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Liposuction of arm and leg lymphoedema

Tissue composition alterations and treatment outcomes

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Liposuction of lymphoedema

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Tobias Karlsson, MD

DOCTORAL DISSERTATION

Clinical Medicine with Focus on Plastic Surgery

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Liposuction of arm and leg lymphoedema – Tissue composition alterations and treatment outcomes

Abstract
Introduction*:* Lymphoedema is a condition most often caused by parasitic infections, trauma, genetic variations or cancer treatment with lymph node dissection. A known consequence of lymphoedema is the formation of adipose tissue in the affected region. In addition, previous studies have indicated excess muscle volume in the affected limb. Liposuction is a method that has been proved to be a safe alternative for removing the excess adipose tissue, and when combined with CCT, the results are maintained over time. In addition, liposuction has been found to decrease the incidence of erysipelas in arm lymphoedema by 87%. The aim of this thesis was to evaluate a possible reduction of erysipelas following liposuction of leg lymphoedema, and assess the tissue composition and characteristics of late-stage lymphoedema in the arm, leg and the gluteal region, and to estimate the long-term outcome of liposuction for arm and leg lymphoedema at two different centres.

Methods*:* Study I: Indocyanine green (ICG) lymphography images were analysed for 28 patients with leg lymphoedema, and dermal backflow to the gluteal region was used as a sign of gluteal lymphoedema. Magnetic resonance imaging (MRI) of the gluteal region was assessed to identify tissue changes in these patients. Study II. The incidence of erysipelas was evaluated before and after liposuction combined with CCT for 124 patients with leg lymphoedema. Study III: Dual energy X-ray absorptiometry (DXA) was used to measure fat, lean and bone in 18 patients with breast cancer-related lymphoedema (BCRL) before and after liposuction. Study IV: MRI was used to measure the area of muscle compartments in 55 arm and leg lymphoedema at three and four positions along the limb, respectively. Studies V and VI: The results of liposuction in combination with CCT for arm (study VI) and leg (study V and VI) lymphoedema were evaluated over a five-year period in Sweden (n=67) and Australia (n=59).

Results*:* Study I: Gluteal lymphoedema was a common condition in late-stage leg lymphoedema (10/28) and might be associated with excess adipose tissue and skin hypertrophy in the gluteal region. Study II: The incidence of erysipelas decreased by 67% after liposuction in combination with CCT from 0.20 bouts/person/year to 0.07 bouts/person/year. Study III: The median 704 ml (interquartile range (IQR):545 – 885) increase in adipose tissue and 651 ml (IQR: 475 – 880) increase in lean volume decreased by 139% (IQR: 125-151) and 54% (IQR: 24 – 83) one year after liposuction, respectively. Study IV: No increase in muscle area in lymphoedematous limbs was generally seen except from one level in arm lymphoedema when the dominant arm was affected. Study V: The 3515 ml (IQR: 2225 – 5455) ml excess volume of the affected leg decreased by 101% (IQR: 84 – 116), p<0.001, one year and 115% (IQR: 98 – 124), p<0.001, five years after liposuction and CCT in Sweden. Study VI: In Australia, the 1061 ml (IQR: 763 – 1599) excess volume in arm lymphoedema decreased by 95% (IQR: 81 – 119) after one year and 98% (IQR: 74 – 120) after five years. For legs, the excess volume of 3447 ml (IQR: 2065 – 5656) decreased by 90% (IQR: 71 – 104) after one year and 72% (IQR: 55 – 91) after five years. However, a large loss to follow-up was seen after five years.

Conclusions*:* DXA showed muscle volume increase in the affected arm that decreased after liposuction. On the other hand, MRI analysis did not find a general increase in muscle area in late-stage lymphoedema, which might be attributed to the presence of muscle tissue increase in only a subpopulation of patients, and/or a possible increase in skin, fluid and fibrosis. Also, gluteal lymphoedema could be a topic for future research. Liposuction is a safe and effective method for reducing excess volume with the results maintained with CCT. In addition to significant volume reductions, the treatment significantly reduced the incidence of erysipelas.

Key words: Lymphoedema, liposuction, gluteal, tissue composition, MRI, ICG, muscle, adipose tissue

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MADE IN SWEDEN

To Louise, Theodor and Noah

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Preface

My endeavours as a doctoral student began at Lund University in March 2018 with Professor Håkan Brorson as my Principal Supervisor. At that time, a former PhD student within the same research group had just finished his thesis and passed his dissertation. Dr Mattias Hoffner's thesis focused mainly on liposuction for arm lymphoedema, and the results presented were regarding reduction in limb volume, skin infections, quality of life, and adipose tissue deposition. We did not know if these results were analogous for leg lymphoedema patients and the focus of my thesis started to take form, with Dr Mattias Hoffner and Professor Jonas Manjer as associate supervisors. The plan for the thesis was formed, and included, in addition to the three papers conducted at Lund University presented here, one project evaluating quality of life after liposuction and controlled compression therapy (CCT) and one project evaluating wear and tear of compression garments. Shortly thereafter, a possibility to apply for a Cotutelle Joint PhD with a research team at Macquarie University in Sydney appeared since Håkan had prior close collaboration with them. This challenge and the possibility for international collaboration led to a thorough application process, which resulted in me being officially enrolled as a PhD student at Macquarie University in August 2020 with Associate Professor Thomas Lam as my principal supervisor. The collaboration resulted in the possibility to explore other projects and research questions. Since we had made good progress in three projects, these were chosen to be included in the thesis and two regarding quality of life and compression garments were excluded. The project concerned with quality of life has particular importance for patients with leg lymphoedema treated with liposuction and CCT, and will be conducted separately from this thesis.

Previous research from the team at Macquarie University focused on lymphatic drainage patterns in leg lymphoedema and the findings showed a frequent pattern of drainage to the gluteal region. However, descriptions of lymphoedema in the gluteal region were rare in the literature. In addition, the progress in study III resulted in questions regarding the presence of increased muscle tissue in late-stage lymphoedema, possibly due to heaviness of the limb, with no consensus in the literature. The possibility to evaluate magnetic resonance imaging data at Macquarie University unlocked the opportunity to get more insight into this question. Therefore, three additional projects were chosen to be completed at Macquarie University, leading to this final thesis.

Context of this Thesis

The first part of the thesis was conducted as a part-time student at Lund University together with the Lymphoedema Research Team at the Department of Plastic and Reconstructive Surgery, Skåne University Hospital in Malmö, Sweden. When the Cotutelle Joint PhD was initiated, I had more time to conduct research full-time. The agreement between the universities stated that at least one year of the research must be conducted at each of the institutions and I planned to work in Sydney between August 2020 and August 2021. However, the COVID-19 pandemic resulted in border closures and travel restrictions, obliging me to work remotely from Sweden instead. The projects were thus modified so they could be conducted from abroad. When the border restrictions ceased, I had the great pleasure of travelling to Sydney and to visit the team I had worked with during this time and experience their clinical practice.

The format for a thesis at Lund University differs slightly from the thesis format at Macquarie University. Therefore, this thesis includes mandatory sections from each format and has been submitted for examination in similar form, with the exact same content, at each institution.

Abstract

Introduction

Lymphoedema is a condition most often caused by parasitic infections, trauma, genetic variations or cancer treatment with lymph node dissection. A known consequence of lymphoedema is the formation of adipose tissue in the affected region. In addition, previous studies have indicated excess muscle volume in the affected limb. Liposuction is a method that has been proved to be a safe alternative for removing the excess adipose tissue, and when combined with CCT, the results are maintained over time. In addition, liposuction has been found to decrease the incidence of erysipelas in arm lymphoedema by 87%. The aim of this thesis was to evaluate a possible reduction of erysipelas following liposuction of leg lymphoedema, and assess the tissue composition and characteristics of late-stage lymphoedema in the arm, leg and the gluteal region, and to estimate the long-term outcome of liposuction for arm and leg lymphoedema at two different centres.

Methods

Study I: Indocyanine green (ICG) lymphography images were analysed for 28 patients with leg lymphoedema, and dermal backflow to the gluteal region was used as a sign of gluteal lymphoedema. Magnetic resonance imaging (MRI) of the gluteal region was assessed to identify tissue changes in these patients.

Study II. The incidence of erysipelas was evaluated before and after liposuction combined with CCT for 124 patients with leg lymphoedema.

Study III: Dual energy X-ray absorptiometry (DXA) was used to measure fat, lean and bone in 18 patients with breast cancer-related lymphoedema (BCRL) before and after liposuction.

Study IV: MRI was used to measure the area of muscle compartments in 55 arm and leg lymphoedema at three and four positions along the limb, respectively.

Studies V and **VI**: The results of liposuction in combination with CCT for arm (study VI) and leg (study V and VI) lymphoedema were evaluated over a five-year period in Sweden (n=67) and Australia (n=59).

Results

Study I: Gluteal lymphoedema was a common condition in late-stage leg lymphoedema (10/28) and might be associated with excess adipose tissue and skin hypertrophy in the gluteal region.

Study II: The incidence of erysipelas decreased by 67% after liposuction in combination with CCT from 0.20 bouts/person/year to 0.07 bouts/person/year.

Study III: The median 704 ml (interquartile range (IQR):545 – 885) increase in adipose tissue and 651 ml (IQR: 475 – 880) increase in lean volume decreased by 139% (IQR: 125-151) and 54% (IQR: 24 – 83) one year after liposuction, respectively.

Study IV: No increase in muscle area in lymphoedematous limbs was generally seen except from one level in arm lymphoedema when the dominant arm was affected.

Study V: The 3515 ml (IQR: 2225 – 5455) ml excess volume of the affected leg decreased by 101% (IQR: 84 – 116), p<0.001, one year and 115% (IQR: 98 – 124), p<0.001, five years after liposuction and CCT in Sweden.

Study VI: In Australia, the 1061 ml (IQR: 763 – 1599) excess volume in arm lymphoedema decreased by 95% (IQR: 81 – 119) after one year and 98% (IQR: 74 – 120) after five years. For legs, the excess volume of 3447 ml (IQR: 2065 – 5656) decreased by 90% (IQR: 71 – 104) after one year and 72% (IQR: 55 – 91) after five years. However, a large loss to follow-up was seen after five years.

Conclusions

DXA showed muscle volume increase in the affected arm that decreased after liposuction. On the other hand, MRI analysis did not find a general increase in muscle area in late-stage lymphoedema, which might be attributed to the presence of muscle tissue increase in only a subpopulation of patients, and/or a possible increase in skin, fluid and fibrosis. Also, gluteal lymphoedema could be a topic for future research. Liposuction is a safe and effective method for reducing excess volume with the results maintained with CCT. In addition to significant volume reductions, the treatment significantly reduced the incidence of erysipelas.

Candidate's Statement

This thesis entitled "Liposuction of arm and leg lymphoedema – Tissue composition alterations and treatment outcomes" is being submitted to Macquarie University and Lund University in accordance with the Cotutelle agreement dated Nov 29th, 2019. No part of the thesis has previously been submitted as part of the requirements for a degree to any other academic institution.

I certify that the thesis has been written by me and to the best of my knowledge and belief, it contains no material previously published or written by another person, unless clearly stated. Also, all sources of information and used literature have been properly acknowledged.

The research has been approved by appropriate institutional human research ethics committees as stated below:

- Study I – Approval from Macquarie University Health Clinical Innovation and Audit Committee (MQCIAC2020001A_2021).
- Study II – Approval from the Swedish Ethical Review Authority (2020-03102).
- Study III – Approval from the Ethics of Human Investigation Committee of Lund University (697 98), and the Radiation Protection Committee of Skåne University Hospital in Malmö (981212)
- Study IV – Approval from Macquarie University Health Clinical Innovation and Audit Committee (MQCIAC2020001A_2021)
- Study V – Approval from the Swedish Ethical Review Authority (2020-03102)
- Study VI – Approval from Macquarie University Human Research Ethics Application (reference numbers: 5201300315 and 52020613914268)

Tobias Karlsson

Signed:__________________________________

List of Papers

I. Gluteal lymphoedema associated with lower extremity lymphoedema. – a preliminary study with indocyanine green lymphography and magnetic resonance imaging.

   Karlsson T, Mackie H, Ho-Shon K, Blackwell R, Weydon-White A, Koelmeyer L, Suami H.

   Journal of Plastic, Reconstructive and Aesthetic Surgery 2022 Oct 18; 20. 88-93 doi: https://doi.org/10.1016/j.bjps.2022.10.029

II. Liposuction and controlled compression therapy reduce the erysipelas incidence in primary and secondary lymphedema.

   Karlsson T, Hoffner M, Brorson H.

   Plastic and Reconstructive Surgery Global Open 2022 May 6; 10(5). doi: 10.1097/GOX.0000000000004314.

III. Liposuction of breast cancer-related arm lymphedema reduces fat and muscle hypertrophy.

   Karlsson T, Karlsson M, Ohlin K, Olsson G, Brorson H.

   Lymphatic Research and Biology 2022 Feb 20(1): 53-63 doi: 10.1089/lrb.2020.0120

IV. Segmental area-based measurements of the muscular compartment in late-stage lymphoedema in the arms and legs.

   Karlsson T, Mackie H, Koelmeyer L, Lam T, Brorson H, Suami H.

   In manuscript

V. Complete reduction of leg lymphoedema following liposuction – a five-year prospective study in 67 patients without recurrence.

   Karlsson T, Hoffner M, Ohlin K, Svensson B, Brorson H.

   Submitted to *Plastic and Reconstructive Surgery*

VI. Liposuction for advanced lymphedema in a multidisciplinary team setting in Australia – five-year follow-up.

Karlsson T, Mackie H, Koelmeyer L, Heydon-White A, Ricketts R, Toyer K, Boyages J, Brorson H, Lam T.

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Abstracts Resulting from this Thesis

Karlsson T, Ohlin K, Svensson B, Hoffner M, Brorson H. Long-term outcomes of liposuction and controlled compression therapy for primary and secondary lymphedema in leg. *27th ISL World Congress of Lymphology*, September 23-26, 2019, Buenos Aires/Iguazú, Argentina, USA.

Karlsson T, Ohlin K, Svensson B, Hoffner M, Brorson. Liposuction of breast cancer-related arm lymphedema effectively reduces excess fat and decreases muscle hypertrophy measured with Dual Energy X-ray Absorptiometry. *27th ISL World Congress of Lymphology*, September 23-26, 2019, Buenos Aires/Iguazú, Argentina, USA.

Karlsson T, Brorson H. Long-term Outcomes of Liposuction and Controlled Compression Therapy for Primary and Secondary Lower Extremity Lymphedema. *Kirurgveckan*, August 23-27, 2021, Göteborg, Sweden.

Karlsson T, Karlsson M, Ohlin K, Olsson G, Brorson H. Liposuction of Breast Cancer-Related Arm Lymphedema Reduces Fat and Muscle Hypertrophy. *Kirurgveckan*, August 23-27, 2021, Göteborg, Sweden.

Karlsson T, Hoffner M, Brorson H. Liposuction in Combination with Controlled Compression Therapy Reduces the Incidence of Erysipelas in Primary and Secondary Lower Extremity Lymphedemas. *Kirurgveckan*, August 23-27, 2021, Göteborg, Sweden.

Karlsson T, Hoffner M, Brorson H. Liposuction in Combination with Controlled Compression Therapy Reduces the Incidence of Erysipelas in Primary and Secondary Lower Extremity Lymphedemas. *28th ISL World Congress of Lymphology*, September 20-24, 2021, Athens, Greece.

Karlsson T, Mackie, H, Ho-Shon K, Suami H. Gluteal Lymphoedema Associated with Advanced Lower Extremity Lymphoedema. - A Study with Indocyanine Green Lymphography and Magnetic Resonance Imaging. *28th ISL World Congress of Lymphology*, September 20-24, 2021, Athens, Greece.

Karlsson T, Mackie, H, Ho-Shon K, Blackwell R, Heydon-White A, Koelmeyer L, Suami H. Gluteal Lymphoedema Associated with Advanced Lower Extremity Lymphoedema. - A Study with Indocyanine Green Lymphography and Magnetic

Resonance Imaging. *14th Australasian Lymphology Association Conference*, May 26-28, Hobart, Australia.

Karlsson T, Mackie, H, Koelmeyer L, Heydon-White A, Ricketts R, Toyer K, Boyages J, Lam T. Liposuction for Lymphedema at Macquarie University Hospital ALERT programme – Five Years of Experience. *14th Australasian Lymphology Association Conference*, May 26-28, Hobart, Australia.

Grants and Awards

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- International Cotutelle Macquarie University Research Excellence Scholarship
- The Swedish Cancer Society, Stockholm
- Skåne County Council's Research and Development Foundation

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- Presidential Prize, *27th ISL World Congress of Lymphology*, September 23-26, 2019, Buenos Aires/Iguazú, Argentina, USA.
- Presidential Prize, *28th ISL World Congress of Lymphology*, September 20-24, 2021, Athens, Greece.

Svensk Sammanfattning

Lymfödem i armar eller ben är en svullnad som orsakas av ett flödeshinder i lymfcirkulationen, cirkulationen som transporterar näringsrik vätska från vävnaden tillbaka till blodcirkulationen. Lymfödem kan succesivt tillväxa och bli mycket stora och kallas i sitt slutstadium för elefantiasis på grund av analogin till en elefants ben. De vanligaste orsakerna till lymfödem är parasitinfektion, trauma eller kirurgisk behandling för cancer. Cirka var tredje kvinna som behandlas kirurgiskt för bröstcancer eller gynekologisk cancer utvecklar lymfödem. Dessutom kan lymfödem vara medfödda.

