Breast Cancer Research and Treatment (2020) 181:635–644
https://doi.org/10.1007/s10549-020-05661-y

CLINICAL TRIAL

Assessing breast lymphoedema following breast cancer treatment using indocyanine green lymphography

Asha Heydon-White[1] · Hiroo Suami[1] · John Boyages[1,2] · Louise Koelmeyer[1] · Karen C. Peebles[3]

Received: 2 March 2020 / Accepted: 28 April 2020 / Published online: 6 May 2020

Abstract

Purpose Breast lymphoedema is a largely unrecognised survivorship issue for women following breast cancer treatment. While a few objective methods have previously been applied to assess breast lymphoedema, none are capable of imaging breast lymphatics or identifying lymphatic morphological changes indicative of breast lymphoedema. The purpose of this study was to determine if indocyanine green (ICG) lymphography, a validated assessment technique in breast cancer-related lymphoedema), can visualise breast lymphatics and identify breast lymphoedema. Additionally, ICG lymphography was utilised to investigate lymphatic drainage pathways of the affected breast following breast-conserving therapy.

Methods Twenty female participants (10 breast lymphoedema and 10 healthy controls) were recruited for this pilot study. All underwent a medical history, physical breast assessment, tissue dielectric constant measures of breast water content, and ICG lymphography.

Results ICG lymphography identified lymphatic morphological changes in all breast lymphoedema participants (dermal backflow patterns = 10, collateral lymphatic drainage = 9) and none in the control group. The dominant lymphatic drainage pathway to the ipsilateral axilla was observed in all control participants but in only four breast lymphoedema participants. Collateral drainage pathways in the breast lymphoedema group were to: parasternal (6/10); contralateral axilla (4/10); intercostal (3/10); and clavicular (2/10) regions.

Conclusion These findings suggest ICG lymphography, through the identification of morphological lymphatic changes, is a potential qualitative objective assessment technique for breast lymphoedema. Furthermore, in this group of breast lymphoedema patients it identified changes to the normal drainage pathway of the breast. Understanding these changes will have implications for clinical management.

Keywords Breast lymphoedema · Breast cancer · Indocyanine green (ICG) · Fluorescence imaging

Abbreviations

BCRL	Breast cancer-related lymphoedema
BCT	Breast-conserving therapy
BMI	Body mass index
ICG	Indocyanine green
MLD	Manual lymphatic drainage
TDC	Tissue dielectric constant

Introduction

Breast-conserving therapy (BCT) involving breast-conserving surgery, and radiotherapy, is routinely used for managing early-stage breast cancer. BCT offers equivalent survival rates to mastectomy and is often the recommended treatment for this patient population [1]. However, despite these benefits, BCT can impede or obstruct breast lymphatic drainage causing breast lymphoedema [2].

Breast lymphoedema is characterised as persistent breast swelling and localised secondary tissue changes such as skin thickening and tissue fibrosis. Swelling occurs as lymphatic dysfunction leads to fluid stasis within the breast

✉ Asha Heydon-White
asha.heydon-white@mqhealth.org.au

1 Australian Lymphoedema Education Research and Treatment Program (ALERT), Department of Clinical Medicine, Faculty of Medicine, Health and Human Sciences, Macquarie University, Level 1, 75 Talavera Rd, Sydney, NSW, Australia

2 Icon Cancer Centre, Sydney, NSW, Australia

3 Department of Health Professions, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, NSW, Australia

subcutaneous tissue. Chronic fluid stasis induces inflammatory tissue responses, and alters both lymphatic vessel histology and surrounding subcutaneous tissue architecture, further impeding lymphatic function [3–6]. Breast lymphoedema has other untoward effects including a predisposition to cellulitis [2, 7, 8] and negative impacts on body image and quality of life [9]. Despite this, breast lymphoedema, unlike breast cancer-related arm lymphoedema (BCRL), has received scant research attention. Given the preference towards BCT for managing early-stage breast cancer, the prevalence of breast lymphoedema may be increasing [10]. Therefore, further studies investigating this condition and the impact BCT has on the lymphatic drainage of the breast are warranted.

