress) across the wall. The third stress is the radial stress σ_{radial}. The fourth stress is the axial stress σ_{axial} caused by longitudinal stretching (24). These stresses are schematically presented in figure 15.

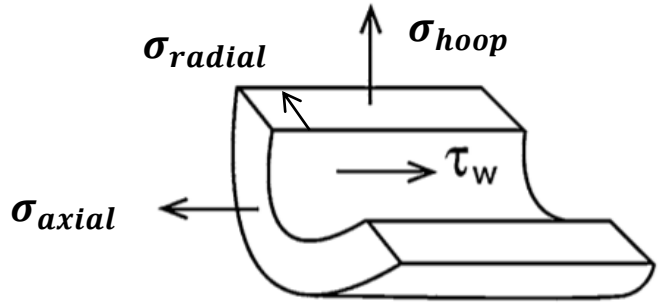

Figure 15: Schematic presentation of the four main stresses acting on the lymphatic wall. Wall shear stress τ_w, circumferential stress σ_{hoop}, radial stress σ_{radial} , and axial stress σ_{axial} . Adapted from (41).

3.2.1. Shear stress

Shear rate is the rate of change of velocity in passing between fluid layers. Dynamic viscosity is the ratio of shear stress and shear rate. There is a direct relationship between shear stress and shear rate in a Newtonian fluid. Shear stress (τ) is the shear rate multiplied by the dynamic viscosity of the lymph. In Poiseuille flow, shear stress can also be expressed by the following formula (42).

$$\tau = \frac{\Delta P r}{2l}$$

Equation 7

$\Delta P = pressure\ drop\ [Pa], r = vessel\ radius\ [m], l = vessel\ length\ [m]$

The shear stress is the highest at the wall ($r = r_i$), also called the wall shear stress (42). An accurate estimation of wall shear rate is necessary to obtain wall shear stress values. However, this is challenging because of the small size of the lymphatic vessels, wall motion, and slow lymph velocities. This indicates the need to use models to estimate wall shear stress from measurements of pressure drop, flow rate, and/or diameter (48). The law of Poiseuille can be assumed to model the mechanics of lymph to estimate wall shear stress from flow (13). As the Womersley number and the Reynolds number are low, the next formulas can be used (33,42). The use of equation 6 results in the following equation.

$$\tau = \frac{4\mu Q}{\pi {r_i}^3} = \frac{4\mu v_{mean}}{r_i}$$

Equation 8

$Q = lymph\ flow\ [m^3/s], \mu = dynamic\ viscosity\ of\ lymph\ [Pa\ \cdot s\ ], r_i = vessel\ inner\ radius\ [m],$
$v_{mean} = average\ velocity\ [m/s]$

The Poiseuille assumption may not always be appropriate because lymphatic vessels are dynamic and have a significant wall motion and Poiseuille flow assumes rigidity (48). In section 3.1.1, a study was mentioned that measured wall and lymph velocity and concluded that Poiseuille flow is valid for most of the contraction cycle (49). The Poiseuille assumption was also tested by comparison to a complex model of a radially expanding and contracting lymphangion and the Poiseuille flow assumption resulted in an error of 4% when estimating the wall shear stress (13,48). To conclude, Poiseuille can accurately estimate wall shear stress in the non-valvular regions of the lymphatic vessels (48).

There are two types of shear stresses in the lymphatic vessels. First, a laminar pulsatile shear stress and second, a reciprocating shear stress in the valvular region where lymph flow is more unstable (19). The fluid shear stress is affected by the transaxial pressure gradient via flow rate. The lymphatic pump is dynamic in nature and causes a wide range of wall shear stress values which makes the use of an average value not fitting to describe the wall shear stress (17). Dixon et al. 2006 (49) measured the lymph velocity in rat mesenteric prenodal collecting lymphatics to estimate the wall shear stress based on Poiseuille flow. This study reports an average shear stress of 0,64±0,14 dyn/cm^2 (= 64 ± 14 mPa) (49).

3.2.2. Circumferential stress

Transmural pressure and external tissue pressure results in the circumferential loading of lymphatic vessels (34). The circumferential stress (σ_{hoop}) can be expressed by the following equation (42).

$$\sigma_{hoop} = \frac{F_c}{hl}$$

Equation 9

$F_c = circumferential\ force\ [N], h = wall\ thickness\ [m], l = vessel\ length\ (axial)\ [m]$

The average transmural pressure affects the circumferential stress (17). The law of Laplace can be used to describe the relation between the transmural pressure and the circumferential stress (42).

$$\sigma_{hoop} = \frac{P_{TM} r_i}{h}$$

Equation 10

$P_{TM} = transmural\ pressure\ [Pa], r_i = vessel\ inner\ radius\ [m]$

The Laplace law gives the average circumferential stress and cannot give information on the distribution of stress across the wall (42). However, this limitation is probably of minor importance as the radius-to-thickness ratio of lymphatic vessels is typically between seven to eight. Hence, a thin-wall assumption and neglecting transmural variations in wall stress is justified (34). A model of the stress distribution of initial lymphatics showed that the distribution of circumferential stresses is influenced by wall stiffness. Higher wall tension develops in compliant lymphatics at the edge of the connection with the stiff tissue basement. The wall tension is lower and more equally distributed in stiffer lymphatics (22).

3.2.3. Radial stress

Radial stress can be assumed negligible compared to circumferential and axial stress (21).