Tidigare resultat från vår forskargrupp visar att lymfödem redan inom ett par år efter debuten leder till fettvävsnybildning, vilket tidigare ej varit känt. Historiskt har lymfödem oftast behandlats med kompression (strumpa eller linda), massage riktat mot vätskekomponenten och/eller kirurgiskt avlägsnande av vävnad med skalpell. De tidiga kirurgiska metoderna var ofta associerade med allvarliga komplikationer såsom lymffistlar, kraftig ärrvävnad, sårbildning och rosfeber. Amputation var i vissa fall nödvändigt på grund av dessa komplikationer. Först efter införandet av fettsugningsmetoden har överskottsfett vid lymfödem kunnat avlägsnas på ett säkert och effektivt sätt. Metoden har ett starkt stöd i litteraturen och resulterar i att patienten blir av med överskottsvolymen i den drabbade armen eller benet med signifikant ökad livskvalitet. Med hjälp av kompressionsstrumpor bibehålls resultatet över tid.

Avhandlingen baseras på sex delarbeten som bland annat utvärderar långtidsresultat av fettsugning som behandling av lymfödem. Därtill har vävnadssammansättning och karaktär av lymfödem studerats i syfte att få en bättre kunskap om tillståndet för att i framtiden kunna optimera behandlingen. Avhandlingen är ett samarbete mellan två ledande kliniker som erbjuder behandling för lymfödem, plastikkirurgiska kliniken vid Skånes universitetssjukhus i Malmö samt Australian Lymphoedema Education, Research and Treatment vid Macquarie University i Sydney, Australien.

Resultat från delarbetena genomförda vid kliniken i Malmö visar sammanfattat att fettsugning vid benlymfödem i genomsnitt leder till komplett reduktion av överskottsvolymen, där resultaten är väl bibehållna över en uppföljningsperiod på fem år med hjälp av kompressionsbehandling. I tillägg till volymreduktionen ses även en minskning av antalet rosfeberattacker med 67% efter behandlingen, vilket är viktigt då rosfeber är en vanlig komplikation vid lymfödem.

En näst intill komplett reduktion av överskottsvolymen för arm- och benlymfödem ses i ett delarbete som utvärderar fettsugning och kompressionsbehandling genomförd på kliniken i Sydney. Där bibehålls resultaten för armlymfödem väl över uppföljningsperioden på fem år, men på grund av ett lågt antal patienter med benlymfödem vid långtidsuppföljningen är det svårt att dra slutsatser om långtidsresultaten för denna patientgrupp. Vissa faktorer skiljer sig mellan Sverige och Australien, vilket kan påverka resultaten av behandling, t ex kostnad för kompressionsstrumpor och klimatets påverkan på kompressionsbehandlingen.

Resultat från övriga delarbeten, där olika avbildningsmetoder använts för att analysera arm- och benlymfödem, visar att benlymfödem har en tendens till att sprida sig till stussen. Vilken effekt och vilka konsekvenser detta har behöver utredas ytterligare. I ett delarbete har vi visat att överskottsvolymen av fett vid armlymfödem i genomsnitt minskade med 139% ett år efter fettsugning. Tidigare studier från vår forskargrupp har visat att muskelvolymen är ökad vid större armlymfödem och ett delarbete visar att vad som sannolikt motsvarar överskott i muskelvolym minskar med 54% ett år efter fettsugning av armlymfödem. Bakomliggande teori är att tyngden av lymfödemet (vätska och fett) leder till att armen eller benet ökar i vikt, vilket leder till ökad belastning på musklerna och därmed ökad volym. Muskelarea i olika skikt av arm- och benlymfödem har undersökts i ett separat delarbete i avhandlingen med hjälp av magnetisk resonansavbildning inom ett begränsat område och en ökning av muskelarea kunde, i motsats till tidigare resultat där muskelvolym i hela armen beräknats, ej ses för större lymfödem.

Sammantaget visar delarbetena i avhandlingen att fettsugning är en säker och effektiv metod för att reducera överskottsvolym för arm- och benlymfödem och samtidigt minskas risken för att utveckla rosfeber. Förhoppningsvis kan avhandlingens tydliga resultat påverka vården i en riktning där fler kliniker kan erbjuda behandlingen och därmed fler patienter kan erhålla en behandling med ökad livskvalitet och minskad symtombörda relaterade till lymfödem.

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My most sincere appreciations go to my wife and kids who have put up with me spending many weekends and evenings living and breathing lymphology. It is not easy being married to a physician. Early mornings, long hours, evenings and weekends on call, vacations away alone on conferences. And when the kids have gone to sleep and the kitchen has been cleaned, PubMed gets most of the attention. I look forward to spending more evenings and weekends giving you all my attention without having to think about studying.

Thanks go to Håkan Brorson who has supported me with a spark of enthusiasm and commitment never before seen in a doctoral supervisor. To Thomas Lam who welcomed the burden of supervising me, a student from the other side of the world he had never met, while being a consultant plastic surgeon in four different hospitals and being a father. To Mattias Hoffner, for lovely meetings and great advice along the way. To Hiroo Suami, for many inspiring and motivating conversations on Zoom, for showing me the beauty of Sydney and demonstrating the stunning lymphatic pathways in reality. To Jonas Manjer for supporting my progress and taking the baton when Håkan retired. I have really enjoyed working with you all. Your supervision has resulted in this thesis and has taught me a lot.

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I thank the whole ALERT team, for being such a welcoming and kind group of colleagues. All of you were always keen to help me out and push me forward (both in my research and on the dance floor).

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Finally, I would like to thank my whole family and friends for supporting me on the long road from starting medical school to where I am today. I thank Surfligan for helping with checking weather reports when I was too busy.

I appreciate and thank every single one of you.

Abbreviations

ALERT	Australian Lymphoedema Education Research and Treatment
APC	Antigen Presenting Cells
BCRL	Breast Cancer-Related Lymphoedema
BIS	Bioimpedance Spectroscopy
BMI	Body Mass Index
CCL	Compression Class
CCT	Controlled Compression Therapy
CDT	Complex Decongestive Therapy
CI	Confidence Interval
CT	Computed Tomography
DXA	Dual energy X-ray Absorptiometry
ICG	Indocyanine Green
IFN-γ	Interferon-γ
IL	Interleukin
ISL	International Society of Lymphology
IQR	Interquartile Range
LDS	Lymphoedema Distichiasis Syndrome
LEL	Lower Extremity Lymphoedema
LTA_4	Leukotriene A_4
LTB_4	Leukotriene B_4
LVA	Lymphovenous Anastomosis
LYMPHA	Lymphatic Microsurgical Preventive Healing Approach
MLD	Manual Lymphatic Drainage
MRI	Magnetic Resonance Imaging

M-S	Mascagni-Sappey
TDC	Tissue Dialectic Constant
TGF-1	Transforming Growth Factor - 1
Th (1 and 2)	T-helper cells
Treg	Regulatory T Cells
VEGFR-C	Vascular Endothelial Growth Factor C
VEGFR-3	Vascular Endothelial Growth Factor Receptor 3
VLNT	Vascularised Lymph Node Transfer

Introduction

The main function of the lymphatic system includes fluid homeostasis, where the lymphatic system transports protein-rich fluid back to the venous system after the fluid is filtered from the arterial capillaries (Oliver, 2004; Alitalo *et al.*, 2005). Furthermore, it has an important role in the activation of an immune response when tissue barriers are breached by pathogens, by facilitating a highway for antigen-presenting cells (APCs) to interact with lymphocytes within lymph nodes (Szuba *et al.*, 2003). Thus, when the lymphatic system is damaged and the transport capacity impaired, fluid is trapped in the tissue and lymphoedema develops. Furthermore, the loss of function in the immune defence results in an increased risk for cutaneous infections such as erysipelas (Dupuy *et al.*, 1999).

The spectrum of lymphoedema manifestation ranges from the subclinical phase with reduced lymphatic flow without obvious swelling, to late-stage lymphoedema with deformed limb contours, excess adipose tissue deposition and subsequent fibrosis and deteriorated skin condition (Executive Committee of the International Society of Lymphology, 2020). In general, lymphoedema has historically not received the attention warranted, and well tolerated and effective treatments are scarce. Research in the field of lymphology have progressed and multidisciplinary centres focusing on the treatment of lymphoedema have been formed. Still, there is no clear consensus on the management of lymphoedema and interregional routines differ widely. To gain further knowledge and understanding of the underlying concepts and thoughts behind different treatment strategies, this thesis has been conducted at two leading centres offering the best possible treatment for patients who are suffering from lymphoedema.

History of research on the lymphatic system and its disorders

Research on the lymphatic system reaches back thousands of years to the ancient days of Hippocrates and can be found in the collection *Hippocratic Corpus*, dated between 500-300 BC, which is a series of books written by numerous authors (Crivellato *et al.*, 2007; Irschick *et al.*, 2019). The first description of lymphatic nodes and vessels is found in the work "on glands", where anatomical locations of lymph nodes are described along with theories about their function in fluid transportation and immunological responses (Crivellato *et al.*, 2007; Craik, 2009). The first description of lymphoedema is dated back to the ninth century when Ali Ibn Sahl Rabban al-Rabban in medieval Persia described the condition Daa al-Fil, meaning elephant disease (Golzari *et al.*, 2012). Important structures of the lymphatic system were found and described during the 16th and 17th centuries, including the thoracic duct and the interconnection between the lymphatics and the blood circulation at the angle between the jugular and subclavian vein (Irschick *et al.*, 2019). Another discovery during this time was made by Gaspar Asellius who found the lacteals draining chyle (lipid rich lymph absorbed from the intestine) from the intestine when performing surgery on a dog soon after it had eaten (Anderson, 1933). However, he could not correctly describe the origin and target for the movement of chyle and thought this fluid was drained from the pancreatic area to the liver. Furthermore, he found the appearance of the new vessels to differ from the arteries and veins and theorised about a separate circulation (Natale *et al.*, 2017). In the 1650s, the discovery that the lymphatics are connected as a system that drains fluid throughout the body was presented (Ambrose, 2006). However, an intense debate arose regarding who first made this finding as this concept was presented around the same time by two different authors. Olof Rudbeck from Uppsala, Sweden, researched the lymphatic system during his medical studies and presented the idea of a lymphatic system during his thesis defence in 1652, but did not publish his findings until 1653 in *Nova exercitatio anatomica exhibens ductus hepaticos aquosos et vasa glandularum serosa* (Ambrose, 2006; Natale *et al.*, 2017). During the same time, the lymphatics were investigated by a Danish professor, Caspar Bartholin, who presented the same theory in his work *Vasa Lymphatica nuper Hafaniae in animantibus inventa et hepatis exsequiae* in 1653, where the terminology for this vascular system, still currently used, was presented for the first time. It was declared that Rudbeck was probably the first to discover the function of the lymphatics, but nevertheless, Bartholin's book was internationally recognised, thus receiving the most acknowledgement.

In the 18th century, further important discoveries and conclusions were made. Fredrik Ruysch from the Netherlands could correctly depict the direction of flow in the lymphatics by demonstrating the structure and role of the lymphatic valves (Natale *et al.*, 2017). An accurate description of the function of the lymphatic system in relation to the blood circulation was presented by William Hunter, one of the famous Hunter brothers from England, when he observed dye not filling the lymphatics after arterial injection but filling the veins, unless dye was injected into the interstitial space. This gave him an understanding that the lymph vessels are separate from the blood vessels and drain the fluid from the tissues surrounding the blood circulation. A beautiful collection of drawings that illustrated the lymphatic system in detail were published in 1787 by anatomist Paolo Mascagni from Italy after years of study on human cadavers (Mascagni, 1787; Natale *et al.*, 2017).

Demonstrating the lymphatic system in the whole body, Mascagni's publication served as a beautiful and accurate atlas of the lymphatics.

Documented treatment strategies started to develop during the end of the 19th century and focused on what today are considered as conservative measures. Conservative treatment with physical therapy was introduced by von Winiwarter as early as 1892 (von Winiwarter, 1892) but the treatment currently in practice known as Complex Decongestive (Physio)therapy (CDT) was established by Földi in 1978 (Foldi *et al.*, 1985). CDT is a programme consisting of skin care, manual lymphatic drainage (MLD), bandaging and exercise followed by a maintenance phase with continuous compression garment usage, skin care and MLD when necessary (Foldi *et al.*, 1985). Light massage was implemented in the method of Winiwarter, and the method of MLD was further developed in 1936 by Vodder and has since evolved into the method performed today (Foldi *et al.*, 1985; Kasseroller, 1998).

Surgical treatments for lymphoedema and elephantiasis were introduced at the beginning of the 20th century, mainly consisting of debulking procedures such as the Kondoleon surgery, first described in 1912 and later modified by Sistrunk (Sistrunk, 1918; Green, 1920). This procedure included long excisions longitudinal to the extremity where skin, subcutaneous tissue and deep fascia were resected, and the wound subsequently closed with sutures. Another procedure presented before the Kondoleon surgery was lymphangioplasty, described and performed by W. Sampson Handley in 1908, where silk threads were buried into the subcutis in order to facilitate drainage of lymphatic fluid through the silk thread, past the area of lymphatic blockage (Handley, 1908; Handley, 1910a, b). During the same period, an extensive debulking procedure was suggested by Major Harvelock Charles in 1912, which included excision of all tissue down to the muscular fascia where skin grafts were placed, but he never presented any results of the procedure (Charles, 1912; Dellon & Hoopes, 1977). Charles also described and performed a debulking procedure of large scrotal lymphoedema (Charles, 1901). Thereafter, a new method by John Homans was presented in 1936 where debulking of the lower leg was performed by staged excision of subcutaneous tissue down to the deep fascia (Homans, 1936). With this method, the overlying skin flaps were spared and sutured on the deep fascia to reduce the possibility of fluid reaccumulating. Another method described by Noel Thompson in 1962 included wide excision of subcutaneous tissue with deep buried dermal flaps based on the basic idea that the flap would create a pathway for lymphatic drainage (Thompson, 1962; Thompson & Wee, 1980). The surgical procedures currently being performed for lymphoedema were introduced in the second half of the 20th century and most often include microsurgery, first suggested as lymphaticovenular anastomosis in 1962 (Jacobson & Suarez, 1962), and liposuction (Brorson & Svensson, 1997a, 1998; Brorson *et al.*, 2008), which will be described in detail in later sections.

Anatomy of the lymphatic system

Lymphatic development

Historically, two different theories on the embryology of the lymphatic system have primarily been acknowledged. The first of them, presented in 1902 by Florence Sabin, claims that the primary lymph sacs are composed of lymphatic endothelial cells originating from cellular budding from veins (Sabin, 1902). The lymphatic vessels are developed from the lymph sacs and protrude peripherally. This concept was established during studies on pig embryos where different dyes were injected into the embryos at different embryological stages. Thus, the lymphatic system in this theory has a venous origin, starting centrally and progressing peripherally. In a theory presented a few years later by Huntington and McClure in 1912, a non-venous origin of the lymphatics was suggested (Huntington & McClure, 1910). This theory, based on histological examinations of cat embryos, states that lymphatic endothelial cells are developed from mesenchymal cells and the vessels form from the union of mesenchymal cavities. In addition to the non-venous origin proposed, this theory differs from Sabin's model in the direction of development since Huntington and McClure suggested a peripheral to central direction of lymphatic growth. During recent years, much research on the origin of the lymphatic system has been conducted, which has provided new knowledge on the genetic factors contributing to lymphatic development. The finding of *Prox1* expression in budding lymphatic endothelial cells in mice models has advanced our understanding of lymphatic evolution (Wigle & Oliver, 1999; Wigle *et al.*, 2002). *Prox1* becomes present in a subpopulation of venous endothelial cells in the cardinal vein, which has been found to be the subpopulation of cells that end up budding to form the primary lymph sacs. Thus, the initiation of lymphatic formation occurs after the initiation of blood vascular system formation; however, these processes also progress simultaneously (Oliver, 2004). The finding of *Prox1* supports the theory of a venous origin proposed by Sabin, a theory that is now generally considered accurate. In addition to facilitating correct budding, *Prox1* has also been indicated to facilitate the transformation of blood vascular endothelial cells into lymphatic endothelial cells (Wigle & Oliver, 1999; Wigle *et al.*, 2002). In mice that have been genetically modified not to express *Prox1*, the resulting budding endothelial cells express blood vascular biomarkers, and the consequence is the absence of lymphatic formation. Further genes have been identified that are important for lymphatic endothelial cell formation, one of which is the vascular endothelial growth factor C (VEGF-C) and its receptor vascular endothelial growth factor receptor 3 (VEGFR-3) (Karkkainen et al., 2004). The presence of VEGF-C has been shown to work as a gradient for the direction of lymphatic endothelial cell migration after budding from the cardinal vein. In the absence of VEGF-C, the subgroup of endothelial cells in the cardinal vein wall do not bud to form the primary lymph sac. The well-defined effect of VEGF-C on lymphangiogenesis has led to attempts at treating lymphoedema with VEGF-C in combination with microsurgery, currently ongoing in a randomised double-blind clinical trial (Clinicaltrials.gov, NCT03658967). However, VEGF-C has also been shown to induce lymphangiogenesis within malignancies such as breast cancer, a process that seems to increase the rate of lymph node metastasis (Skobe *et al.*, 2001).

Since the discovery of the role of *Prox1* and VEGF-C, new evidence from several studies has suggested that the veins are not the only source of lymphatics (Semo *et al.*, 2016). For example, lymphatic endothelial cells in rostral lymph sacs in tadpoles have been shown to derive from lymphangioblasts (Ny *et al.*, 2005). Furthermore, specific angioblasts in the ventral cardinal vein in zebrafish have been found to develop into lymphatic endothelial cells as well as arterial and venous endothelial cells, thus not being venous in origin but still residing in the venous wall (Nicenboim *et al.*, 2015). These cells have been found to travel straight from the late plate mesoderm to the cardinal vein and seem to mature into lymphatic endothelial cells by a molecular pathway originating in the endoderm leading to *Prox1* expression. This signalling pathway has also been shown to exist in human vascular progenitors. Additionally to these findings, lymphangioblasts of unknown origin have been found to participate in the development in zebrafish facial lymphatics (Okuda *et al.*, 2012). Even in higher vertebrates such as the mouse, lymphatic progenitors of non-venous origins have been found, in this case in the lymphatic system of the heart (Klotz *et al.*, 2015). Although these discoveries question the sole venous origin theory of lymphatic vessels, it remains to be proved that these findings can be generalised to human lymphatic development.

