Trials

STUDY PROTOCOL

Open Access

Topical tacrolimus for the amelioration of breast cancer-related lymphedema (TACLE Trial): a study protocol for a randomized, double-blind, placebo-controlled phase II/III trial

Frederik Gulmark Hansen[1,2*], Mads Gustaf Jørgensen[1,2], Jørn Bo Thomsen[1,2] and Jens Ahm Sørensen[1,2]

Abstract

Background Breast cancer-related lymphedema is a chronic condition affecting 15–30% of breast cancer patients, resulting from treatment-related inflammation and fibrosis primarily mediated by CD4 + T-cells. Tacrolimus, an immunomodulator, has shown efficacy in reducing lymphedema in both animal models and an initial clinical trial. This study aims to validate these findings in a larger cohort, hypothesizing that tacrolimus will reduce lymphedema volume, fibrosis, and fluid retention while improving quality of life.

Methods This multicenter, double-blinded, randomized placebo-controlled trial will enroll 80 women with breast cancer-related lymphedema stages I and II. Participants will be randomized 1:1 to receive either 0.1% tacrolimus ointment or a placebo ointment for 12 months. Primary outcome will be the change in lymphedema volume measured at baseline, 6 months, and 12 months. Secondary outcomes include quality of life assessed via SF- 36, DASH, and LYMPH-Q Upper Extremity questionnaires; lymphedema index via bioimpedance spectroscopy; lymphatic function and flow via indocyanine green lymphangiography; and skin fibrosis measurement. Assessments will take place at baseline, 3, 6, 9, and 12 months.

Discussion This trial will provide robust data on the efficacy of topical tacrolimus in reducing BCRL volume and improving patient quality of life. Positive results could establish tacrolimus as a standard treatment for BCRL, potentially enhancing clinical outcomes for affected patients. The findings will also contribute to understanding the role of immunomodulation in lymphedema management.

Trial registration This trial is registered with the EU Clinical Trials Information System (CTIS) under EU CT Number: 2023–503644 - 13–00 (approved 16.05.2024) and ClinicalTrials.gov under identifier NCT06306274 (registered 12.03.2024).

Keywords Breast cancer-related lymphedema, Breast cancer, Tacrolimus, Inflammation, Quality of life, Randomized controlled trial

*Correspondence:
Frederik Gulmark Hansen
frederik.c.gulmark.hansen@rsyd.dk
Full list of author information is available at the end of the article

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	Topical tacrolimus for the amelioration of breast cancer-related lymphedema (TACLE Trial): a study protocol for a randomized, double-blind, placebo-controlled phase II/III trial
Trial registration {2a and 2b}	EU CT Number: 2023–503644-13–00 ClinicalTrials.gov: NCT06306274
Protocol version {3}	Version 1.5, 22.04.24.
Funding {4}	Danish Cancer Society Danish Cancer Research Fund Lægeforeningens Forskningsfond Overlægerådets Forskningsfond Dagmar Marshalls Fond Simon Spies Fonden Else og Mogens Wedell Wedellsborgs Fond Tømrerhandler Vilhelm Bangs Fond
Author details {5a}	Frederik Gulmark Hansen[1,2] Mads Gustaf Jørgensen[1,2] Jørn Bo Thomsen[1,2] Jens Ahm Sørensen[1,2] 1Research Unit for Plastic Surgery, Odense University Hospital, Odense, Denmark [2]Clinical Institute, University of Southern Denmark, Odense, Denmark
Name and contact information for the trial sponsor {5b}	Sponsor, Jens Ahm Sørensen, MD, Professor, PhD Department of Plastic Surgery, Odense University Hospital Odense C, Denmark T: +45 6541 2436 e-mail: jens.sorensen@rsyd.dk
Role of sponsor {5c}	Sponsor has played a significant role in the study design, collection, management, analysis, and interpretation of data, as well as the writing of the report and the decision to submit the report for publication. As the main supervisor of the project and the leader of the research unit, the sponsor has ultimate authority over most aspects of the study.

Introduction

Background and rationale {6a}

Breast cancer-related lymphedema (BCRL) is a feared and life-long sequelae to breast cancer treatment affecting 15–30% of patients treated for breast cancer, depending on the treatment [1]. Symptoms of BCRL include limb swelling, pain, trophic skin changes, feeling of heaviness, restricted range of motion, and emotional distress, which all compromise activities of daily living and quality of life (QoL) [2]. BCRL is caused by an insufficiency of the lymphatic capillaries which leads to disruption of lymphatic flow when the lymphatic load exceeds the transport capacity in the lymphatic vessels. It leads to retention and accumulation of excess water containing filtered plasma proteins, extravascular blood cells, and stromal cell products in the axilla and limb, which results in an inflammation process that ultimately culminates in fibrosis and deposition of adipose tissue [3].

Lymphedema can be staged according to International Society of Lymphology (ISL) [4]. Stage 0, subclinical lymphedema. A latent phase where swelling is not apparent despite an impaired lymphatic transport. Swelling emerges in stage 1 due to accumulation of protein-rich fluid but subsides with limb elevation. Pitting may be present. The swelling is more permanent in stage II and do not subside with limb elevation. Pitting is present in the early stage II and subcutaneous fat and fibrosis is formed in the later stage II. Stage III is lymphostatic elephantiasis and is accompanied by trophic skin changes and continued fibroadipose tissue formation. Major risk factors for the development of BCRL include lymphadenectomy, radiation therapy, and higher BMI (> 25) [5, 6].

Conservative management of BCRL includes complete decongestive therapy (CDT), manual lymphatic drainage (MLD), and compression therapy [3, 4]. Surgical treatment modalities such as lymphovenous anastomosis and lymph node transplantation are currently used to treat BCRL; however, the evidence on the effect is sparse [7]. Ultimately, no definite curative treatment of BCRL is available [4].

The literature suggests that CD4 + T-cells play a critical role in the development of BCRL by promoting inflammation and fibrosis and by inhibiting lymphangiogenesis through the production of interferon-γ, interleukin- 4, and transforming growth factor-β1 [8, 9]. Furthermore, a correlation between the infiltration of CD4 + T-cells and the degree of fibrosis and severity of lymphedema have been shown [10, 11].

