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VASCULARISED LYMPH NODE TRANSFER AND LYMPHATIC GROWTH FACTORS IN THE TREATMENT OF LYMPHEDEMA

Eeva Rannikko (previously Sulo)



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To my all and everything

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Faculty of Medicine

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EEVA RANNIKKO: Vascularised Lymph Node Transfer and Lymphatic

Growth Factors in the Treatment of Lymphedema

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ABSTRACT

Lymphedema is a progressive disease that can significantly deteriorate the quality of life of patients if not treated appropriately. The conventional treatment for lymphedema has been conservative, with the continuous use of compression garments and physiotherapy. My dissertation aims to further assess the possibilities, limitations and proper indications for lymph node transfer surgery in patients with lymphedema. Lymph node transfer and lymphaticovenous anastomosis have been considered as a possible a cure for lymphedema. Conservative treatment is not intended to be a cure, only for alleviating the symptoms of lymphedema. During lymph node transfer surgery, excess scar tissue from the axillary area of the affected arm is removed, and new immunologically active adipose and lymphatic tissues are transferred to the site to restore the lymphatic environment of the axilla and the lymphatic flow of the affected arm. The transferred lymph nodes function as pumps to further activate the lymph flow. Lymph node transfer improves the volumetry results of the affected arm, lymphoscintigraphy results, tissue dielectric constant results, and decreases rate of cellulitis infections. Treatment with a specific lymphatic growth factor (adenoviral vascular growth factor C (VEGF-C)) was further assessed in a phase II multicenter prospective study after one year of follow-up. This experimental medicinal treatment showed beneficial results in ameliorating lymphedema, but the results were inconclusive.

Lymph node transfer seems to benefit patients with early stage lymphedema. Lymph node transfer seems to increase the quality of life in lymphedema patients and improve lymphedema symptoms more than clinical measurements would suggest. Patient selection is a key factor for successful lymph node transfer surgery. Lymph node transfer has shown both objective and subjective benefits for lymphedema patients, and it is a possible cure for lymphedema when patient selection is performed properly.

KEYWORDS: Lymph node transfer, Reconstructive surgery, Growth factor therapy, Lymphedema

TURUN YLIOPISTO

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TIIVISTELMÄ

Lymfedeema on imunestekierron häiriö ja etenevä tauti, joka saattaa heikentää potilaan elämänlaatua merkittävästi, jos sitä ei hoideta asianmukaisesti. Tavanomaisesti lymfedeeman hoito on konservatiivista sisältäen säännöllisen kompressio-tekstiilien käytön ja fysioterapian. Väitöskirjani tavoitteena on ollut arvioida imusolmuke siirtoleikkausten mahdollisuuksia, rajoituksia ja indikaatioita lymfedeemapotilailla. Imusolmuke siirtoleikkausten ja imusuoni-laskimoanastomoosileikkausten tavoitteena on ollut lymfedeeman parantava hoito. Konservatiivinen hoito ei pyri parantamaan lymfedeemaa, vaan sen tavoitteena on lievittää oireita. Imusolmuke siirtoleikkauksen aikana poistetaan ylimääräinen arpikudos kainalon alueelta ja tuodaan tilalle terveeseen immunologiseen ympäristön omaavaa rasva- ja imukudosta auttamaan käden lymfaattista kiertoa palautumaan normaaliksi. Lisäksi siirretyt imusolmukkeet toimivat pumppuina, jotka tehostavat ja normalisoivat käden imunestekiertoa entisestään. Imusolmuke siirtoleikkaukset näyttävät vähentävän sairastuneen käden tilavuutta, parantavan lymfoskintigrafian tuloksia, kudostenestemittautuloksia ja vähentävän selluliitti-infektioita sairastuneessa kädessä. Väitöskirjani osana on myös kokeellinen lääkkeellinen kasvutekijähoito VEGF-C-pistoksin annettuna imusolmukeleikkaukseen. Tässä prospektiivisessä monikeskustutkimuksessa kävimme läpi faasin II tuloksia yhden vuoden seurannan jälkeen. Kasvutekijähoito näyttää parantavan sairastuneen käden rakennetta terveempään suuntaan, mutta tulokset eivät olleet tilastollisesti merkitseviä.

Imusolmuke siirtoleikkaus vaikuttaa auttavan eniten niitä varhaisvaiheen lymfedeemapotilaita, joilla turvotus on lievää. Imusolmuke siirtoleikkaus parantaa potilaiden elämänlaatua ja subjektiivisesti vähentää lymfedeeman oireita enemmän kuin kliiniset mittaukset antavat ymmärtää. Tämän vuoksi potilasvalinta on erittäin tärkeää onnistuneen imusolmuke siirtoleikkauksen kannalta. Imusolmuke siirtoleikkaukset ovat auttaneet potilaita sekä objektiivisesti että subjektiivisesti ja se voi olla oikein valituille potilaille parantava hoito.

AVAINSANAT: Imusolmuke siirto, Korjaava kirurgia, Kasvutekijähoito, Lymfedeema

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Abbreviations

ADB	Arm dermal backflow
AE	Adverse event
BCRL	Breast cancer-related lymphedema
BMI	Body mass index
CD4	CD4 positive helper lymphocyte
CD8	CD8 positive killer lymphocyte
CHFUS	Conventional high-frequency ultrasound
CI	Confidence interval
DC	Dendritic cell
DIEP	Deep inferior epigastric perforator
ELISA	Enzyme-linked immunosorbent assay
G-CSF	Plasma granulocyte
HFUS	High-frequency ultrasound
ICG	Indocyanine green
ICG-L	Indocyanine green lymphangiography
IL-4	Interleukin-4
IL-10	Interleukin-10
IL-13	Interleukin-13
IMA	Internal mammary artery
ISL	International Society of Lymphology
LEC	Lymphatic endothelial cell
LTA ₄	Leukotriene A ₄
LTB ₄	Leukotriene B ₄
LyQoLI	Lymphedema quality of life inventory
LVA	Lymphaticovenous anastomosis
MBq	Megabecquerel
MDA	MD Anderson classification
MMRM	Mixed model repeated measures
MRI	Magnetic resonance imaging
ms-TRAM	Muscle-sparing transverse rectus abdominis musculocutaneous
NSAID	Nonsteroidal anti-inflammatory drug
PAL	Photoacoustic lymphangiography
POD	Postoperative day

SCIA	Superficial circumflex iliac artery
SCIP	Superficial circumflex iliac perforator
SIEA	Superficial inferior epigastric artery
SPECT/CT	Single photon emission computed tomography with computed tomography
TDC	Tissue dielectric constant
TI	Transport Index
Th1	Helper T-cell type 1
Th2	Helper T-cell type 2
3D	Three-dimensional
TGF- β 1	Transforming growth factor beta 1
TNF- α	Tumor necrosis factor alpha
UHFUS	Ultra-high frequency ultrasound
VEGF	Vascular endothelial growth factor
VEGF-C	Vascular endothelial growth factor C
VEGFR-3	Vascular endothelial growth factor receptor 3
VLNT	Vascularised lymph node transfer

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

- I **Eeva H. Sulo**, Pauliina Hartiala, Tiina P. Viitanen, Maija T. Mäki, Marko P. Seppänen, Anne M. Saarikko. Risk of donor-site lymphatic vessel dysfunction after microvascular lymph node transfer. *Journal of Plastic, Reconstructive and Aesthetic Surgery*, 2015 68:551–558.
- II **Eeva H. Rannikko**, Sinikka H. Suominen, Anne M. Saarikko, Tiina P. Viitanen, Maija T. Mäki, Marko P. Seppänen, Erkki A. Suominen, Pauliina Hartiala. Long-term Results of Microvascular Lymph Node Transfer: Correlation of Preoperative Factors and Operation Outcome. *Plastic and Reconstructive Surgery – Global Open*. January 2021; Volume 9 - Issue 1 - p e3354
- III **Eeva H. Rannikko***, Ida-Maria Leppäpuska*, Mervi Laukka, Anne M. Saarikko, Pauliina Hartiala. Short duration of upper extremity lymphedema correlates with a favorable cytokine response after lymph node transfer surgery. *Lymphology*. 2022;55(2): 54–64. *Equal contribution
- IV **Eeva H. Rannikko**, Susanna Pajula, Sinikka H Suominen, Juha Kiiski, Maria R Mani, Martin Halle, Ilkka S Kaartinen, Outi Lahdenperä, Tinna H Arnardottir, Susanna M Kauhanen, Heli Kavola, Marja Majava, Tarja S Niemi, Nina M Brück, Maija T Mäki, Marko P Seppänen, Anne M Saarikko, Pauliina Hartiala. Phase II study shows potential benefit of adenoviral vascular endothelial growth factor C (VEGF-C) and lymph node transfer in lymphedema. Accepted for publication in *Plastic and Reconstructive Surgery*.

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1 Introduction

The lymphatic system is responsible for proper fluid transmission and the logistics of numerous cytokines and growth factors that are crucial to the normal function of the human body (Oliver *et al.*, 2002; Liao *et al.*, 2015). Although it does not get much appraisal when it is functioning normally, it can cause devastating symptoms of pain and discomfort and worsen the quality of life of patients with a dysfunctional lymphatic system (Mercier *et al.*, 2019; Fish *et al.*, 2020). Disruption of the lymphatic system can be primarily caused by congenital deformities (Martin-Almedina *et al.*, 2021) or secondarily following, for example, filariasis or iatrogenic damage to the lymph vessels and nodes due to procedures such as axillary surgery (Grada *et al.*, 2017). A common cause for axillary surgery, lymph node biopsy, or evacuation is the treatment of metastatic breast cancer (Grada *et al.*, 2017). Lymphatics can also be damaged by other breast cancer-related treatments such as radiotherapy and chemotherapy (Ribeiro Pereira *et al.*, 2017). Lymphedema risk increases when patient undergoes multiple treatment modalities, for example, axillary surgery, radio- and chemotherapy (Ribeiro Pereira *et al.*, 2017). Breast cancer is the most common cancer in women (Wilkinson *et al.*, 2022); hence many women are at risk of developing lymphedema (Ribeiro Pereira *et al.*, 2017). The cumulative incidence of clinically manifested lymphedema after breast cancer treatment is 5.6–41.1% (DiSipio *et al.*, 2013; Gowda *et al.*, 2021; Shen *et al.*, 2023).

Damage to the lymphatic system is expected during axillary surgery to a certain extent, but it is known that lymphatic vessels have a tremendous ability to regenerate (Alitalo, 2011; Jiang *et al.*, 2018). However, it is not fully known which patients will develop chronic lymphedema. Initially, lymphedema starts with the accumulation of excess fluid in the affected limb, and then progresses gradually to form excess fibroadipose tissue, which is non-reversible non-pitting edema (Lymphology, 2003). Excess fluid accumulation and symptoms arising from swelling of the affected arm in the early stages of lymphedema can be treated with regular compression garment use and physical therapy. In the late stages, excess fibroadipose tissue cannot however be treated with conservative treatment alone (Chang *et al.*, 2021). Liposuction can reduce the volume and width of the affected limb, but the results only persist are only lasting if regular, daily compression garment use is continued

after liposuction (Schaverien *et al.*, 2018). Lymphedema also heightens the risk of recurrent soft tissue infections, such as cellulitis (Cheng *et al.*, 2022). The pathophysiology of lymphedema has been extensively studied in recent years; however, the mechanisms of lymphedema is still somewhat unknown, and it is difficult to predict which patients develop lymphedema after iatrogenic lymphatic damage. Fibrosis and scarring are two known factors responsible for the inhibition of lymphatic regeneration (Warren *et al.*, 2007; Maruccia *et al.*, 2019). Therefore, it is logical that factors related to chronic inflammation and fibrosis, such transforming growth factor $\beta 1$ (TGF- $\beta 1$) and interleukin 10 (IL-10), have been of major interest in recent studies (Peranteau *et al.*, 2008; Shi *et al.*, 2013; Leppäpuska *et al.*, 2021; Baik *et al.*, 2022). In addition, the shift towards a helper cell type 2-lineage (Th2)-dominant CD4 positive helper lymphocyte (CD4 positive) T-cell cytokine response is speculated to be a part of the development of chronic lymphedema (Avraham *et al.*, 2013; Mehrara *et al.*, 2021). The lymphatic system is not only responsible for the logistics of lymph and tissue fluids, but also produces a vast amount of cytokines and growth factors. Some cytokines, such as, tumor necrosis factor α (TNF- α) and vascular endothelial growth factor C (VEGF-C), have been identified as prolymphangiogenic. (Jamie C. Zampell *et al.*, 2012) On the other hand, TNF- α is also involved in the proinflammatory route and is responsible for the stimulation of VEGF-C formation by fibroblasts (Ristimaki *et al.*, 1998). Experimental models have proven that transient overexpression of VEGF-C is efficient for in the maturation of collecting lymph vessels (Szuba *et al.*, 2002; Visuri *et al.*, 2015). Hence, VEGF-C has been utilized in growth factor therapy through adenovirus type 5-based gene therapy (Hartiala *et al.*, 2020; Leppäpuska *et al.*, 2022). Initially explored in a porcine model with encouraging outcomes, it has progressed to phase I and II trials involving human subjects. Other immunomodulatory approaches for treating lymphedema include ketoprofen (Rockson *et al.*, 2018), interleukin 13 (IL-13), and interleukin 4 (IL-4) blockers (Babak J. Mehrara *et al.*, 2021). In mouse models, lymphedema has been associated with an inflammatory gene expression profile similar to that of inflammation (Aschen *et al.*, 2012).

Surgical interventions have been developed to cure lymphedema (Babak J Mehrara *et al.*, 2021). Surgical interventions include lymphaticovenous anastomosis (LVA), lymphatico-lymphatic anastomosis and vascularised lymph node transfer (VLNT). These interventions can be combined with simultaneous breast reconstruction and liposuction (Babak J. Mehrara *et al.*, 2021). VLNT surgery has yielded positive results (Schaverien *et al.*, 2018).

A definitive cure for lymphedema is has yet to be found, although surgical interventions have shown promising results (Schaverien *et al.*, 2019). This dissertation aimed to elucidate the possible benefits and complications of VLNT surgery and identify the patients who gain most from the VLNT procedure. In

addition, the effect of investigational VEGF-C growth factor treatment in conjunction with VLNT surgery was under investigated. The results of VLNT surgery are often analyzed using by objective measurements, as should be, but in addition, the aim was also to investigate the subjective results of the procedure on patients in everyday life.

2 Review of the Literature

2.1 The Lymphatic System

The lymphatic system consists of lymph nodes and connecting lymph vessels (Suami *et al.*, 2018). Lymphoid tissue in an embryo begins to develop during the first trimester of pregnancy. The embryonic veins protrude and form a lymph sac, and the mesenchymal tissue in the sac induces an interaction between lymphoid tissue-inducer cells and mesenchymal organizer cells. (Blum *et al.*, 2006) Eventually, the lymph sacs form lymph nodes and vessels from lymphatic endothelial cells (LECs) (Blum *et al.*, 2006). Since the lymphatic system develops in various directions, it is a complex network throughout the body. The lymphatic network is also highly variable. (Hsu *et al.*, 2016)

The lymphatic system continues to grow and develop after birth and fully develops in childhood (Blum *et al.*, 2006). After puberty, the size of the lymph nodes starts to decrease (Oliver *et al.*, 2002). Lymph nodes gradually degenerate due to aging. In the elderly, the lymph nodes are small and consist of only a small amount of active lymphoid tissue. (Alitalo *et al.*, 2005) The lymph vessels are blind-ended capillaries (diameter 50–200 μm) when they start in tissues and progress into collecting vessels consisting of smooth muscle cell layering and valves (Ambler *et al.*, 2022). See figure 1.

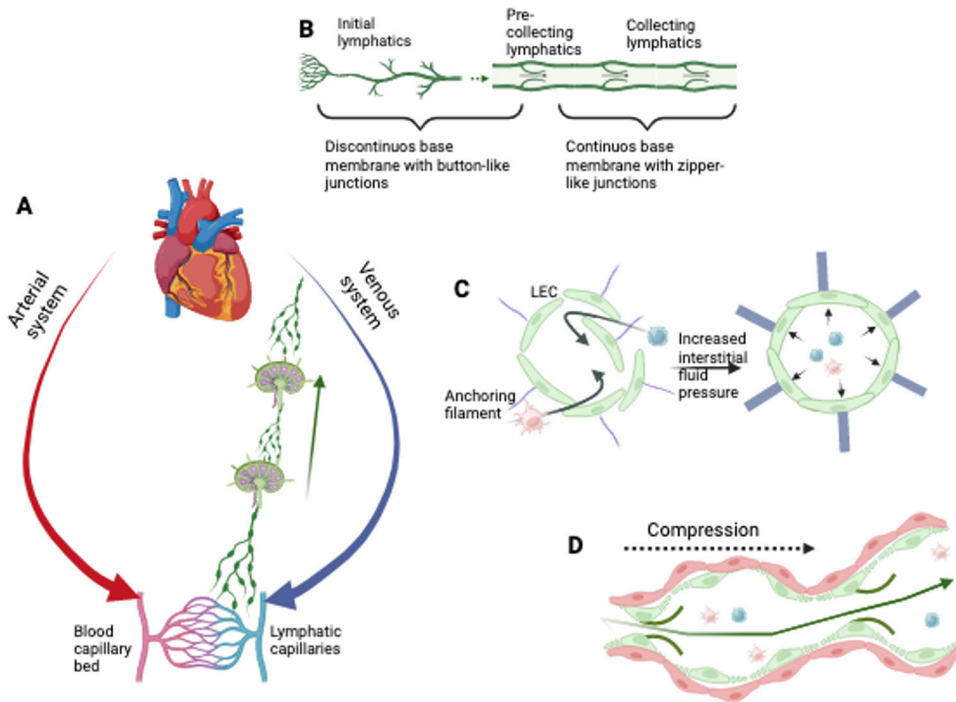


Figure 1. An illustration depicting the intricate anatomy of the lymphatic vessels. **A:** The lymphatic vessels form an integral part of the circulatory system. Excess fluid permeates into the lymphatic capillaries and proceeds through initial lymphatic vessels, gradually advancing into larger collecting lymph vessels. Subsequently, the lymph fluid traverses the thoracic duct to join the venous system. **B:** Initial lymphatics feature button-like junctions, facilitating the entry of interstitial fluid, macromolecules, and immune cells into the lymphatic vessels. **C:** LECs possess anchoring filaments that serve dual purposes: orienting the LECs to facilitate the entry of interstitial components into the lymphatic vessels and stabilizing the composition of lymphatic vessels to prevent leaks by binding to the surrounding extracellular matrix. **D:** Coordinated contractions of smooth muscle cells within the lymphatic vessel wall, combined with the presence of intraluminal valves, culminate in the unidirectional transport of lymph fluid. Modified from Angeli et al. 2023. Created by author in Biorender.com.