The true incidence of breast lymphoedema is unknown, with a wide interval presented in the literature (9–70%) [8, 11–15]. One reason contributing to the paucity of research and varied incidence rates is the lack of a standardised method of assessment. The majority of studies assess breast lymphoedema using patient reported symptoms (e.g. breast pain, heaviness) and physical examination of clinical signs (e.g. swelling, erythema) [8, 15–17]. While these methods are useful in the busy clinical environment, they are inherently subjective. A few studies have used more objective methods including tissue dielectric constant (TDC) measurements to measure breast tissue water [14, 18] and conventional diagnostic imaging (e.g. mammography, [19] MRI, [20] and ultrasound [2, 10, 16, 21]). These methods are better for quantifying breast swelling and subsequent tissue changes; however, they do not allow direct visualisation of the lymphatic system. This is important as abnormal lymphatic morphology suggestive of lymphatic dysfunction precedes the clinical signs of lymphoedema [22].

Indocyanine green (ICG) lymphography offers a potential method for visualising breast lymphatics and identifying abnormal morphologies which could facilitate earlier diagnosis and intervention. In the area of breast cancer, ICG lymphography has gained momentum as a valid and reliable tool in assessing and monitoring BCRL [23, 24]. In this technique, a lymphatic specific tracer (ICG dye) is injected intradermally where it binds to albumin, [25] and is absorbed into lymphatic vessels. When excited with diode light, ICG emits near-infrared fluorescence which is filtered and recorded with a charge-coupled video camera, thereby allowing real-time imaging of lymph flow and lymphatic vasculature. In patients with BCRL, lymphatic morphological changes are characterised by dermal backflow patterns, indicating retrograde movement of lymph to capillary vessels, [26] and/or collateral drainage pathways that function to re-route lymph from areas of obstruction towards functional lymphatics [27].

Since the fundamental mechanisms of lymphoedema are similar in the arm and breast, ICG lymphography was considered to offer a promising minimally invasive technique for assessing breast lymphoedema. To our knowledge, no studies have used ICG lymphography to do this. Thus, the purpose of this study was to determine if ICG lymphography could be used to visualise the pattern and path of lymphatic drainage in patients with breast lymphoedema. We hypothesised that patients with breast lymphoedema would show dermal backflow. We also hypothesised that lymphatic drainage pathways of the breast may be altered in breast lymphoedema patients, since the primary lymphatic drainage pathway of the breast to ipsilateral axillary lymph nodes [28] is targeted during BCT.

Methods

Study participants

Twenty female participants in two groups (10 breast lymphoedema and 10 healthy controls) aged 19 to 81 years were included in this pilot study. Women with breast lymphoedema were recruited from breast cancer clinics at Macquarie University Hospital or local lymphoedema clinics. Healthy control participants, with no history of breast cancer, were recruited from the wider community.

To be included in the breast lymphoedema group, participants had to have: (i) a single episode of unilateral, non-metastatic breast cancer; (ii) axillary surgery (sentinel node biopsy or axillary lymph node dissection); (iii) completed BCT at least 6 months prior (excluding Herceptin or hormonal therapy); (iv) no active cancer; and (v) current symptoms of breast lymphoedema. Irrespective of group, participants were excluded if they: (i) had any other medical conditions that may cause breast lymphoedema (e.g. congestive cardiac failure, or current breast infection); (ii) had previous breast augmentation surgery; or (iii) were pregnant or breast feeding. Participants were also excluded if they had any contraindications to ICG dye such as hyperthyroidism or a benign thyroid tumour, and/or anaphylaxis to iodine or shellfish [29].