Topical tacrolimus is an immunomodulating macrolide used in the treatment of multiple skin diseases, including atopic dermatitis, vitiligo, and psoriasis [12–14]. Tacrolimus inhibits calcineurin's phosphatase activity in T-cells, which ultimately leads to decreased T-cell activation, proliferation, and differentiation [15].

A trial conducted on mice with induced lymphedema treated with topical tacrolimus showed a decrease in lymphedema volume as well as reduced inflammatory infiltration, reduced degree of fibrosis, and increased

lymphangiogenesis and lymphatic function after treatment with topical tacrolimus [16]. Based on this trial, we conducted a clinical trial where 18 females with BCRL stage I and II (ISL) were treated with topical tacrolimus for 6 months [17]. The results from this trial showed a significant volume reduction, decrease in excess fluid, and an increase in QoL. The trial design proved to be feasible and topical tacrolimus proved to be safe in the patients.

Based on the promising results from these trials, we now want to conduct a randomized placebo-controlled trial to confirm these findings. We hypothesize that tacrolimus ointment reduces lymphedema volume, degree of fibrosis, and excessive fluid and that the treatment increases QoL in the patients.

Objectives {7}

The objective of this trial is to evaluate and confirm the previously observed effect of topical tacrolimus on stage I and II breast cancer-related lymphedema (BCRL). Specifically, we aim to compare the impact of tacrolimus ointment to a placebo ointment on various parameters including lymphedema volume, QoL, lymphatic flow and function, skin fibrosis, and excess water accumulation in the affected limb. The primary endpoint, assessed at the 12-month follow-up, focuses on achieving a minimum of 10% reduction in lymphedema volume, a threshold based on findings from our pilot trial [17], where a similar reduction was observed alongside improved QoL. While volume reduction is an important objective measure, we acknowledge that it does not necessarily correlate directly with QoL improvement [18]. We hypothesize that this volume reduction will lead to a significant improvement in both subjective and objective symptoms associated with lymphedema. Additionally, we anticipate that patients may experience a reduction in the need for other concomitant lymphedema treatment modalities.

Trial design {8}

The trial is designed as a multicenter, double-blinded, randomized placebo-controlled superiority trial with two parallel groups and a primary endpoint of lymphedema volume. Randomization will be performed as block randomization with a 1:1 allocation. The population of the trial will include of women with BCRL ISL stage I or II. A total of 80 patients will be included. The patients will be treated with tacrolimus ointment or placebo once daily for 6 months followed by a maintenance period of 2 weekly applications for 6 months. Patients will be assessed at baseline and after 6 and 12 months.

Methods: participants, interventions and outcomes

Study setting {9}

The patients will have a 12-month follow-up period at either of the centers: Department of Plastic Surgery, Odense University Hospital, Denmark; Department of General- and Plastic Surgery, Lillebaelt Hospital, Vejle, Denmark; or Department of Plastic- and Breast Surgery, Zealand University Hospital, Roskilde, Denmark.

Eligibility criteria {10}

Inclusion criteria

Patients eligible for the trial must comply with the following: female, age ≥ 18 years, BCRL ISL stage I or II, pitting edema, postmenopausal or use of contraceptive drugs,[1] healthy opposite arm, L-Dex score ≥ 10, lymphedema volume[2] > 10% of healthy arm, comprehension of Danish. Patients who meet any of the following must be excluded from the trial: pregnant,[3] breast-feeding, or aiming to conceive within the next year, bilateral breast cancer, contralateral lymphadenectomy, allergy to tacrolimus, macrolides, or iodine, pacemaker known kidney or liver disease, defect skin-barrier on the affected arm, diagnosed immunodeficiency or treated with immunosuppressive medicine, no previous surgical treatment for lymphedema.

Who will take informed consent? {26a}

Patients referred to us or who contact us will receive both oral and written information about the project, along with an assessment of their eligibility criteria. Written information will be included in the summons letter for their first appointment, where eligibility criteria will also be assessed. Before any assessment takes place, the principal investigator (PI) or another designated medical doctor will provide written and oral information to the patients. Patients will be informed of their right to 24 h of consideration time before deciding whether they wish to participate. Participants will be informed of their right to withdraw from the study at any time without consequence. Clear procedures for withdrawal will be outlined in both written materials and during the informed consent process. Participants can express their desire to withdraw verbally or in writing to the research team. Any further participation in the study will cease.

[1] Absence of menstruation in at least 12 consecutive months or continuous usage of contraceptive drugs (Spiral, birth-control pills, implant, transdermal patches, vaginal ring, or depot injection).

[2] Lymphedema volume: difference in volume between the lymphedema arm and the healthy arm; measured with water displacement volumetry.

[3] At inclusion, a pregnancy test will used to ensure that patients of childbearing age are not pregnant.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Not applicable. There is no collection of data or biological specimens for ancillary studies.

Interventions

Explanation for the choice of comparators {6b}

In our previous study, tacrolimus was tested without a comparator to assess feasibility and initial efficacy in reducing BCRL. The study provided promising results but lacked a comparator to definitively determine the effect of tacrolimus. To gain certainty on the potential efficacy of tacrolimus, we have chosen to use a placebo ointment as the comparator in this trial.

The placebo ointment has been specifically developed to mimic the appearance, texture, and application of tacrolimus ointment, ensuring that any observed effects can be attributed to the active ingredient rather than the vehicle or the application process. The use of a placebo comparator is essential to eliminate bias and establish a clear understanding of tacrolimus's impact compared to no active treatment.

Intervention description {11a}

Included patients will be treated with either the investigational medicinal product (IMP) tacrolimus ointment (0.1% tacrolimus) or placebo ointment for 12 months. The concentration of 0.1% is based on our pilot trial, which showed a clinically meaningful reduction in lymphedema volume and improved quality of life with minimal adverse effects. This concentration has a well-established safety profile in dermatological use, with low systemic absorption (< 1.0 ng/mL), and a preclinical study supports its efficacy in reducing inflammation and fibrosis. A lower dose was not considered due to uncertainty about its effectiveness. Topical tacrolimus is currently solely approved in the treatment of atopic dermatitis by the Danish Medicines Agency; thus, this intervention will be an off-label treatment. The patients will be instructed how to apply the ointment in a thin layer covering the axilla, arm, and hand once daily throughout the trial. This regime leans towards the current recommendations for treatment of atopic dermatitis and already has an established safety profile. Participants will be instructed to not to use compression-sleeves or any other compression treatment until 2 h after application; avoid direct sun exposure to the applicated site until 2 h after application and minimize exposure from the sun during treatment; avoid ultraviolet (UV) light from tanning beds and treatment with UV-A or UV-B light in combination with psoralens (PUVA); use sunscreen on the application site and/or to stay in the shade to avoid direct sun exposure; and not to use emollient cream on the application site 2 h before and 2 h after tacrolimus application; fertile females on contraceptive drugs are recommended to continue the use of contraceptives for at least 3 weeks after last treatment.