3.2.4. Axial stress

Downstream and upstream parts of the vessel pull the vessel longitudinally, which is the axial loading of the lymphatic vessel. This axial loading causes axial stress (27). The average axial stress can be described by the following equation with the thin-wall assumption mentioned in section 3.2.2. (21,34).

$$\sigma_{axial} = \frac{F_z}{\pi h(2r_i + h)}$$

Equation 11

$F_z = axial\ force\ [N], r_i = vessel\ inner\ radius\ in\ loaded\ configuration\ [m],$
$h = wall\ thickness\ in\ loaded\ configuration\ [m]$

3.3. Compliance of lymphatic vessels

Compliance quantifies the relation between gradients of pressure and volume. The volume increases when the transmural or distending pressure is increased and vice versa. Compliance C is expressed in the following equation (42).

$$C = \frac{\Delta V}{\Delta P_{TM}}$$

Equation 12

$\Delta P_{TM} = transmural\ pressure\ [Pa], \Delta V = change\ in\ volume\ [m^3]$

The inverse of compliance is elastance or stiffness which represents the structural rigidity of the vessel (16,42). Pressure-volume or pressure-radius relationship measurements can be used to

derive compliance. The slope around a chosen working point is the compliance in a nonlinear pressure-volume relation. In biological organs the pressure-volume relation is convex to the volume axis (42). Study on the wall compliance of rat diaphragmatic initial lymphatic vessels has revealed two populations of vessels, namely highly compliant vessels (C=6,7±1,6 nl $mmHg^{-1}$) and stiffer vessels (C=1,5±0,4 nl $mmHg^{-1}$) which is represented in figure 16. This could be ascribed to differences in stiffness of the surrounding tissue and the relative percentage of stiff tissue in the vessel wall (22).

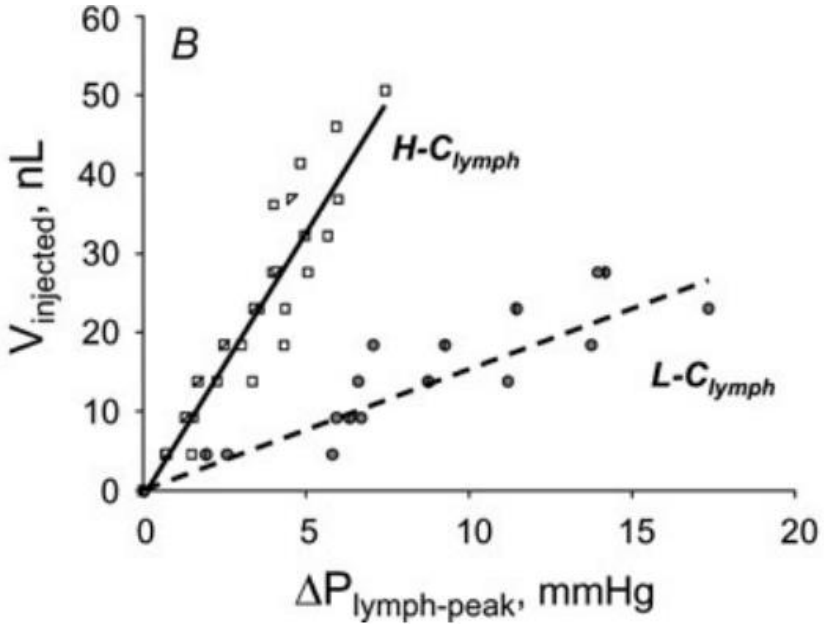

Figure 16: Two populations with a different compliance. H-C_{lymph}, high compliance. L-C_{lymph}, low compliance (22).

Literature on human pelvic lymphatic vessels shows a highly nonlinear pressure-diameter relationship, measured in cannulated and pressurized isolated vessels. Animal experimental data shows that at low pressure lymphatic vessels are more compliant than arterial or venous vessels which reduces the loss of contractile pumping work to deform the vessel between contraction and distension. Athanasiou et al. 2017 (16) investigated the passive biomechanics of human pelvic lymphatic vessels. Lymphatic vessels showed to be characterized by a transition in which the rigidity (i.e. the slope of the pressure-diameter curve) significantly increases. The vessels are stiffer and higher pressure results only in minimal diameter changes after this transition zone. This rigidity increase prevents fluid accumulation and damage due to overstretching the vessels. In unstretched vessels this transition zone occurs around 5 cmH_2O (=3,7 mmHg). Rat data shows that the sharpness of transition between low and high stiffness zones is similar to humans. This may mean that a nonlinear strain-stiffening response is common in different species and lymphatic locations and independent of vessel size. Elongation of the vessels results in a smoother transition, decreased slope differences between the low and high stiffness zones, and a higher pressure and diameter at which the transition occurs. This is shown in figure 17. When extrinsic pumping mechanisms elongate lymphatic vessels, lymphatic muscle cells will undergo greater difficulty to

contract the vessel. However, these mechanisms also compress the lymphatic diameter which may lead to propulsion and pumping (16).

