Evidence Synthesis in the Treatment for Breast Cancer - related Lymphedema

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PhD Thesis

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Barcelona, 2024

Acknowledgments

I extend my deepest gratitude to my mother, whose endless support, boundless love, and invaluable guidance have been a constant source of strength throughout my life's journey.

To my sisters and brothers, their steadfast presence and willingness to provide care and help whenever I needed it have meant the world to me. Their support has been a valuable pillar in my life.

I am sincerely grateful to my direct supervisors, Dr. Jaume Masia and Dr. Xavier Bonfill, for their continuous assistance, mentorship, and guidance throughout this challenging yet rewarding journey. Their wisdom and encouragement have been instrumental in shaping my academic and professional career.

My deepest appreciation goes to all the co-authors of this work for their outstanding collaboration, dedication, and contributions. It is through your collective efforts and support; we have accomplished this work together.

Special thanks to Dr. Fekri Dureab, Ana Isabel Quindós Sendino & Diego Armando Buitrago Mora for their assistance and support throughout the project. Their insights and recommendations have significantly enriched my research journey.

The capacity to learn is a gift; the ability to learn is a skill; the willingness to learn is a choice.

Brian Herbert

List of Abbreviations

Number	Abbreviation	Details
1	ASC	Autologous stem cells transplantation
2	BCRL	Breast cancer - related lymphedema
3	CONSORT	Consolidated Standards of Reporting Trials Statement
4	DCT	Decompressive congestive therapy
5	EBM	Evidence based medicine
6	ESWT	Extra corporal shock wave therapy
7	GEM	Global Evidence Mapping
8	KT	Kinesio tape
9	LN	Lymph node
10	LV	Lymph vessels
11	LVA	Lymphaticovenular anastomosis
12	MLD	Manual lymphatic drainage
13	PICOS	Population, Intervention, Comparison, Outcome and Study Type
14	PRISMA-P	Preferred reporting items for systematic reviews and metanalysis protocol
15	Qol	Quality of life
16	RCTs	Randomised clinical trials
17	SGB	Satellite ganglionic block
18	SNB	Sentinel node biopsy

19	SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
20	SRs	Systematic reviews
21	TIDieR	Template for Intervention Description and Replication
22	VLNT	Vascularized lymph node transfer

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Abstract

Background: Women treated for breast cancer are facing a lifetime risk of developing lymphedema, which occurs in up to 40% of this population. In the past, breast cancer-related lymphedema (BCRL) treatments were relied mainly on conservative therapy. However, surgical treatments are alternative options that could be highly beneficial, and notably there is a major advancement in surgical instruments and techniques.

The aim of this thesis is to identify, describe and organize of the currently available evidence in the treatment for BCRL, and to assess the risk of bias of the surgical randomized clinical trials (RCTs) and systematic reviews (SRs).

Methods: We conducted two evidence mapping reviews according to the methodology proposed by Global Evidence Mapping (GEM). We have made systematic search in MEDLINE, EMBASE, CENTRAL (Cochrane), and Epistemonikos from the year 2000 onward. In the second review, we assessed the risk of bias for the surgical RCTs and SRs using the RoB-2 and ROBIS tools, respectively. Results were summarized in narrative and tubular forms.

Results: After the last search update, a total of 272 studies were included, of which 225 were nonsurgical studies and 47 studies addressed the surgical treatment for BCRL, of these surgical studies, only two RCTs and eight SRs met our eligibility criteria. The overall risk-of-bias assessments of these studies were rated as with some concerns (six outcomes) and high risk (three outcomes) for the measured outcomes among the RCTs, and as a high risk of bias (five studies) and low risk (three studies) for the included SRs.

Conclusions: The overall evidence in the literature on surgical treatment for BCRL is low, as there are few published RCTs and SRs, and the risk-of-bias assessment for the majority was rated as high risk of bias or with some concerns. High-quality studies in the surgery field are needed to measure the real effectiveness of the applied treatment and to improve evidence-based decision-making by surgeons and patients.

Resumen

Antecedentes: Las mujeres con cáncer de mama tienen un riesgo de hasta el 40% de desarrollar linfedema de por vida. En el pasado, los tratamientos de linfedema relacionados con el cáncer de mama (BCRL) se fundamentaban en la terapia conservadora. No obstante, las intervenciones quirúrgicas representan alternativas que podrían ser altamente beneficiosas, destacando el notable progreso en los instrumentos y técnicas quirúrgicas.

El objetivo de esta tesis es identificar, describir y organizar la evidencia actualmente disponible en el tratamiento del linfedema relacionado con el cáncer de mama, y evaluar el riesgo de sesgo de los ensayos clínicos aleatorizados (ECAs) y revisiones sistemáticas (RSs) sobre el tratamiento quirúrgicos.

Métodos: Se realizaron dos revisiones de mapeo de evidencia según la metodología propuesta por el Global Evidence Mapping (GEM). Se realizaron búsquedas sistemáticas en MEDLINE, EMBASE, CENTRAL (Cochrane) y Epistemonikos a partir del año 2000. En la segunda revisión, se evaluó el riesgo de sesgo para los ECAs y los RS quirúrgicos utilizando las herramientas RoB-2 y ROBIS, respectivamente. Los resultados se resumieron en forma narrativa y tabular.

Resultados: Después de la última actualización de la búsqueda, se incluyeron un total de 272 estudios, de los cuales 225 fueron no quirúrgicos y 47 estudios abordaron el tratamiento quirúrgico para BCRL, de estos estudios quirúrgicos, solo dos ECAs y ocho RS cumplieron criterios de elegibilidad. Las evaluaciones generales de riesgo de sesgo de estos estudios se clasificaron con algunas dudas de sesgo (seis desenlaces) y alto riesgo de sesgo (tres desenlaces) para los que están en ECAs, y un alto riesgo de sesgo (cinco estudios) y bajo riesgo (tres estudios) para los RSs incluidos.

Conclusiones: La evidencia general en la literatura sobre el tratamiento quirúrgico para BCRL es baja, ya que hay pocos ECAs y RSs publicados, y la evaluación del riesgo de sesgo para la mayoría se calificó como de alto riesgo de sesgo o con algunas dudas de sesgo. Se necesitan estudios de alta calidad en el campo de la cirugía para medir la efectividad real del tratamiento aplicado y para mejorar la toma de decisiones por parte de cirujanos y pacientes, basada en la evidencia.

1. Introduction

1.1 Pathophysiology of the lymphatic system

Blood vessels form a closed circulatory system, whereas lymphatic vessels form a one-way conduit for tissue fluid and leukocytes. In most vertebrates, the main function of lymphatic vessels is to collect excess protein-rich fluid that has extravasated from blood vessels and transport it back into the blood circulation. Lymphatic vessels have an important immune surveillance function, as they import various antigens and activated antigen-presenting cells into the lymph nodes and export immune effector cells and humoral response factors into the blood circulation.[1]

Briefly, the lymphatic system has 3 major functions: (1) the preservation of fluid balance; (2) host defence; and (3) a nutritional function, as intestinal lymphatics are responsible for fat absorption. Defects in lymphatic function can lead to lymph accumulation in tissues, dampened immune responses, connective tissue and fat accumulation, and tissue swelling known as lymphedema.[2]

The lymphatic system is arguably the most neglected bodily system, as a result, its contribution to human health and disease is not well understood. The recent advances in research have greatly improved our understanding of the biology behind pathophysiology of the lymphatic system, and led an innovation in the diagnostic techniques, therapeutic interventions, and targeted treatment strategies aimed at addressing various lymphatic disorders.[3]

1.2 Lymphedema definition

Lymphedema is a chronic disease characterized by massive lymphatic fluid stasis, swelling and fibroadipose deposition.[4] In these conditions, progressive adipose deposition and tissue fibrosis result in increased limb volume, heaviness, functional difficulties, increased susceptibility to infection and rare but fatal secondary tumours.[5]

Several clinical findings suggest that the pathology of lymphoedema is a multi-step sequence in which lymphatic injury is the initiator of these events.[4] This hypothesis is supported by the fact that for example lymphedema develops only in a subset of patients who undergo lymphadenectomy and not uniformly in all patients who suffer lymphatic injury.[6] Moreover, some patients develop lymphoedema even after an apparently trivial injury to the lymphatic system, suggesting that even minor disturbances of lymphatic function can initiate the pathological sequence.[7] Finally, in most patients, lymphedema develops slowly, usually months or years after the initial surgical injury, suggesting that secondary events are necessary for the development of this pathological process.[8]

1.3 Classification of lymphedema

There are two general classifications of lymphedema: primary and secondary.

- Primary lymphedema develops because of a congenital and/or hereditary defect. Characterized by aplasia, hypoplasia or dysplasia of the lymphatic network, primary lymphedema is one of the so-called rare diseases. Although 10% of cases are congenital, most cases are detected before the age of 35, and in a high percentage due to an intercurrent event such as a sprain or infection.[9] Although rarer, some primary lymphedemas are familial forms such as Milroy and Meige syndromes. Primary lymphedema may also be part of more complex malformations such as Klinefelter, Turner or Noonan syndromes.[9]

- Secondary lymphedema accounts for 99% of lymphedema cases in adults worldwide.[10] The most common cause of acquired lymphedema in developed countries is iatrogenic, predominantly reflecting the large group of patients in whom lymphatic trauma is a direct consequence of surgical and radiotherapeutic interventions for cancer.[10] In addition, lymphedema can also be acquired from other forms of lymphatic vascular trauma. These include burns and large or circumferential wounds to the limb, infection, tumour blockage, chronic venous insufficiency, immobility, or tourniquet effects,[11] but estimates of relative prevalence are difficult to determine.[10]

1.4 Breast cancer-related lymphedema

Breast cancer-related lymphedema (BCRL) is a major complication experienced by a large number of breast cancer survivors. More than 1.38 million women worldwide were estimated to be diagnosed with breast cancer in 2008, accounting for 23% of all diagnosed cancers among women.[12] Given that the 5-year survival rate for breast cancer is up to 90%, experiencing breast cancer is ultimately about quality of life.[13] Women treated for breast cancer are facing a lifetime risk of developing lymphedema, which occurs in up to 40% of this population and negatively affects breast cancer survivors' quality of life.[14,15]

According to the literature, about 20% of women will develop arm lymphedema after breast cancer, this estimation is the average incidence of studies that have been included in several systematic reviews of lymphedema after breast cancer, the incidence is about four times higher in women who had an axillary-lymph-node dissection than it was in those who had Sentinel node biopsy (SNB).[16,17] Other risk factors that aggravate the condition are adjuvant radiation, docetaxel chemotherapy, infection, iatrogenic injury, and obesity. Consequently, developing lymphedema leads to a chronic condition that is usually challenging to treat.[18–23]

Understanding BCRL as a chronic and progressive condition has led to increased weight on early detection, preventive strategies, and tailored therapeutic approaches include both surgical and non-surgical interventions designed to alleviate its impact.[24]

1.5 BCRL treatment options

Treatment for BCRL has mostly been symptomatic in nature and designed mainly to prevent swelling progression and have long been based on conservative therapy, such as manual lymphatic drainage, compression therapy, exercise programs, and skin care, these conservative measures are mainly aimed at alleviating lymphedema symptoms, reduce swelling and prevent disease progression without definitive curative intent.[25–27]

Surgical treatments are alternative options that could be highly beneficial, especially for patients who are not responsive to standard conservative therapy, which includes mostly reductive and reconstructive techniques. Both excisional and reconstructive surgical approaches have been described in the treatment of BCRL.[25–27]

Reductive or nonphysiologically procedures include the Charles operation and liposuction. These strategies are most often performed in a later stage of disease when there are no remaining functional lymphatic vessels.[28,29] Reconstructive options, on the other hand, are physiological operations that aim to restore lymphatic flow to aid in lymphatic drainage from the affected extremity. These include lymphaticovenular anastomosis (LVA) and vascularized lymph node transfers (VLNT), which currently have promising results for treating the early stages of lymphedema.[30–34]

Nonetheless, the optimal sequence, timing, and combination of these treatment modalities in BCRL management are still areas of ongoing research and arguments. Most importantly, the integration of the best evidence, patient preferences and the physician expertise, present the best core pf evidence-based medicine.[35]

1.6 The current evidence

In the past few decades, a major advancement in the lymphatic imaging and surgical instruments and techniques have recalled broader interest among surgeons in the pursuit of definitive treatment for lymphedema, prompting extensive research efforts to identify and assess the efficacy of treatment options aimed at mitigating its impact.[36]

Surgical techniques have improved significantly, which aim to prevent the lymphedema development and to provide adequate treatment strategies, this done by the meticulous axillary lymph node dissection, the SNB and the advanced development of microsurgery and supermicrosurgery techniques, which have broadly increased the treatment options for BCRL.[37]

However, despite advancements in treatment options, challenges persist in optimizing the management of BCRL, including the need for standardized protocols, personalized approaches, and long-term follow-up assessments. Until today there is no strong evidence of which type of treatment is superior to the other, together with existing gaps and the lack of consensus, making it very hard to give a clear evidence-based recommendation for patients with BCRL.[24, 38]

1.7 Types of reviews

Systematic reviews (SR) are studies that use a systematic and explicit method to identify, analyse and synthesize empirical evidence, and to answer a specific research question.[39] Although, the expansion of evidence-based practice across sectors has led to an increasing the diversity of review types.[40]

Example of reviews` types are:

- Critical review: Aims to demonstrate writer has extensively researched literature and critically evaluated its quality. Goes beyond mere description to include degree of analysis and conceptual innovation. Typically results in hypothesis or model.

- Literature review: generic term, published materials that provide examination of recent or current literature. Can cover wide range of subjects at various levels of completeness and comprehensiveness.

- Mapping review/systematic map: Map out and categorize existing literature from which to commission further reviews and/or primary research by identifying gaps in research literature.

- Rapid review: Assessment of what is already known about a policy or practice issue, by using systematic review methods to search and critically appraise existing research.

- Scoping review: Preliminary assessment of potential size and scope of available research literature. Aims to identify nature and extent of research.

- Systematic review: Seeks to systematically search for, appraise and synthesis research evidence, often adhering to guidelines. It may or may not include metanalysis.

- Meta-analysis: Technique that statistically combines the results of quantitative studies to provide a more precise effect of the results.

However, the diversity of terminology used means that the full potential of these review types may be lost amongst a confusion of indistinct and misapplied terms.[40]

2. Justification of the thesis

Given the significant limitation in the available evidence, particularly in the specialized field of lymphedema and the shortage of comprehensive knowledge and practical experience in the treatment of BCRL, underlines the necessity of innovative methodologies to effectively synthesize the available evidence.

This research aimed to highlight the currently available evidence in the treatment of BCRL using novel tool for evidence synthesis which is the evidence mapping. The use of evidence mapping represents a significant departure from traditional systematic reviews, offering a new and dynamic approach. This research provides a descriptive and visual approach for better understanding of the current state in the treatment of BCRL.

Furthermore, the strategic application of evidence mapping enables the presentation of diverse sources of evidence, ranging from systematic reviews and clinical trials to observational studies and case reports, facilitating a comprehensive view of the treatment landscape. This approach has the potential to clarify patterns and identify current knowledge gaps or inconsistencies in the evidence if existing.

In it is core, the use of evidence mapping methodology in this research seeks to provide a complete overview in relation to BCRL treatment, address the gaps, provide a user-friendly approach to interpreting evidence, and finally support evidence-informed decision by surgeons and patients.

3. Hypothesis

The existing body of evidence does not definitely demonstrate the superiority of any specific treatment modality for BCRL over other alternative intervention.

4. Objectives

4.1 Main objective

The aim of this thesis is to identify, describe and organize of the currently available evidence in the treatment for BCRL, and to assess the risk of bias of the surgical RCTs and SRs.

4.2 Secondary objectives

1- To identify the gap of knowledge.

2- To enumerate the limitations and constraints that exist in the field of BCRL treatment.

3- To give detail description for the RCTs and SRs on surgical treatments for BCRL.

4- To provide recommendations for the future research needs.

5. Compendium of publications

5.1 Clarifications of the methodology

Two evidence mapping reviews were conducted according to the methodology proposed by Global Evidence Mapping (GEM),[41] and adhered to the Preferred Reporting Items for Systematic Reviews and Metanalysis (PRISMA)—Extension for Scoping Reviews.[42] All methods were specified a priori in a protocol.

PICOS framework (population, intervention, comparison, outcome and study design) was used to formulate the eligibility criteria in this thesis.[43]

The search strategy was conducted in MEDLINE (via PubMed) and EMBASE (via Ovid), Epistemonikos, and the Cochrane Library from the year 2000 onward. The date of the baseline search in the first article was on July 5th 2020, and the updated search in the second article was on October 22nd 2021.

The risk-of-bias assessment was done only for high evidence studies addressing the surgical intervention (SRs and RCTs). For RCTs, the Cochrane risk-of-bias tool for randomized trials—version 2 (RoB-2) was used for the assessment of each outcome in the RCTs.[44] SRs were assessed by the ROBIS tool.[45]

As recommended in the Cochrane Handbook for Systematic Reviews of Interventions,[46] a flow chart for the whole process of study selection was elaborated based on the Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA-P diagram).[47]

5.2 First article

Ali M. Al-Sakkaf, Jaume Masia, Ariadna Auladell-Rispau, Aliaa I. Shamardal, Luis Vasconcello-Castillo, Ivan Sola, Xavier Bonfill. Evidence Mapping of the Treatments for Breast Cancer–related Lymphedema. Journal of Plastic and Reconstructive Surgery PRS (Global Open). January 2022. DOI: 10.1097/GOX.0000000000004045

OPEN

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ORIGINAL ARTICLE
Breast

Evidence Mapping of the Treatments for Breast Cancer–related Lymphedema

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Background: Women treated for breast cancer are facing a lifetime risk of developing lymphedema, which occurs in up to 40% of this population. There is a lack of evidence and limited knowledge regarding the treatment of breast cancer–related lymphedema (BCRL). The aim of this study was to identify, describe, and organize the currently available evidence in the treatment of BCRL.

Methods: We conducted an evidence mapping review study according to the methodology proposed by Global Evidence Mapping. We performed a systematic search in Medline, Embase, Central (Cochrane), and Epistemonikos, from 2000–2020. We included studies about all treatment types for BCRL, including surgical and nonsurgical treatment. Results were summarized in narrative and tabular forms.

Results: A total of 240 studies were included in this mapping review, distributed as follows: 147 experimental studies [102 randomized clinical trials (RCTs) and 45 quasi-experimental clinical trials], 48 observational studies (34 prospective and 14 retrospective studies), and 45 systematic reviews (17 of them with metanalysis). Most of the RCTs were on nonsurgical interventions. Only two RCTs addressed surgical intervention.

Conclusions: In the last 20 years, there were an average of 12 publications per year on the treatment of BCRL. Recently this lack of attention has been partially corrected, as the majority were published in the past 5 years. However, most of them were on nonsurgical interventions. Well-designed RCTs on surgery are needed to measure the effectiveness of the applied interventions. *(Plast Reconstr Surg Glob Open 2022;10:e4045; doi: 10.1097/GOX.0000000000004045; Published online 18 January 2022.)*

INTRODUCTION

More than 1.38 million women worldwide were estimated to be diagnosed with breast cancer in 2008, accounting for 23% of all diagnosed cancers among women.[1] Given that the 5-year survival rate for breast cancer is now 90%, experiencing breast cancer is ultimately about quality of life.[1] Women treated for breast cancer face a lifetime risk of developing lymphedema, which occurs in up to 40% of this population and negatively affects breast cancer survivors' quality of life.[2–5]

Patients with lymphedema have a significantly decreased quality of life, with frequent infections, reduced range of motion, and a cosmetic deformity that is difficult to conceal.[6] Treatment for breast cancer–related lymphedema (BCRL) has mostly been symptomatic in nature and designed mainly to prevent swelling progression.[7] BCRL treatment might involve (1) surgical treatments, which currently include the different microsurgery techniques and liposuction, or (2) nonsurgical treatments, which might include pharmacotherapy, diet, exercise, and physiotherapy options.

In the past decades, major advancements in the lymphatic imaging and surgical instruments and techniques have rekindled broader interest among surgeons in the pursuit of a more effective treatment for lymphedema.[8] Nonsurgical

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Received for publication November 10, 2021; accepted November 16, 2021.

DOI: 10.1097/GOX.0000000000004045

Disclosure: *The authors have no financial interests to declare in relation to the content of this article.*

treatments are taking part in both prevention and treatment of BCRL, and they include a great variety of options, mainly physiotherapy like complex physical therapy, intermittent pneumatic compression, or compressive garments.[9]

The limited knowledge regarding the treatment of BCRL, together with the insufficient standardization of the different therapeutic options, warrants highlighting the available evidence using an innovative approach provided by evidence mapping, combined with a detailed description of the available randomized clinical trials (RCTs) published in the literature.

Evidence mapping allows a visual understanding of the evidence base of any treatment, apart from supporting the process of decision-making by facilitating information in a user-friendly format.[10] Furthermore, it is the best study design when there is an abundance and a diversity of research and an excellent way to identify gaps in a topic area.[10]

Thus, the main objective of this study was to identify, describe, and organize the currently available evidence in the treatment of BCRL, with an additional focus on RCTs, especially in relation to surgical interventions. In addition, the study aimed to identify the existing gaps of knowledge and to provide recommendations for future research.

Takeaways

Question: There is a lack of evidence and limited knowledge regarding the treatment of breast cancer–related lymphedema.

Findings: A total of 240 studies were included in this mapping review, most of them nonsurgical interventions. Among these studies, there were 102 randomized clinical trials, with only two RCTs addressing surgical interventions

Meaning: More surgical randomized clinical trials are needed in the future to measure the real effectiveness of the applied interventions.

PATIENTS AND METHODS

Study Design

An evidence mapping study was conducted according to the methodology proposed by Global Evidence Mapping.[11] This evidence mapping adhered to the PRISMA-Extension for Scoping Reviews.[12] All methods were specified a priori in a protocol (available on request).

Eligibility Criteria

We have used the PICOS framework (population, intervention, comparison, outcome and study design) to formulate the eligibility criteria.[13] We considered eligible patients (older than 18 years old) with BCRL. We included all treatment types for BCRL, including surgical and nonsurgical treatment. We considered studies with all kinds of comparison and studies without comparison groups. Due to the nature of the study, all types of outcomes were eligible. We included the following study designs from the year 2000 to 2020: RCTs, quasi-experimental clinical trials, observational studies (retrospective, prospective), and systematic reviews (SRs) with or without metanalysis. We selected the most updated publication when we identified studies published on the same topic and by the same team. We excluded animal studies, in vitro studies, single case reports, case series, letters to the editor, narrative reviews, studies including different types of edema or mixed edema, studies including less than 10 patients or reviews with fewer than three studies and studies addressing other than treatment of BCRL or addressing both prevention and treatment together.

Search Strategy

The search strategy was conducted in MEDLINE (via PubMed), Embase (via Ovid), Cochrane Central Register of Controlled Trials (CENTRAL) via The Cochrane Library, and Epistemonikos. A search algorithm was designed, including a combination of controlled vocabulary, Medical Subject Headings (MeSH) descriptors, free text terms and thesaurus terms when available, with no language restriction. We did not search for grey literature.

Study Selection and Data Extraction

The studies retrieved by titles and abstract were uploaded to Mendeley and then managed with the software Rayyan QCRI. After removing duplicates, three reviewers (AMA, AIS, and LVC) independently screened all titles and abstracts, with each article being screened by at least two reviewers. Afterward, a full-text screening was done by the same reviewers who confirmed eligibility based on the inclusion and exclusion criteria. Disagreements between two reviewers were resolved mainly by the third reviewer or by consensus. For each individual study, data extraction was conducted separately by the two reviewers (AMA and AIS). Results were then compared, and in case of disagreement, the third reviewer (LVC) acted as a referee to reach consensus.

Data Synthesis and Analysis

The obtained results are presented in a narrative and visual format using tables, graphs, and a bubble plot. A flow chart for the whole process of study selection was elaborated based on the PRISMA-P diagram.[14] The analysis of the selected studies was divided in two parts: a general mapping description of all studies included in this mapping review, and a detailed description of the included RCTs.

RESULTS

Studies Selection

The flow chart of the studies selection is shown in Figure 1. The search yielded a total of 4993 studies. After removing 1751 duplicates, we proceeded with 3242 studies to screen by title and abstract. In total, 2889 studies were excluded for not being related to the review's main topic. Then, a full-text review was done for 353 studies. After the resolution of discrepancies by consensus between researchers, we excluded 106 studies. Similarly, seven studies where the full-text was missing were also excluded

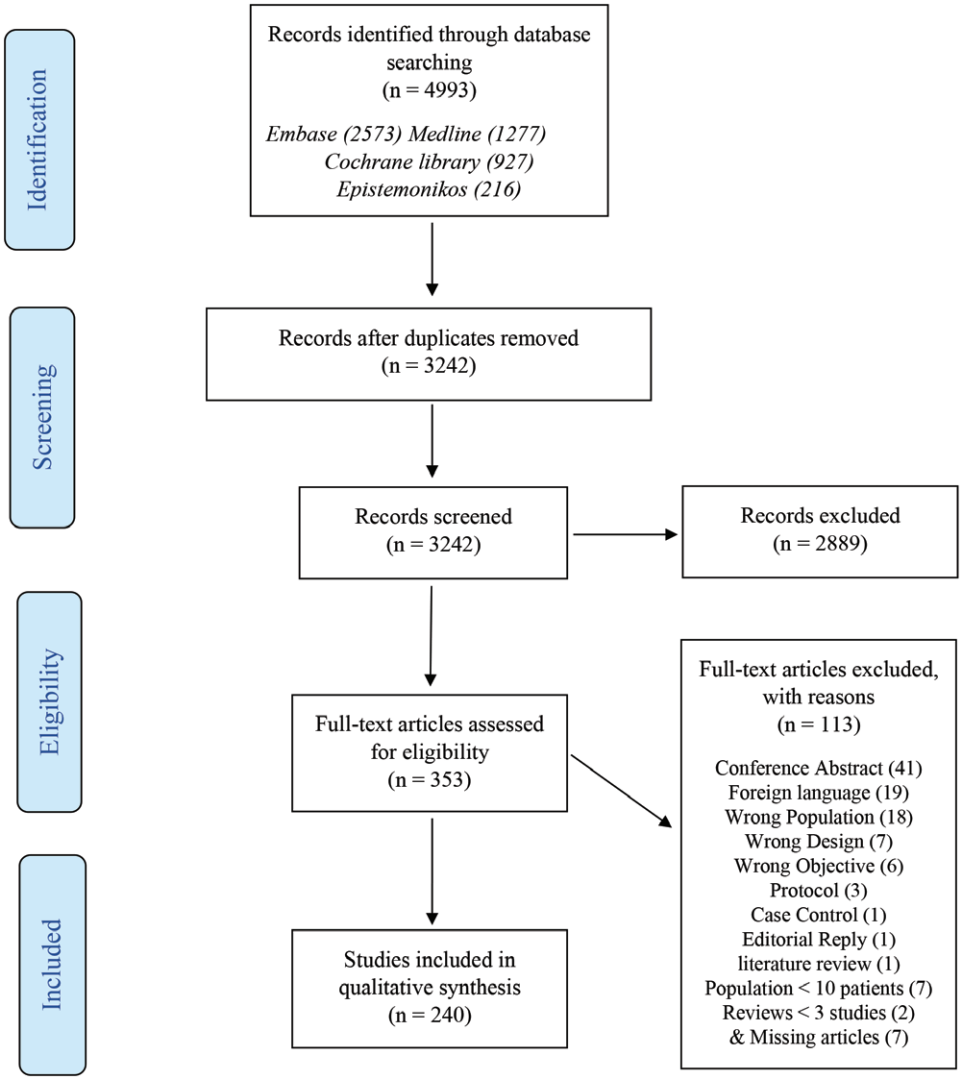


Fig. 1. PRISMA flow diagram and selection process of studies on BCRL.

from the descriptive analysis. Finally, a total of 240 studies were included in this mapping review.

The main reason for excluding studies was that the articles were published as conference abstracts (41); other reasons included foreign languages (other than English and Spanish) (19), wrong population (18), wrong design (7), wrong objective (6), published protocol (3), case report (1), editorial reply (1), literature review (1), population less than 10 patients (7), reviews including less than three studies (2), and the aforementioned missing full-text (7).

