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Lymphatic Function Decreases over Time in the Arms of Breast Cancer Patients following Treatment

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Background: In patients with breast cancer-related lymphedema, distinct lymphatic patterns and changed lymphatic contractile function have been described, but it is unknown how these characteristics change over time and to what extent they appear before clinical edema is detectable. Recently, we described the lymphatic morphology and function in a cohort of breast cancer patients shortly after radiation therapy (RT). In the current study, we investigate lymphatic function and morphology in the same cohort after 1 year of follow-up.

Methods: The study population consisted of 28 breast cancer patients investigated 12 months after adjuvant locoregional RT. Lymphatic contraction frequency (CF), propulsion velocity, and the morphology of lymphatic vessels in the upper extremities were described in vivo using near-infrared fluorescence imaging. Lymphatic stress test was performed using hyperthermia.

Results: At 1 year after RT, (n = 28) 46% of the patients presented with lymphatic morphological abnormalities with a degree of dermal backflow and 21% had developed clinical breast cancer-related lymphedema. In the ipsilateral arm, CF was 23% lower than in the contralateral arm ($P = 0.04$). Since primary examination, CF in the ipsilateral arm decreased by 40% ($P = 0.03$), whereas no change was observed in the contralateral arm. During hyperthermia, the ipsilateral arms with lymphatic complications were not able to increase CF as the remaining subgroups.

Conclusions: Lymphatic function in the ipsilateral arm deteriorated over time after adjuvant breast cancer therapy. Furthermore, the presence of abnormal torturous lymphatic vessels in asymptomatic arms appeared to be associated with weak lymphatic reserve pumping capacity. *(Plast Reconstr Surg Glob Open 2022;10:e4507; doi: 10.1097/GOX.0000000000004507; Published online 16 September 2022.)*

INTRODUCTION

More than one in five women surviving breast cancer experience breast cancer-related lymphedema (BCRL), which is associated with physical and psychological impairment.[1–3] Axillary surgery with lymph node dissection and radiotherapy significantly increases the risk of BCRL.[3–5] However, remarkably, the incidence of BCRL keeps increasing up to 10 years after cancer treatment.[6,7]

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The mechanisms leading to insufficient lymphatic drainage are still poorly understood and cannot only be contributed to resection of lymph nodes since problems may occur after months to years of freedom from symptoms. Near-infrared fluorescence (NIRF) imaging is a novel and noninvasive imaging technique that has enabled us to visualize human subcutaneous lymphatic vessels in real time. NIRF imaging allows characterization of superficial healthy and abnormal lymphatic vessels, and it has recently been validated to quantify dynamic functional properties of lymphatic transport.[8,9]

In women diagnosed with BCRL, distinct pathological lymphatic patterns have been described at all stages using NIRF imaging.[10] Moreover, studies also indicate changed lymphatic contractile function.[10–12] However, it is unknown

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how lymphatic functional and morphological characteristics change over time and to what extent these subclinical indications appear before clinical edema is detectable. It has been shown that early detection and treatment of subclinical BCRL in breast cancer survivors is crucial in delaying debut and preventing further progression, which may have implications for quality of life.[13,14]

Recently, an explorative study by Alstrup et al[15] investigating the lymphatics in the upper extremities in a cohort of consecutive node-positive early breast cancer patients was published. Shortly after patients completed surgery and radiation therapy (RT), no remarkable difference in functional or microcirculatory quantities was observed. Eight patients presented with changed lymphatic morphology.

We investigated the same patient cohort as a follow-up investigation of the functional and morphological state of the lymphatic vasculature in the arms of these patients a year after treatment, aiming to further characterize and distinguish the course of lymphatic dysfunction in breast cancer patients. Furthermore, the contractile reserve capacity of the lymphatic vessels during a hyperthermic stress test was assessed.[8,9]

We hypothesized that the lymphatic contractile function would change over time as consequence of cancer treatment and increase vulnerability to developing BCRL.

MATERIAL AND METHODS

Ethical Approval

The Regional Committee on Health Research Ethics of the Central Denmark Region (1-10-72-193-18) has approved this study. The study is registered on ClinicalTrials.gov (identifier: NCT03572998). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, revised in 2013, and all participants provided written informed consent before enrollment. This study meets the STROBE guidelines.

Study Design and Population

The study was designed as a prospective cohort study, setup as a follow-up on a previously examined cohort from September 2018 to December 2019 in a primary lymphatic examination at Aarhus University Hospital (AUH) by Alstrup et al.[15] The population consisted of 32 consecutive women with unilateral breast cancer, who all completed both surgery and locoregional RT less than 6 months before the primary lymphatic examination. Surgical procedure consisted of either lumpectomy or mastectomy, including either sentinel node biopsy or axillary lymph node dissection. All patients participated in the Danish Breast Cancer Group (DBCG) RT Skagen trial 1 (NCT02384733). For detailed information about patient's adjuvant radio-, chemo- and endocrine therapy as well as patient recruitment and exclusion criteria, we refer to the primary study.[15] If clinical BCRL had developed before the primary examination, the patient was excluded, while subsequent BCRL development before or after the follow-up examination was accepted. Therefore, BCRL patients consisted of a group of patients that at the time of follow-up examination already had or later were at risk of BCRL.

In this current follow-up study, we examined the same cohort of patients from October 2019 to June 2020, which was between 6 and 12 months after completion of the first lymphatic examination and approximately a year after the end of RT. Therefore, in this study, we report data from the primary examination previously published by Alstrup et al, to compare with our findings in lymphatic function and morphology.