Experimental protocol

Participants attended a two-hour session at the ALERT Lymphoedema Clinic, Macquarie University. Participants completed questions about breast lymphoedema, general medical history, and anthropometric measurements including height and weight, from which Body Mass Index (BMI) was calculated. Participants were then positioned supine to minimise the effects of extrinsic forces on the lymphatic system (e.g. gravity), and underwent: i) physical assessment of breast lymphoedema; ii) TDC measures of breast water content; and iii) ICG lymphography, as follows.

Physical assessment of breast lymphoedema

Breast assessment, undertaken by an accredited lymphoedema therapist, involved breast observation and palpation. Each breast quadrant (upper outer, upper inner, lower outer and lower inner) was assessed for swelling, erythema, and tissue fibrosis.

TDC measures of breast water content

A hand-held TDC device (MoistureMeterD Compact®, Delfin Technologies Ltd., Finland) was used to measure breast tissue water content. The principle of this technique has been described by others [14, 30]. In brief, the device emits a high frequency (300 MHz) signal which is absorbed or reflected by tissue water to an effective depth of 2.5 mm [30]. TDC measurements, which are calculated from the reflected signal, are automatically converted by the device into a tissue water percentage (0–100%) and displayed on the screen. TDC measurements were taken in triplicate in each breast quadrant. We reported the: (i) mean tissue water percentage at each quadrant; (ii) whole breast tissue water percentage (average of each quadrant); and (iii) whole breast TDC ratio in breast lymphoedema participants (ratio of affected to unaffected breast). A previous study has reported a whole breast TDC ratio of ≥ 1.40 to be indicative of breast lymphoedema [14].

ICG lymphography

ICG lymphography was performed by HS and AHW. Both have extensive experience in this technique, completing more than 600 assessments of limb lymphoedema in the previous two years. It was performed on the affected breast of breast lymphoedema participants and bilaterally in control participants to determine if any asymmetries existed between sides.

The technique for imaging the breast was similar to that used in the arm [31]. Injection sites were marked on the participant's skin, specifically, the anterior upper torso (sites 1 and 2), and the lower outer quadrant of the breast (site 3), as shown in Fig. 1. The anatomical basis of these injection sites was based on comprehensive three dimensional radiographic tracing of breast lymphatic anatomy in human cadavers (for details see Suami et al. [28]). Prior to injection, to reduce discomfort we cooled each site using the CoolSense™ device (CoolSense Medical Ltd., Tel Aviv) [32]. A small volume (0.1 – 0.3 ml) of ICG dye (Verdye 25 mg, Aschheim-Dornach, Germany), reconstituted with 5 ml of 0.9% sodium chloride was injected (using a 30 gauge needle) intradermally at sites 1 and 2. Immediately following injections continuous lymphatic scanning with a near-infrared camera (Hamamatsu PDE-Neo II, Hamamatsu Photonics, Japan) [33] was conducted. Manual lymphatic drainage (MLD) massage was administered if lymphatic drainage pathways remained undetermined after 20 min. If ICG did not extend to the breast following injections at sites 1 and 2, a further injection was administered at site 3. Imaging