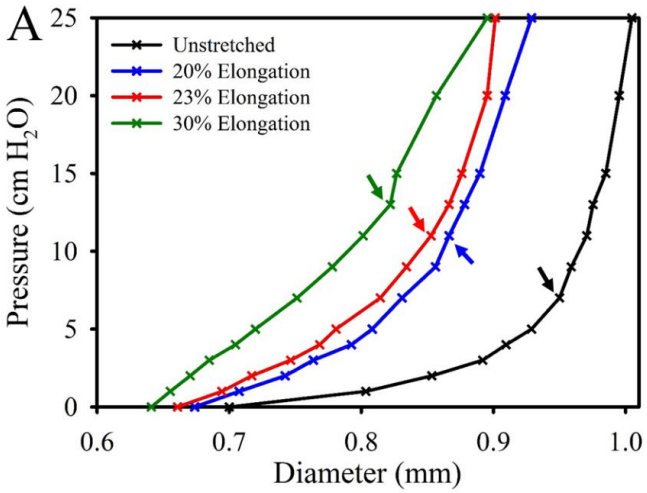

Figure 17: Pressure-diameter relationship at different stages of elongation.
The arrows indicate the transition between low stiffness and high stiffness responses (16).

A simulation of a chain of lymphangions showed that the compliance of a lymphangion also might depend on the diameter. The compliance is maximal at intermediate diameters and decreases with the presence of greatly positive or negative transmural pressure. Both low and high diameters result in low compliance due to lymphangion collapse and strain-stiffening response respectively (35).

3.4. Modulus of elasticity

The Young's modulus of elasticity (E) is a material property and is the slope of the linear relation between stress (σ) and strain (ε). In biological materials, this relationship is typically not linear and the local slope results in the incremental elastic modulus (42). The lymphatic wall movement has two components. First its active movement due to muscular activity and second its passive movement responding as an elastic membrane to the fluid pressures (20). The ability of the vessel to distend in response to mechanical load is related to the contractile function of the lymphatic vessel. Lymphatics are anisotropic and undergo large deformation under physiological loading (21). The compliance can be used to estimate the Young's modulus. The measurement of wall thickness, radius, and pressure are also necessary for this estimation (42). The compliance of a non-pumping lymphangion has been measured in a bovine mesenteric collecting vessel and the calculated Young's modulus value was 1200±700 N/m^2. However, the Young's modulus measured in pumping lymphangions was significantly larger (20).

3.5. Permeability

A study on rat mesenteric collecting vessels has shown that the lymphatic wall is permeable to macromolecules (24,44). Reports have been made of a median apparent solute permeability to albumin of rat mesenteric collecting vessels of $3{,}5\pm1{,}0\cdot10^{-7}$ cm s^{-1} (44). Data on permeability of rat

mesenteric collecting lymphatic vessels suggests that fluid and solute move together through common routes and that albumin flux is outward if the luminal pressure is higher than ~2 cmH_2O (=1,5 mmHg). The permeability of collecting vessels can be regulated by atrial natriuretic peptides (ANP) and brain natriuretic peptides (BNP) (24). The effect of ANP and BNP measured in rat mesenteric collecting vessels was respectively a 2±0,4 and 2,7±0,8 fold increase in permeability to serum albumin (45). Shear stress can also influence the permeability of collecting lymphatic vessels. Increases in shear stress (e.g. downstream of a localized lymph formation increase) in an in vitro study of lymphatic endothelial cells, decreases the permeability (13,24). Quantitative data on the permeability of the initial lymphatics has not been found. Adaptations of permeability of the initial lymphatics is hypothesized to optimize lymph formation. However, the physiological function of permeability of the collecting vessel wall is less clear (24).

4. Altered biomechanics in lymphedema

The alteration of the biomechanical properties in lymphedema is one of the least studied areas of the lymphatic biomechanics. However, understanding this alteration is crucial in improving the efficacy of treatment as most treatments are mechanical in nature (e.g. compression garments, manual lymph drainage) (13). This section discusses the effects of lymphedema on permeability, pressure, contraction, and morphology.

4.1. Effects on permeability

There have been reports of the impact of inflammatory agents on the permeability in lymphatic endothelial cell cultures (24). An in vitro study on lymphatic endothelial cells of rat mesenteric collecting vessels observed an increase in permeability to albumin of 25 to 250% in response to inflammatory cytokines, depending on the specific cytokine (43). The maximum diameter of particles that can enter the initial lymphatics in a rat was observed to be between 0,5 µm and 0,8 µm both in normal and inflammatory conditions. However, during inflammation quantum dots with a diameter of 10 nm could escape back into the interstitium, the transportation direction is thus reversed. Fluid is less efficiently cleared during inflammation than if the lymphatics were not permeable in an outward direction. There is more fluid in the tissue due to increased blood vessel permeability which in combination with decreased lymph transport increases the lymphedema. Furthermore, inflammatory mediators remain longer in the tissue because they are not easily transported in the leaking lymphatics, which increases the inflammatory response (29).

4.2. Effects on pressure

Transmural pressure is suggested to be elevated in lymphedema and increases as lymphedema progresses (13,34). The elevated transmural pressure in the affected area disrupts the mechanical state of the lymphatic vessel which could have an impact on the shear stress sensitivity. Models

suggest that the flow-mediated dilatation plays an important role in increasing the lymph transport during periods of increased lymph flow (33). Data from human studies on edematous limbs suggests that the outflow pressure (afterload) is significantly and chronically elevated in peripheral lymphatic trunks (47).