Criteria for discontinuing or modifying allocated interventions {11b}

If a patient experience unexpected adverse reactions out of this period, PI and sponsor will decide if the patients should pause the treatment for 7 days or if they should discontinue the treatment. Treatment with tacrolimus ointment is in general well-tolerated and is not expected to have an increased risk of serious adverse events due to the low systemic absorption of tacrolimus at this dosage (< 1.0 ng/mL).

Strategies to improve adherence to interventions {11c}

The patients will be asked to note whenever they forget or do not apply the ointment. The number of days the patients have not used the ointment will be recorded at every follow-up consultation to assess adhesion to intervention (compliance). The patients will be requested to deliver any unused or partially used drug to investigator with the purpose of appropriate disposal and destruction, and to confirm adhesion to intervention. The investigational medicinal product (IMP) is given to the patients at baseline and ad hoc dependent on the individual use.

Relevant concomitant care permitted or prohibited during the trial {11d}

Patients enrolled in the trial are allowed to concomitant lymphedema treatment (e.g., compression, MLD, CDT); however, patients must not use compression garments 72 h prior to consultations. Surgical or medicinal treatments (experimental or not) for lymphedema are not allowed. We do not find any ethical concerns regarding the restriction of surgical or experimental treatments, as this ensures that any observed effects can be attributed to the intervention. Additionally, the 72-h pause in compression therapy before consultations follows standard clinical practice and has not been associated with worsening of lymphedema symptoms.

Provisions for post-trial care {30}

Although the local application of topical tacrolimus has a very low systemic availability, a comprehensive post-treatment follow-up corresponding to 3–5 half-lives of tacrolimus, estimated at approximately 75 h, is deemed relevant to detect any potential adverse reactions related to the treatment. Thus, during the last 10 days prior to their final follow-up appointment, patients will be instructed to refrain from applying the ointment. This enables us to assess any post-treatment adverse events.

The patients are covered by the Danish patient insurance system and will be covered by the Danish act on the right to complain and receive compensation and will be covered by the right to compensation for treatment and medicine-related injuries, should any unexpected injuries of incidents should occur during the trial.

Outcomes {12}

Primary outcome measure

Lymphedema volume Lymphedema volume is defined as the difference in volume between the healthy arm and the lymphedema arm. Lymphedema volume is measured with water displacement volumetry (WDV), a precise and validated method [19]. This method follows from the Archimedes principle, a physical law of buoyancy. We will use Bravometer (Novuqare BV, PJ Horst, NL). In brief, the patient lowers the arm in a basin filled with water, which cause an equal volume of water to displace into another basin. The amount of displaced water is then measured in grams and converted to milliliters (1:1). Lymphedema volume is measured at baseline and at 3, 6, 9, and 12 months. Lymphedema volume as primary endpoint will be evaluated at 12-month follow-up.

Secondary outcome measures

Patient-Reported Outcome Measures (PROM) PROM is a tool to understand and optimize treatment based on patients' subjective measures. It is key in understanding and setting goals of achievement in treating patients. PROMs are obtained using questionnaires. We intend to use three independent questionnaires, validated and translated to Danish, to assess QoL:

> The 36-Item Short Form survey (SF- 36) is a generic quality-of-life questionnaire that includes eight domains on general health: physical functioning, body pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy or fatigue, and general health problems [20]. The questions are answered on a three-, five-, and six-step scales with binary (yes/no) answers depending on the question.
>
> Disability of the Arm Shoulder and Hand (DASH) is a questionnaire developed for patients with disabilities of the upper limb [21, 22]. It includes 30 questions about the patient's symptoms and function of the upper limb. The questions are answered on a five-step scale from *not difficult* to *impossible*.
>
> LYMPH-Q Upper Extremity is a disease specific questionnaire developed for women who have developed lymphedema after breast cancer [22, 23]. It contains six independently functioning scales measuring arm symptoms, function, appearance, psychological function, and satisfaction with information and with arm sleeves. The seven scales vary from nine to 15 items and are all answered on a four-step scale.

All questionnaires are answered on iPads in-clinic at baseline, 3, 6, 9, and 12 months.

Bioimpedance spectroscopy (BIS) BIS is a method to measure the impedance of the extracellular fluid in an extremity [24]. With the use of SOZO® (ImpediMed, Brisbane, Australia) along with the manufacturer's software, we will measure how the body impedes current flow in the upper extremities though electrode pads where the patients place their hands and feet. The measured outcome is lymphedema index (L-Dex). L-Dex >10 is defined as lymphedema in this study. L-Dex values within the interval (−)10–10 are considered normal, and values >10 are considered abnormal [25]. The following variables are furthermore measured by the device: extracellular fluid, intracellular fluid, total body water, fat mass, active tissue mass, extracellular mass, and skeletal muscle mass. The variables will be reported in percentage (%) of total body water or body weight accordingly. BIS will be measured at baseline and at 3, 6, 9, and 12 months.

Indocyanine green lymphangiography (ICG-L) Indocyanine green (ICG) emits near infrared fluorescence and allows for a real-time imaging of lymphatic function and flow when using a near infrared camera. ICG-L is achieved by injecting 0.1 mL ICG (2.5 mg/mL Verdye, Diagnostic Green, Aschheim, Germany) subcutaneously in the web space between the thumb and index finger, between the 3rd and 4th finger, and at the ulnar border of the palmaris longus tendon at wrist level. Fluorescent imaging of the lymph vessels and lymph flow is obtained using HyperEye Medical System (MNIRC- 501, HEMS; Mitzuho Co., Tokyo, Japan). ICG-L scans will be recorded at 0, 10, and 60 min after injection. ICG-L scans are graded individually by two senior consultants according to the 5-step MD Anderson (MDA) scale, which we previously used to validate ICG-L [26]. In cases of discrepancy, a consensus agreement will be reached after reevaluation. ICG-L will be done at baseline and at 12 months.