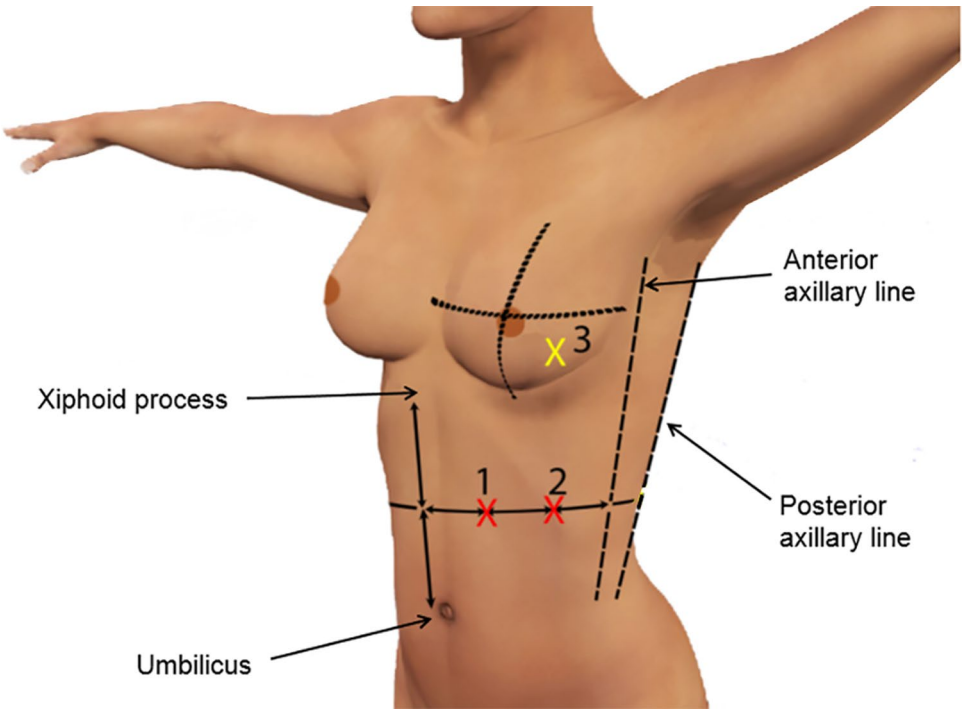

Fig. 1 ICG lymphography breast lymphoedema injection sites. Injection sites 1 and 2: the mid-point between the xiphoid process and umbilicus was identified. The horizontal distance from this mid-point to the anterior axillary line was then divided into thirds. The first injection site was marked at the distance of the first third, with the second at the second third. Injection site 3: located at the outer edge of the lower outer quadrant of the affected breast

was considered complete once lymphatic drainage pathways of the breast were identified and images became stable (no further extension of dermal backflow).

Throughout the scanning procedure, felt pens were used to mark lymphatic vessels and areas of dermal backflow on participant's skin. Scanned images were captured using a digital video recorder (MDR-600HD: Ikegami Tsushinki Co., Ltd, Japan) and still photographs were taken using both the near-infrared camera system and a digital camera. Montage images of near-infrared photographs were created by HS using Photoshop CC, Adobe Systems. Video recordings and images were stored securely for off-line analysis.

The protocol for analysing ICG lymphography data was developed in our clinic by Suami et al. [31] Analysis focused on identifying lymphatic vasculature, dermal backflow, and drainage pathways of the breast. Lymph node regions relevant to the breast were termed; ipsilateral axilla, contralateral axilla, clavicular (supraclavicular and infraclavicular lymph node region), parasternal (internal mammary lymph node region), and intercostal (lateral upper torso of affected side). Descriptive characteristics of ICG lymphography drainage pathways and area of dermal backflow were presented as individual data.

Statistical analysis

Demographic information, participant characteristics, and measures of tissue water percentage are presented as means ± standard deviation (or number and percentage). For statistical analysis, social statistics package (SPSS version 25, Surrey, UK) was used. Significance was set at $p < 0.05$ for all comparisons.

Demographic and anthropometric data were compared using an unpaired *T* test. A general linear model was employed to compare tissue water percentages between breast quadrants and sides (left and right, or affected and unaffected) for each group. When significant side-by-quadrant interactions were observed, further analysis using post hoc contrasts (Bonferroni Dunn test) was undertaken to determine between-side and between-quadrant effects. Tissue water percentage was further compared between quadrants and groups (affected breast versus control, unaffected breast versus control). In this comparison, control participants left and right quadrant data were averaged as no difference existed between the two breasts. Post hoc contrasts were examined for significant group-by-quadrant interactions.

Results

All participants completed the study and none experienced any adverse reactions. Participant characteristics are shown in Table 1. Both participant groups were of similar age and BMI (pooled mean age 51 ± 15 y and BMI, 26 ± 4 kg m^{-2}).