4.3. Effects on contraction

Studies on chronic lymphedema patients have shown evidence of lymphatic contractile dysfunction. The vessels of the patients exhibited weak contractions and lymphatic pump failure. However, it is difficult to determine whether contractile dysfunction leads to lymphedema or lymphedema overloads the contractile capacity of the lymphatics (31). The changes of the contractility pattern are described for different stages of lymphedema in a human leg. Active contractility is observed to be irregular in early stages of lymphedema. While lymphatics are still able to contract in further stages, the generated pressures are insufficient to propel lymph forward. The most advanced stages of lymphedema show no spontaneous lymph flow and only rudimentary pulse waves could be observed (32).

Contractility could also possibly alter in response to changes in mechanical load. A study on rat mesenteric collecting vessels concludes that the lymphatic vessels adapt to output pressure elevations. The contractility increases to be able to continue to transport lymph during edemagenic or gravitational loads (47). Another study on rat mesenteric collecting vessels induced edemagenic stress by a saline infusion. The vessels increased the lymph transport to adapt to this stress. Increases in flow rate, contraction frequency, and shear stress were also noted (25).

The afterload is elevated in lymphedema as mentioned in section 4.2, which has an influence on flow parameters and contractility pattern. Caulk et al. 2016 (34) developed a lumped parameter model that shows that the functional flow parameters (ejection fraction, contractile amplitude, and time-averaged outflow) decrease with sustained increase in afterload. Ultimately, the net flow rate and time-averaged shear stress will be reduced (34). The alterations of these parameters are shown in figure 18.

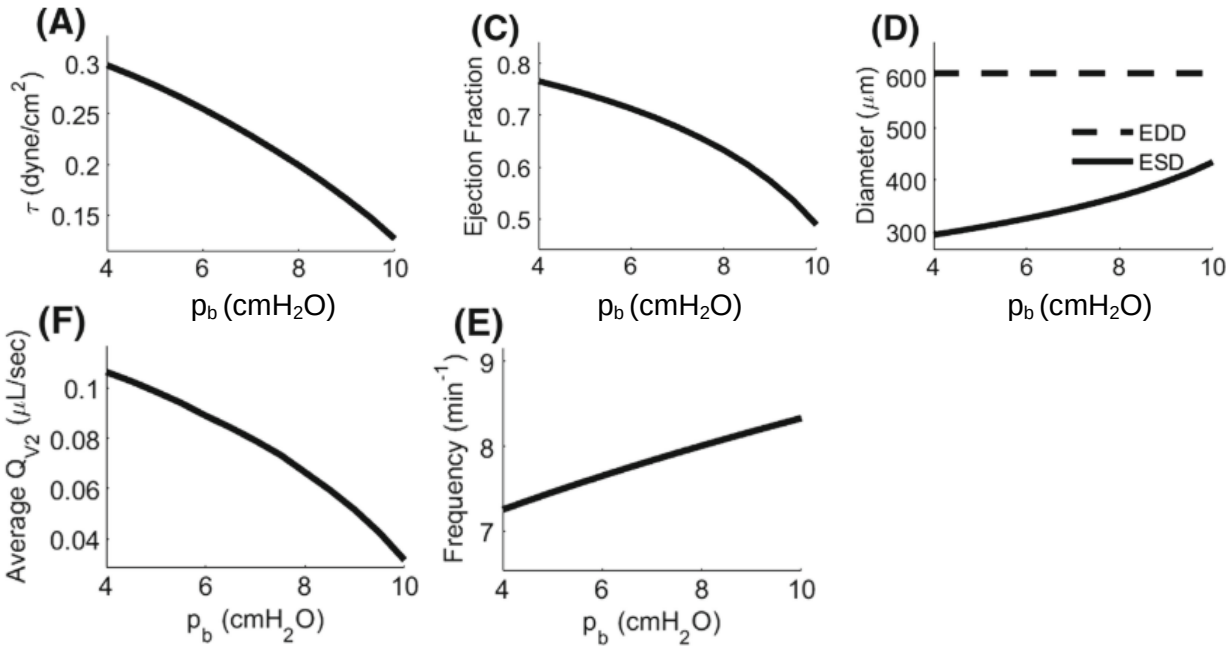

Figure 18: Functional flow parameters decrease with sustained increase in afterload (p_b). A: Time-averaged shear stress decreases. C: Ejection fraction decreases. D: Contractile amplitude decreases. EDD= end diastolic diameter. ESD= end systolic diameter F: Average flow rate decreases. E: Contractile frequency increases (34).

In response to these alterations, the contractility will increase which restores the time-averaged shear stress and increases the ejection fraction. The circumferential stress will decrease but is not completely restored and will remain 20% higher than the homeostatic value. The average outflow rate will be compensated by these changes. The timescale of the adaptation of contractility ranges from minutes to days. This acute adaptation of contractile function is shown in figure 19 (34).