Skin fibrosis measurement Skin fibrosis is measured with SkinFibroMeter (SFM) (Delfin Technologies Ltd.,

Finland). The SFM measures the force (N) of the resistance in the indurated skin caused by fibrosis through an indenter at the tip of the probe. The indenter is pressed shortly against the skin five times repeatedly. The contralateral arm is used as reference for the measurements. SFM have previously been used and have proven to correlate significantly with lymphedema stage [27].

Participant timeline {13}

	STUDY PERIOD					
	Enrollment	Post-allocation				
Timepoint	Allocation	Baseline	3 months	6 months	9 months	12 months
ENROLLMENT:						
Elegibility screen	X					
Informed consent	X					
Allocation	X					
INTERVENTIONS:						
Tacrolimus		←	—	—	—	→
Placebo		←	—	—	—	→
ASSESSMENTS:						
Baseline characteristics		X				
Medical anamnesis		X				
Arm volume		X		X		X
SF-36 questionnaire		X	X	X	X	X
DASH questionnaire		X	X	X	X	X
LYMPH Q questionnaire		X	X	X	X	X
Bioimpedance spectroscopy		X		X		X
ICG lymphangiography		X				X
Skin fibrosis measurements		X		X		X
Safety (adverse events)			X	X	X	X

Sample size {14}

Based on the results from our pilot trial, we expect to observe a 10% decrease in lymphedema volume with a standard deviation of 15%. Given these assumptions, a two-sided test with 80% power and a significance level of 0.05, we need 74 patients with 37 patients in each group. We expect a drop-out rate at between 5 and 10% (7.5%) which leaves us at 80 patients in total.

Recruitment {15}

Patients will be recruited through various channels to ensure broad access to potential participants. Recruitment efforts include:

1. Referral from healthcare professionals: Patients will be referred to the department as part of the normal workflow, as we already treat lymphedema. Referrals may come from physiotherapists, doctors, and other healthcare professionals involved in the care of patients with lymphedema.

2. Hospital promotion and patient associations.
3. Referral from general practitioners: Patients who wish to participate can obtain a referral from their general practitioner to our department.
4. Self-referral: Patients interested in participating can contact us directly via the email provided on the information materials.

Patients will be informed of their right to 24 h of consideration time before deciding whether they wish to participate. Participants will be informed of their right to withdraw from the study at any time without consequence. Clear procedures for withdrawal will be outlined in both written materials and during the informed consent process. Participants can express their desire to withdraw verbally or in writing to the research team. Any further participation in the study will cease. The screening of possible participants and enrollment will extend over 18 months or until the target of 80 patients is achieved.

Assignment of interventions: allocation

Sequence generation {16a}

The randomization lists used in REDCap will be generated by a data manager at OPEN (Open Patient data Explorative Network, J. B. Winsløws Vej, Odense, Denmark), using the Sealed Envelope system [28]. Block randomization with random block sizes of 4 and 8 will ensure an equal distribution in both arms. No strata will be used for randomization.

Concealment mechanism {16b}

To ensure concealment, block sizes will not be disclosed. Moreover, REDCap will not release the outcome of the randomization until the patient has been included and baseline measurements have been completed. The randomization-lists will remain undisclosed for the investigators until the end of the trial. However, a hard copy of the randomization list will be available in case of system failure and the need for emergency unblinding.

Implementation {16c}

At inclusion, patients will be randomly assigned to either experimental or control group with a 1:1 allocation using computer generated randomization through REDCap [29] hosted by OPEN. PI and delegated personnel will be responsible for inclusion and allocation to intervention.

Assignment of interventions: blinding

Who will be blinded {17a}

The tacrolimus ointment and the placebo will be labeled for randomization and then sealed and delivered at the four centers in identical boxes to ensure blinding of the assessor at the enrolment and follow-up consultations. The participants will not be able to compare placebo tubes to the tacrolimus tubes during the trial.

Procedure for unblinding if needed {17b}

The investigators are encouraged to maintain the blinding throughout the trial. In the event of an emergency, the decision to unblind rests with the investigator. Unblinding is not necessarily a reason for study drug discontinuation. PI will have access to a hard copy of the blinding list, should it be necessary.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Data will be obtained by PI or personnel delegated by PI or sponsor. The following demographic and baseline information will be collected at enrolment: age, BMI, menopausal status, medical history (breast cancer treatment details, previous lymphedema treatments, comorbidities), and lymphedema-specific parameters (disease duration, initial limb volume difference, L-Dex score, skin fibrosis measurements, and lymphatic function via ICG-lymphangiography). The data on breast cancer treatment will be obtained through the patient's medical record. The data on the primary and secondary outcomes will be obtained and entered directly in the eCRF at baseline and 3-, 6-, 9-, and 12-month follow-up consultations. The primary outcome measured with WDV will be noted directly in the eCRF upon measurement. The questionnaires will be answered online via REDCap (available through a link sent to the patients' Digital Post; an integral part of the national digital service infrastructure in Denmark) and the answers are uploaded directly to the eCRF. Results from SOZO® (L-Dex and body composition) will be transferred directly to a cloud-based database, from where it will be entered in the eCRF. Skin fibrosis measurements will be performed in-clinic and registered directly into the eCRF. Information on adhesion to intervention and adverse events will be obtained at 3-, 6-, 9-, and 12-month follow-ups at registered in the eCRF.

Plans to promote participant retention and complete follow-up {18b}

The patients will receive an e-mail reminder 1 week prior to each visit to increase retention to the study.

Data management {19}

In this study, all data will be entered and stored electronically in the electronic case report form (eCRF) through REDCap. Data will be analyzed at the end of the project and will be stored 25 years afterwards.

Confidentiality {27}

Sensitive personal data in this study is treated confidentially according to the Danish act on processing of personal data and the Danish Health Act. Applications for the study are reported to the Regional Data Processing Record, Danish National Committee on Health Research Ethics, and Danish Medicines Agency before starting.