Characteristics of All Included Studies

Publication Year and Language

We observed a marked increase in the number of publications in the past 5 years; 139 (58%) of the published studies in the treatment of BCRL were from 2016 to 2020 (Table 1). As defined in the eligibility criteria, we included only studies published in English and Spanish. Overall, only two studies were published in Spanish, and the rest (238 studies) were all in English.

Countries

The published studies were distributed among different countries worldwide. The United States of America had the highest number of publications (32) followed by Turkey (19), Australia (17), China (16), South Korea (14), Brazil (12), the United Kingdom (12), and Iran (10). The rest of the countries had fewer than 10 published studies (Fig. 2).

Study Design

We identified 147 experimental studies (102 RCTs and 45 quasi-experimental clinical trials), 48 observational studies (34 prospective studies and 14 retrospective studies), and 45 SRs (28 SRs without metanalysis and 17 with metanalysis) (Table 2).

Intervention Type

The identified studies included different types of intervention (42 surgical treatment and 198 nonsurgical treatment). Most of the surgical interventions were combined with a nonsurgical treatment, such as garment, exercise, or

Table 1. Distribution of the Total Studies and RCTs on BCRL by Publication Year

Years	Total Studies, N (%)	RCTs, N (%)
2000–2004	15 (6.25)	9 (9)
2005–2009	33 (13.75)	13 (13)
2010–2014	53 (22)	28 (27)
2015–2020	139 (58)	52 (51)
Total	240 (100)	102 (100)

others. The distribution of the intervention type according to the study design is described in Figure 3.

Characteristics of the RCTs

Publication Year and Language

Out of the total RCTs, 52 (51%) were published in the past 5 years, and they all were published in the English language (Table 1).

Countries

During the last 20 years, RCTs have been published in many different countries, the main ones being Australia (12), the USA (11), Turkey (8), South Korea (8), the UK (7), China (5), and Poland (5). The remaining countries published fewer than five clinical trials.

Population Characteristics and RCTs Design

These RCTs included patients with BCRL affecting the ipsilateral arm, but three studies described breast or chest lymphedema secondary to breast conservative treatment or mastectomy. Three trials studied BCRL only in overweight or obese patients. All were parallel with two arm groups, but there were five crossover design trials, and five RCTs comparing three arm groups.

We encountered some RCTs that included the same population and methodology protocol and were performed by the same team but measuring different outcomes and/or describing subgroup analysis. These studies have been included as separate studies and analyzed independently.

Intervention Type

Overall, most of the RCTs were on nonsurgical treatment, and there was a large diversity in nonsurgical

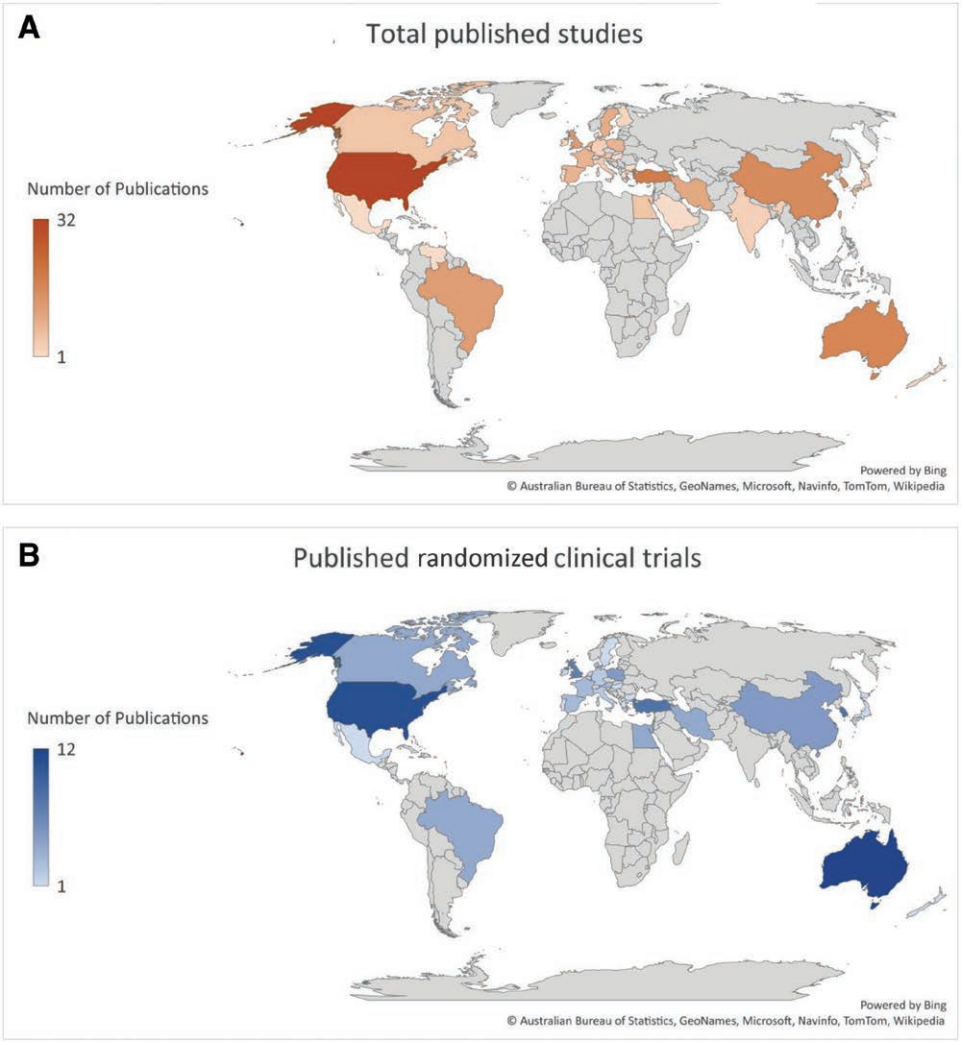


Fig. 2. Geographic distribution of the published studies on BCRL. A, Total number of studies B, Randomized clinical trials.

Table 2. Frequency of the Study Design among Total Studies in the Treatment of BCRL

Study Design	No. Studies (%)
Interventional	147 (61.25)
RCTs	102 (42.5)
Quasi experimental	45 (18.75)
Observational	48 (20)
Prospective	34 (14.16)
Retrospective	14 (5.84)
Systemic reviews	45 (18.75)
SR without meta-analysis	28 (11.67)
SR with meta-analysis	17 (7.08)
Total	240 (100)

treatment options, mostly regarding physiotherapy treatments, such as different kinds of sleeves/bandages, kinesio tape, manual lymphatic drainage, pneumatic compression pump, decongestive compression therapy, exercise protocols (active, resistant, aqua exercise), yoga, weight loss, acupuncture or different pharmacotherapy, laser therapy, satellite ganglionic block, etc.

Among the nonsurgical treatment trials, we also found two studies addressing autologous stem cell transplantation (ASC), which is considered a minimally invasive medical intervention; both studies were done in a surgery setting (Table 3).

There were only two RCTs on surgical treatment: the first trial about lymphovenous anastomosis (LVA), and the second trial about vascularized lymph node transfer (VLNT). The characteristics of the included RCTs on surgical intervention are described in Table 4.

Measured Outcomes

There was a wide variety of outcomes measured in these trials and most of the trials focused on more than one outcome. The main outcome measured was the arm volume and circumference, followed by lymphedema symptoms like heaviness or pain, arm function and range of movements, and quality of life. Other measured outcomes were patient adherence, satisfaction, safety and adverse events, skin changes, infection and inflammatory markers, cost of treatments, or intervention duration (Fig. 4).

Effect of Intervention

Most of the RCT results favored toward intervention (59). Some other studies favored toward the comparison group (7), and the rest of the trials had no difference of effect by applying the intervention (37). The therapeutic results according to the intervention type among the RCTs are described in Figure 5.

DISCUSSION

As we perceived that the available evidence of the treatment for BCRL is not proportional to the importance of this health condition, which is considered as significant problem for women who had been treated for breast cancer, we decided to conduct a mapping review to identify and to make a broad picture of the current situation of this important topic.

There is a variety of methodological standards to develop a mapping review, but we decided to follow the Global Evidence Mapping initiative because it is very rational and systematic.[11] This methodology includes three core tasks: setting the topic area's boundaries and the context in question, searching and selecting relevant studies, and reporting on search results and study characteristics.[11]

In the last 20 years, we identified 240 publications about the treatment for BCRL, which means an average of 12 publications per year. Recently, this lack of attention has been partially corrected when the majority of these articles (58%) were published in the past 5 years. We assume that this is due to the availability of new treatment options and techniques in the recent years, as well as an increase in the interest of clinical research among

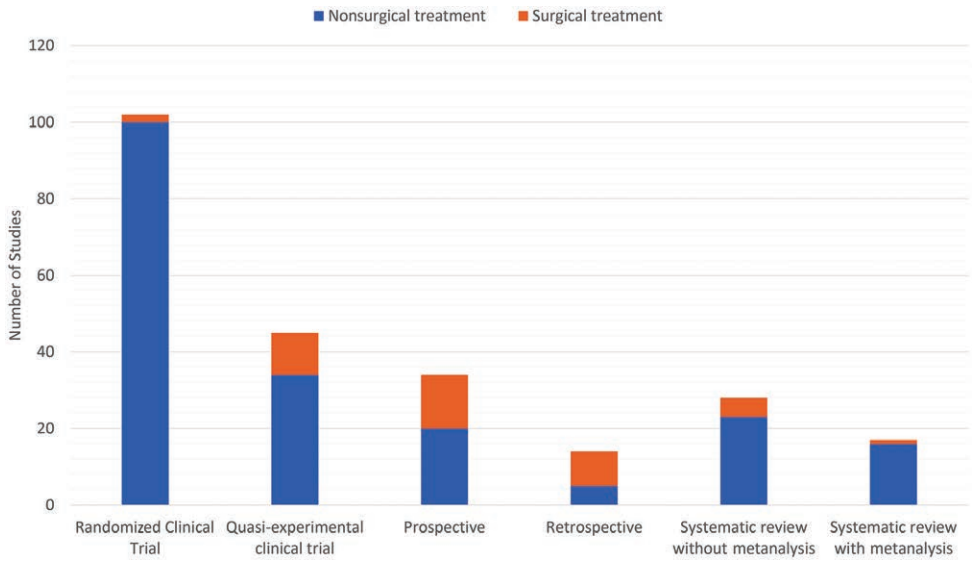


Fig. 3. Distribution of the intervention type according to the study design among the total studies (total: n = 240) on BCRL.

Table 3. Frequency of the Specific Treatment on BCRL according to the Intervention Type among the RCTs

Intervention Type	Specific Treatment	No. RCTs
Surgical treatment (N = 2)	Lymphovenous anastomosis (robot-assisted)	1
	Vascularized lymph node transfer	1
Nonsurgical treatment (N = 100)	Exercise (active, resistant, aqua exercise)	18
	Compression sleeve/bandage	14
	Kinesio tape	10
	Manual lymphatic drainage	10
	Pneumatic compression pump	10
	Pharmacotherapy	8
	Laser therapy/electrical therapy	8
	Yoga	4
	Acupuncture	3
	Decongestive compression therapy	3
	Satellite ganglionic block	3
	Diet and weight loss	3
	Autologous stem cells transplantation	2
	Hyperbaric oxygen therapy	1
	Extra corporal shock wave therapy	1
	Others	2
Total		102

health professionals about the effectiveness and safety of these treatments. Due to the extreme importance of BCRL and its physical and psychosocial consequences, more research is needed in this field to strengthen the evidence base and ensure patients receive clinically effective treatment.[17]

We have observed in this review that surgical interventions in these articles were mainly distributed between microsurgical techniques (LVA, vascularized lymph node transfer) and liposuction. These surgical procedures focused on re-establishing the function of the lymphatic system and reducing the volume, respectively, which might reflect the common surgical practice nowadays.[18] On the other hand, there was great diversity of nonsurgical interventions, and usually there was a mix of different options that have been used to treat a considerably large number of patients with BCRL.[19]

Our results show that the country with the most research on this topic was the USA, followed by three main countries: Turkey, Australia, and China. Moreover, there was significant variability among the countries in the treatment options that have been assessed in these studies, which supports the diversity of the treatment worldwide.[20, 21]

In our findings, of the total 240 articles that we have identified, about 81% were primary studies, which include a considerably large number of interventional studies (61%) and observational studies (20%). However, about 19% of the total publications were SRs, which might indicate a considerable gap in secondary studies. Even though well-conducted SRs are considered higher quality evidence than other studies in decision-making for clinical practice and health policy,[22] it was not the goal of this study to analyze these SRs.

Due to the fact that RCTs are highly controlled and managed studies, and their level of evidence is higher than other types of primary studies,[23] on this mapping review, we have decided to focus more on these RCTs and to provide more description of their data.

Of the total 102 RCTs that we have identified, there were 100 RCTs on nonsurgical treatment and only two studies addressing surgical treatment,[15,16] which indicates the insufficient number of RCTs in the field of lymphedema surgery. This could be mainly due to the usual and general difficulties to design RCTs to assess the surgical procedures compared with pharmaceutical agents.[24] Therefore, despite the progressive use of the different surgical interventions in clinical practice, there is currently not enough high-quality research to assess their effectiveness.[25] This highlights the current need for well-designed RCTs to compare the effectiveness of the broad range of these surgical modalities, as well as in relation to nonsurgical therapy.

Sometimes the scarcity of RCTs has been justified due to the difficulty of blinding, but this requirement is not absolutely necessary in designing RCTs and could be overcome with alternative designs to maximize the validity and to reduce the chance of assessment bias.[26] However, we assumed that well-designed non-randomized prospective studies were conducted on surgical intervention.

We observed that many RCTs described combined treatment options for patients with BCRL. Likewise, we have identified that studies addressing the surgical interventions were also accompanied by garments and/or physiotherapy. This signifies the importance of a multidisciplinary team in lymphedema treatment provided by a group of healthcare professionals.[27]

Among the total number of RCTs, three studies assessed exclusively the treatment effectiveness in overweight and

Table 4. Characteristics of the Included Randomized Clinical Trials on Surgical Intervention for BCRL

Study	Country	Title	No. Patients	Intervention	Comparison Group	Outcome Measured	Reported Results
van Mulken et al[15]	Netherlands	First-in-human robotic supermicrosurgery using a dedicated microsurgical robot for treating breast cancer-related lymphedema: a randomized pilot trial	20	Robot-assisted supermicrosurgical lymphatico-venous anastomosis LVA	Manual supermicrosurgical lymphatico-venous anastomosis LVA	Upper limb volume, quality of life, duration of surgery, and quality of anastomosis	No difference
Dionyssiou et al[16]	Greece	A randomized control study of treating secondary stage II breast-cancer–related lymphoedema with free lymph node transfer	36	Vascularized lymph node transfer, physiotherapy, and compression	Physiotherapy and compression alone	Upper limb volume, infection, and lymphedema symptoms	Favors toward intervention

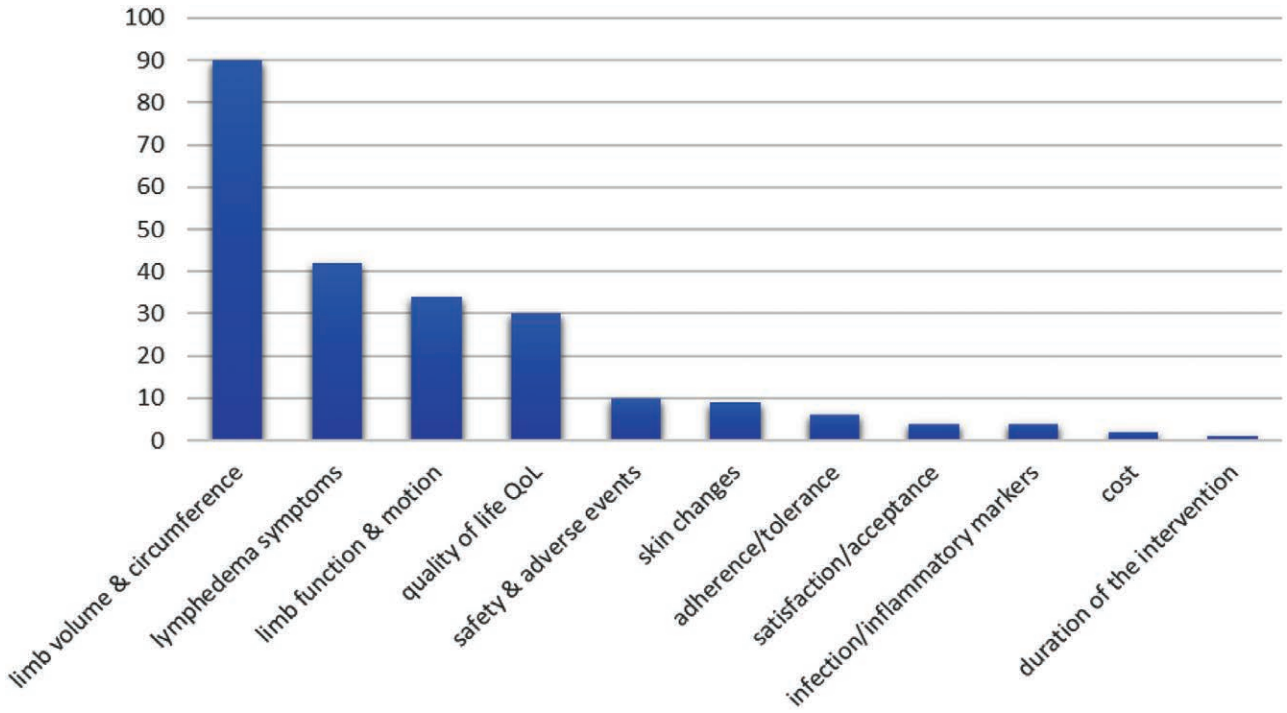
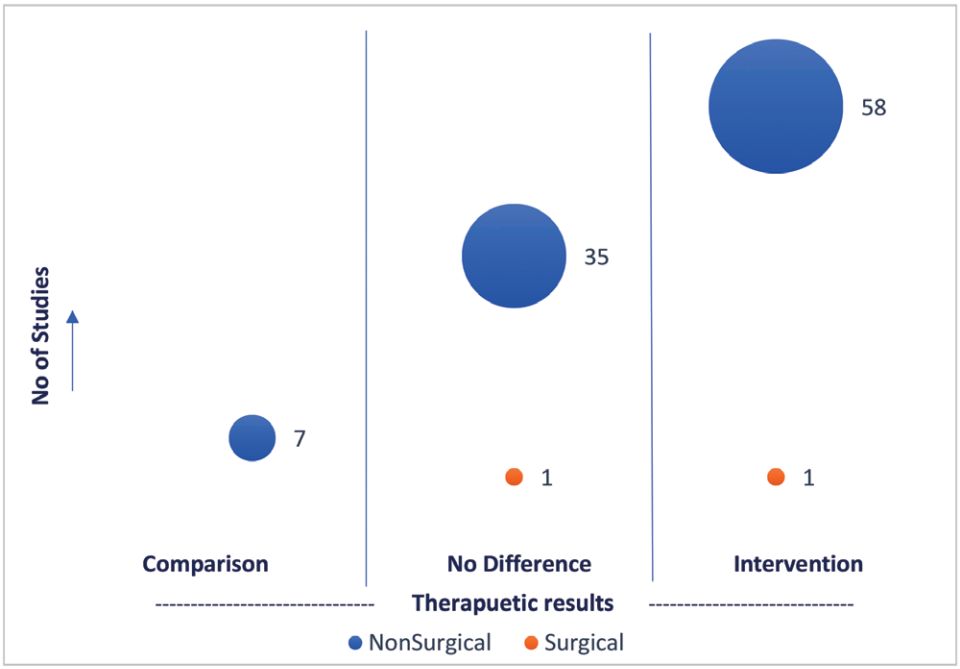


Fig. 4. Distribution of the measured outcomes in the randomized control trials on BCRL.



- Therapeutic results: Favors toward comparison / No difference effect / Favors toward intervention
- Total studies N = 102; Nonsurgical studies N = 100, Surgical studies N = 2

Fig. 5. Evidence mapping of the therapeutic results according to the type of intervention among the randomized clinical trials on BCRL.

obese patients.[28–30] Given the fact that obese individuals have three times the risk of developing lymphedema compared with the non-obese population,[31] we assumed that this population was well represented in the totality of RCTs. Moreover, there were three RCTs that assessed the treatment effectiveness for lymphedema of the breast and chest, which could combine the arm lymphedema.[32–34] Unfortunately, these lymphedema sites are usually missed during the clinical assessment and treatment of BCRL.

There was a wide heterogeneity among the published studies in terms of population characteristics, intervention types, measured outcomes, and study design. This should be addressed clearly for future research to provide evidence about the treatment effectiveness and could be complemented by SRs if the primary studies are valid and available in number.

Even though there was a great variability of the measured outcomes in these RCTs and the majority focused on more than one outcome, almost 90% of them measured the limb volume and circumference, which is considered the most relevant objective and subjective outcome for patients' follow-up after the applied intervention. It depends mainly on the detectable volume and circumference difference between the involved and the uninvolved limb.[35]

As there were no reported harmful interventions, we classified the conclusions of the RCT results as favoring toward the intervention (59 articles), toward comparison (only seven articles) or no different effect (36 articles). We have presented these results on a bubble plot to obtain a broader outlook of the available evidence (Fig. 5).

This study presents some limitations. Firstly, the absence of the methodological quality assessment of primary studies that is not conducted in a mapping review. Secondly, the nature of the mapping review is descriptive and not to provide sufficient evidence to support the applied intervention. And finally, the mapping review requires additional expertise for creating the visual output.

Among the strengths of this study, we have made an extensive search using standard methodology that contributes to the descriptive objectives. Moreover, there was a consistency between the reviewers, and the screening part was done in a systematic way by three different reviewers, which ensures confidence of the reported results.

Although the quality assessment part is not a core task of evidence mapping,[10] we took into consideration the methodological quality when we defined the eligibility criteria by excluding low evidence studies such as case reports. Also, we presented the results in a relatively easy way to interpret and understand. The results of this evidence mapping review might be used to address more focused research in the future, particularly in the field of surgery. Finally, this might be the first evidence mapping about therapeutic interventions for BCRL, as per our knowledge.

CONCLUSIONS

In the last 20 years, there were an average of 12 publications per year on the treatment of BCRL, even though lymphedema is considered an important health problem for women who undergo breast cancer treatment. Recently this lack of attention has been partially corrected, as the majority of the studies were published in the past 5 years. However, most of these studies were on nonsurgical interventions.

Most of the RCTs focused on nonsurgical treatment, and only two RCTs addressed the effectiveness of surgical treatment. Therefore, well-designed RCTs on surgical interventions are needed to measure their real effectiveness before wider use in regular clinical practice.

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5.3 Second article

Ali M. Al-Sakkaf, Xavier Bonfill, Sofia Ardiles-Ruesjas, Josefina Bendersky-Kohan, Ivan Sola, Jaume Masia. Risk-of-bias assessment of the randomized clinical trials and systematic reviews on surgical treatments for breast cancer-related lymphedema: A mapping review. Journal of Plastic, Reconstructive and Aesthetic Surgery JPRAS. May 2023. DOI: 10.1016/j.bjps.2023.05.002

Journal of Plastic, Reconstructive & Aesthetic Surgery 84 (2023) 134–146

JPRAS
An International Journal of Surgical Reconstruction
www.JPRASurg.com

Review

Risk-of-bias assessment of the randomized clinical trials and systematic reviews on surgical treatments for breast cancer-related lymphedema: A mapping review

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Received 17 August 2022; Accepted 2 May 2023

KEYWORDS
Evidence mapping;
Risk-of-bias assessment;
Breast cancer-related lymphedema

Abstract *Background:* Breast cancer treatment is the principal cause of lymphedema in the upper extremities. Breast cancer-related lymphedema (BCRL) treatments were previously based on conservative therapy; surgical treatments are alternative options that could be highly beneficial, especially for patients who are not responsive to conservative therapy. The main aim of this study was to describe and critically assess the risk of bias of randomized clinical trials (RCTs) and systematic reviews (SRs) on surgical treatment for BCRL.
Methods: We conducted an evidence mapping review according to the methodology proposed by Global Evidence Mapping (GEM). An update was done for our previous systematic search in MEDLINE, EMBASE, CENTRAL (Cochrane), and Epistemonikos from the year 2000 onward. We assessed the risk of bias for the RCTs and SRs using the RoB-2 and ROBIS tools, respectively.
Results: Two surgical RCTs and eight SRs were found among the 47 surgical studies that met the eligibility criteria. The overall risk-of-bias assessments of these studies were rated as some concerns (six outcomes) and high risk (three outcomes) for the measured outcomes among the RCTs and as a high risk of bias (five studies) and low risk (three studies) for the included SRs.

Abbreviations: BCRL, Breast cancer-related lymphedema; RCTs, Randomized control trials; SRs, Systematic reviews; GEM, Global Evidence Mapping; LVA, lymphaticovenular anastomosis; VLNT, vascularized lymph node transfers

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https://doi.org/10.1016/j.bjps.2023.05.002

Conclusions: The overall evidence in the literature on surgical treatment for BCRL is low, as there are few published RCTs and SRs, and the risk-of-bias assessment for the majority was rated as high risk of bias or with some concerns. High-quality studies are needed to improve evidence-based decision-making by surgeons and patients.

Contents

Background

Lymphedema is defined as the abnormal collection of lymphatic fluid within subcutaneous structures. In advanced countries, damage to the lymphatic system due to cancer or its treatment is the most common cause of secondary lymphedema. In the upper extremities, breast cancer treatment is the principal cause of lymphedema.[1]

According to the literature, the incidence of breast cancer-related lymphedema (BCRL) depends on the type of axillary treatment; axillary lymph node dissection results in lymphedema in up to 53.5% of cases, and sentinel lymph node biopsy results in lymphedema in up to 15.8% of cases.[2,3] Other risk factors that aggravate the condition are adjuvant radiation, docetaxel chemotherapy, infection, iatrogenic injury, and obesity. Consequently, developing lymphedema leads to a chronic condition that is usually challenging to treat.[4-10]

BCRL treatment options have long been based on conservative therapy, such as compression garments/bandages and manual lymph drainage. These conservative measures are mainly aimed at alleviating lymphedema symptoms without curative intent. Surgical treatments are alternative options that could be highly beneficial, especially for patients who are not responsive to standard conservative therapy, which includes mostly excisional and reconstructive techniques.[11-13]

Both excisional and reconstructive surgical approaches have been described in the treatment of BCRL. Excisional or nonphysiological procedures include the Charles operation and liposuction. These strategies are most often performed in a later stage of disease when there are no remaining functional lymphatic vessels.[14,15] Reconstructive options, on the other hand, are physiological operations that aim to restore lymphatic flow to aid in lymphatic drainage from the affected extremity. These include lymphaticovenular anastomosis (LVA) and vascularized lymph node transfers (VLNT), which currently have promising results for treating the early stages of lymphedema.[16-20]

Our team previously conducted a mapping review on all treatments for BCRL, without assessing the risk of bias of the included studies, and did not focus on surgical treatment.[21] Therefore, based on our previous mapping findings and the limited knowledge of the quality of the available research, the

main aim of this study was to describe and critically assess the risk of bias of randomized clinical trials (RCTs) and systematic reviews (SRs) on surgical treatment for BCRL. Other objectives are to identify gaps in knowledge, enumerate the limitations and constraints that exist in this field, and provide recommendations for future research needs.

Methods

An evidence mapping review was conducted according to the methodology proposed by Global Evidence Mapping (GEM)[22] and adhered to the Preferred Reporting Items for Systematic Reviews and Metanalysis (PRISMA)—Extension for Scoping Reviews.[23] All methods were specified a priori in a protocol *(available on request)*.

Eligibility criteria

We updated our search strategy based on our previous mapping work.[21] It was built on the population, intervention, comparison, outcome, and type of study (PICOT) framework to formulate the eligibility criteria.[24] We considered eligible patients (older than 18 years) with BCRL. Those who had either surgical or nonsurgical treatments for BCRL were initially eligible. Due to the nature of this study, we included studies with any type of comparison and those without a comparison group. All outcomes were eligible for this mapping review. This mapping review included all published studies in full text from the year 2000 onward, including SRs with or without metanalysis, RCTs, quasi-experimental clinical trials, and observational studies (prospective and retrospective studies), to have a broader look at the available evidence in this field. When several studies published on the same topic and by the same team were identified, we considered the most recent publication. We excluded animal studies, in vitro studies, single case reports, case series, letters to the editor, narrative reviews, studies including different types of edemas or mixed edema, studies including less than 10 patients or reviews with fewer than three studies, and studies addressing other than treatment of BCRL or addressing both prevention and treatment together.