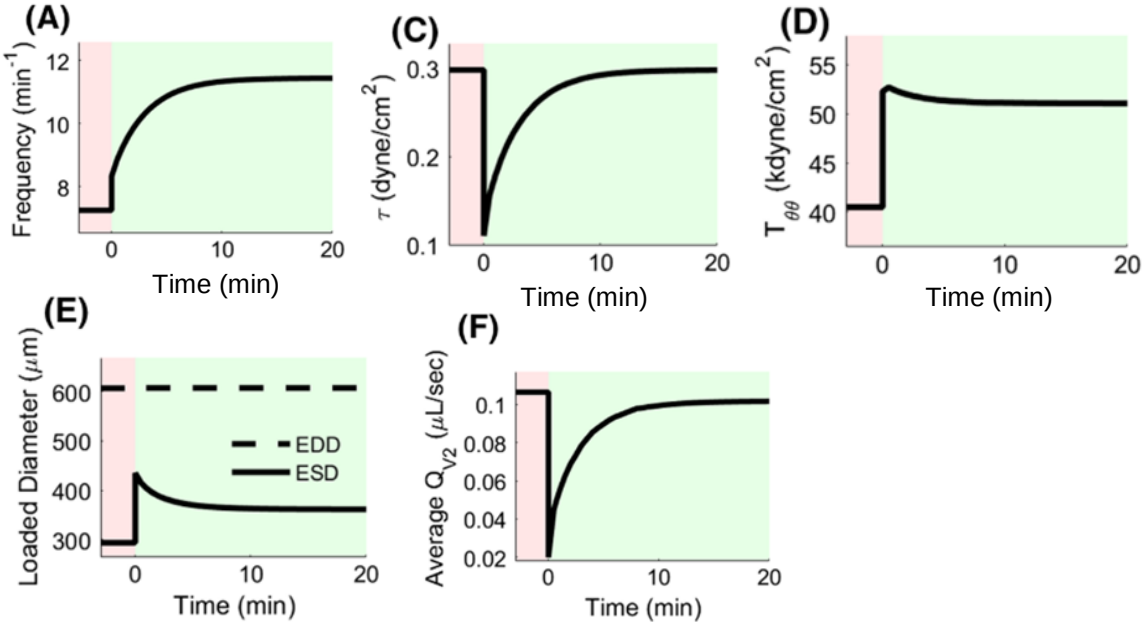

Figure 19: Acute adaptation of contractile function in response to a sustained increase in afterload. A: Contractile frequency increases to restore the shear stress. C: Shear stress. D:Circumferential stress. E: Decrease in end systolic diameter (ESD) which increases the ejection fraction. F: Average outflow rate (34).

As edema progresses, the vessels will decompensate and the pump function will decline (47). The continuous overloading of the lymphatics with lymph, dilates the vessels which results in insufficient valves. This leads to a large standing fluid column if the extremity is positioned in a dependent position (i.e. switching from a horizontal to a vertical position of the limb increases the hydrostatic pressure) (32,47). In normal conditions the lymph pressure is not affected by position changes of the limb (32). The pump function will decline further due to an elevation of the outflow pressure (47).

4.4. Morphological changes

The lymphatic vessels remodel to adapt to changes in the mechanical environment. A deficit in lymphatic pumping results in lymphatic expansion due to compensatory lymphangiogenesis. However, this mechanism doesn't resolve the pumping failure (31). The vessel wall thickens and the unloaded midwall diameter increases in response to the increased circumferential stress (see section 4.3). Vessels may adapt and become stronger pumps in a period of a few days, but simulations of lymphatic remodelling suggest that long-term growth and remodelling may result in reduced flow rate (34).

DISCUSSION & CONCLUSION

Lymphedema is a prevalent and important disease which can lead to functional impairment, physical deformity, and compromised quality of life. Unfortunately, no curative treatment is available at this moment. Expanding our knowledge on the lymphatic system and lymphedema is crucial in the development of new treatments. Recent advances have been made through multi-disciplinary biomedical engineering approaches such as computational modelling. Until now, no overview of the biomechanical properties of the lymphatic system in combination with an overview of the alterations in lymphedema can be found. Such overview could be a starting point for readers interested in the lymphatic system with either an engineering or a medical background. A literature study was conducted to give rise to this overview.

The main conclusions from the literature study on the biomechanical properties of the lymphatic system can be summarized as follows. The type of flow can be described with the help of the Reynolds number, the Womersley number, and the law of Poiseuille. The Reynolds number in the lymphatic system is typically less than one and can be higher in close presence of valves. However, the viscous effects remain dominant and the flow is laminar. The Womersley number is also low which means that the Poiseuille law can be used as a reasonable estimate for the flow. Studies suggest that the conditions for the validity of the Poiseuille assumption are fulfilled for most of the contraction cycle and in the non-valvular regions of the lymphatic vessel. Poiseuille flow can thus be used as an approximation for the velocity distribution. Vessel contraction and the velocity pattern is found to be 180° out of phase. Flow rate is modulated by transmural pressure and the contraction frequency. The maximum mean flow rate is generated when the vessel is most compliant. The lymph pump is coordinated by a time delay between contractions of adjacent lymphangions on the one hand and between subsequent contractions of the same lymphangion on the other hand. Pumping was found to be more effective with this pump coordination mechanism. The lymphatic valves have an important role in providing a net pumping effect. The transvalvular pressure gradients, responsible for valve closure and depending on vessel distension, and viscous forces control valve gating. The valves show a delayed response to changes of flow. Valve opening and closing lags the pressure gradient. The valve geometry, valve aspect ratio, and valve stiffness are optimized to allow forward flow and prevent backward flow. There are four main stresses acting on the lymphatic wall, namely shear stress, circumferential stress, radial stress, and axial stress. The law of Poiseuille can be used to estimate wall shear stress from flow. This Poiseuille flow assumption is tested in a complex model of a contracting lymphangion and seems a good estimate to determine wall shear stress in non-valvular regions. The average transmural pressure can affect the circumferential stress and this relation can be described by the law of Laplace. Only the average circumferential stress can be obtained and not the distribution of stress across the wall. However,

the transmural variations of wall stress can be neglected due to a small radius-to-thickness ratio typical for lymphatic vessels. The radial stress can assumed to be negligible compared to the circumferential and axial stresses. Lymphatic vessels show a highly nonlinear pressure-diameter relationship. The vessels are characterized by a transition in which the rigidity significantly increases. Elongation of the vessels results in changes of this transition. The Young's modulus is larger in pumping than non-pumping lymphangions. The lymphatic wall is permeable to macromolecules and the permeability can be regulated by shear stress and specific peptides.

