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Breast

Lymphatic Function in the Arms of Breast Cancer Patients—A Prospective Cohort Study

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Background: Lymphedema is a highly feared complication of breast cancer treatment, but the underlying complex mechanisms are still unknown. Thus, we investigated the lymphatic morphology and contractility in the lymphatic vessels of arms of high-risk breast cancer patients treated for node-positive early breast cancer.

Methods: In this prospective cohort study 32 women treated for unilateral node-positive breast cancer were enrolled and studied 36 ± 23 days after loco-regional radiotherapy. Near-infrared fluorescence imaging was used to assess morphology and function of the superficial lymphatic vessels. Strain-gauge plethysmography was performed to evaluate the capillary filtration of fluid.

Both arms were investigated, with the non-treated arm acting as control. The patients were questioned about the presence of lymphedema yearly and finally 574 ± 118 days after ended radiotherapy.

Results: Morphologically, 25% of the treated arms expressed lymphatic vessel abnormalities compared to the control arms ($p = 0.0048$). No difference in functional parameters (maximal pumping pressure, $p = 0.20$; contraction frequency, $p = 0.63$; contraction velocity, $p = 0.55$) was found between the treated and control arms. Patients who later developed lymphedema had a difference in velocity compared to those who did not develop lymphedema ($p = 0.02$). The capillary filtration rate was similar between the two arms ($p = 0.18$).

Conclusions: Peripheral lymphatic vessels were morphologically changed in the ipsilateral arm in 25% of the patients and patients who later developed lymphedema showed an early increase in velocity. Other functional parameters and capillary filtration were unchanged in this early phase. These discrete changes might be early indicators of later development of lymphedema. *(Plast Reconstr Surg Glob Open 2021;9:e3779; doi: 10.1097/GOX.0000000000003779; Published online 25 August 2021.)*

INTRODUCTION

Breast cancer is the most common and most deadly cancer among women.[1] Recent advances in the treatment of breast cancer have increased the attention on the lifelong complications following this disease, including lymphedema. Approximately one in five breast cancer patients experience breast cancer-related lymphedema (BCRL) with estimates ranging from 13% to 65%.[2,3] Why lymphedema occurs as a consequence of breast cancer treatment is still not completely understood; however, axillary lymph node dissection (ALND) and radiotherapy have been recognized as major risk factors.[4–6]

The consequences of BCRL can be severe, both physical and psychological. Examples include a decrease of physical strength and range-of-motion of the shoulder, and sensibility in the arm as well as an increased feeling of pain and heaviness. Anxiety, depression, sexual

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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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List of products, devices, and drugs used: *Indocyanine Green (ICG); 785 nm 450 mW laser (PowerTechnology, Ark.); Electron-multiplier charge-coupled device camera (C9100-13 Hamamatsu, Japan); Hokanson sphygmomanometer cuff (Marcom Medical Denmark); Hokanson E20 Rapid cuff inflator, Hokanson AG101 air source, SC10 cuff; (Marcom Medical, Denmark); Strain gauge plethysmography (Hokanson EC6 and Hokanson E20; Marcom Medical, Denmark); Analog-to-digital converter (ADInstruments, Oxford, United Kingdom); LabChart 7 software; Custom-written LabView program (National Instruments, Tex.); and Stata/SE 15.1 for Mac (StataCorp, Tex.).*

dysfunction, reduced body confidence, and a lowering of the general quality of life have also been observed in these patients.[7–12]

The current treatment of lymphedema consists primarily of bandaging, compression garments, manual lymphatic drainage, and exercise. Although the current treatment options can help patients with lymphedema to some degree, the patients still have to undergo lifelong treatment to manage the condition.[13,14] The discovery of an intrinsic contractile function in the lymphatic vessels[15] combined with the development of new imaging techniques, such as near-infrared fluorescence (NIRF) imaging, has recently lead to an adequate in vivo functional description and hitherto unseen insight into lymphatic morphology.[16] Decreased functional parameters and abnormal morphology have been shown in patients with BCRL indicating that the lymphatic vasculature plays a role in the development of this condition.[17–19] Yet, no studies have examined if these functional and morphological findings are present before the development of BCRL. Thus, this prospective cohort study aimed to examine the baseline lymphatic function and morphology in women who have recently undergone and completed treatment for breast cancer.