Search strategy

The search strategy was conducted in MEDLINE (via PubMed) and EMBASE (via Ovid), Epistemonikos, and the Cochrane Library from the year 2000 onward. A search algorithm was designed, including a combination of controlled vocabulary, the use of MeSH descriptors, free-text term, and thesaurus term when available, adapting it accordingly for each database, with no language restriction, and no gray literature was searched. The last update was done on 22nd of October 2021 *(the search strategy is attached as* Supplementary material*)*.

Study selection and data extraction

The studies were retrieved by titles and abstract and were uploaded to *Mendeley* and then managed with *Rayyan QCRI software*. After removing duplicates, three reviewers (AMA, AIS, and LVC) independently screened all titles and abstracts, with each article screened by at least two reviewers. Afterward, full-text screening was done independently by the same three reviewers who confirmed eligibility based on the inclusion and exclusion criteria. Disagreements between the two reviewers were resolved mainly by the third reviewer. At this step, the reasons for exclusion were recorded.

For each study, data extraction was conducted separately by the two reviewers in a predesigned spreadsheet (AMS and AIS). The results were then compared, and in case of disagreement, the third reviewer (LVC) acted as a referee to reach consensus. All extracted data were recorded in a data extraction sheet using *Microsoft Excel*.

Assessment of risk of bias

Methodological assessment of risk of bias was independently assessed by three reviewers (AMA, SAR, and JBK). Each article was assessed blindly by two reviewers (AMA and SAR), and any disagreement in the results was resolved by the third reviewer (JBK). The risk-of-bias assessment was done only for high evidence studies addressing the surgical intervention (SRs and RCTs); for that reason, we did not consider the necessity of assessing the risk of bias for the nonrandomized studies.

For RCTs, the Cochrane risk-of-bias tool for randomized trials—version 2 (RoB-2) was used for the assessment of each outcome in the RCTs.[25] The domains included in the RoB-2 are as follows: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in measurement of the outcome, and bias in selection of the reported result. The risk-of-bias judgment for the RCTs was then assigned to one of three levels to each domain: low risk of bias, some concerns, or high risk of bias.[25]

SRs were assessed by the ROBIS tool.[26] The tool is completed in three phases: phase 1 consists of assessing the relevance *(this was optional and not applied in this article)*; phase 2 consists of identifying concerns with the review process, covering four domains: study eligibility criteria, identification and selection of studies, data collection and study appraisal, and synthesis and findings; and phase 3 consists of judging the risk of bias and assessing the overall risk of bias in the interpretation of review findings and whether this considered limitations identified in any of the phase 2 domains. The risk-of-bias judgment for SRs is then assigned as low risk, high risk, or unclear concern.[26]

Data synthesis and analysis

As recommended in the *Cochrane Handbook for Systematic Reviews of Interventions*,[27] a flow chart for the whole process of study selection was elaborated based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA-P diagram).[28] The obtained results were presented in a narrative and visual format using tables, figures, and a bubble plot. The bubble plot was created to illustrate the study designs in relation to their risk-of-bias assessment; the color of the figures indicated the study design (RCTs or SRs); the size of the figure reflected the number of population or number of studies in the included RCTs and SRs, respectively; and their positions in the graph were based on their overall risk-of-bias assessment.

The analysis of the selected studies was divided into two parts: first, a general mapping presentation of the included surgical studies included in this review, providing a more detailed description of the available SRs and RCTs and, second, an assessment of the risk of bias of the RCTs and SRs addressing the surgical intervention, using the RoB-2 and ROBIS tool, respectively. Due to the large amount of data collected from the eligible studies, we focused mainly on the important results that contributed to the objectives of this article.

Results

Search results

The flowchart of the study selection of the baseline research and update is shown in Figure 1. The search after the last update yielded a total of 5663 studies. After removing 1919 duplicates, we proceeded with 3744 studies to screen by title and abstract. In total, 3355 studies were excluded because they were unrelated to the review's main topic. Then, a full-text review of 389 studies was conducted. After the resolution of discrepancies by consensus between researchers, we excluded 110 studies. Similarly, seven studies in which the full text was missing were also excluded from the descriptive analysis. Finally, a total of 272 studies were included, of which 225 were nonsurgical studies and 47 studies addressed the surgical treatment for BCRL. Of these surgical studies, only two RCTs and eight SRs were critically assessed for the purpose of this mapping review.

The main reason for excluding studies was that the articles were published as conference abstracts (41). Other reasons included foreign languages (other than English and Spanish) (19), wrong population (18), wrong design (10), wrong objective (7), published protocol (3), case report (1), editorial reply (1), literature review (1), population < 10 patients (7), reviews including < 3 studies (2), and the aforementioned missing full text (7).

Surgical studies on treatment for BCRL

There were 47 studies addressing surgical intervention (39 primary studies and eight secondary studies), which

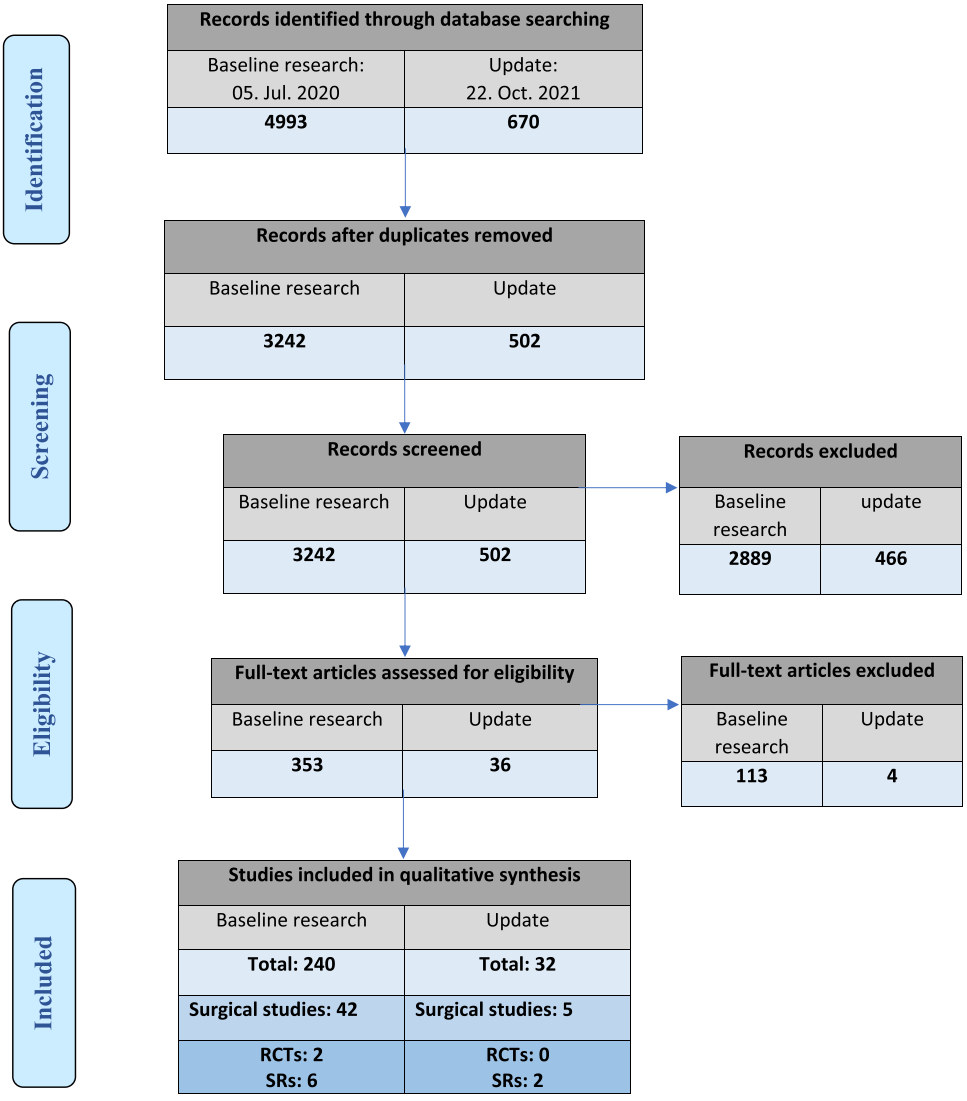

Figure 1 PRISMA flow diagram and selection process of studies on surgical treatments for BCRL (baseline research and update).

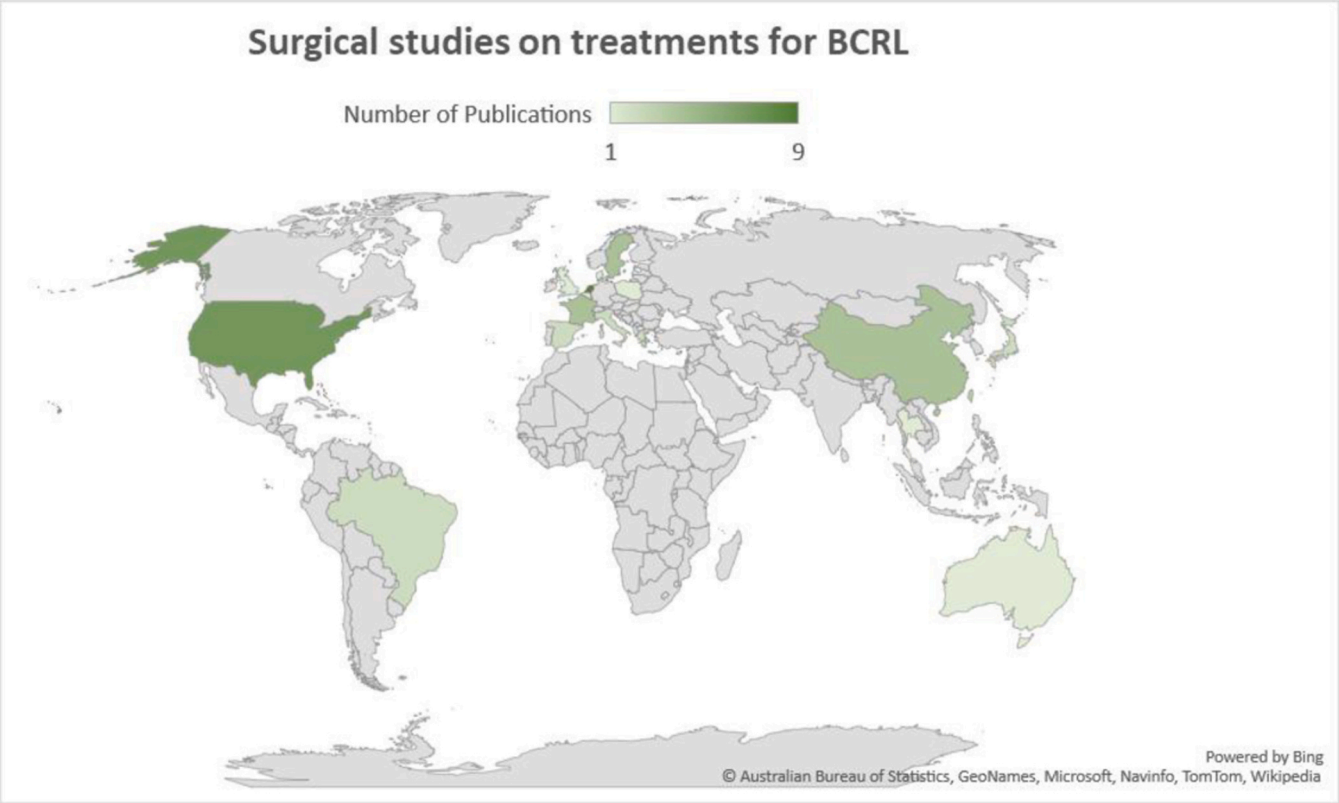

Figure 2 Geographic distribution of total published studies on surgical treatments for breast cancer-related lymphedema (BCRL).

included 15 experimental studies (13 quasi-experimental clinical trials and two RCTs), 24 observational studies (14 prospective cohorts and 10 retrospective cohorts), and eight SRs with and without meta-analysis.

These published studies were geographically distributed among the following countries: the Netherlands had the highest number of publications (9), followed by the United States of America (7), France (4), Sweden (4), China (4), Taiwan (3), Japan (2), Brazil (2), Spain (2), Italy (2), Greece (2), the United Kingdom (1), Belgium (1), Denmark (1), Poland (1), Thailand (1), and Australia (1) (see Figure 2).

RCTs' characteristics

The first RCT, by *Dionyssiou* et al.,[29] was conducted in Greece and compared the VLNT to the conservative measurements, and the second RCT, by *Van Mulken* et al.,[30] conducted in the Netherlands, compared the robotic versus the manual LVA. Both RCTs assessed different outcomes. *Dionyssiou* et al. mainly assessed three patients' outcomes: upper limb volume, infection episode, and subjective symptoms using the subjective analog scaling system.[31] *Van Mulken* et al. assessed various outcomes, including four patients' outcomes: the daily use of compression garment, the need for manual lymphatic drainage, the arm circumference using the mean upper extremity lymphedema index (mean UEL index), and quality of life using a validated health questionnaire, the mean Lymphedema Functioning, Disability and Health questionnaire (Lymph-ICF).[32] Furthermore, this RCT assessed two surgeons' related outcomes: the duration of surgery and quality of anastomosis using the Structured Assessment of Microsurgery Skills (SAMS)[33] and the University of Western Ontario Microsurgical Skills Acquisition Instrument (UWOMSA) scoring[34] (see Table 1).

SRs' characteristics

Among the eight SRs on surgical treatments for BCRL,[35-42] three SRs performed quantitative assessment (metanalysis).[36,37,41] Three were conducted in the Netherlands,[35,36,41] three in the United States of America,[37,39,40] one in Greece[42], and one in Brazil.[38] There was heterogeneity in the included study designs, mainly among case series, case reports, prospective studies, retrospective studies, and nonrandomized trials. Two SRs did not mention the type of the included study design. The range of included studies was from five to 17 studies. One SR included a total of 67 studies, but only 13 were described in the qualitative synthesis, which were addressed in our results.[42] These SRs addressed different surgical interventions, such as VLNT,[38,41] LVA,[3] both VLNT and LVA,[35,42] or combined treatment such as autologous reconstruction with VLNT[37,40] or lipoaspiration with VLNT.[39] The only common outcome that was measured in all included SRs was limb volume, although different outcomes were also assessed, such as subjective symptoms, quality of life, infectious episodes, complications, and discontinuation of conservative treatments (see Table 1).

Matrix of evidence

We created a matrix of evidence to show the SRs linked to the included primary studies and the overlaps of the

Table 1 Characteristics of the included SRs and RCTs on surgical treatments for BCRL.

Randomized Clinical Trials

Number	Study ID	Country	Title	N. of population	Intervention	Comparison	Measured outcomes	Reported results
1	Dionyssiou et al.[29]	Greece	A randomized control study of treating secondary stage II breast cancer-related lymphoedema with free lymph node transfer	36 patients	Vascularized lymph node transfer (VLNT), physiotherapy and compression	Physiotherapy and compression alone	1. Upper limb volume 2. Infection episodes 3. Subjective symptoms	Results favor toward intervention
2	Mulken et al.,[30]	Netherlands	First-in-human robotic supermicrosurgery using a dedicated microsurgical robot for treating breast cancer-related lymphedema: a randomized pilot trial	20 patients	Robot-assisted supermicrosurgical lymphaticovenous anastomosis (LVA)	Manual supermicrosurgical lymphaticovenous anastomosis (LVA)	1. Daily use of compression garment 2. Manual lymphatic drainage 3. Mean lymph - ICF 4. Mean UEL Index 5. Duration of surgery 6. Quality of anastomosis	No difference between both comparative groups

Systematic Reviews

Number	Study ID	Country	Title	N. of studies	Intervention	Metanalysis	Measured outcomes	Reported results
1	Penha et al.[35]	Netherlands	Microsurgical techniques for the treatment of breast cancer-related lymphedema: a systematic review	10 studies (Case series)	Lymph node transplantation (VLNT), lymph vessels transplantation, or derivative lymphatic surgery (LVA).	No	1. Limb volume or circumference 2. Lymph flow by lymphoscintigraphy 3. Symptoms relief 4. Discontinuation of post operative conservative therapy 5. Complications	Consist positive finding with regards to limb volume reduction and limited complications
2	Cornelissen et al.[36]	Netherlands	The effect of lymphaticovenous anastomosis in breast cancer-related lymphedema: A review of literature	15 studies (10 prospective and 5 retrospective studies)	Lymphaticovenous anastomosis (LVA)	Yes	1. Limb volume or circumference 2. Quality pf life	Heterogeneous results in volume/ circumference reduction and improvement of quality of life in most of the studies

(*continued on next page*)

Table 1 (*continued*)

3	Siotos et al.[37]	USA	Delayed breast reconstruction on patients with upper extremity lymphedema a systematic review of the literature and pooled analysis	8 (7 case series and 1 case report)	Delayed autologous breast reconstruction with or without lymph node transfer (VLNT)	Yes	Any outcomes reported	The VLNT composite of the autologous breast reconstruction might be the largest contributing factor leading to lymphedema improvement
4	Ribeiro et al.[38]	Brazil	Lymph node transplantation in the management of post-mastectomy lymphedema: a systematic review with metanalysis	10 studies	Autologous lymph node transplantation (VLNT)	No	Limb volume	There is a considerable reduction in limb volume higher than 50%
5	Forte et al.[39]	USA	Lipoaspiration and lymph node transfer for treatment of breast cancer-related lymphedema: a systematic review	5 studies (3 prospective studies, 1 case series and 1 case report)	Lipoaspiration and venous lymph node transfer (VLNT)	No	Limb volume	Meaningful volume reduction was achieved in all cases, patients who underwent lymph node transfer first followed by lipoaspiration appear to have the best results
6	Forte et al.[40]	USA	Lymph node transfer combined with deep inferior epigastric perforators and transvers rectus abdominis myocutaneous procedures	6 studies	Autologous breast reconstruction with Lymph node transfer (VLNT)	No	1. Limb volume or circumference 2. Symptoms relief	Most of the studies demonstrated subjective symptoms improvement, also good results with limb circumference size and infectious episodes

(*continued on next page*)

Table 1 (*continued*)

7	Winter et al.[41]	Netherlands	A systematic review and metanalysis of vascularized lymph node transfer for breast cancer-related lymphedema	17 studies (non-randomized trials)	vascularized lymph node transfer (VLNT)	Yes	1. Limb volume 2. Quality of life 3. Infectious episodes 4. Complication 5. Discontinuation of compression garment	Evidence from all studies suggest that VLNT reduce volume up to 40%, although in few studies it has also some affect in improving quality of life, infectious episodes, the use of compression garment and complication rate
8	Gasteratos et al.[42]	Greece	Microsurgical techniques in the treatment of breast cancer-related lymphedema: a systematic review of efficacy and patient outcomes	67 studies, but only 13 studies addressed in the synthesis (6 Prospective studies, 3 retrospective studies, 2 RCTs, 1 SRs and 1 narrative review)	LVA, LYMPHA technique and VLNT	No	1. All patients' outcomes 2. Efficacy of microsurgical techniques 3. Complications	Microsurgical techniques can be beneficial to many patients with BCRL

*SRs: Systematic reviews, RCTs: Randomized clinical trials, BCRL: Breast cancer-related lymphedema, LVA: Lymphatic venous anastomosis, VLNT: Vascularized lymph node transfer, LYMPHA: Lymphatic microsurgical preventive healing approach.

primary studies between these SRs. Because there was heterogenicity in the objectives and in the assessed surgical interventions of the SRs, we found that a total of 55 primary studies were included in all SRs, but only 19 studies overlapped between two or more SRs. *Saaristo* et al.[43] overlapped in five SRs: *Chang* et al.[44] overlapped in four SRs; *Lin* et al.[18], *Becker* et al.[17], *Damstra* et al.[45], *De Brucker* et al.[46], and *Montag* et al.[47] overlapped in three SRs; and the rest overlapped in two SRs (see Table 2).

Risk-of-bias assessment for RCTs

Based on the RoB-2, nine outcomes were assessed in the two included RCTs.

Three outcomes were assessed in the RCT of *Dionyssiou* et al.[29] Two were rated as having a high risk of bias (limb volume and subjective symptoms) and one had some concerns (infection episodes). Three RoB-2 domains, 'the randomization process domain, deviation from intended

Table 2 The overall SRs on surgical treatments for BCRL: the overlaps matrix of their included studies and their overall risk of bias assessment.

Systematic Reviews / Included Studies	Penha, et al. 2013	Cornelissen, et al. 2018	Siotos, et al. 2018	Ribeiro, et al. 2019	Forte, et al. 2019	Forte, et al. 2020	Winter, et al. 2021	Gasteratos, et al. 2021¶
Lin et al, 2009	■			■			■	
Becker et al, 2008	■			■				
Becker et al, 2006	■			■			■	
Saaristo et al, 2012	■		■	■		■	■	
Baumeister et al, 2002	■							
Weiss et al, 2002	■							
Furukawa et al, 2011	■							
Chang et al, 2010	■	■	■					■
Damstra et al, 2009	■	■						■
Yamamoto et al, 2003	■							
Koshima et al, 2000		■						■
Auba et al, 2010		■						
Mihara et al, 2012		■						
Ayestaray et al, 2013		■						
Chang et al, 2013		■						■
Chen et al, 2015		■						
Torrisi et al, 2015		■						
Gennaro et al, 2016		■						
Cornelissen et al, 2018		■						
Engel et al, 2017		■						
Lee et al, 2017		■						
Poumellec et al, 2017		■						■
Winter et al, 2017		■						■
De Brucker et al, 2016			■			■	■	
Chen et al, 2014			■			■		
Blanchard et al, 2012			■					
Lee et al, 2012			■					
Khan et al, 2011			■					
Fosnot et al, 2015			■					
Leppapuska et al, 2019					■			
Agok et al, 2018					■			
Cook et al, 2016					■			
Nicoli et al, 2015				■	■			
Granzow et al, 2014					■		■	
Dancey et al, 2013						■		
Nguyen et al, 2015						■	■	
Montag et al, 2019				■		■	■	
Cheng et al, 2013				■				
Dionyssiou et al, 2016				■			■	
Gratzon et al, 2017				■			■	
Liu et al, 2018				■			■	
Akita et al, 2017							■	
Aljaaly et al, 2018							■	
Engel et al, 2017							■	
Gharb et al, 2011							■	
Maruccia et al, 2019							■	
Patel et al, 2014							■	
Yang et al, 2017							■	
Jorgensen et al, 2018								■
Feldman et al, 2015								■
Boccardo et al, 2019								■
Hahamoff et al, 2019								■
Winter et al, 2019								■
Mulken et al, 2020								■
Becker et al, 2012								■

¶ Gasteratos et al.,[42]; 67 studies were included in the study, but only 13 studies were addressed in the study synthesis.
$ The colors of the systematic review reveal the risk of bias assessment, where: green is low risk of bias and red high risk of bias.
*SRs: Systematic reviews, BCRL: Breast cancer-related lymphedema.
The included SRs, the overlaps matrix and their risk of bias assessment.

Table 3 RoB-2 assessment (per outcome) of the randomized clinical trials on surgical treatments for BCRL.

RCTs	Experimental	Comparator	Outcome	Weight	D1	D2	D3	D4	D5	Overall
Dionyssiou et al.[29]	LNT + Physiotherapy	Physiotherapy	Limb volume	1	!	-	+	!	+	-
Dionyssiou et al.[29]	LNT + Physiotherapy	Physiotherapy	Infection episodes	1	!	!	+	!	+	!
Dionyssiou et al.[29]	LNT + Physiotherapy	Physiotherapy	Subjective symptoms	1	!	!	+	-	+	-
Mulken et al.,[30]	Robot-assisted LVA	Manual LVA	Daily use of compression garment	1	!	!	+	+	+	!
Mulken et al.,[30]	Robot-assisted LVA	Manual LVA	Manual lymphatic drainage	1	!	!	+	+	!	!
Mulken et al.,[30]	Robot-assisted LVA	Manual LVA	Mean lymph - ICF	1	!	!	+	+	+	!
Mulken et al.,[30]	Robot-assisted LVA	Manual LVA	Mean UEL index	1	!	!	-	+	+	-
Mulken et al.,[30]	Robot-assisted LVA	Manual LVA	Duration of surgery	1	!	!	+	+	+	!
Mulken et al.,[30]	Robot-assisted LVA	Manual LVA	Quality of the anastomosis	1	!	!	+	+	+	!

¶ **Domains explanation** D1: Randomization process, D2: Deviation from intended intervention, D3: Missing outcome data, D4: Measurement of the outcome, and D5: Selection of the reported results.
$ **The colors indication** Green: Low risk, Yellow: Some concerns, Red: High risk of bias.
* BCRL: Breast cancer-related lymphedema, LVA: Lymph-venous anastomosis, LNT: Lymph node transfer, Lymph ICF: Lymphedema Functioning, Disability and Health Questionnaire, and UEL Index: Upper extremity lymphedema index.

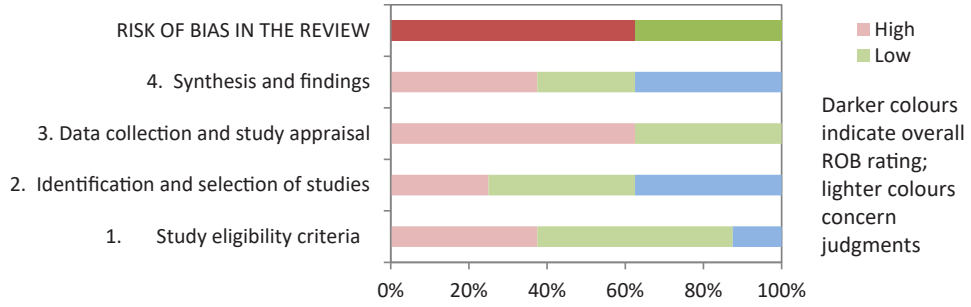

Figure 3 ROBIS risk-of-bias assessment of the systematic reviews (SRs) on surgical treatments for breast cancer-related lymphedema (BCRL); overall and per-domain percentages. * Total number of the included SRs = 8.

intervention domain, and measurement of the outcome domain', had a probability of introduced bias in all RCT outcomes and downgraded the rating to some concerns or high risk of bias.

Six outcomes were assessed in the RCT of *Van Mulken* et al.[30] Five were rated as with some concerns (daily use of compressive garment, use of manual lymphatic drainage, the mean lymph - ICF, duration of surgery, and quality of the anastomosis) and one as high risk of bias (mean UEL index). Two RoB-2 domains, 'the randomization process domain and deviation from intended intervention domain', were rated as having some concerns regarding the probability of introduced bias in all RCT outcomes. In addition, "the missing outcome data" domain was rated as having a high risk of bias in the mean UEL index outcome, downgrading this outcome to a high risk of bias (see Table 3).

Risk-of-bias assessment for SRs

Based on the ROBIS tool, five SRs (5/8, 62.5%) were rated as high risk of bias[37-40,42] and three (3/8, 37.5%) were rated as low risk of bias.[35,36,41]

The five SRs rated as having a high risk of bias were downgraded because there was a probability of introducing bias in more than one domain. All rated as high risk of bias in the 'data collection and study appraisal' domain (5/8, 62.5%),[37-40,42] three in the 'study eligibility criteria' domain (3/3, 37.5%),[38,39,42] three in the 'synthesis of finding' domain (3/3, 37.5%),[38-40] and two in the 'identification and selection of studies' domain (2/8, 25%).[38,40] Finally, 50% of all included SRs had rated the domain of 'study eligibility criteria' as having a low risk of bias,[35-37,41] which was the best rated ROBIS domain (see Figure 3).