The patients' names and social security numbers will be kept classified when publishing results of this study. Participants will be informed that the relevant authorities will be given access to their data with the purpose of inspection, monitoring, and auditing.

The signed consent forms will be kept with investigator. Investigator is also required to show the consent forms if demanded by patients or relevant authorities. The GCP unit in Odense, Danish Medicines Agency, and other relevant authorities are given direct access to data and documentation by investigator.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Not applicable. There will not be taken any biological samples in this study.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The Breast Q, DASH, and SF- 36 raw scores will be transformed into scores ranging from 0 (worst) to 100 (best) using the original scoring key. Analyses will be chosen according to the specific outcome. Numerical data will be assessed for normality. If they meet the normality assumptions, we will use mixed effects linear regression. If not, mixed effect linear regression with bootstrapped standard errors will be used. Continuous parametric variables will be expressed as mean ± standard deviation, and categorical variables will be expressed as frequency and percentage (%).

Interim analyses {21b}

In this trial, no interim analyses are planned due to the expectation that participant inclusion will be completed in a short period of time, resulting in clusters of results being available within the same month or two.

Based on findings from our pilot trial and the established safety profile of tacrolimus as a safe and well-tolerated drug, we do not anticipate significant safety concerns. Nevertheless, any participant experiencing adverse events incompatible with continuing the study will be discontinued from the trial to ensure their safety.

Since no interim analyses will be performed, there will be no interim results reviewed. The sponsor will ultimately have the final decision-making authority regarding the continuation or termination of the trial based on overall safety and efficacy data collected during the study.

Methods for additional analyses (e.g., subgroup analyses) {20b}

We will conduct subgroup analyses to assess the treatment effect of tacrolimus based on lymphedema duration, categorizing patients into 2-year intervals to explore whether disease duration influences treatment response. Additionally, we will examine whether the treatment effect varies based on the tissue composition features of the lymphedema, specifically distinguishing between fibroadipose tissue and fluid retention if possible.

The pathology of lymphedema, particularly the chronic aspects involving fibrosis and adipose tissue formation, is not fully understood. We hypothesize tacrolimus to be more effective in the earlier stages of lymphedema where fluid retention is predominant. These analyses will help determine if the treatment should be targeted to a specific subpopulation of lymphedema patients based on the duration of their condition and tissue composition features. Based on findings from our pilot trial and the homogeneity of the study population, we do not anticipate significant confounding variables that would necessitate adjustment in our primary analysis. However, we will remain vigilant for any unforeseen confounders that might arise during the study and will address them as needed.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

The study aims to prevent missing data given the nature of the study. Data from our previous trial demonstrated high compliance, with 77.8% of participants adhering to over 97% of the treatment regimen and the remaining participants maintaining compliance rates between 78 and 92.8%, and we expect a similar compliance in this trial. At random missing data will be handled by the mixed linear models used in the statistical analyses, and we will conduct sensitivity analyses to assess the impact of different missing data assumptions, ensuring the robustness of our findings. All randomized patients will be included in the final analysis based on an intention-to-treat approach, analyzing them according to their original treatment assignment regardless of adherence.

Plans to give access to the full protocol, participant-level data and statistical code {31c}

The full protocol will be available through the Clinical Trials Information System (CTIS). Making the protocol accessible through CTIS ensures transparency and allows other researchers to understand the study design and methodologies used, thereby facilitating replication and further research.

Participant-level data will be made available to qualified researchers upon reasonable request following the publication of the primary results. Access will be granted under a data sharing agreement to ensure the confidentiality and appropriate use of the data. Interested researchers can request access by contacting the principal investigator.

The statistical code used for the analyses will not be publicly available. However, it will be thoroughly reviewed and approved by a qualified statistician during the analysis and manuscript preparation stages.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

The sponsor has overall responsibility for the study. The primary investigator (PI) is responsible for the day-to-day management of the trial and coordination with other trial sites. The sponsor and PI maintain daily contact to ensure smooth trial operations. Each trial site has a designated responsible trial site investigator who reports to the PI and sponsor. The PI is responsible for updating all members of any protocol changes or important trial information as it arises.

The Trial Steering Committee consists of the PI, sponsor, and two additional clinicians and researchers (one professor and one postdoctoral researcher), all of whom have extensive experience with clinical trials and lymphedema treatment. The roles of the sponsor and the two additional members are to provide supervision and guidance. The TSC meets approximately bimonthly to oversee trial progress and make key decisions.

The PI, with the support of a qualified statistician from OPEN, is responsible for data management. The data management team communicates on an ad hoc basis and will hold a formal meeting once all data is collected after the last patient's last visit to review and finalize the data.

Composition of the data monitoring committee, its role and reporting structure {21a}

The Good Clinical Practice (GCP) unit in Odense, Region of Southern Denmark will be responsible for monitoring, will audit the study, and oversee that the study is conducted in accordance with the ethical principles in the Declaration of Helsinki, ICH-GCP guidelines, and current legislation. The GCP unit will make a risk assessment and a monitoring plan in cooperation with sponsor, PI, or a delegate. Personnel from the Danish National Committee on Health Research Ethics, the Danish Medicines Agency, and from other Danish health authorities can monitor and audit the study.

Adverse event reporting and harms {22}

An adverse event (AE) is defined as any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment. All AEs reported by the patients will be registered in the CRF. AEs will be registered after the patients have given their consent and are enrolled in the study. Mild skin irritation located to the application site, erythema, warm feeling, pain, paresthesia, rash, and flushing are common adverse reactions to tacrolimus. Mild burning sensation and itching occur regularly in mild to intermediate intensity and are accelerated by sun exposure and alcohol. These symptoms often fade after a week of treatment.

All AEs that meet the criteria for a serious adverse event (SAE) during the trial will be reported to the Danish Medicines Agency. SAE is defined as any untoward medical occurrence that at any dose requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, results in a congenital anomaly or birth defect, is life-threatening, or results in death. PI will report all SAE to sponsor within 24 h.

The product resumé for tacrolimus "Accord" will be used as reference to all reported adverse events. The participants can report adverse events and reactions throughout the project and will receive information on how and who to contact if necessary. PI is responsible for assessment of adverse events and reactions and responsible for assessment of relatedness of AEs. All participants will be evaluated regarding to adverse events and reactions up until the end of the project.