2.1.1 Immune Response

The lymphatic system is an essential component of the immune response in the body. Lymph vessels function as messengers of leukocytes and antigens to secondary draining lymphoid organs, such as lymph nodes. (Ambler *et al.*, 2022; Angeli *et al.*, 2023) Primary and secondary adaptive immune responses occur in lymph nodes. The primary immune response occurs when the body encounters an antigen for the first time, and a secondary adaptive immune response occurs after the initial exposure when the same antigen is presented at a later time for the second or subsequent time. (Alitalo, 2011) The lymphatic system is responsible for transporting and presenting

antigens to lymph nodes and naïve T-cells. Naïve T-cells further develop into either CD4 or CD8 positive killer lymphocyte (CD8) T-cells. (Alitalo, 2011) The blind-ended lymphatic capillaries collect substances that can be presented as antigens, which are then transported to the lymph node by collecting lymph vessels. Lymph nodes are highly compartmentalized and have several specialized regions. Both CD4 and CD8 T-cells are found in the lymph nodes. (Angeli *et al.*, 2023) Macrophages and dendritic cells (DCs) regulate LEC permeability by secreting cytokines. Th2 type cytokine release causes a fibrotic and inflammatory response and prevents lymphangiogenesis. (Angeli *et al.*, 2023) TNF- α is a cytokine that induces the formation of another pro-lymphangiogenic factor, VEGF-C. An inflammatory response is required for the initiation of proper wound healing and lymphangiogenesis after surgery. However, chronic inflammation is associated with profibrotic cytokines such as TGF- β 1 and IL-10. Scar and fibrosis are known factors in lymphedema; hence, the type of cytokine response after trauma or iatrogenic damage to the lymphatic system is pivotal to the development of lymphedema. (Shi *et al.*, 2013)

2.1.2 Transport of Fluids and Macromolecules

The lymphatic system absorbs proteins and fluids from blood vessels and transports them back into the blood circulation (Alitalo, 2011). The lymphatic capillaries are lined with discontinuous basement membranes forming button-like junctions, which act as one-way valves with 2 μ m size pores (Oliver *et al.*, 2020). The lymphatic system is responsible for transporting 8–12 litres of fluid per day (Scallan *et al.*, 2016). Understandably, if this process is faulty, these proteins and fluids accumulate in the periphery of the limbs and cause lymphedema, cellulitis infections and pain. (Alitalo, 2011) Collecting lymph vessels are lined with smooth muscle cells and tight zipper-like endothelial cells with decreased permeability (Oliver *et al.*, 2020).

2.1.3 Transport of Lipids

Absorption of dietary fat is also a function of the lymphatic system (Alitalo, 2011). If dietary fats are not absorbed, they are wasted, and the nutrition of the patient is at risk. (Oliver *et al.*, 2020) Fatty acids and monoglycerides are wrapped in proteins, and cholesterol, and absorbed as chylomicrons (200-1000nm in diameter) into the lymphatic capillaries through button-like junctions (Thorup *et al.*, 2023). Chylomicrons are then transported from the intestinal mucosa via the lymphatic system to the blood stream and form chyle (Thorup *et al.*, 2023). In addition, the lymphatics in the periphery are involved in the collection of cholesterol. It has been shown, that impaired lymphatic flow leads to accumulation of cholesterol in the

affected area, resulting in adipose hypertrophy accompanied with fibrotic tissue. This causes irreversible swelling and hardening of the affected areas. (Azhar *et al.*, 2020)

2.2 Anatomy of the Lymphatic System

The lymphatic system is a complex network of small lymph vessels and numerous lymph nodes located at various sites (Hsu *et al.*, 2016). The lymphatic system also involves the spleen, thymus, bone marrow, and the digestive system. Lymph node clusters are commonly found in the neck, axillary, inguinal, and abdominal areas. (Hsu *et al.*, 2016) Lymphatics can be divided into three categories; soft tissue lymphatics, intestinal, and liver lymphatics. The intestinal and liver lymphatics are responsible for most of the lymph fluid, with the soft tissue lymphatics in the limbs and skin generating only approximately 20% of the lymph fluid in the body (Hsu *et al.*, 2016). The axillary and inguinal lymph nodes are responsible for the lymphatic function of the limb (Hsu *et al.*, 2016). The lymph capillaries are small and blind-ended. They lack pericytes and continuous basement membrane and are connected to the extracellular matrix. (Alitalo, 2011)

2.2.1 Upper Limb and Axillary Area

The lymphatic network of the upper limb can be divided into different pathways as well as superficial and deep networks. There are three pathways in the forearm; medial, radial, and ulnar. The proximal part of the arm is divided into two pathways, medial and lateral. The medial pathway of the forearm drains the volar part of the arm. The radial and ulnar pathways drain the dorsal arm on their respective sides. (Suami *et al.*, 2018; Ma *et al.*, 2019; Granoff *et al.*, 2022) The proximal medial pathway follows the basilic vein and the lateral pathway follows the cephalic vein. The medial forearm pathway always connects to the medial proximal pathway. The radial and ulnar forearm pathways can be connected to lateral or medial proximal pathways. (Granoff *et al.*, 2022)

The axilla has the regional lymph nodes of the upper limb. Some of these are sentinel nodes, and the number of lymph nodes varies by patient. (Ma *et al.*, 2019) Sentinel nodes are the main nodes draining from the region (Ma *et al.*, 2019).

2.2.2 Abdominal, Inguinal Area and Lower Limb

The superficial lymphatic collectors are located immediately below the subdermal venules above the fascia of Scarpa (Tourani *et al.*, 2013). The abdominal wall area is divided into the upper and lower area. The lower part of the abdominal wall drains

into the superolateral inguinal lymph nodes (Tourani *et al.*, 2013). The deep lymphatic system drains into the iliac lymph nodes (Tourani *et al.*, 2013).

The lymphatic system in the lower limb is also divided into deep and superficial layers. The superficial lymphatic system follows the great saphenous vein and the small saphenous vein. (Hsu *et al.*, 2016) The lymph from below the knee drains into the deep popliteal lymph nodes; however, the lymph fluid from the anterior surface of the lower limb drains into the superficial inguinal lymph nodes (Hsu *et al.*, 2016). In the thigh area, there are three distinct lymphatic pathways; one superficial medial pathway, one deep pathway following the superficial femoral vessels and another deep lymphatic pathway between the sciatic nerve and the profunda femoris vessels (Hsu *et al.*, 2016). It has been demonstrated that there are a few critical sentinel lymph nodes in the inguinal area that are responsible for the majority of lymph fluid transport from the lower limb (van der Ploeg *et al.*, 2009; Cesmebasi *et al.*, 2015). These critical lymph nodes are usually located in the superficial inguinal area, often medial to femoral vessels (van der Ploeg *et al.*, 2009).

2.3 Lymphangiogenesis

The regeneration of lymph vessels, also known as lymphangiogenesis, is regulated by multiple growth factors and cytokines (Tammela *et al.*, 2010). Lymph vessels have a tremendous ability to regenerate after an injury (A Yan *et al.*, 2011). The use of growth factors such as VEGF-C, to induce lymphangiogenesis has been studied as a treatment for lymphedema (Szuba *et al.*, 2002; Sommer *et al.*, 2012; Visuri *et al.*, 2015). Another possibility might be blocking of lymphangiogenesis-inhibiting signals. It has been speculated without evidence that growth factors that induce lymphangiogenesis could potentially contribute to tumor growth or metastasis. Blocking the lymphangiogenesis-inhibiting signals would bypass the speculated idea of inducing tumor growth. (Viitanen *et al.*, 2015; Rauniyar *et al.*, 2018) Previous studies (J C Zampell, *et al.*, 2012; Avraham *et al.*, 2013) have shown that normal VEGF-C and other lymphangiogenic cytokines can be observed in patients with impaired lymphatic function. Therefore, lymphangiogenesis is a multifactorial process that requires both lymphangiogenic growth factors and a lack of inhibitory factors (Weitman *et al.*, 2013).

2.4 Lymphedema

Lymphedema is the accumulation of excess tissue fluid into the affected limb due to the disruption of lymph fluid flow (Rockson, 2021). Lymphedema can be hereditary or acquired; and either primary or secondary (Rockson, 2021). Lymphedema starts with the accumulation of fluid and pitting edema, but if not treated accordingly,

excess fibroadipose tissue is formed resulting in permanent non-pitting edema and an increase in the width of the affected limb (Rockson, 2021). Lymphedema increases the risk of cellulitis infections (Cheng *et al.*, 2022). See figure 2.

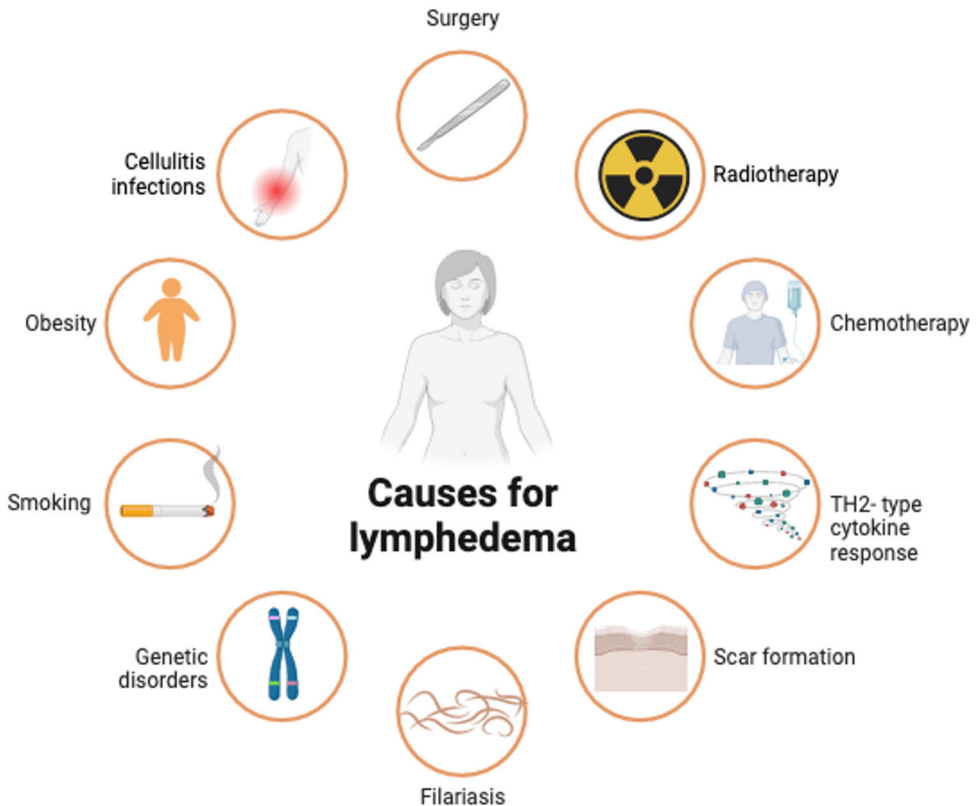


Figure 2. General causes for lymphedema. Created by author in Biorender.com.

2.4.1 Primary Lymphedema

Primary lymphedema is a rare genetic condition that includes either dysfunction of the lymphatic vessels or non-existing lymphatic vessels. In either case, the disruption in lymph flow is caused by a predisposed cause. (Brouillard *et al.*, 2021) It is a rare condition (Brouillard *et al.*, 2021). Primary lymphedema is more difficult to treat because the lymphatic system is not constructed normally, and it is usually systemic rather than local, compared to as in secondary lymphedema. (Brouillard *et al.*, 2021) Several gene mutations cause primary lymphedema. In Milroy's disease (congenital hereditary lymphedema) the impairment is linked to mutations in vascular endothelial growth factor receptor 3 (VEGFR-3). (Brouillard *et al.*, 2021)

2.4.2 Secondary Lymphedema

Secondary lymphedema is caused by a factor, disease, or iatrogenic damage to a previously normal lymphatic system (Bernas *et al.*, 2022). Iatrogenic damage is a frequent cause of lymphedema in industrialized countries. This is caused by iatrogenic damage and subsequent scar and fibrosis formation that disrupts normal lymph fluid flow leading to and result in lymphedema (Bernas *et al.*, 2022). The most common iatrogenic damage is breast cancer-related surgery, involving either axillary lymph node biopsy or evacuation (Grada *et al.*, 2017). Recent studies have demonstrated that the reduction of axillary surgery does not compromise the effectiveness of breast cancer treatment when combined with neoadjuvant and adjuvant therapies (Gentilini *et al.*, 2023; Schwartz T., 2024). Consequently, the approach to axillary surgery in breast cancer treatment has evolved significantly, de-escalating from complete lymph node dissection to sentinel lymph node biopsy, and ultimately, in some cases, to the omission of axillary surgery altogether (Montagna *et al.*, 2023). In 2022, the incidence of new breast cancer cases in Finland was 173.34 per 100,000 individuals (0.17%) as reported by the Finnish Cancer Registry. Globally, the age-standardized incidence rate of breast cancer in 2022 was 186.5 per 100,000 individuals (0.19%), according to the Global Cancer Observatory (Globocan). Non-iatrogenic causes of secondary lymphedema include infections, such as filariasis, an infection by parasitic worms (Drews *et al.*, 2021). Because the lymphatic system has been normal prior to secondary damage, treatment possibilities are more abundant than in primary lymphedema with non-existing lymphatics (Lee *et al.*, 2017; Schaverien *et al.*, 2019).

2.4.2.1 Filariasis

Filariasis is globally the most common cause of secondary lymphedema (Drews *et al.*, 2021). Filariasis is a neglected parasitic tropical disease caused by parasitic microscopic, thread-like worms that destroy the lymphatic vessels of the affected area. Infection is transmitted from person to person via mosquitoes. (Drews *et al.*, 2021) The incidence of lymphatic filariasis has decreased due to vigorous mass drug administration in endemic areas; however, 51 million people were affected in 2018. Filariasis is the most common cause of permanent disabilities worldwide. It is still endemic to some parts of Africa and Southeast Asia. (Drews *et al.*, 2021) There are few medical treatments for filariasis, and the aim is to destroy the parasitic worms in the lymphatic system. However, the development of new medical treatments is underway, and hopefully these will not have as many frequent side effects as previously used drugs (ivermectin, diethylcarbamazine). (Boniface *et al.*, 2019)

2.4.2.2 Breast Cancer-Related Lymphedema (BCRL)

Breast cancer treatment, including iatrogenic damage due to surgery and other treatments such as radiotherapy and neoadjuvant treatment, can result in secondary lymphedema (Cheng *et al.*, 2022). In many cases, different treatment modalities are used in combination thus increasing the risk of BCRL (Cheng *et al.*, 2022). Damage to the lymphatic system disrupts normal lymph flow in the affected limb (Oliver *et al.*, 2020). Breast cancer treatment often entails either axillary lymph node biopsy or dissection, which may remove the draining lymph nodes from the axillary area, resulting in failure of the lymph flow pump of the affected limb. Breast cancer treatment also causes scarring and fibrosis due to an inflammatory response that can disrupt the lymph flow. (Cheng *et al.*, 2022) It is not fully known why some patients does develop an anti-lymphangiogenic inflammatory response after breast cancer treatment and why some do not (Peranteau *et al.*, 2008). In some cases, scar removal might be sufficient to restore lymph flow in the affected limb without any other treatment (Warren *et al.*, 2007; Maruccia *et al.*, 2019).

2.4.3 Pathophysiology of Lymphedema

2.4.3.1 Vascular Endothelial Growth Factors (VEGFs)

Many VEGFs have a part in the development and regeneration of lymph vessels, also known as lymphangiogenesis (Liao *et al.*, 2015). However, VEGF-C has shown the highest potential as an inducer of lymphangiogenesis (Hartiala *et al.*, 2010; Rauniyar *et al.*, 2018). This has been studied in various animal models (Szuba *et al.*, 2002; Visuri *et al.*, 2015). VEGF-C induces the growth of capillary lymph vessels, which stabilize into true collecting lymph vessels via an intrinsic differentiation and maturation program (Rauniyar *et al.*, 2018). VEGF-C moreover is a pro-lymphangiogenic cytokine that inhibits fibrosis and scarring (Brown *et al.*, 2023). VEGF-C can be administered locally or systemically via injection or as an adjunct to engineered biomaterials (Weitman *et al.*, 2013). In vivo gene delivery by adeno-associated virus transmission has been evaluated as the most efficient method for recombinant VEGF-C-expression (Frueh *et al.*, 2016; Suami *et al.*, 2016; Cornelissen *et al.*, 2017; Rauniyar *et al.*, 2018).

2.4.3.2 The Role of Inflammation

The inflammatory process has been proven to play a significant role in the development of lymphedema and regeneration of lymphatic vessels (Ghanta *et al.*, 2015). Growth factors and cytokines related to chronic inflammation and fibrosis,

such as TGF- β 1 and IL-10, are being vigorously researched (Shi *et al.*, 2013). TGF- β 1 is a potent anti-lymphangiogenic agent that induces a decrease in LEC proliferation and migration, impairs formation of lymphatic tubules, and downregulates lymphatic-specific gene expression (Rauniyar *et al.*, 2018; Baik *et al.*, 2022). TGF- β 1 inhibition may promote lymphangiogenesis and serve as an alternative for clinical applications (Weitman *et al.*, 2013; Rauniyar *et al.*, 2018). Furthermore, TGF- β 1 links lymphangiogenesis with inflammatory pathways by regulating tissue fibrosis and scarring during the later stages of wound healing (Baik *et al.*, 2022). IL-10 is an anti-inflammatory cytokine that protects against TGF- β 1 induced fibrosis (Baik *et al.*, 2022). Studies have shown a beneficial increase in IL-10 levels after VLNT (Viitanen *et al.*, 2015). A fibrotic Th2 inflammatory cytokine response is thought to be involved in the development of lymphedema (Azhar *et al.*, 2020). The Th2 cytokine response is induced by CD4 helper cells and macrophages (J C Zampell, *et al.*, 2012). Macrophages play an anti-fibrotic role in lymphedema and regulate Th2 differentiation, either directly or indirectly (Avraham *et al.*, 2013). Disruption of lymphatic flow and accumulation of interstitial fluids, proteins, and cholesterol have been shown to result in an inflammatory response. This Th2 type inflammatory response causes adipose hypertrophy and fibrosis (Azhar *et al.*, 2020; Angeli *et al.*, 2023; Brown *et al.*, 2023). Hence, it is logical that regular and continuous use of compression garments treats lymphedema and hinders the progression of lymphedema into the later stages with accumulation of fibrotic adipose and connective tissue by minimizing excess fluid accumulation in the early stages. Mouse models have shown that IL-4 or IL-13 can neutralize antibody inhibition of Th2 differentiation to treat or prevent lymphedema (Ghanta *et al.*, 2015). TNF- α and VEGF-C have shown pro-lymphangiogenic properties. TNF- α stimulates the production of VEGF-C by fibroblasts. (Visuri *et al.*, 2015) Furthermore, lymphedema has been associated with a gene expression profile similar to that of inflammation in a mouse model (Aschen *et al.*, 2012; Jamie C. Zampell *et al.*, 2012).