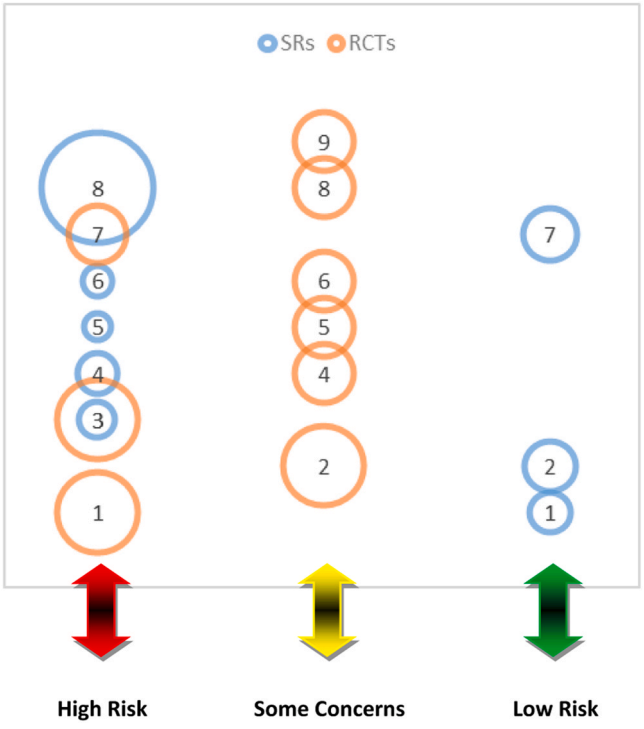

Figure 4 Bubble plot for the overall risk of bias of the systematic reviews and randomized clinical trials outcomes on surgical treatments for BCRL. Studies descriptions: Systematic reviews (Blue circles): 1: Penha et al.[35], 2: Cornelissen et al.[36], 3: Siotos et al.[37], 4: Ribeiro et al.[38], 5: Forte et al.[39], 6: Forte et al.[40], 7: Winter et al.[41], 8: Gasteratos et al.[42]. Randomized clinical trials (Orange circles): 1-3: Dionyssiou et al.[29] (Outcomes; Limb Volume, Infection Episodes & Subjective symptoms, respectively), 4-9: Van Mulken et al.[30] (Outcomes; Daily use of compressive garment, Manual lymphatic drainage, Mean lymph - ICF, Mean UEL Index, Duration of surgery & Quality of the anastomosis, respectively). * SRs: systematic reviews, RCTs: randomized clinical trials, BCRL: breast cancer-related lymphedema.

The overall risk-of-bias assessment for both RCTs and SRs in relation with the number of population or number of studies in the included RCTs and SRs, respectively, has been demonstrated in a bubble plot figure for an overall visual presentation of the results (see Figure 4).

Discussion

The main objective of this research was to describe and critically assess the risk of bias of RCTs and SRs on the surgical treatment for BCRL. To achieve this purpose, we conducted a systematic mapping review, which allowed a visual understanding of the evidence base of any treatment, apart from supporting the process of decision-making by facilitating information in a user-friendly format. Furthermore, it is the best study design to identify gaps of knowledge in any research topic.[48]

Our previous mapping review was conducted to provide an overview of the current situation in the treatment for BCRL but did not focus on the surgical treatment and did not include the risk-of-bias assessment.[21] Furthermore, after updating our previous search in all the databases, our findings result in only two RCTs and eight SRs were among the 47 surgical studies that met the eligibility criteria.

The overall risk-of-bias assessment of the two surgical RCTs[29,30] was rated as some concerns (six outcomes) and high risk (three outcomes) of bias for the measured outcomes among the included RCTs using the RoB-2 and high risk of bias (five studies) and low risk (three studies) for the eight included SRs[35-42] using the ROBIS tool. In addition to the low-quality SRs and RCTs published in the surgical treatment for BCRL, there was a significant heterogeneity in the assessed intervention, the measured outcomes, and the included studies in the case of SRs.

A study with a similar scope was an SR conducted by *Chang* et al.[49] that addressed surgical treatment and prevention for secondary lymphedema. In general, it showed that there was evidence to support some efficacy of LVA and VLNT, but their evidence was mainly based on observational studies and expert consensus. Other SRs that were involved in our results had positive findings on surgical interventions but were based mainly on case series,[35,37] observational studies,[36,39,42,] and nonrandomized trials,[41] and some did not mention which study design they included, probably not including high-quality studies as we assumed.[38,40] Generally, there is a lack of level 1 evidence to support the efficacy of the applied intervention.

Chang et al.[49] assessed the risk of bias for their two included RCTs: one on surgical prevention[50] and another on surgical treatment.[29] The latter RCTwas also assessed in our study, but in contrast, they used a different risk-of-bias tool; nevertheless, they reached a conclusion similar to ours. They rated that RCT[29] with a high risk of bias regarding performance and detection biases, which is comparable to our rating as high risk of bias in the deviation from the intended intervention and measurement of the outcome in the RoB-2. Similar to our finding, *Gasteratos* et al.[42] included in their results one similar RCT on surgical treatment,[30] but they did not assess the risk of bias in their included studies.

There are more promising surgical treatments in the field of BCRL, and demonstrating the effectiveness of these interventions has become more challenging. However, both patients and surgeons need high-quality information about treatment outcomes to inform decision-making.[51] Surgeons now have to adopt more scientific methodologies and evidence-based strategies to improve the standards of care for patients undergoing surgery.[52] Based on our results, the overall evidence in the literature on surgical treatment for BCRL is low, and the limited number of well-designed RCTs in this field is an established barrier that needs to be addressed.

The constraints to conducting high-quality studies in surgery are attributed to the challenges related to the implementation of well-designed studies, the nature of the interventions, and the lack of methodological experience among surgeons. *Ergina* et al.[53] highlighted the difficulties in evaluating surgical innovations, especially in comparison to pharmacological research, which usually contributes to uncertainty about the risk of biases and has led to skepticism about the value of surgical research. Yet, this is applicable by understanding the processes of evaluation in

surgery and creating alternative designs to maximize validity and reduce the chance of bias.[53]

This study has some limitations. First, the search strategy years were from 2000 onward because we assumed that the evolution of the surgical treatments occurred in the previous two decades. Second, the mapping review usually requires additional expertize to create the visual output. Finally, in this kind of study, there is a probable risk of publication bias.

Among the strengths of this study, the systematic screening was performed by three independent reviewers to ensure the reliability of the reported results. The methodological quality assessment consideration was adequately done by defining the eligibility criteria and identifying the risk of bias of the studies. The graphic presentation of the results was made to be relatively easy to interpret and understand. Moreover, the findings of this research illustrate the gaps in the literature and provide a clear picture of future needs in research in the field of surgery.

The shortage of strong evidence in the surgical treatment for BCRL makes the implications of this work in research and practice significantly important and indicates the need to conduct higher quality studies in this field, which can guide health policy and clinical decision-making.

Conclusion

The overall evidence in the literature on surgical treatment for BCRL is low because there are only two RCTs and eight SRs among the 47 published studies. The risk-of-bias assessment for the RCTs outcomes and most SRs were rated either as high risk of bias or with some concerns, and only three SRs were rated as low risk of bias.

High-quality RCTs on different surgical interventions for BCRL should be conducted to measure their real effectiveness, risks, and complications and to compare their benefit with other nonsurgical treatments. Moreover, better quality SRs on BCRL surgical treatments are needed to improve evidence-based decision-making by surgeons and patients.

Conflicts of interest

All remaining authors have declared no conflicts of interest.

Acknowledgments

The authors thank Aliaa I. Shamardal and Luis Vasconcello-Castillo for their participation in the baseline research screening and data collection.

Ethical approval

Not required.

Funding

This mapping review did not receive any funding support.

Disclosure

The first author Ali M. Al-Sakkaf is a PhD candidate at the Surgery and Morphological Sciences program, Universitat Autònoma de Barcelona, Spain.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bjps.2023.05.002.

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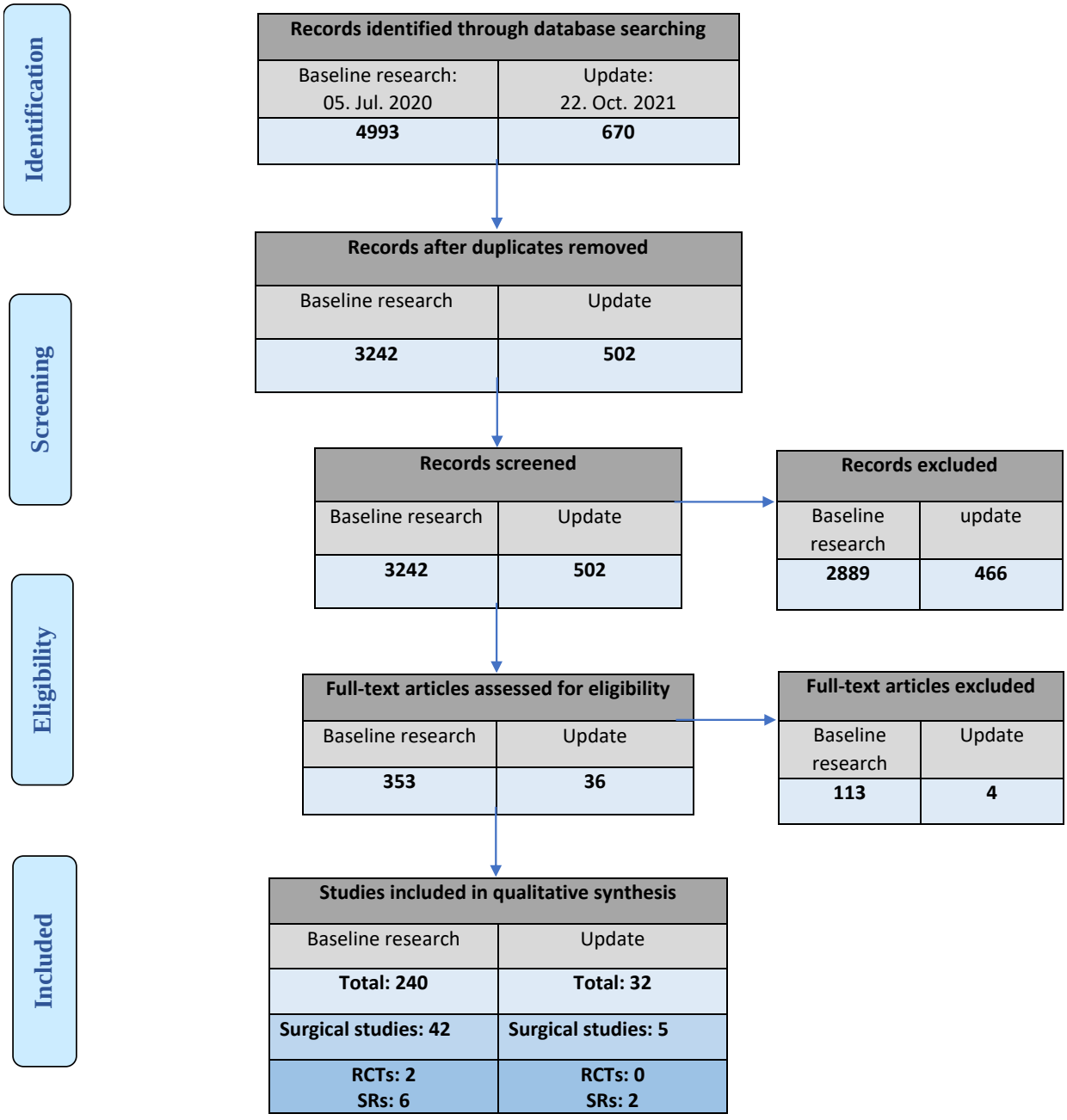
6. Overall Summary of Results

The thesis presented as a compendium of the two publications, outline the main finding are described and summarized below. However, for a complete interpretation of results, the reader is referred to see the full publications text.

6.1 Studies selection

After the last update of our systematic searching for articles from 2000 onward, this research produced a total of 5663 studies. The flow chart of the study selection of the baseline research and update is shown in the following PRISMA Flow diagram:



The baseline results of the first publication focused on providing an overall description of all studies addressing treatments for BCRL, including details of the available RCTs:

6.2 Characteristics of all included studies

- **Publication years and languages**

We observed a marked increase in the number of publications in the recent years; 139 (58%) of the published studies in the treatment of BCRL were from 2016 to 2020. As defined in the eligibility criteria, we included only studies published in English and Spanish. Overall, only two studies were published in Spanish, and the rest (238 studies) were all in English.

- **Countries**

The published studies were distributed among different countries worldwide. The United States of America had the highest number of publications (32) followed by Turkey (19), Australia (17), China (16), South Korea (14), Brazil (12), the United Kingdom (12), and Iran (10). The rest of the countries had fewer than 10 published studies.

- **Study design**

We identified 147 experimental studies (102 RCTs and 45 quasi-experimental clinical trials), 48 observational studies (34 prospective studies and 14 retrospective studies), and 45 SRs (28 SRs without metanalysis and 17 with metanalysis).

- **Intervention types**

The identified studies included different types of intervention (42 surgical treatment and 198 nonsurgical treatment). Most of the surgical interventions were combined with a nonsurgical treatment, such as garment, exercise, or others.

6.3 Characteristics of all RCTs

- **Publication year and languages**

Out of the total RCTs, 52 (51%) were published in the recent 5 years, and they all were published in the English language.

- **Countries**

From the year 2000 till 2020, RCTs have been published in many different countries, the main ones being Australia (12), the USA (11), Turkey (8), South Korea (8), the UK (7), China (5), and Poland (5). The remaining countries published fewer than five clinical trials.

- **Population characteristics and RCTs design**

These RCTs included patients with BCRL affecting the ipsilateral arm, but three studies described breast or chest lymphedema secondary to breast conservative treatment or mastectomy. Three trials studied BCRL only in overweight or obese patients. All were parallel with two arm groups, but there were five crossover design trials, and five RCTs comparing three arm groups.

We encountered some RCTs that included the same population and methodology protocol and were performed by the same team but measuring different outcomes and/or describing subgroup analysis. These studies have been included as separate studies and analysed independently.

- **Intervention types**

Overall, most of the RCTs were on nonsurgical treatment, and there was a large diversity in nonsurgical treatment options, mostly regarding physiotherapy treatments, such as different kinds of sleeves/bandages, kinesio tape, manual lymphatic drainage, pneumatic compression pump, decongestive compression therapy, exercise protocols (active, resistant, aqua exercise), yoga, weight loss, acupuncture or different pharmacotherapy, laser therapy, satellite ganglionic block, etc.

Among the nonsurgical treatment trials, we also found two studies addressing autologous stem cell transplantation (ASC), which is considered a minimally invasive medical intervention; both studies were done in a surgery setting.

There were only two RCTs on surgical treatment: the first trial about lympho-venous anastomosis (LVA), and the second trial about vascularized lymph node transfer (VLNT).

- **Measured outcomes**

There was a wide variety of outcomes measured in these trials and most of the trials focused on more than one outcome. The main outcome measured was the arm volume and circumference, followed by lymphedema symptoms like heaviness or pain, arm function and range of movements, and quality of life. Other measured outcomes were patient adherence, satisfaction, safety and adverse events, skin changes, infection and inflammatory markers, cost of treatments, or intervention duration.

- **Effect of intervention**

Most of the RCT results favoured toward intervention (59). Some other studies favoured toward the comparison group (7), and the rest of the trials had no difference of effect by applying the intervention (37)

After the last update, the results of the second publication were focused on the studies addressing the surgical treatments for BCRL and assessing the risk of bias of the RCTs and SRs:

6.4 Surgical studies on treatment for BCRL

There were 47 studies addressing surgical intervention (39 primary studies and eight secondary studies), which included 15 experimental studies (13 quasi- experimental clinical trials and two RCTs), 24 observational studies (14 prospective cohorts and 10 retrospective cohorts), and eight SRs with and without meta-analysis.

These published studies were geographically distributed among the following countries: the Netherlands had the highest number of publications (9), followed by the United States of America (7), France (4), Sweden (4), China (4), Taiwan (3), Japan (2), Brazil (2), Spain (2), Italy (2), Greece (2), the United Kingdom (1), Belgium (1), Denmark (1), Poland (1), Thailand (1), and Australia (1).

- **RCTs' characteristics**

The first RCT, by Dionyssiou et al,[48] was conducted in Greece and compared the VLNT to the conservative measurements, and the second RCT, by Van Mulken et al,[49] conducted in the Netherlands, compared the robotic versus the manual LVA. Both RCTs assessed different outcomes. Dionyssiou et al,[48] mainly assessed three patients' outcomes: upper limb volume, infection episode, and subjective symptoms using the subjective analogue scaling system.[50] Van Mulken et al,[49] assessed various outcomes, including four patients' outcomes: the daily use of compression garment, the need for manual lymphatic drainage, the arm circumference using the mean upper extremity lymphedema index (mean UEL index), and quality of life using a validated health questionnaire, the mean Lymphedema Functioning, Disability and Health questionnaire (Lymph-ICF).[51] Furthermore, this RCT assessed two surgeons' related outcomes: the duration of surgery and quality of anastomosis using the Structured Assessment of Microsurgery Skills (SAMS)[52] and the University of Western Ontario Microsurgical Skills Acquisition Instrument (UWOMSA) scoring.[53]

- **SRs' characteristics**

Among the eight SRs on surgical treatments for BCRL,[54-61] three SRs performed quantitative assessment (metanalysis).[55,56,60] Three were conducted in the Netherlands,[54,55,60] three in the United States of America,[56,58,59] one in Greece,[61] and one in Brazil.[57] There was heterogeneity in the included primary studies designs, mainly contained case series, case reports, prospective studies, retrospective studies, and nonrandomized trials. Two SRs did not mention the type of the included study design. The range of included studies was from five to 17 studies. One SR included a total of 67 studies, but only 13 were described in the qualitative synthesis, which were addressed in our results.[61] These SRs addressed different surgical interventions, such as VLNT,[57,60] LVA,[55] both VLNT and LVA,[54,61] or combined treatment such as autologous reconstruction with VLNT [56,59] or lipo-aspiration with VLNT.[58] The only common outcome that was measured in all included SRs was limb volume, although different outcomes were also assessed, such as subjective symptoms, quality of life, infectious episodes, complications, and discontinuation of conservative treatments.

- **Matrix of evidence**

We created a matrix of evidence to show the SRs linked to the included primary studies and the overlaps of the primary studies between these SRs. Because there was heterogenicity in the objectives and in the assessed surgical interventions of the SRs, we found that a total of 55 primary studies were included in all SRs, but only 19 studies overlapped between two or more SRs. Saaristo et al,[62] overlapped in five SRs; Chang et al,[63] overlapped in four SRs; Lin et al,[32] Becker et al,[31] Damstra et al,[64] De Brucker et al,[65] and Montag et al.[64] overlapped in three SRs; and the rest overlapped in two SRs.

6.5 Risk of bias assessments

- **Risk-of-bias assessment for RCTs**

Based on the RoB-2, nine outcomes were assessed in the two included RCTs. Three outcomes were assessed in the RCT of Dionyssiou et al.[48] Two were rated as having a high risk of bias (limb volume and subjective symptoms) and one had some concerns (infection episodes). Three RoB-2 domains, 'the randomization process domain, deviation from intended intervention domain, and measurement of the outcome domain', had a probability of introduced bias in all RCT outcomes and downgraded the rating to some concerns or high risk of bias.

Six outcomes were assessed in the RCT of Van Mulken et al.[49] Five were rated as with some concerns (daily use of compressive garment, use of manual lymphatic drainage, the mean lymph – ICF, duration of surgery, and quality of the anastomosis) and one as high risk of bias (mean UEL index). Two RoB-2 domains, 'the randomization process domain and deviation from intended intervention domain', were rated as having some concerns regarding the probability of introduced bias in all RCT outcomes. In addition, "the missing outcome data" domain was rated as having a high risk of bias in the mean UEL index outcome, downgrading this outcome to a high risk of bias.

- **Risk-of-bias assessment for SRs**

Based on the ROBIS tool, five SRs (5/8, 62.5%) were rated as high risk of bias[56-59,61] and three (3/8, 37.5%) were rated as low risk of bias.[54,55,60] The five SRs rated as having a high risk of bias were downgraded because there was a probability of introducing bias in more than one domain. All rated as high risk of bias in the 'data collection and study appraisal' domain (5/8, 62.5%),[56-59,61] three in the 'study eligibility criteria' domain (3/3, 37.5%)[57,58,61] three in the 'synthesis of finding' domain (3/3, 37.5%),[57-59] and two in the 'identification and selection of studies' domain (2/8, 25%).[57,59] Finally, 50% of all included SRs had rated the domain of 'study eligibility criteria' as having a low risk of bias,[54-56,60] which was the best rated ROBIS domain.

7. Overall Summary of the Discussion

As we perceived that the available evidence of the treatment for BCRL is not proportional to the importance of this health condition, which is considered as significant problem for women who had been treated for breast cancer and compromising their quality of life, we decided to provide a broad picture of the current situation of this important topic and thereby shedding light on areas requiring more attention.

The main objective of this research is to identify, describe and organize of the currently available evidence in the treatment for BCRL, and to assess the risk of bias of the surgical RCTs and SRs. To achieve this purpose, we conducted a systematic mapping review, which allowed a visual understanding of the evidence base of any treatment, apart from supporting the process of decision-making by facilitating information in a user-friendly format. Furthermore, it is the best study design to identify gaps of knowledge in any research topic.[67]

There is a variety of methodological standards to develop a mapping review, but we decided to follow the GEM initiative. because it is very rational and systematic.[41] This methodology includes three core tasks: setting the topic area's boundaries and the context in question, searching and selecting relevant studies, and reporting on search results and study characteristics.[41] Additionally, we planned to ass the risk of bias of high quality studies, which is the RCTs and SRs, for that reason together with the interest of the authors, we did not consider the necessity to assess the risk of bias of other kind of studies.

Following our recent search update, our comprehensive review process yielded a total of 272 studies were included, of which 225 were nonsurgical studies and 47 studies addressed the surgical treatment for BCRL (which include 39 primary studies and eight secondary studies), there is a clear tendency to publish more non-surgical studies and more primary studies, underscores significant gaps in the representation of surgical studies and secondary studies within the current body of literature.

Nevertheless, we have observed a growing tendency towards increased publications in the field of surgery in recent years. We attribute this trend to the evolution of new treatment modalities and techniques in the field of BCRL surgical treatments, as well as the rise in surgeons' engagement with clinical research, indicating a growing interest in contributing to the scholarly discourse within their field. However, it's noteworthy that despite this growing trend, our findings revealed a relatively few numbers of RCTs and SRs among the pool of eligible surgical studies. Specifically, only two RCTs and eight SRs met our eligibility criteria.

The significant limited number of RCTs could be mainly due to the general difficulties to design RCTs to assess the surgical procedures, if we compare it with pharmaceutical agents.[68] Therefore, despite the wide use of the different surgical interventions in clinical practice, there is currently not enough high-quality research to assess their effectiveness.[69] This highlights the current need for well-designed RCTs to compare the effectiveness of these surgical interventions, as well as to assess its relation to nonsurgical treatment.

Conducting RCTs in surgery presents unique challenges because of the inherent complexity and variability in surgical procedures, patient populations, and outcomes. Factors such as surgeon expertise, patient comorbidities, and surgical techniques can significantly influence results, making it challenging to standardize interventions across study groups. Additionally, ethical considerations arise concerning counterbalance, as surgeons may prefer certain procedures or techniques based on their experience or beliefs. Furthermore, the need for large sample sizes to detect clinically meaningful differences in outcomes adds to the complexity of surgical trials.[68-70]

In some case, the scarcity of surgical RCTs been justified due to the difficulty of applying blinding, as it may be challenging to conceal treatment allocation from both patients and surgeons, but this requirement is not inherently necessary in designing RCTs and could be overcome with alternative designs to maximize the validity and to reduce the chance of assessment bias.[70] However, we assume that there are well-designed non-randomized prospective studies conducted on surgical intervention.

Through the analysis of the published surgical studies, we encountered a wide heterogeneity in terms of population demographics, measured outcomes and the included primary studies in the case of SRs. This observed heterogeneity underscores the importance of addressing these variations comprehensively in future publications and should be complemented by SRs if the primary studies are valid and available in number. On the other hand, the applied surgical interventions were mainly microsurgical techniques (LVA and/or vascularized lymph node transfer) and liposuction, which reflect the common surgical practice nowadays, these surgical procedures focus on re-establishing the function of the lymphatic system and reducing the volume, respectively.[66]

The evaluation of the risk of bias in surgical research is vital to ensure the validity and reliability of study findings. In the context of our work, the overall risk-of-bias assessment of the two surgical RCTs[48,49] was rated as some concerns (six outcomes) and high risk of bias (three outcomes) for the measured outcomes among the included RCTs using the RoB-2, and high risk of bias (five SRs) and low risk (three SRs) for the eight included SRs[54-61] using the ROBIS tool.

A study with a similar scope was a SR conducted by Chang et al.[71] it addressed surgical treatment and prevention for secondary lymphedema. In general, it showed that there was evidence to support some efficacy of LVA and VLNT, but their evidence was mainly based on observational studies and expert consensus. Also, the SRs that were involved in our results had positive findings on surgical interventions but were based mainly on case series,[54,56] observational studies,[55,58,61] and nonrandomized trials,[60] and some did not mention which study design they included, probably not including high-quality studies as we presumed.[57,59] Obviously, there is a lack of level 1 evidence to support the efficacy of the applied intervention.

Chang et al[71] assessed the risk of bias of their two included RCTs, one was on surgical prevention[72] and another on surgical treatment.[48] The latter RCT was also assessed in our study, but in contrast, they used a different risk-of-bias tool; nevertheless, they reached a conclusion similar to ours. Change et al[71] rated that RCT[48] with a high risk of bias regarding performance and detection biases, which is comparable to our rating as high risk of bias in the deviation from the intended intervention and measurement of the outcome in the RoB-2 tool.

Based on our results, the overall evidence in the literature on surgical treatment for BCRL is low, and the limited number of well-designed research in this field is an established barrier that needs to be addressed. Ergina et al[73] highlighted the difficulties in evaluating surgical innovations, which are attributed to the challenges related to the implementation of well-designed studies and the lack of methodological experience among surgeons, that usually contributes to uncertainty about the risk of biases and has led to scepticism about the value of surgical research. Yet, this is applicable by understanding the processes of evaluation in surgery and creating alternative designs to reduce the chance of bias.[73]

Another important fact, that research in surgery often requires significant resources, including funding for equipment, staff, and infrastructure for the implementation of the intervention, accordingly, limited resources together with the logistical and practical constraints can hinder the accomplishment of large-scale, well-designed studies in surgery.[74] Eventually, the need of high-quality research in surgery, particularly in the context of BCRL surgical treatment, are crucial for advancing patient care and optimizing outcomes.