Functional and structural lymphatic anatomy

To grasp the underlying pathology of lymphoedema and the possible approaches to treating this condition, it is crucial to understand the anatomy of the lymphatic system. As previously described, the lymphatics transport protein-rich fluid from tissues into the venous system, passing through lymph nodes on the way. On the other hand, one study showed that the protein concentration in lymph was lower than in plasma (Bates *et al.*, 1993). The fluid originates from the blood circulation and is filtered from the blood capillaries to the interstitium. Previously, it was thought that the filtration occurred on the arterial side of the capillary and due to Starling forces, there was a reabsorption of fluid on the venous side. However, newer evidence points to the fact that in most tissues under normal conditions there is a continuous filtration throughout the whole length of the capillary and the filtered fluid returns to the blood circulation exclusively through the lymphatic system (Levick & Michel, 2010; Mortimer & Rockson, 2014). The fluid enters the lymphatic system in the lymphatic capillary meshwork, composed of blind ended vessels surrounding the blood transporting capillaries located in the dermis. The lymphatic capillaries are composed of a single endothelial cell layer that have a scattered basal lamina and open interendothelial connections (Leak & Burke, 1968; Leak, 1970). Adjacent endothelial cells have scattered button-like connections where endothelial flaps overlap, leaving the space between junctions open for fluid, and possibly, cell transport (Baluk *et al.*, 2007). The endothelial cells are attached to the surrounding connective tissue by anchoring filaments (Leak & Burke, 1968; Leak, 1970). When fluid accumulates in the neighbouring tissue, expanding the connective tissue, the endothelial cells are pulled apart by the anchoring filaments leading to further opening of the intercellular junctions. Thus, fluid and particles can pour into the lumen of the lymphatic capillaries.

It is when fluid has entered the lymphatic system that it is first called lymph. From the capillary meshwork, the lymph flows to the deeper and larger lymphatic precollectors that have intraluminal valves, preventing the backwards flow of lymph to the capillaries (**Figure 1**). The precollectors drain lymph into the even larger lymphatic collectors that transport lymph to the lymph nodes. These larger lymphatic vessels also have intraluminal valves as well as a layer of smooth muscle cells that enables vessel contraction and lymph flow (Oliver, 2004). External to the smooth muscle layer, an outer layer of extracellular matrix and fat cells is present, which contains small blood vessels (Arkill *et al.*, 2010). In contrast to the connections between endothelial cells at the end of lymphatic capillary meshwork, lymphatic collectors have continuous zipper-like connections without the presence of the interendothelial openings found in lymphatic capillaries (Baluk *et al.*, 2007). Similar to cardiac, skeletal and smooth muscle cells, the contractility of lymphatic muscle cells seems to be driven by calcium channels, also called L-type calcium channels or I_{CaL} (Atchison & Johnston, 1997). In addition to calcium channels, ATP-sensitive potassium channels (Mizuno *et al.*, 1999) and calcium sensitive potassium channels (Cotton *et al.*, 1997) seem to play an important role in the contractility in lymphatic smooth muscle cells.

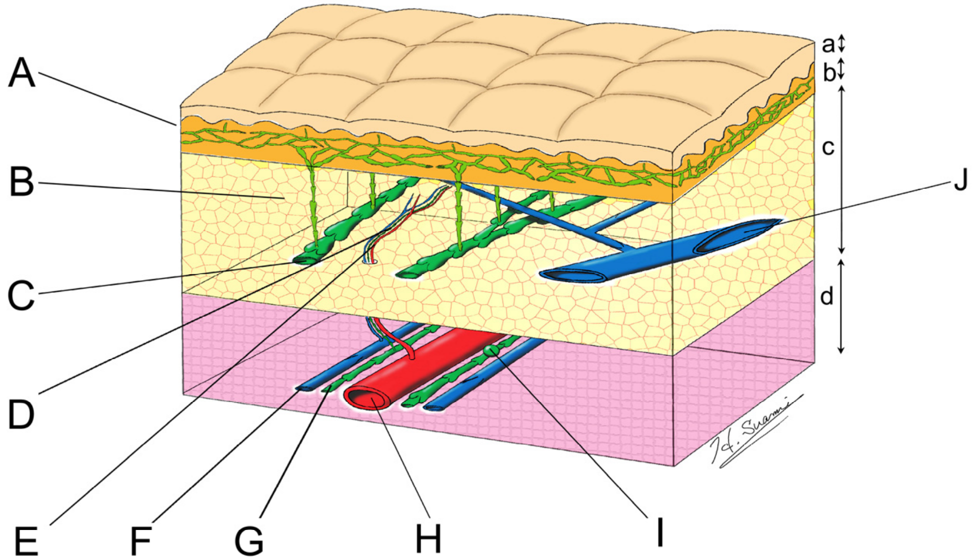

Figure 1. Illustration of the skin and the lymphatic system. A. Lymphatic capillary. B. Precollector. C. Superficial lymphatic collector. D. Perforating lymphatic vessel. E. Perforating artery. F. Deep vein. G. Deep lymphatic collector. H. Artery. I. Interval lymph node. J. Superficial vein. a. Epidermis. b. Dermis. c. Fat. d. Muscle. Produced and copyright by Hiroo Suami, reprinted with permission.

The segment between two valves is called a lymphangion, which is the functional unit that may either contract separately or uniformly with the neighbouring segments (Armenio *et al.*, 1981). Lymphangion contractions work in two dimensions by both narrowing the lumen as well as contracting longitudinally (Olszewski & Engeset, 1980). This mechanism is referred to as intrinsic contractility. A contraction phase occurs when pressure rises to a threshold level (varies widely) and consists of multiple contraction waves or pulsations. The frequency and strength of contractions seem to be augmented by lymphatic load (Smith, 1949; McHale & Roddie, 1976) and increase for example when the body is moved from a supine to a standing position as well as during muscular movements (Olszewski & Engeset, 1980). Furthermore, lymphatic vessels are innervated by the sympathetic nervous system with both α-receptors and β-receptors that increase or decrease lymphatic contraction, respectively (McHale, 1990). Further agents have been shown to influence contractions in human lymphatics, including prostaglandin $F\alpha_2$ and 5-hydroxytryptamine (Sjöberg & Steen, 1991). In addition to the intrinsic contractility, extrinsic forces also contribute to the propulsion of lymph along lymphatic vessels as well as into lymphatic vessels (Negrini *et al.*, 1994; Negrini *et al.*, 2004; Moriondo *et al.*, 2005). These forces include, among others, respiratory movements, heart and arterial pulsations and skeletal muscle actions, which result in an increased external pressure.

Lymphatic collectors are either located in the subcutaneous layer (superficial collecting vessels) or they run below the deep muscle fascia (deep collecting vessels), and these two systems run parallel to but separate from each other. The deep lymphatics are believed to account for transportation of around 10% of the total lymphatic fluid emerging from the extremities (Szuba & Rockson, 1997). Both the superficial and the deep collecting vessels connect with lymph nodes before returning the lymph back to the venous system. There are two types of lymph nodes, the regional lymph nodes, and the interval lymph nodes (Suami & Scaglioni, 2018). Regional lymph nodes are located in regions with multiple lymph nodes, such as inguinal lymph nodes and axillary lymph nodes, that connect with lymphatic pathways originating and draining from a specific area. The interval lymph nodes, such as the popliteal lymph node, are located along deep lymphatics in the limbs and are passed by the lymphatics as they continue towards the regional lymph nodes. The lymph reaches the lymph nodes through several afferent lymphatics, that each connect to a lymphoid lobule, the structural units that form the lymph node (Willard-Mack, 2006). The lymph flows from each afferent vessel and pass through multiple nodal sinuses, including the subcortical, transverse and medullary sinuses, and merge in a collective solitary efferent lymphatic vessel. The blood enters the lymph node through an artery adjacent to the efferent lymphatic vessel in the lymphatic hilus and flow to the lymphatic cortex, subsequently leaving the node through veins following the pathway of the arteries (Bélisle & Sainte-Marie, 1990). APCs enter lymph nodes through the afferent lymphatic system, containing antigens gathered in the tissue and presenting them to lymphocytes to evoke an immune response. APCs present their antigens to B-lymphocytes in round follicles located in the superficial cortex (Willard-Mack, 2006), whereas T-lymphocytes encounter antigens on APCs at the reticular network in the paracortex (Gretz *et al.*, 1996).

The lymphatics from the lower extremities originate in the foot and are divided into four different routes depending on where the lymphatics run past the ankle (Shinaoka *et al.*, 2020). These routes are the posteromedial, the anteromedial, the anterolateral and the posterolateral. The posterolateral pathway connects to a popliteal lymph node and joins the deep lymphatics. The other pathways normally connect to inguinal lymph nodes. The lymphatics of the lower extremities convene in the left and right lumbar trunks that, together with the intestinal trunk, form the thoracic duct that transports lymph from the lower body, left thorax and left arm, and returns it to the blood circulation at the angle between the left jugular vein and the left subclavian vein (Loukas *et al.*, 2007; Hsu & Itkin, 2016) (**Figure 2**). A widening of the thoracic duct can be found at the distal end called the *cisterna chyli*. This anatomical landmark measures about 2x1cm and is commonly located where the intestinal trunk and the left lumbar trunk join, but the anatomy varies widely (Loukas *et al.*, 2007).

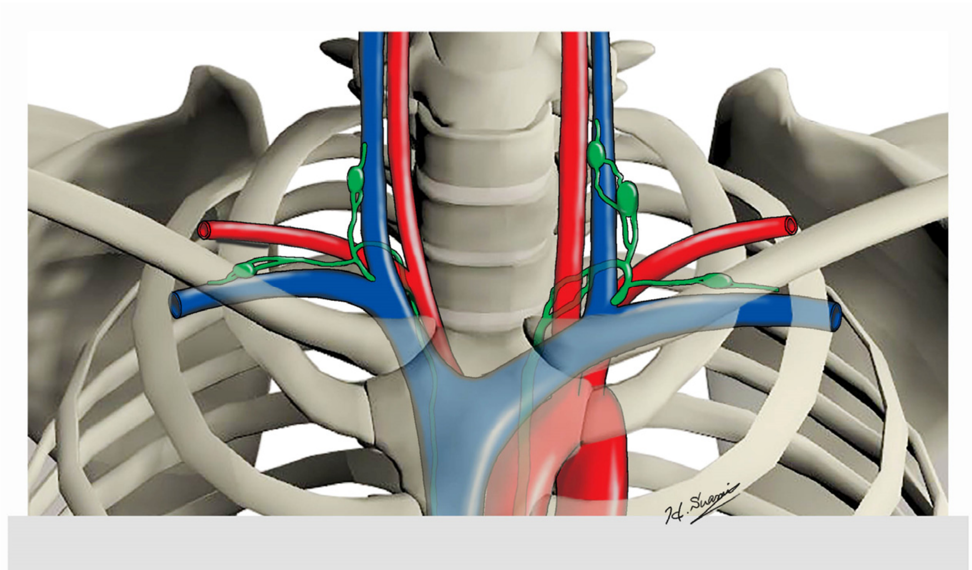

Figure 2. Illustration of the lymphatic and venous connections as the thoracic duct drains into the veins in the angle between the left jugular and subclavian vein. The lymphatics from the right arm, right hemithorax and right side of the head and neck drain into the angle between the right jugular and subclavian vein. Produced and copyright by Hiroo Suami, reprinted with permission.

In the upper extremities, the lymphatics originate in the hand and flow through lymphatic collectors that connect to lymph nodes in the axilla (Suami & Scaglioni, 2018). An additional pathway exists in some patients called the Mascagni-Sappey (M-S) delto-pectoral pathway running alongside the cephalic vein in the lateral upper aspect of the arm and most often draining into supraclavicular nodes (Mascagni, 1787; Sappey, 1874; Johnson *et al.*, 2020). This pathway was first illustrated in 1787 by Paulo Mascagni and again a century later by Philbert Constant Sappey and might present a natural defence against lymphoedema. The M-S pathway has been documented to exist in around three out of four women diagnosed with breast cancer (Johnson *et al.*, 2020). Another example of a lymphatic pathway that does not pass the axilla is the delto-tricipital pathway to the supraclavicular area (Amore *et al.*, 2016). An illustration of the major lymphatic drainage regions and the lymphatic pathways is shown in **Figure 3**.

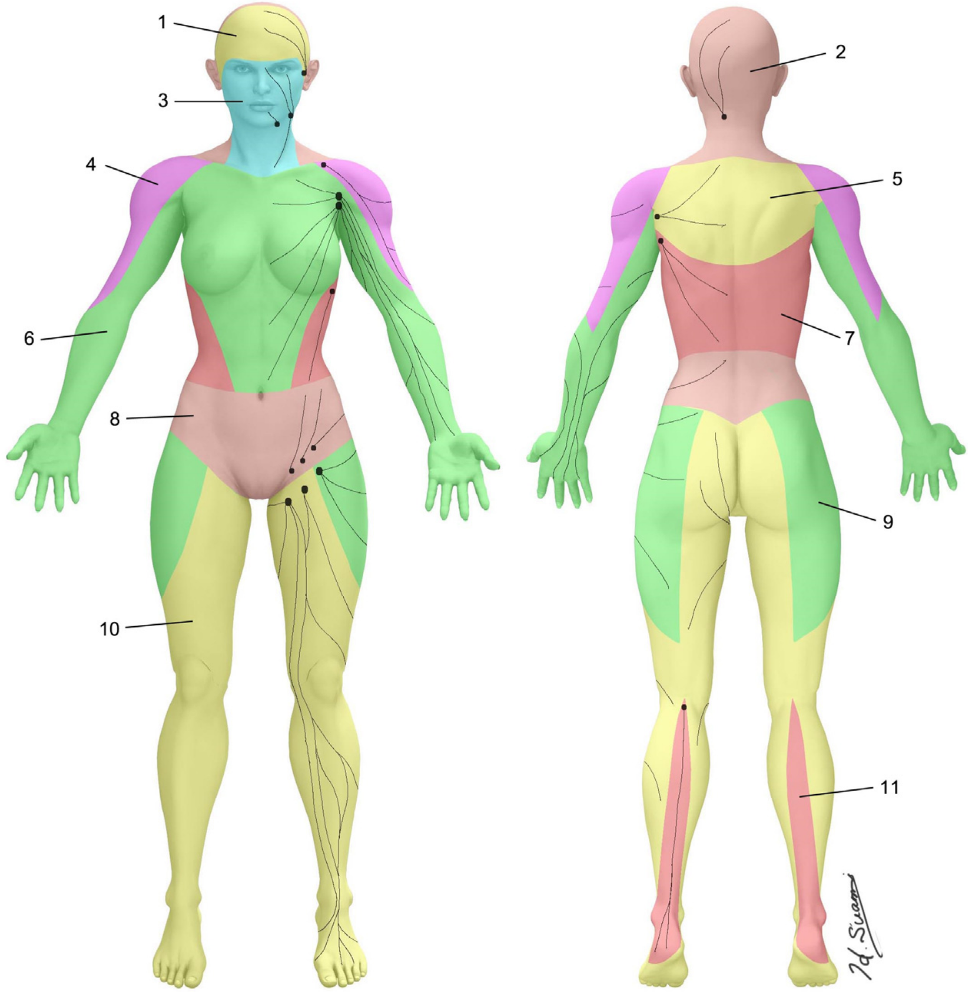

Figure 3. The 11 major lymphatic drainage regions, also called lymphosomes, throughout the body (Suami & Scaglioni, 2018). The classical lymphatic pathways from each lymphosome and their respective regional lymph nodes are also shown. 1. Temporal. 2. Occipital. 3. Submental. 4. Subclavicular. 5. Subscapular. 6. Lateral axillary. 7. Pectoral. 8. Superior inguinal. 9. Lateral inguinal. 10. Inferior inguinal. 11. Popliteal. Produced and copyright by Hiroo Suami, reprinted with permission.

Lymphoedema

Pathophysiology

When describing the pathophysiology of lymphoedema, it is reasonable to separately describe primary and secondary lymphoedema. First, primary lymphoedema is considered to be genetic in origin and can manifest at any time from birth to adulthood (Schook *et al.*, 2011). There are multiple different conditions leading to primary lymphoedema, many with separate clinical presentations, and a brief overview of the classifications as well some types of primary lymphoedema are presented. The classification presented in this thesis was first described by Connell et al. in 2010 (Connell *et al.*, 2010) and has been further developed since (Connell *et al.*, 2013; Gordon *et al.*, 2020). In their publication from 2020, a detailed flow chart is available, which provides assistance in categorising primary lymphoedema. Principally, this classification consists of five different subtypes including vascular or lymphatic malformations, syndromes, lymphoedema with visceral and/or systemic involvement, disorders with onset under one year of age and disorders with onset after one year of age. The subtype with vascular or lymphatic malformations can be associated with other anomalies such as Klippel-Trénaunay syndrome or be isolated such as simple lymphangiomas. Common syndromes include Turner and Noonan syndromes, which both feature involvement of the lymphatic system, resulting in lymphoedema. In primary lymphoedema with a systemic and/or a visceral component, in addition to lymphoedema in some or all parts of the body, failure of the lymphatics in organs such as the heart and lungs might result in pericardial or pleural oedema. The group with early onset includes those with Milroy disease, which in addition to lymphoedema in the lower extremities (most often present at birth), also might be characterised by hydrocele and protruding veins in the legs (Brice *et al.*, 2005). Milroy disease is caused by a mutation in the gene coding for VEGFR-3, a receptor of most importance in lymphangiogenesis, as previously described. The final group of primary lymphoedema is late onset lymphoedema, which includes both Meige disease, the most common form of primary lymphoedema, as well as lymphoedema distichiasis syndrome (LDS) (Connell *et al.*, 2013). Meige disease is solely presented by lymphoedema in the lower half of the legs without accompanying conditions and often presents during or after puberty (Rezaie *et al.*, 2008). In LDS, the lower extremities are also affected by lymphoedema, but this is accompanied by an additional set of eyelashes (distichiasis) (Brice *et al.*, 2002). In the vast majority of cases, this condition is known to be caused by a mutation in the *FOXC2* gene, which has been shown to impair the development of lymphatic valves and to disturb lymphatic capillary development in mice models (Petrova *et al.*, 2004).