2.4.4 Clinical Staging of Lymphedema

Lymphedema can be categorized as mild, moderate, or severe. Various evaluation scales have been developed. The most frequently used method is the International Society of Lymphology (ISL)-scale. (Lymphology, 2003) Stages illustrated in figure 3.

International Society of Lymphology (ISL) staging

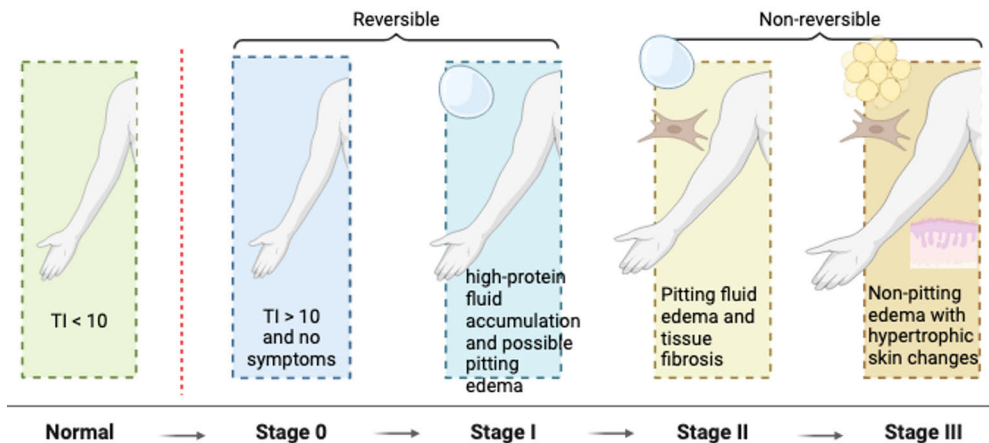


Figure 3. The ISL stages of lymphedema. Created by author in Biorender.com. (TI = Transport index)

Other evaluation scales used in secondary lymphedema are the International Lymphedema Framework (ILF)-scale and the Dreyer scale (MacGregor et al, 2006; Dreyer G *et al.*, 2002). The ILF-scale is similar to the ISL-scale but is split into five stages and has divided the stage 2 into 2A and 2B stages. In clinical practice, the staging is adequate in three stages; mild, moderate and severe and therefore the ISL-scale is sufficient when the latent and subclinical stage 0 is included (Douglass et al. 2019). The Dreyer scale is designed for evaluating lymphedema associated with filariasis, and therefore not so used in the developed countries (Dreyer G *et al.*, 2002).

2.4.4.1 International Society of Lymphology (ISL) Scale

Stage 0: Latent or subclinical condition where swelling is not evident despite impaired lymph transport.

Stage I: Early accumulation of fluid relatively high in protein content (e.g. in comparison with “venous” edema) that subsides with limb elevation. Pitting may occur.

Stage II: Pitting may or may not occur as tissue fibrosis develops. Limb elevation alone rarely reduces tissue swelling.

Stage III: Lymphostatic elephantiasis where pitting is absent. Hypertrophic skin changes, such as acanthosis, fat deposits, and warty overgrowths, often develop.

2.4.5 Diagnostics of Lymphedema

Lymphedema has few definitions, but it is important to recognize the difference between simple excess adipose tissue accumulation with normal lymphatics compared to lymphedema with excess fluid and fibroadipose tissue after a dysfunctional lymphatic system (Babak J. Mehrara *et al.*, 2021). The lymphedema stage greatly determines the treatment method (Babak J. Mehrara *et al.*, 2021). Hence, it is very important to have proper diagnosis and staging of lymphedema. In Finland, lymphedema is usually diagnosed and staged by volumetry, tissue dielectric constant (TDC) measurements, and lymphoscintigraphy. Other countries also use imaging, such as indocyanine green lymphangiography (ICG-L) and magnetic resonance imaging (MRI). Other emerging imaging modalities are also used worldwide, such as, three-dimensional (3D) imaging, photoacoustic lymphangiography (PAL) and high-frequency ultrasound (HFUS).

2.4.5.1 Clinical Diagnosis

Lymphedema is typically diagnosed by measuring the volume of the affected limb (Babak J. Mehrara *et al.*, 2021). Volumetry is considered the gold standard, where the limb width is measured at standard intervals, and the volume of the limb is derived with a truncated cone model provided by Brorson and Höijer (Brorson *et al.*, 2012). The unaffected limb is used as a control and a 2 cm difference in limb width or over 200 ml volume difference between the contralateral limbs is diagnostic for lymphedema (Brorson *et al.*, 2012). The volume of the limbs can also be measured using water displacement method (Karlsson *et al.*, 2021). In this method, the limb is submerged into a container filled with a known amount of water, and the amount of water that spills over from the container into another surrounding container is weighed (Karlsson *et al.*, 2021). The accuracy of this method can be improved by adding ethanol to the water solution (Hughes *et al.*, 2008). The displaced amount of water can also be measured by keeping the container on an electronic scale and dividing the change in weight of the container by the density of the water or solution (Hughes *et al.*, 2008). This method is not popular in Finland but is used in some countries.

2.4.5.2 Indocyanine Green Lymphangiography (ICG-L)

ICG-L is a quite new method of evaluating lymphatic flow during surgery or follow-up after surgery (Jørgensen *et al.*, 2021). First, ICG is injected subcutaneously and intradermally at the first and third web spaces, the area between digits or phalanges. Immediately after injection, the first scan is performed. The second and third scans are performed at 10 and 60 minutes, respectively. (Liu *et al.*, 2022) The third scan

provides lymphedema stage determined by the Arm Dermal Backflow (ADB) scale and MD Anderson classification (MDA). (Liu *et al.*, 2022) ICG-L is a fast and reliable method for visualizing lymphatic flow or dysfunction in real-time (Jørgensen *et al.*, 2021).

2.4.5.3 Lymphoscintigraphy

Lymphoscintigraphy, also known as isotopic lymphangiography, is a standard method for staging lymphedema and is included in the ISL scale (Lymphology, 2003). It utilizes a technetium-labelled sulfur nanocolloid (99mTc-Nanocoll, GE Healthcare) to visualize the lymphatic vessels and track the lymphatic flow (Villa *et al.*, 2019). First, an intradermal injection of the isotope (0.1–0.2 ml) is administered at the webspaces of either the upper or lower limb. After five minutes, the first imaging with an Infinia Hawkeye single photon emission computer tomography with computed tomography (SPECT/CT) (General Electric Medical Systems, Milwaukee, WI, USA) is performed followed by further imaging at 15, 30, 45, 60, and 120 minutes. Images are then assessed by nuclear medicine physicians, resulting in a semiquantitative transport index (TI). (Villa *et al.*, 2019) The contralateral limb is used as a control. TI is formulated by assessing the following criteria; lymphatic transport kinetics, radiopharmaceutical distribution pattern, time in which lymph nodes appear, and visualization of lymph nodes and lymph vessels (Kleinhans *et al.*, 1985). TI is considered pathological when it is >10 (Kleinhans *et al.*, 1985).

2.4.5.4 Tissue Dielectric Constant (TDC)

The stage of lymphedema can also be analyzed by TDC (Karlsson *et al.*, 2021), measured using the MoistureMeterD Compact device (Delfin Technologies, Kuopio Finland). It measures the tissue water content from the skin surface down to a depth of 2.5 mm using a microwave field (Karlsson *et al.*, 2021) and converts it into a water percentage (0–100%). TDC measurement is an especially good method for assessing the fluid component of lymphedema; therefore, it might provide more accurate diagnostics at the early stages of lymphedema (Karlsson *et al.*, 2021). The TDC measurements are taken from standard locations, similar to volumetry. The contralateral limb of the patient is used as a control.

2.4.5.5 Magnetic Resonance Imaging (MRI)

To achieve proper patient selection and perform surgery with good precision with minimal complications in a reasonable time period, adequate imaging of the lymphatics and accurate staging of lymphedema are required. Therefore, MRI is a

good option. MRI provides high-resolution images of the lymphatic anatomy and surrounding soft tissues, with the possibility of highlighting edema, fat, or contrast agents without any ionizing radiation. (Salehi *et al.*, 2023) MRI can be used preoperatively for staging lymphedema, patient selection, and planning of the surgery, and postoperatively to assess the results of the surgery and to re-evaluate the stage of lymphedema (Kim *et al.*, 2020; Cellina *et al.*, 2021). MRI can be performed with or without the injection of a contrast agent (Arrive *et al.*, 2017).

2.4.5.6 Other Imaging Techniques

3D imaging is an alternative method for evaluating the lymphedema stage and postoperative results (Antonio J. Forte *et al.*, 2021). 3D imaging provides the volume of the limb with the assistance of a software (Landau *et al.*, 2018). The results appear to be similar to those of the volumetry or water displacement method (Landau *et al.*, 2018; Antonio J. Forte *et al.*, 2021). Another novel method for imaging lymph vessels is PAL (Suzuki *et al.*, 2022). In the PAL method, near-infrared lasers repeatedly irradiate the desired area and cause the tissues to transmit specific photoacoustic waves back to the ultrasound transducer, resulting in an image (Suzuki *et al.*, 2022). It provides high-resolution (0.2mm) images of the lymphatic vessels in 3D and the detailed structure of the dermal backflow (Suzuki *et al.*, 2022). One emerging imaging technique in lymphatic surgery planning is HFUS (Hayashi *et al.*, 2019, 2022; Bianchi *et al.*, 2020). Conventional high-frequency ultrasound (CHFUS) and ultra-high frequency ultrasound (UHFUS) are available options. UHFUS is more accurate in assessing small lymphatics and hence, is more promising in assessing the lymphatics. One of the several advantages of UHFUS is that ultrasound does not expose the patient to any potentially harmful radiation. UHFUS provides high-resolution images. (Hayashi *et al.*, 2019)

2.4.6 Quality of Life Among BCRL Patients

The quality of life among patients with BCRL is known to be lower than that in the non-affected population (Fish *et al.*, 2020). However, the quality of life of these patients seems to improve after VLNT or other surgical approaches, even when the lymphedema symptoms remain (Fish *et al.*, 2020). BCRL affects body image and causes discomfort, pain and difficulty in finding suitable clothes. BCRL can also cause disability at work and, therefore, significantly decrease the income of BCRL patients. (Penha *et al.*, 2016)

2.5 Conservative Treatment of Lymphedema

Conservative treatment is still the corner stone for patients with BCRL (Babak J. Mehrara *et al.*, 2021), although surgical interventions have extensively expanded the treatment possibilities. Conservative treatment aims to reduce fluid accumulation in patients with BCRL and slow down the lymphedema progression (Martín *et al.*, 2011). It is possible to stop the progression when conservative treatment starts preemptively or at the early stages of lymphedema (ISL stages 0–I), but in the later stages (ISL stages II–III), the excess fibrotic and adipose tissue cannot be removed without liposuction or surgery (Babak J. Mehrara *et al.*, 2021). Conservative treatment includes manual lymphatic drainage with physiotherapeutic manoeuvres and/or compression garment therapy as well pneumatic intermittent pump treatment. It is also important to manage other risk factors for lymphedema, such as obesity, smoking, and recurrent cellulitis infections to prevent lymphedema or its progression (Babak J. Mehrara *et al.*, 2021).

2.5.1 Manual Lymphatic Drainage

The aim of manual lymphatic drainage is to manually drain the accumulated fluid component of lymphedema from the affected limb (Ezzo *et al.*, 2015). It can be achieved using a specific massage technique by trained physiotherapists at regular intervals (Ezzo *et al.*, 2015). However, it should be taken into consideration that this method does not provide long-lasting results and alleviates the symptoms of lymphedema only temporarily. Also, it is time-consuming and not feasible for all patients to use. (Ezzo *et al.*, 2015)

2.5.1.1 Compression Therapy

Regular compression therapy using compression garments is the baseline treatment for lymphedema. Compression garments are custom-made with exact individual measurements for each patient, and should be used as much as possible. The compression garments must be tight enough to offer adequate support and compression. (Brorson *et al.*, 2012) Compression garments are usually a compression sleeve with a glove when needed for the arms and compression stockings up to the knee or inguinal area for the lower limb. Compression garments are used for up to 24 hours per day and are only removed during a shower. To be effective, compression garment use requires constant compression in the affected area. Thus, compression garments should be renewed regularly, approximately every three to six months, or four to six times a year. Intermittent compression garment use does not adequately treat lymphedema. (Damstra *et al.*, 2009)

2.5.1.1.1 Intermittent Pneumatic Compression Treatment

One way to enhance the compression treatment is intermittent pneumatic compression treatment (Franks *et al.*, 2015). This treatment uses a mechanical pump to enhance the drainage of the accumulated excess fluid from the affected limb. The pumping manoeuvre starts from the distal end of the limb and gradually pumps excess fluid to the proximal site of the limb until the trunk area, where it is secreted out of the body. The pump simulates the pumping action of manual lymphatic drainage by physiotherapists. (Franks *et al.*, 2015) However, it has been shown to decrease the limb volume of the affected arm more and with better long-term results than manual lymphatic drainage (Franks *et al.*, 2015). Similarly to manual lymphatic drainage, intermittent pneumatic compression treatment reduces the excess fluid component of lymphedema during the treatment but does not prevent the fluid from accumulating again afterward. Therefore, the effect of the treatment is temporary and only sufficient if used on a regular basis. Intermittent pneumatic compression treatment requires the patient to stay immobile for the duration of the treatment making it infeasible to continue this treatment regularly for years without disrupting the patient's normal life. (Franks *et al.* 2015)

2.6 Surgical Treatment of Lymphedema

Surgical methods aim to cure lymphedema, since conservative methods have not been able to provide this (Chang *et al.*, 2021). However, the results vary between studies. The necessary surgical procedures to alleviate lymphedema differ, because for each patient the stage of lymphedema and the underlying cause of lymphedema are a unique combination. In addition, surgery has its limits and boundaries, and those should be respected to prevent it from causing more harm than gain to the patient. For example, scarring is a known risk factor for lymphedema, and surgery should be performed only if it is probable that the patient has less scar tissue postoperatively than preoperatively (Warren *et al.*, 2007; Peranteau *et al.*, 2008; Shi *et al.*, 2013). Previous studies have shown that scar removal alone can be used to treat lymphedema. Scar removal normalizes lymph flow by removing the mechanical pressure from the lymph vessels (Maruccia *et al.*, 2019). Additionally, scar removal and surgical treatment of lymphedema may induce a beneficial inflammatory response that promotes lymphangiogenesis (Viitanen *et al.*, 2015). In stage I and II lymphedema, reconstructive and possibly curative surgical methods, such as LVA and/or VLNT, are used. However, in the irreversible stage III lymphedema liposuction (or rarely, reductive surgery) is also needed because the excess adipose and fibrous tissue cannot be removed with LVA or VLNT alone (Chang *et al.*, 2021; Leppäpuska *et al.*, 2019). Furthermore, medical treatments are under investigation

to determine whether they enhance the results of reconstructive methods like LVA and VLNT (Weitman *et al.*, 2013; Rauniyar *et al.*, 2018).

2.6.1 Reductive Surgery

Fibrotic adipose tissue at the later stages of lymphedema (ISL stages II–III) cannot be removed without either reductive surgery or liposuction (Hoffner *et al.*, 2018). Reductive surgery involves the excision of excess skin and soft tissues, including lymphedematous fibrous and adipose tissue. It is a very invasive method that causes extensive visible scarring and should be considered only in severe cases (Gallagher *et al.*, 2018). One reductive method is the Charles or Homans procedure, where the excess skin and subcutaneous tissue is excised, and full thickness skin grafts are used to cover the defect. This procedure is understandably very invasive, carries a risk of severe complications, and can cause greater discomfort and worse results than the pre-existing lymphedema (Miller TA, 1980). The modified Charles procedure involves negative wound pressure treatment to reduce postoperative complications and enhance graft take (van der Walt JC et al, 2009). However, excess tissue should be addressed during lymphedema treatment, because excess tissue can cause severe difficulties in mobility and significantly worsen the patient quality of life (Fish *et al.*, 2020).

2.6.2 Liposuction

Dry- or wet liposuction is the standard method for removing excess adipose tissue in stage III lymphedema (Schaverien *et al.*, 2018). It is preferred over reductive surgery because it is less invasive and requires only small incisions (Schaverien *et al.*, 2018). Liposuction does not remove excess skin or fibrotic tissue; therefore, it is important to correctly identify the preoperative composition of excess tissue in the affected limb (Lymphology, 2003). Liposuction can also be combined with other surgical techniques, such as VLNT or LVA, but the combination treatment is technically more challenging and requires good planning and an experienced surgeon (Brorson, 2016; Leppäpuska *et al.*, 2019). It is important to avoid damaging the reconstructed LVA or the lymphatic pathways that are supposed to regenerate after VLNT surgery during liposuction (Chang et al, 2021; Leppäpuska *et al.*, 2019).

2.6.3 Lymphaticovenous Anastomosis (LVA)

Lymphedema patients with identifiable lymph vessels and accompanying veins in the affected area may undergo treatment with LVA (Scaglioni *et al.*, 2017). These patients should have been diagnosed with lymphatic dysfunction and dermal

backflow (Scaglioni *et al.*, 2017). LVA can also be combined with VLNT or liposuction (Scaglioni *et al.*, 2017). LVA directs abruptly lymph flow to the adjunct vein and hence reduces dermal backflow and accumulation of lymphatic fluid in the affected area. LVA requires supermicrosurgery because lymph vessels are very small in diameter (50–200 μm). During surgery, lymphatic vessels are identified with ICG-L and/or blue dye. (Damstra *et al.*, 2009) In most studies, LVA reduces the volume of the affected limb (Onoda *et al.*, 2022). The number of anastomoses is not relevant or does not seem to affect the results and should be decided individually for each patient based on the anatomy and impairment of the lymphatic system (Onoda *et al.*, 2022). Upper limb volumetry showed approximately -5 to 50% volume change in the affected limb after LVA (Yang *et al.*, 2020; Onoda *et al.*, 2022). It is crucial to optimize compression garment use before and after LVA to gain the most from the procedure. The use of compression garments and manual lymphatic therapy is difficult to standardize before LVA because the patients might be entering the proper treatment protocol for lymphedema in various stages and with variable instructions. The use of compression garments and manual lymphatic therapy can be supervised and standardized more easily after LVA; therefore, this might enhance the results for LVA, even though the improved results could be due to standardized conservative treatment. (Mihara *et al.*, 2024)

2.6.4 Lymphatico-lymphatic Bypass

Lymph vessels can also be anastomosed to other lymph vessels; however, this is technically challenging because the lymph vessels are small and fragile. Often, lymph vessels are widely damaged in the affected area, and a viable lymph vessel recipient can be difficult to find. (Torrise *et al.*, 2015) This method is not widely used because LVA is preferred. In LVA veins adjacent to the affected area are usually available, not damaged, and function better because they are larger in size (Scaglioni *et al.*, 2017). Another possible treatment option is lymphatic bypass with an autologous lymph vessel graft. (Baumeister *et al.*, 1986) In a method suggested by Baumeister *et al.* (Baumeister *et al.*, 1986), disrupted lymph flow is restored using a lymph vessel graft harvested from a healthy thigh transferred to the affected area. However, this may induce donor-site complications, and is not an option for all patients (Wallmichrath *et al.*, 2023).