There are more promising surgical treatments in the area of BCRL, and demonstrating the effectiveness of these interventions has become more challenging. However, both patients and surgeons need high-quality evidence about treatment outcomes to inform decision-making.[75] Surgeons now should adopt more scientific methodologies and evidence-based strategies in research to improve the standards of care for patients undergoing surgery.[76]

In research, the applied intervention should be clearly described and reported, so other researchers can replicate or build on research findings.[77] The intervention description for non-pharmacological interventions is frequently insufficient, one analysis of trials and reviews found that 67% of descriptions of drug interventions were adequate compared with only 29% of non-pharmacological interventions.[78] A recent study of 137 interventions, from 133 trials of non-drug interventions, found that only 39% of interventions were described adequately in the primary paper or any references, appendices, or websites.[79]

The landscape of clinical trial reporting standards is anchored by essential guidelines such as the Consolidated Standards of Reporting Trials (CONSORT) statement, it provides recommendations intended to guide authors on providing the information necessary to include in the published report of an RCT. It facilitates the complete and transparent reporting and assists in the critical evaluation and interpretation. A small number of CONSORT extension statements contain expanded guidance about describing non-pharmacological interventions.[80]

In parallel, the guidance for content of trial protocols, SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials), provides some recommendations for describing interventions in protocols. Complementing CONSORT, SPIRIT contributes to the meticulous planning and documentation of trial methodologies, fostering methodological rigor and facilitating accurate interpretation.[81]

Expanding upon these foundational frameworks, TIDieR (Template for Intervention Description and Replication), is an extension of the CONSORT and the SPIRIT statements with the objective of improving the completeness of reporting, and ultimately the replicability of interventions and comprises a structured checklist of 12 items, TIDieR can be considered a useful tool to describe any type of non-pharmacological intervention regardless of the methodological design used.[77]

We believe surgical research can be greatly improved, and evolution in surgical care and interventions will become safer, more efficient and better in the future. Our research team has started to conduct an extensive investigation in the field of Lymphedema, actively contributing to the collection of evidence for its surgical treatment while ensuring the quality of our research. Thus far, we have successfully completed a comprehensive cross-sectional study, conducted three experimental animal trials, and we are currently immersed in designing a pilot randomized clinical trial. Together, we are working in conducting consensus article on lymphedema reconstructive surgery based on Delphi methodology criteria.[82]

Some of the published articles related to these works are provided as annexes, showcasing the breadth and depth of our team's ongoing efforts in advancing the understanding of surgical treatment of Lymphedema. *Annex 5*

7.1 Potential strengths

Evidence mapping review has been used, as it provides a comprehensive and systematic review of available evidence, enabling the organization and visualization of all kinds of studies and presenting a holistic view of the current state of surgical interventions for BCRL. By visually presenting existing evidence, evidence mapping helps in identifying gaps in the literature. It is valuable tool to organize areas where research is absent or less or if there are contradictory results, which guide the future research directions and highlighting areas requiring further investigation.[83, 84]

Furthermore, it supports evidence-informed decision-making by surgeons and patients. It plans to realise the breadth and depth of current evidence, facilitating informed decisions in this case for the surgical interventions for BCRL management. Evidence mapping synthesized data in a user-friendly format, making the complex data to more accessible and easier for interpretation and utilization to a wide range of beneficiaries.[85,86]

Based on all the advantages of evidence mapping, it guides for future research and policy development, it contributes to shape the research priorities, and helps in the formulation of evidence-based guidelines in the field of BCRL surgical management. Although evidence mapping is not replacing systematic reviews and meta-analyses, it is also important to mention that evidence mapping can be complementary to systematic reviews and can be the first step before starting more in-depth systematic reviews.[87,88]

7.2 Limitations

Unlike systematic reviews or meta-analyses, evidence mapping mainly focuses on descriptive and visual presentation of current evidence. It may not provide quantitative data synthesis, such as effect sizes or pooled estimates of the effect of treatments, which are important for evaluating the efficacy of the interventions. Also, evidence mapping depends on the published research, which may lead to publication bias, and provide studies with positive finding or statistically significant results.[89]

Additionally, it provides a holistic picture of the current evidence, which is subject to progress over time, as new studies may publish in the future and varying the available evidence and making it sometimes outdated. The heterogeneity in data among the included studies in mapping studies, may lead to challenges for researchers in data extraction and synthesis across studies.[83]

And regarding the surgical treatment for BCRL, evidence mapping may have some limitation to identify some specific surgical details and their outcomes due to the complexity and the wide range of interventions. Finally, evidence mapping may also require experts for the synthesis of the results and understanding of the clinical context related to the assessed interventions.[84]

8. Conclusions

The overall evidence in the literature on surgical treatment for BCRL is low, even though lymphedema is considered an important health problem for women who undergo breast cancer treatment. Recently this lack of attention has been partially corrected, as there is a marked increase in the publication in the past few years. However, most of these studies were on nonsurgical interventions.

There are only two RCTs and eight SRs among the 47 published studies on BCRL surgical treatment. The risk-of-bias assessment for the RCTs outcomes and most SRs were rated either as high risk of bias or with some concerns, and only three SRs were rated as low risk of bias.

High-quality RCTs on different surgical interventions for BCRL should be conducted to measure their real effectiveness, risks, and complications and to compare their benefit with other nonsurgical treatments. Moreover, better quality SRs are also needed to improve evidence-based decision-making by surgeons and patients.

9. Future Lines

Incorporation of evidence-based medicine into surgery

The recent emphasis of the incorporation of evidence-based medicine into surgical practice underscores the important role of rigorous research in guiding the clinical decision-making process. Looking ahead, surgeons will be encouraged to critically appraise the existing body of evidence, integrating findings into their clinical practice, and contributing to the generation of new knowledge through research endeavours.

Validation of observed outcomes and informed decision-making

Within this context, surgical procedures like LVA, VLNT and Liposuction, collectively offer promising results for enhancing the quality of life for patients with BCRL, providing tailored solutions for its treatment. In clinical practice, surgeons often rely on observed outcomes to evaluate the effectiveness of these techniques for their patients. However, consolidating evidence from existing literature not only validates these observed outcomes but also support the informed decision for surgeons and guide for treatment planning.

Clinical applications of evidence mapping

In regard to the clinical applications, it supports surgeon in the selection and recommendation for the surgical intervention and tailored treatment plans for each patient. The visual presentation can help the clear communication between surgeons and patients, and it involve patients in shared decision-making processes regarding their treatment.

Prioritization of research efforts and future research direction

Evidence mapping can serve as a catalyst for shaping future research in surgery field. It can support the health system to allocate the resources efficiently and optimizing the utilization and the distribution of the resources and patient care delivery. Spotting the gaps or areas lacking evidence, helps researchers to prioritize the research efforts in areas where high evidence studies are really needed, and subsequently evidence mapping guide future research direction.

Development of evidence-based guidelines or protocols

The synthesized evidence derived from evidence mapping can serve as a base for developing evidence-based guidelines or protocols. As we refine our understanding of BCRL treatment strategies, this can help in setting up a standardized practices and guidelines in the field of surgical interventions for BCRL.

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References following the Vancouver - citing and referencing

11. Annexes

11.1 Annex 1: Data extraction table

ITEM	DESCRIPTION
STUDY ID	Unique description for each study.
JOURNAL	The name of the journal where the study has been published.
YEAR	The year of the study publication.
COUNTRY	Country of the study (International= more than one country).
AUTHOR	The name of the authors.
DESIGN	The design of the study. Randomized clinical trial, Quasi-experimental trials, Prospective, Retrospective, Systematic reviews with or without Metanalysis.
OBJECTIVES	The main objective of the study.
POPULATION	Population included in the study.
N. OF PARTICIPANT / N. OF STUDIES	Number of the included patients/ Number of the included studies (in case of systematic reviews and metanalysis).
INTERVENTION	Types and names of the used surgical and non-surgical treatment.
COMPARISON	Type of comparison if exist.
OUTCOME	Outcomes measured.
RESULTS	Main study results on the treatment effect. (favours towards intervention, favours toward comparison or no difference).

11.2 Annex 2: Search strategy

- **EMBASE search strategy:**

Embase.com

Last update: 22/10/2021

#1	'breast cancer-related lymphedema'/exp AND [embase]/lim	579
#2	'breast tumor'/exp AND [embase]/lim	522258
#3	((breast NEXT/1 tumor*):ab,ti) AND [embase]/lim	26986
#4	((breast NEXT/1 tumour*):ab,ti) AND [embase]/lim	4782
#5	((breast NEXT/1 neoplasm*):ab,ti) AND [embase]/lim	923
#6	((breast NEXT/1 cancer*):ab,ti) AND [embase]/lim	384734
#7	#2 OR #3 OR #4 OR #5 OR #6	561175
#8	'lymphedema'/exp AND [embase]/lim	19349
#9	lymphedema:ab,ti AND [embase]/lim	10146
#10	lymphoedema:ab,ti AND [embase]/lim	2822
#11	((arm NEXT/1 edema):ab,ti) AND [embase]/lim	256
#12	((arm NEXT/1 oedema):ab,ti) AND [embase]/lim	63
#13	((limb NEXT/1 edema):ab,ti) AND [embase]/lim	868
#14	((limb NEXT/1 oedema):ab,ti) AND [embase]/lim	318
#15	#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14	22096
#16	#7 AND #15	4928
#17	#1 OR #16	4957
#18	'systematic review'/exp AND [embase]/lim	306754
#19	#17 AND #18	210
#20	#17 NOT #19	4747
#21	('randomized controlled trial'/exp OR 'randomized controlled trial') AND [embase]/lim	756573
#22	'single-blind method'/exp AND [embase]/lim	37174
#23	'double-blind method'/exp AND [embase]/lim	169213
#24	'crossover procedure'/exp AND [embase]/lim	58797
#25	(random*:ti,ab OR factorial*:ti,ab OR crossover*:ti,ab OR ((cross NEXT/1 over*):ti,ab) OR placebo*:ti,ab OR assign*:ti,ab OR allocat*:ti,ab OR volunteer*:ti,ab) AND [embase]/lim	2020219
#26	((doubl* NEXT/5 blind*):ti,ab) AND [embase]/lim	208644
#27	((singl* NEXT/5 blind*):ti,ab) AND [embase]/lim	28664
#28	#21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27	2196300

#29	('animals'/exp OR 'invertebrate'/exp OR 'animal experiment'/exp OR 'animal model'/exp OR 'animal tissue'/exp OR 'animal cell'/exp OR 'nonhuman'/exp) AND [embase]/lim	23103224
#30	('human'/exp OR 'human cell'/exp) AND [embase]/lim	17394890
#31	#29 NOT #30	5708334
#32	#28 NOT #31	1956841
#33	#20 AND #32	845
#34	#17 NOT (#19 OR #33)	3902
#35	'cohort analysis'/exp AND [embase]/lim	707214
#36	'longitudinal study'/exp AND [embase]/lim	118393
#37	'prospective study'/exp AND [embase]/lim	616647
#38	'evaluation and follow up'/exp AND [embase]/lim	2579095
#39	'follow up'/exp AND [embase]/lim	1601113
#40	longitudinal*:ab,ti AND [embase]/lim	325033
#41	prospective*:ab,ti AND [embase]/lim	1075170
#42	((follow NEXT/1 up):ab,ti) AND [embase]/lim	1498809
#43	cohort:ab,ti AND [embase]/lim	989062
#44	#35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43	4402181
#45	#34 AND #44	1633
#46	#19 OR #33 OR #45	2688
#47	(#19 OR #33 OR #45) AND [1-7-2020]/sd NOT [1-11-2021]/sd	408

- **MEDLINE search strategy**

Pubmed.gov

Last update: 22/10/2021

#1	Breast cancer lymphedema [MeSH]	283
#2	Breast neoplasm [MeSH]	313.926
#3	Breast* [ti] OR Breast neoplasm* [tiab] OR Breast cancer* [tiab] OR Breast tumor* [tiab] OR Breast tumour* [tiab]	401.980
#4	#2 OR #3	455.292
#5	Lymphedema [MeSH]	13.008
#6	Lymphedema* [tiab] OR Lymphoedema* [tiab] OR Arm edema [tiab] OR Arm oedema [tiab] OR Limb edema [tiab] OR Limb oedema [tiab]	12.284
#7	#5 OR #6	17.731
#8	#4 AND #7	3.466
#9	#1 OR #8	3.479
#10	Systematic [sb]	206.117
#11	“Clinical trials as topic” [mesh:noexp] OR “Randomized controlled trial”[pt] OR “Controlled clinical trial”[pt] OR Randomized[tiab] OR Placebo[tiab] OR Randomly[tiab] OR Trial[ti]) NOT (“animals”[Mesh] NOT “humans”[Mesh])	1.301.093
#12	“Comparative Study” [pt] OR “Multicenter Study” [pt] OR” Clinical Trial” [pt] OR “Evaluation Studies” [pt] OR “Prospective Studies” [Mesh] OR “Follow-Up Studies” [Mesh] OR trial*[tiab] OR Prospective*[tiab] OR compare*[tiab] OR Cohort[tiab]	7.616.140
#13	#9 AND #10	160
#14	#9 NOT #13	3.319
#15	#14 AND #11	442
#16	#9 NOT (#13 OR #15)	2.877
#17	#16 AND #12	1.087
#18	#13 OR #15 OR #17	1.689
#19	#13 OR #15 OR #17 Filter: 01.07.2000 – 01.11.2021	210

- **CENTRAL search strategy**

Cochranelibrary.com

Last update: 22/10/2021

#1 MeSH descriptor: [Breast cancer lymphedema] explode all trees 77
#2 MeSh descriptor: [Breast neoplasm] explode all trees 13.847
#3 (Breast neoplasm): ti,ab,kw OR (Breast cancer):ti,ab,kw OR (Breast tumor):ti,ab,kw OR (Breast tumour):ti,ab,kw 39.286
#4 #2 OR #3 40.083
#5 MeSH descriptor: [Lymphedema] explode all trees 656
#6 (Lymphedema): ti,ab,kw OR (Lymphoedema):ti,ab,kw OR (Arm edema) :ti,ab,kw OR (Arm oedema):ti,ab,kw OR (Limb edema):ti,ab,kw OR (Limb oedema):ti,ab,kw 3533
#7 #5 OR #6 3648
#8 #4 AND #7 1064
#9 #1 OR #8 1064
(Reviews 13 – Trials 151)
#10 #1 OR #8 Filter: 01.07.2000 – 01.11.2021 1
(Reviews 1 - Trials 0)

- **Epistemonikos search strategy**

Epistemonikos.org

Last update: 22/10/2021

(title: (Breast cancer lymphedema) OR abstract: (Breast cancer lymphedema))
Filter: 01.07.2020 – 01.11.2021:
Total references: 51
(Reviews and primary studies)

11.3 Annex 3: Descriptive data of all RCTs

Number	Year	Author	Title of paper	Population	Type & Name of Intervention	Comparison Group	Outcome measured	Results
1	2020	T.J.M., van Mulken, et al	First-in-human robotic supermicrosurgery using a dedicated microsurgical robot for treating breast cancer-related lymphedema: a randomized pilot trial	20	robot-assisted supermicrosurgical lymphatico-venous anastomosis LVA	manual supermicrosurgical lymphatico-venous anastomosis LVA	upper limb volume, quality of life, duration of surgery, and quality of anastomosis	no difference
2	2016	D., Dionyssiou, et al	A randomized control study of treating secondary stage II breast cancer-related lymphoedema with free lymph node transfer	36	vascularized lymph node transfer (LNT) and physiotherapy and compression	physiotherapy and compression alone	upper limb volume, infection, and lymphedema symptoms	favours towards intervention
3	2008	Hou, Chuanqiang, et al	Autologous bone marrow stromal cells transplantation for the treatment of secondary arm lymphedema: a prospective controlled study in patients with breast cancer related lymphedema	50	autologous bone marrow stromal cells (BMSCs)	complex decongestive therapy (CDT)	upper limb volume and pain	favours towards intervention
4	2011	Maldonado, Gerardo Enrique Muñoz, et al	Autologous stem cells for the treatment of post-mastectomy lymphedema: a pilot study	20	autologous stem cells (ASC)	compression sleeve therapy (CST)	Upper extremity volume, pain sensitivity, and mobility	favours towards intervention

5	2019	Z.-F., Xiong, et al	Sliding cupping along meridian for lymphedema after breast cancer surgery: A randomized controlled trial	60	sliding-cupping along meridian with short-stretch elastic bandage wrapping	comprehensive detumescence therapy	upper limb circumference and volume	favours towards intervention
6	2011	R.J., Damstra, et al	Compression therapy in breast cancer-related lymphedema: A randomized controlled study of relation between volume and interface pressure changes	36	low pressure bandages	high pressure bandages	upper limb volume and pressure tolerance	no difference
7	2020	E., Tastaban, et al	Role of intermittent pneumatic compression in the treatment of breast cancer-related lymphoedema: a randomized controlled trial	76	complex decongestive treatment + intermittent pneumatic compression	complex decongestive therapy (CDT)	upper limb volume, feeling tightness and heaviness	favours towards intervention
8	2019	A., Cacchio, et al	Effectiveness and safety of a product containing diosmin, coumarin, and arbutin (Linfadren¬Æ) in addition to complex decongestive therapy on management of breast cancer-related lymphedema	50	Liinfardin + CDT	complex decongestive therapy (CDT)	upper limb volume and drug safety	favours towards intervention
9	2013	A., Smykla, et al	Effect of Kinesiology Taping on breast cancer-related lymphedema: A randomized single-blind controlled pilot study	56	Kinesiology Taping (KT)	Quasi KT or MCT (multi-layered compression therapy)	upper limb circumference and volume	favours towards comparison
10	2015	H., Uzkeser, et al	Efficacy of manual lymphatic drainage and intermittent pneumatic compression pump use in the treatment of lymphedema after mastectomy: a randomized controlled trial	31	intermittent pneumatic compression pump	manual lymphatic drainage	upper limb circumference, volume, dermal thickness, and pain	no difference
11	2018	M., Tambour, et al	Manual lymphatic drainage adds no further volume reduction to Complete Decongestive Therapy on breast cancer-related lymphoedema: a multicentre, randomised, single-blind trial	77	Complete Decongestive Therapy (CDT) including manual lymphatic drainage (MLD)	complex decongestive therapy (CDT)	upper limb circumference, volume of the arm, heaviness and tension, and general health status	no difference

12	2009	S.C., Hayes, et al	Exercise and secondary lymphedema: Safety, potential benefits, and research issues	32	supervised group exercise	habitual activities	upper limb circumference and volume	no difference
13	2019	M.B., Ligabue, et al	Efficacy of self-administered complex decongestive therapy on breast cancer-related lymphedema: a single-blind randomized controlled trial	41	self-administrated complex decongestive therapy (sa CDT)	usual care	upper limb volume and pain	favours towards intervention
14*	2020	M., Navaei, et al	The Effects of Synbiotic Supplementation on Antioxidant Capacity and Arm Volumes in Survivors of Breast Cancer-Related Lymphedema	88	synbiotic supplementation	placebo	upper limb volume and oxidative markers	favours towards intervention
15	2015	W., Lee, et al	Effects of stellate ganglion block on breast cancer-related lymphedema: Comparison of various injectates	32	Stellate ganglion block (SGB) with 0.5% bupivacaine 5 mL	Stellate ganglion block (SGB) with 0.5% bupivacaine 4.5 mL + 20 mg of triamcinolone 0.5 mL or 0.5% bupivacaine 4 mL + 40 mg of triamcinolone 1 mL	upper limb circumference, physical, emotional, and social functioning	favours towards comparison
16*	2016	J., Buchan, et al	A Randomized Trial on the Effect of Exercise Mode on Breast Cancer-Related Lymphedema	41	resistance exercise	aerobic-based exercise	upper limb circumference, function, lymphedema symptoms and quality of life	no difference
17	2017	J.-H., Park	The effects of complex exercise on shoulder range of motion and pain for women with breast cancer-related lymphedema: a single-blind, randomized controlled trial	69	complex exercise	conventional decongestive therapy	shoulder range of motion and pain	favours towards intervention
18	2019	V., Pajero Otero, et al	Kinesio taping versus compression garments for treating breast cancer-related lymphedema: a randomized, cross-over, controlled trial	30	Kinesiology Taping (KT)	compression garments	upper limb volume, range of motion, self-perception of comfort, and lymphedema symptoms	favours towards intervention

19	2019	H.W., Han, et al	Sodium selenite alleviates breast cancer-related lymphedema independent of antioxidant defence system	26	sodium selenite dissolved in normal saline	only normal saline	upper limb volume and blood levels of oxidative markers	favours towards intervention
20	2018	G.D., Baxter, et al	Low level laser therapy for the management of breast cancer-related lymphedema: A randomized controlled feasibility study	17	low level laser therapy (LLLT) and conventional therapy	conventional therapy alone	upper limb circumference, lymphedema symptoms, psychological impacts, function, and patient's adherence and satisfaction	no difference
21	2019	K.H., Schmitz, et al	Effect of Home-Based Exercise and Weight Loss Programs on Breast Cancer-Related Lymphedema Outcomes among Overweight Breast Cancer Survivors: The WISER Survivor Randomized Clinical Trial	351	home-based exercise or weight loss intervention or both	facility-based lymphedema care	upper limb volume	favours towards comparison
22	2018	M., Karafa, et al	The effect of different compression pressure in therapy of secondary upper extremity lymphedema in women after breast cancer surgery	96	inelastic multi-layer bandages at pressure 31-40 and at pressure 41-60	inelastic multi-layer bandages at pressure 21-30	upper limb circumference	favours towards intervention
23	2019	C., Wang, et al	Moxibustion as a Therapy for Breast Cancer Related Lymphedema in Female Adults: A Preliminary Randomized Controlled Trial	48	moxibustion	compression garments	upper limb circumference and fatigue	favours towards intervention
24*	2020	S., Vafa, et al	The effects of synbiotic supplementation on serum inflammatory markers and edema volume in breast cancer survivors with lymphedema	88	synbiotics Capsules	placebo	upper limb volume, inflammatory markers, serum leptin concentration	favours towards intervention

25	2009	K.H., Schmitz, et al	Weightlifting in women with breast-cancer-related lymphedema	141	weightlifting	no weightlifting	upper limb volume, exacerbations of lymphedema, lymphedema symptoms, and muscle strength	favours towards intervention
26	2003	C.J., Carati, et al	Treatment of postmastectomy lymphedema with low-level laser therapy: A double blind, placebo-controlled trial	61	low-level laser therapy (LLLT)	placebo	upper limb volume, extracellular tissue fluid distribution, dermal tonometry, and range of movement	favours towards intervention
27	2013	K., Johansson, et al	Water-based exercise for patients with chronic arm lymphedema: a randomized controlled pilot trial.	29	water-based exercise	normal daily exercise	upper limb volume, shoulder range of movement and adherence	favours towards intervention
28	2002	J., Sitzia, et al	Manual lymphatic drainage compared with simple lymphatic drainage in the treatment of post-mastectomy lymphoedema	28	manual lymphatic drainage (MLD)	simple lymphatic drainage (SLD)	upper limb volume	favours towards intervention
29	2010	L., Gothard, et al	Randomised phase II trial of hyperbaric oxygen therapy in patients with chronic arm lymphoedema after radiotherapy for cancer	58	hyperbaric oxygen (HBO) therapy	best standard care	upper limb volume and quality of life	no difference
30	2017	M.A., Storz, et al	Photobiomodulation therapy in breast cancer-related lymphedema: a randomized placebo-controlled trial	40	active laser therapy	placebo	upper limb volume, pain, quality of life and grip strength	no difference
31	2013	I.S., Dayes, et al	Randomized trial of decongestive lymphatic therapy for the treatment of lymphedema in women with breast cancer	103	complex decongestive therapy (CDT)	compression therapy (elastic compression garments alone)	upper limb volume	no difference
32	2016	M., Park, et al	Comparison of effectiveness between complex decongestive therapy and stellate ganglion block in breast cancer related lymphedema patients: A prospective randomized study	38	stellate ganglion block (SGB)	complex decongestive therapy (CDT)	upper limb circumference, volume, and quality of life	no difference

33	2012	A., Loudon, et al	The effect of yoga on women with secondary arm lymphoedema from breast cancer treatment	40	Yoga exercise program	best standard care	upper limb volume, lymphoedema symptoms, quality of life, range of motion of the arm and thoracic spine, shoulder strength, and weekly and daily physical activity	favours towards intervention
34	2018	R., Kizil, et al	Is Continuous Passive Motion Effective in Patients with Lymphedema? A Randomized Controlled Trial	30	continuous passive motion (CPM) and complete decongestive therapy (CDT)	complex decongestive therapy (CDT)	upper limb volume, shoulder range pf motion (ROM) and function	no difference
35	2012	J., Bracha, et al	The immediate effect of upper arm exercise compared with lower or combined upper and lower arm exercise on arm volume reduction in6women with breast cancer related lymphedema: A randomized preliminary study	16	lower arm exercise	upper arm exercise & upper arm exercise was followed by lower arm exercise	upper limb volume	favours towards intervention
36	2016	D., Melgaard	What is the effect of treating secondary lymphedema after breast cancer with complete decongestive physiotherapy when the bandage is replaced with Kinesio Textape? - A pilot study	10	complete decongestive physiotherapy (CDP) and Kinesio Textape	complete decongestive physiotherapy (CDP) and bandage	upper limb circumference, quality of life, costs, and working environment for the physiotherapist	favours towards intervention
37	2018	N.M., Abdelhalim, et al	Comparison of extracorporeal shock waves therapy versus intermittent pneumatic compression therapy in breast cancer-related lymphedema	43	Extracorporeal shockwave therapy (ESWT)	intermittent pneumatic compression therapy (IPCT)	upper limb circumferences, skin folds thickness, and handgrip strength	favours towards intervention
38	2014	C.A., Smith, et al	A feasibility study to examine the role of acupuncture to reduce symptoms of lymphoedema after breast cancer: a randomised controlled trial	20	acupuncture	routine treatment	extracellular fluid, lymphoedema symptoms, well-being, and safety	favours towards intervention

39	2006	O., Wilburn, et al	A pilot, prospective evaluation of a novel alternative for maintenance therapy of breast cancer-associated lymphedema	10	Flexitouch™ (Pneumatic compression pump)	self-massage	upper limb volume, patient's acceptance, and quality of life	favours towards intervention
40	2019	G., Ergin, et al	Effectiveness of kinesio taping on anastomotic regions in patients with breast cancer-related lymphedema: A randomized controlled pilot study	32	Kinesio Taping (KT) and complex decongestive physiotherapy (CDP)	complex decongestive therapy (CDT)	upper limb volume	no difference
41	2013	S.H., Ridner, et al	A pilot randomized trial evaluating low-level laser therapy as an alternative treatment to manual lymphatic drainage for breast cancer-related lymphedema	46	low-level laser therapy (LLLT)	manual lymphatic drainage (MLD) or manual lymphatic drainage (MLD) and low-level laser therapy (LLLT)	upper limb volume, lymphedema symptoms, and quality of life (QOL)	no difference
42	2019	S.A., Tantawy, et al	Comparative Study Between the Effects of Kinesio Taping and Pressure Garment on Secondary Upper Extremity Lymphedema and Quality of Life Following Mastectomy: A Randomized Controlled Trial	66	Kinesiology Taping (KT)	pressure garment	upper limb circumference, Shoulder Pain and function, hand grip strength, and quality of life	favours towards intervention
43	2016	A., Loudon, et al	The effects of yoga on shoulder and spinal actions for women with breast cancer-related lymphoedema of the arm: A randomised controlled pilot study	23	Yoga exercise program	best standard care	Lumbo-pelvic posture, range of motion (ROM) in the shoulder and spine, and shoulder strength	favours towards intervention
44	2017	H.Ö., Şener, et al	Effects of clinical pilates exercises on patients developing lymphedema after breast cancer treatment: A randomized clinical trial	60	clinical Pilates exercises	standard exercises	upper limb circumferences, function, and quality of life	favours towards intervention

45	2004	L., Gothard, et al	Double-blind placebo-controlled randomised trial of vitamin E and pentoxifylline in patients with chronic arm lymphoedema and fibrosis after surgery and radiotherapy for breast cancer	68	oral dl-alpha tocopheryl acetate (Vit E) and pentoxifylline	placebo	upper limb volume	no difference
46	2007	C., Shaw, et al	Randomized controlled trial comparing a low-fat diet with a weight-reduction diet in breast cancer-related lymphedema	64	weight loss (either reduced energy intake or low-fat diet)	control	upper limb volume	favours towards intervention
47	2013	E., Jeffs, et al	Randomised controlled trial to determine the benefit of daily home-based exercise in addition to self-care in the management of breast cancer-related lymphoedema: A feasibility study	23	home-based exercise combined with standard lymphoedema self-care	standard lymphoedema self-care alone	upper limb volume, quality of life, arm function and range of shoulder movement.	favours towards intervention
48	2009	R.W.L., Lau, et al	Managing postmastectomy lymphedema with low-level laser therapy	21	low-level laser therapy (LLLT)	laser irradiation	upper limb volume and tissue resistance, joint movement, and lymphedema symptoms	favours towards intervention
49	2016	A., Petkov, et al	Improving the quality of life through effects of treatment with low intensity extremely low-frequency electrostatic field with deep oscillation¬Æ in patients with breast cancer with secondary lymphedema to patients treated with standard lymph equipment	21	lymphatic drainage with Deep Oscillation (manual lymphatic drainage)	standard lymphatic drainage	upper limb volume, pain, shoulder range of movement, movement of the neck and volume of the chest	favours towards intervention
50*	2017	S., Mestre, et al	Interest of an auto-adjustable nighttime compression sleeve (MOBIDERM¬Æ Autofit) in maintenance phase of upper limb lymphedema: the MARILYN pilot RCT	40	night-use arm sleeve (MOBIDERM® Autofit)	control	upper limb volume, quality of life, lymphedema symptoms, sleep quality and safety	favours towards intervention