Studies on the altered biomechanics in lymphedema are scarce. The following conclusions can be made based on the limited studies. Inflammatory agents result in increased permeability of the lymphatic wall in an outward direction which results in a decreased efficiency of fluid clearing. The transmural pressure is elevated in lymphedema and increases as lymphedema progresses. The outflow pressure (afterload) is significantly and chronically elevated in edematous limbs. Chronic lymphedema patients have lymphatic contractile dysfunction. Different stages of lymphedema are characterized by changes in contractility pattern. The contractility will increase in response to a decrease in functional flow parameters caused by a sustained afterload elevation. This change of contractility will maintain the average outflow rate. However, as lymphedema progresses, the vessels will decompensate and the pump function will decline. The decompensation of the vessels is associated with dilatation of the vessels which results in valve deficiency. Lymphatic vessels can remodel to adapt to changes in the mechanical environment. Lymphatic expansion, vessel wall thickening, and increased vessel diameter will not resolve the pumping failure in long-term, despite possible temporary beneficial effects in the acute stage of the adaptation.

The scarcity of studies found on the biomechanics of the lymphatic system and especially on the altered biomechanics in lymphedema indicate the need for further research. The complexity of the lymphatic system in combination with lack of experimental data is noticeable in the abundance of assumptions and simplifications used in current computational models. These assumptions must be tested and further validated to enhance the available models (e.g. the validation of the Poiseuille flow assumption in a lymphangion with valves). Not all elements known to play a role in lymphatic physiology and pumping are incorporated in the existing models. More complex models of vessel networks including hydrostatic forces, external compression forces, neural modulation, and modulation by humoral agents are necessary for a more thorough study of the lymphatic system. The clinical significance of some simulations is not yet understood. Valve stiffness appears to have an important role in lymphatic pumping performance in simulations, however whether this is a relevant mechanism in the onset or progression of lymphedema is unclear (15). The elevated transmural pressure in lymphedema could have an effect on the shear stress sensitivity but the impact on the lymphatic function is not yet experimentally demonstrated (33). Permeability of the

wall of the collecting vessel is observed, but the physiological function is unknown. Some subjects are still controversial, such as the impact of active contraction on valve gating. Some subjects, such as axial stress or radial stress are hardly studied. The found studies on the altered biomechanics in lymphedema only cover the effects on permeability, pressure, contraction, and morphology but no information has been found on compliance, valve gating, pump coordination or other topics discussed in the biomechanics of the normal lymphatic system. The exact pathway of onset and progression of lymphedema is not yet determined. Several processes such as inflammation, fibrosis, and dysregulated lymphangiogenesis are known to play a role in the pathophysiology of lymphedema but the detailed mechanisms or cause and effect are unknown. Simulations that study and combine these processes could possibly help to understand the pathophysiology of lymphedema. To conclude, the research on the lymphatic system has still a long way to go to fully understand lymphedema and ultimately find a curative treatment for this debilitating disease.

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APPENDICES

1. Lymphatic modelling

Computational modelling in combination with experimental verification can expand our knowledge of the physiology of the lymphatic system. Models that could predict changes in the system due to local changes of load could help to increase the understanding of the development of lymphedema. Ultimately, the goal is to develop new and more effective treatments of lymphedema (4,13). Models can integrate sets of data, compare competing hypotheses time- and cost-efficiently, and direct the setup and interpretation of experiments (34). Models can thus complement the experimental evidence and deliver further insights when experiments are unfeasible (30). However, there are several challenges in computational modelling of the lymphatic system. A severe difficulty is the scarcity of experimental data. The lymphatic system is less studied relative to the cardiovascular system and only recently has basic data on vessel behaviour become available. Measurements are difficult, especially non-invasive in humans (23). Locating and handling of lymphatic vessels is challenging (15). Lymphatic vessels are (microscopically) small, have thin transparent walls, and transport transparent fluid at low velocities which makes them difficult to identify (15,23). Furthermore, the vessels are quite fragile (15). To conclude, the complex character of the lymphatic system combined with the scarcity of experimental data on anatomy and physiology results in a significant challenge to model with mathematical methods (4).

1.1. Lumped parameter model

Lumped parameter models are described by ordinary differential equations or algebraic equations. It reduces a distributed system to a discrete one. These models are idealised and cannot describe the flow in detail, but they can result in useful insights into the behaviour of the studied system (4). Lumped parameter models are the most frequently published type of models on the lymphatic system. This is a useful model due to the lack of data to support detailed distributed models (23). However, also in lumped parameter modelling, many assumptions are necessary due to the lack of data (34).