The breast lymphoedema participants' pathology and treatment history are summarised in Table 2. The majority (~ 70%) had T1, invasive ductal breast cancer involving the upper outer quadrant. Chemotherapy treatment was received by 80% of participants, all of which included a Taxane. All received whole breast radiation therapy (most with boost) and 50% underwent radiation to the regional lymph nodes. All breast lymphoedema participants reported clinically confirmed swelling in their affected breast and frequently reported breast heaviness (70%) and/or discomfort (70%). Fibrosis was palpated in the affected breast of most (80%), but erythema was not commonly seen (30%) or reported (20%). None of these signs or symptoms were evident or reported in the unaffected breast (breast lymphoedema group) or control group.

The TDC measures of tissue water percentage are shown in Table 3. There were significant side-by-quadrant interactions between the control group and affected breast, the unaffected and affected breasts in the breast lymphoedema group, but not between the control and the unaffected breasts. Post hoc analysis revealed more tissue water in all quadrants of the affected breast, than unaffected and control breasts. Higher tissue water content was also evident in the lower compared to the upper quadrants of the affected breast (e.g. mean tissue water was 55 ± 14% and 44 ± 9% in the lower outer and upper outer quadrant, respectively, $p < 0.05$). By contrast, water content was higher in the inner versus outer

Table 1 Participant characteristics

	Controls ($n = 10$)	Breast lymphoedema ($n = 10$)
Age (years)	45 ± 11 (range 19–58)	58 ± 15 (range 36–81)
Height (m)	1.65 ± 0.05	1.58 ± 0.06*
Weight (kg)	69 ± 10	65 ± 14
BMI (kg m^{-2})	25 ± 3	26 ± 5
Dominant arm (right)	9 (90)	9 (90)

Values are means ± SD (or %)

BMI body mass index

*$p < 0.05$ compared to control group

Table 2 Breast cancer pathology and treatment characteristics for breast lymphoedema group

	Breast lymphoedema ($n=10$)
Pathology	
Type of cancer	
Invasive ductal	8 (80)
Invasive lobular	1 (10)
Other	1 (10)
Tumour location	
Upper outer quadrant	7 (70)
Upper inner quadrant	0 (0)
Lower outer quadrant	1 (10)
Lower inner quadrant	1 (10)
Upper outer quadrant and Lower inner quadrant	1 (10)
Tumour staging	
pT1	8 (80)
pT2	2 (20)
Nodal surgery	
Sentinel node biopsy	6 (60)
Axillary lymph node dissection	4 (40)
Adjuvant treatment	
Radiotherapy area treated	
WBI	5 (50)
WBI + SCF + IMC	1 (10)
WBI + SCF/ICF	1 (10)
WBI + SCF/ICF + IMC + axilla	3 (30)
Radiotherapy boost to primary	9 (90)
Chemotherapy	8 (80)
Taxane based chemotherapy	8 (80)

Data are number and percentage. Tumour staging reported using Tumour Node Metastases (TMN) staging for breast cancer

WBI whole breast irradiation, *SCF* supraclavicular fossa lymph nodes, *ICF* infraclavicular fossa lymph nodes, *IMC* internal mammary chain lymph nodes

Table 3 Comparison of mean breast tissue water percentage in control and breast lymphoedema participants

Breast quadrant	Control ($n=10$)	Breast lymphoedema ($n=10$)	
	Average for both breasts	Unaffected	Affected
Upper outer quadrant	36 ± 6	32 ± 5	44 ± 9
Upper inner quadrant	39 ± 7*	36 ± 5*	45 ± 8
Lower outer quadrant	36 ± 6	33 ± 4	55 ± 14*
Lower inner quadrant	41 ± 5	39 ± 5*^{T}	53 ± 12*^{U}

Values are means ± SD

*$p < 0.05$ compared to upper outer quadrant

$^{U}p < 0.05$ compared to upper inner quadrant

$^{T}p < 0.05$ compared to lower outer quadrant

quadrants for both the control and unaffected breasts (e.g. mean tissue water was 39 ± 7% and 36 ± 6% in the upper inner and upper outer quadrant, respectively, $p < 0.05$). The TDC ratio was ≥ 1.40 in half the breast lymphoedema group (Table 4).