51	2020	S.H., Ridner, et al	A Randomized Clinical Trial Comparing the Impact of a Web-Based Multimedia Intervention Versus an Educational Pamphlet on Patient Outcomes in Breast Cancer Survivors with Chronic Secondary Lymphedema	160	Web-based Multimedia Intervention (WBMI)	hard copy educational pamphlet	upper limb volume, function, lymphedema symptom, psychological well-being, and cost	favours towards intervention
52	2014	A., Bergmann, et al	Physiotherapy in upper limb lymphedema after breast cancer treatment: A randomized study	66	manual lymphatic drainage (MLD), skin care, bandaging and remedial exercises	skin care, bandaging and remedial exercises only	Upper limb volume	no difference
53	2010	S., Haghighat, et al	Comparing two treatment methods for post mastectomy lymphedema: Complex decongestive therapy alone and in combination with intermittent pneumatic compression	112	Modified CDT (MCDT) combined with Intermittent Pneumatic Compression (IPC)	complex decongestive therapy (CDT)	Upper limb volume	favours towards comparison
54	2019	R., Deacon, et al	Does the speed of aquatic therapy exercise alter arm volume in women with breast cancer related lymphoedema? A cross-over randomized controlled trial	18	slow aquatic exercise in the form of modified Ai Chi	conventional (faster pace) aquatic therapy	Upper limb volume	favours towards intervention
55	2012	M.F.G., Godoy, et al	Synergic effect of compression therapy and controlled active exercises using a facilitating device in the treatment of arm lymphedema	20	active exercises using this facilitating device and compression sleeve	active exercises using this facilitating device only	upper limb volume	favours towards intervention
56	2019	V., Pujol-Blaya, et al	Effectiveness of a precast adjustable compression system compared to multi-layered compression bandages in the treatment of breast cancer-related lymphoedema: a randomized, single-blind clinical trial	42	precast adjustable compression system	multi-layered compression bandages	upper limb volume and lymphedema symptoms	no difference
57	2012	M., King, et al	Compression garments versus compression bandaging in decongestive lymphatic therapy for breast cancer-related lymphedema: A randomized controlled trial	21	compression garments	compression bandaging	upper limb volume, lymphedema symptoms, and upper extremity function	favours towards comparison

58	2010	M.K., McClure, et al	Randomized controlled trial of the breast cancer recovery program for women with breast cancer-related lymphedema	49	Breast cancer recovery program (Exercise sessions + self-monitoring home program)	control	Decreased swelling, active range of motion (AROM), mood, quality of life (QoL), and adherence.	favours towards intervention
59	2010	D.S., Kim, et al	Effect of active resistive exercise on breast cancer related lymphedema: A randomized controlled trial	40	Active resistive exercise after complex decongestive physiotherapy	complex decongestive therapy (CDT)	Circumferences of the upper limbs and quality of life	favours towards intervention
60	2012	S.H., Ridner, et al	A randomized clinical trial comparing advanced pneumatic truncal, chest, and arm treatment to arm treatment only in self-care of arm lymphedema	42	truncal/chest/arm advanced pneumatic compression therapy	arm only pneumatic compression	self-reported symptoms, function, arm impedance ratios, circumference, volume, and trunk circumference	no difference
61	2016	C., Yao, et al	Effects of warm acupuncture on breast cancer related chronic lymphedema: A randomized controlled trial	30	acupuncture and moxibustion	diosmin tablets	upper limb circumferences, range of motion, quality of life, clinical safety, and adverse events.	favours towards intervention
62	2011	M.T., Ahmed Omar, et al	Treatment of post-mastectomy lymphedema with laser therapy: Double blind placebo control randomized study	50	active laser therapy	placebo	upper limb volume, shoulder mobility and hand grip strength	favours towards intervention
63	2004	Cluzan, R V, et al	Efficacy of BN165 (Ginkor Fort) in breast cancer related upper limb lymphedema: a preliminary study.	48	BN165 (Ginkor Fort)	placebo	Symptoms of discomfort, side effect and indirect functional lymphatic flow	favours towards intervention
64	2010	Tidhar, Dorit, et al	Aqua lymphatic therapy in women who suffer from breast cancer treatment-related lymphedema: a randomized controlled study.	48	Aqua lymphatic therapy (ALT)	self-massage	Safety, adherence, limb volume, and quality of life	favours towards intervention

65	2014	Letellier, Marie-Eve, et al	Breast cancer-related lymphedema: a randomized controlled pilot and feasibility study.	25	Aqua lymphatic therapy (ALT) T in addition to a home land-based exercise program	home land-based exercise program	Arm volume, arm disability, pain, and quality-of-life	favours towards intervention
66	2018	Luz, Roberta Pitta Costa, et al	Complex Therapy Physical alone or Associated with Strengthening Exercises in Patients with Lymphedema after Breast Cancer Treatment: A Controlled Clinical Trial.	42	complex physical therapy (CPT)	complex decongestive therapy (CDT)	Upper limb strength, volume, and shoulder range of motion	no difference
67	2019	Park, Myung Woo, et al	Comparison Between the Effectiveness of Complex Decongestive Therapy and Stellate Ganglion Block in Patients with Breast Cancer-Related Lymphedema: A Randomized Controlled Study.	38	stellate ganglion block (SGB)	complex decongestive therapy (CDT)	upper limb circumference, volume, and bioimpedance in the upper extremity	no difference
68	2019	Arinaga, Yoko, et al	The 10-Min Holistic Self-Care for Patients with Breast Cancer-Related Lymphedema: Pilot Randomized Controlled Study.	43	BCRL self-care program	usual care	L-Dex (the lymphedema index)	favours towards intervention
69	2019	Pasyar, Nilofar, et al	Effect of yoga exercise on the quality of life and upper extremity volume among women with breast cancer related lymphedema: A pilot study.	40	Yoga exercise program	Routine care of lymphedema clinic	Upper limb edema volume and quality of life	favours towards intervention
70	2016	Taradaj, J, et al	The influence of Kinesiology Taping on the volume of lymphoedema and manual dexterity of the upper limb in women after breast cancer treatment.	82	Kinesiology Taping (KT)	multi-layered compression bandages	Limb size, grip strength and range of motion	favours towards comparison
71*	2016	Singh, Ben, et al	Compression use during an exercise intervention and associated changes in breast cancer-related lymphedema.	41	aerobic based exercise	resistance based exercise	Lymphedema symptoms	no difference

72	2014	Pekyavaş, Nihan Özünlü, et al	Complex decongestive therapy and taping for patients with postmastectomy lymphedema: a randomized controlled study.	45	Kinesio Tape® and Complex Decongestive Therapy (CDT)	complex decongestive therapy (CDT)	Lymphedema symptoms and upper extremity circumference	favours towards intervention
73	2002	Williams, A F, et al	A randomized controlled crossover study of manual lymphatic drainage therapy in women with breast cancer-related lymphoedema.	31	manual lymphatic drainage (MLD)	simple lymphatic drainage (SLD)	Upper limb volume. Skin thickness, lymphedema symptoms and quality of life	favours towards intervention
74	2015	Singh, B, et al	Effect of compression on lymphedema during resistance exercise in women with breast cancer-related lymphedema: Randomized, cross-over trial.	25	compression with moderate load resistance exercise	moderate load resistance exercise without compression	Upper limb circumference and lymphedema symptoms	no difference
75	2020	Kilmartin, Laurie, et a	Complementary low-level laser therapy for breast cancer-related lymphedema: a pilot, double-blind, randomized, placebo-controlled study.	22	low-level laser therapy (LLLT)	placebo	lymphedema symptoms, symptom distress, and limb volume	favours towards intervention
76	2017	Rezende, Monique Silva, et al	Blood Flow Velocity in Brachial and Subclavian Vessels Immediately After Compressive Procedures for Treatment of Post cancer Therapy Lymphedema in Breast Cancer: A Randomized Blind Clinical Trial.	20	Kinesiology Taping (KT)	elastic compression and functional compressive bandaging	Blood flow	favours towards comparison
77	2012	Belmonte, Roser, et al	Efficacy of low-frequency low-intensity electrotherapy in the treatment of breast cancer-related lymphoedema: a cross-over randomized trial.	36	low-frequency low-intensity electrotherapy	manual lymphatic drainage	lymphoedema volume, pain, heaviness and tightness, and quality of life	no difference
78	2014	Loudon, Annette, et al	Yoga management of breast cancer-related lymphoedema: a randomised controlled pilot-trial.	28	Yoga exercise program	Usual care	arm volume, tissue induration, levels of sensations, pain, fatigue, and quality of life	favours towards intervention

79	2009	Szolnoky, G, et al	Intermittent pneumatic compression acts synergistically with manual lymphatic drainage in complex decongestive physiotherapy for breast cancer treatment-related lymphedema.	27	intermittent pneumatic compression pump	manual lymphatic drainage	Upper limb volume, and lymphedema symptoms	favours towards intervention
80	2012	Fife, Caroline E, et al	A randomized controlled trial comparing two types of pneumatic compression for breast cancer-related lymphedema treatment in the home.	36	Advance Pneumatic compression devices (APCD)	Standard Pneumatic compression devices (SPCD)	upper limb volume	favours towards intervention
81	2009	Damstra, Robert J, et al	Compression therapy in breast cancer-related lymphedema: A randomized, controlled comparative study of relation between volume and interface pressure changes.	36	low pressure bandages	high pressure bandages	upper limb volume, discomfort, and quality of life	favours towards intervention
82	2009	Pilch, U, et al	Influence of compression cycle time and number of sleeve chambers on upper extremity lymphedema volume reduction during intermittent pneumatic compression.	57	IPC cycle times: 90:90s	IPC cycle times: 45:15s	upper limb circumference	no difference
83	2005	Johansson, K, et al	Low intensity resistance exercise for breast cancer patients with arm lymphedema with or without compression sleeve.	31	exercise program with compression sleeve	exercise program without compression sleeve	upper limb volume	no difference
84	2010	Kasseroller, Renato G, et al	A prospective randomised study of alginate-drenched low stretch bandages as an alternative to conventional lymphologic compression bandaging.	61	alginate semi-rigid bandage	conventional low-stretch compressive bandaging	upper limb and the subjective sensations of the skin	no difference

85	2004	McNeely, Margaret L, et al	The addition of manual lymph drainage to compression therapy for breast cancer related lymphedema: a randomized controlled trial.	50	manual lymph drainage massage (MLD) with multi-layered compression bandaging (CB)	multi-layered compression bandaging (CB) alone	upper limb volume	no difference
86	2008	Jahr, Silke, et al	Effect of treatment with low-intensity and extremely low-frequency electrostatic fields (Deep Oscillation) on breast tissue and pain in patients with secondary breast lymphoedema.	21	manual lymphatic drainage (MLD) supplemented by Deep Oscillation®	manual lymphatic drainage	subjective pain, circumference, range of movement of the shoulder and the cervical spine, and breast volume	favours towards intervention
87	2002	Szuba, Andrzej, et al	Decongestive lymphatic therapy for patients with breast carcinoma-associated lymphedema. A randomized, prospective study of a role for adjunctive intermittent pneumatic compression.	23	intermittent pneumatic compression (IPC) and decongestive lymphatic therapy (DLT)	decongestive lymphatic therapy (DLT) alone	upper limb volume, elasticity of the skin, joint mobility, and safety	favours towards intervention
88	2000	Andersen, L, et al	Treatment of breast-cancer-related lymphedema with or without manual lymphatic drainage--a randomized study.	42	manual lymphatic drainage (MLD) and standard therapy	standard therapy alone	upper limb volume and lymphedema symptoms	no difference
89	2007	Shaw, Clare, et al	A randomized controlled trial of weight reduction as a treatment for breast cancer-related lymphedema.	21	dietary advice	booklet on general healthy eating	upper limb volume	favours towards intervention
90	2019	Bahtiyarca, Zeynep Tuba, et al l	The addition of self-lymphatic drainage to compression therapy instead of manual lymphatic drainage in the first phase of complex decongestive therapy for treatment of breast cancer-related lymphedema: A randomized-controlled, prospective study.	24	self-lymphatic drainage (SLD) and compression bandaging (CB)	compression bandaging	upper limb circumference, function, quality of life, and anxiety-depression	no difference

91	2017	Ha, Kyung-Jin, et al	Synergistic Effects of Proprioceptive Neuromuscular Facilitation and Manual Lymphatic Drainage in Patients with Mastectomy-Related Lymphedema.	55	Manual lymphatic drainage (MLD) and proprioceptive neuromuscular facilitation (PNF)	Manual lymphatic drainage (MLD) alone and proprioceptive neuromuscular facilitation (PNF) alone	Upper limb volume, shoulder range of motion (ROM), pain, depression, and axillary arterial blood flow	favours towards intervention
92	2019	Omar, Mohammed T A, et al	Low-Intensity Resistance Training and Compression Garment in the Management of Breast Cancer-Related Lymphedema: Single-Blinded Randomized Controlled Trial.	60	low-intensity resistance exercises	exercises and compression garment	Upper limb volume, lymphedema symptoms, shoulder mobility and function.	no difference
93	2003	McKenzie, Donald C, et al	Effect of upper extremity exercise on secondary lymphedema in breast cancer patients: a pilot study.	14	exercise program	control	Upper limb circumference, volume, and quality of life	no difference
94	2009	Tsai, Han-Ju, et al	Could Kinesio tape replace the bandage in decongestive lymphatic therapy for breast-cancer-related lymphedema? A pilot study.	41	modified decongestive lymphatic therapy (DLT) with Kinesio tape (K-tape)	standard decongestive lymphatic therapy (DLT) with bandage	upper limb circumference, lymphedema-related symptoms, quality of life, and patients' acceptance to the bandage or tape	no difference
95	2017	Osório, F, et al	Satisfaction with a therapeutic sleeve for arm lymphedema secondary to breast cancer treatment: Controlled crossover trial.	46	new compressive sleeves PRADEX®	traditional compressive sleeves	efficacy and patients' comfort	favours towards intervention
96	2018	Bao, Ting, et al	Acupuncture for breast cancer-related lymphedema: a randomized controlled trial	82	acupuncture	standard care	upper limb circumference, volume, safety, and tolerance	no difference
97	2016	Chmielewska, D D, et al	Intermittent pneumatic compression in patients with postmastectomy lymphedema	21	intermittent pneumatic compression (IPC) and exercise	intermittent pneumatic compression therapy (IPCT)	upper limb circumference and hand function	no difference

98*	2017	Mestre, S, et al	An Auto-Adjustable Night Garment to Control Early Rebound Effect of Edema Volume after Intensive Phase of Decongestive Lymphedema Therapy	40	auto adjustable autofit night arm sleeve (MOBIDERM A)	control	upper limb volume	favours towards intervention
99	2019	Johansson, K, et al	Compression Treatment of Breast Edema: A Randomized Controlled Pilot Study	56	sports bra of compression type	standard bra	Breast volume and associated symptoms and upper limb volume	no difference
100	2018	Collins, S, et al	Kinesiology taping for breast lymphoedema after breast cancer treatment: a feasibility randomised controlled trial	14	kinesiology tape (KT) and standard care	standard care	Safety and acceptability	favours towards intervention
101	2011	Kim, B H, et al	The Effect of Sodium Selenite on Breast Cancer-Related Lymphedema	40	sodium selenite with complex decongestive physiotherapy (CDPT)	complex decongestive therapy (CDT)	Upper limb volume and quality of life	favours towards intervention
102	2013	Uzkeser, H, et al	Intermittent pneumatic compression pump in upper extremity impairments of breast cancer-related lymphedema	25	intermittent pneumatic compression (IPC) and standard care	standard care	Upper limb range of movement and shoulder dysfunction	no difference

**Studies number (14 & 24 / 16 & 71 / 50 & 98) each pair of these published studies include same population and methodology protocol but measuring different outcome and/or includes subgroup analysis and analysed separately.*

#Studies 1 & 2 are surgical interventions studies, studies 3-102 are non-surgical interventions studies.

11.4 Annex 4: Risk of bias assessment tools

➢ Cochrane risk-of-bias tool for randomized trials—version 2 (RoB-2) for RCTs

The domains of bias are:

1- bias arising from the randomization process;

2- bias due to deviations from intended interventions;

3- bias due to missing outcome data;

4- bias in measurement of the outcome; and

5- bias in selection of the reported result.

Bias domains included in version 2 of the Cochrane risk-of-bias tool for randomized trials, with a summary of the issues addressed:

Bias domain	Issues addressed*
Bias arising from the randomization process	Whether: • the allocation sequence was random; • the allocation sequence was adequately concealed; • baseline differences between intervention groups suggest a problem with the randomization process.
Bias due to deviations from intended interventions	Whether: • participants were aware of their assigned intervention during the trial; • carers and people delivering the interventions were aware of participants' assigned intervention during the trial.

When the review authors' interest is in the effect of assignment to intervention:

- (if applicable) deviations from the intended intervention arose because of the experimental context (i.e. do not reflect usual practice); and, if so, whether they were unbalanced between groups and likely to have affected the outcome;
- an appropriate analysis was used to estimate the effect of assignment to intervention; and, if not, whether there was potential for a substantial impact on the result.

When the review authors' interest is in the effect of adhering to intervention:

- (if applicable) important non-protocol interventions were balanced across intervention groups;
- (if applicable) failures in implementing the intervention could have affected the outcome;
- (if applicable) study participants adhered to the assigned intervention regimen;
- (if applicable) an appropriate analysis was used to estimate the effect of adhering to the intervention.

Bias due to missing outcome data

Whether:

- data for this outcome were available for all, or nearly all, participants randomized;
- (if applicable) there was evidence that the result was not biased by missing outcome data;
- (if applicable) missingness in the outcome was likely to depend on its true value (e.g. the proportions of missing outcome data, or reasons for missing outcome data, differ between intervention groups).

Bias in measurement of the outcome	Whether: • the method of measuring the outcome was inappropriate; • measurement or ascertainment of the outcome could have differed between intervention groups; • outcome assessors were aware of the intervention received by study participants; • (if applicable) assessment of the outcome was likely to have been influenced by knowledge of intervention received.
Bias in selection of the reported result	Whether: • the trial was analysed in accordance with a pre-specified plan that was finalized before unblinded outcome data were available for analysis; • the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple outcome measurements within the outcome domain; • the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple analyses of the data.

➢ ROBIS tool for SRs

The tool is completed in three phases: (1) assess relevance (optional), (2) identify concerns with the review process, and (3) judge risk of bias in the review.

Summary of phase 2 ROBIS domains, phase 3, and signaling questions:

	Phase 2				Phase 3
	1. Study eligibility criteria	**2. Identification and selection of studies**	**3. Data collection and study appraisal**	**4. Synthesis and findings**	**Risk of bias in the review**
Signaling questions	1.1 Did the review adhere to predefined objectives and eligibility criteria?	2.1 Did the search include an appropriate range of databases/ electronic sources for published and unpublished reports?	3.1. Were efforts made to minimize error in data collection?	4.1. Did the synthesis include all studies that it should?	A. Did the interpretation of findings address all of the concerns identified in domains 1 to 4?
	1.2 Were the eligibility criteria appropriate for the review question?	2.2 Were methods additional to database searching used to identify relevant reports?	3.2. Were sufficient study characteristics available for both review authors and readers to be able to interpret the results?	4.2. Were all predefined analyses reported or departures explained?	B. Was the relevance of identified studies to the review's research question appropriately considered?
	1.3 Were eligibility criteria unambiguous?	2.3 Were the terms and structure of the search strategy likely to retrieve as many eligible studies as possible?	3.3. Were all relevant study results collected for use in the synthesis?	4.3. Was the synthesis appropriate given the nature and similarity in the research questions, study designs, and outcomes across included studies?	C. Did the reviewers avoid emphasizing results on the basis of their statistical significance?
	1.4 Were all restrictions in eligibility criteria based on study characteristics appropriate?	2.4 Were restrictions based on date, publication format, or language appropriate?	3.4. Was risk of bias (or methodologic quality) formally assessed using appropriate criteria?	4.4. Was between-study variation minimal or addressed in the synthesis?	
	1.5 Were any restrictions in eligibility criteria based on sources of information appropriate?	2.5 Were efforts made to minimize error in selection of studies?	3.5. Were efforts made to minimize error in risk of bias assessment?	4.5. Were the findings robust, for example, as demonstrated through funnel plot or sensitivity analyses? 4.6. Were biases in primary studies minimal or addressed in the synthesis?	
Judgment	Concerns regarding specification of study eligibility criteria	Concerns regarding methods used to identify and/or select studies	Concerns regarding methods used to collect data and appraise studies	Concerns regarding the synthesis	Risk of bias in the review

11.5 Annex 5: Insights from our team's lymphedema publications

- Campos JL, Pons G, Rodriguez E, Al-Sakkaf AM, Vela FJ, Pires L, et al. Popliteal Vascular Lymph Node Resection in the Rabbit Hindlimb for Secondary Lymphedema Induction. J Vis Exp. 2022; 30, (189). DOI: 10.3791/64576

- Campos JL, Pires L, Vela FJ, Pons G, Al-Sakkaf AM, Sánchez-Margallo FM. Lymphaticovenous anastomosis in rabbits: A novel live experimental animal model for supermicrosurgical training. JPRAS. 2024. DOI: 10.1016/j.bjps.2024.04.023

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Popliteal Vascular Lymph Node Resection in the Rabbit Hindlimb for Secondary Lymphedema Induction

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Campos, J.L., Pons, G., Rodriguez, E., Al-Sakkaf, A.M., Vela, F.J., Pires, L., Jara, M.J., Sánchez-Margallo, F.M., Abellán, E., Masiá, J. Popliteal Vascular Lymph Node Resection in the Rabbit Hindlimb for Secondary Lymphedema Induction. *J. Vis. Exp.* (189), e64576, doi:10.3791/64576 (2022).

Date Published

November 30, 2022

DOI

10.3791/64576

URL

jove.com/video/64576

Abstract

Lymphedema is a common condition often associated with cancer and its treatment, which leads to damage to the lymphatic system, and current treatments are mostly palliative rather than curative. Its high incidence among oncologic patients indicates the need to study both normal lymphatic function and pathologic dysfunction. To reproduce chronic lymphedema, it is necessary to choose a suitable experimental animal. Attempts to establish animal models are limited by the regenerative capacity of the lymphatic system. Among the potential candidates, the rabbit hindlimb is easy to handle and extrapolate to the human clinical scenario, making it advantageous. In addition, the size of this species allows for better selection of lymphatic vessels for vascularized lymph node resection.

In this study, we present a procedure of vascular lymph node resection in the rabbit hindlimb for inducing secondary lymphedema. Anesthetized animals were subjected to circumferential measurement, patent blue V infiltration, and indocyanine green lymphography (ICG-L) using real-time near-infrared fluorescence, a technique that allows the identification of single popliteal nodes and lymphatic channels. Access to the identified structures is achieved by excising the popliteal node and ligating the medial and lateral afferent lymphatics. Special care must be taken to ensure that any lymphatic vessel that joins the femoral lymphatic system within the thigh without entering the popliteal node can be identified and ligated.

Postoperative evaluation was performed at 3, 6, and 12 months after induction using circumferential measurements of the hindlimb and ICG-L. As demonstrated during follow-up, the animals developed dermal backflow that was maintained until the 12th month, making this experimental animal useful for novel long-term evaluations in the management of lymphedema. In conclusion, the approach described here is

feasible and reproducible. Additionally, during the time window presented, it can be representative of human lymphedema, thus providing a useful research tool.

Introduction

Lymphedema is a chronic condition that deserves special attention, owing to its worldwide incidence, lack of curative and standardized treatment, and serious impact on patients' quality of life[1,2].

In developed countries, lymphedema is mainly acquired and is secondary to breast cancer, owing to the high prevalence of this malignancy; the cumulative incidence of breast cancer-related lymphedema 10 years after surgery can reach up to 41.1%[3]. However, diseases such as melanoma, gynecological cancers, genitourinary tumors, and head and neck neoplasms are also associated with a high incidence of this disease[4]. Regional lymph node resection, as part of the necessary oncological treatment to increase survival rates, leads to the disruption of functional lymphatic drainage. In some cases, this results in compensatory mechanisms that prevent or delay the onset of lymphedema[5]. However, when chemotherapy and radiotherapy are administered, these mechanisms are not able to compensate for the change, and lymphedema resultantly occurs. This has a negative impact on patients' quality of life, affecting their functional, social, and psychological well-being[6,7].

The need for an effective cure for lymphedema requires understanding of the physiopathology of the lymphatic system, as well as a deep insight into the complex cellular mechanisms and their responses in both normal and dysfunctional lymphatic systems[8,9,10]. Such insights can be obtained initially from experimental animal models that can reproduce chronic human diseases[11].

Many attempts have been made to replicate lymphedema in experimental animal models; however, most of them have been hindered by some limitations, including the inability to reproduce chronic lymphatic insufficiency in a stable animal model, the costs of the study, and most importantly, the great regenerative capacity of the lymphatic system, which enables it to restore circulation[12,13].

This study presents the experimental approach for surgically inducing stable acquired lymphedema using the rabbit hindlimb. Based on literature review, this animal can be considered optimal for the development of lymphedema because of the consistent anatomy of its hindlimb lymphatic system, which includes a single popliteal node that drains the hindlimb and reaches the main femoral lymphatic system in the leg[14,15].

The specific anatomy of the rabbit's hindlimb allows for the reproduction of the surgical procedures performed in humans to induce secondary lymphedema. Therefore, this procedure can be used for microsurgical training and preclinical research to extrapolate the results to human medicine.

Protocol

All procedures were approved by the ethical committee of the Jesús Usón Minimally Invasive Surgery Center and the welfare guidelines of the regional government, which are based on European legislation.

1. Presurgical and surgical preparation

1. House nine female New Zealand white rabbits weighing 4-4.5 kg and aged 4 months in separate cages maintained at a temperature of 22-25 °C, with free access to food and water. Make sure that the cages contain a polysulfone tray with a surface area of 3 m^2 and a height of 40 cm, as well as a bed with wood shavings.
    1. Identify the cages with the project code and animal identification number.
    2. Acclimate the animals for 1 week before surgery to prevent stress-induced problems. Collect preoperative laboratory values of blood samples to ensure that each animal is in good health prior to anesthesia.
2. Ensure all rabbits follow a 12 h fast before each surgical procedure.
    1. After premedication, preoxygenate the rabbits using a face mask (Hall mask) for 5 min with 100% oxygen and a fresh gas flow of 3-5 L/min. Perform the co-induction phase with midazolam (0.3 mg/kg) and propofol (10 mg/kg) intravenously.
3. Intubate the rabbits with 3.0-3.5 endotracheal tubes, with pneumotaponation, connected to a semi-closed circular circuit linked to a ventilator with a flow of fresh gases at 1 L/min for an initial 5 min, and subsequently set at 0.5 L/min.
    1. Perform maintenance anesthesia by inhalation of sevoflurane at a concentration of 3%-3.5% set on the vaporizer.
4. Administer a continuous infusion of Ringer's lactate solution (2-4 mL/kg/h) through the marginal vein of the ear to the anesthetized rabbits throughout the surgical procedure.
    1. Use a protective eye ointment to protect the ocular surface.
5. General anesthesia monitoring: use a rectal thermometer to monitor the temperature at 38.7-39.7 °C, inspect the mucous membrane color, and monitor the O_2saturation at >95% and the heart rate at 180-240 bpm using a rabbit pulse oximeter.
6. Place a thermal support so the animal maintains a constant temperature throughout the procedure.
7. Administer ketorolac (1.5 mg/kg) plus tramadol (3 mg/kg) intravenously for intraoperative analgesia.
8. Administer antibiotics (7.5 mg/(kg·day) enrofloxacin subcutaneously [s.c.]) before surgery and 5 days after surgery, as well as postoperative analgesia (10 µg/(kg·day) buprenorphine s.c.) for 5 days.
9. Place the rabbits in the supine position and shave the animal's hindlimb and inguinal areas. Place the animal in dorsal/supine recumbency and clip the hair from the hindlimb and inguinal areas.
10. Perform skin antisepsis by applying 0.5% chlorhexidine and 70% ethanol to the previously shaved skin. Once the area has been disinfected, cover the rabbit with a sterile cloth, except for the left hindlimb.

2. Popliteal vascular lymph node resection surgery (Figure 1)

1. Infiltrate 0.2-0.3 mL of indocyanine green (ICG) intradermally in the second and third interdigital spaces of the left hindlimb. Massage, gently flex, and extend the hindlimb for a few minutes to facilitate the uptake of the

dye into the lymphatic vessels. Use the contralateral limb as a control.

2. Use a real-time, near-infrared fluorescence camera to visualize and mark (using a surgical marker) the lymphatic vessels crossing at the knee level and the popliteal lymph node (PLN) on the skin (**Figure 2**).

3. Inject patent blue V (0.2 mL) into the interdigital area for subsequent identification of the lymphatic vessels and lymph nodes.

4. Once the PLN is identified using a real-time, near-infrared fluorescence camera (**Figure 3**), create a 2 cm incision in the center of the popliteal fossa, longitudinal to the long axis of the hindlimb through the ischial vein, which is visible through the skin.