After participating in the follow-up examination, patients were followed regarding potential development of BCRL. They were contacted on September 1, 2020 by phone and on January 18, 2021 and finally on July 6, 2021 BCRL status was validated through medical records.

The arm adjacent to the treated breast was labeled "ipsilateral," whereas the nontreated side was labeled "contralateral," enabling patients to serve as their own control.

In this study, we defined BCRL as clinically evident lymphedema in the arm or hand diagnosed and described in the *Electronic Patient Journal* by experts at the lymphedema clinic, AUH. We used the lymphedema criteria defined in the DBCG RT Skagen trial 1. The definition of arm lymphedema was greater than or equal to 10% increased arm circumference measured 15 cm proximal and/or 10 cm distal of the olecranon on the ipsilateral arm compared with the contralateral arm. If the patient used an arm sleeve, she was asked to not wear this 24 hours before measurement. Measurements were supplemented by patient reported outcome measures with questions of subjective sensations like heaviness and numbness of the arm. This definition is in harmony with the After Mapping of the Axilla: Radiotheray or Surgery (AMAROS) trial.[16]

All examinations were completed under similar conditions standardized by examining all subjects in a supine position in the same examination room with a fixed temperature of 22°C ± 2°C. Before we commenced the protocol, patients rested in the bed acclimatizing to position and temperature for at least 10 minutes. The mean duration of each examination was approximately 4.5 hours with all examinations initiated at the same time of day.

Endpoints

Our primary endpoint was lymphatic function quantified by contraction frequency (CF), lymph propulsion velocity, and response to volume stress by hyperthermia, while our secondary endpoint was lymphatic morphology characteristics.

Study Procedure

NIRF Imaging

We used the same electronic equipment including camera and imaging software and same fluorophore technique and concentrations like in the primary examination.[15] We refer to this study for detailed information. This setup has previously been used by the Department of Cardiothoracic and Vascular Surgery, AUH.[8,9,17]

Table 1. Functional and Morphological Data of the Ipsilateral and Contralateral Arms of the Patients at the Two Examinations: Primary and Follow-up

	Primary Examination		Follow-up Examination	
Endpoints	**Ipsilateral, N = 32**	**Contralateral, N = 32**	**Ipsilateral, N = 28**	**Contralateral, N = 28**
NIRF imaging				
CF, min^{-1} (n)	0.9±0.5 (30)	0.8±0.4 (30)	0.5±0.3 (28)*†	0.7±0.3 (28)
Contraction velocity, cm/s (n)	1.1±0.4 (25)	1.0±0.2 (28)	1.1±0.6 (21)*	0.8±0.2 (21)†
Morphological abnormalities, n (%)	8 (25)	0 (0)	13 (46)	0 (0)

Data reported as means ± SDs and/or absolute numbers and percentages of patients.
The number of participants (n) is reported within brackets after SD.
Data from the primary examination was previously published by Alstrup et al.[15]
*Significant ($P < 0.05$) difference between the ipsilateral and contralateral arms.
†Significant ($P < 0.05$) difference between the primary and follow-up examination in the same arm.

Table 2. Functional and Morphological Data of the Ipsilateral Arm in BCRL and Non-BCRL Patients and Patients with or without Lymphatic Abnormalities

Endpoints	BCRL, n = 6	Non-BCRL, n = 22	w/ Abnormal, n = 13	w/o Abnormal, n = 15	P^*	$P^\dagger$
NIRF imaging						
CF, min^{-1} (n)	0.7±0.3 (6)	0.5±0.3 (22)	0.6±0.4 (13)	0.5±0.3 (15)	0.221	0.459
Contraction velocity, cm/s (n)	1.7±0.8 (3)	1.1±0.5 (18)	1.2±0.7 (8)	1.1±0.6 (13)	—	0.932
Morphological abnormalities, n (%)	5 (83)	8 (36)	—	—	0.056	—

Data reported as means ± SDs and/or absolute numbers and percentages of patients.
The number of participants (n) is reported within brackets after SD.
The labels of the groups "W/ abnormal" and "W/o abnormal" relate to whether subcutaneous morphological lymphatic vessel abnormalities in the ipsilateral arm were observed.
Statistics for groups below four participants were excluded.
*Significant ($P < 0.05$) difference between BCRL and non-BCRL patients.
†Significant ($P < 0.05$) difference between patients with or without lymphatic abnormalities.
W/ abnormal, with subcutaneous morphological lymphatic vessel abnormalities.
W/o abnormal, without subcutaneous morphological lymphatic vessel abnormalities.

Injection and Baseline Sequence

These procedures were conducted identically as in the primary study.[15]

Hyperthermia Sequence

The forearm was submerged in 38°C warm water for 5 minutes. Shortly hereafter (1–2 minutes), a 6-minute recording was completed.

Data Analysis and Statistics

All sequences were analyzed by the same protocol guideline by two investigators as in the primary study with identical blinding process.[15] All recorded baseline and hyperthermia sequences were analyzed for CF and velocity of the lymphatic package in a custom-written LabView program (National Instruments, Tex.). Analysis was done by measuring the intensity of emission in various regions of interest placed over the visible most distal part of the lymphatic vessels. All sequences showed at least one lymphatic propulsion in a proximal direction, but not all visible lymphatic vessels produced lymphatic movement in this time window. A contraction, seen as a movement of a lymph package, was defined as a transient intensity increase, a peak, measured in the region of interest. The frequency and velocity of passing lymphatic packages were estimated and calculated for all visible lymphatic vessels, and an average was calculated. If any discordance regarding morphology or functional data was present, the sequence was reanalyzed by the two investigators, and if there was continued disagreement, a third investigator's evaluation was acquired.