METHODS

Ethics 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, 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. Participants were included at Aarhus University Hospital, Denmark, in the period from September 2018 to December 2019.

The population consisted of 32 consecutive women with unilateral breast cancer who all underwent both surgery and completed locoregional radiotherapy before the lymphatic examination. Surgical procedure consisted of either lumpectomy or mastectomy including sentinel node biopsy or ALND. Patients who had adjuvant chemotherapy (three cycles pirubicin/cyclophosphamide followed by three cycles paclitaxel, total 18 wks) started the systemic therapy within 1 month after the last surgery, and the radiotherapy was initiated 2 weeks after the last chemotherapy. Patients who had surgery and no chemotherapy started radiotherapy 4–6 weeks after the last surgery. If endocrine therapy was recommended, this was prescribed already before radiotherapy. All patients participated in the DBCG RT Skagen trial 1 (NCT02384733). In that trial, patients treated with locoregional radiotherapy are randomized between 50 Gy/25 Fr versus 40 Gy/15 Fr, and the primary endpoint is arm lymphedema at 3 years (dbcg.dk). They were recruited during their course of radiotherapy. Patients with previous breast cancer, ductal carcinoma in situ of the breast, bilateral breast cancer, previous radiation therapy to the chest region, or metastasis of their breast cancer were not eligible. If clinical lymphedema had developed before the examination the patient was also excluded. Both arms of the participants were examined. The arm adjacent to the treated breast was labeled "ipsilateral arm" and the opposite arm was labeled "contralateral arm." The patients served as their own controls. The examination was done after the completion of radiotherapy and no later than half a year after completion. All patients underwent the same procedure and were under similar conditions, on the same segment of the arm, and in a supine position. The temperature was kept constant at 21 ± 1°C during the examination.

Patients were contacted regarding lymphedema status yearly. The last contact was on January 18, 2021, and was validated through their medical records.

Endpoints

Our primary endpoint consisted of the lymphatic functional parameters. Secondary outcomes included morphological abnormalities and plethysmography.

Study Procedure

Half an hour before the arrival of the patient, the fluorescent dye, indocyanine green (ICG) (Diagnostic Green GmbH, Germany), was dissolved in sterile water and diluted with isotonic saline. A 0.1 mL injection of 0.025% ICG dissolved in saline, equivalent to a 25-µg dose per injection, was injected intradermally three times on each hand using a 30-G needle. The injections were given dorsally between the second and third proximal phalanges, between the fourth and fifth proximal phalanges, and the last on the palmar wrist. The fluorophore was excited using a custom-designed 785 nm 450mW laser (PowerTechnology, Ark.), with a 780 ± 28 nm band-pass filter to minimize light leakage. The emitted light from the fluorophore was collected with an electron-multiplier charge-coupled device camera (C9100-13 Hamamatsu, Japan) with a Navitar lens (25 mm f0.95) and two 835 nm ± 15 nm (>OD5) band-pass filters mounted in front and behind.

Three sequences were recorded of each arm: injection sequence, baseline frequency and velocity, and maximal

pumping pressure. Last, the capillary filtration rate (CFR) was measured using strain gauge plethysmography.

Injection Sequence

The recording was initiated, and the injection of the fluorophore began. The small cutaneous lymphatic vessels collected the injected fluorophore and the morphology of the collecting lymphatic vessels was visualized.

Baseline Frequency and Velocity

Ten minutes after the injection sequence, the baseline frequency and velocity sequence was started and the flow was recorded for 6 minutes on the forearm.

Maximal Pumping Pressure

A tourniquet was placed distally on the forearm to occlude the lymphatic flow. Subsequently, the lymphatic vessels proximal to the tourniquet were emptied manually. Then, a Hokanson sphygmomanometer cuff (Marcom Medical Denmark) was placed proximally to the tourniquet and inflated to a pressure of 80 mm Hg. The strap was then removed. This ensured that all lymphatic fluid that passed the cuff originated distally from the cuff. The pressure was then lowered every fifth minute by 5 mm Hg until the lymphatic vessels were able to generate a pressure equal to or higher than the current pressure employed by the cuff (Hokanson E20 Rapid cuff inflator, Hokanson AG101 air source, SC10 cuff; Marcom Medical, Denmark). When this equilibrium was achieved or surpassed, the lymphatic fluid passed the cuff and filled the lymphatic vessels proximal to the cuff. The pressure level at this point was noted as the maximal pumping pressure.