    1. To obtain real-time panoramic images of the lymphatic system with the real-time, near-infrared fluorescence camera, use the optical head equipped with a class 1 laser as the excitation light source and a near-infrared sensitive camera, from the ankle to the knee of the hindlimb of the animal.

    2. Visualize the lymphatic vessels above the muscle fascia by resecting the subcutaneous fat[5]. The lymphatic vessels appear blue due to the patent blue V staining in step 2.3.

    3. Use microsurgical forceps to stretch the incision and expose the PLN, including the vascular and afferent lymphatic pedicles. Ensure clear visibility of all the lymphatic and vascular structures (**Figure 4**).

    4. Identify the PLN, with a diameter of 0.8 mm, under the ischial vein and between the biceps femoris and medial hamstring muscles.

5. Identify the two main lymphatic vessels on the medial aspect of the PLN. These vessels are located parallel to the distal saphenous vein and divide into a network of microvessels as they approach the PLN (**Figure 5**).

6. Dissect the lymph node pedicle while avoiding damage to the surrounding tissues and vessels (**Figure 6**).

7. Ligate the medial artery (a branch of the popliteal artery) and the lateral saphenous vein distally and proximally using 10/0 nylon non-absorbable sutures.

8. Identify and cauterize the two groups of afferent lymphatic vessels that directly join the femoral lymphatic system within the thigh, but do not enter the PLN (**Figure 7**).

    NOTE: The first group corresponds to the medial afferent lymphatic vessels that drain lymph from the upper leg and calf. The second group is composed of lymphatic vessels in the lower extremity musculature. These vessels run along the gastrocnemius muscle, together with the saphenous vein.

9. Confirm complete disruption of the lymphatic system by repeating real-time near-infrared fluorescence imaging.

10. Remove surrounding fatty tissue entirely to avoid possible lymphangiogenesis.

11. Suture the skin incision with 4-0 polyglycolic acid (PGA) absorbable braided sutures (with a 16 mm 3/8 triangular needle) using a continuous intradermal pattern to avoid postoperative auto-mutilation.

12. House the rabbits individually in cages after surgery; keep them under surveillance and at a room temperature between 16 and 22 °C.

3. Postoperative evaluation

1. Perform postoperative assessments at 3, 6, and 12 months after induction.
2. Anesthetize the rabbits following the steps used previously (steps 1.2-1.7).
3. Measure the perimeters of the hindlimbs of the anesthetized rabbits with a tape measure. Take measurements every 2 cm, with the first point being at the ankle and the last at the knee. Calculate the total volume using the truncated cone formula.
4. Use indocyanine green lymphography (ICG-L) for assessing lymphatic function.
    1. Infiltrate 0.2-0.3 mL of ICG intradermally into the second and third interdigital spaces and gently massage for 1 min to facilitate ICG uptake into the lymphatic vessels.
5. Collect images after 15 min using the near-infrared fluorescence system to assess dermal backflow.
6. Once the follow-ups have been completed, euthanize the rabbit following the same anesthetic protocol as that used in the intervention. Once the desired anesthetic plane is achieved, administer intravenous potassium chloride into the auricular vein at an average rate of 2 meq/kg.

Representative Results

Nine rabbits underwent lymphedema induction in this study, however, three rabbits died during the immediate postoperative period and could not be evaluated. Study data were obtained at 3, 6, and 12 months postoperatively by three independent researchers. Circumferential hindlimb measurements and ICG-L were performed under general anesthesia to assess the lymphatic system function and dermal backflow.

The data obtained by ICG-L at 3 months postoperatively showed dermal backflow and the absence of PLNs in the left hindlimbs of the rabbits, compatible with the disruption of normal lymphatic system function. ICG-L results at 6 and 12 months postoperatively showed dermal backflow and dilated lymphatic channels distal to the popliteal fossa during surgical exploration (**Figure 8**).

No evidence of clinical lymphedema was found upon examination or circumferential hindlimb measurements. The increase in the volume of the left hindlimb was not significant ($p < 0.05$) compared to that of the right hindlimb (control) (**Figure 9**). All results obtained from the left hindlimb were compared with those obtained from the right hindlimb of the same animal, which was used as the negative control.

In the described surgical procedure, after PLN resection and ICG application, no compromised blood flow was observed in the hindlimb, no alteration in vital parameters was reported during anesthesia, and no pain or lameness was described. All parameters were assessed according to the guidelines for quantifying pain, stress, and distress in laboratory animals[16,17,18,19].

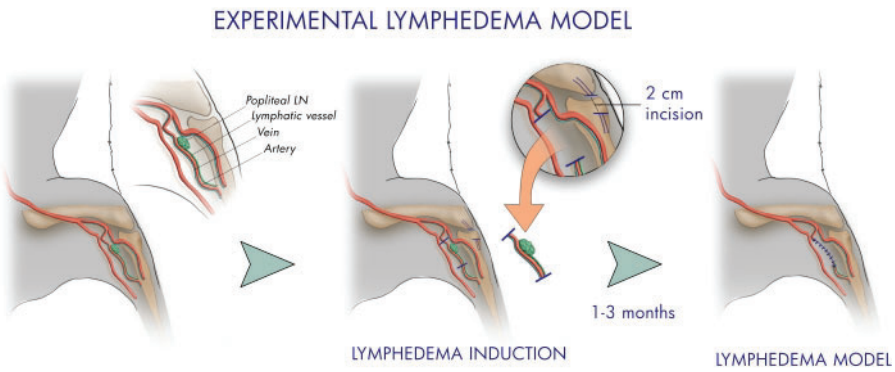

Figure 1: Experimental surgical procedure for the induction of lymphedema. Detailed protocol for the creation of a vascular lymph node resection in the left hindlimb of the rabbit for the induction of secondary lymphedema. Abbreviations: LN = lymph node; VLN = vascular lymph node. Please click here to view a larger version of this figure.

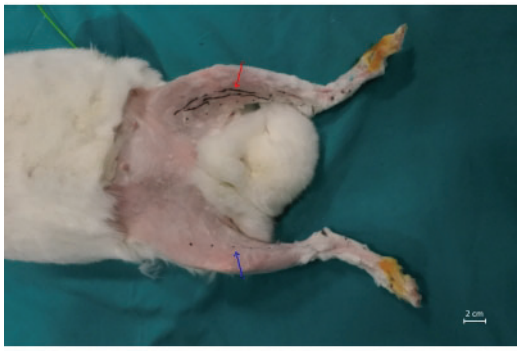

Figure 2: Preoperative markings of rabbit hindlimbs. Skin marks on the left hindlimb show the measurement points every 2 cm (blue arrow) and a healthy lymphatic system drawn with a surgical marker (red arrow) observed by ICG-L. Scale bar = 2 cm. Abbreviation: ICG-L = indocyanine green lymphography. Please click here to view a larger version of this figure.

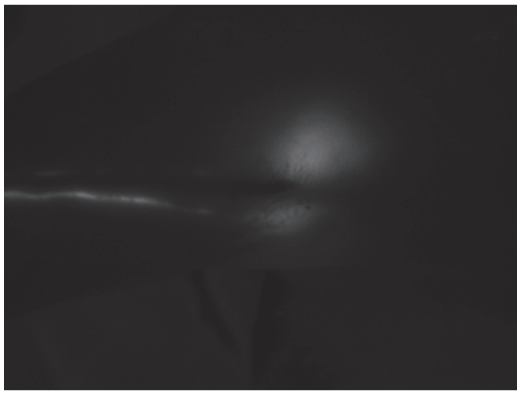

Figure 3: Preoperative ICG-L showing the popliteal lymph node and afferent lymphatic vessels from the rabbit hindlimb. Abbreviation: ICG-L = indocyanine green lymphography. Please click here to view a larger version of this figure.

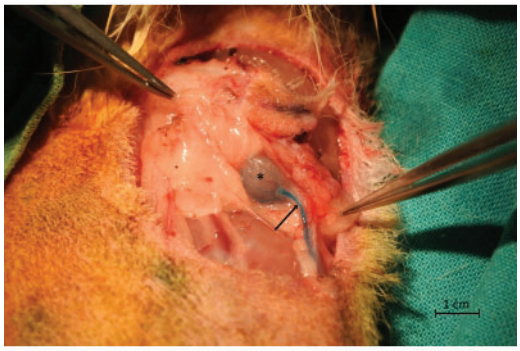

Figure 4: Lymphatic and vascular structures. Popliteal lymph node (asterisk) with an afferent lymphatic vessel (arrow). Please click here to view a larger version of this figure.

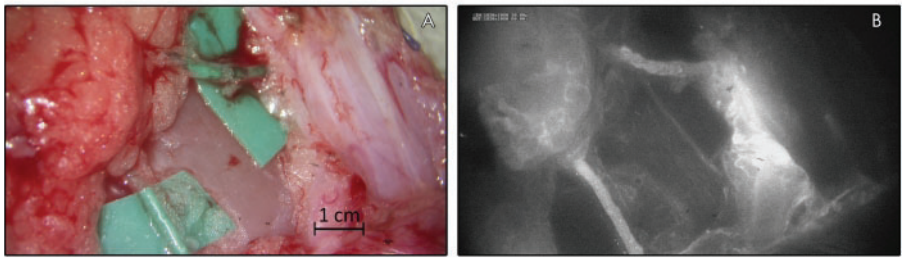

Figure 5: Principal lymph vessels. (**A**) Identification of the two main lymphatic vessels running parallel to the saphenous vein distal to the PLN by surgical microscopy without ICG. (**B**) Identification of the two main lymphatic vessels running parallel to the saphenous vein distal to the PLN with ICG. Scale bar = 1 cm. Abbreviations: PLN = popliteal lymph node; ICG = indocyanine green. Please click here to view a larger version of this figure.

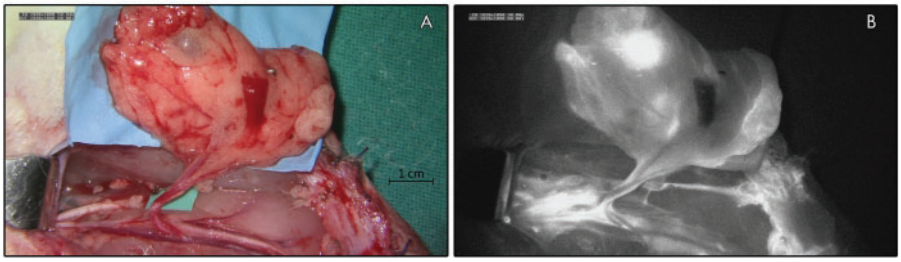

Figure 6: PLN pedicle. (**A**) Identification of the PLN pedicle by surgical microscopy without ICG. (**B**) Identification of the PLN pedicle with ICG. Scale bar = 1 cm. Abbreviations: PLN = popliteal lymph node; ICG = indocyanine green. Please click here to view a larger version of this figure.

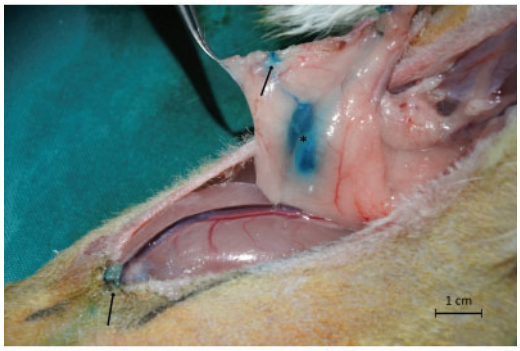

Figure 7: Two groups of afferent lymphatic vessels that do not enter the PLN but directly join the femoral lymphatic system within the thigh. Popliteal lymph node (asterisk) with an afferent lymphatic vessel transected (arrows). Scale bar = 1 cm. Please click here to view a larger version of this figure.

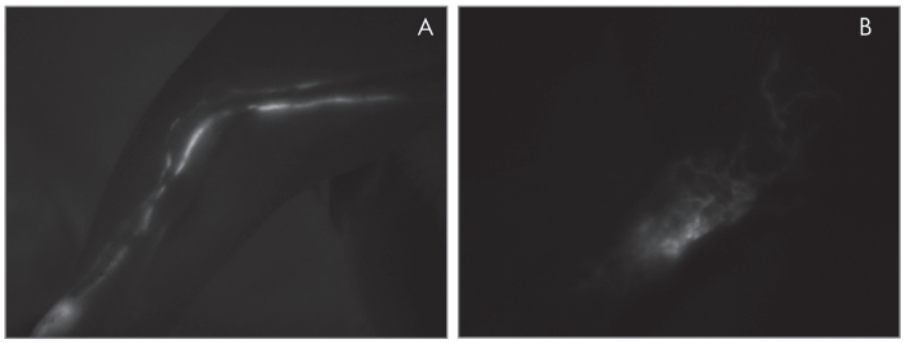

Figure 8: Preoperative and postoperative ICG-L. (**A**) Preoperative ICG-L showing healthy linear lymphatics in the hindlimb.**(B**) Postoperative ICG-L showing dermal backflow pattern in the rabbit lymphedematous hindlimb. Abbreviation: ICG-L = indocyanine green lymphography. Please click here to view a larger version of this figure.

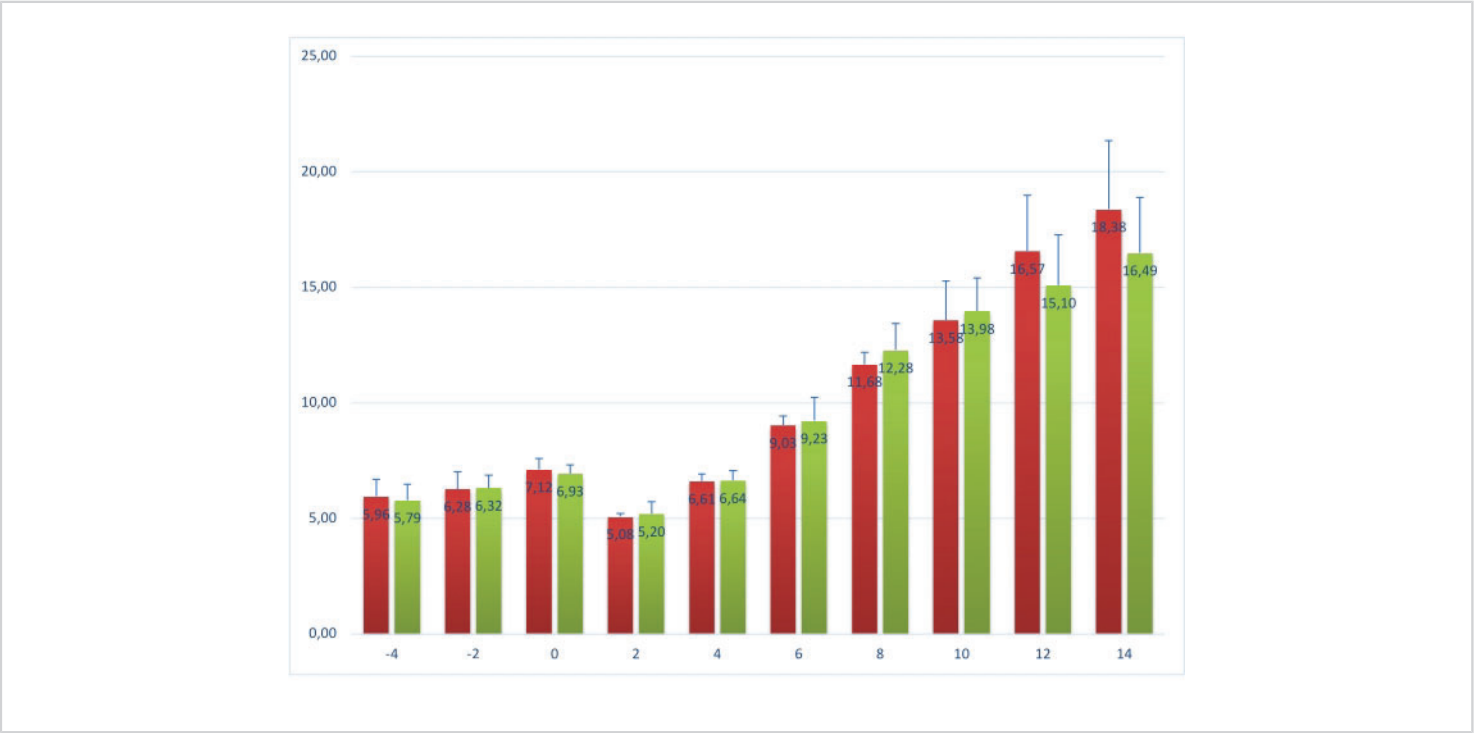

Figure 9: Circumferential hindlimb measurements. The red column chart corresponds to the right hindlimb measurements, while the green column chart corresponds to the left hindlimb measurements. The increase in volume of the left hindlimb was not significant ($p < 0.05$) compared to the control right hindlimb. Please click here to view a larger version of this figure.

Discussion

Resection of the PLN in an experimental animal is a relatively new procedure that can induce secondary lymphedema in the limbs for assessment and study. After lymph node resection, there is a period of alteration of lymphatic system functionality, lymph accumulation, and histological changes of lymphatic vessels that appear dilated. When this lymph accumulation reaches adequate levels, the characteristic dermal backflow of lymphedema, similar to that observed in humans, can be observed using objective techniques such as ICG-L. The development of this technique may create a cost-effective preclinical research animal, which may help prevent and treat this pathophysiological process.

Intraoperatively, patent blue V application can identify the presence or absence of flow through the lymphatic vessels of the hindlimbs[5]. The disadvantage of this technique is that the lymphatic vessels can only be observed intraoperatively. Therefore, a real-time near-infrared fluorescence camera technology is used, which is an excellent method that allows surgeons to identify, map, and quantify lymph flow in the lymphatic channels of the application site without the need for surgery. Nowadays, this technology is considered the gold standard for lymphedema diagnosis.

In this study, all lymphatic vessels that were observed and identified by ICG and patent blue V were patent. After resection of the PLN, the left hindlimbs presented dermal

backflow at 3, 6, and 12 months after induction and linear functional lymphatic vessels were not present. This predicted good lymph stasis in the left hindlimb during the total study period. These results suggest the successful creation of secondary lymphedema in an experimental animal over a longer period than in previous studies[5]. However, this technique has its limitations. To obtain reliable results, all vascular and lymphatic structures of the PLN must be ligated and removed, as well as the surrounding fatty tissue, to avoid possible lymphangiogenesis. In addition, because of the physiology of the lymphatic system in rabbits[15,20], no evidence of clinical lymphedema was demonstrated upon examination or circumferential hindlimb measurements, in contrast to other studies using the same experimental animal[5].

A real-time near-infrared fluorescence camera was used during follow-up. This technology, with its high sensitivity, represents a valuable technique for real-time mapping of lymphatic vessels and lymph nodes, as verified here. Patent blue V is more widely used but is less specific and limited by the fact that lymphatic vessels can only be observed under intraoperative microscopic vision, whereas ICG allows the detection of these same vessels and lymph nodes without the need for any surgical intervention.

The present study demonstrated that the rabbit is a reliable, reproducible, and affordable experimental animal for the surgical induction of lymphedema. The consistent lymphatic system anatomy of the hindlimbs with a unique PLN permits the induction of long-term and stable lymphedema. This makes the rabbit a useful animal for both lymphedema experimentation and research.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgments

This research project was performed at the Jesús Usón Minimally Invasive Surgery Center (CCMIJU), which is part of the ICTS Nanbiosis. The study was performed with the assistance of the following Nanbiosis units: U21, experimental operating room, and U22, animal housing. This work was supported by Hospital de la Santa Creu i Sant Pau. This work has been partially funded by the Junta de Extremadura, the European Regional Development Fund (Grant Number GR21201). The funder played a role in the study design, data collection, analysis, decision to publish, and manuscript preparation. Special thanks are extended to María Pérez for preparing the figures and to the Microsurgery Department of JUMISC for providing constant encouragement.

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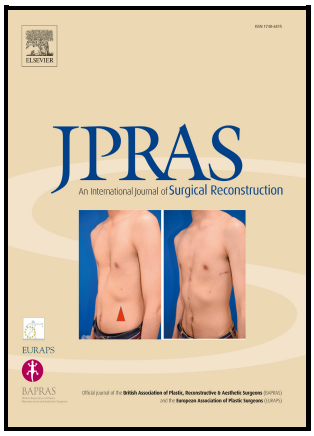
Lymphaticovenous anastomosis in rabbits: A novel live experimental animal model for supermicrosurgical training



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PII: S1748-6815(24)00209-2

DOI: https://doi.org/10.1016/j.bjps.2024.04.023

Reference: PRAS8911

To appear in: *Journal of Plastic, Reconstructive & Aesthetic Surgery*

Received date: 5 December 2023
Accepted date: 5 April 2024

Please cite this article as: José Luis Campos, Laura Pires, Francisco Javier Vela, Gemma Pons, Ali M. Al-Sakkaf, Francisco Miguel Sánchez-Margallo, Elena Abellán and Jaume Masiá, Lymphaticovenous anastomosis in rabbits: A novel live experimental animal model for supermicrosurgical training, *Journal of Plastic, Reconstructive & Aesthetic Surgery,* (2024) doi:https://doi.org/10.1016/j.bjps.2024.04.023

Lymphaticovenous anastomosis in rabbits: A novel live experimental animal model for supermicrosurgical training

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Previous presentations:

None

Summary

Background: Lymphaticovenous anastomosis is widely used in lymphedema management. Although its effectiveness in reducing edema in patients can be clinically

observed, evaluating the long-term outcomes of this technique can be complex. This study established an animal model to assess the lymphaticovenous anastomosis technique at 15- and 30-days post-surgery, utilizing indocyanine green lymphography, Patent Blue V, and histopathological examination.

Methods: An experimental model was established in the hindlimbs of ten rabbits using the popliteal vein and afferent lymphatic vessels in the popliteal area. The subjects were divided into two groups: the first (n=5) underwent patency assessment at 0 and 15 days, and the second (n=5) at 0 and 30 days, resulting in 20 anastomoses. Patency was verified at 0, 15, and 30 days using indocyanine green and Patent Blue V. Histopathological examinations were performed on the collected anastomosis samples.

Results: The patency rate was 90% (19/20) initially, 60% (6/10) at 15 days post-surgery, and 80% (8/10) at 30 days. The average diameter of lymphatic vessels and veins was 1.0 mm and 0.8 mm, respectively. The median number of collateral veins was three; the median surgical time was 65.8 min. Histopathology revealed minimal endothelial damage and inflammatory responses due to the surgical sutures, with vascular inflammation and thrombosis in a single case. Local vascular neoformations were observed.

Conclusion: This study highlights the reliability and reproducibility of rabbits as an experimental model for training in lymphaticovenous anastomosis technique due to the accessibility of the surgical site and the dimensions of their popliteal vasculature.

Keywords

lymphedema; supermicrosurgery; lymphaticovenous anastomosis; rabbit animal model

Introduction

Lymphedema is a chronic pathological condition characterized by the extensive stagnation of lymphatic fluid, inflammatory responses, and deposition of fibroadipose tissue.[1] Progressive accumulation of adipose tissue and fibrotic changes in the affected area lead to a noticeable increase in limb volume, a sensation of heaviness, impaired functionality, heightened vulnerability to infections, and secondary tumor development.[2] These factors are detrimental to patients' overall quality of life, negatively affecting their functional capabilities, social interactions, and psychological well-being.[3,4]

The conventional management of lymphedema relies on conservative and nonsurgical interventions.[5,6] Nonetheless, the implementation of microsurgical techniques such as lymphaticovenous anastomosis (LVA) and vascularized lymph node transfer has demonstrated notable efficacy in reducing limb volume, mitigating cellulitis risk, reducing reliance on compression garments, and minimizing the need for cellulitis treatment.[6,7]

Several studies have attempted replicating LVA in live experimental animal models to facilitate surgeon training. However, to our knowledge, only a few training models have been reported for LVA. Most procedures conducted in live experimental animal models involve the hindlimbs of dogs,[8,9] the connection between saphenous lymphatic vessels and saphenous veins in pigs,[10] and the linking of peritoneal lymph ducts with iliolumbar veins,[11–13] as well as saphenous lymphatic vessels with saphenous veins in rats.[14,15]

Our previous investigations show rabbits are optimal candidates for inducing secondary lymphedema and performing surgical procedures, including popliteal lymph node excision.[16,17] The distinctive anatomical attributes of the rabbit hindlimb render it amenable to the faithful reproduction of surgical techniques practiced in human patients.[18] Consequently, rabbits are frequently used as models for microsurgical training and preclinical investigations, thereby facilitating the extrapolation of research findings to human medicine.[19,20] This study aimed to implement an experimental methodology for the surgical application of the LVA technique using lymphatic vessels and popliteal veins located in the popliteal region of rabbit hindlimbs. This methodology aims to establish a training platform for surgeons in the field of supermicrosurgery.

Materials and methods

After obtaining approval from the institutional review board, this study was conducted at the Jesús Usón Minimally Invasive Surgery Center (Cáceres, Spain). All parameters were assessed according to guidelines for quantifying pain, stress, and distress in laboratory animals.[21–24]

This investigation was designed to assess and implement a supermicrosurgical training methodology for LVA in an animal model. The study was conducted using a cohort of ten healthy one-year-old male rabbits (New Zealand White rabbits; Granja San Bernardo, Tulebras, Navarra, Spain), in which surgical procedures were performed on both hindlimbs. The study population was randomly divided into two groups using Microsoft Excel® Microsoft 365 MSO (Version 2207 Build 16.0.15427.20248), each comprising five animals, resulting in ten LVAs within each group. Indocyanine green

(ICG) lymphography was used to establish the anastomotic site and evaluate the patency of the LVA within the popliteal fossa of both hindlimbs. For each animal, two digital video cameras equipped with an infrared filter (Pentero 800s [ZEISS, Jena, Germany] and Fluobeam [Fluoptics, Grenoble, France]) were used to capture and record the ICG contrast (25 mg; Verdye; Diagnostic Green Limited, Westmeath, Ireland) (Figure 1). Patent Blue V (PBV) (2.5 g/100 mL; Bleu Patente V Sodique; Guerbet, Villepinte, France) was used to visualize the lymphatic vessels intraoperatively in the anastomosis zone. Supermicrosurgical instruments were used for all surgical procedures (EMI Set A-supermicro; Mitaka Europe Gmbh, Kurfürstendamm, Berlin, Germany). Follow-up, euthanasia, and sample collection procedures were performed at 15- and 30-day post-surgery. Two independent researchers collected the data.

The co-induction phase of anesthesia was initiated by administering midazolam (5 mg/mL; Normon SA, Madrid, Spain) at a dose of 0.5 mg/kg and propofol (10 mg/mL; Propomitor; Orion Pharma, Spoo, Finland) at a dose of 10 mg/kg via infusion into the ear marginal vein. All rabbits were subsequently intubated using 3.0–3.5 endotracheal tubes connected to a semi-closed circuit, maintaining sevoflurane (1000 mg/g; SevoFlo; Zoetis Belgium, Luvain-la-Neuve, Belgium) at a concentration of 3.9–4.5%. During the intraoperative phase, analgesia was achieved by administering ketorolac (30 mg/mL; Normon SA) at a dose of 1.5 mg/kg and tramadol (50 mg/mL; Normon SA) at a dose of 3 mg/kg.

In the postoperative period, buprenorphine (300 μg/mL; Bupaq; Richter Pharma, Wels, Austria) was administered at a dose of 30 mg/kg, along with meloxicam (5 mg/mL; Meloxidyl; Ceva Santé Animale, Libourne, France) at a dose of 2 mg/kg and

enrofloxacin (10 mg/mL; Baytril; Bayer Animal Health Gmbh, Leverkusen, Germany) at a dose of 5 mg/kg, and continued for five days post-surgery. An anesthetic protocol identical to that described previously was employed during the 15- and 30-day postoperative follow-up assessments. Euthanasia was performed using intravenous potassium chloride (20 mmol/10 mL; B. Braun Medical SA, Barcelona, Spain) at an average rate of 2 mEq/kg into the auricular vein.