The model created by Reddy et al. (39) is considered to be the earliest (1977) computational model of a large part of the lymphatic system (20,39). It is a simplified one-dimensional model based on the Navier-Stokes equation (4). In 1986, Drake et al. (37) created an electrical analogue model of the lymphatic vessels (4,37). Quick et al. (52) developed a lumped model in 2008 using a simplified algebraic equation based on the time-varying elastance concept (i.e. ratio of transmural pressure and volume) which was originally developed for the ventricles of the heart (4,38,52). Venugopal et al. (38) refined this concept in 2010 (4,38). Bertram et al. (35) modelled in 2010 a chain of

lymphangions and studied the pumping behaviour (35). This model is schematically represented in figure 20. This model included a pressure dependent valve resistance (4). Valve resistance varies hysteretically with transmural and transvalvular pressure in the model of Davis et al. in 2011 (23,40). Jamalian et al. (50) modelled in 2013 a chain of lymphangions (23,50). In 2014, Bertram et al. (51) developed a model of a multi-lymphangion vessel. This model includes a refractory period between contractions, nonlinear pressure-diameter relationship, hysteric and transmural pressure dependent valve gating, and dependence of active tension on muscle length (51). Caulk et al. (34) simulated in 2016 local control of the contraction rate, tone, and stress induced remodelling with the use of generalized vascular regulatory properties (23,34). Bertram et al. (53) modelled in 2016 a chain of lymphangions to study the interaction of contraction timing with the valve properties and to determine the configuration that results in the greatest flow (23,53). In 2016, Jamalian et al. (30) modelled a bifurcating network of collecting vessels and each vessel consists of multiple lymphangions (30).

The lumped parameter models have shown that sequential contraction (i.e. a time delay between contractions) is more advantageous than synchronized contraction (30,35,53). The models have studied the parameters that affect lymphatic output such as external pressure and contraction frequency and the parameters that affect the pressure gradient necessary to open and close lymphatic valves (40,50). Lumped parameter models have also been used to simulate acute and long-term adaptation of lymphatic vessels to changes in the mechanical environment (34). The lumped parameter models have revealed useful information on the biomechanics of the lymphatic system and can help to develop more complex and accurate models and to propose relevant experiments.

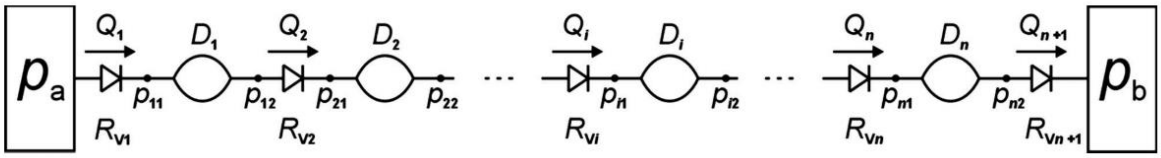

Figure 20: Schematic representation of an example of a lumped parameter model of a chain of lymphangions developed by Bertram et al. Nonreturn valve with Q=flow rate and R_V= flow resistance. Contractile segments with D=diameter. P= downstream and upstream pressure (35).

1.2. Continuum model

Continuum models are described by partial differential equations. Lymphatic continuum models are scarce (4). In 2008, Macdonald et al. (20) refined the model of Reddy et al. but retained the continuum nature of the equation (4,20). This model can reproduce the pumping behaviour and studies the contraction pattern and flow generation (20). The discussed models (lumped parameter and continuum) all assume a one-dimensional laminar flow with a parabolic velocity profile (4). Rahbar & Moore (48) challenged this assumption in 2011 with the development of a three-

dimensional model of a contracting lymphangion (4,48). This model doesn't include valves and inertial effects. As discussed in section 3.2.1, the assumption of Poiseuille flow to estimate shear stress is reasonable, however this still needs to be validated in a model of a lymphangion with valves (4). In 2018, Ballard et al. (15) developed a three-dimensional, fully coupled fluid-solid interaction model, shown in figure 21, to study valve behaviour and the consequences to lymphatic function. This model gives insights on how abnormal valve morphologies can lead to lymphatic dysfunction (15). Mendoza and Schmid-Schönbein (36) modelled in 2003 the initial lymphatics and the primary valves. They showed that the overlapping endothelial cells in the initial lymphatics function as the primary valve system which is necessary for the unidirectional lymph transport (36).

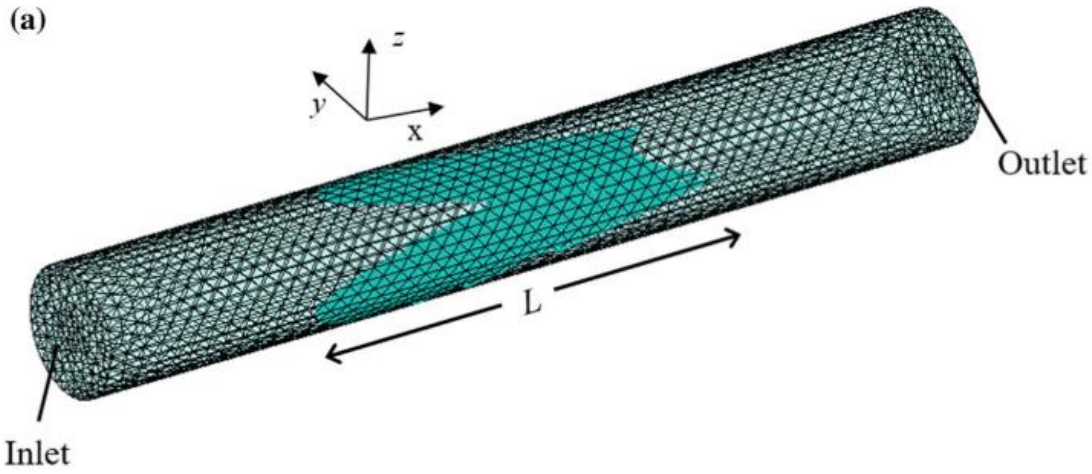

Figure 21: Ballard et al. (15) modelled a three-dimensional lymphangion consisting of a cylindrical vessel and valves. L= valve length (15).