Secondary lymphoedema, in contrast to primary lymphoedema, arises when an external factor disrupts the normal flow of lymph. Examples are surgical removal of lymph nodes in cancer treatments, radiotherapy, trauma, skin infections as well as lymphatic filariasis caused by the nematode *Wochereria bancrofti* (the most common cause of lymphoedema globally) (Szuba & Rockson, 1998; Babu & Nutman, 2014; Organisation Mondiale de la Santé, 2019). In these examples the lymphatic pathway is damaged, blocked or impaired due to postinfectious fibrosis. The result is typically a lymphoedema in the body part that is drained by the impaired lymphatic pathway. In surgical lymph node removal in the inguinal area from conditions such as cervical cancer, endometrial cancer and malignant melanoma, the lymphoedema develops in the ipsilateral lower extremity (Cormier *et al.*, 2010). In treatment for breast cancer, including surgical lymph node removal in the axilla and radiotherapy, the ipsilateral arm is at risk for BCRL (DiSipio *et al.*, 2013). However, the exact mechanism of lymphoedema development is still unclear because some patients do not develop BCRL until several years after surgery. A genetic predisposition in lymphangiogenic, inflammatory and transmembrane protein coding genes has been suggested to exist for developing BCRL (Kapellas *et al.*, 2022). An interesting finding in a prospective surveillance study measuring lymphatic drainage in the ipsilateral and contralateral arm at seven and 30 months after surgery for breast cancer showed that patients that end up developing BCRL have increased lymphatic drainage in both arms at seven months compared to those not developing BCRL, with a significant decrease in lymph flow in the lymphoedematous arm at 30 months (Stanton *et al.*, 2009). This might indicate that some people have a high base-rate of interstitial fluid turnover, thus increased lymphatic flow, which can lead to a higher risk for developing BCRL. The deterioration in lymphatic transport is proposed to come from an increased resistance to lymph flow in all patients after surgical removal of lymph nodes, which leads to overload in lymphatic capacity for people with a high fluid turnover, finally resulting in intrinsic failure and lymph stasis.

Risk factors for developing secondary lymphoedema after breast cancer include number the of lymph nodes removed, mastectomy, high body-mass index (BMI), radiotherapy, taxane-based chemotherapy, metastases in lymph nodes as well as lack of physical activity (DiSipio *et al.*, 2013; Kilbreath *et al.*, 2016). Subclinical lymphoedema (increased fluid fraction in the affected arm without an increase in circumference) found with bioimpedance analysis was discovered preoperatively in patients with breast cancer (Iyigun *et al.*, 2018). Risk factors include a high number of lymph nodes with metastasis (>8) as well as BMI >30. Preoperative subclinical lymphoedema was also shown to significantly correlate with clinical lymphoedema after surgery.

A general model for the manifestation of lymphoedema was proposed by Andrzej Szuba and Stanley G Rockson in a review from 1997 (Szuba *et al.*, 2003). Starting with any occurrence that impedes lymph flow, such as an obstruction, loss of valvular function or abnormal lymphatic development, the lymphatic impairment subsequently worsens, finally leading to the clinical features associated with lymphoedema. These clinical features include excess fluid in the subcutaneous tissue and skin, excess adipose tissue, fibrosis and hypertrophy of the skin (Daróczy, 1995; Idy-Peretti *et al.*, 1998; Brorson *et al.*, 2006b; Brorson *et al.*, 2009). When the diameter of the lymphatic vessel increases, the valves require a higher pressure gradient in order to close (Davis *et al.*, 2011). This results in a higher tendency of the valves to remain open in distended lymphatic vessels and thus, a higher tendency to be incompetent. Histological changes in lymphatic collecting vessels in the lower extremities have been investigated in patients treated with lymph node removal due to gynaecological cancers (Mihara *et al.*, 2012b). The findings show that as the outflow of collecting vessels is impaired and the pressure inside lymphatic vessels increases, a number of histological changes occur. The junctions between endothelial cells become incompetent, enabling leakage of lymph fluid into the surrounding tissue. Also, smooth muscle cells proliferate noticeably, which, in combination with an increased amount of collagen fibres, reduces the lumen size drastically, finally leading to a stiff fibrotic vessel with a reduced capability for intrinsic lymph transport. The reduced contractility of lymphatic vessels in lymphoedema has been shown to occur in humans (Olszewski, 2002).

Immunological changes have also been implied to have an important role in the development of lymphoedema. Lymphatic obstruction seems to lead to an accumulation of $CD4^+$ cells, which correlates with lymphoedema formation and fibrosis in mice models (Zampell *et al.*, 2012c; Avraham *et al.*, 2013). The increase in $CD4^+$cells consists both of T-helper 1 (Th1) and T-helper 2 (Th2) cells; however, on closer evaluation it seems to be the Th2 cells that facilitate lymphoedema formation. Hindering the differentiation into Th2 cells by inhibiting interleukin (IL)-4 and IL-13 prevented lymphoedema formation, increased lymphatic drainage, and reduced the amount of fibrosis. The reduction in Th2 cells lowers the expression of transforming growth factor-1 (TGF-1), an inhibiter of lymphangiogenesis, a response that seems partly to be responsible for these physiological changes. Furthermore, T cells have been found to secrete interferon-γ (IFN-γ), which downregulates lymphangiogenesis by suppressing the expression of *Prox-1* in lymphatic endothelial cells (Kataru *et al.*, 2011). Other cells that have been found to be upregulated in lymphoedema are the regulatory T (Treg) cells, which in contrast to Th2 cells have a protective effect on the development of lymphoedema (Gousopoulos *et al.*, 2016). When Treg cells are decreased, mediators such as TGF-1, IL-4, IL13 and IFN-γ are increased, leading to excess fibrosis and lymphatic dysfunction. However, when Treg cells are amplified, lymphoedematous changes are prevented or improved.

The macrophage is another cell type that increases in number in response to lymphatic injury and lymphoedema, specially the M2 differentiated type (Ghanta *et al.*, 2015). The effect macrophages have on the pathophysiology in lymphoedema was seen when macrophages were reduced in mice models. In these mice, an increase in the Th2 cell count was observed in combination with a lower expression of VEGF-C as well as increased fibrosis (Zampell *et al.*, 2012b; Ghanta *et al.*, 2015). Thus, it is believed that M2 macrophages reduce the inflammatory responses in lymphoedema as well as prevent fibrosis.

Ketoprofen, a nonsteroidal anti-inflammatory drug improved the physiological features of lymphoedema in mice models, which is believed to action through a downregulation of leukotriene B_4 (LTB_4) (Nakamura *et al.*, 2009; Tian *et al.*, 2017).

In lymphoedema models, low levels of LTB_4 were initially observed shortly after lymphatic injury, and were correlated with an increase in lymphangiogenesis; however, LTB_4 levels rose quickly to levels that had the opposite effect on lymphatic growth. Thus, antagonising LTB_4 is an interesting approach to treat lymphoedema. Similar effects were seen when using Bestatin, an inhibitor of leukotriene A_4 (LTA_4) hydrolase that converts LTA_4 into LTB_4.

Obesity is linked to an impaired lymphatic function, inflammation surrounding lymphatic vessels as well as a decrease in the expression of lymphatic markers (*Prox1* and VEGFR-3) in lymphatic endothelial cells (Greene *et al.*, 2015; García Nores *et al.*, 2016). When the expression of T-helper cells is downregulated in obese mice, the lymphatic contractility and lymphatic flow increase with an increase in the density of lymphatic capillaries and a decrease in the perilymphatic inflammation (Torrisi *et al.*, 2016). Thus, T-helper cells seem to play an important role in the development of obesity-induced lymphatic dysfunction. However, the mechanism behind the opposite phenomenon, adipogenesis, in the presence of lymph stasis, is not fully understood. In a rat model, lymph stasis increases the expression in factors promoting adipogenesis, such as CCAAT/enhancer binding protein-alpha and adiponectin, and an increase in both the number of adipocytes and size of fat droplets has been seen (Aschen *et al.*, 2012; Zampell *et al.*, 2012a). Another mediator that is increased in lymphoedema is IL-6, which is released from inflammatory cells, and evidence suggests that IL-6 has a regulatory role in controlling adipose deposition in lymphoedema (Cuzzone *et al.*, 2014). When IL-6 is downregulated in mice models, the adipose tissue deposition in lymphoedema is increased, resulting in the hypothesis that IL-6 has a downregulatory role on adipogenesis.

Epidemiology

The overall incidence of secondary, cancer-associated lymphoedema is reported to be 21% in breast cancer (with four times higher incidence after axillary clearance compared to sentinel node biopsy), 25% in gynaecological cancers, 11% in urological cancers and 3% as well as 18% in malignant melanoma located on the upper and lower extremities, respectively (Cormier *et al.*, 2010; DiSipio *et al.*, 2013). However, the incidences vary widely depending on the methods used for lymphoedema diagnosis, type of study and length of follow-up. Since breast cancer is the second most common type of cancer in the global population (after lung cancer) and is estimated to have been diagnosed in more than two million females in 2018 (Bray *et al.*, 2018), BCRL is believed to be the most common cancer-associated cause of lymphoedema. The incidence of BCRL is highest during two years after treatment but lymphoedema can present at any time after cancer treatment (Rockson & Rivera, 2008; DiSipio *et al.*, 2013). However, studies have shown that implementing an early surveillance programme to detect subclinical lymphoedema after breast cancer treatment can decrease the rate of clinical lymphoedema significantly (Soran *et al.*, 2014; Koelmeyer *et al.*, 2019; Koelmeyer *et al.*, 2020a). In these studies, fluid changes in the arms were monitored with bioimpedance spectroscopy (BIS) and if subclinical lymphoedema was found (increased fluid content but no apparent swelling in the arm), early interventions such as compression garments and physical activity were started. Furthermore, randomised clinical trials are currently ongoing in which no sentinel lymph node biopsy at all is tested for clinical early stages of breast cancer (Gentilini & Veronesi, 2012; Reimer *et al.*, 2017). Also, the procedure of axillary clearance is persistently being questioned, and randomisation to avoid this procedure when finding up to three macrometastases in sentinel lymph node biopsies is also being tested (Reimer *et al.*, 2017). In combination, these aspects could reduce the incidence of BCRL in the future.

Cervical cancer is the fourth most common cancer in women, estimated to have affected 570 000 women globally in 2018 (Bray *et al.*, 2018) and thus possibly the major cancer-associated aetiology of leg lymphoedema among women, with a post-treatment incidence of around 13-33% (Cormier *et al.*, 2010; Ohba *et al.*, 2011; Kim *et al.*, 2012; Hayes *et al.*, 2017).

A major non-iatrogenic cause of secondary lymphoedema is the condition referred to as lymphatic filariasis, an infection by a parasite that lodges in the lymphatic vessels and causes lymphatic injury (Case *et al.*, 1991). The parasites responsible for causing lymphatic filariasis are the nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, all transmitted to humans through mosquitoes (Babu & Nutman, 2014; Organisation Mondiale de la Santé, 2019). Worldwide, the disease is endemic in tropical and subtropical countries and was estimated to affect 120 million people in 2020, with 90 % of the infections being caused by the nematode *Wuchereria bancrofti* (Ottesen, 2000). The nematode resides within the lymphatic vessels, causing lymphatic dilation, thrombosis, lymphatic wall and valve enlargement as well as immunologic reactions and fibrosis (Case *et al.*, 1991). Due to the debilitating effect lymphatic filariasis has on people's lives, a global programme has been implemented by the World Health Organization to mass administer drugs to people in endemic areas in order to eliminate this disease (Organisation Mondiale de la Santé, 2019). Up to 2018, more than 910 million people worldwide had been administered drugs to eradicate this infection.

Compared to secondary lymphoedema, primary lymphoedema is less common and the incidence is reported to be around 1.15/100 000 in people under the age of 20 per year (Smeltzer *et al.*, 1985). Other studies have indicated a prevalence of 1 in 5000-6000 in the UK, with a higher prevalence among females (Dale, 1985; Cooper & Bagnall, 2016). Some studies of prevalence, still frequently quoted in modern articles, are more than 35 years old, indicating the scarce information currently available and that modern, larger scale studies are needed in this area. However, there might be obstacles to the methodology of studies reporting primary lymphoedema prevalence. Using questionnaires answered by health care providers treating people with lymphoedema in a given population has the potential to neglect all persons with lymphoedema that are unknown to the health care providers (Keeley & Moffatt, 2018). Thus, the prevalence of lymphoedema is possibly underestimated.

Much research is available on lymphoedema in the arms and legs, however, a previous study using ICG lymphography indicated dermal backflow extending to the gluteal region in 22% of cancer-related leg lymphoedema (Koelmeyer *et al.*, 2020b). In addition, another study using lymphoscintigraphy found lymphatic drainage pathways extending to the gluteal region and draining to the common iliac lymph nodes in 29% of patients with leg lymphoedema (Bourgeois & Leduc, 2021). Gluteal lymphoedema is a topic rarely described in the literature, which could simply be because the gluteal region is viewed as a part of the leg and not seen as a separate region when assessing lymphoedema. However, liposuction of leg lymphoedema is performed up to the level of the groin and only occasionally in the gluteal region (Brorson *et al.*, 2015a) and it is difficult to achieve an optimal compression with garments due to the oval shape of the gluteal regions. If gluteal lymphoedema is a common problem for leg lymphoedema patients, more research into tissue composition, characteristics, causes and possible treatments could be warranted. An old publication from 1950 presents a case of 'Elephantiasis buttocks' caused by lymphatic filariasis (Bhattacharjea, 1950). The patient was suffering significantly from the condition. The treatment of choice was antibiotics and surgical excision. Gluteal lymphoedema has also been linked to lymphangioleiomyomatosis and hidradenitis suppurativa (Hoshika *et al.*, 2013; Micieli & Alavi, 2018). Perhaps more clinically relevant, in an outpatient lymphoedema clinic in Japan, gluteal oedema was present in 21% of patients attending the clinic (Dai *et al.*, 2019).

Diagnosis

When diagnosing lymphoedema, it is wise to take into consideration co-morbidities that can affect the condition, such as venous disease, heart failure, kidney disease, as well as malignant diagnoses or tumour recurrence (Executive Committee of the International Society of Lymphology, 2020). There are numerous imaging modalities to assess the lymphatic system if lymphoedema is suspected. In addition, a genetic evaluation can add further information in a possible primary lymphoedema. The gold standard to image the lymphatic vessels, lymph nodes as well as the lymphatic transport is by lymphoscintigraphy (Pappalardo & Cheng, 2020). Radiotracer, susceptible to uptake by the lymphatic vessels, is injected distally in the limb and images are taken at different time intervals to evaluate lymphatic function; however, a specific protocol for the procedure has not been widely accepted (Executive Committee of the International Society of Lymphology, 2020; Pappalardo & Cheng, 2020). This method is a successor to the previously used method of lymphangiography, where the radio-opaque solution was injected directly into the lymphatic vessel to be analysed (Kinmonth *et al.*, 1955). Other modalities to image the lymphatics are MRI and computed tomography (CT). A method with increasing popularity is ICG lymphography, or near infrared fluorescent imaging, which has the advantage of real-time mapping where a radiotracer is injected distally in the limb and a near infrared camera shows radiotracer transport (Suami *et al.*, 2012; Suami *et al.*, 2019). The diagnosis of lymphoedema is made by finding dermal backflow on ICG lymphography (Chang *et al.*, 2013; Nguyen *et al.*, 2017). Dermal backflow is seen as a rerouting of the lymph flow from the collecting vessels to more superficial smaller lymphatics when a lymphatic obstruction is present. The classification of lymphoedema on ICG lymphography is done according to the MD Andersson classification scale, and ranges from stage 0 with normal lymphatics and no dermal backflow to stage 4 with extensive dermal backflow in the whole limb including the hand/foot and no patent lymphatics, and stage 5 with no movement of the dye (Chang *et al.*, 2013; Nguyen *et al.*, 2017). Furthermore, methods such as DXA and BIS are used to estimate the tissue composition in lymphoedematous limbs (Gjorup *et al.*, 2017; Koelmeyer *et al.*, 2020c). BIS measures impedance for different frequencies and primarily assesses extracellular fluid to detect or follow changes in fluid balance (Kushner, 1992; Koelmeyer *et al.*, 2020c; Ward, 2021). DXA analysis of lymphoedematous arms has found, in addition to a 73% increase in fat volume, a 47% increase in lean tissue in the affected arm (Brorson *et al.*, 2009). There are some doubts about what lean tissue on DXA actually accounts for but it probably includes muscle, fluid, skin and viscera (Clarys *et al.*, 2010). A theory is that increased load from excess volume in lymphoedema affects the muscles and leads to muscular hypertrophy. However, from DXA analysis it cannot be certain that the increase found in lean tissue represents excess muscle volume, and it could reasonably also be from increased skin volume. When lymphoedematous limbs have been investigated with other imaging modalities, conflicting results have been presented. Some studies have not found any significant increase in muscle volume in the lymphoedematous limb with MRI, CT or DXA (Collins *et al.*, 1995; Dylke *et al.*, 2013; Lu *et al.*, 2014; Borri *et al.*, 2017; Yoo *et al.*, 2017), while other studies have indicated an increase in muscle volume in some patients (Case *et al.*, 1992; Sen *et al.*, 2018). Small excess volumes and a low number of included patients are two issues causing difficulties in drawing conclusions from these studies. Other results have shown that, although no significant increase in preoperative muscle volume could be found with water/fat MRI, the water/muscle volume of the subfascial compartment decreased after liposuction, and the authors theorise that this is from the decreased load on the limbs (Hoffner *et al.*, 2018b; Trinh *et al.*, 2019).