Suspected unexpected serious adverse reactions (SUSAR) cover unexpected adverse reactions, and serious adverse events assumed to have an association to the intervention.

PI will report SUSARs to sponsor as soon as possible after confirmation of the SUSAR at a consultation and registering it in the CRF. Sponsor will be responsible for assessment of expectedness and, therefore, whether an event is a SUSAR and furthermore assessment of the causality with the use of the product resumé for tacrolimus and is responsible for reporting the SUSAR to EudraVigilance as soon as possible and not later than 7 days of after sponsor's acknowledgement of a lethal or life-threatening SUSAR at not later than 15 days after the

acknowledgement of a non-lethal and non-life-threatening SUSAR. Sponsor will report all relevant information about sponsor's and investigator's follow-up on their reporting. All reports must contain a comment on any possible consequences for the project.

Sponsor will make a list of all suspected serious adverse reactions that has occurred during the period of the project including a report on safety of the participants once a year. This job may be delegated to investigator.

Frequency and plans for auditing trial conduct {23}

The trial will be audited and monitored according to the following plan:

Initiation visits: Each participating center will undergo an initiation visit to ensure readiness and compliance with trial procedures. Key areas of focus will include the maintenance of screening and identification lists, delegation logs, and investigational medicinal product (IMP) handling procedures.
Monitoring visits:

- The first monitoring visit will be conducted shortly after the inclusion of the first two participants at each center.
- Subsequent monitoring visits will be scheduled based on the inclusion rate and the center's needs, with increased frequency during the inclusion period.
- The GCP unit will maintain at least annual contact with each center, ensuring ongoing compliance and addressing any issues promptly.

Data and protocol compliance: Monitoring will involve verifying that the protocol is correctly implemented, and that data quality is maintained. This includes checking all registered data and ensuring protocol-specific actions are followed for the first two participants and for a total of 10% of participants at each center.
Final monitoring visits: A final monitoring visit will be conducted at the sponsor's location after all other centers have completed their final visits. The GCP coordinator will verify that the Trial Master File contains all relevant documents for archiving.
Evaluation of monitoring plan: The monitoring plan will be continuously evaluated and revised as necessary based on findings from GCP unit monitoring, central monitoring, or audits. This includes addressing changes such as protocol amendments, significant non-compliance, inadequate data quality, or major changes in project staff.

The monitoring process will be independent of the investigators and the sponsor to ensure unbiased oversight and adherence to regulatory requirements.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Any modifications to the protocol that may impact the conduct, safety, design, population, or procedures of the study will require a formal modification (either substantial modification or non-substantial modification) to the protocol. Any modification must be approved by the relevant authorities before being implemented in the study.

Dissemination plans {31a}

Irrespective of the outcome of the clinical trial, within 1 year from the end of the clinical trial, sponsor will submit to the EU database (CTIS) a summary of the results of the clinical trial and will be accompanied by a summary written in a manner that is understandable to laypersons. Furthermore, results from the study will be sought to be published in an international medical journal. The study will be registered at www.clinicaltrials.gov and CTIS. Results of the project will be available for the participants through PI.

Discussion

Conducting a multicenter, double-blinded, randomized placebo-controlled trial involves practical and operational challenges, particularly in recruiting and retaining participants. To address this, we will use targeted recruitment strategies, including referrals from healthcare professionals, hospital promotions, patient associations, and self-referrals. Ensuring participant retention is equally important; thus, we will implement regular follow-up reminders and clear communication of study benefits.

Resource management is critical for the trial's success. Each trial site will have adequate personnel, equipment, and facilities. To ensure uniformity, the same two assessors will perform all measurements across all centers. Coordination among sites will be managed through regular communication and coordination meetings. Data management will use eCRFs in REDCap, ensuring secure and accurate data collection. Our experience from the pilot trial has prepared us well for this study, and the GCP unit will audit the process to ensure quality data collection and execution. Protocol deviations will be documented and promptly addressed.

The primary outcome of this trial is the change in lymphedema volume, a key parameter directly reflecting the fluid accumulation and fibroadipose tissue deposition, defining lymphedema. By measuring this change,

we can assess the effectiveness of tacrolimus in reducing the swelling characteristic of this condition.

QoL is a secondary outcome, capturing the broader impact of lymphedema on patients' well-being. Including QoL reflects the importance of addressing both physical symptoms and overall QoL, as lymphedema affects emotional and social aspects of patients' lives.

Additional secondary outcomes include assessments via SF- 36, DASH, and LYMPH-Q questionnaires; lymphedema index (L-Dex); ICG-L; and skin fibrosis measurements. These outcomes provide a comprehensive evaluation of tacrolimus treatment, offering valuable clinical and research insights. This evaluation will help determine the effectiveness of tacrolimus in reducing lymphedema volume, improving QoL, and addressing secondary symptoms. This data can guide clinicians in making informed decisions about incorporating tacrolimus into standard treatment protocols for BCRL. From a research perspective, the study may contribute to understanding the mechanisms by which tacrolimus affects lymphedema.

Our pilot trial revealed several limitations, including its unblinded design, small sample size, lack of long-term follow-up, and absence of a control group. These issues have been addressed by incorporating a double-blind, placebo-controlled design, increasing the sample size, and including a 12-month follow-up period.

If tacrolimus proves effective, it will offer a treatment for patients suffering daily from BCRL. The results may also be extrapolated to other BCRL stages and could lead to investigations of tacrolimus treatment for secondary lymphedema due to other diseases, such as malignant melanoma, gynecological cancers, prostate cancer, and lymphomas. Expanding research to these conditions could significantly broaden the treatment's impact.

This study is not expected to have significant ethical issues. It aims to provide new information on the medicinal treatment of BCRL, offering participants potential relief. All participants will benefit from increased attention to their condition and QoL. The known adverse events of tacrolimus are manageable compared to its potential benefits.

This trial will provide robust data on the efficacy of topical tacrolimus in reducing BCRL volume and improving patient QoL. Positive results could establish tacrolimus as a standard treatment, enhancing clinical outcomes for affected patients. The findings will also contribute to understanding the role of immunomodulation in lymphedema management.

Future research should focus on the long-term effects of tacrolimus and explore its potential applications in other forms of lymphedema. By addressing these practical and operational issues, we aim to conduct a successful trial that advances the treatment of breast cancer-related lymphedema, ultimately improving patient outcomes and QoL.