Adequate Imaging Quality

All analyzed sequences had at least one lymphatic propulsion in the distal-to-proximal direction; however, lymphatic propulsion was not observed in all fluorescent vessels. In all the analyzed maximal pumping pressure sequences, the lymphatic fluid surpassed the cuff and filled the vessels proximal of the cuff.

Capillary Filtration Rate

The CFR ($\mu L \times 100\,ml^{-1}$ tissue $\times\ min^{-1}$) of the arms was measured using a strain gauge plethysmography setup (Hokanson EC6 and Hokanson E20; Marcom Medical, Denmark) connected to a PC using an analog-to-digital converter (ADInstruments, Oxford, United Kingdom) and analyzed using LabChart 7 software. Initially, a sphygmomanometer cuff was placed around the brachium of the participant. The cuff was then inflated to a pressure of 20 mm Hg and increased with 10 mm Hg every 3 minutes until a pressure of 80 mm Hg was reached. The change in circumference was recorded continuously by the strain gauge, placed distal to the cuff. Initially, the increase in venous pressure resulted in a rapid, nonlinear increase in the volume of the arm, due to venous distension. Subsequently, the greater hydrostatic pressure in the capillaries increased the CFR, resulting in a modest linear increase in the circumference of the arm, due to an increase in the filtration and thus the interstitial fluid volume.

Morphology

Each sequence was blinded and evaluated by two investigators independently for the presence of abnormalities. If there was any discordance over whether lymphatic abnormalities were present, a third investigator's evaluation was acquired. Normal lymphatic vessels of the forearm were characterized as ascending in a distal-proximal direction in a straight manner. Morphological abnormalities were characterized according to a previous study[18] (Fig. 1).

Data Analysis and Statistics

All recorded sequences were analyzed in a custom-written LabView program (National Instruments, Tex.). Regions of interest (ROIs) were plotted in the recorded lymphatic vessels. The intensity of the fluorescence at the ROIs was measured and allowed for the calculation of the contraction frequency and the velocity. Contractions were seen as a transient increase in intensity and by counting the number of events over time, the contraction frequency (events/minute) could be calculated. The velocity (cm/s) was calculated by timing seconds spent by the lymph packet traveling a set distance of at least 5 cm between two ROIs. Results are reported as mean ± SD for continuous data and as absolute numbers and percentages of participants for binary data.

Differences between groups were calculated using paired and unpaired Student's t-test as well as two-way ANOVA for Gaussian distributed data and Wilcoxon and Mann–Whitney test for non-normal distributed data. Some data achieved a Gaussian distribution through reciprocal transformation, and this was done before any data analysis. Chi-square test and Fisher's exact test were used for binary data. Results were considered statistically significant when the P value was less than 0.05. Statistical analysis was performed using Stata/SE 15.1 for Mac (StataCorp, Tex.). Based on a mean pumping pressure found in an NIRF validation study of 59 mm Hg and an SD of 12.7 a total of 23 or more study subjects were targeted to be included in this clinical trial.[16] The probability that the study would detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments was 10 mm Hg, was 80%.

RESULTS

Thirty-three patients who completed radiotherapy were included. One had developed lymphedema before the examination day and as such was excluded. The total number of participants was 32 patients. The time since ended radiotherapy was 36 ± 23 days.

An average of 2.76 ± 0.92 and 2.82 ± 0.83 lymphatic vessels were analyzed in the ipsilateral arm and contralateral arm, respectively. Demographic data of the patients are summarized in Table 1.

Table 2 shows the functional and morphological data of the patients. The ipsilateral arm had a higher occurrence of abnormalities of their peripheral lymphatic vessels compared to the contralateral arm. There was no difference in maximal pumping pressure, contraction frequency, and contraction velocity between the ipsilateral arm and the contralateral arm.