A new method of mapping the lymphatic system is under evaluation called photoacoustic lymphangiography (Kajita *et al.*, 2020; Suzuki *et al.*, 2020). This method also requires the use of cutaneous ICG injections but has the advantage of creating high resolution 3D images where lymphatics down to 0,2mm in diameter can be visualised in the whole subcutaneous compartment. However, the usefulness of this imaging modality is still to be explored.

Another method used to monitor and detect lymphoedema involves using the tissue dialectic constant (TDC) (Mayrovitz, 2022). A portable, handheld, measuring device is held in contact with the skin and estimates a measurement of the superficial water content. The device transmits an ultra high-frequency electromagnetic signal of 300 MHz from a probe, and part of the signal is subsequently reflected back from the tissue. The reflection is measured and used to calculate the TDC (Nuutinen *et al.*, 2004). The method has been validated with good reliability in determining superficial water content in human limbs and has potential for early detection of lymphoedema after surgical cancer treatments (Nuutinen *et al.*, 2004; Jensen *et al.*, 2012; Mayrovitz, 2022).

Figure 4. Prominent lymphoedema showing deep pitting. The arm swelling consists generally of fluid, caused by an accumulation of lymph (left). No pittining is evident allthough hard pressure is applied by the thumb for one minute, and the only visible effect is a slight reddening of the skin (right). At this stage, the lymphoedema consists mostly of adipose tissue and “oedema” is an incorrect description of the condition as no, or a minimal amount of lymph is present in the aspirate when liposuction is performed. Copyright by Håkan Brorson, reprinted with permission from J Lymphoedema 2008; 1: 38-47.

Without using imaging to assess lymphatic transport or compositional changes, simple diagnostic tools can be used in assessing lymphoedema. The first is the classical pitting test (Sanderson *et al.*, 2015). The clinician uses a finger and presses firmly on the extremity for up to one minute and assesses the dent created on the surface. A large dent indicates fluid in the subcutaneous tissue, also known as pitting oedema (Brorson *et al.*, 2015a; Kogo *et al.*, 2017). If no or minimal pitting is present in a patient with lymphoedema and significant excess volume, excess fat is likely in the limb (**Figure 4**). Another clinically relevant test when suspecting lymphoedema in the lower extremities is the Stemmer sign (Stemmer, 1999; Goss & Greene, 2019). The clinician tries to pinch the skin directly proximal to the metatarsophalangeal joint on dig II on the foot. If unable to pinch the skin with the fingertips, the Stemmer sign is positive (**Figure 5**). Lymphoedema affects the feet, and increased fat/fibrosis/fluid in this area causes the positive test. The test has high sensitivity to detect lymphoedema but low specificity and should be used in combination with other clinical assessments.

Figure 5. The Stemmer sign performed on a patient with lympoedema of the right leg. The examiner cannot pinch the skin of the base of the toe. A positive Stemmer sign is a sensitive predictor for lymphoedema (right foot). If the examiner can pinch a thin fold, the sign is negative (left foot) Copyright by Imke Wallenius, reprinted with permission.

Staging

The severity of lymphoedema ranges from the subclinical stage where swelling is not present to a state of excessive fat/fibrosis with skin thickening and verrucous overgrowth, also known as elephantiasis (Executive Committee of the International Society of Lymphology, 2020). One of the most common classifications of clinical lymphoedema stages has been developed by the International Society of Lymphology (ISL) which is mainly based on the physical appearance of the limb as well as simple palpable changes in tissue composition. It consists of four main stages with the addition of a severity grading.

Stage 0 is the latent or subclinical phase of lymphoedema with impaired lymphatic transport, but the limb has not yet increased in volume. Also, the amount of fluid content in the limb can be changed in this stage and patients may complain of subjective symptoms. It can be transitory and may exist for long time before oedema develops.

In stage I, clinical lymphoedema is present with a swelling consisting of protein-rich fluid, showing pitting if pressure is applied on the skin. Elevating the limb is enough to remove the oedema.

Stage II indicates a lymphoedema that is more manifest than the previous stage and elevation does not reduce the oedema. Early in this stage, pitting is still evident but as the amount of fat and fibrosis increases, pitting may be reduced in the later phase in this stage.

At the end of the scale, in stage III lymphoedema there is additional fat and fibrosis where different skin manifestations are present such as hyperpigmentation, verrucous overgrowths and thickened skin. This stage can lead to extreme excess volume and is referred to as elephantiasis due to the resemblance of the limb to an elephant's leg. Pitting may be absent.

In addition to the stages (I-III), the severity grade is added by classification according to the excess volume (calculated as the excess compared to the contralateral limb). The three grades are minimal, moderate and severe, which are defined as 5-20%, 20-40% or above 40% excess volume, respectively.

Skin infections

It is well known that lymphoedema increases the risk for developing skin infections, such as erysipelas and cellulitis, in the affected limb. These infections are most often caused by streptococcal infection and are localised to the dermis and subcutis (Bonnetblanc & Bedane, 2003). Cellulitis and erysipelas normally present with similar symptoms such as redness, local heat and pain accompanied by fever. The infections differ somewhat in the demarcation where erysipelas has a clear border to the elevated affected area and cellulitis has more diffuse redness due to a deeper infection (Bisno & Stevens, 1996). The decreased lymphatic transport capacity in lymphoedema with impaired clearance of pathogens seems to be one of the underlying risk factors for skin infections (Duvanel *et al.*, 1989; Damstra *et al.*, 2008), and lymphoedema has been found to be the greatest risk factor for erysipelas in the lower extremities. The lower extremities are the location most often affected by skin infections and up to 85% of cases occur here. Erysipelas in leg lymphoedema has an incidence of 36-48% (Thompson & Wee, 1980; Deng *et al.*, 2015; Mihara *et al.*, 2016) and lymphoedema is also found to be the greatest risk factor for recurrence of infection as well as bacteraemia from skin infections (Tay *et al.*, 2014; Sapuła *et al.*, 2020).

The infection in itself also seems to impair lymphatic function and flow (de Godoy *et al.*, 2000). Clinical trials have found that mice infected with methicillin-resistant Staphylococcus aureus show decreased contractile function of the lymphatics and a reduced lymph flow (Jones *et al.*, 2018). The lymphatic dysfunction in these mice remained 120 days after infection, indicating permanent damage to lymphatic function. The dysfunction was found to be caused by damage to lymphatic muscle cells from bacterial toxins.

Treatment

Lymphoedema is associated with heaviness, restriction of movement, pain, implications for daily and professional life as well as a psychological impact and financial impairment (Brorson *et al.*, 2006a; Pyszel *et al.*, 2006; Boyages *et al.*, 2016; Boyages *et al.*, 2017; Kalfa *et al.*, 2019; Klernäs *et al.*, 2020). The basic conservative management of lymphoedema is CDT which in the initial stage includes skin care, MLD, bandaging and exercise followed by a continuous period with compression garment usage, skin care as well as MLD (Foldi *et al.*, 1985; Executive Committee of the International Society of Lymphology, 2020). Later research has found that MLD has no effect on volume reduction or prevention of lymphoedema and can be omitted, and a focus on bandaging is what is most important for reducing excess volume (Wanchai & Armer, 2021; De Vrieze *et al.*, 2022a; De Vrieze *et al.*, 2022b). The aim of the treatment is thus to remove the excess fluid in the limb as well as to hinder any aggravation of the condition. In early stages of the disease when no fibrosis or excess fat is present, simple use of compression garments can be a satisfactory option. However, the continuous use of compression garments can be demanding in warm and tropical countries. Other attractive treatment modalities in early-stage lymphoedema include microsurgical procedures where a possible route for lymphatic drainage is created by different means. Examples include lymphaticovenous anastomosis (LVA) (O'Brien *et al.*, 1990; Campisi *et al.*, 2010; Mihara *et al.*, 2012a; Qiu *et al.*, 2020), lymphatic vessel transplantation (Baumeister & Frick, 2003; Felmerer *et al.*, 2012; Baumeister *et al.*, 2016) and vascularised lymph node transplantation (VLNT) (Cheng *et al.*, 2012; Cheng *et al.*, 2013). Single or multiple LVAs are performed using functional lymphatic collecting vessels that are anastomosed to nearby located veins of equal or larger size in an end-to-end or end-to-side fashion (Qiu *et al.*, 2020). Lymphatic vessel transplantations are often done by harvesting patent lymphatic collectors on the medial aspect of the thigh that can be up to 30 cm in length (Baumeister *et al.*, 2016). These are thereafter anastomosed with patent lymphatics both distal and proximal to the lymphatic obstruction. The VLNTs are most often harvested as free flaps and inserted either proximally or distally on the lymphoedematous limb with microsurgical anastomosis of a local artery and vein for blood supply (Scaglioni *et al.*, 2018). The nodes can be harvested from inguinal, submental, supraclavicular, omental or lateral thoracic lymph nodes. These procedures have in common that they all try to increase lymphatic drainage to reduce the excess volume of the limb, along attemting to reduce or remove the need for compression garments (Granzow *et al.*, 2014b; Brown *et al.*, 2022). The results from studies evaluating microsurgical procedures vary and are sometimes difficult to compare due to different measurement methods and follow-up protocols (Scaglioni *et al.*, 2018). A meta-analysis evaluating VLNT for the treatment of BRCL has indicated an excess volume reduction of 40%, decreased skin infection rates and improved quality of life, but the study design of some included studies and potential confounders raise concern about the results (Winters *et al.*, 2022). For LVA, the postoperative reduction of excess volume after treatment for BCRL is around 30% (Forte *et al.*, 2020). Since microvascular reconstructions are often combined with postoperative use of compression garments it is difficult to evaluate if the excess volume reduction is caused by the surgery per se or by the use of compression garments. Indications exist for reductions in compression garment wear after microsurgical procedures, which would be a great asset in the treatment of lymphoedema (Forte *et al.*, 2020; Winters *et al.*, 2022).

When lymphoedema progresses into a state with increased fat and fibrosis, other methods are needed to achieve a complete reduction in excess volume. As previously described, extensive debulking procedures have historically been used widely, but suffered sometimes from dreaded complications resulting in amputation of the limb (Miller, 1980). Thus, a less invasive method of removing the excess fat has been implemented by using liposuction, with the results maintained with CCT (Brorson & Svensson, 1997a, 1998; Brorson, 2000; Brorson *et al.*, 2008; Brorson, 2016; Hoffner *et al.*, 2018a). Results show a complete reduction of the exess volume in patients treated with liposuction and CCT both in lymphoedematous arms and legs.

The method of liposuction, sometimes referred to as suction-assisted lipectomy, has been included in treatment combinations with microsurgical procedures with the aim of both treating the solid and fluid components of late-stage of lymphoedema. This combination has the theoretical possibility to achieve a significant reduction in excess volume in combination with a reduced demand for compression garments (Granzow *et al.*, 2014a; Nicoli *et al.*, 2015; Campisi *et al.*, 2017; Brazio & Nguyen, 2021). Furthermore, microsurgical procedures have started to be implemented and performed at the same time as the oncologic lymph dissection in the axilla and inguinal region to prevent the development of cancer-associated lymphoedema (Boccardo *et al.*, 2014; Boccardo *et al.*, 2016). The method, called the lymphatic microsurgical preventive healing approach (LYMPHA), includes LVAs using the afferent lymphatics, running to the nodal region being dissected, and anastomosing these to nearby veins. Promising results at the four-year follow-up have been presented with the LYMPHA technique after axillary dissection for breast cancer treatment, with an incidence of lymphoedema of 4% (Boccardo *et al.*, 2014).

Due to the invasive nature of liposuction and the need to have functional lymphatics for microsurgical procedures, some centres have taken precautions in order not to harm the lymphatic vessels during liposuction (Campisi *et al.*, 2017). These precautions include preoperative mapping of the lymphatic system with ICG lymphography to avoid the larger collecting lymphatics during liposuction. However, several studies have proved that liposuction performed in a longitudinal fashion does not harm the larger superficial lymphatic vessels (Brorson *et al.*, 1998; Frick *et al.*, 1999; Haddad Filho *et al.*, 2009; Greene *et al.*, 2017). In addition, it has also been shown that liposuction for lymphoedema does not impair the blood circulation in the limb (Brorson & Svensson, 1997b).

Liposuction has become a popular treatment strategy for late-stage lymphoedema, and it has been implemented in standard care in centres worldwide, including in Sweden, Australia, Netherlands, the United Kingdom and the USA (Brorson & Svensson, 1997a; Damstra *et al.*, 2009; Schaverien *et al.*, 2012; Boyages *et al.*, 2015; Greene & MacLellan, 2016; Stewart & Munnoch, 2018; Granoff *et al.*, 2020; Granoff *et al.*, 2021). Postoperative excess volume reductions range between 101 and 118% for arm lymphoedema and between 90 and 115% for leg lymphoedema, results that can be maintained with the use of compression garments. Postoperative results after liposuction and CCT in two patients are shown in **Figures 6** and **7**. Furthermore, liposuction has been found to reduce the incidence of erysipelas in arm lymphoedema by 87% (Lee *et al.*, 2016) as well as to increase quality of life after surgery (Brorson *et al.*, 2006a; Hoffner *et al.*, 2017; Klernäs *et al.*, 2020).

A detailed description of the liposuction technique and the CCT regimen is presented in later sections of this thesis.

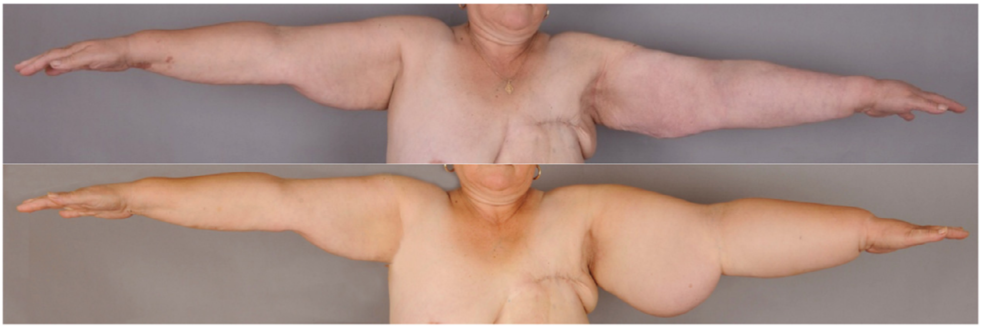

Figure 6. A 57-year-old woman with a non-pitting breast cancer-related lymphoedema of her left arm, that measured 4 325 ml in excess volume, and had existed for five years (bottom). A total reduction in the preoperative excess volume was seen one year after liposuction (top). (Hoffner *et al.*, 2018a). Copyright by Håkan Brorson, reprinted with permission.

Figure 7. A 32-year-old woman with a non-pitting secondary lymphoedema in her right leg, that measured 7 070 ml in excess volume, and had lasted for 12 years following treatment of a synovial sarcoma in the right groin (left). A total reduction in the excess volume was seen six months after liposuction (right). (Brorson H. Liposuction Normalizes Lymphedema Induced Adipose Tissue Hypertrophy in Elephantiasis of the Leg – A Prospective Study with a Ten-Year Follow-Up. *Plast Reconstr Surg*: 2015; 136 (4S–1 Suppl): 133-134 doi: 10.1097/01.prs.0000472449.93355.4a). Copyright by Wolter Kluwer Health Inc., reprinted with permission.

Aims

The overall aim of the thesis was to assess liposuction and CCT for late-stage lymphoedema and to compare treatment outcomes from different international regions where health care settings vary. Excess volume reduction was not the only outcome of interest and skin infection rates for leg lymphoedema were also assessed. We also sought to find a better understanding of tissue composition changes in both arm and leg lymphoedema, before and after liposuction.

Specific Aims

I. To determine whether extension of lymphoedema to the gluteal region seen on ICG lymphography is associated with gluteal subcutaneous tissue changes visible with MRI.

II. To determine if liposuction and CCT for leg lymphoedema reduce the incidence of erysipelas in the affected leg.

III. To follow soft-tissue composition one year after liposuction and CCT in patients with BCRL.

IV. To measure the muscular compartment in late-stage lymphoedema in the arms and legs with MRI at different positions along the limb, and compare with the healthy limb.

V. To assess the five-year outcome of liposuction and CCT for leg lymphoedema when performed at the Department of Plastic and Reconstructive Surgery, Skåne University Hospital, Malmö, Sweden.

VI. To assess the five-year outcome of liposuction and CCT for both arm and leg lymphoedema when performed at the ALERT clinic, Macquarie University Hospital, Sydney, Australia.

Materials and Methods

Patients

Due to ethical considerations, no study included patients from both institutions. Studies II, III and V included patients treated with liposuction and CCT at the Department of Plastic and Reconstructive Surgery, Skåne University Hospital, Malmö, Sweden. Studies I, IV and VI included patients assessed for, or treated with, liposuction and CCT at the ALERT clinic, Macquarie University, Sydney, Australia.

Study I

Twenty-eight patients with unilateral leg lymphoedema were included in the study. All patients had been assessed with both ICG lymphography of the affected leg and MRI of both legs at the ALERT clinic, Macquarie University, between August 2017 and March 2021. No more than 12 months were allowed to have passed between the assessments.

Study II

Patients with unilateral leg lymphoedema treated with liposuction and CCT at Skåne University Hospital were included. Between 1993 and 2020, 124 patients were operated on and met the study´s inclusion criteria, which consisted of a minimum of 10% excess volume, no or minimal pitting on examination, no sign of active cancer, no skin infection at the time of surgery and acceptance of lifelong use of compression garments. All patients had received conservative treatment before surgery and presented with excess adipose tissue, naturally unresponsive to any conservative treatment. At the first visit to our clinic, patients were asked about previous bouts of erysipelas and this information was also checked in hospital charts when available.