2.6.5 Vascularised Lymph Node Transfer (VLNT)

The aim of VLNT is to remove harmful scar tissue from previous surgery and transfer lymph nodes and healthy adipose tissue with beneficial cytokines and

growth factors that promote lymphangiogenesis in the affected area (Becker, 2016; Schaverien *et al.*, 2018).

The most frequently used donor site for the VLNT flap is the groin and lower abdomen area; either the deep inferior epigastric perforator (DIEP), superficial inferior epigastric artery (SIEA), or muscle-sparing transverse rectus abdominis musculocutaneous (ms-TRAM) flap (Becker, 2016). The lower abdomen serves as an ideal donor site for flaps due to several advantages. The surgical scar is easily concealable, providing a cosmetically favourable outcome. Additionally, accessibility to lymph nodes and vasculature is relatively straightforward. Moreover, an ample supply of skin and subcutaneous tissues adjacent to the lymph nodes facilitates the reconstruction of a new breast, if required (Chang *et al.*, 2018; Schaverien *et al.*, 2018). The lymph nodes in the donor site that lie superficially drain lymph from the lower abdomen; however, the deeper and more medial lymph nodes are critical for the lymph flow of the donor lower limb. Hence, the number of harvested lymph nodes should be limited to the superficial lymph nodes, and no surgery should be extended medial to the femoral vessels. (van der Ploeg *et al.*, 2009; Cesmebasi *et al.*, 2015)

Other possible and used donor sites for the lymph node flap are the supraclavicular, submental, lateral thoracic, gastroepiploic, and jejunal mesenteric regions. These are not commonly used in Finland, but are more frequently used in countries with higher number of patients. (Cheng *et al.*, 2012; Ciudad *et al.*, 2016; Coriddi *et al.*, 2017; Ooi *et al.*, 2017; Poccia *et al.*, 2017; Nicoli *et al.*, 2018; Scaglioni *et al.*, 2018; Schaverien *et al.*, 2018)

The supraclavicular lymph node flap consists of lymph nodes harvested from the cervical Vb area (deep cervical lymph nodes below the anterior cricoid arch and posterior to the sternocleidomastoid muscle), and is based on the perforator of the transverse cervical vessels and branches of the external jugular vein. The lymph nodes drain the lung, esophagus, and thyroid with donor site lymphedema still being extremely rare. (Mardonado *et al.*, 2017) The downside to this flap is the small size and small number of harvested lymph nodes when compared to other possible donor sites. In addition, donor site anatomy can vary significantly and includes always critical structures that need to be avoided during surgery, thus increasing the technical difficulty level of surgery. In this approach, scars can easily be hidden. (Mardonado *et al.*, 2017, Cheng *et al.*, 2012; Cheng *et al.*, 2017; Poccia *et al.*, 2017)

Submental flaps have been used since the 1990s in head and neck reconstruction as free or pedicled flaps because they are reliable and versatile. Cheng *et al.* (Cheng *et al.*, 2012) introduced this technique in VLNT surgery. A branch of the facial artery, the submental artery, is the basis of the flap. This flap is not as variable in anatomy as the supraclavicular flap, and the risk of donor site lymphedema is even lower. In addition, it is useful in lower limb lymphedema because the donor site is

distant from the affected area, and there is no risk need of the worsening of lymphedema in the lower limb, as can be the case if the flap is harvested from the groin area. (Cheng *et al.*, 2017) However, careful dissection of the flap is necessary to avoid damage to the marginal mandibular nerve, since it can cause asymmetry in facial movements, particularly when opening the mouth, smiling, or grimacing. (Cheng *et al.*, 2017; Nonomura *et al.*, 2018) In addition, the scar is located on the mandible and is not easily hidden, and the vessel pedicle is short. (Cheng *et al.*, 2017)

The lateral thoracic lymph node flap involves lymph nodes from the lower section of the axilla and is nourished by the lateral thoracic artery. The lateral thoracic artery can be absent as a genetic variant; however, the thoracodorsal artery supplies the lymph nodes. This flap is reliable, and in most cases, perforators to the skin also allow a skin paddle with the lymph node flap. (Tinhofer *et al.*, 2017; Schaverien *et al.*, 2018) The scar in the axilla is easily hidden and the anatomy is quite consistent, making it a good candidate for VLNT surgery. However, axillary surgery carries the risk of donor site lymphedema in the upper limb; therefore, reverse lymphatic mapping is necessary to avoid critical lymph nodes during harvest. (Tinhofer *et al.*, 2017; Schaverien *et al.*, 2018)

2.7 Medical Trials Regarding Lymphedema Therapeutics

Lymphedema development is multifactorial (Alitalo *et al.*, 2005). Although surgical methods have provided beneficial and promising results, the results have varied, with only some patients experiencing significant relief from surgery (Fish *et al.*, 2020). Bioengineering and medical trials have been conducted to improve the results of surgical techniques (Weitman *et al.*, 2013; Rauniyar *et al.*, 2018).

2.7.1 Nanofibrillar Collagen Scaffold

Nguyen *et al.* demonstrated an approach using a mechanical structure that enhances the process of lymphangiogenesis, which can be combined with VLNT, LVA, and/or liposuction (Nguyen *et al.*, 2021). The nanofibrillar collagen scaffold is referred to as BioBridge™. It has been investigated in a clinical trial by Nguyen *et al.* and Deptula *et al.* (Nguyen *et al.*, 2021; Deptula *et al.*, 2022) Deptula *et al.* (Deptula *et al.*, 2022) provided a new triple therapy algorithm for optimizing the treatment protocol of late stage (ISL II–III) lymphedema for each patient treated with BioBridge™. Preoperative lymphatic mapping determines the treatment modality (liposuction, LVA, or VLNT) that the patient receives in combination with BioBridge™ treatment. The BioBridge™ scaffolds are placed percutaneously into the subcutaneous layer. This method has been shown to reduce volume and dermal

backflow, with the gain of new lymphatic collectors. (Deptula *et al.*, 2022; Nguyen *et al.*, 2022) The mechanism behind the promising results of BioBridge™ treatment is under discussion. It has been speculated that the improvement of the lymph flow is due to the scaffold providing a path of lesser resistance for the lymph fluid, not necessarily new lymph vessels (Shuck *et al.*, 2021). BioBridge™ treatment has also been shown to increase cell survival and inhibit inflammation, both of which may improve lymphedema surgery outcomes (Huang *et al.*, 2013; Rochlin *et al.*, 2020).

2.7.2 Growth Factor Therapy

VEGF-C have shown promising results as a growth factor treatment in clinical trials (Hartiala *et al.*, 2020; Leppäpuska *et al.*, 2022). VEGF-C induces lymphangiogenesis and enhances the survival, function, and integration of transplanted lymph nodes into the lymphatic network (Szuba *et al.*, 2002; Visuri *et al.*, 2015). The aim of VEGF-C treatment is to restore the normal anatomy and function of both collecting and capillary lymphatic vessels. This improves lymph flow, ameliorates edema and enhances immune protection in the affected area. (Szuba *et al.*, 2002) VEGF-C is a good candidate for therapeutic agents because it can be delivered as a recombinant protein, as well as by a viral vector, or as a naked plasmid (Weitman *et al.*, 2013). It is practical because it can be administered in multiple ways; directly or released on demand from bioengineered matrices or biodegradable microparticles (Weitman *et al.*, 2013). In vivo and animal model experiments have shown that an adeno-associated virus expressing recombinant VEGF-C is the most efficient delivery method (Visuri *et al.*, 2015). The experimental medicinal product Lymfactin® by Herantis Pharma is a gene therapy that uses an adenovirus type 5 expressing human VEGF-C, that can be used in combination with VLNT (Hartiala *et al.*, 2020; Leppäpuska *et al.*, 2022).

A phase I study by Hartiala et al. (Hartiala *et al.*, 2020) combined Lymfactin® treatment with VLNT to treat BCRL. The results demonstrated that Lymfactin® was safe and well-tolerated with no dose-limiting toxicities. An average reduction of 46% in the excess arm volume after a 12-month follow-up and TI was improved in 7/12 patients (Leppäpuska *et al.*, 2022). Discontinuing the compression garment use for a week preoperatively resulted in statistically significant arm swelling, but after surgery and Lymfactin® treatment, the swelling was less and not statistically significant. Quality of life was significantly improved in these patients during follow-up (Leppäpuska *et al.*, 2022).

2.7.3 Neutralizing Antibody Inhibition of Th2 Differentiation/IL-4 and IL-13

Lymphedema is associated with a fibrotic Th2 type inflammatory response orchestrated by Th2 type CD4 helper T-cells and macrophages (J C Zampell *et al.*, 2012; Avraham *et al.*, 2013). Macrophages play an anti-fibrotic role in lymphedema and directly or indirectly regulate Th2 differentiation (Ghanta *et al.*, 2015). A study by Ghanta *et al.* (Ghanta *et al.*, 2015) provided evidence that lymphedema-associated macrophages are a major source of VEGF-C, and that impaired macrophage response after lymphatic injury results in decreased lymphatic function. Neutralizing antibody inhibition of Th2 differentiation using IL-4 or IL-13, that are cytokines necessary for the differentiation of naïve CD4 positive cells to the Th2 lineage, is effective in both the treatment and prevention of lymphedema in a mouse tail model. Mice treated in this manner showed decreased fibroadipose tissue deposition, decreased inflammation, improved lymphatic collecting vessel pumping capacity, decreased lymphatic leakiness, and overall improved lymphatic function. (J C Zampell *et al.*, 2012; Avraham *et al.*, 2013)

Mehrara *et al.* (Babak J. Mehrara *et al.*, 2021) conducted a phase I, open-label trial using QBX258, an experimental drug consisting of two humanized monoclonal antibodies that inhibit IL-4 (VAK296) and IL-13 (QAX576). Patients with BCRL were treated once a month with intravenous infusion of QBX258 for 4 months. QBX258 treatment was demonstrated to be safe, and most adverse events (AEs) were minor and self-limiting. Treatment with QBX258 improved quality of life and alleviated both skin stiffness and histological changes in the lymphedematous limb. Drug treatment significantly decreased keratinocyte hyperplasia, mast cell infiltration, and the expression of Th2 inducing cytokines in the skin. However, no significant improvements in volumetry or bioimpedance measurements were observed. (Babak J. Mehrara *et al.*, 2021)

2.7.4 Nonsteroidal Anti-inflammatory Drugs in Lymphedema Treatment

In experimental studies the altered histopathology of lymphedema has been reversed in a more favorable fashion with ketoprofen, a nonsteroidal anti-inflammatory drug (NSAID) (Tian *et al.*, 2017). This effect has been indicated to be specifically due to the inhibition of the 5-lipoxygenase metabolite, Leukotriene B₄ (LTB₄). Low LTB₄ concentrations have been demonstrated to promote lymphangiogenesis in both *in vitro* and *in vivo* experimental animal models, whereas lymphatic growth and function are impeded at high concentrations. Interestingly, LTB₄ concentrations are increased in both experimental animal models and human clinical lymphedema patients. It is speculated that during the first few days after surgery and during the

initial wound-healing period, LTB₄ produced at low concentrations has an important function in promoting angiogenesis and lymphangiogenesis. However, with the progression of lymphedema and concurrent increase in LTB₄ concentration, a shift from initial lymphangiogenesis-stimulating to anti-lymphangiogenic effects is observed. These findings suggest that LTB₄ may be a promising drug target for the treatment of acquired lymphedema. (Tian *et al.*, 2017)

Rockson *et al.* (Rockson *et al.*, 2018) reported beneficial effects of ketoprofen in the treatment of patients with lymphedema. The study hypothesis was first tested in an open-label exploratory trial, followed by a placebo-controlled trial. No serious AEs were observed. A reduction in skin thickness, improvement in histopathology and reduction in plasma granulocyte (G-CSF) expression were observed compared to the placebo group. However, limb volumes and bioimpedance results were similar between the groups. (Rockson *et al.*, 2018) While treatment with ketoprofen in this study was reported to be safe with no serious AEs, it is important to note that prolonged NSAID use is associated with cardiovascular, renal, and gastrointestinal risks ([FDA, 2021](#); [U.S. Prescribing information, 2021a](#)).

Another oral medical therapeutic option for lymphedema is acebilustat. It is researched in a clinical phase II study by Rockson *et al.* (ClinicalTrials.gov NCT05203835) (Elborn *et al.*, 2018) Acebilustat has an anti-inflammatory mechanism by inhibiting Leukotriene A₄ (LTA₄) hydrolase and modulating LTB₄ (Elborn *et al.*, 2018).

3 Aims

- I To evaluate the risk of donor site complications of VLNT (study I).
- II To define the preoperative parameters helping patient selection for the best outcome for patients in VLNT surgery.
- III To analyze the postoperative factors influencing lymphedema symptoms after VLNT surgery.
- IV To evaluate the efficacy, safety, and tolerability of growth factor treatment.

4 Patients and Methods

This thesis included four articles with total of 135 patients. All patients were female and had secondary iatrogenic lymphedema. All patients signed a written consent form to participate after reading the patient information newsletter. All studies were approved by the Ethical committee of Turku University Hospital. All included patients were cancer-free preoperatively. Patient characteristics are shown in table 1.

Table 1. Summary of the preoperative patient characteristics in all of the studies I–IV.

	PREOPERATIVE PATIENTS CHARACTERISTICS					
	ARTICLE I		ARTICLE II	ARTICLE III	ARTICLE IV	
	Original method	Modified method			Lymfactivin	Plasebo
Number of patients (n)	13	16	67	18	18	18
Age (years)	55 (±10)	51 (±8)	52 (±8)	52 (±10)	56 (±5)	56 (±10)
BMI (kg/m²)	29 (±3.6)	26.6 (±2.8)	26.5 (±3.3)	28.2 (±3.2)	27.9 (±2.5)	27.4 (±3.9)
Diabetes	1 (7.7)	1 (6.3)	2 (3.0)	1 (5.6)	0 (0.0)	0 (0.0)
Smoking	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Duration of preoperative lymphedema (months)	not available	not available	42 (±30)	37 (±21)	28 (±14)	38 (±13)
Follow-up time (months)	54 (±17)	25 (±6)	70 (±17)	79 (±7)	12	12

Data are presented as number of patients (percentages) or mean (±SD).

Study I included two groups; the original method group (13 patients) and modified method group (16 patients). Study II involved 67 patients. In study I and II 18 patients were the same. Study III had 18 patients and study IV had two groups, the Lymfactivin® group (18 patients after one patient excluded due to flap loss) and the placebo group (18 patients after two patients excluded due to flap loss), in a total of 39 patients. See figure 4.

	General	Breast cancer -related	Lymph node metastasis	Lymphedema status	Other
Inclusion	F/M aged 18 to 70 years	Stage N1-N2a	≤ 9 axillary lymph nodes	≤ 5 years	
	BMI 18-32 kg/m ²	≥ 2 years of breast cancer surgery and/or the end of chemotherapy and/or radiotherapy		The compression garment used in the affected arm	
		No recurrent or active breast cancer or any other malignancy		The presence of pitting edema in the affected arm without compression garment	
				The volume of the affected arm is ≥ 10 % greater than the unaffected arm following 7 days without compression garment	
Exclusion	Current treatment of immunosuppressive drugs	Stage T4 and/or	N2b/N3		Evidence or history of other neoplasm (except basal cell ca or cervical in situ ca)
	History of drug abuse				
	Human immunodeficiency virus - or acquired immunodeficiency syndrome-related illness				
	history of hepatic dysfunction, cirrhosis or hepatitis				

Figure 4. The inclusion and exclusion criteria for study IV. Modified from study IV.

Studies I, II, and III were retrospective studies, and study IV was a prospective, randomized, and multicenter study. The patients’ medical history were obtained from patient data bases. Preoperative and postoperative results were gathered during study and follow-up visits. The following data were collected; age at operation, type of operation, body mass index (BMI), smoking, preoperative duration of lymphedema, pre- and postoperative number of cellulitis episodes, volumetry measurements, limb circumference measurements, TDC measurements, pre- and postoperative use of antibiotics for cellulitis, number of postoperative seroma aspirations, lymphoscintigraphy results, and lymphedema quality of life inventory (LyQoLI) results. See table 2 for the baseline characteristics in study IV.

Table 2. The baseline characteristics in study IV. Modified from study IV.

	Lymfactivin (n=18)	Placebo (n=18)
Age (years)	56±5	56±10
BMI (kg/m²)	27.9±2.5	27.4±3.9
Tumour grade (n (%))		
grade 1	8 (44)	6 (33)
grade 2	9 (50)	8 (44)
grade 3	1 (6)	4 (22)
Number of Metastatic lymph nodes (n (%))		
N0	1 (5.5)	0 (0)
N1	16 (89)	15 (83)
N2a	1 (5.5)	3 (17)
Radiation therapy (n (%))	17 (94)	18 (100)
Chemotherapy (n (%))	18 (100)	18 (100)
Hormonal therapy (n (%))	11 (61)	14 (78)
Trastutsumabi (n (%))	0 (0)	3 (17)
Duration of lymphedema preoperatively (months)	28±14	38±13
LQOLI score (physical)	14±7	13±6
LQOLI score (psychosocial)	25±9	15±7
LQOLI score (practical)	19±8	13±6
LQOLI score (total)	58±22	40±16
Patients with preoperative erysipelas (n (%))	1 (5.5)	3 (17)
Excess volume of the affected arm with compression (%)	16±10	19±10
Excess volume of the affected arm without compression (%)	20±9	21±10
TI-value of the affected arm	30.4±14.0	29.3±16.0
TDC-value of the affected arm with compression (%)	1.34±0.23	1.28±0.17
TDC-value of the affected arm without compression (%)	1.36±0.21	1.31±0.20
VLNT (n (%))	10 (56)	10 (56)
VLNT with BR (n (%))	8 (44)	8 (44)

Values are reported as mean±sd, except for the number of patients marked as (n (%)).

In study I the data were gathered from March 2007 to June 2013, in study II from May 2007 to April 2015, in study III from May 2011 to February 2013, and in study IV from June 2018 to December 2019. In study I the average age at operation was 55 ± 10 years in the original group and 51 ± 8 years in the modified group, in study II 52 ± 8 years, in study III 52 ± 10 years, and in study IV 56 ± 5 years in the Lymfactivin® group and 56 ± 10 years in the placebo group. The average follow-up time in study I was 52 ± 17 months for the original group and 23 ± 6 months for the modified group, in study II 70 ± 17 months, in study III 79 ± 7 months, and for study IV 12 months. See the previous table 1.