Analyzed data were stored in Microsoft Excel 2019 (16.33), and all statistical analyses and graphical presentation of the data were managed using GraphPad Prism 6 and Stata/SE 15.1.

Data were tested for normality and presented as mean ± standard deviation (SD) for continuous data and for binary data as absolute numbers and percentages of participants. In Tables 1–3, the number of participants (n) was reported within brackets after SD, because some sequences were unfit for velocity analysis.

Data were tested for significance in difference between groups with a paired and unpaired Student *t* test as well as two-way ANOVA for normally distributed data. Fisher exact test was used for binary data. Significance level was set to 0.05 in all tests. Sample size was calculated and described in the primary study.[15]

Table 3. Hyperthermia. CF in the Ipsilateral and Contralateral Arms during Hyperthermia Divided into Subgroups

Groups	Ipsilateral Arm, N = 28	Contralateral Arm, N = 28	*P*
Joint group, min^{-1} (n)	0.8±0.4 (28)	1.0±0.3 (28)	0.026
W/ abnormal, min^{-1} (n)	0.8±0.5 (13)	1.1±0.2 (13)	0.042
W/o abnormal, min^{-1} (n)	0.8±0.4 (15)	1.0±0.4 (15)	0.267
BCRL, min^{-1} (n)	0.5±0.4 (6)	1.1±0.1 (6)	0.016
Non-BCRL, min^{-1} (n)	0.9±0.4 (22)	1.0±0.4 (22)	0.257

Data reported as means ± SDs and absolute participant numbers.
The number of participants (n) is reported within brackets after SD.
P values between the ipsilateral and contralateral arms during hyperthermia.
The labels of the groups "W/ abnormal," "W/o abnormal," "BCRL," and "non-BCRL" relate to the ipsilateral arm, since no abnormalities or edema were observed in the contralateral arm.

RESULTS

Participant Characteristics

Twenty-nine women completed the follow-up examination. One patient was excluded postanalysis because frequency data were deviating with more than five SDs from mean (Fig. 1).

Mean total follow-up time was 787 ± 114 days. Patient demographics are summarized in Table 4. The average number of vessels analyzed per patient was 3.2 ± 1.1 with no difference between ipsilateral and contralateral arms ($P = 0.71$).

Functional and Morphological Results during Rest

Patients were divided into two groups based on appearance of the lymphatic vessels: with (w/) or without (w/o) tortuous vessels and dermal backflow[11] (Fig. 2). In total, 46% of patients presented lymphatic morphologic abnormalities with a degree of dermal backflow. All lymphatic abnormalities were observed in the ipsilateral arm.

Table 1 shows functional and morphological results of NIRF imaging in the ipsilateral and contralateral arms during both primary and follow-up examination (Table 1). At follow-up, the lymphatic CF was lower in the ipsilateral arm ($P = 0.04$) (Fig. 3A), while lymphatic propulsion velocity was higher (0.01) both compared with the contralateral arm.

During the period from the primary examination to follow-up, there was a significant reduction in CF in the ipsilateral arm ($P = 0.03$) (Fig. 3B), whereas no significant change was observed in the contralateral arm ($P = 0.71$). Comparing the CF differences from the primary to