Study III

The study included 18 patients with BCRL that had previously been analysed with DXA prior to liposuction (Brorson *et al.*, 2009). All patients were treated with liposuction and CCT and postoperative measurements were carried out with DXA after three and 12 months. The same inclusion criteria as for study II were used. As part of their breast cancer treatment, 16 patients had a modified radical mastectomy and two patients had partial mastectomies with axillary clearance. Fifteen patients had received adjuvant radiotherapy and four patients had received adjuvant chemotherapy.

Study IV

All patients that had been assessed for liposuction at the ALERT clinic between May 2012 and May 2017 and had undergone an MRI as part of this assessment were included. Unilateral arm and leg lymphoedema were included and satisfactory resolution on MRI images for both limbs (arms or legs) was required. This led to the inclusion of 55 patients; 28 arm lymphoedema patients and 27 leg lymphoedema patients.

Study V

The study included 67 patients with leg lymphoedema that had been treated with liposuction and CCT at the Department of Plastic and Reconstructive Surgery, Skåne University Hospital, between September 1993 and May 2017. Inclusion criteria were unilateral leg lymphoedema with at least 10% larger volume in the affected leg compared to the healthy leg, conservative treatments had not resulted in satisfactory reduction, no or minimal pitting on examination, and patients who agreed to continue with lifelong CCT. Patients were excluded if they had signs of active cancer, wounds or ongoing infection. All patients were followed for five years.

Study VI

The study included 59 patients that had been treated with liposuction and CCT at the ALERT clinic, Macquarie University, between May 2012 and May 2017. Twenty-nine patients had arm lymphoedema and 30 patients had leg lymphoedema. All patients were assessed by a multidisciplinary team prior to liposuction treatment and the patients eligible for liposuction had stage II-III ISL lymphoedema, suffered significant morbidity from the condition, had no sign of active cancer, had an excess volume of at least 20%, had excess adipose tissue and minimal fluid as measured with MRI, had previously tried conservative treatments and agreed to continue with lifelong CCT.

Methods

Liposuction and CCT

Skåne University Hospital

Prior to treatment with liposuction, the lymphoedema was treated with optimised compression garments or other conservative methods to reduce the fluid part of the lymphoedema, leaving mostly excess adipose tissue. In addition, the patient had to be fully compliant with compression therapy since the liposuction does not treat the underlying pathology of lymphoedema and the condition will return without optimal compression therapy. A few weeks prior to the procedure, the measurements of the healthy contralateral arm or leg were taken and used to order three new sets of garments (see details of compression garments below). One of them was sterilised to be applied directly after liposuction.

In the operating theatre, with the patient under general anaesthesia, the limb was first exsanguinated with Esmarch bandages and a tourniquet placed on the most proximal part of the limb. A scalpel was used to make 10-20 small incisions along the limb and power-assisted liposuction (Lipomatic, Nutational Infrasonic Liposculpture; Euromi, Andrimont, Belgium) was performed using specially made cannulas 15-25 cm long and with a diameter of 3-4 mm. The cannula orientation was always longitudinal to the limb to minimise lymphatic injury. A tape measure was used to check the progress and the healthy limb was used as a reference. When an adequate amount of adipose tissue had been removed, the compression garment was applied up to the distal edge of the tourniquet, which was removed. Liposuction of the proximal part of the limb previously under the tourniquet was performed using the tumescence method, with 1000-2000 ml saline mixed with 1 mg adrenaline and 20 ml Lidocaine 2% (Xylocaine; AstraZeneca PLC, London, United Kingdom) per 1000 ml saline. The aspirate was collected in canisters of 2000 ml, which were left for sedimentation for 24 hours. When liposuction was finished, the compression garments were pulled up as proximally as possible and the incisions were left open for drainage and secondary healing. Prophylactic antibiotics with isoxazolylpenicillin were given, intravenously on the day of surgery and orally for an additional ten days. Patients with penicillin allergy received clindamycin instead.

The above-mentioned liposuction technique with tourniquet and tumescence was implemented from 1996 for arms and from 1998 for legs. Patients treated prior to

1996 and 1998, respectively, had the ‘dry technique’, meaning liposuction without a tourniquet or tumescence.

Compression garments ordered for arms included JOBST Elvarex (Essity, Sweden) compression class (CCL) 2 sleeves and standard interim gloves (Cicatrix interim, Thuasne Begat, France) together with a gauntlet (a glove without fingers but with a thumb) on top of the interim glove. For legs, a garment with a panty (JOBST Elvarex, CCL 3, Essity, Sweden) with a leg-long compression garment (JOBST Elvarex, CCL 2, Essity, Sweden) on top was used during the day but the CCL 2 garment was removed at night. For legs, three CCL 3 garments and two CCL 2 garments were ordered preoperatively, with one CCL 3 sterilised to be used after surgery. The compression garment applied during surgery was removed after two days and the patient had a shower. After lubrication of the skin, a new set of garments was put on with the sterilised garments disposed of due to their reduced compression capabilities. For legs, the leg-long CCL 2 was added to the CCL 3 garment with a panty during the day. The compression garments were changed again after another two days, and the patient was discharged. In the first postoperative week the compression garments were changed every second day and thereafter every day, with the used set washed to be used the next day.

The garments, which were made-to-measure, were renewed every three to six months and follow-ups were conducted at two weeks, one, three, six, nine and 12 months. For legs, six to eight sets of garments were needed for one year and for arms, four sets were normally needed. When complete reduction and a steady state were reached, follow-ups were scheduled every 12 months and garments were ordered for a whole year. Patients with a high level of physical activity required more sets. When 24 months had passed, the patient was referred to their regional therapist for continued monitoring with yearly reporting of volume measurements. For patients without a complete reduction after 12 months, visits were scheduled every three months to order new compression garments.

Macquarie University

The main concept of liposuction was identical in the two centres, but some minor specifics differed. Another power-assisted liposuction (PAL®) system (Microaire, Charlottesville, VA, USA) was used with commercially available ‘spiral’ cannulas. The compression garment sleeve was identical for arms with the addition of a flat-knit standard ready-to-wear glove (Medi, Germany). For legs, Velcro wraps (Easy-wrap, Haddenham, UK, covering the foot and Ready-wrap, Lohmann and Rauscher, Germany, for the rest of the leg) were used and applied in the operating theatre. Foam was applied to the ankle, shin and back of the knee. The wraps were removed within one week and exchanged for one-legged pant style compression garments (JOBST Elvarex class 3, Essity, Sweden). When comfortable with changing garments by themselves, patients were discharged and returned to the standard garment change every day. The follow-up regimen was similar to that in Sweden, with visits scheduled at two to six weeks, three, six, nine, 12, 18 and 24 months and thereafter with the patient's regional therapist. Garments were replaced every three to six months and when measurements remained stable, every six months.

Measurements

Volume measurements

At Skåne University Hospital, volume measurements were taken using plethysmography, a water displacement technique, where the limb was lowered into a canister filled with water and the outflow collected (Kettle *et al.*, 1958; Stanton *et al.*, 2000; Brorson *et al.*, 2015b). The outflow was weighed and measured to the nearest 5 g, equivalent to 5 ml. When referred back to regional therapists, volume measurements were taken using circumferential measurements every 4 cm along the limb with the volume calculated using the formula for a truncated cone which has been found to correlate well with plethysmography (Sander *et al.*, 2002; Taylor *et al.*, 2006; Brorson & Hoijer, 2012; Brorson *et al.*, 2015b). The same method was used at ALERT, Macquarie University, with the arm on a measuring board and the measurement starting at the ulnar styloid for arms and the lateral malleolus for legs.

Bioimpedance spectroscopy

BIS was used to measure extracellular fluid using the L-Dex® U400 (ImpediMed, Brisbane, Australia). Values are presented as L-Dex units. This unit is a ratio between the affected and the healthy limb with the normal range lying between -10 to 10.

Dual Energy X-ray Absorptiometry

DXA (DXA total body scan, DPX-L version 3.2; Lunar, Madison, WI) was used to assess the body composition of the affected and healthy arms. Patients were put into a supine position with foam between the arm and torso. The measured region of interest was between the distal end of the hand and a position 40 cm proximal to the ulnar styloid, and the same region was measured with plethysmography. With the standard Lunar software, fat mass, bone mineral content and lean mass were measured with the machine calibrated daily. Lean mass was assumed to mostly consist of muscle in the arm and the densities for fat (0.92 g/ml) (Fidanza *et al.*, 1953), muscle (1.06 g/ml) (Mendez, 1960; Segal *et al.*, 1986; Ward & Lieber, 2005) and bone (3.15 g/ml) (Lide, 2008) were used to calculate volumes.

Magnetic Resonance Imaging

The details of the Macquarie Medical Imaging at Macquarie University Hospital protocol for MRI assessment of lymphoedema have previously been published (Sen *et al.*, 2018). A 3T MRI machine (3.0T Siemens Versio) was used. The images were used in the clinic to quantify the amount of subcutaneous excess fat in the affected limb and to exclude a large amount of free fluid prior to liposuction. Both arm and leg lymphoedema were assessed. Images were deidentified and saved as JPEG files for analysis. For all images, the ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018) software was used to perform all the included area measurements with the software calibrated with a pre-existing scale in each image.

For study I, a transversal T1-weighted image at the proximal portion of the great trochanter was used with the subcutaneous area measured in cm^2. The gluteal cleft was used as a central border separating the left and right sides. In addition, a STIR image was used to analyse the presence of hyperintense skin which indicates skin thickening.

For study IV, three transversal levels were used for arm lymphoedema and four transversal levels were used for leg lymphoedema. For arms, these levels were eight cm proximal to the humeroulnar joint, eight cm distal to the humeroulnar joint and 15 cm distal to the humeroulnar joint. For legs, these levels were 30 cm proximal to the tibiofemoral joint, 15 cm proximal to the tibiofemoral joint, 15 cm distal to the tibiofemoral joint and 30 cm distal to the tibiofemoral joint. At each level, the outer border of the muscular compartment was marked, and the area measured in cm^2. Both the affected and healthy limbs were measured at all levels and the excess muscle area was calculated as the difference between the affected and healthy sides. T1-weighted images were routinely used for measurements but when excess fluid was present near the deep facia, STIR images were used instead.

Indocyanine Green Lymphography

ICG lymphography was used in the ALERT clinic to assess the drainage routes in lymphoedema and to possibly guide MLD and personalise conservative treatment according to the findings (Koelmeyer *et al.*, 2020b). A near infrared camera system (PDE Neo II; Hamamatsu Photonics K.K., Hamamatsu, Japan) was used. For legs, four injection sites were chosen in the foot to visualise all possible drainage routes along the limb. The ICG protocol consists of three phases. The first phase was to see if any spontaneous movement of the dye occurred along the lymphatics. In the second phase, MLD was performed by an accredited lymphoedema therapist until no further movement of the dye was observed. The third phase was to document and gather the data. The ICG operator then categorised the lymphoedema according to the MD Andersson classification scale from 0 to 5. When dermal backflow extended from the leg up to the gluteal region, the patient was diagnosed as having gluteal lymphoedema.

Ethics

Ethical considerations regarding the use of registry data include management of personal information and confidentiality. When handling and analysing data, all personal information was de-identified and a project ID was assigned to each participant. In theory, when reporting results from a treatment programme developed and implemented by a principal supervisor, there might be an underlying incentive to report positive results. In this field, surgeons' opinions historically seem to have been divided; either they believed in microsurgery, or they believed in liposuction. Many studies reviewed for this thesis have not adopted any standardised presenting format, and results sometimes seem to be cast in a better light than they should be. Subjective results, lack of data and different measurement methods result in the impossibility of comparing studies. The problem with not being able to compare outcomes still exists in this field and could hinder the development of optimal treatment strategies. It is the researcher´s and clinician´s duty and moral obligation to objectively assess and present unbiased and interpretable data that can drive progress and help patients, not to convey the superiority of one favourite treatment. With our studies, especially the ones assessing treatment outcomes, we try to be precise in our methodology, and to present data that is clear, consistent and that can easily be extracted.

Studies I and IV was approved by Macquarie University Health Clinical Innovation and Audit Committee (MQCIAC2020001A_2021) and no consent to participate was necessary since all data was obtained in routine clinical care and no additional intervention was required for patients.

Written information about the projects was distributed to all participants in studies II and V and an opt-out method was used for study inclusion. Both studies were approved by Swedish Ethical Review Authority (2020-03102).

Written informed consent was obtained for patients included in study III and ethical approval was obtained from the Ethics of Human Investigation Committee of Lund University (697 98), and the Radiation Protection Committee of Skåne University Hospital in Malmö (981212).

Written informed consent was also retrieved for patients included in study VI and the study was approved by Macquarie University Human Research Ethics Application (reference numbers: 5201300315 and 52020613914268).

Statistical Analysis

Statistical analyses were performed for all studies using IBM SPSS Statistics for Macintosh (Version 27.0. Armonk, NY: IBM Corp, released 2020). To test for normal distribution of the primary data, the Shapiro-Wilk test was used, and histograms were included in the output. The normal distribution could not be confirmed in the majority of the data, and non-parametric tests were used. Thus, results are presented as median and IQR or numbers and percent. To assess for correlations between continuous variables, the Spearman correlation coefficient was used. To assess differences in continuous variables between groups, the Mann-Whitney test was used, and to assess nominal data between groups, the Chi-Square test was used. To analyse changes over time for continuous variables such as preoperative to postoperative values, or related measures, the Wilcoxon signed-ranks test was used. A p-value under 0.05 was considered as statistically significant.

In study II, the incidence of erysipelas was calculated with all bouts of erysipelas experienced for all patients in total, divided by the total number of person years at risk. Period prevalence was defined as the proportion of patients that experienced at least one bout of infection during the intended period. When analysing for any significance between preoperative and postoperative incidence, the preoperative and postoperative incidence for each patient was calculated and compared using Wilcoxon signed-rank test. Also, the preoperative prevalence was compared to the postoperative prevalence using McNemars exact test.

In addition to the above-mentioned analyses, study III included Bland-Altman plots (Bland & Altman, 1986). This plot is another method of assessing correlation. With this method, the agreement between DXA and plethysmography was tested. The Bland-Altman plots show the average value of two measurements for each patent on the x-axis and the difference between the two measurements on the y-axis, each dot indicating one patient. In addition, 95% confidence intervals (95% CI) for the difference between the two measurements were calculated.

Main Results

Study I

Out of 28 patients, 18 had secondary lymphoedema and ten had primary lymphoedema. All patients had late-stage lymphoedema (ISL stage II-III). When assessed with ICG lymphography, ten patients had dermal backflow to the gluteal region on the ipsilateral side. **Figure 8** shows an example of a patient with this finding. No demographics were significantly associated with gluteal lymphoedema but there was a trend of more gluteal lymphoedema in patients with secondary lymphoedema compared to patients with primary lymphoedema.

Figure 8. ICG lymphography drainage patterns in a patient with left sided leg lymphoedema. Dermal backflow is present in the whole limb and spreads proximally to the gluteal region (arrow) (Karlsson *et al.*, 2022c).

On MRI, an increase in the subcutaneous tissue in the gluteal region on the affected side was visible with a median increase of 13% (IQR: 4 – 30). Even if the subcutaneous tissue was larger in patients with gluteal lymphoedema on ICG lymphography, with a median increase on the affected side corresponding to 20% (IQR: 7 – 38) compared to 11% (IQR: 3 – 23) in the group without gluteal lymphoedema, this difference was not statistically significant. The increase in subcutaneous tissue in the gluteal region was moderately correlated to total excess leg volume ($r_{Spearman}$=0.50, p=0.007). **Figure 9** shows an MRI image of a patient with visible increase signal intensity in the skin, representing skin hypertrophy. This finding was significantly more common in the gluteal lymphoedema group (89%) than in the non-gluteal lymphoedema group (29%).

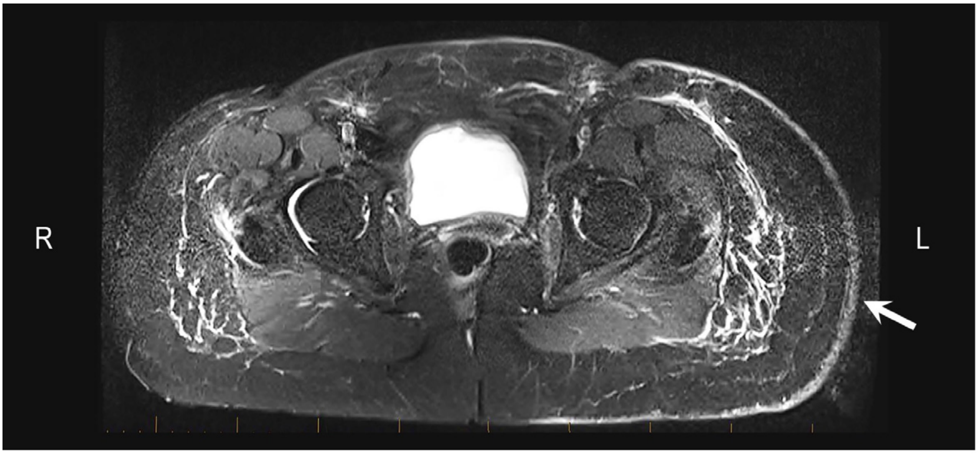

Figure 9. MRI image of a patient with left sided lymphoedema and increased signal intensity in the skin on the left side (arrow) (Karlsson *et al.*, 2022c).

Study II

With a total of 335 bouts combined for all patients before liposuction and 1680 person years at risk, the preoperative incidence rate was 0.20 bouts/person/year. This dropped to 53 bouts in 763 person years at risk and an incidence rate of 0.07 bouts/person/year after liposuction. This represents a statistically significant 65% decrease. With 64 patients experiencing erysipelas before liposuction and 28 patients experiencing it after, the period prevalence dropped significantly from 52% to 23%. **Figure 10** shows a flow chart of the included patients and the number of patients that experienced erysipelas during the study period.