Trial status

The approved protocol is version 1.5, dated April 22nd, 2024. Recruitment is expected to begin on September 1st and enrollment will extend over a maximum of 18 months or until the target of 80 patients is achieved.

Abbreviations

TACLE Trial	Topical Tacrolimus for the Amelioration of Breast Cancer-Related Lymphedema Trial
BCRL	Breast cancer-related lymphedema
CD4 +	Cluster of differentiation 4 positive
QoL	Quality of life
CTIS	Clinical Trials Information System
ISL	International Society of Lymphology
GCP	Good Clinical Practice
PI	Primary investigator
TSC	Trial Steering Committee
IMP	Investigational medicinal product
MLD	Manual lymphatic drainage
CDT	Complete decongestive therapy
PUVA	Psoralen ultraviolet A
eCRF	Electronic case report form
REDCap	Research Electronic Data Capture
OPEN	Open Patient data Explorative Network
BIS	Bioimpedance spectroscopy
L-Dex	Lymphedema index
ICG-L	Indocyanine green lymphangiography
MDA	MD Anderson (scale)
SFM	SkinFibroMeter
WDV	Water displacement volumetry
SF- 36	36-Item Short Form survey (Survey)
DASH	Disability of the Arm Shoulder and Hand (Survey)
PROM	Patient-Reported Outcome Measure
AE	Adverse event
SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reaction

Acknowledgements

Not applicable.

Authors' contributions {31b}

Frederik Gulmark Hansen (FGH): FGH is the chief investigator. He conceived the study, led the proposal and protocol development, and is responsible for the day-to-day management of the trial. FGH drafted the manuscript and coordinated with other centers. Mads Gustaf Jørgensen (MGJ): MGJ contributed to the study design and protocol development. He provided expertise in lymphedema treatment and supervised the clinical aspects of the trial. MGJ also substantively revised the manuscript. Jørn Bo Thomsen (JBT): JBT assisted in the study design and provided substantial input into the methodology. He contributed to the revision of the manuscript. Jens Ahm Sørensen (JAS): JAS is the sponsor and provided overall supervision and guidance. He played a significant role in study design and methodology. JAS also reviewed and substantively revised the manuscript. All authors (FGH, MGJ, JBT, JAS) have made substantial contributions to the conception and design of the work, have drafted the work or substantively revised it, and have approved the submitted version. Furthermore, all authors agree to be personally accountable for their own contributions and ensure that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Funding {4}

Open access funding provided by University of Southern Denmark This trial is supported by the following sources of funding:
- Danish Cancer Society
- Danish Cancer Research Fund

- Lægeforeningens Forskningsfond
- Overlægerådets Forskningsfond
- Overlægerådets Forskningsfond
- Simon Spies Fonden
- Else og Mogens Wedell Wedellsborgs Fond
- Tømrerhandler Vilhelm Bangs Fond

None of these funding bodies have any influence on the design of the study; the collection, analysis, or interpretation of data; or the writing of the manuscript. They will not influence the trial in any way. The funding is solely to support the financial aspects of conducting the trial.

Data availability {29}

The final trial dataset will be accessible to the principal investigator (PI) and the research team involved in the study. Additionally, a qualified statistician from the OPEN (Open Patient data Explorative Network) will have access to the dataset for the purposes of data analysis. There are no contractual agreements that limit access to the final trial dataset for investigators. All data will be handled in accordance with the relevant data protection regulations, ensuring confidentiality and integrity. If necessary, data sharing agreements will be put in place to govern the sharing of data with qualified researchers upon reasonable request, following the publication of the primary results. These agreements will ensure the confidentiality and appropriate use of the data.

Ethics approval and consent to participate {24}

This study has been approved by The Danish Medicines Agency and the Medical Research Ethics Committees (MREC) through the Clinical Trials Information System (CTIS). The committee's reference number for this approval is EU CT Number: 2023 - 503644 - 13 - 00. Written, informed consent to participate will be obtained from all participants prior to any study-related procedures.

Consent for publication {32}

Consent form in Danish.

Version 1.3
CTIS No: 2023-503644-13-00
Date 10.05.2024

(S1)

Informeret samtykke til deltagelse i et sundhedsvidenskabeligt forskningsprojekt.

Forskningsprojektets titel: *Tacrolimussalve til behandling af lymfødem efter brystkræft*

Engelsk: Topical tacrolimus for the amelioration of breast cancer-related lymphedema: a randomized, double-blind, placebo-controlled phase II/III trial (TACLE Trial)

Erklæring fra forsøgspersonen:

Jeg har fået skriftlig og mundtlig information og jeg ved nok om formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til at deltage i forskningsprojektet, og har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn: _____

Dato: _____ Underskrift: _____

Ønsker du at blive kontaktet i forbindelse med fremtidige studiet, hvor din deltagelse vil være relevant?:

Ja _____ (sæt x) Nej _____ (sæt x)

Ønsker du at blive informeret om forskningsprojektets resultat samt eventuelle konsekvenser for dig?:

Ja _____ (sæt x) Nej _____ (sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget.

Efter min overbevisning er der givet tilstrækkelig information til, at der kan træffes beslutning om deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____ Underskrift: _____

Projektidentifikation: (Fx komiteens Projekt-ID, EudraCT nr., versions nr./dato eller lign.)

Side **6** af **7**

Competing interests {28}
The authors declare that they have no competing interests.

Author details
[1]Research Unit for Plastic Surgery, Odense University Hospital, Odense, Denmark. [2]Clinical Institute, University of Southern Denmark, Odense, Denmark.