2. Lymphatic properties

Table 2 gives an overview of experimental values of lymphatic properties. The studied species and type of lymphatic vessel are specified in this table. Some of these values are discussed above in more detail. Other values such as density and viscosity are necessary to calculate for example the Reynolds or Womersley number.

Table 2: Overview of experimental values of lymphatic properties.

Property	Experimental value	Studied specimen	Reference
Viscosity	1,23 mPas	Dog thoracic duct	(23)
Density	1,0097 g/ml	Dog thoracic duct	(23)
Valve length	230-2800 µm	Human leg vessels, rat mesenteric vessel	(15)
Vessel diameter	100-2800 µm	Bovine mesentery collecting vessels, human leg vessels, rat mesenteric vessel	(15)
Young's modulus	1200±700 N/m^2	Bovine mesentery collecting vessels	(20)
Lymph velocity in collecting lymphatics (average)	0,87±0,18 mm/s	Rat mesenteric prenodal collecting vessel	(49)
Lymph velocity in initial lymphatics	10 µm/s-0,51 mm/s	Human initial lymphatics	(28)
Median apparent solute permeability to albumin	$3,5±1,0·10^{-7}$ cm s^{-1}	Rat mesenteric collecting vessel	(44)

3. List of abbreviations

ANP	Atrial natriuretic peptide
ATP	Adenosine triphosphate
BIS	Bioimpedance spectroscopy
BMI	Body mass index
BNP	Brain natriuretic peptide
CD4	Cluster of differentiation 4
CDT	Complex decongestive therapy or complete decongestive therapy
CT	Computed tomography
DP	Diffuse pattern
DVT	Deep venous thrombosis
ECM	Extracellular matrix
EDD	End diastolic diameter
ESD	End systolic diameter
ESWT	Extracorporeal shock wave therapy
FITC	Fluorescein isothiocyanate
HLA	Human leukocyte antigen
ICG	Indocyanine green
IF	Interstitial fluid
IFN-γ	Interferon gamma
IL-13	Interleukin 13
IL-4	Interleukin 4
IL-6	Interleukin 6
iNOS	Induced nitric oxide synthase
IPC	Intermittent pneumatic compression
ISL	International society of lymphedema

LLLT	Low level laser therapy
LP	Linear pattern
LVA	Lymphaticovenous anastomoses
LVB	Lymphovenous bypass
LYMPHA	Lymphatic microsurgical preventing healing approach
MFBIA	Multi-frequency bioimpedance analysis
MLD	Manual lymph drainage
MRI	Magnetic resonance imaging
NIRF	Near infrared fluorescence
NO	Nitric oxide
PBM	Photobiomodulation
SAPL	Suction-assisted protein lipectomy
SFBIA	Single frequency bioimpedance analysis
SP	Splash pattern
TGF-β	Transforming growth factor beta
Th1	T helper 1
Th17	T helper 17
Th2	T helper 2
Tregs	T regulatory cells
VEGF-C	Vascular endothelial growth factor C
VLNT	Vascularized lymph node transfer
VOLT	Vascularized omentum lymphatic transplant

4. List of symbols

$P_c = local\ capillary\ blood\ pressure\ [Pa]$
$P_i = interstitial\ fluid\ hydrostatic\ pressure\ [Pa]$
$\sigma = Staverman's\ osmotic\ reflection\ coefficient$
$\pi_p = colloid\ osmotic\ pressure\ of\ plasma\ [Pa]$
$\pi_g = colloid\ osmotic\ pressure\ of\ underside\ of\ glycocalyx\ [Pa]$
$Re = Reynolds\ number$
$\rho = lymph\ density\ [kg/m^3\ ]$
$\nu = mean\ velocity\ of\ lymph\ [m/s]$
$D = vessel\ inner\ diameter\ [m]$
$\mu = dynamic\ viscosity\ of\ lymph\ [Pa \cdot s]$
$\alpha = Womersley\ number$
$\omega = angular\ frequency = 2\pi f$
$f = frequency\ [Hz]$
$r_i = vessel\ inner\ radius\ [m]$
$v = velocity\ [m/s]$
$r = vessel\ radius\ [m]$
$Q = lymph\ flow\ [m^3/s]$
$\Delta P = pressure\ drop\ [Pa]$
$l = vessel\ length\ [m]$
$K = valve\ bending\ stiffness\ parameter$
$\delta = gap\ distance$
$A = aspect\ ratio$
$\tau_{wall} = wall\ shear\ stress[Pa]$
$\sigma_{hoop} = hoop\ stress[Pa]$
$\sigma_{radial} = radial\ stress\ [Pa]$
$\sigma_{axial} = axial\ stress[Pa]$
$\tau = shear\ stress[Pa]$
$F_c = circumferential\ force\ [N]$
$h = wall\ thickness\ [m]$
$P_{TM} = transmural\ pressure[Pa]$
$F_z = axial\ force\ [N]$
$C = compliance$
$\Delta V = change\ in\ volume\ [m^3]$
$E = Young's\ modulus\ of\ elasticity\ [Pa]$
$\sigma = stress\ [Pa]$
$\varepsilon = strain$