4.1 Evaluation of Lymphatic Vessel Function

Evaluation of lymphedema stage and determining the root cause of the disruption of lymph flow are critical for choosing the most appropriate treatment method for lymphedema patients. In addition, it is important to assess which patients do not benefit from lymphatic surgery, to prevent any additional harm to the patients. Therefore, accurate and high-quality imaging of the lymph vessels and lymph flow is crucial. Different measurement options are illustrated in figure 5.

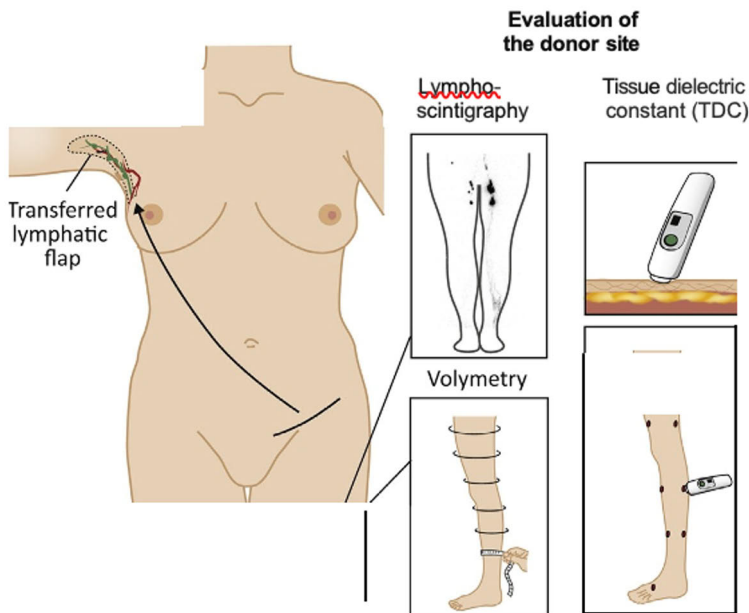


Figure 5. The methods of lymphedema measurement. Modified from Viitanen et al. 2012 and adapted from study I. Author's own modification. Permission to use the figure.

4.1.1 Lymphoscintigraphy (Studies I–IV)

Lymphoscintigraphy was performed pre- and postoperatively in all patients, when feasible. 40 megabecquerel (MBq) of technetium-labeled sulfur nanocolloid (^{99m}Tc -Nanocoll; GE Healthcare Ltd, Italy) in the volume of 0.1–0.2 ml is injected intradermally between digits or phalanges. SPECT-CT was performed using an Infinia Hawkeye (General Electric Medical Systems, Milwaukee, Wis., USA). Images were then analyzed by nuclear medicine specialists, and semiquantitative TI was calculated. TI is derived from the speed of lymphatic transport, distribution

pattern of the images, time at which lymph nodes appear during imaging, and whether the lymph nodes and lymph vessels are visualized during imaging. TI is considered pathological when it is >10 . (Kleinhans *et al.*, 1985)

4.1.2 Circumference Measurements (Studies II and III) and Volumetry (Studies I–IV)

All patients underwent pre- and postoperative circumference measurements in the upper and lower limbs. The measurement sites were standardised. The circumference of the limbs of the patient was measured at 4 cm intervals, from the distal end of the radius to the axilla in the upper limb and from the distal end of the lateral malleolus to the groin in the lower limb. The circumference measurements were inserted into a truncated cone model by Brorson and Höijer (Brorson *et al.*, 2012) to determine the volume of the limb. The truncated cone model is an excel-based calculation formula. All patients did not have all the measurement points required for volume determination by the truncated cone model. These patients had the arm circumference measurements from the same sites analysed during the follow-up. The contralateral limb of the patient was used as a control.

4.1.3 TDC Measurements (Studies I, II, III and IV)

TDC was measured in all patients using the MoistureMeterD Compact device (Delfin Technologies, Kuopio Finland). It measures the tissue water content from the skin surface to a depth of 2.5 mm using a microwave field. It converts the result into a water percentage (0–100%). The water percentage represents the fluid component of lymphedema. The contralateral arm was used as a control.

4.1.4 Cytokine Level Measurements (Study III)

On the first, second, fifth, or sixth postoperative days (PODs), a seroma sample was collected from the drainage pouches draining the operated axilla. The samples were centrifuged and frozen. Enzyme-linked immunosorbent assay (ELISA) analysis was performed as described below, and the concentrations of VEGF-C, IL-10, TNF- α , and TGF- β 1 were determined. The change of cytokine concentration levels during the first six PODs were analysed.

4.1.5 ELISA-analysis (Study III)

The quantitative levels of cytokines (IL-10, TNF- α , TGF- β 1, and VEGF-C) were analyzed by ELISA-analysis. A commercial kit (Human IL-10, TNF- α , TGF-

VEGF-C ELISA KIT; Thermo Fisher Scientific, Bender MedSystems GmbH, Austria) was used. The ELISA analysis included the capture of the antigen by immobilizing the antigen on the surface of polystyrene microplate wells with an antibody conjugate (100 μ l per well). The coating took one hour at room temperature. The microplate wells were then washed and blocked with blocking buffer (300 μ l per well) for one hour at room temperature. Standards and samples were then distributed into microplate wells (100 μ l per well). These wells were then incubated for one to two hours with simultaneous continuous tremor (~500 rpm) at room temperature. A detection antibody was administered to the wells and incubated for two hours at room temperature with mild tremor (~500 rpm). Finally, the wells were incubated with the substrate for 30 minutes or more (until the desired color intensity) at room temperature. The absorbance of the supernatant was measured at 450 nm and 540 nm using a microplate reader (Victor multilabel counter, Wallac, Finland). The result was the subtracted value of the measurement at 540 nm subtracted from the measurement at 450nm. The subtracted value yielded the final result (pg/mL) from the standard curve. The concentration change of the cytokine during the follow-up was the concentration of the cytokine on the 6th POD subtracted from the concentration on the 1st POD.

4.1.6 General Score (Study III)

A general score was generated to assess the benefits of surgery. It entails three components; the change in the TI value, the change in the mean circumference difference, and the change in use of compression garments during the follow-up period. All components were assigned the value between zero to two depending on the results. See table 3 for detailed formulation of the general scores.

Table 3. The description of the general score used in study III. Modified from study III.

The Component Values Used to Generate the General Score of Change for the Subjects			
Points	Transport index change	Circumference diff. change (cm)	The use of compression garments (hours/day)
0	< 0,0	< 0,0	23
1	0,1–10,0	0,1–2,0	> 0–< 23
2	> 10,0	> 2,0	0

Volumetry results were used to confirm the circumference difference scores in patients who underwent volumetry. The patients were divided into three groups depending on the general score results; group 1 (0–2 points) did not benefit from the operation, and group 2 (3–4 points) had some benefit, and group 3 (5–6 points) benefitted the most from the operation.

4.2 Surgical Technique and Postoperative Care (Studies I–IV)

VLNT was performed as described below. First, preoperatively the lower abdominal wall perforators and pedicle vessels were searched and marked with the guidance of a portable Doppler ultrasound device (Huntleigh dopplex D900). To visualize the lymph nodes, 0,5 ml of Patent Blue (Guerbet, Roissy CdG Cedex, France) was injected intradermally in the lower abdominal wall just above the iliac crest five minutes before the first skin incision. The lymphatic flap was dissected from the lateral to the medial direction, and the SIEA and superficial circumflex iliac perforator (SCIP) vessels were identified and preserved.

The original method in study I included SIEA vessels as a second pedicle and multiple lymph nodes, whereas, in the modified method the dissection of the lymphatic flap was limited lateral to the femoral artery and only one palpable lymph node was harvested in order to prevent donor-site complications. See figure 6. However, it is probable that lymph nodes that were too small to be palpable were included in the lymphatic flap in both methods. In studies I and II both original and modified method were utilised but in studies III and IV all patients were operated with the modified method.

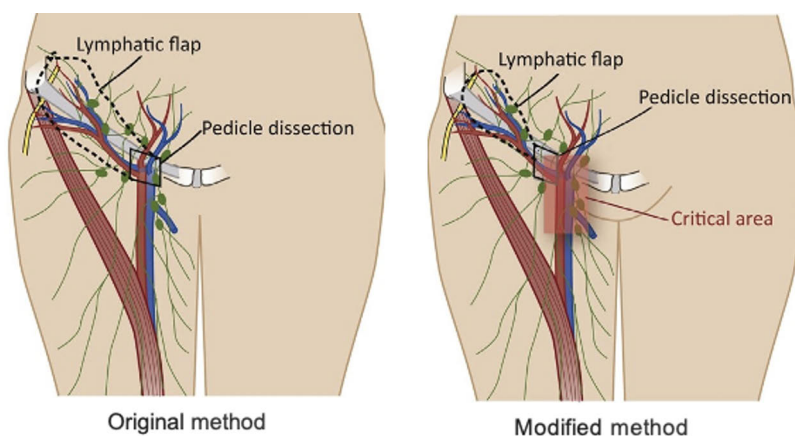


Figure 6. The different surgical fields of the original and modified method in study I. Modified from Viitanen et al. 2012 and adapted from study I. Author's own modification, permission for the use of the figure.

The lymphatic flap also consists of lymphatic vessels and adipose tissue from the anterior iliac spine to the origin of the superficial circumflex iliac artery (SCIA) vessel pedicle. The patients needing breast reconstruction had VLNT with an abdominal microvascular DIEP or an ms-TRAM flap. The lower abdominal skin and subcutaneous tissue were raised, and the related vessel pedicles were searched and carefully dissected to harvest a long arterial and venous pedicle for successful microvascular anastomosis in the axilla after raising the flap (Allen Jr and Cheng, 2016). In study IV the lymphatic flap and VLNT were raised as in the modified method, but the administration of Lymfactin® was made after disconnecting the flap from the abdomen, but prior to attaching it to the axilla.

In all patients except the patients operated on in Tampere in study IV, the thoracodorsal vessels were the recipient vessels of microvascular anastomosis. In Tampere, the DIEP flap was anastomosed to the internal mammary artery (IMA) vessels. The patients receiving lymphatic flap had second anastomosis to the serratus branches of the thoracodorsal artery and vein, if possible and needed. Sometimes the lymphatic flap did not have large enough arteries or veins to undergo anastomosis, or the blood flow between the DIEP and lymphatic flap was assessed by the operating surgeon to be sufficient without the second anastomoses. Importantly, all scar tissue were removed from the axillary area prior to anastomosis. The lymph node flap was positioned to the axilla such a way, that it covered the axillary plexus. Some of the patients were monitored with a Licox (Integra, New Jersey) oxygen monitoring device for five days; otherwise, the flap was monitored regularly with an audible handheld Doppler ultrasound device and by monitoring the color of the skin and capillary refill reaction. All patients used a compression bandage around their lower abdomen and inguinal area to prevent seroma formation and tension on the wound in the lower abdomen for at least four weeks. All patients were also recommended to use compression garments regularly for 24h per day for at least six months postoperatively. The patients also had a pre- and postoperative week-long trial without compression garments, if possible, and the difference in the volume of the affected limb between the beginning of the discontinuation of the compression garment use and after the 1-week pause was recorded. If the patient did not postoperatively have an increase in volumetry results during this 1-week trial of discontinuing the compression garment, the patient was allowed to reduce or discontinue the use of compression garments. However, if the 1-week trial without compression garment induced swelling of the affected arm, the patient was required to continue with regular compression garment use. In study I, the volume of the lower limbs was assessed with volumetry to assess the risk of donor site lymphedema. Patients in the studies I–III underwent manual lymphatic drainage postoperatively for at least three months, some patients continued for years, if needed. The practice of regular manual lymphatic drainage has since been stopped,

since the government does not support it financially anymore. Patients can however undergo manual lymphatic drainage on their own expenses, if they want to.

4.3 Statistical Analyses

Statistical analyses were performed using Excel, GraphPad PRISM, and/or SPSS. A statistical analysis specialist was consulted for proper statistical analyses for each study. The analyses were conducted by the author or another member of the research group. Statistical significance was set with the p-value of <0.05 .

The pairwise comparison within one group between the donor and non-donor sites was made in study I. Additionally, between groups, the donor and non-donor sites were compared individually.

Unpaired or 2-tailed Student's t-test was used for normally distributed continuous variables. The postoperative measurements were correlated with the preoperative and patient- and operation-related parameters to identify factors predicting a good surgical outcome. Correlation analysis was performed using Pearson or Spearman correlation coefficients, depending on data normality. Data normality was determined using the Kolmogorov-Smirnov test. Categorical data (different compression garment use according to preoperative measurements or general score groups according to cytokine response) were analyzed using Kruskal-Wallis analysis of variance followed by Dunn's multiple comparison test in studies II and III.

Mixed model repeated measures (MMRM) analysis of covariance was used for the volume and TDC results in study IV. These values were analyzed for changes from the baseline, and the baseline values were used as covariates. LyQoLI results were analyzed using ANOVA according to the change from baseline after the follow-up.

5 Results

5.1 Donor-site Symptoms and Lymphatic Function after VLNT Surgery

No symptomatic donor-site lymphedema was observed in our studies I–IV, irrespective of the surgical method (original vs. modified). In study I, even with the volume difference of +16,8% between the patients' limbs no donor-site lymphedema was found. In study I, TI was abnormal in two patients in the donor limb and five patients had greater TI in the donor limb. None of the patients however reported clinical symptoms of lymphedema. See table 4.

Table 4. The individual results of patients in study I. Adapted from study I.

	Patient	Age at operation	Transport index		Volume (ml)		Delfin (%)		Clinical symptoms	Follow-up (Months)
			Non-donor	Donor	Non-donor	Donor	Non-donor	Donor		
Original method	1	56	4.6	8.4					None	84
	2	40	0.2	12.8	9419	11000	33	38	None	77
	3	51			8388	8731	40	40	None	76
	4	62	0.2	10.8	11768	11328	41	38	None	55
	5	55	5.8	5.4	8075	7921	45	46	None	55
	6	50			7127	6900			None	54
	7	51	0.2	0.2	6338	6408	39	36	None	48
	8	65	0.2	0.2	10537	10486	37	36	N,P	44
	9	60	0.2	4.2	7759	7397			None	39
	10	56	0.2	0.2	9251	9997	39	40	None	37
	11	37	0.2	9.2	929	929			N	36
	12	74	0.2	4.2	9489	9883	48	45	None	36
	Modified method	13	61	7.8	4.8	9975	10462			None
14		53			7629	8647			None	32
15		49	0.6	0.2	8057	8311	44	40	N	32
16		50	5.4	5.4	8994	9471	40	38	N	31
17		56	0.2	9.8	8960	9280	45	46	N	29
18		59	0.12	0.12	8000	8246			None	28
19		55			10433	10708			None	27
20		47			10168	10212	39	40	None	27
21		49			7817	7749	42	36	None	20
22		31	0.2	0.2					None	20
23		47	0.2	0.2	10357	9982	39	38	N	20
24		52	0.2	0.8	9196	9077	40	41	N,P	20
25		56	5.4	5.4	7434	7197	41	41	N	19
26		68	0.2	0.2	10487	10961			None	19
27		50	0.2	4	12209	11320	45	44	N,S	18
28		44	0.2	0.2	10819	10792	46	44	N	18
29	51	0.2	4.6	10733	11604	38	37	N	11	

Symptoms = P = pain, N = numbness/decreased sensation, S = slow wound healing.

5.1.1 Donor-limb Volumetry (Study I)

The patients who underwent the modified method had a smaller average volume difference between the contralateral limbs than those who underwent the original method. However, this difference was not statistically significant. It seems that the best strategy to prevent lymphedema is to limit surgery to the area lateral of the femoral artery. See tables 4 and 5 for detailed information on the patients included in study I.

Table 5. The summary of the results in study I. Adapted from study I.

	Volumetry	Lymphoscintigraphy		Tissue dielectric constant	
	ml	Ti-value		%	
		Donor	Non-donor	Donor	Non-donor
Original method	199 ± 540	5.5 ± 4.2	1.8 ± 2.7	40 ± 4	40 ± 4
Modified method	151 ± 463	2.6 ± 3.1	1.1 ± 1.9	40 ± 3	42 ± 3

The range of volume differences between the donor and non-donor limbs was smaller in the modified method group (-7.3% to +13.3% (+151 ± 463 ml)) than in the original method group (-4.7% to +16.8% (+199 ± 540 ml)). Patients with VLNT alone (+270 ± 456 ml) had a greater average volume difference between the patients' arms after the follow-up than VLNT patients with simultaneous breast reconstruction (+138 ± 509 ml). These results were not statistically significant either in the comparison of limb volume difference ($p = 0.77$) or the comparison of the donor-limb volume difference ($p = 0.40$) between the two groups.

5.1.2 Donor-limb Lymphoscintigraphy (Study I)

Mean TI value was smaller in the modified method group 2.6 ± 3.1 (range 0.12 to 9.8) than in the original method group 5.5 ± 4.2 (range 0.2 to 12.8.). See the previous tables 4 and 5 for detailed data. Slightly elevated TI values were observed in four patients in both groups (original method 4.2 to 9.2, modified method 0.8 to 9.8), but they were still within the normal range of TI values. Only two patients had TI over the normal limit in the donor limb (TI 12.8 and 10.8), both of which were in the original method group. Although this indicates slightly abnormal lymphatic function in the donor limb, these patients had no symptoms of lymphedema. The comparison of TI values of the contralateral limbs was statistically significant ($p = 0.018$) in the original method group. In addition, the comparison of the TI values of the donor limb between the groups (original vs. modified method) was statistically significant ($p = 0.04$). The average TI value of the donor limb was greater in the patients who had VLNT alone (6.4 ± 4.5) in comparison to the VLNT patients with simultaneous breast reconstruction (3.1 ± 3.3).

5.1.3 Donor-limb TDC Measurements (Study I)

The donor limb was at most 5% greater in the original method group and 1% in the modified method group than the non-donor limb. See table 5 for detailed data. In the original method group, the average TDC was $40 \pm 4\%$ in both donor and non-donor limbs. The average TDC was within the normal range in both groups. The average TDC was smaller in the donor limb ($40 \pm 3\%$) than in the non-donor limb ($42 \pm 3\%$) in the modified method group. The difference between donor and non-donor limbs was statistically significant ($p = 0.042$) in the modified group. Patients who underwent VLNT alone had a greater TDC value than those who underwent simultaneous breast reconstruction. The comparison of donor limb TDC between the groups was not statistically significant ($p = 0.16$). See tables 4 and 5 for further information.

5.1.4 Donor-limb Postoperative Symptoms (Studies I–IV)

Donor-site complications included poor wound healing, cellulitis infection, and pain or numbness in the lower abdomen or upper thigh. The numbness of the operated area decreased over time in all patients. In study I postoperative seroma fluid drainage was needed for an average of six days (range, 3–10 days). In studies I–II 15–24% of the patients required needle aspiration of seroma build-up postoperatively after removal of the drainage. After follow-up, none of the patients reported lymphedema symptoms in the donor limb. See table 6 for a summary of donor-site complications in all of the studies.