Received: 27 August 2024 Accepted: 1 April 2025
Published online: 08 April 2025

References

1. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. Lancet Oncol. 2013;14(6):500–15.
2. Brunelle CL, Roberts SA, Shui AM, Gillespie TC, Daniell KM, Naoum GE, et al. Patients who report cording after breast cancer surgery are at higher risk of lymphedema: results from a large prospective screening cohort. J Surg Oncol. 2020;122(2):155–63.
3. Lawenda BD, Mondry TE, Johnstone PA. Lymphedema: a primer on the identification and management of a chronic condition in oncologic treatment. CA Cancer J Clin. 2009;59(1):8–24.
4. Executive Committee of the International Society of L. The diagnosis and treatment of peripheral lymphedema: 2020 consensus document of the International Society of Lymphology. Lymphology. 2020;53(1):3–19.
5. McLaughlin SA, Brunelle CL, Taghian A. Breast cancer-related lymphedema: risk factors, screening, management, and the impact of locoregional treatment. J Clin Oncol. 2020;38(20):2341–50.
6. Bundred N, Foden P, Todd C, Morris J, Watterson D, Purushotham A, et al. Increases in arm volume predict lymphoedema and quality of life deficits after axillary surgery: a prospective cohort study. Br J Cancer. 2020;123(1):17–25.
7. Markkula SP, Leung N, Allen VB, Furniss D. Surgical interventions for the prevention or treatment of lymphoedema after breast cancer treatment. Cochrane Database Syst Rev. 2019;2(2):CD011433.
8. Ogata F, Fujiu K, Matsumoto S, Nakayama Y, Shibata M, Oike Y, et al. Excess lymphangiogenesis cooperatively induced by macrophages and CD4(+) T cells drives the pathogenesis of lymphedema. J Invest Dermatol. 2016;136(3):706–14.
9. Ghanta S, Cuzzone DA, Torrisi JS, Albano NJ, Joseph WJ, Savetsky IL, et al. Regulation of inflammation and fibrosis by macrophages in lymphedema. Am J Physiol Heart Circ Physiol. 2015;308(9):H1065–77.
10. Avraham T, Zampell JC, Yan A, Elhadad S, Weitman ES, Rockson SG, et al. Th2 differentiation is necessary for soft tissue fibrosis and lymphatic dysfunction resulting from lymphedema. FASEB J. 2013;27(3):1114–26.
11. Zampell JC, Yan A, Elhadad S, Avraham T, Weitman E, Mehrara BJ. CD4(+) cells regulate fibrosis and lymphangiogenesis in response to lymphatic fluid stasis. PLoS ONE. 2012;7(11):e49940.
12. Cury Martins J, Martins C, Aoki V, Gois AF, Ishii HA, da Silva EM. Topical tacrolimus for atopic dermatitis. Cochrane Database Syst Rev. 2015;7:CD009864.
13. Malecic N, Young H. Tacrolimus for the management of psoriasis: clinical utility and place in therapy. Psoriasis (Auckl). 2016;6:153–63.
14. Seneschal J, Duplaine A, Maillard H, Passeron T, Andreu N, Lassalle R, et al. Efficacy and safety of tacrolimus 0.1% for the treatment of facial vitiligo: a multicenter randomized, double-blinded, vehicle-controlled study. J Invest Dermatol. 2021;141(7):1728–34.
15. Tsuda K, Yamanaka K, Kitagawa H, Akeda T, Naka M, Niwa K, et al. Calcineurin inhibitors suppress cytokine production from memory T cells and differentiation of naive T cells into cytokine-producing mature T cells. PLoS ONE. 2012;7(2):e31465.
16. Gardenier JC, Kataru RP, Hespe GE, Savetsky IL, Torrisi JS, Nores GD, et al. Topical tacrolimus for the treatment of secondary lymphedema. Nat Commun. 2017;8:14345.
17. Gulmark Hansen FC, Jørgensen MG, Sørensen JA. Treatment of breast cancer-related lymphedema with topical tacrolimus: a prospective, open-label, single-arm, phase II pilot trial. J Breast Cancer. 2023;26:26.
18. Jorgensen MG, Toyserkani NM, Hansen FG, Bygum A, Sorensen JA. The impact of lymphedema on health-related quality of life up to 10 years after breast cancer treatment. NPJ Breast Cancer. 2021;7(1):70.
19. Brorson H, Hoijer P. Standardised measurements used to order compression garments can be used to calculate arm volumes to evaluate lymphoedema treatment. J Plast Surg Hand Surg. 2012;46(6):410–5.
20. Treanor C, Donnelly M. A methodological review of the Short Form Health Survey 36 (SF-36) and its derivatives among breast cancer survivors. Qual Life Res. 2015;24(2):339–62.
21. Park JE, Jang HJ, Seo KS. Quality of life, upper extremity function and the effect of lymphedema treatment in breast cancer related lymphedema patients. Ann Rehabil Med. 2012;36(2):240–7.
22. Klassen AF, Tsangaris E, Kaur MN, Poulsen L, Beelen LM, Jacobsen AL, et al. Development and psychometric validation of a patient-reported outcome measure for arm lymphedema: the LYMPH-Q upper extremity module. Ann Surg Oncol. 2021;28(9):5166–82.
23. Madsen CB, Poulsen L, Jørgensen MG, Lorenzen MM, Tsangaris E, Klassen A, et al. Advanced translation and cultural adaption of the LYMPH-Q upper extremity module from English to Danish. Eur J Plast Surg. 2022;45(4):617–22.
24. Shah C, Vicini F, Beitsch P, Laidley A, Anglin B, Ridner SH, et al. The use of bioimpedance spectroscopy to monitor therapeutic intervention in patients treated for breast cancer related lymphedema. Lymphology. 2013;46(4):184–92.
25. Fu MR, Cleland CM, Guth AA, Kayal M, Haber J, Cartwright F, et al. L-dex ratio in detecting breast cancer-related lymphedema: reliability, sensitivity, and specificity. Lymphology. 2013;46(2):85–96.
26. Jørgensen MG, Toyserkani NM, Hansen FCG, Thomsen JB, Sørensen JA. Prospective Validation of Indocyanine Green Lymphangiography Staging of Breast Cancer-Related Lymphedema. Cancers (Basel). 2021;13(7):1540. https://doi.org/10.3390/cancers13071540.
27. Sun D, Yu Z, Chen J, Wang L, Han L, Liu N. The value of using a SkinFibroMeter for diagnosis and assessment of secondary lymphedema and associated fibrosis of lower limb skin. Lymphat Res Biol. 2017;15(1):70–6.
28. Sealed Envelope Ltd. 2024. Simple randomisation service. [Online] Available from:https://www.sealedenvelope.com/simple-randomiser/v1/. Accessed 5 Apr 2025.
29. Harvey LA. REDCap: web-based software for all types of data storage and collection. Spinal Cord. 2018;56(7):625.

Publisher's Note