Before surgery, the study subjects were placed in the prone position, and meticulous hair depilation of their hindlimbs up to the groin region was performed, followed by skin antisepsis. To visualize the vessels and lymph node, we administered 0.2 mL of PBV intradermally into the second and third interdigital spaces of both hindlimbs. After injection, the area was gently massaged, and controlled flexion and extension movements of the hindlimbs were performed for a few minutes to facilitate dye absorption into the lymphatic vessels. Open surgery was performed in the popliteal fossa to identify the popliteal vein and afferent lymphatic vessels leading to the popliteal lymph nodes. These two vascular structures were meticulously dissected from the arterial and collateral venous networks, and their diameters were measured using a surgical ruler. The distal end of the lymphatic vessel was ligated to prevent unintentional leakage of ICG and PBV during subsequent surgical assessments. A comparable precaution was applied to the proximal end of the vein to ensure readiness for anastomosis.

The lymphatic vessel and popliteal veins were cut, and LVAs were performed under a surgical microscope, facilitating the unidirectional flow of lymph from the distal lymphatic vessel to the proximal popliteal vein. End-to-end anastomosis was

meticulously performed using six or eight 11-0 monofilament nylon non-absorbable sutures (Covidien; Mansfield, MA, USA) (Figure 2). ICG lymphography and PBV were used to evaluate the lymphatic fluid flow from the lymphatic vessel to the popliteal vein and to confirm the patency of the anastomosis. The skin incision was sutured using 4-0 polyglycolic acid absorbable sutures (Aragó; Barcelona, Spain) in a continuous intradermal pattern and a single-stitch technique to mitigate the risk of postoperative self-mutilation.

Follow-up examinations were conducted at 15- and 30-day postoperatively. A longitudinal incision was made at the same anatomical site as in the initial surgical procedure, enabling the assessment of anastomotic patency via ICG lymphography and PBV (Figures 3 and 4). Following data acquisition, samples of the LVAs were harvested for subsequent histopathological analysis, and the subjects were euthanized using ethical procedures.

Hematoxylin and eosin staining was used to histologically examine the region approximately 0.5 cm from the anastomotic site. Histological sections were digitally scanned using the Aperio GT 450 DX system (Leica Biosystems, Barcelona, Spain). The assessed parameters included endothelial loss, thrombosis, superficial fibrin deposition, presence of fibrin within the neointima or media, calcification, semi-quantitative appraisal of neointimal thickness at the anastomotic regions, neoangiogenesis (evaluated semi-quantitatively), and the extent and distribution of inflammation.

Results

Ten rabbits were divided into two groups to undergo the supermicrosurgical LVA technique in both hindlimbs, totaling 20 LVAs. The initial group, comprising five rabbits, underwent euthanasia 15 days post-surgery. The second group, comprising five rabbits, was euthanized after 30 days.

In the described supermicrosurgical procedure, following the execution of the LVA technique, there were no apparent impairments in hindlimb blood perfusion, no deviations in vital physiological parameters recorded under anesthesia, and no pain or lameness.

The mean weight of the rabbits was 5.3 (range, 4.5–6.0) kg. The mean diameters of the 20 lymphatic vessels and veins were 1.0 (range, 0.6–1.5) mm and 0.8 (range, 0.3–2.0) mm, respectively. The median number of collateral veins within the primary vein was three (range, 0–6). The initial postoperative patency rate for the LVAs was 90% (19/20 patients). The patency rates 15 and 30 days after surgery were 60% (6/10) and 80% (8/10), respectively. The mean time from the initial cutaneous incision to the evaluation of LVA patency was 65.8 (range, 45–180) min (Table 1).

During both follow-up evaluations, we observed the progressive formation of fibrotic tissue encircling the anastomotic sites, rendering visual inspection of the sutures challenging. One of the subjects engaged in self-mutilation at the incision site, resulting in the unavailability of LVA 15 days post-surgery.

Histological analysis

Mild endothelial loss was evident in three cases, without any discernible presence of fibrin in the de-endothelialized regions (Figure 5). Thrombosis or superficial fibrin deposition was observed in a single case (Figure 6). None of the samples exhibited calcification, and the neointimal thickness was less than that of the medial wall. Neoangiogenesis was uniform in all samples, displaying varying degrees of intensity, generally ranging between six and ten neoformed vascular structures (Figure 7). Signs of inflammation ranging from mild to moderate were observed in all cases. In addition, lymphatic valves were present, and the structure of the lymphatic ducts closely approximated normalcy.

Discussion

Attaining proficiency in microsurgery is imperative to achieving expertise as a skilled microsurgeon. Numerous publications have addressed microsurgery training, providing valuable insights and strategies for improving technical skills in this specialized field.[25] Conventional training programs focusing on acquiring technical microsurgical skills employ silicone tubes and animal models, including chicken vessels and rat femoral arteries or veins.[26–28] These models provide valuable hands-on experience and are effective tools for honing microsurgical techniques and developing the dexterity and precision required in this demanding field.

The diameter of the lymphatic vessels involved in LVA procedures is usually <1 mm, requiring advanced skills compared to conventional microvascular anastomosis.[29] Supermicrosurgery of lymphatic vessels presents distinct and challenging characteristics compared to microsurgery.[30] Transparent lymphatic vessels pose difficulties in defining the boundary between the adventitia and surrounding connective tissue.[31] Additionally,

the soft and delicate nature of lymphatic ducts makes their management challenging, necessitating the adoption of an atraumatic surgical technique.[32]

Utilizing the LVA surgical technique in live experimental animal models is a novel approach with the potential to provide surgical training for surgeons. Advancements in this technique have the potential to establish a preclinical model, aiding in the prevention and treatment of pathophysiological processes such as lymphedema.[33]

Various authors have documented the use of animal models to conduct LVA, specifically in dogs,[8,9] pigs,[10] and rats.[11–15] Despite instances in which the diameters of lymphatic vessels and veins in these animal models resemble those in humans,[11] there are several drawbacks regarding their management and housing. Among these, rat models have been extensively investigated. Rat models are affordable and easily housed; however, any surgical intervention performed on these small experimental animals carries inherent risks to their survival, attributable to the surgical procedure and anesthesia. Furthermore, there is a potential risk of self-mutilation at the surgical site and challenges associated with postoperative monitoring.

We believe that the use of rabbits as training animal models surpasses other options because of their optimal average size for handling and housing.[34] Moreover, rabbits possess anatomical characteristics that allow the replication of surgical techniques performed in humans.[16,18] In particular, despite the presence of distinct superficial afferent lymphatic vessels in the right and left hindlimbs of rabbits, the deep vascular bundle within the popliteal fossa, comprising the popliteal artery, popliteal vein, and afferent lymphatic vessels, is a favorable site for LVA. This site features larger

lymphatic vessels and offers convenient access facilitated by excising the subcutaneous fat, without necessitating dissection of adjacent muscle tissue. Furthermore, there are no major vascular structures nearby at risk of injury, thereby avoiding potential risks to the animal's life. Additionally, postoperative follow-ups can be conducted, as these procedures pose minimal risk to the rabbits' lives and exhibit remarkable healing capacity.

The mean diameter of the lymphatic vessels and popliteal veins in this particular model was 1.0 mm and 0.8 mm, respectively. When comparing these calibers with other animal models, the mean diameter of lymphatic vessels in rats ranges from 0.61 mm to 0.24 mm.[11–15] In comparison, it was approximately 1 mm in pigs.[10] Unfortunately, no available data exist regarding the diameters of the lymphatic vessels and veins in dogs.[8,9] Regarding the veins, our observations indicate that the mean diameter in rats ranges from 0.37 mm to 0.81 mm.[11–15] Regrettably, there is a dearth of data on vein diameter in pigs. In comparison, our animal model exhibited a larger lymphatic vessel/venous size than the previously mentioned animal models, facilitating vascular dissection and rendering it more manageable even for inexperienced surgeons. This provides a significant opportunity for refining skills in dissecting lymphatic vessels, veins, and arteries.

During the surgical procedure, PBV was used to assess the presence or absence of flow within the hindlimb lymphatic vessels.[35] However, this technique has limitations because it can only be performed intraoperatively. To overcome this limitation, we utilized real-time near-infrared fluorescence imaging technology, a superior method for identifying, mapping, and quantifying lymphatic flow in the lymphatic channels at the

ICG application site without requiring invasive surgical intervention.[36] This innovative approach enables real-time visualization of lymphatic dynamics and provides valuable insights for research and clinical applications.

Following the anastomosis, a patency test was conducted to evaluate the presence of countercurrent lymph flow in the LVA region, thereby determining the success or failure of the procedure. Subsequently, the patency of the anastomosis was confirmed using ICG lymphography. Furthermore, the use of PBV enabled direct visual observation of the flow of the dye from the lymphatic vessel to the popliteal vein. Regarding the duration of the anastomosis procedure, the average time from the first incision to the patency evaluation was 65.8 min, consistent with the findings of earlier investigations.[11,12,15]

We observed a smooth endothelial transition without exposure of the subendothelial tissue layers in cases where permeability was achieved. Conversely, in the three impermeable samples, endothelial loss was observed without fibrin. Thrombosis and fibrin in the medial or neointimal layer were identified in only one non-permeable sample. At the same time, inflammation was observed in all cases, primarily due to the local suture thread reaction. Collectively, these factors appear to be significant contributors to long-term patency.

Our findings demonstrated favorable lymphatic patency in the hindlimbs of rabbits throughout the study period following the implementation of LVA procedures. These results indicate the promising establishment of an experimental animal model suitable for training purposes.

This study had some limitations. This technique requires live animals and should be minimized by ethical considerations.[37] From an ethical standpoint, employing this model during the advanced stages of training after practice with ex vivo supermicrosurgery models or inert materials is preferable.[26,38–40] Another limitation inherent to this model is the variability in the observed diameters of veins and lymphatic vessels; notably, in some instances, the afferent lymphatic vessel exhibits a larger diameter than the popliteal vein, thereby introducing heightened complexity into the surgical procedure.

In conclusion, this study provides reasonable evidence that rabbits are a reliable and reproducible experimental animal model for performing LVA. The anatomy of the lymphatic system of the popliteal area of the hindlimb enables the practice of supermicrosurgical techniques and the improvement of dissection skills owing to the comparatively larger size of vascular structures compared to alternative animal models. Furthermore, it provides a surgical field readily accessible by excising the subcutaneous fat layer without necessitating entry into the musculature of the hindlimb.

Ethics

All experimental procedures conducted in this study were approved by the Ethics Committee of the Jesús Usón Minimally Invasive Surgery Center (Cáceres, Spain) and the Government of the Junta de Extremadura (EXP-20231219), in accordance with European legislation.

Funding

This work has been funded by the Jesús Usón Minimally Invasive Surgery Center.

Conflict of interest statement

None.

Acknowledgments

We express our sincere appreciation to the teams of the Microsurgery Department, Anesthesia Department, and operating theatre technicians at JUMISC for their unwavering support and motivation. We are grateful to Maria Perez for her valuable contributions to figure preparation.

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Figure legends

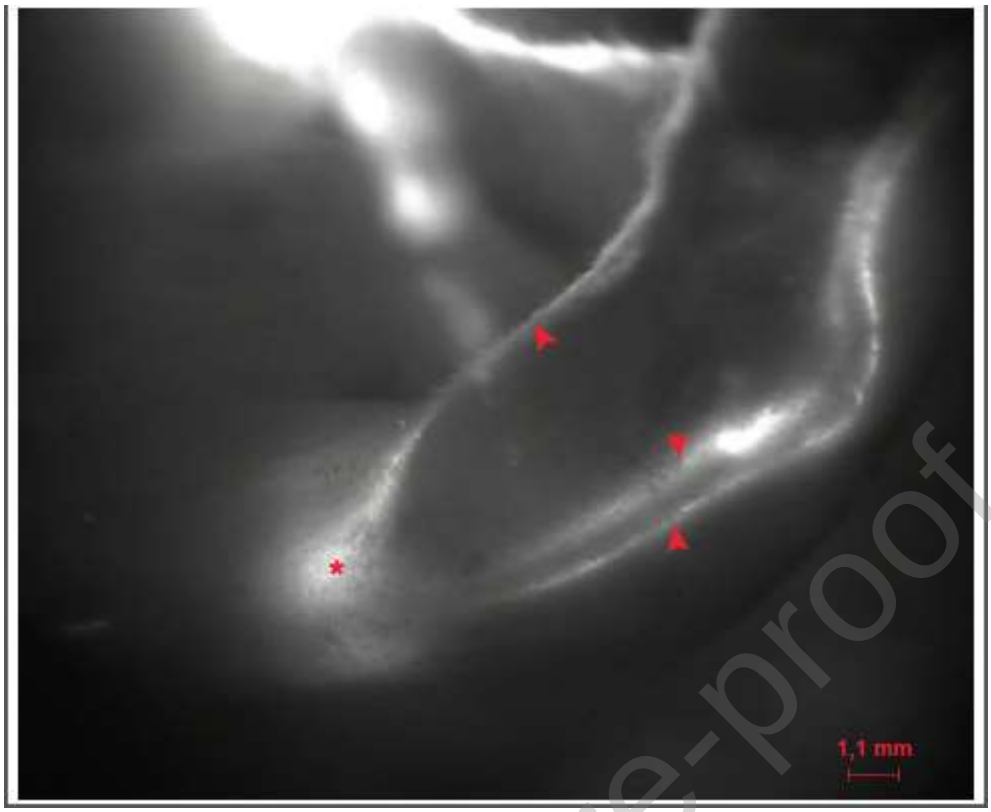

Figure 1. Preoperative findings in a rabbit model before lymphaticovenous anastomosis. Viability of the popliteal lymph node (red asterisks) and hindlimb lymphatic vessels (red arrowheads) was confirmed via ICG lymphography.

ICG, indocyanine green

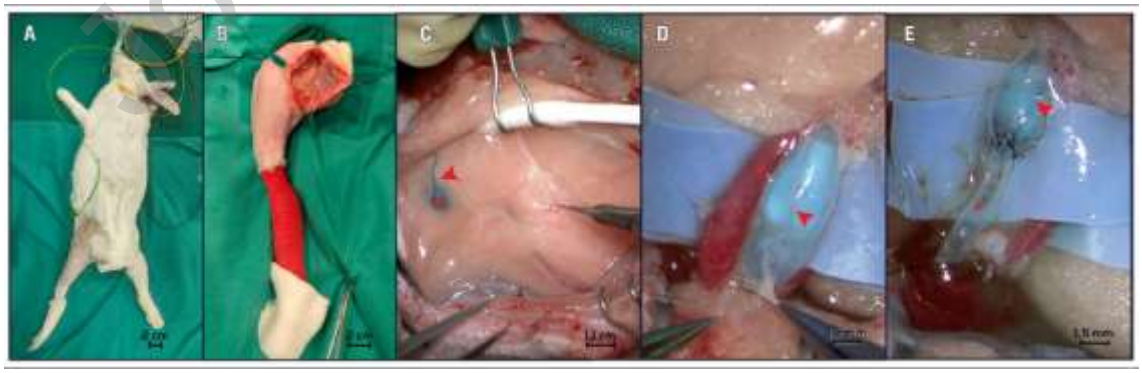

Figure 2. A, Positioning and general anesthesia of the rabbit with the left hindlimb shaved preoperatively. B, Surgical approach in the popliteal area. C, Identification of

the popliteal lymph node (red arrowheads) using PBV. D, Popliteal vein (red asterisks) and afferent lymphatic vessel (red arrowheads) identification using PBV. E, Lymphaticovenous anastomosis was performed between the afferent lymphatic vessel (red arrowheads) and the popliteal vein (red asterisks) using the end-to-end technique with single stitches. Patency was assessed using PBV.

PBV, Patent Blue V

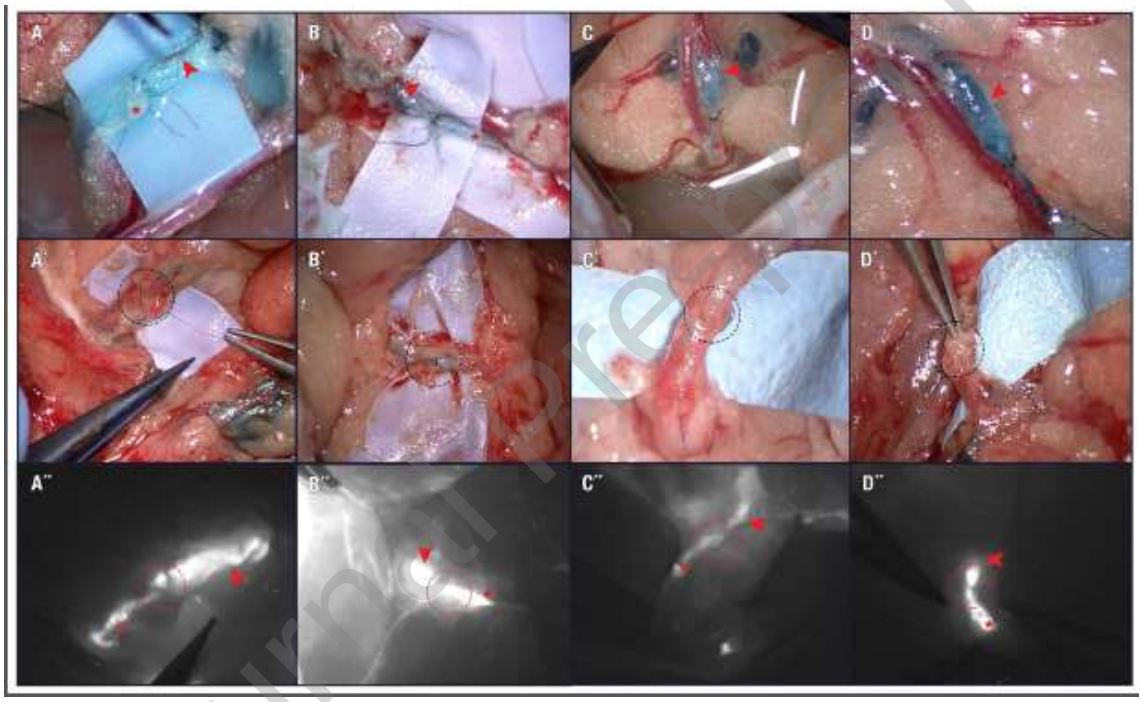

Figure 3. Follow-up and anastomotic patency assessment at 15 days post-surgery using ICG and PBV. A, Patent lymphaticovenous anastomosis confirmed by PBV on day 0. The afferent lymphatic vessel (red arrowheads) and the popliteal vein (red asterisks) are visible. A', Lymphaticovenous anastomoses observed under a surgical microscope 15 days post-surgery. Scar tissue is evident surrounding the anastomotic site (black circle). A'', Assessment of anastomotic patency using ICG (red circle). Lymphatic fluid is observed flowing from the lymphatic vessel (red arrowheads) into the vein (red asterisks).

ICG, indocyanine green; PBV, Patent Blue V

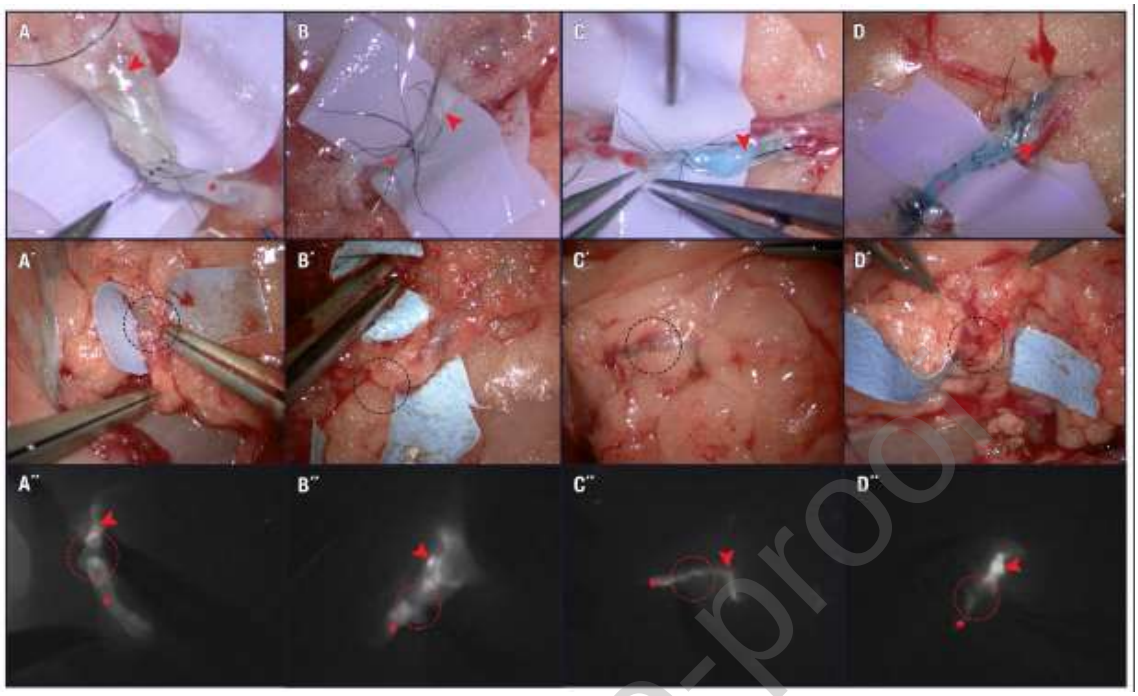

Figure 4. Follow-up and anastomotic patency assessment at 30 days post-surgery using ICG and PBV. A, Patent lymphaticovenous anastomosis confirmed by PBV on day 0. The afferent lymphatic vessel (red arrowheads) and the popliteal vein (red asterisks) are visible. A', Lymphaticovenous anastomoses observed under surgical microscope 30 days post-surgery. Scar tissue is evident surrounding the anastomotic site (black circle). A", Assessment of anastomotic patency using ICG (red circle). Lymphatic fluid is observed flowing from the lymphatic vessel (red arrowheads) into the popliteal vein (red asterisks).

ICG, indocyanine green; PBV, Patent Blue V

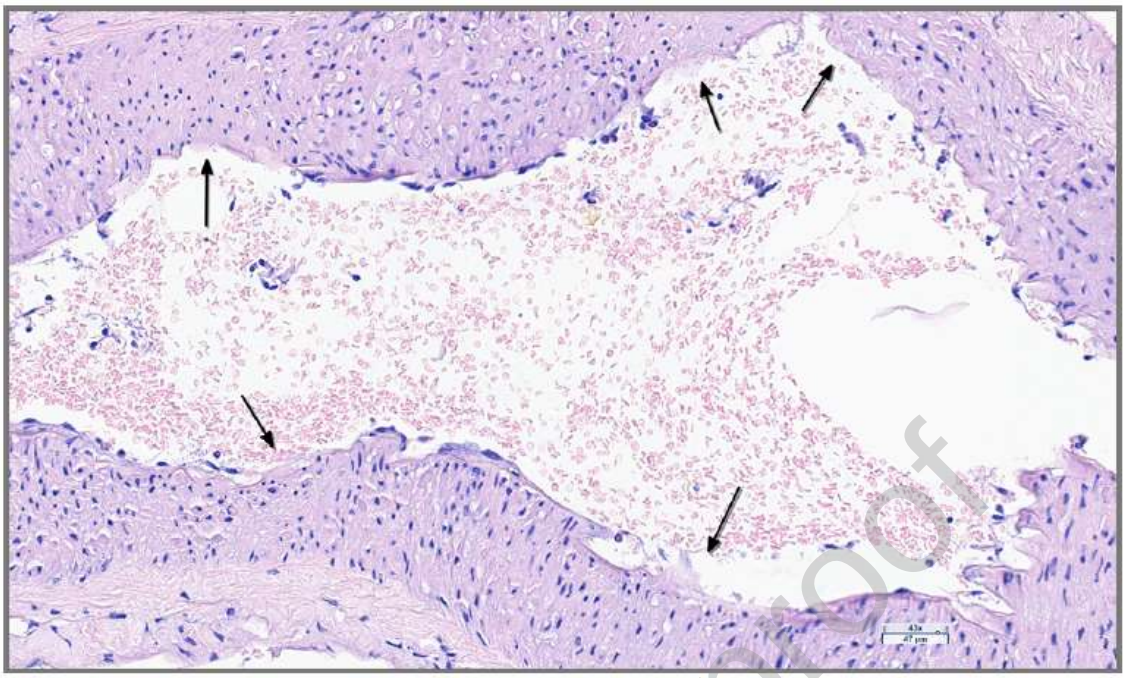

Figure 5. Cross-sections of the lymphatic vessel in a patent case (hematoxylin and eosin staining) reveal a mild area of endothelial loss (black arrowheads).

Figure 6. Cross-sections of the lymphatic vessel (hematoxylin and eosin staining) show vascular thrombosis with associated inflammation.

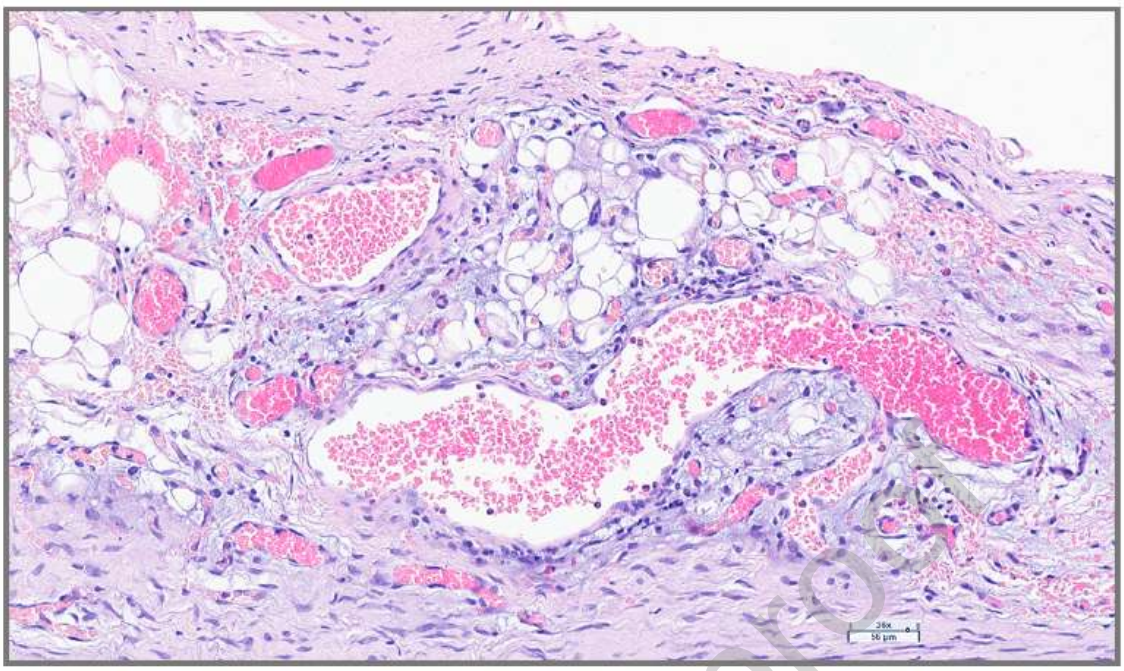

Figure 7. Digital microscopic image (Aperio GT 450 DX system) displaying neoangiogenesis in all cases, typically with 6-10 newly formed vascular structures.

Tables

Table 1 Description of the experimental rabbit lymphaticovenous anastomosis model

Animal number	Right hindlimb lymphatic vessel (mm)	Left hindlimb lymphatic vessel (mm)	Right hindlimb vein (mm)	Left hindlimb vein (mm)	Right anastomotic patency (0/15/30)	Left anastomotic patency (0/15/30)
1	1.5	1.5	0.5	2	⊘/⊖/×	⊖/⊖/×
2	1	2	0.5	1.5	⊘/⊖/×	⊘/⊘/×
3	1.2	1.5	0.8	0.9	⊘/⊘/×	⊘/⊘/×
4	1	1.2	0.8	1	⊘/⊘/×	⊘/⊖/×
5	1	1.1	1.5	0.5	⊘/⊘/×	⊘/⊘/×
6	0.6	0.6	0.9	0.5	⊘/×/⊖	⊘/×/⊘

7	0.7	1	0.6	1.2	⊘/×/⊘	⊘/×/⊖
8	0.9	0.9	0.3	0.3	⊘/×/⊘	⊘/×/⊘
9	0.7	0.7	0.3	0.9	⊘/×/⊘	⊘/×/⊘
10	1	0.9	0.6	0.3	⊘/×/⊘	⊘/×/⊘
Main	1.0		0.8		90/60/80 (%)	

LVA, lymphaticovenous anastomosis; 0, immediately after LVA; 15, 15 days later; 30, one month later; ⊘, permeable LVA; ⊖, non-permeable LVA; ×, no follow-up.

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