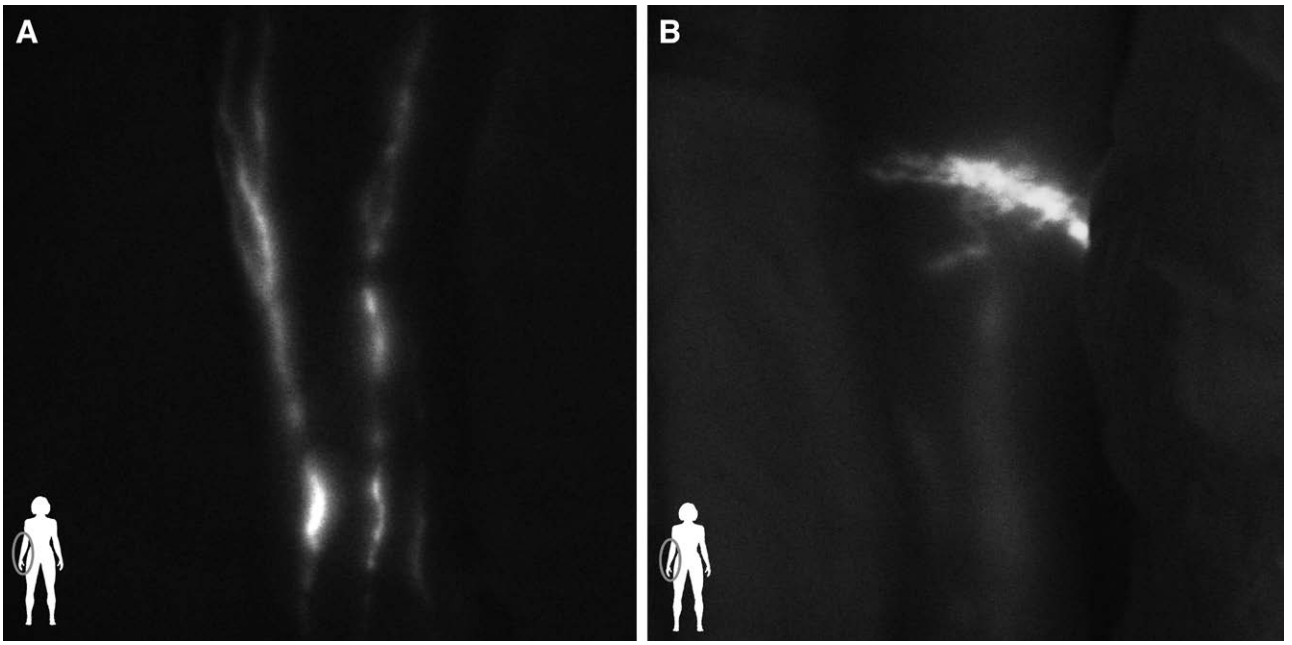

Fig. 1. Images of lymphatic vessels. A, Ipsilateral forearm presenting with normal lymphatic morphology. B, Ipsilateral forearm presenting with lymphatic abnormalities.

Table 3 shows the functional and morphological data of the ipsilateral arm of patients destined to develop BCRL and patients who did not develop BCRL. No difference was observed regarding maximal pumping pressure and contraction frequency. A difference was observed in contraction velocity ($P = 0.02$). The CFR is illustrated in Figure 2. No difference between the two arms was observed.

All patients who presented with lymphatic abnormalities underwent ALND, whereas 50% of the patients who did not present with lymphatic abnormalities underwent ALND ($P = 0.01$). No other difference was observed between these groups. The number of observations differs due to some sequences (NIRF and plethysmography) being inadequate for proper analysis.

Table 1. Characteristics of Participants Who Completed Breast Cancer Treatment

	Breast Cancer-treated Patients, n = 32	Patients Destined to Develop BCRL, n = 6	Non-BCRL Patients, n = 26
Demographics			
Age, y	53 ± 12	46 ± 8	55 ± 13
Weight, kg	74 ± 14	63 ± 8	77 ± 14
Height, cm	167 ± 6	165 ± 5	168 ± 6
Body mass index, kg/m^2	26 ± 5	23 ± 3	27 ± 5
Axillary surgical type, n (%)			
Sentinel node	12 (38)	0 (0)	12 (46)
ALND	20 (63)	6 (100)	14 (54)
Lymph nodes removed	12 ± 9	14 ± 4	12 ± 9
Operation, n (%)			
Mastectomy	9 (28)	1 (17)	8 (31)
Lumpectomy	23 (72)	5 (83)	18 (69)
Chemotherapy, n (%)	24 (75)	5 (83)	19 (73)
Endocrine therapy, n (%)	28 (88)	5 (83)	23 (88)
Radiation treatment n (%)			
50 Gy/25 fractions	14 (44)	3 (50)	11 (42)
40 Gy/15 fractions	18 (56)	3 (50)	15 (58)
Time since treatment, days	35 ± 23	39 ± 19	35 ± 24
Follow-up time since treatment, days	574 ± 118	622 ± 82	563 ± 123

Data reported as means ± SDs or absolute numbers and percentages of patients.