Table 6. Listing of donor-site complications in all of the studies I–IV.

		DONOR-SITE COMPLICATIONS						
		Seroma aspirations (n)	Skin necrosis (n)	Wound infection (n)	Pain of the thigh (n. cutaneuos femoris) (n)	Numbness of the thigh or abdomen (n)	Delayed wound healing (n)	Lymphedema symptoms (n)
ARTICLE I	Original Method	4	0	2	1	2	0	0
	Modified Method	3	0	0	1	9	1	0
ARTICLE II		10	1	4	2	1	10	0
ARTICLE III		5	0	1	1	1	3	0
ARTICLE IV	Lymfactin	1	0	1	0	0	0	0
	Plasebo	3	0	4	8	4	3	0

n = number of patients

5.2 Affected Arm Symptoms and Lymphatic Function after VLNT (Studies I–IV)

After VLNT, patients experienced improvements in TI results, arm circumference, and volume. The improvement was constant for up to two years, after which there was slight worsening of the results, although remaining better than the preoperative results. The need for the use of compression garments reduced postoperatively. The rate of cellulitis infections was lower postoperatively, but in study II four patients had postoperative cellulitis infection with no previous cellulitis infection. In study III patients with preoperative cellulitis infections were more likely to continue using compression garment use postoperatively compared to patients without preoperative cellulitis infections. Subjectively, in study II the function of the arm was improved by 51% of the patients. In study III the total general score (mean±SD) of 3.0 ± 1.2 suggests that patients benefit from VLNT.

5.2.1 Arm Volumetry (Studies II–IV)

In study II the average volume difference between contralateral arms decreased from 416 ± 432 ml to 267 ± 285 ml postoperatively. In study III the mean (\pm SD) volume of the affected arm decreased from 522.1 ± 449.6 ml to 233.0 ± 388.2 ml postoperatively. Furthermore, the median reduction in excess volume increased during follow-up in all studies. In study IV the median reduction in excess volume with compression garment use was in the Lymfactin® group 107 ± 139 ml (21.5%, IQR 3.8–41.8) and in the placebo group 93 ± 157 ml (19.5%, IQR -5.2–42.3). Without compression the median reduction in excess volume was 136 ± 189 ml (30.0%, IQR 12.0–55.5) in the Lymfactin® group and 164 ± 133 ml (23%, IQR 13.0–45.0) in the placebo group. Please refer to figures 7–9 for further details.

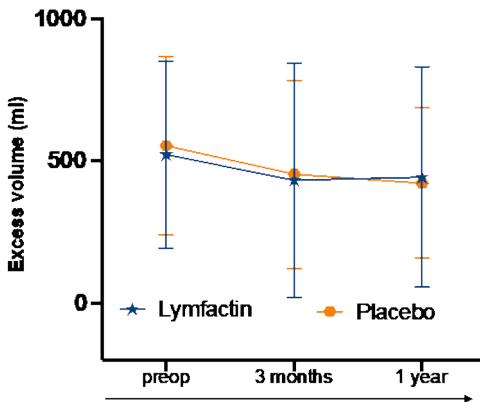


Figure 7. The (mean \pm SD) volume difference of the patients' arms in study II. Adapted from study II.

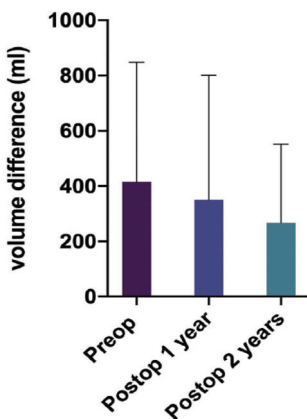


Figure 8. The (mean \pm SD) excess volume of the affected arm with compression in study IV. Modified from study IV.

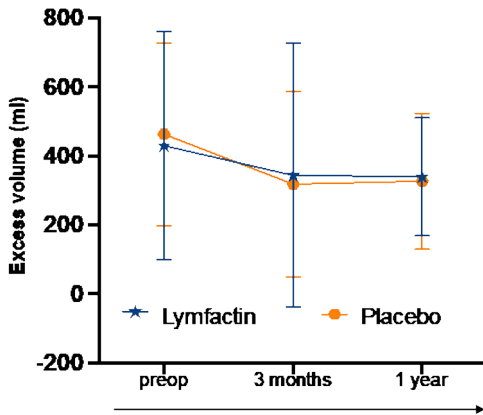


Figure 9. The (mean ± SD) excess volume of the affected arm without compression in study IV. Modified from study IV.

In study II the patients with smaller volume difference between their arms before the surgery had statistically significant correlation ($r = 0.6364$; 95% Confidence interval (CI), 0.0792–0.8906; $p = 0.0299$) with smaller volume difference also after the surgery in study II ($r = 0.6364$; 95% CI 0.0792–0.8906; $p = 0.0299$). However, the change in arm volume during follow-up was not statistically significant ($p = 0.452$) (study II).

5.2.2 Arm Circumference Difference (Studies II–III)

The average arm circumference difference decreased after follow-up (study II: 3.2 ± 2.6 cm to 2.5 ± 1.7 cm; study III: 2.5 ± 2.1 cm to 1.1 ± 1.3 cm). In study II, a larger arm circumference difference before the surgery correlated ($r = 0.6905$; 95% CI, 0.3448–0.8713; $p = 0.0008$) with a larger arm circumference difference also after the surgery in study II ($r = 0.6905$; 95% CI 0.3448–0.8713; $p = 0.0008$). See figures 10–11.

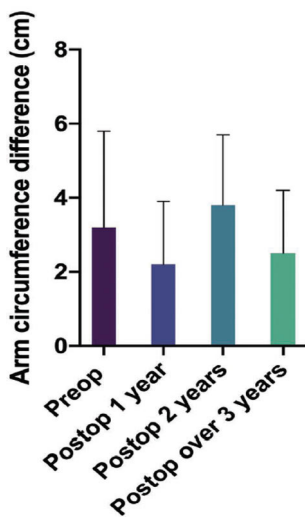


Figure 10. The correlation of arm circumference difference between pre- and postoperative results in study II. Adapted from study II.

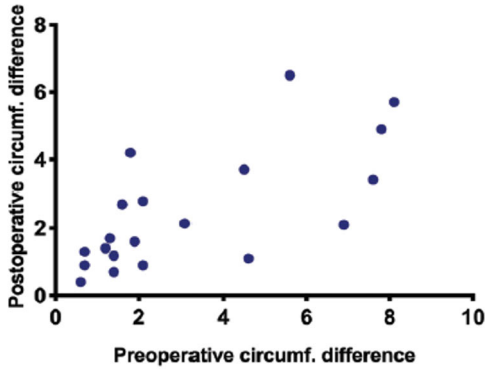


Figure 11. The (mean \pm SD) arm circumference difference of the patients' arms in study II. Adapted from study II.

5.2.3 Arm Lymphoscintigraphy (Studies II–IV)

In study II, a smaller preoperative TI had a statistically significant correlation with greater reduction of TI during follow-up, indicating that patients with later stages of lymphedema could benefit from VLNT. In study II, the average TI decreased from 29.3 ± 14.4 to 20.9 ± 14.2 during follow-up of two years. See figure 12. In study III, over half of the patients (65%) had an improvement in the TI during the follow-up. TI remained below the preoperative level throughout the follow-up, but after two years, there was an increase as observed in study II.

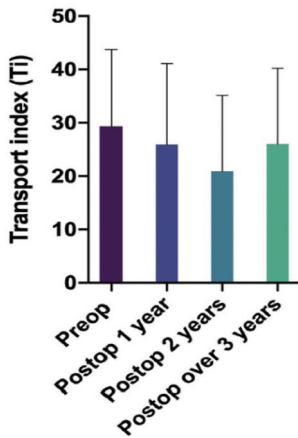


Figure 12. The (mean \pm SD) TI in study II. Adapted from study II.

In study IV, preoperative TI did not differ significantly ($p = 0.58$) between the Lymfactin® and placebo group. In study IV, the mean TI decreased in the placebo group, indicating better lymphatic function by 10.54% (IQR 0–6.65) after one year of follow-up, and surprisingly, increased in the Lymfactin® group by 16.95% (IQR 4.445–0.775). See figure 13. However, these differences in study IV were not statistically significant ($p = 0.73$).

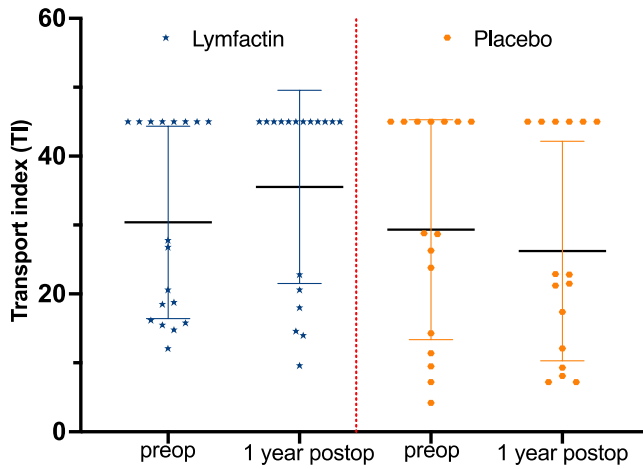


Figure 13. The (mean± SD) lymphoscintigraphy results after one year of follow-up in study IV. Modified from study IV.

As seen in volumetry results, in study II a smaller TI preoperatively correlated with smaller postoperative TI ($r = 0.5497$; 95% CI, 0.1278–0.8031; $p = 0.0120$). In study II, the patients with VLNT alone had a greater reduction in TI during the follow-up than the VLNT patients with simultaneous breast reconstruction. This difference was statistically significant ($p = 0.03$). In study II, the patients with larger TI values before the surgery had greater reduction in TI value during the follow-up in correlation analysis ($r = 0.4636$; 95% CI, 0.02656–0.7519; $p = 0.0395$), see figure 14.

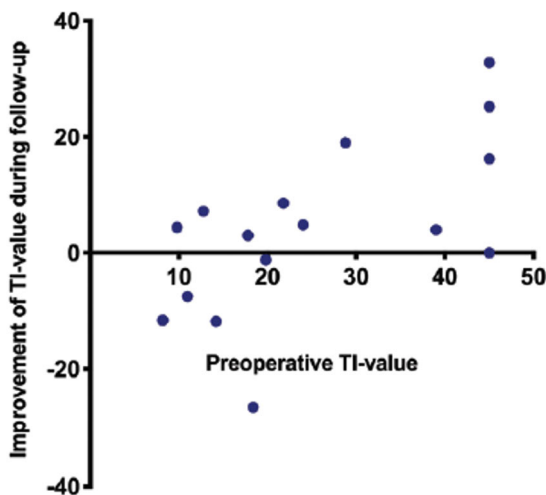


Figure 14. The correlation of preoperative TI value and the improvement of the TI value during follow-up in study II ($p = 0.0395$). Adapted from study II.

5.2.4 TDC Ratio of the Arms (Study IV)

In study IV a statistically significant baseline effect was found in the MMRM analysis of covariance with the TDC ratios and compression treatment. In addition, the reduction in the TDC ratio was significantly greater ($p = 0.020$) in the Lymfactin® group than that in the placebo group after the follow-up of 12 months. This result in study IV indicated a more favorable composition of the lymphedematous limb in the Lymfactin® group than in the placebo group.

Patients in the Lymfactin® group had a greater mean change in TDC ratio of the whole arm (-0.136 ± 0.03) during the follow-up compared to the patients in the placebo group (0.006 ± 0.025) (Lymfactin® - placebo = -0.142 ; 95% CI -0.220 to -0.065). Furthermore, the Lymfactin® group exhibited a significantly greater reduction in the TDC ratio compared to the placebo group during the follow-up period ($p = 0.015$).

Different areas of the arm (above the elbow, below elbow, and whole arm) had a different reaction to the treatment after follow-up ($p = 0.08$). As a statistically significant result, the mean change of TDC ratio was greater in the Lymfactin® group (-0.043 ± 0.029) compared to the placebo group (0.066 ± 0.029) (Lymfactin® - placebo = -0.109 ; 95% CI -0.189 to -0.028) during the follow-up period. See figure 15.

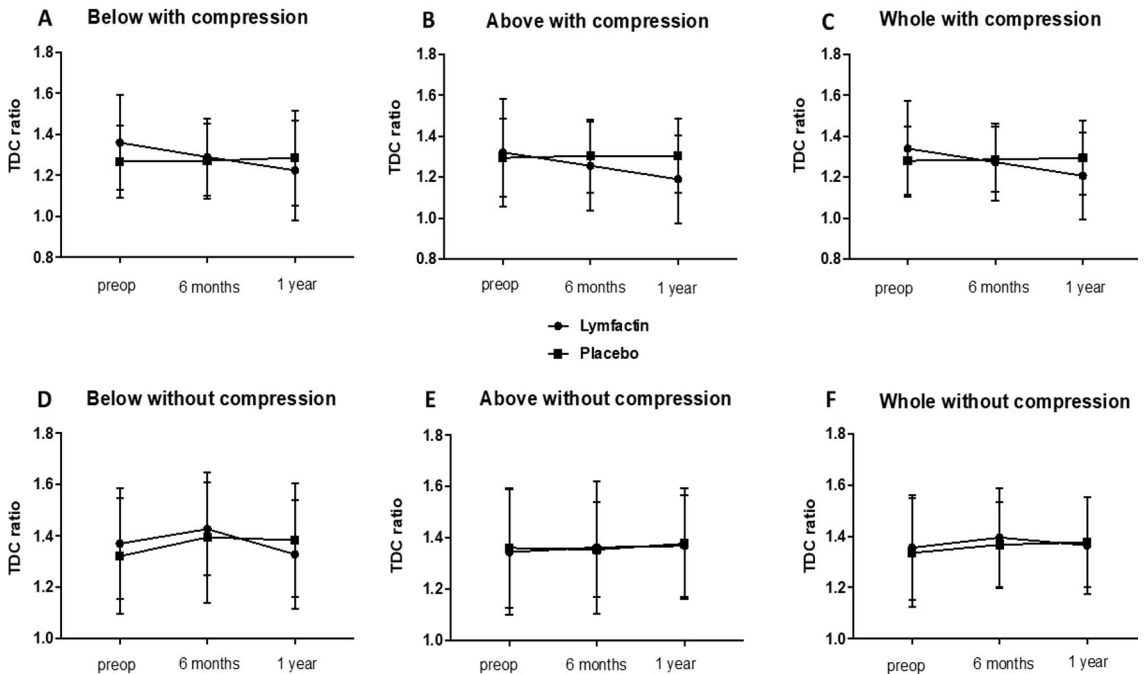


Figure 15. TDC ratio (TDC value of the affected arm divided by the value of the normal arm) results in different regions of the affected arm with or without compression in study IV. Modified from study IV.

5.2.5 General Score (Study III)

The general score was an evaluation tool that our research group developed. It was used in study III. It has not been validated and was only used in a supportive role in evaluating the results. In study III, the total general score (mean±SD) of 3.0 ± 1.2 suggests that patients benefited from VLNT. The mean ± SD general score of arm circumference of 1.0 ± 0.7 in study III supported the beneficial results seen in arm circumference difference. Furthermore, the general score for the TI component supports the improved TI results observed in study III (mean ± SD 1.0 ± 0.9).

5.2.6 Compression Garment Therapy of the Affected Arm (Studies I–IV)

All patients were required to wear compression garments regularly before the operation, and compression garment use was continued for a minimum of six months after the surgery. All patients in study IV continued the use of compression garments throughout the follow-up of 12 months. The requirement was to wear the compression garments regularly for 24 hours per day, except for those without compression measurements pre- and postoperatively requiring a one-week pause. This one-week trial was performed to assess the severity of lymphedema and the results of the treatment.

Patients were allowed to discontinue the use of compression garments altogether, if the patients' improved results persisted after discontinuing the use of compression garment use for one week. The following results were considered; TI, arm circumference, pitting edema, incidence of postoperative cellulitis, and overall swelling of the arm. In studies II–III, 30–42% of the patients were able to discontinue the use of compression garments after the follow-up. See figure 16.

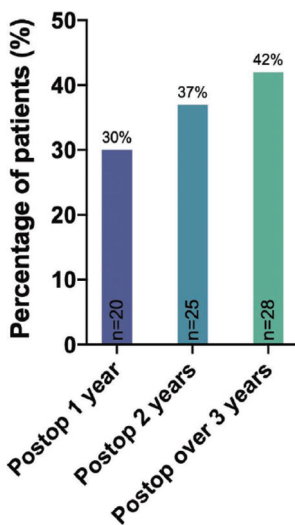


Figure 16. The percentage of patients able to discontinue the use of compression garments during follow-up in study II. Adapted from study II.

On average, in study II compression garments were discontinued 13 ± 12 months after surgery. In addition, in studies II–III 16–28% of the patients were able to reduce the time of use of the compression garments. In study III, patients who were able to discontinue the use of compression garments after the surgery had significantly lower incidence of cellulitis infections before the surgery than the patients who needed to continue the use of compression garments after the surgery. This also included patients who were able to reduce the use of compression garments after the surgery.

In study III the general score of 1.2 ± 0.9 (mean \pm SD) for compression garment use also supports these positive results.

5.2.7 Symptoms and Day-to-day Function of the Affected Arm (Studies II–III)

In studies II–III pain was one of the symptoms of lymphedema before the surgery in 24–61% of the patients, and 75–89% of them reported a decrease in pain after the surgery. In studies II–III subjective day-to-day functions, such as fine motor skills, the endurance of the arm, and the ease of finding suitable clothes, were easier in 76–94% of the patients after the surgery. See detailed information in tables 7 and 8.

Table 7. Patient variables in study II. Adapted from study II.

Variable	Patients (n = 67)
Postoperative complications	34 (50.7)
Immediate	17 (25.4)
Delayed (arm)	19 (28.4)
Delayed (groin)	16 (23.9)
Preoperative cellulitis	13 (19.4)
Postoperative cellulitis (n = 66)	7 (10.6)
Preoperative TI value (n = 20)	29.3 (\pm 14.4)
Postoperative TI value (n = 20)	26.0 (\pm 14.2)
Preoperative affected arm volume difference (n = 12), ml	416 (\pm 432)
Postoperative affected arm volume difference 24 mo postoperatively (n = 12), ml	267 (\pm 285)
Preoperative affected arm circumference difference (n = 20), cm	3.2 (\pm 2.6)
Postoperative affected arm circumference difference (n = 20), cm	2.5 (\pm 1.7)
Subjectively improved function of the affected arm	51 (76.1)
Able to discontinue the use of compression garment	28 (41.8)
Follow-up time, mo	70 (\pm 17)

Data are presented as number of patients (percentages) or mean (\pm SD).

Table 8. Summary of patients in study III. Adapted from study III.