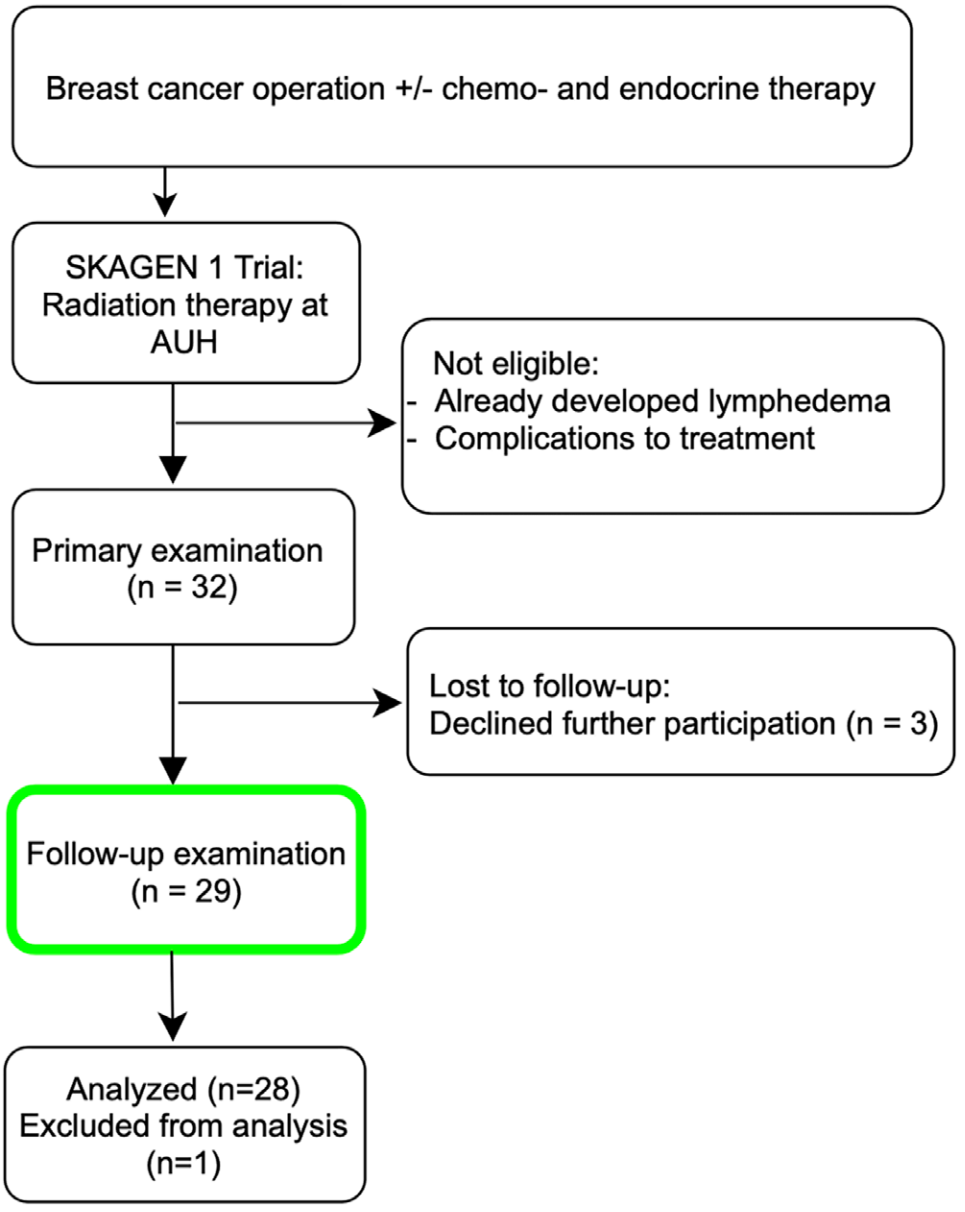

Fig. 1. Flow diagram of trial recruitment and examination.

Table 4. Characteristics of Participants Who Completed Breast Cancer Treatment

Characteristics	Breast Cancer Treated Patients, n = 28	BCRL Patients, n = 6	Non-BCRL Patients, n = 22	*P*
Demographics				
Age, years	55±11	47±7	58±11	0.037
Weight, kg	74±15	71±20	75±14	0.581
Height, cm	166±6	161±5	168±6	0.012
Body mass index, kg/m²	27±5	28±8	27±4	0.697
Currently smoking, n (%)	3 (11)	1 (17)	2 (9)	0.539
Axillary surgical type, n (%)				
Sentinel node	10(36)	0 (0)	10 (45)	0.049
ALND	18 (64)	6 (100)	12 (55)	
Lymph node removed	12±9	14±6	12±10	0.566
Surgery, n (%)				
Mastectomy	7 (25)	1 (17)	6 (27)	0.522
Lumpectomy	21 (75)	5 (83)	16 (73)	
Chemotherapy, n (%)	20 (71)	4 (67)	16 (73)	0.568
Endocrine therapy, n (%)	25 (89)	4 (67)	21 (95)	0.107
Radiation treatment, n (%)				
50 Gy/25 fractions	12 (43)	3 (50)	9 (41)	0.521
40 Gy/15 fractions	16 (57)	3 (50)	13 (59)	
Time since treatment, days				
Primary examination	35±23	30±18	37±25	0.512
Follow-up examination	312±66	328±70	308±66	0.507
Total follow-up time since treatment, days	787±114	820±99	778±118	0.436

Data reported as means ± SDs or absolute numbers and percentages of patients.
P values between BCRL and non-BCRL patients.
ALND, axillary lymph node dissection.

follow-up examination between the ipsilateral and contralateral arms, a real distinction was not clear ($P = 0.09$).

Table 2 shows NIRF imaging results in the ipsilateral arm comparing four subgroups at follow-up: BCRL, non-BCRL patients, and whether patients presented with lymphatic abnormalities (Table 2). No difference was observed comparing NIRF functional data between subgroups.

Lymphatic Contraction Frequency after Hyperthermia

Table 3 shows lymphatic CF in both arms after hyperthermia exposure. Comparisons in subgroups were performed between the ipsilateral and contralateral arms during hyperthermia (Table 3).

Regarding the change in CF from normothermia to hyperthermia, the joint group performed a significant increase in both arms (ipsilateral, $P = 0.01$; contralateral, $P \leq 0.01$) (Fig. 4A).

When stratifying on BCRL and morphological vessel status during hyperthermia, CF was lower in the ipsilateral arm in patients with lymphatic complications than that in the contralateral arm. Furthermore, no significant CF increase was observed from normothermia to hyperthermia in the ipsilateral arms with lymphatic complications (w/ abnormal, $P = 0.27$ and BCRL, $P = 0.32$).

Contrary, groups with no lymphatic complications (non-BCRL and patients with normal lymphatic vessel pattern) showed no difference between the ipsilateral and contralateral arms during hyperthermia. Furthermore, both arms were able to increase CF significantly from normo- to hyperthermia (w/o abnormal ipsilateral, $P = 0.01$ and contralateral, $P = 0.02$) (non-BCRL ipsilateral, $P < 0.01$ and contralateral, $P < 0.01$) (Fig. 4B).

Finally, the ipsilateral arm of BCRL patients performed a lower CF than the ipsilateral arm of non-BCRL patients ($P = 0.03$).