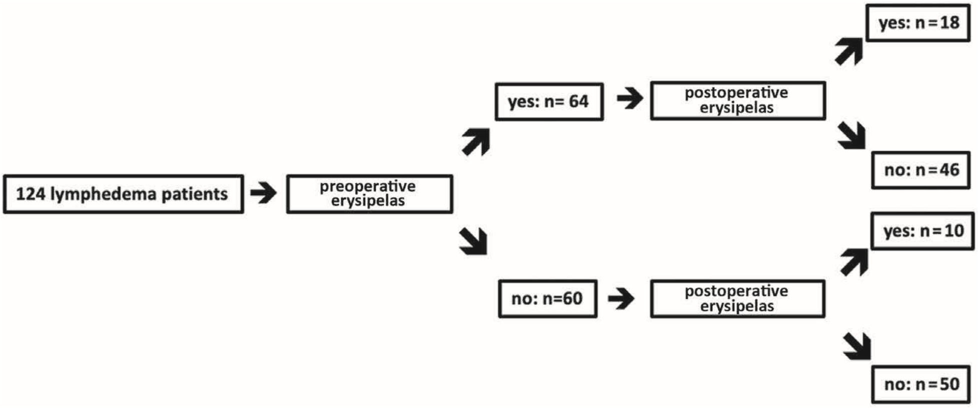

Figure 10. Illustrative flowchart indicating how many patients experienced erysipelas during the study period (Karlsson *et al*., 2022a).

In addition to the reduction in infection rates, the median 3158 ml (IQR: 2114 – 4650) preoperative excess volume of the affected leg and 34% (IQR: 24 – 44) larger volume than the healthy leg, decreased to -5 ml (IQR: -430 – 568) one year after liposuction.

Study III

The median excess arm volume before liposuction was 1425 ml (IQR: 1049 – 1538) measured with DXA and 1213 ml (IQR: 1014 – 1676) measured with plethysmography. Three months after liposuction, the excess volume had decreased to 193 ml (IQR: 81 – 295) and 165 ml (IQR: 70 – 233), respectively. One year after liposuction, the excess volume had decreased to 2 ml (IQR: -90 – 139) and -73 ml (IQR: -180 – 59), respectively. **Figure 11** illustrates the changes in total arm excess volumes over the study period.

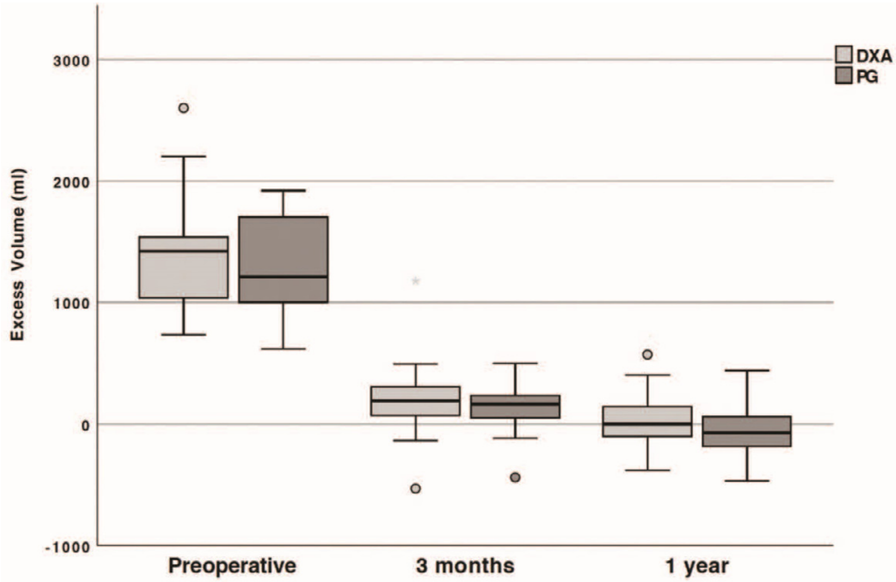

Figure 11. Preoperative excess total volume (ml) before and after surgery, measured with DXA and PG. The line within the box represents median excess volumes, box length represents the IQR, and whiskers represent the lowest and highest values except outliers (circles). Reductions measured with both DXA and PG were statistically significant at 3 months ($p < 0.001$ and $p < 0.001$, respectively) and 1 year ($p < 0.001$ and $p < 0.001$, respectively). DXA, dual energy X-ray absorptiometry; PG, plethysmography (Karlsson *et al.*, 2022b).

Fat Volume

Measured with DXA, the excess fat volume compared to the healthy arm decreased from 704 ml (IQR: 545 – 885) before liposuction to -196 ml (IQR: -258 – 112) after three months and -269 ml (IQR: -420 – -166) after one year. This corresponds to a 139% (IQR: 125-151) decrease in adipose tissue after one year. The results indicate that a lower amount of adipose tissue remained in the affected arm compared to the healthy arm after surgery. **Figure 12** shows these changes in excess fat volume.

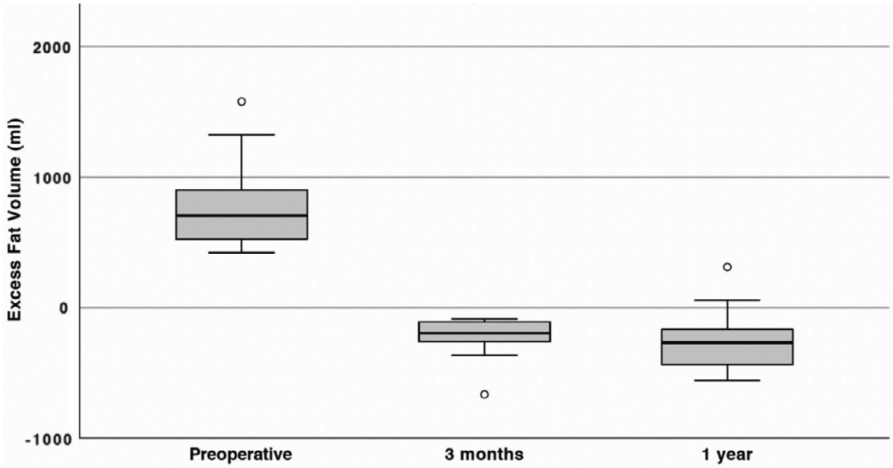

Figure 12. Excess fat tissue volume (ml) before and after surgery. The line within the box represents median excess volumes, box length represents the IQR, and whiskers represent the lowest and highest values except outliers (circles). Values obtained with DXA, n = 18. The median volume reductions from preoperative to 3 months, as well as preoperative to 1 year, show statistical significance ($p < 0.001$ and $p < 0.001$, respectively) (Karlsson *et al.*, 2022b).

Lean Volume

The excess lean volume compared to the healthy arm reduced from 651 ml (IQR: 475 – 880) before liposuction to 362 ml (IQR: 288 – 549) and 338 ml (IQR: 104 – 446) three months and one year after surgery, respectively. This corresponds to a 54% (IQR: 24 – 83) decrease in excess lean volume one year after surgery. Thus, a significant decrease in the lean volume was seen after liposuction and CCT. **Figure 13** shows these changes over the study period.

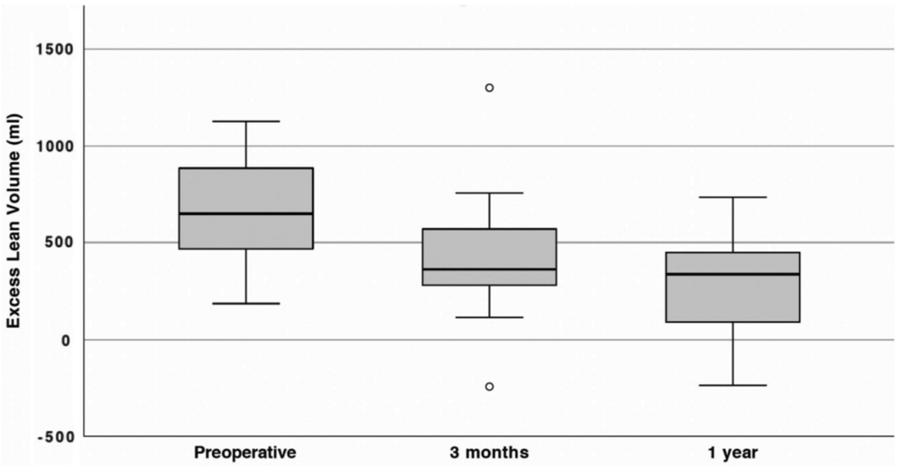

Figure 13. Excess lean tissue volume (ml) before and after surgery. The line within the box represents median excess volumes, box length represents the IQR, and whiskers represent the lowest and highest values except outliers (circles). Values obtained with DXA, n = 18. The median volume reductions from preoperative to 3 months, as well as preoperative to 1 year, show statistical significance ($p = 0.016$ and $p = 0.0013$, respectively) (Karlsson *et al.*, 2022b).

Bone Mineral Volume

With bone mineral volume, only very small changes were observed. The median excess bone mineral volume was 2.1 ml (IQR: 0.4 – 3.4) before surgery, which changed to 2.4 ml (IQR: 1.1 – 3.7) after three months and 1.0 ml (IQR: -0.5 – 2.7) after one year. Only the volume at one year after surgery was significantly different from the preoperative value.

DXA Compared to Plethysmography

In summary, plethysmography and DXA differed slightly, with plethysmography giving somewhat larger volumes. The largest difference between the two methods was for volumes of the affected arm at three months after liposuction, with an average difference of 8% (95% CI: 1-15).

Study IV

Twenty-eight patients had arm lymphoedema and 27 patients had leg lymphoedema. For arm lymphoedema patients, the median excess volume of the affected arm was 1054 ml (IQR: 758 – 1578). For legs, the median excess volume of the affected leg was 3663 ml (IQR: 2638 – 5886). The median muscle area measurements for arm lymphoedema are presented in **Table 1**. No significant difference in muscle area was found between the affected and healthy arm except in one location in dominant arms, where the difference was 7.9 % (IQR: 1.5 – 12.4). The median muscle area measurements for leg lymphoedema are presented in **Table 2**. In the two distal locations on the leg, there were a significant -5.2% (IQR: -9.4 – -0.5) and -4.0 % (IQR: -8.9 – 1.2) difference in muscle area.

Arm segmental measurements

	Affected arm (cm²)	Non-affected arm (cm²)	Difference (%)	Sign.#
All patients (n=28)				
8 cm proximal*	26.3 (24.6 – 29. 7)	26.0 (24.9 – 29.0)	1.8 (-3.3 – 4.6)	0.412
8 cm distal*	30.5 (28.3 – 32.9)	30.5 (27.4 – 32.2)	0.5 (-4.5 – 7.9)	0.456
15 cm distal*	17.5 (14.9 – 19.7)	17.1 (15.6 – 20.0)	0,0 (-6.5 – 5.5)	0.770
Total	74.1 (67.6 – 81.8)	73.3 (68.0 – 80.6)	2.4 (-2.5 – 4.0)	0.304
Dominant arm affected (n=9)				
8 cm proximal*	24.8 (22.5 – 27.4)	24.8 (23.5 – 25.3)	2.6 (-3.0 – 5.2)	0.374
8 cm distal*	29.6 (27.2 – 32.5)	27.4 (24.9 – 30.2)	7.9 (1.5 – 12.4)	**0.028**
15 cm distal*	15.9 (14.6 – 17.7)	16.4 (14.9 – 17.4)	0.7 (-6.0 – 6.2)	0.953
Total	70.3 (65.0 – 78.1)	68.0 (64.1 – 72.8)	3.8 (-1.4 – 8.1)	0.110
Non-dominant arm affected (n=13)				
8 cm proximal*	27.1 (25.9 – 30.5)	27.4 (25.9 – 29.1)	0.4 (-4.7 – 3.7)	0.917
8 cm distal*	31.8 (26.3 – 32.9)	31.1 (28.3 – 35.3)	-2.1 (-9.1 – 2.5)	0.308
15 cm distal*	18.1 (14.9 – 19.5)	17.5 (15.2 – 21.1)	-1.5 (-7.2 – 4.7)	0.477
Total	77.3 (65.8 – 82.8)	75.6 (69.5 – 85.4)	0.4 (-10.2 – 3.0)	1.000

Table 1. Segmental area-based measurements of the muscular compartment in arms.
**In relation to the humeroulnar joint.*
#P-value from non-parametric Wilcoxon signed rank test.
All values presented as median (IQR). cm= centimetre. IQR= interquartile range.

Leg segmental measurements

	Affected leg (cm²)	Non-affected leg (cm²)	Difference (%)	Sign.#
All patients (n=27)				
30 cm proximal*	122.7 (109.1 – 136.0)	121.2 (109.9 – 136.9)	-0.4 (-4.6 – 5.8)	0.829
15 cm proximal*	101.2 (94.0 – 115.2)	98.7 (90.1 – 113.1)	0.3 (-4.8 – 8.3)	0.904
15 cm distal*	62.8 (56.4 – 67.9)	65.2 (59.0 – 70.5)	-5.2 (-9.4 – -0.5)	**0.001**
30 cm distal*	20.7 (19.1 – 24.6)	21.6 (18.9 – 23.6)	-4.0 (-8.9 – 1.2)	**0.017**
Total	311.4 (281.0 – 335.6)	308.3 (282.6 – 335.3)	-2.0 (-4.9 – 2.7)	0.280

Table 2. Segmental area-based measurements of the muscular compartment in legs.
**In relation to the tibiofemoral joint.*
#P-value from non-parametric Wilcoxon signed rank test.
All values presented as median (IQR). cm= centimetre. IQR= interquartile range.

Study V

Out of the 67 patients, 36 patients had primary lymphoedema and 31 had secondary lymphoedema, where gynaecological cancer was the most common cause. The median preoperative excess volume was 3513 ml (IQR: 2225 – 5455) in the affected leg (**Figure 14**) and the ratio to the healthy leg was 1.35 (IQR: 1.25 – 1.53). The excess volume decreased significantly during the follow-up. At one year after surgery, the excess volume had decreased by 101% (IQR: 84 – 116) and at five years after surgery, the excess volume had decreased by 115% (IQR: 98 – 124). The postoperative percentage reduction of excess volume was negatively correlated to the preoperative excess volume, indicating that patients with smaller lymphoedema had a higher reduction rate in excess volume after surgery. The median aspirated volume was 3770 ml (IQR: 2240 – 5140) and contained 85% fat (IQR: 79 – 91). The median aspirate when the tourniquet was used measured 2080 ml (IQR: 1150 – 3205) and contained 100% fat (IQR: 90 – 100). No major complications were experienced and in total, five patients required blood transfusions after surgery. No blood transfusions were required after October 2006.

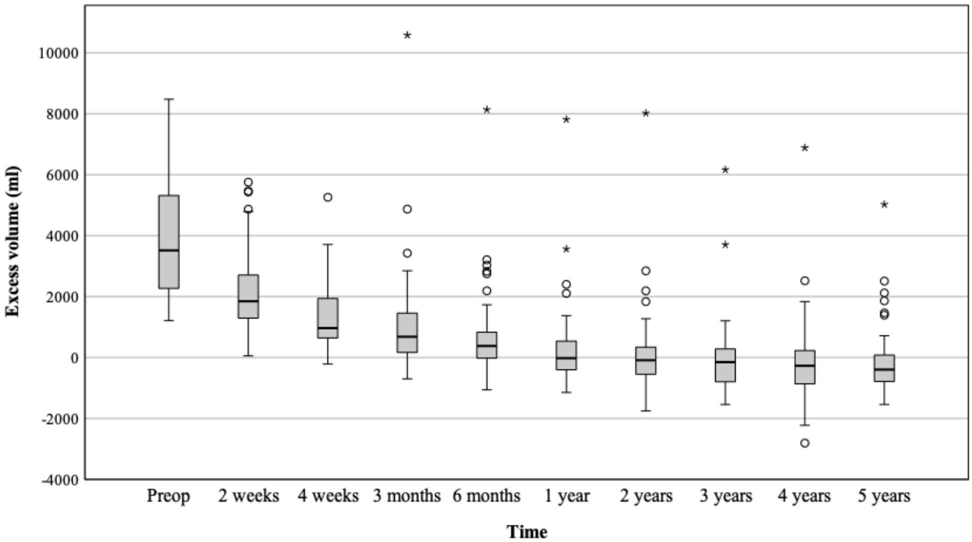

Figure 14. Excess volumes of the affected leg during the study period. One patient representing an extreme outlier lies outside of the visualized field in the graph. The missing values for this patient in the graph are: preop: 20 805 ml, two weeks: 12 575 ml, and four weeks: 12 695 ml. The edge of the box illustrates the interquartile range with the median as the line within the box. Whiskers indicate the lowest and highest values without outliers (circles) and extreme outliers (stars).

Study VI

All 29 patients with arm lymphoedema had secondary lymphoedema. The surgical outcomes are presented in **Table 3**. For arms, the excess volume before liposuction was 1061 ml (IQR: 763 – 1599) which significantly reduced by 95 % (IQR: 81 – 119) after one year by 98 % (IQR: 74 – 120) after five years. However, as illustrated in **Table 3**, there was a large loss to follow-up after five years. The fluid component as measured with L-Dex decreased during the follow-up for arm lymphoedema patients from the preoperative L-Dex score of 41 (IQR: 32 – 60) to 27 (IQR: 21 – 38) after one year and to 20 (IQR: 14 – 28) after five years.

Twelve out of the 30 patients with leg lymphoedema had primary lymphoedema and 18 had secondary lymphoedema. The excess volume before liposuction was 3447 ml (IQR: 2065 – 5656) which was reduced by 90 % (IQR: 71 – 104) after one year and by 72 % (IQR: 55 – 91) after five years. Thus, there was a slight increase in median excess volume from one year to five years after surgery. No difference in surgical outcome was observed for patients with primary or secondary lymphoedema. The fluid component in leg lymphoedema did not significantly change during the study period; only a trend with slightly lower L-Dex scores was observed after surgery.