DISCUSSION

We present for the first time the vascular filtration and lymphatic function in patients who recently underwent locoregional radiotherapy for early node-positive breast cancer and before showing any clinical signs of lymphedema. At an average of 1 month after radiotherapy, one-quarter of patients disclosed abnormal lymphatic morphology at the lower part of their ipsilateral arm, whereas no abnormalities were detected on the contralateral arms. There were no changes in functional parameters including maximal pumping pressure, contraction frequency, and CFR. However, those who developed clinical BCRL within the follow-up period (BCRL-destined) showed a higher lymphatic velocity compared to those who did not develop clinical BCRL (non-BCRL). This indicates that the peripheral lymphatic vasculature responds to the operation, lymphatic node dissection, and subsequent radiation with both morphological and functional changes.

The formation of lymphedema occurs when there is a mismatch between the amount of fluid filtered into and the amount of fluid transported away from the interstitial space.[20] Increased vascular filtration[21] as well as a decreased lymphatic contractility of the lymphedematous arm[18,22] have been reported in patients who have developed BCRL. It is unknown whether these findings were present before the breast cancer diagnosis, developed after the axillary surgery and radiation treatment, or are merely a product of an already developed lymphedema.

Table 2. Functional and Morphological Data of the Ipsilateral and Contralateral Arm of Breast Cancer Patients

	Ipsilateral Arm, n = 32	Contralateral Arm, n = 32	*P*
NIRF imaging			
Maximum pumping pressure, mm Hg	48 ± 15*	46 ± 14*	0.1964
Contraction frequency, min^{-1}	0.9 ± 0.5*	0.8 ± 0.4*	0.6313
Contraction velocity, cm/s	1.1 ± 0.4‡	1.0 ± 0.2†	0.5504
Morphological abnormalities, n (%)	8 (25)	0 (0)	0.0048

Data reported as means ± SDs or absolute numbers and percentages of patients.
*n = 30.
†n = 28.
‡n = 25.

Our findings of unchanged lymphatic contractility and capillary filtration immediately after breast cancer treatment indicate that the lymphatic changes observed in BCRL patients are not an immediate consequence of the breast cancer treatment. More likely, the inhibition of lymphatic vessel function occurs over a timespan which more often than not exceeds the timespan in this study.[23]

Degrading lymphatic contractility will eventually render the lymphatic vessels incapable of providing the necessary movement of fluid away from the arm. In support of this hypothesis, a recent study found pathological changes in the composition of the vessel walls in the collecting lymphatic vessels.[24] These formations occurred before the onset of lymphedema and were a consequence of the increased endolymphatic pressure that follows lymphadenectomy.

The earliest pathological change in the lymphatic vessels in patients with subclinical stages of lymphedema is dilatation of the lymphatic vessels.[24] Radiation therapy could lead to inflammatory changes in the lymphatic vasculature and cause the dilatation of the vessels. At this point, we do not know if the velocity in the vessels is constant. The dilatation of the vessels could explain why we observe an increase in contraction velocity in the BCRL-destined patients. Lymphatic vessels are divided into smaller subunits, called lymphangions, by unidirectional valves.[25] These valves are essential for maintaining the unidirectional flow and preventing backflow.[25] In a contracting lymphangion, the outflow pressure is greater than

Table 3. Functional and Morphological Data of the Ipsilateral Arm of Patients Destined to Develop BCRL and Patients Who Have Not Developed BCRL

	BCRL-destined, n = 6	Non-BCRL, n = 26	*P*
NIRF imaging			
Maximum pumping pressure, mm Hg	49 ± 5	47 ± 3	0.7320
Contraction frequency, min^{-1}	1.1 ± 0.3	0.8 ± 0.1	0.1623
Contraction velocity, cm/s	1.5 ± 0.6*	1.0 ± 0.2†	0.0219
Morphological abnormalities, n (%)	3 (50)	5 (19)	0.1479

Data reported as means ± SDs or absolute numbers and percentages of patients.
*n = 5.
†n = 20.