DISCUSSION

This prospective longitudinal study is the first to document that lymphatic function and morphology deteriorate during the first year after breast cancer surgery, adjuvant RT, and systemic therapy. The collecting lymphatic vessels

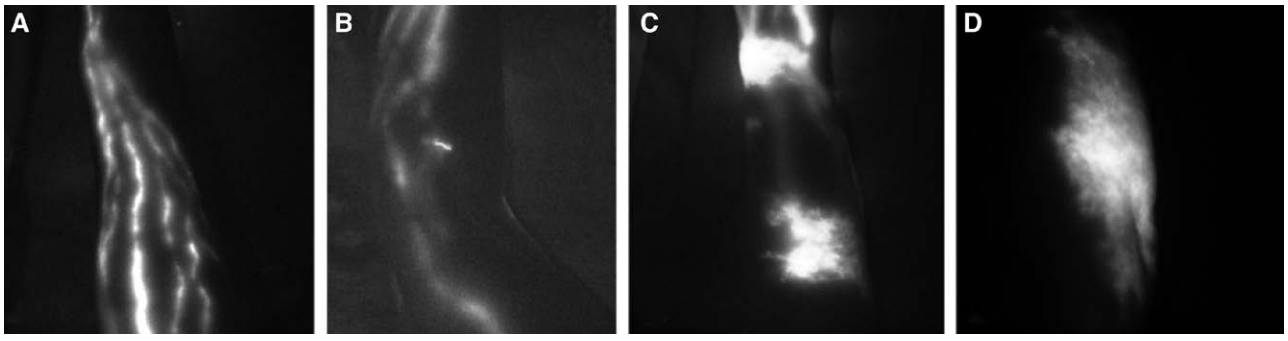

Fig. 2. Lymphatic vessel morphology. A, Linear pattern with fairly straight, distinguishable vessels, which is considered a normal pattern. B, Illustration of a single tortuous vessel. C, Stardust pattern with more tortuous vessels and lymphatic rerouting. D, Diffuse pattern with a large area of lymphatic rerouting and dermal backflow with indistinguishable vessels.

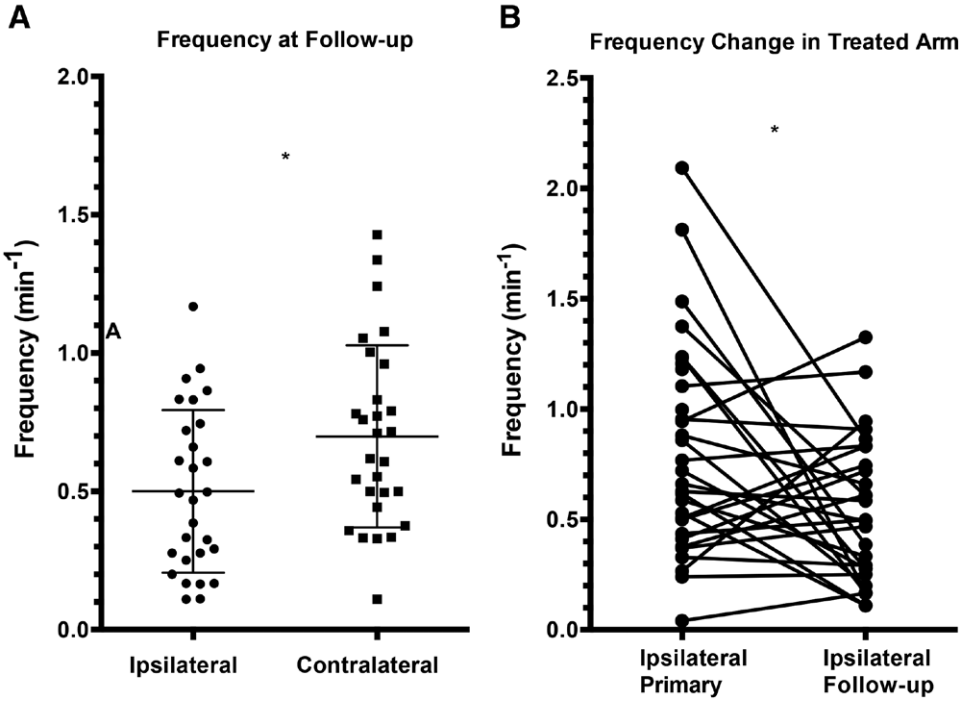

Fig. 3. Contraction frequency. A, Lymphatic CF at follow-up in the ipsilateral and contralateral arms. B, Change in lymphatic CF in the ipsilateral arm from primary to follow-up examination. (*) indicates $P < 0.05$. N = 28.