ID	Age at operation	Duration of lymphedema preoperatively (months)	General Score				Cellulitis (n)		Function of the limb		Pain symptom
			Tl-value	Circumference difference	Compression garment use	In total	preop	postop	Improved (n=19/20)	Improved (n=18/20)	
1	38	6	1	1	0	2	1	0	X	X	
2	74	31	2		2	4	3	1	X	X	
3	61	48	1	2	0	3	0	0	X	X	
4	53	16		1	2	3	2	0			
5	49	18	0	0	2	2	0	0	X	X	
6	50	72	1	0	2	3	0	0	X	X	
7	56	84	1	0	1	2	0	0	X	X	
8	59	10		2	0	2	0	0	X	X	
9	60	36	0		2	2	0	0	X	X	
10	55	60	2	1	1	4	0	0	X	X	
11	47	69	2	1	2	5	0	0	X	X	
12	38	24	0	2	0	2	0	0	X	X	
13	68	51	0	2	0	2	0	0	X	X	
14	50	27	0	1	2	3	0	0	X	X	
15	49	16	0	1	0	1	0	0	X		
16	31	20	1	1	2	4	0	0	X	X	
17	47	27	0	2	0	2	0	0	X	X	
18	52	35	2	0	0	2	0	0	X	X	
19	44	30	2	2	1	5	0	0	X	X	
20	52	24	2	1	2	5	0	0	X	X	
MEAN ± SD	52±10	79±7	35±21	0,9±0,9	1,1±0,8	1,1±0,9	2,9±1,2	0,3±0,8	0,1±0,2		

5.2.8 Lymphedema Quality of Life Inventory (LyQoLI) (Study IV)

Before the treatment, the patients in study IV in the Lymfactivin® group had a worse quality of life based on higher LyQoLI scores compared to the placebo group in the total, psychosocial, and practical domains (total 57.78 vs. 40.11, $p = 0.010$; psychosocial 25.0 vs. 14.9, $p < 0.001$; practical 18.67 vs. 12.67, $p = 0.017$). Physical LyQoLI scores did not differ significantly before surgery (14.11 vs. 12.56, $p = 0.480$).

In both groups, all domains of the mean LyQoLI score improved from the preoperative scores during the follow-up. However, the only statistically significant reduction in the mean was in the psychosocial LyQoLI domain (43.12% and 31.70%, $p = 0.031$). It seems that the psychosocial aspects of quality of life improved after VLNT. The mean LyQoLI scores are shown in figure 17.

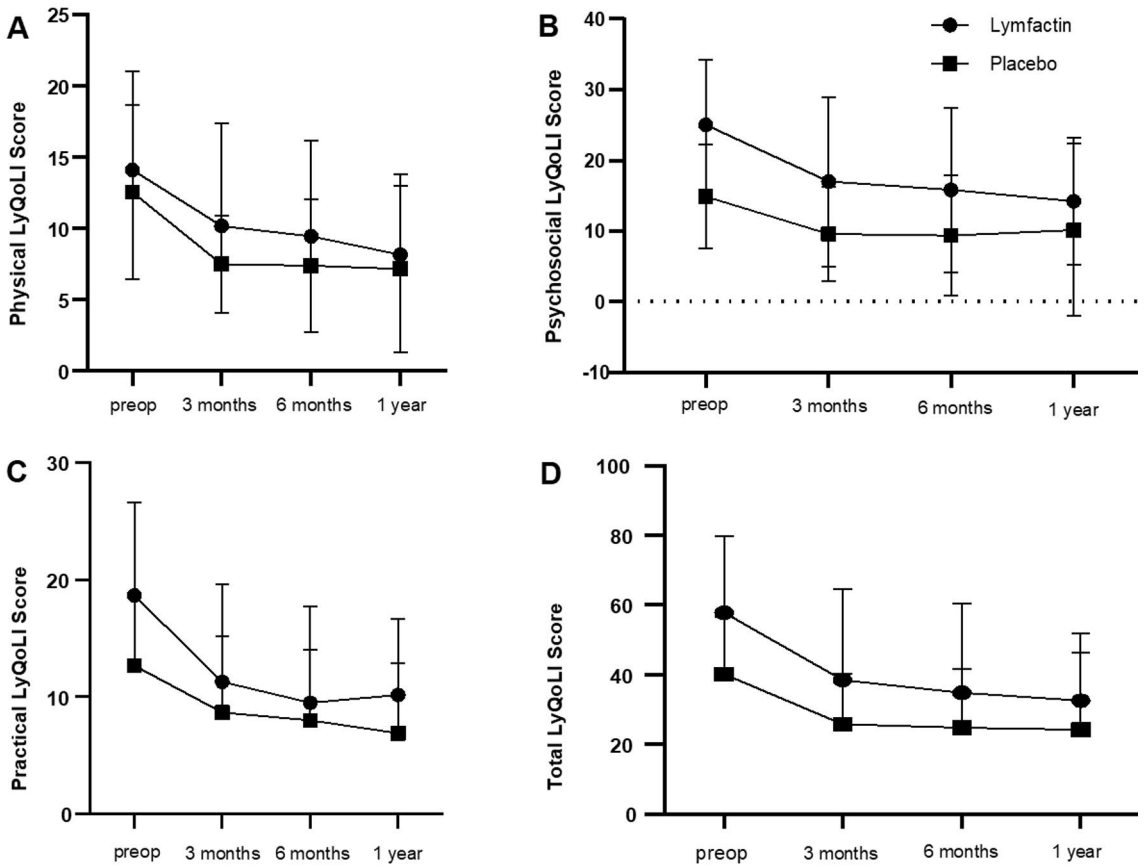


Figure 17. The mean LyQoLI scores by the three main domains (A: Physical, B: Psychosocial and C: Practical and total sum of the three domains (D). Modified from study IV.

5.2.9 Cellulitis Infection of the Affected Arm (Studies II–III)

In studies II–III the incidence of cellulitis infection reduced during follow-up (study II: preoperative $0.20 \pm 0.55/y$ (13/67 patients, 19,4%) to postoperative $0.02 \pm 0.08/y$ (7/66 patients, 10,6%); study III: preoperative $0.06 \pm 0.15/year$ (3/18 patients, 16,7%) to postoperative $0.01 \pm 0.03/year$) (1/18, 5,6%). See figure 18.

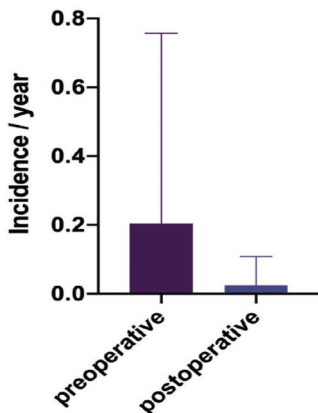


Figure 18. The (mean ± SD) incidence of cellulitis infection ($p= 0.0998$) in study II. Adapted from study II.

However, in study II four patients had cellulitis infection after surgery, even though they had no previous cellulitis infection. In study II cellulitis infections occurred on an average of 10 ± 8 months after the surgery. In study II prophylactic antibiotics were used by 10% of the patients before the surgery, and four of them were able to discontinue the prophylaxis after the surgery.

5.2.10 Complications of the Arm and Adverse Events (AEs)

Postoperative flap-related complications in studies I–IV included hematoma, re-anastomosis, and medial breast reconstruction flap necrosis. In study II 28% of the complications occurred immediately, one to two days after the surgery, and 16% of them were delayed, more than one week after the surgery. In studies I–II one breast reconstruction flap (the same patient) was lost on the 12th POD, but the VLNT flap was salvaged. In studies I–II all VLNT flaps survived throughout the follow-up period. Summary of recipient site complications is seen in table 9.

Table 9. A summary of recipient site complications in all of the studies I–IV.

ARTICLE		RECIPIENT SITE COMPLICATIONS									
		Immediate					Delayed				
		Re-anastomosis (n)	Hematoma evacuation (n)	Flap loss (n)	Swelling of the axilla (n)	Wound infection (n)	Skin necrosis (n)	Cellulitis (n)	Delayed wound healing (n)	Wound infection (n)	Skin necrosis (n)
I	Original method	0	4	0	0	2	1	2	2	1	0
	Modified method	1	3	1 (BR-flap)*	0	1	2	1	2	1	0
II		6	7	1 (BR-flap)*	1	1	5	7	1	0	2
III		0	2	0	0	0	0	1	2	0	1
IV	Lymfactivin	0	0	1 #	1	2	0	1	0	1	1
	Placebo	1	3	2 #	0	1	0	1	0	5	0

n = number of patients, * = same patient, # = excluded from the analysis

In study IV twenty patients experienced complications, that could be causally associated with the given treatment. The AEs were evenly distributed between the groups. In both groups a transient elevation of liver enzymes was observed but normalized during the follow-up in all patients. See table 10.

Table 10. The number of different AEs in study IV.

ADVERSE EVENTS	Lymfactin (n = 19)	Placebo (n = 20)
Spontaneous hematoma	0	1
Coronary artery disease	0	1
Goitre, hyperparathyroidism	0	2
Keratitis, vitreous detachment	2	0
Constipation, diarrhea, abdominal pain, reflux	11	12
Pyrexia, general pain, asthenia, swelling of feet	8	12
Allergy	3	0
Erysipelas , urinary tract infection, common cold, flu, shingles, tooth infection, sinusitis, yeast, paronychia	35	24
Postoperative wound complication, procedural pain, wound dehiscence, flap necrosis	13	15
Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin abnormal	50	55
Vitamin B complex deficiency, hypercalcemia, hypokalemia	1	2
Back pain, extremity pain, arthralgia, neck pain	18	28
Benign nevus, lipoma	1	1
Headache, syncope, dizziness	30	23
Insomnia	3	3
Breast pain	1	0
Cough, oropharyngeal pain, pneumothorax, pulmonary embolism	10	7
Blister, eczema, rash	12	5
Dental prosthesis user	0	1
Aortic anastomosis, tooth extraction, appendectomy, colectomy, hematoma evacuation	1	10
Arterial thrombosis, lymphedema, hematoma , venous thrombosis on limb	3	5
Non-study related adverse events	175	178
Study-related adverse events	27	29

n = number of adverse events, bolded events could be study-related

Table includes also the patients excluded from the analysis (due to flap loss).

In study IV, four patients in the Lymfactin® group had one serious AE, and nine patients in the placebo group had a total of 11 serious AEs after the treatment. A serious AE include AEs in which the patient is required to be hospitalized or prolongation of existing hospitalization, result in persistent or significant disability or incapacity or if the investigator considers the AE to be serious. In study IV three patients were excluded due to flap loss (one in the Lymfactin® group and two in the placebo group). See table 11.

Table 11. The number of SAEs in the study IV.

SERIOUS ADVERSE EVENTS (SAE)		
	Lymfactivin (n= 19)	Plasebo (n=20)
Cellulitis	1	1
Blockage of artery and vein to free flap	1	1
SCIP-flap loss	1	2
Infection	1	2
Wound edge necrosis	1	1
Abdominal wound dehiscence	0	1
Planned laparoscoped right sided hemicolectomy	0	1
Chest pain	0	1
Pulmonary embolism	0	1
Lower extremity venous thrombosis	0	1
Number of patients with SAE	4	11

All reported as n = number of patients

Table includes also the patients excluded from the analysis (due to flap loss).

5.3 Cytokine and Growth Factor Response after VLNT (Study III)

In study III, the inflammatory profile shifted towards an unfavorable, profibrotic, and antilymphangiogenic Th2 type response in patients with a longer preoperative duration of lymphedema. This suggests that a shorter duration of preoperative lymphedema could predict better results from VLNT surgery.

5.3.1 Wound Exudate Analysis (Study III)

In study III during the first six PODs, the concentrations of IL-10 and TNF- α increased, whereas those of TGF- β 1 and VEGF-C decreased. However, these differences were not statistically significant. See figure 19 for detailed data.

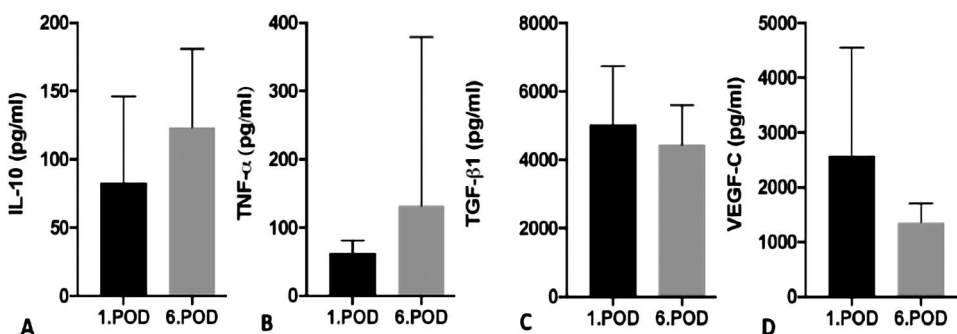


Figure 19. The (mean \pm SD) concentration of IL-10 (A), TNF- α (B), TGF- β 1 (C) and VEGF-C (D) in wound exudate at 1st and 6th POD in study III. Adapted from study III.

In study III the change in VEGF-C concentration during the first six PODs did not correlate with the outcome of the surgery. In study III a nearly statistically significant or statistically significant correlation was found between a shorter duration of lymphedema before the surgery and the increase of IL-10 and TNF- α during the first six PODs (IL-10: $r = 0.495$, 95% CI -0.001 – 0.795 , $p = 0.051$; TNF- α : $r = 0.737$, 95% CI 0.282 – 0.921 , $p = 0.006$), and decrease in TGF- $\beta 1$ ($r = -0.613$, 95% CI -0.863 – -0.123 , $p = 0.020$). See figure 20.

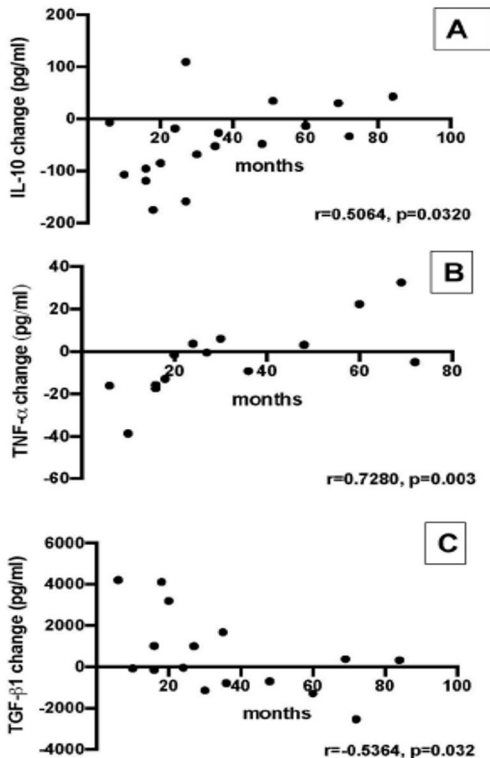


Figure 20. The correlation between the change of IL-10 (A), TNF- α (B) and TGF- $\beta 1$ (C) (1st–6th POD) and the duration of preoperative lymphedema (months) in study III. Adapted from study III.

In study III patients with greater age had a statistically significant correlation with the increase in TGF- $\beta 1$ concentration ($r = -0.584$; 95% CI -0.851 – 0.077 , $p = 0.029$) during the first six PODs.

In study III the increase of concentration of TNF- α during the first six PODs correlated statistically significantly with a greater total of general score ($r = 0.775$; 95% CI 0.327 – 0.939 , $p = 0.005$). See figure 21. This may indicate a better result in these patients, with an increase in TNF- α levels during the first six PODs.

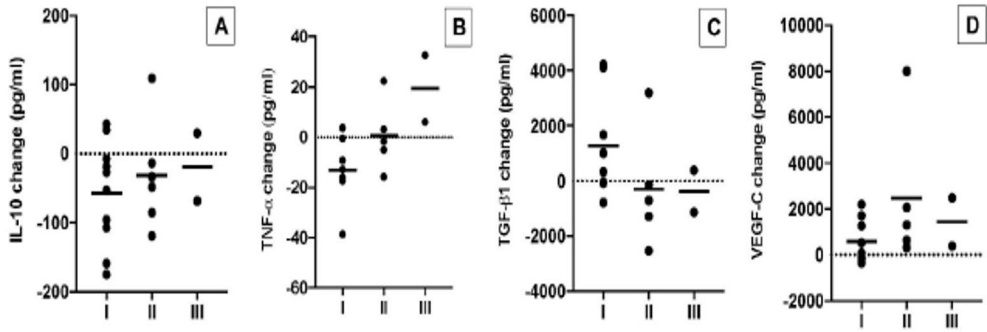


Figure 21. The correlation between the change of TNF- α (1st-6th POD) and the total general score in study III. Adapted from study III.

6 Discussion

VLNT surgery is an interesting field of research because it is a relatively new technique and has the potential to be a cure for lymphedema (Schaverien *et al.*, 2019). However, the cause of lymphedema remains a mysterious and intricate web of different factors. The aim of this thesis was to subjectively and objectively evaluate the clinical effect of VLNT, refine the patient selection process, and study the possibilities of adjacent medicinal treatment with growth factor treatment.

6.1 The Clinical Effect of VLNT

Patients with BCRL are known to have a worse quality of life than patients without BCRL (Fish *et al.*, 2020). This can be easily understood as lymphedema is a progressive disease that influences all aspects of life. It can cause pain, discomfort, low self-esteem, difficulties in finding suitable clothing, need for continuous compression garment use, and cause recurrent cellulitis infections with the need for prophylactic antibiotics, as well as the inability to work. (Fish *et al.*, 2020) VLNT surgery improves the quality of life of patients with BCRL. VLNT reduces the need for compression garment use, cellulitis infection rate, and pain by reducing the volume of the affected limb. (Fish *et al.*, 2020)

Scar removal alone improves lymphatic flow in the affected arm and alleviates lymphedema symptoms (Warren *et al.*, 2007; Maruccia *et al.*, 2019). All of these beneficial effects were corroborated in the studies included in this thesis. However, not all of the patients in our studies gained the desired effect from VLNT surgery, which is why we aimed to identify the patients that most likely benefit from the surgery. In our findings, patients with early stages of lymphedema and a short duration of lymphedema before VLNT surgery had the best results. Patients with a shorter duration of lymphedema might have a more adaptive immune system and more functional lymphatic system in the affected area. In addition, it is plausible that patients with earlier stages of lymphedema, by definition, lymphedema is still in its reversible stage and hence VLNT surgery can improve lymphatic flow and function more (Rockson *et al.*, 2019; Michopoulos *et al.*, 2021).

Our studies did not find a conclusive correlation between age and VLNT surgery results. It might be speculated that younger patients could have a more alert and

adaptive immune system, and hence a more mouldable immunological environment with better prospects of healing after VLNT surgery. (Sayan *et al.*, 2022; Liu *et al.*, 2023)

In all studies, the need for compression garment use was reduced. This is an important finding, since compression garments are a visible treatment and require a lot of commitment from the patient. The mere reduction of compression garment use and, of course, the discontinuation of the use of compression garments can have a massive impact on the quality of life of patients after VLNT surgery.