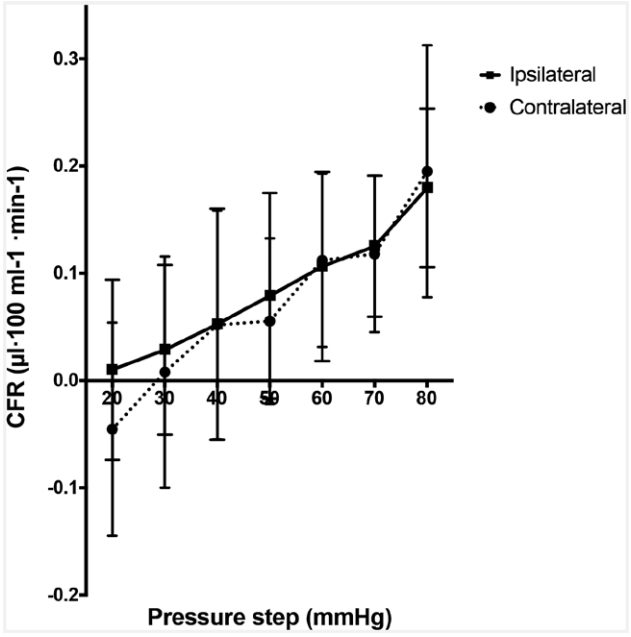

Fig. 2. CFR of the ipsilateral and contralateral arm of breast cancer-treated patients.

the inflow pressure which leads to closure of the valves.[26] When the luminal diameter increases, the valve's ability to close is compromised.[25–27] When the valves stay open, the resistance for the fluid to move into the next lymphangion is decreased and could explain the increased velocity in BCRL-destined patients. This increase in velocity is, however, not an indication of more lymphatic fluid being transported since the valve mechanism preventing backward flow is also compromised.

Incompetent lymphatic valves could also explain the morphological abnormalities observed in a quarter of the ipsilateral arms. An insufficient closure of the valves could lead to lymphatic fluid flowing backward and explain the "dermal backflow" pattern[28] we observe (Video 1). (**See Video 1 [online]**, which displays injection of indocyanine green intradermally. A, Dorsal injection on the ipsilateral arm showing a characteristic dermal backflow pattern. B, Dorsal injection on the corresponding contralateral arm showing normal uptake into the lymphatic vessels.)

Lymphatic abnormalities are well described in lymphedematous arms and have also been observed in patients treated for breast cancer with no lymphedema.[18,28] It is proposed that these early morphological abnormalities could be an indicator of subclinical lymphedema.[19] Only three of the six patients who were identified as BCRL-destined patients expressed lymphatic abnormalities. It has to be taken into consideration that the follow-up is relatively short and many more patients may develop abnormalities with time.

It is noteworthy that all patients who presented with lymphatic abnormalities had ALND which is considered a major risk factor for developing BCRL.[2] This suggests that the causality of early lymphatic abnormalities and BCRL could be similar. It is, however, important to notice that five of the eight patients who demonstrated lymphatic

morphological abnormalities were not identified as BCRL-destined patients. We suggest early lymphatic morphological abnormalities to be regarded as a possible risk factor for developing BCRL and not necessarily a precursor for BCRL.

Limitations

Due to low tissue penetration of the fluorescence, only the superficial lymphatic vessels are visualized. Also, some lymphatic vessels of the forearm might not be connected to the three injection sites of the hand and wrist and as such might not be recognized, leaving a possibility of underestimating the total amount of lymphatic vessels present in the arm.

In addition, the vessels involved in the lymphatic abnormalities were often marked unfit for functional measurements. These vessels might have a lower contractility compared to the vessels not involved in the lymphatic abnormality. As such, the average contractions per minute measured in the arms with lymphatic abnormalities might have been overestimated.

Since breast cancer treatment involves systemic treatments such as chemotherapy, endocrine therapy and zoledronic acid, both arms were affected by these treatments. A possible effect of the systemic treatment on the functionality of the lymphatic vessels would therefore have been unnoticed. This is also true regarding the possible systemic reaction to the local axillary treatment.

Only six patients were identified as destined to develop BCRL which limits the power of the statistics regarding this subgroup. The risk of type-2 errors is evident and as such interpretation of the data should be done cautiously; however, the study opens for generating hypotheses.