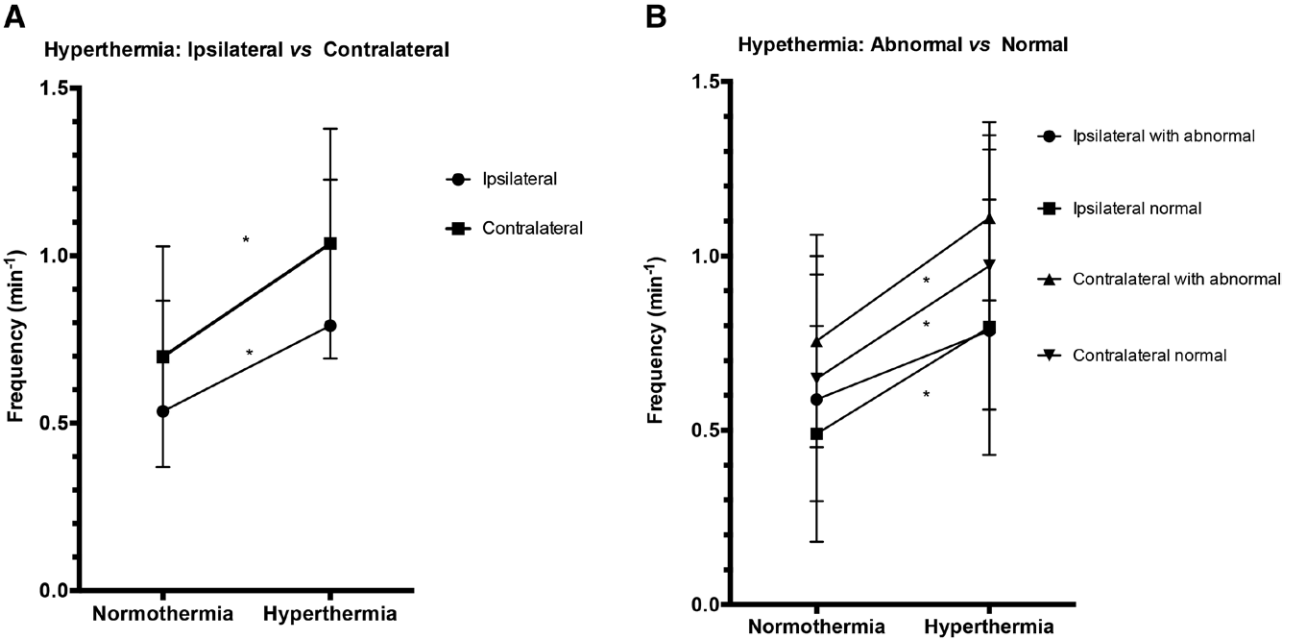

Fig. 4. Hyperthermia. A, Change in lymphatic CF from normothermia to hyperthermia in the ipsilateral (●) and contralateral (■) arms in the joint group. B, Change in the lymphatic CF from normothermia to hyperthermia in both the ipsilateral and contralateral arms separated on whether patients presented lymphatic morphologic abnormalities in the ipsilateral arm or not. Thus, "contralateral with abnormal (▲)" indicates that it is coupled to the "ipsilateral with abnormal (●)" but the contralateral arm itself does not show any morphologic abnormalities. Contralateral normal (▼) and ipsilateral normal (■). (*) indicates $P < 0.05$. N = 28.

in the arms of this patient cohort had a 23% lower CF in the ipsilateral arm than the contralateral arm, and CF was reduced by 40% at follow-up compared with the primary examination. Almost half of the patients presented morphological lymphatic abnormalities in the ipsilateral arm, and after complete follow-up, 21% of patients had developed BCRL. Subgroups with lymphatic complications were found to have a reduced reserve capacity of lymphatic contraction, being unable to increase CF in response to stressful hyperthermic conditions, while

patients with a normal, linear pattern of collecting vessels responded expectedly with an increased CF as showed in the previous studies.[8,18]

Decreasing Lymphatic Contractility Could Be Associated with Impaired Vessel Integrity

Under normal conditions, approximately 8 L of protein-rich fluid is filtered daily from the blood capillaries to interstitial compartments, which is reabsorbed to recirculate in the systemic blood system to maintain fluid balance.[19,20] As proposed by Levick and Michel,[21] the tissue fluid balance depends critically on lymphatic drainage and to a much lesser extent on venous reabsorption. Thus, tissue fluid accumulation may be caused by a mismatch between capillary filtration and lymphatic drainage.

In this study, we have revealed decreasing lymphatic drainage capability in the upper extremity of breast cancer patients expressed by significantly lower lymphatic CF than in the contralateral arm. Even lower CFs of $0.30 \pm 0.30\ min^{-1}$ were found in another group of BCRL patients, suggesting that BCRL and low CFs are correlated.[12]

In 2012, Mihara et al[22] characterized biopsies of lymphatic vessels from patients who developed lymphedema after inguinal lymphadenectomy. They showed how the histochemical composition and macroscopic appearance correlated to lymphedema stage with normal vessels progressing to end-stage sclerotic dysfunctional vessels. They found that the smooth muscle cells surrounding lymphatic vessels were increasingly infiltrated by collagen fibers, in turn limiting the contractility of the lymphatic vessels.[22] We were not able to directly correlate a declining CF with decreasing lymphatic function and BCRL development, which partly could be explained by the restricted population size as well as noise from interindividual CF differences. In light of the findings by Mihara et al,[22] it is possible that the progression of declining CF correlates to the histological changes in lymphatic vessel composition after increased endolymphatic pressure. It remains to be answered whether a declining inotropy is a direct cause of impaired contractility and vessel integrity or merely an adaptation to new pressure challenges.

Abnormal Lymphatic Vessel Morphology May Be Linked to Dysfunctional Valves and BCRL Development

Lymphatic vessels are known to be spontaneously contracting[23,24] and have bicuspid valves that divide the vessel into small functional units called lymphangions. When a series of coordinated lymphangions contract, the valves are crucial for maintaining unidirectional downstream flow preventing backward flow. The functional integrity of the valves highly depends on the diameter of the vessel.[25–27] If or when the lymphatic vessels dilate as a response to increased endolymphatic pressure after lymphadenectomy, the valves of impaired segments may not close adequately, allowing lymph to flow backward. In our study, we observed lymphatic morphologic abnormalities in the forearm approximately 30–50 cm peripherally from the actual lymphatic injury in the field of surgery and RT. After injecting the fluorophore, it traveled proximally via straight collecting vessels, but at some point, leaking backward into bundles of torturous vessels eventually extravascularising into interstitial compartments like described in several studies.[10–12] (**See Video 1 [online],** which demonstrates dermal re-routing and dermal backflow pattern.) (**See Video 2 [online]**, which demonstrates a normal lymphatic vessel pattern.) The underlying exact mechanism remains to be described, but we speculate that in early stages of BCRL, in confined areas of the lymphatic vasculature more susceptible to increased pressure, valves may become inadequate allowing backward flow into lymphatic capillaries, while lymph stasis contributes to leaking into interstitial compartments.