No major surgical complications were reported for the whole study cohort and the only minor complications included pressure wounds on the skin from compression garments. Five patients experienced this, but no surgical invention was necessary for healing.

Table 3. Not avaliable online due to copyright by publisher. Full table will be avaliable open access in *Plastic and Reconstructive Surgery* when the final version of paper VI is published.

Discussion

Methodological Considerations and Results

Gluteal Lymphoedema

The number of patients having gluteal dermal backflow on ICG lymphography (36%) in this material was marginally higher than the previous finding of 22% in cancer-related leg lymphoedema (Koelmeyer *et al.*, 2020b). Patient selection and the number of included patients could influence these numbers. However, the extension of dermal backflow to the gluteal region seems to be a common condition in late-stage lymphoedema and the implications for patients are still to be investigated. With only a handful of studies mentioning lymphoedema in the gluteal region, this might be an area for future research. The current study presents gluteal lymphoedema as a separate entity and emphasises that future studies should evaluate its clinical aspects. One possible theory is that a significant increase in the subcutaneous tissue in one side of the buttocks results in a bad posture, for example when sitting, and could potentially cause lower back pain and reduced quality of life.

The study evaluates extension of dermal backflow to the gluteal region by distal injections of ICG in the limb. Thus, the normal drainage routes in the gluteal regions were not visualised when no extension of dermal backflow was found on ICG. The lymph in the gluteal region is normally drained to the inguinal lymph nodes and an obstruction of the inguinal lymph nodes could also impede lymph drainage from the gluteal region and cause gluteal dermal backflow, indicating lymphoedema. Therefore, some patients without a sign of gluteal lymphoedema on ICG lymphography with our protocol might still have the condition. This could possibly explain why no significant difference in the subcutaneous area was found between the groups and why the gluteal subcutaneous area was moderately correlated to total leg excess volume. In addition, hypertrophy in the gluteal skin on the affected side was found in five out of 17 patients without gluteal lymphoedema. In future studies, additional injections of ICG dye could be considered in the gluteal region to evaluate this theory.

Liposuction and Controlled Compression Therapy Outcomes

The liposuction procedure has been performed for lymphoedema patients for more than 25 years at Skåne University Hospital in Malmö and the surgeon, Håkan Brorson, has vast experience in this treatment modality. Previous studies have proved the effects of the treatment, and centres worldwide have been able to achieve similar results after implementation of the procedure. The current studies showed a complete reduction of the excess leg volume by a median 100% (study II) and 101% (study V) reduction in excess volume after one year and 115% after five years (study V). Comparable results were obtained in Macquarie University in Sydney where the reduction in excess leg volume was 90% and 72% after one and five years, respectively (study VI). Obvious differences between the two centres are the health care system, costs for patients and the climate. With the cost of the surgical and postoperative treatment being covered by the government in Sweden but not in Australia, the availability of the treatment differs, and some patients cannot afford to be treated. In addition, the hot and humid climate in Sydney affects the usage of compression garments and patients must have discipline and compliance to wear compression garments for 24 hours a day, all year. Also, in Sweden the leg patients wear two layers of flat-knitted garments on top of each other to optimise compression.

In addition to the reduction seen in excess volume for leg lymphoedema patients, the incidence of erysipelas reduced by 65% which is comparable to the previously reported incidence reduction of 87% in arm lymphoedema (Lee *et al.*, 2016). Recurrent, potentially life threatening skin infections are troublesome for patients and frequent use of antibiotics can both drive antibiotic resistance and cause side-effects. In addition, the infection may require hospital admission and the treatment constitutes a cost for both patients and society. Other studies have indicated a much higher incidence of erysipelas than the present study with up to six bouts/person/year (Mihara *et al.*, 2014; Deng *et al.*, 2015; Mihara *et al.*, 2016; Aljaaly *et al.*, 2019; Koide *et al.*, 2020). The information about skin infection incidence was often presented as a secondary outcome in these studies and information about the observation period was frequently missing.

Tissue Composition in Late-Stage Lymphoedema

It is generally accepted that lymphoedema leads to adipose tissue deposition in the affected limb. This has been verified in several studies (Brorson *et al.*, 2006b; Brorson *et al.*, 2009; Hoffner *et al.*, 2018b; Trinh *et al.*, 2019) and the affected arm in 18 BCRL patients had on average 73% more fat compared to the healthy arm. The same study indicated a 47% increase in lean volume and suggested that this increase was due to muscle hypertrophy from the added weight of the arm. As previously discussed, there are conflicting reports about the presence of excess muscle in lymphoedema and no agreement in the literature exists. If the muscle is hypertrophied due to the increased load on the arm, a total reduction of the excess volume after liposuction should in time result in the muscle returning close to the original size. The results from study III support this theory. While the total excess volume reduced by 106 % one year after liposuction, the excess lean volume reduced by 54 % from the initial 651 ml. Thus, a substantial amount of excess lean tissue was still present after one year. Lean tissue on arm DXA measures muscle, fluid, and skin and the modality cannot differentiate one component from another. Thus, to find which component was increased in these patients, another imaging modality was required.

MRI can delineate the muscle compartment in arm and leg lymphoedema and can also calculate whole limb muscle volumes separately from other tissue components (Sen *et al.*, 2018). However, the geographical challenges when conducting these studies resulted in the limitation that it was only possible to measure sectional areas instead of volumes, which reduced the statistical power of the assessment. Using total limb volumes would be the gold standard and to further subcategorise into sectional volumes could possibly show whether regional differences in muscles exist throughout the limb. The fact there was no increase in muscle area in any of the locations throughout the leg in study IV strongly contradicts the above-mentioned theory of increased muscle volume. Rather, a small decrease in muscle area distally in the affected leg was seen. This could possibly be associated with gait patterns and a reduced use of muscles in the calf of the affected leg. For arms, only a small increase in muscle area was found on one location when the dominant arm was affected but the increase was not significant for the total measured muscle area in the arm. It is not possible to conclude whether this is a general increase or if this is due to hand dominance. Therefore, the indications from study III concerning increased muscle volume cannot be proved with this methodology. The increase in lean volume seen in study III could also constitute fluid, skin or fibrosis instead of muscle. The fluid content in the aspirate from liposuction was low, and major differences of free fluid in the muscular compartments in the affected and healthy limb are unlikely, due to the similar muscular areas. As lymphoedema are known to induce fibrotic changes, this could explain the part of lean volume increase seen with DXA that did not reduce during the one-year follow-up. Methods to quantify fibrosis with automatised, formula-based, CT calculations are currently being developed (Son *et al.*, 2022).

Study Limitations

Methodological limitations include retrospective study designs which may result in missing data and no possibility to assess other data than what has previously been gathered. Another obvious limitation includes loss to follow-up during the COVID-

19 pandemic in Australia with closed international and interstate borders and local restrictions. Many patients were residents in other states or from New Zealand, resulting in fewer patients with the possibility for long-term follow-up.

Regarding collection of bouts of erysipelas, patients were asked at the first visit about previous bouts of erysipelas since the lymphoedema debut. Some patients had had lymphoedema for many years before being treated with liposuction and the exact number might be difficult to remember. Some patients had received prescriptions from their physician for antibiotics to take as needed in the case of erysipelas and used them at home when symptoms started. Medical charts were checked for bouts requiring hospital admission, but bouts treated by the patients themselves at home were also included. When relying on the recollections of the patients, the actual number of bouts might be overestimated or underestimated. In contrast, if only bouts documented in patients' charts were included in the study, such as bouts treated with admission to hospital, many bouts would be missed, and the data would underestimate the burden of erysipelas on this patient cohort.

Other limitations include the depth of field of ICG lymphography. Since the method uses a fluorescent dye visualised with a near infrared camera, lymphatics to a depth of approximately 1 cm can be visualised (Suami *et al.*, 2012). Deeper lymphatic pathways cannot be visualised, which could have added additional information to the study on gluteal lymphoedema.

As previously discussed, there are uncertainties about the repeatability and validity of DXA in the literature (Clarys *et al.*, 2010). Comparing mass from DXA with the weighted mass after anatomic dissection resulted in questionable correlations, especially for lean tissue. However, since study III included measuring excess tissue compared to the healthy arm, with a low coefficient of variation (Brorson *et al.*, 2009), tissue compositional changes before and after liposuction regarding lean tissue strongly indicated excess volume of one or more lean tissue components, such as muscle or skin.

Subsequently, some limitations regarding the methodology when assessing the muscle area with MRI also exist. Not including the more proximal arm muscles that might be more exposed to an increased weight of the arm exposes the study to potential type 2 errors and could potentially miss a hypertrophy of these muscles. Also, since the outer border of the muscle compartment was measured, other structures such as bone, blood vessels, fluid and fat (Hoffner *et al.*, 2018b) between muscles could also affect the differences between the sides. Similar limitations are obvious in the study assessing gluteal lymphoedema. The subcutaneous tissue was measured on one transversal image at the upper level of the greater trochanter. Since only one level has been assessed, no conclusion on the total volume of the gluteal region can be made. The gluteal subcutaneous volume could be assessed in future studies. MRI would be a great method to measure this volume; however, other more clinically available volume measurements such as plethysmography or circumference might be neither practical nor accurate. The skin was assessed by noting presence of an increased signal intensity as an indication of hypertrophy of the skin. However, to accurately measure the skin depth and characteristics, a different MRI setting with specific skin coils should be used (Idy-Peretti *et al.*, 1998).

Statistical Considerations

One essential first step when assessing clinical data is to check if the data is following the normal distribution curve. There are many methods to check for normal distribution, including histograms, skewness, kurtosis, the Shapiro-Wilks test and the Kolmogorov-Smirnov test. The used methods included the Shapiro-Wilks test for a statistical analysis and histograms for a visual assessment. Since most data did not follow a normal distribution for the included studies, non-parametric analyses were used. For non-parametric tests, the significance is based on p-values. Using multiple tests in the same study increases the risk for type 1 errors since a p-value of 0.05 is statistically correct in 19 out of 20 tests performed. A Bonferroni correction can be applied in these cases but then, the risk for type 2 errors increases. If the included number of patients is low and the analysis is of an experimental nature to be assessed in future studies, then type 2 errors should arguably be avoided rather than avoiding significant results where no actual significance exists. In many cases, parametric tests, such as the t-test, give more informative results with 95% CI. These not only indicate if the test is statistically significant, but also show the span of the CI. It is also true that the population mean statistically lies outside of the 95% confidence interval in one out of 20 tests performed, but the interval adds extra information for the reader.

The Bland-Altman plot is an informative method to assess the agreement between two types of measurements (Bland & Altman, 1986). The standard correlation such as the Pearson correlation coefficient might result in a perfect correlation even if the two methods show a clinically relevant difference. The ordinary methods do not express the size of the disagreement, or the range of the calculated 95 percentiles of the disagreement, which Bland-Altman plots display. These plots were used to compare DXA with plethysmography, since it is important to highlight clinically relevant differences between the methods that can be anticipated.

Conclusions

Overall, this thesis has demonstrated convincing postoperative results of liposuction and CCT in both arm and leg lymphoedema and has highlighted interesting findings from studies on tissue characteristics and composition. The major conclusions are summarised as follows:

- Late-stage lymphoedema in the leg can cause altered lymphatic drainage to the gluteal region, which in turn might indicate subcutaneous tissue changes, including excess adipose tissue and skin hypertrophy, in this area. 'Gluteal lymphoedema' is a topic for future studies, which should examine its severity and implications for patients.
- The increase in lean tissue previously found in BCRL with DXA decreased by 54% one year after liposuction, which could be attributed a postoperative reduction in muscle size due to less load, and/or reduced volume of skin, fluid and other components.
- No general increase in muscle area on MRI could be found in late-stage lymphoedema using the current method.
- Liposuction and CCT for arm and leg lymphoedema were found to treat the swelling in lymphoedema and led to a total, or near total, reduction in excess volume. The results were maintained during the studied five years with the use of CCT. Regional differences might exist that have a direct impact on the treatment outcomes.
- Liposuction and CCT of leg lymphoedema reduce the incidence rate of erysipelas by 65%, which is comparable to the results of this treatment for arm lymphoedema.

Clinical Implications and Future Perspectives

As previously stated, liposuction, when performed by a trained and experienced surgeon in a multidisciplinary team setting, is generally considered as a safe and effective method for removing the excess adipose tissue. It is still important to continuously evaluate the progress and results of a treatment, and the five-year results from Studies II and V may reassure leg lymphoedema patients about the expected outcomes from this treatment. Many centres around the world have implemented this technique, but since many aspects of health care services in different countries differ, such as financial aspects, availability, socioeconomic status and climate, it is important for each centre to separately analyse and present their results. The loss to follow-up experienced in study VI generates some questions concerning the long-term outcomes as the presented results undoubtedly could have ben affected by the reduced number of patients. However, the presented data show clear and significant results in the first years after surgery and also support the safety of the procedure. Also, they imply a need to investigate the background of loss to follow-up, to possibly improve the programme in the future. Data are continuously collected as part of clinical routine, and long-term follow-up in a larger cohort of patients will be conducted in the future.

As discussed at the beginning of this thesis, clinical outcomes are not only about actual postoperative results such as volumes, infection rates or complications. Probably, the most important aspect concerns the results perceived by the patient, in terms of quality of life. Previous studies have shown improved quality of life for patients treated both at Skåne University Hospital in Malmö and at Macquarie University Hospital in Sydney (Brorson *et al.*, 2006a; Boyages *et al.*, 2015; Hoffner *et al.*, 2017; Klernäs *et al.*, 2020). However, the quality of life data for the leg patients in study V has not been assessed, which would add valuable information about the postoperative results for this cohort and put the excess volume reductions and reduced amount of skin infections into context. In Sydney, there was a change in the assessment tools used to assess quality of life during the study period and therefore, the data was incomplete and could not be evaluated for the included cohort. New data with the use of validated lymphoedema-specific quality of life scales will be published in future studies.

In the future, it would be interesting to perform volume calculations on muscle compartments in whole limbs using MRI for both arms and legs, including the same patients as in study IV, or a similar cohort as in study III. These patients had large, late-stage lymphoedema, and if accurate volume measurements are performed and no muscle hypertrophy is found, other aspects such as skin, fluid or fibrosis should probably be considered. A regional control group with similar BMI and age, could be used to evaluate the impact arm dominance has on muscle volume.

Gluteal lymphoedema is a condition rarely discussed in the medical literature. The shortage of information on this topic could be because lymphoedema in the leg is considered to be the same entity as gluteal lymphoedema, without a need to separate the two. It could also be because gluteal lymphoedema is a condition without clinical relevance and because the leg is more affected than the gluteal region due to its more distal location, in combination with the gravitational effects. We have introduced a method to diagnose gluteal lymphoedema, and the only approach to assess the magnitude, physical implications and effect on quality of life is to further scrutinise this topic in future studies. Other methods to diagnose and assess gluteal drainage could be magnetic resonance lymphography with the possibility to observe the lymphatic drainage in the gluteal region in three dimensions as well as the deeper lymphatic vessels (Gennaro *et al.*, 2017). However, a reasonable first step could be a qualitative study considering patients' experience of swelling in the buttocks related to late-stage leg lymphoedema, and drawing conclusions about clinical implications. Gluteal lymphoedema is an interesting aspect of future studies, and if not researched, the possibility to improve treatment and outcomes for a group of patients is potentially lost. With a significant increase in gluteal volume on one side, every time a patient sits down, a clear postural effect on the lower spine could be anticipated. Liposuction of the gluteal region should perhaps be considered in more patients.

Outside this thesis, many interesting topics around lymphoedema pathophysiology and treatment are currently being studied. The role that inflammation has on lymphoedema formation and associated tissue changes generally seems to be accepted. Studies on anti-inflammatory drugs to prevent lymphoedema have in some studies shown encouraging results in animal trials; however, the clinical relevance is still to be proved (Nakamura *et al.*, 2009; Tian *et al.*, 2017). If a clinically safe drug could stop the deterioration of lymphatic vessels and hinder the formation of excess adipose tissue and fibrosis, then microsurgical procedures could theoretically be more effective and eliminate the need for compression therapy in early stages of lymphoedema.

Promoting lymphangiogenesis when performing microsurgical procedures such as VLNT is an appealing approach since the effect of VLNT requires new, afferent lymphatic routes into lymph nodes for lymphatic drainage. Therefore, VEGF-C is tested as a drug in combination with VLNT in BCRL treatment, and a long-term phase II trial is currently ongoing (Clinicaltrials.gov, NCT03658967). The phase I trial that evaluated this treatment combination over a 24-month period showed a decrease of excess volume of 46% after one year and overall improved quality of life over the study period (Leppäpuska *et al.*, 2022). In addition to using VEGF-C as a direct treatment therapy, therapies acting on the intracellular VEGF-C signalling pathway have shown promising lymphangiogenic effects in mice models without the side-effects seen with directs use of VEGF-C (Kataru *et al.*, 2021).

Finally, what might be one of the most important aspects in improving health care in the future is prevention of lymphoedema after cancer treatment and other secondary lymphoedema. Implementation of early surveillance and intervention programmes with BIS after breast cancer treatment has shown promising results for averting lymphoedema development (Koelmeyer *et al.*, 2019; Koelmeyer *et al.*, 2020a). The LYMPHA protocol with direct microsurgical LVA during lymph node dissection has the potential to further decrease the number of patients ending up with lymphoedema (Boccardo *et al.*, 2014; Boccardo *et al.*, 2016). Secondary lymphoedema due to filariasis is globally the most common cause of lymphoedema, and a programme to eliminate this disease has been implemented for many years (Organisation Mondiale de la Santé, 2019). Although progress has been made in lymphatic research, it is still critical to educate patients about lymphoedema and self-management, as well as educate physicians and health care providers about diagnosis, treatment and available resources.

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