CONCLUSIONS

A quarter of the breast cancer patients demonstrated lymphatic morphological abnormalities immediately after completed locoregional radiotherapy. A change in function could not be shown at this point, however, patients destined to develop BCRL did have a higher contraction velocity when compared to patients who were not destined to develop BCRL. These two findings suggest that early changes in the lymphatic function and morphology could be a predictor of the risk of later diminishing lymphatic function and eventual lymphatic failure leading to lymphedema. This study also indicates that lymphatic changes leading to lymphatic failure occur over longer timespans than covered by this study. Larger studies following the lymphatic changes in breast cancer-treated patients closer and for a longer period of time is highly encouraged as it could help enlighten the pathophysiology of the development of BCRL.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68:394–424.
2. 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.
3. Gärtner R, Jensen MB, Kronborg L, et al. Self-reported arm-lymphedema and functional impairment after breast cancer treatment–a nationwide study of prevalence and associated factors. *Breast.* 2010;19:506–515.
4. Ahmed RL, Schmitz KH, Prizment AE, et al. Risk factors for lymphedema in breast cancer survivors, the Iowa Women's Health Study. *Breast Cancer Res Treat.* 2011;130:981–991.
5. Deo SV, Ray S, Rath GK, et al. Prevalence and risk factors for development of lymphedema following breast cancer treatment. *Indian J Cancer.* 2004;41:8–12.
6. 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.
7. Erickson VS, Pearson ML, Ganz PA, et al. Arm edema in breast cancer patients. *J Natl Cancer Inst.* 2001;93:96–111.
8. Ridner SH. Quality of life and a symptom cluster associated with breast cancer treatment-related lymphedema. *Support Care Cancer.* 2005;13:904–911.
9. Ridner SH. Pathophysiology of lymphedema. *Semin Oncol Nurs.* 2013;29:4–11.
10. Smoot B, Wong J, Cooper B, et al. Upper extremity impairments in women with or without lymphedema following breast cancer treatment. *J Cancer Surviv.* 2010;4:167–178.
11. Swedborg I, Wallgren A. The effect of pre- and postmastectomy radiotherapy on the degree of edema, shoulder-joint mobility, and gripping force. *Cancer.* 1981;47:877–881.
12. Vassard D, Olsen MH, Zinckernagel L, et al. Psychological consequences of lymphoedema associated with breast cancer: a prospective cohort study. *Eur J Cancer.* 2010;46:3211–3218.
13. Smile TD, Tendulkar R, Schwarz G, et al. A review of treatment for breast cancer-related lymphedema: paradigms for clinical practice. *Am J Clin Oncol.* 2018;41:178–190.
14. Piller N, Carati C. The diagnosis and treatment of peripheral lymphedema. *Lymphology.* 2009;42:146–147.
15. Telinius N, Mohanakumar S, Majgaard J, et al. Human lymphatic vessel contractile activity is inhibited in vitro but not in vivo by the calcium channel blocker nifedipine. *J Physiol.* 2014;592:4697–4714.
16. 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.
17. 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.
18. 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.

19. Akita S, Nakamura R, Yamamoto N, et al. Early detection of lymphatic disorder and treatment for lymphedema following breast cancer. *Plast Reconstr Surg.* 2016;138:192e–202e.
20. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev.* 1993;73:1–78.
21. Jensen MR, Simonsen L, Karlsmark T, et al. Microvascular filtration is increased in the forearms of patients with breast cancer-related lymphedema. *J Appl Physiol (1985).* 2013;114:19–27.
22. Modi S, Stanton AW, Svensson WE, et al. Human lymphatic pumping measured in healthy and lymphoedematous arms by lymphatic congestion lymphoscintigraphy. *J Physiol.* 2007;583(Pt 1):271–285.
23. McDuff SGR, Mina AI, Brunelle CL, et al. Timing of lymphedema after treatment for breast cancer: When are patients most at risk? *Int J Radiat Oncol Biol Phys.* 2019;103:62–70.
24. Mihara M, Hara H, Hayashi Y, et al. Pathological steps of cancer-related lymphedema: histological changes in the collecting lymphatic vessels after lymphadenectomy. *PLoS One.* 2012;7:e41126.
25. Breslin JW, Yang Y, Scallan JP, et al. Lymphatic vessel network structure and physiology. *Compr Physiol.* 2018;9:207–299.
26. 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.
27. 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.
28. 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.