Like in the primary study as well as several others, especially in patients with lymphatic complications, we observed a higher lymphatic propulsion velocity in the ipsilateral arm compared contralaterally ($P = 0.01$).[15,28,29] This may be caused by the lymphatic vessels being slightly more contracted and with reduced compliance due to collagen deposition, so that if the lymphatic vessel diameter decreases, the velocity of the fluid increases during a constant flow.

Furthermore, in our study, 38% of patients with lymphatic abnormalities had developed BCRL and more may develop BCRL later, indicating that these lymphatic structural changes may be associated with a lymphatic disorder. Rasmussen et al[12] observed similar lymphatic architectural changes in all symptomatic limbs investigated. Accordingly, studies suggest that by visualizing morphological changes early after cancer treatment, lymphatic disorder could be detected before serious fluid accumulation, and importantly, early treatment has proven to prevent or limit BCRL.[10]

Decreased Ability to Mobilize Reserve Capacity of Lymphatic Contraction

Inducing local hyperthermia has previously been demonstrated to increase lymphatic CF in human extremities and is used to stress the lymphatic vessels.[8] Increased local skin temperature increases blood flow, capillary permeability, and fluid filtration into interstitial compartments, thus increasing the fluid load on the lymphatic vasculature. The purpose of this test was to indirectly disclose the lymphatic vessel's ability to mobilize reserve capacity of contraction. In previous studies, inducing hyperthermia has proven to increase lymphatic CF by 32%–135% in healthy subjects.[8,9,18]

In line with above-mentioned theories, it was highly interesting that BCRL patients and patients with lymphatic morphological abnormalities were not able to increase CF significantly (Table 1), whereas their own contralateral arms as well as remaining subgroups demonstrated expected increases in CF. Mohanakumar et al[28] found that Fontan patients, a particular group of patients with congenital heart disease prone to lymphatic complications, have similar difficulties in increasing CF after inducing hyperthermia.

Our results suggest that the lymphatic vasculature of patients with morphological changes was already challenged and operated in the range of maximal functional capacity during normothermic conditions possibly due to

vessel wall degeneration and valve insufficiencies. Hence, it was not able to increase activity even further during extra fluid load. This supports the hypothesis that patients with lymphatic abnormalities may be in a subclinical asymptomatic state with increased vulnerability to developing symptomatic BCRL.

In future studies, it would be interesting to try to determine a potential threshold of lymphatic dysfunction associated with clinically relevant lymphadenopathy, useful in the clinic to target candidates who could benefit from early treatment.

Study Limitations

The indocyanine green-based NIRF technique is limited to visualizing superficial collecting lymphatic vessels within a depth of a few centimeters below the skin. Deeper lying lymphatics, for instance, around muscle tissue, were, therefore, not included in the measurements.

We measured the lymphatic function at 1-year follow-up, but lymphatic changes could potentially appear later, since some patients develop lymphedema up to 10 years after breast cancer treatment. Therefore, this study can not be regarded as a long-term study, and thus, it would be relevant to continue following this study population to detect late complications beyond the follow-up of this study.

The diagnosis of BCRL is difficult since it is a slowly progressive and potentially reversible condition with varying symptomatology. Many diagnostic methods have been proposed, yet today no internationally acknowledged definition or diagnostic methods exist.[3] We defined BCRL as when diagnosed by experienced personnel at the lymphedema clinic, AUH, following the lymphedema definition from the DBCG RT Skagen trial 1. This is an inexpensive method, reliable when used by trained assessors. Currently, it may be the most appropriate method in the long-term evaluation because it detects size change and interlimb size differences irrespective of tissue composition of the lymphedema. However, it has little sensitivity detecting preclinical lymphedema and does not take patient's body composition into account.

Finally, a limitation of this study is patients being their own controls, since many facets of breast cancer treatment would potentially affect the lymphatic system systemically including the control arm. Thus, to strengthen this study, a matched control group or a pretreatment lymphatic examination would have been appreciated. Speculations about the influence of different chemotherapy regimens on lymphatic function also need further investigation.

The prospective study design with 12 months of follow-up in the same cohort and only three patients lost to follow-up was a clear advantage of this study. Furthermore, patients were consecutive and treated at a single center after identical guidelines (DBCG).

CONCLUSION

In this longitudinal prospective study of a cohort of node-positive early breast cancer patients following locoregional RT, we are the first to demonstrate that lymphatic contractile function in the treated arm diminishes over time. Furthermore, the presence of abnormal torturous bundles of lymphatic vessels and degrees of dermal backflow of lymphatic fluid in asymptomatic arms seem to be associated with weak lymphatic reserve pumping capacity. Thus, this study provides evidence that the lymphatic function and morphology in the treated arm are affected at 1-year follow-up by the cancer treatment and put forward overall suggestions of pathological causality, but the exact mechanisms remain to be described.

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ETHICAL APPROVAL STATEMENT

The Regional Committee on Health Research Ethics of the Central Denmark Region (1-10-72-193-18) has approved this study. The study is registered on ClinicalTrials.gov (identifier: NCT03572998). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, revised in 2013.