6.2 The Role of Inflammation and Fibrosis

The causes of lymphedema are multifactorial. Scar and mechanical obstruction are one part (Warren *et al.*, 2007), but a critical part of the development of lymphedema is the immunological environment of the affected area. The immunological environment is influenced and orchestrated by macrophages and cytokines, such as VEGF-C, TNF- α , IL-10 and TGF- β 1. Iatrogenic damage due to surgery, radiotherapy or chemotherapy results in different cytokine responses. (Liu *et al.*, 2023) When the cytokine response shifts towards an inflammatory Th2 type, it results in a profibrotic and anti-lymphangiogenic response. This indicates more and possibly excess scarring and fewer new lymph vessels. Both factors elevates the risk of lymphedema. (Avraham *et al.*, 2013)

In our studies, we observed the same Th2 type response in patients with less benefit from VLNT (study III). Cytokines were analyzed in easily accessible drainage fluid. Fluid drainage is commonly used in breast cancer surgery; therefore, perhaps the individual cytokine response of the patient might be analyzed during breast cancer surgery and thereby possibly identify patients with a higher risk of lymphedema development. Such patients could be good candidates for VLNT surgery, if needed. For example, after breast cancer treatment and axillary surgery, scars are present in all patients, but only some of them will develop lymphedema. It is known that scar removal during VLNT surgery is crucial to restore lymph flow and remove mechanical obstruction, in addition to allowing new lymphatic vessels to find functioning lymphatic vessels. This provides a “fresh start” for the immune system and can facilitate a healthy nonfibrotic immunological environment. (Warren *et al.*, 2007; Maruccia *et al.*, 2019)

6.3 Donor Site Morbidity

VLNT surgery is a major procedure that results in extensive scars in both the recipient and donor sites. Surgery requires long general anesthesia and a relatively long hospital stay and absence from work. (Becker, 2016) Thus, it is important to

consider the donor site and other possible morbidities. Donor site complications include seroma build-up requiring draining with a needle and syringe, wound infections, wound dehiscence, or pain/numbness in the upper thigh (Viitanen *et al.*, 2012). Some of these complications occur immediately during recovery, whereas others present later. Since patients already have a relatively long recovery time even with no complications, it is a major setback for the patient to have wound dehiscence and absence from work or a prolonged need for assistance or care with the wounds. This could be one of the factors affecting the quality of life after VLNT surgery, and even the perception of subjective results from VLNT surgery.

Fortunately, in our studies, no donor site lymphedema was reported, but such cases have been described (Pons *et al.*, 2013; Vignes *et al.*, 2013; Demiri *et al.*, 2018). As described in the articles by Pons *et al.* and Vignes *et al.*, their patients had a probable genetic predisposition for lymphedema (Pons *et al.*, 2013; Vignes *et al.*, 2013). In study I, lymphedema symptoms in the donor limb were not observed, although some of the patients had subclinical lymphedema according to TI, volumetry and TDC measurements. Some patients had abnormal measurements in the non-donor limb, which may indicate a genetic predisposition for lymphedema.

As previously described, our study group modified the technique for harvesting the VLNT flap to minimize the risk of donor-site complications (Viitanen *et al.*, 2012). Our modified method (studies I-IV) limits surgical dissection lateral to the femoral vessels to prevent disruption of critical sentinel lymph nodes of the lower limb (van der Ploeg *et al.*, 2009). Sentinel lymph nodes are responsible for most of the lymph fluid flow from the limb, and any damage, such as restrictive scarring may result in symptomatic donor-site lymphedema (Pan, le Roux *et al.*, 2011). This more conservative lymph node harvest resulted in smaller volume, TI, and TDC measurements postoperatively compared to the preoperative situation in study I.

Seroma build-up is a common complication after VLNT surgery and can be a nuisance for the patient. An atraumatic surgical technique and avoidance of wide dead spaces inside the wound by closing the wound in multiple layers, including subcutaneous stitching, may reduce seroma formation after VLNT surgery (Granzow, 2018).

6.4 Lymphatic Growth Factors and Cytokines

As described previously, cytokines play an important role in lymphedema development and the healing of lymphatic flow after damage. VEGF-C is a growth factor responsible for the maturation and differentiation of lymphatic vessels (Szuba *et al.*, 2002; Visuri *et al.*, 2015). In addition, the cytokines TGF- β 1, IL-10, TNF- α , IL-13, and IL-4 are involved in lymphatic regeneration (J C Zampell, Avraham, *et al.*, 2012). Lymphangiogenesis requires a helper cell type 1 (Th1) cytokine response

that facilitates a noninflammatory and nonfibrotic response (Szuba *et al.*, 2002; Tammela *et al.*, 2010).

In study III, better results were observed in patients with increased levels of VEGF-C and TNF- α and decreased levels of IL-10 and TGF- β 1 during the first six PODs. This result confirms the benefits of the Th1-type response. Some new medicinal treatments, such as growth factor therapy and ketoprofen, have been suggested to modify the immune response after surgery, with promising results (Szuba *et al.*, 2002; Tervala *et al.*, 2015; Tian *et al.*, 2017; Rockson *et al.*, 2018; Leppäpuska *et al.*, 2022). These treatments could be an excellent add-on for VLNT surgery to make it more successful.

Few possible growth factors and administration profiles have been studied, but adeno-associated viruses expressing recombinant VEGF-C have shown the most promise (Szuba *et al.*, 2002; Alan Yan *et al.*, 2011; Sommer *et al.*, 2012; Visuri *et al.*, 2015). This growth factor treatment was initiated after extensive previous research (Hartiala *et al.*, 2010; Hartiala *et al.*, 2020). It was studied in phase I (Hartiala *et al.*, 2020; Leppäpuska *et al.*, 2022) and phase II studies (study IV). Although the results were not conclusive in study IV, a lot of potential was observed.

VEGF-C is a known factor involved in lymphangiogenesis (Szuba *et al.*, 2002). The aim of medicinal treatment for lymphedema is to turn back time to the timepoint when the immunological environment is friendly and pro-lymphangiogenic. In my opinion, it is logical to implement immunologically positive behavior of VEGF-C to the unfriendly affected site and use it as a so-called peace offering in a hostile immunological situation. Study IV showed a shift to a more favorable composition of the affected arm with growth factor treatment, which corroborates the idea of VEGF-C growth factor treatment. It has been under discussion whether growth factor treatment imposes a risk of a neoplasm, but no evidence has been found in previous studies (Skobe *et al.*, 2001; Visuri *et al.*, 2015). In study IV, as a precaution, growth factor was injected into the flap itself and administered before the flap was anastomosed to the patient (Hartiala *et al.*, 2020).

One approach for the medicinal treatment of lymphedema is the inhibition of cytokines IL-4 and IL-13 (Babak J. Mehrara *et al.*, 2021). The idea is to prevent the Th2 type cytokine response by inhibiting IL-4 and IL-13 using neutralizing antibodies. IL-4 and IL-13 are required for the formation of Th2 cells. (Babak J Mehrara *et al.*, 2021) In my opinion, this is an interesting approach. The method is conservative, with only monthly intravenous infusions for four months without any surgery. Although it showed improvements in the composition of the affected arm, it did not affect volumetry or TDC measurements. The results are promising and show the benefit of altering the immunological environment of the affected arm; however to provide a cure for lymphedema or substantially alleviate it, surgical approaches should also be implemented. It could possibly work together with VLNT

or liposuction in later stages of lymphedema. However, further research and phase II trials should be conducted to draw any final conclusions.

Rockson *et al.* (Rockson *et al.*, 2018) studied the possibility of anti-inflammatory drug treatment with ketoprofen. The idea is to inhibit the 5-lipoxygenase metabolite, LTB₄ (Nakamura *et al.*, 2009; Rockson *et al.*, 2018). This method is easy to administer and its cost is low compared to other medicinal treatments for lymphedema. Ketoprofen as an NSAID, can increase the risk of cardiovascular, renal, and gastrointestinal complications, which limits its use. Ketoprofen could be a good add-on for VLNT surgery and provide assistance for the postoperative healing process. However, low levels of LTB₄ during the first few PODs are beneficial for lymphangiogenesis and should be considered during the administration of ketoprofen (Rockson *et al.*, 2018). Another option for the medicinal treatment of LTA₄ and LTB₄ is acebilustat (Elborn *et al.*, 2018). This idea is the same as that of ketoprofen and is now under research in a phase II study. However, acebilustat is not as commonly and widely used as ketoprofen; therefore, it should undergo more scrutiny regarding side effects and possible AEs.

6.5 Limitations

The study populations in our studies I-IV are small and have multiple treatment profiles. All of these factors cause difficulty in finding statistically significant results in our analyses. Not all patients were able to attend all study visits and undergo all study measurements as planned, which further decreases the statistical power of the analysis. Additionally, the measurement protocol and the data gathered during the study visits have evolved over the years, making it difficult to compare results from different studies or within a study. The lack of validated questionnaire of lymphedema symptoms and quality of life (except study IV) is a limitation, since lymphedema symptoms may vary and, as a result, it is difficult to objectively measure them.

7 Future Directions

Lymphedema treatment has the potential for future inventions (Brown *et al.*, 2023). Some of these possibilities include futuristic ideas, such as organ on-chip, bioengineering, 3D-printing, and stem cell therapy (Nosenko *et al.*, 2016; Rauniyar *et al.*, 2018; Li *et al.*, 2020; Ogunleye *et al.*, 2020; Sung *et al.*, 2022).

Prophylactic lymphatic surgery is also a new idea (Ciudad *et al.*, 2022). The reasoning for prophylactic lymphatic surgery is to prevent lymphedema to ever happen and thus preventing the possibly devastating and debilitating symptoms of lymphedema (Ciudad *et al.* 2022). In prophylactic lymphatic surgery, the lymphatic vessels of the axilla are visualized during the sentinel lymph node biopsy or lymph node evacuation with ICG-L and if a lymphatic vessel disruption is seen, and a suitable lymphatic vessel and a vein for LVA are present, LVA is performed to prevent the disruption of lymphatic flow and lymphedema that follows. The incidence of lymphedema reduced in the study by Ciudad *et al.* (Ciudad *et al.*, 2022), but it did not eradicate lymphedema. Therefore, it is not yet established as a working method (Ciudad *et al.* 2022).

Organ-on-chip is a method in which a microenvironment is developed to mimic the *in vivo* environment of the human body. This allows for *in vivo*-like studies of drugs and other medicinal products without the ethical burden of testing in humans. (Nosenko *et al.*, 2016) Organ on-chip could help to identify the medicinal treatments that should proceed to human trials more quickly than the conventional processes of medicine development.

Bioengineering has some implications; however, the boundaries of its possibilities are not yet known. Bioengineering may provide a way to enhance the results of lymphatic surgery or even treat lymphedema without surgery (Weitman *et al.*, 2013; Sung *et al.*, 2022). Bioengineering suits lymphedema treatment quite well, at least in theory, since the treatment of lymphedema and alleviation of its symptoms requires changes in immunological processes. Bioengineering can enhance immunological changes to a greater extent than surgery alone. However, bioengineering should be performed safely and AEs should be known before the use of any method. This requires longitudinal and extensive further research; therefore, conclusive results are yet to be obtained.

3D-printing is also a promising and interesting method (Ogunleye *et al.*, 2020; Lee *et al.*, 2023). If 3D-printing is available and able to provide functional lymphatic structures, the reconstructive possibilities increase exponentially because a donor site is no longer needed and the limits of the human body would be stretched. There are already investigational biomimetic 3D-printers, that can produce soft tissue, cartilage and bone tissue (Brinter®, Santa Monica, CA, USA/ Turku, Finland). This 3D-printing is interesting, because it eliminates the need for donor site and the risk of donor site morbidity. Normalization of the anatomy after iatrogenic damage could possibly be performed with lesser risks and recovery time. If 3D-printing can be performed with the patient's own cells, the lymphatic graft would also be an autologous graft with no risk of graft-versus-host response and no need for immunosuppressants, as is the case for in allografts. 3D-printing could also be a never-ending supply for grafts if a patient loses a previous graft for some reason. However, the reason for graft failure should be addressed before using a 3D-printed graft again. 3D-printing does not remove any excess tissue from the affected area, and the product must be inserted surgically, so 3D-printing alone cannot be a solution for lymphedema treatment. It could potentially be utilized either in conjunction with VLNT or as a component of LVA surgery.

There is also an ongoing study of hydrophobic tubes as in silicone tubes inserted into the affected limb for draining the limb of excess fluid in severe cases of lower limb lymphedema (Olszewski *et al.* 2015). Results showed a decrease in the width of the limb two weeks after the surgery; however, the results varied, and the number of patients was small (20 patients, only eight with a follow-up of two years). Silicone tubes require a collaborative force, for example, intermittent pneumatic compression or compression garments (Olszewski *et al.* 2015). This method might not bring any new innovations to the field of lymphedema treatment, since it seems quite invasive method for mild improvements and require continuing vigorous compression garment use.

Stem cell therapy has also shown beneficial results in some studies on other disease entities, but it might enhance the ability to regenerate and heal after iatrogenic damage or reconstructive surgery (Li *et al.*, 2020; Antonio Jorge Forte *et al.*, 2021). Stem cells may alter the immunological environment in a manner similar to that of the medicinal treatment of lymphedema.

As it has been established that lymphedema is a multifactorial disease, it is unlikely that just one treatment modality is going to cure lymphedema. VLNT helps with mechanical obstruction and brings healthy lymph nodes and adipose tissue with positive cytokines and stem cells to the affected area, but medicinal treatment could enhance the results by boosting the alteration of the immunological environment towards prolymphangiogenic. In addition, liposuction may be required in later stages of lymphedema to remove excess adipose and connective tissue and to normalize the

width of the affected arm. However, organ-on-chip research could steer bioengineering research into safe waters, and as a result, provide a treatment that improves the composition of the affected arm and facilitates a better response to any of the surgical methods.

8 Summary

This thesis has added information on VLNT and the cytokine response underlying lymphedema development. VLNT is safe and more often provides an aid for lymphedema symptoms, such as pain and discomfort even when the objective measurements are not showing benefit from the surgery. Donor site lymphedema symptoms were not observed in our studies. A few patients had abnormal TI values and +16,8% volume difference of the arms but none of the patients' experienced symptoms of donor site lymphedema.

Proper patient selection is a key component of successful VLNT, since patients with a shorter preoperative duration of lymphedema and milder stage of lymphedema seem to benefit from VLNT more. They have more beneficial cytokine response and less cellulitis postoperatively. The awareness of lymphedema after breast cancer treatment should be increased among breast cancer patients and general practitioners. The sooner we diagnose a patient with lymphedema and start conservative treatment, the better the chances we have to cure lymphedema by VLNT or other treatments afterwards. Additionally, the lymphatic system should be taken into consideration during breast cancer surgery and avoid all unnecessary surgical trauma.

VLNT improves the symptoms and lymphatic function of the affected arm, but growth factor treatment seems to further improve the composition of the affected arm, thereby improving the quality of life of the patients. Further research may pave the way for the implementation of growth factor treatment and other medicinal therapies in the management of lymphedema.

The indications for axillary surgery in breast cancer treatment are changing and the trend is to avoid axillary surgery whenever it is feasible (Gentilini *et al.*, 2023). This most welcome de-escalation of axillary surgery is interesting also from a lymphedema research point-of-view. Since axillary surgery is a known risk factor for lymphedema, the number of patients with lymphedema is supposed to decrease. (Gentilini *et al.*, 2023) However, lymphedema is not going to be eradicated, and more subtle indicators or risk factors for lymphedema development must be identified to prevent and treat lymphedema also in the future.

A summary of the findings of this thesis is available in figure 22.

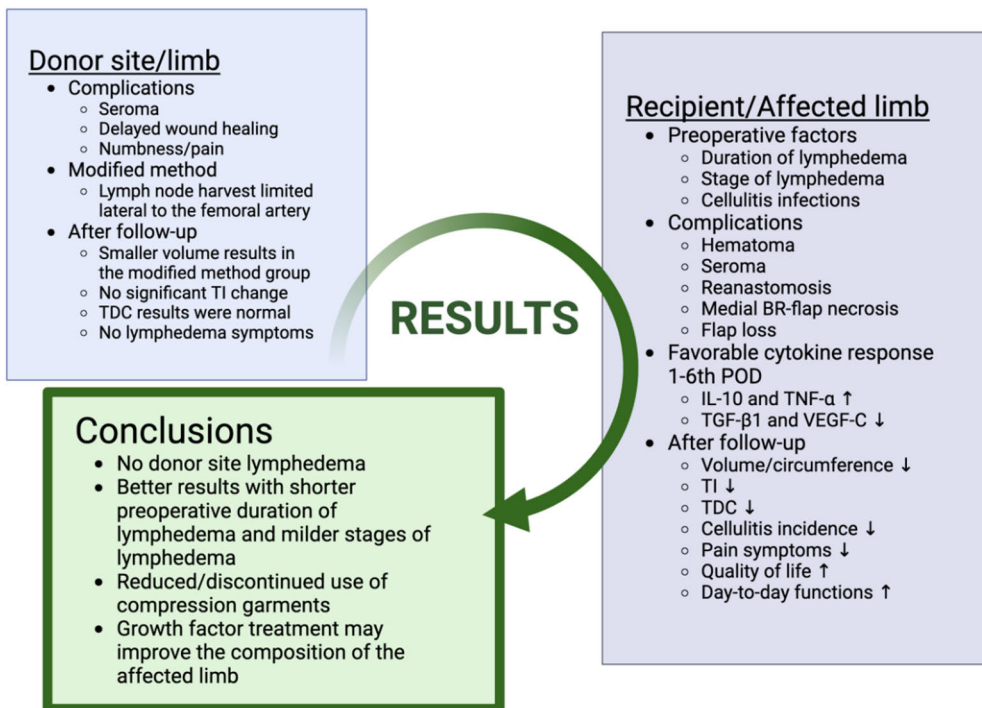


Figure 22. Summary of the findings in this dissertation. Figure includes explanation of the most frequent complications, difference of surgical method, and the key results of the donor-site in left blue box; the preoperative factors influencing the results, most frequent complications and the cytokine response after VLNT, and the key findings in the recipient site in the right-side blue box. Green box include the conclusions of this thesis. Created by author in Biorender.com.

9 Conclusions

- I After VLNT surgery, no lymphedema was detected at the donor site (study I).
- II Patients with a shorter preoperative duration and milder stages of lymphedema had better results (studies II and III).
- III After undergoing VLNT surgery, the necessity for using compression garments was decreased or eliminated (studies II and III).
- IV Combining VLNT with medicinal growth factor treatment may improve the composition of the affected arm (study IV).

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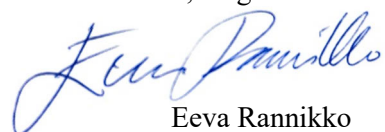
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