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REFERENCES

1. Ridner SH. Quality of life and a symptom cluster associated with breast cancer treatment-related lymphedema. *Support Care Cancer*. 2005;13:904–911.
2. Taghian NR, Miller CL, Jammallo LS, et al. Lymphedema following breast cancer treatment and impact on quality of life: a review. *Crit Rev Oncol Hematol*. 2014;92:227–234.
3. DiSipio T, Rye S, Newman B, et al. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. *Lancet Oncol*. 2013;14:500–515.
4. Gillespie TC, Sayegh HE, Brunelle CL, et al. Breast cancer-related lymphedema: risk factors, precautionary measures, and treatments. *Gland Surg*. 2018;7:379–403.
5. Tsai RJ, Dennis LK, Lynch CF, et al. The risk of developing arm lymphedema among breast cancer survivors: a meta-analysis of treatment factors. *Ann Surg Oncol*. 2009;16:1959–1972.
6. Ribeiro Pereira ACP, Koifman RJ, Bergmann A. Incidence and risk factors of lymphedema after breast cancer treatment: 10 years of follow-up. *Breast*. 2017;36:67–73.
7. Armer JM, Stewart BR. Post-breast cancer lymphedema: incidence increases from 12 to 30 to 60 months. *Lymphology*. 2010;43:118–127.
8. Kelly B, Mohanakumar S, Telinius N, et al. Function of upper extremity human lymphatics assessed by near-infrared fluorescence imaging. *Lymphat Res Biol*. 2020;18:226–231.
9. Groenlund JH, Telinius N, Skov SN, et al. A validation study of near-infrared fluorescence imaging of lymphatic vessels in humans. *Lymphat Res Biol*. 2017;15:227–234.
10. Akita S, Mitsukawa N, Tokumoto H, et al. Regional oxygen saturation index: a novel criterion for free flap assessment using tissue oximetry. *Plast Reconstr Surg*. 2016;138:510e–518e.
11. Yamamoto T, Yamamoto N, Doi K, et al. Indocyanine green-enhanced lymphography for upper extremity lymphedema: a

novel severity staging system using dermal backflow patterns. *Plast Reconstr Surg*. 2011;128:941–947.
12. Rasmussen JC, Tan IC, Marshall MV, et al. Human lymphatic architecture and dynamic transport imaged using near-infrared fluorescence. *Transl Oncol*. 2010;3:362–372.
13. Shah C, Arthur DW, Wazer D, et al. The impact of early detection and intervention of breast cancer-related lymphedema: a systematic review. *Cancer Med*. 2016;5:1154–1162.
14. Soran A, Ozmen T, McGuire KP, et al. The importance of detection of subclinical lymphedema for the prevention of breast cancer-related clinical lymphedema after axillary lymph node dissection; a prospective observational study. *Lymphat Res Biol*. 2014;12:289–294.
15. Alstrup M, Johannessen AL, Mohanakumar S, et al. Lymphatic function in the arms of breast cancer patients-a prospective cohort study. *Plast Reconstr Surg Glob Open*. 2021;9:e3779.
16. Donker M, van Tienhoven G, Straver ME, et al. Radiotherapy or surgery of the axilla after a positive sentinel node in breast cancer (EORTC 10981-22023 AMAROS): a randomised, multicentre, open-label, phase 3 non-inferiority trial. *Lancet Oncol*. 2014;15:1303–1310.
17. Mohanakumar S, Telinius N, Kelly B, et al. Morphology and function of the lymphatic vasculature in patients with a Fontan circulation. *Circ Cardiovasc Imaging*. 2019;12:e008074.
18. Olszewski W, Engeset A, Jaeger PM, et al. Flow and composition of leg lymph in normal men during venous stasis, muscular activity and local hyperthermia. *Acta Physiol Scand*. 1977;99:149–155.
19. von der Weid PY, Zawieja DC. Lymphatic smooth muscle: the motor unit of lymph drainage. *Int J Biochem Cell Biol*. 2004;36:1147–1153.
20. Breslin JW. Mechanical forces and lymphatic transport. *Microvasc Res*. 2014;96:46–54.
21. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res*. 2010;87:198–210.
22. Mihara M, Hara H, Araki J, et al. Indocyanine green (ICG) lymphography is superior to lymphoscintigraphy for diagnostic imaging of early lymphedema of the upper limbs. *PLoS One*. 2012;7:e38182.
23. Telinius N, Drewsen N, Pilegaard H, et al. Human thoracic duct *in vitro*: diameter-tension properties, spontaneous and evoked contractile activity. *Am J Physiol Heart Circ Physiol*. 2010;299:H811–H818.
24. Telinius N, Majgaard J, Mohanakumar S, et al. Spontaneous and evoked contractility of human intestinal lymphatic vessels. *Lymphat Res Biol*. 2017;15:17–22.
25. Bertram CD, Macaskill C, Moore JE Jr. Simulation of a chain of collapsible contracting lymphangions with progressive valve closure. *J Biomech Eng*. 2011;133:011008.
26. Breslin JW, Yang Y, Scallan JP, et al. Lymphatic vessel network structure and physiology. *Compr Physiol*. 2018;9:207–299.
27. Davis MJ, Rahbar E, Gashev AA, et al. Determinants of valve gating in collecting lymphatic vessels from rat mesentery. *Am J Physiol Heart Circ Physiol*. 2011;301:H48–H60.
28. Mohanakumar S, Kelly B, Turquetto ALR, et al. Functional lymphatic reserve capacity is depressed in patients with a Fontan circulation. *Physiol Rep*. 2021;9:e14862.
29. Tan IC, Maus EA, Rasmussen JC, et al. Assessment of lymphatic contractile function after manual lymphatic drainage using near-infrared fluorescence imaging. *Arch Phys Med Rehabil*. 2011;92:756–764.e1.