Representative ICG lymphography images of lymphatic drainage patterns and pathways for both groups are shown in Fig. 2. In all control participants, ICG dye was injected at sites 1 and 2, and flowed in a linear pattern towards the ipsilateral axillary region. No side-to-side differences or dermal backflow was observed.

For the majority of breast lymphoedema participants, ICG dye was injected only at sites 1 and 2. Three required an additional injection (site 3). ICG dye flowed towards the ipsilateral axilla in 40% of participants, all of which had a lower TDC ratio (Table 4). Irrespective of whether breast lymphoedema participants drained to the ipsilateral axilla or not, 90% exhibited collateral drainage pathways to parasternal (6/10), contralateral axilla (4/10), intercostal (3/10), or clavicular (2/10) regions (Table 4). Lymphatic drainage of the breast to inguinal regions was not observed in any participants. Participants who had undergone axillary lymph node dissection (4/10) did not drain to the ipsilateral axilla, and three demonstrated lymphatic drainage to the contralateral axilla. Drainage to the parasternal region was observed equally in participants with a whole breast TDC ratio above or below 1.40. Those with a TDC ratio ≥ 1.40 were more likely to drain to clavicular and contralateral axillary regions, while those with a TDC ratio < 1.40 to intercostal regions (Table 4). All breast lymphoedema participants had dermal backflow in their affected breast. This involved the entire breast in all participants with a high TDC ratio, and two participants with a low TDC ratio.

Discussion

This study is the first to utilise ICG lymphography to examine breast lymphoedema. We report the following novel findings. First, ICG lymphography identified dermal backflow in all breast lymphoedema, but no control participants. Second, breast lymphatic drainage pathways differed between groups. In the control group, lymph drained from the breast to the ipsilateral axilla region in 100% of participants. In the breast lymphoedema group, only 40% drained to the ipsilateral axilla and 90% drained to other regions. These findings support our hypotheses and demonstrate that ICG lymphography can be used to directly visualise the pattern and pathways of lymphatic drainage in individuals with breast lymphoedema. They also have implications for

Table 4 Breast lymphoedema participant treatment characteristics and objective assessment results ranked according to TDC ratio

Case	Lymph node treatment		Whole breast TDC ratio	Lymphatic drainage					Dermal backflow in breast
	Surgery	Regional node irradiation		Ipsilateral axilla	Parasternal	Intercostal	Clavicular	Contralateral axilla	
1	ALND	IMC + SCF	(1.81)	-	-	-	+	+	Whole breast
2	SNB	Nil	(1.77)	-	+	-	-	-	Whole breast
3	SNB	Nil	(1.64)	-	+	-	-	+	Whole breast
4	ALND	IMC + ICF/SCF + Axilla	(1.46)	-	+	-	+	-	Whole breast
5	ALND	ICF/SCF	(1.45)	-	-	-	-	+	Whole breast
6	SNB	Nil	(1.39)	+	+	+	-	-	Whole breast
7	ALND	IMC + ICF/SCF + Axilla	(1.16)	-	-	+	-	+	Lower half
8	SNB	IMC + ICF/SCF + Axilla	(1.15)	+	+	+	-	-	Whole breast
9	SNB	Nil	(1.07)	+	-	-	-	-	Lower outer quadrant
10	SNB	Nil	(1.03)	+	+	-	-	-	Lower half

Breast lymphoedema cases listed in descending TDC ratio value. Dotted line delineates participants with a TDC ratio ≥ 1.40 which is reported by Johansson and colleagues as indicative of breast lymphoedema [14]

TDC tissue dielectric constant, *ICG* indocyanine green, *SNB* sentinel node biopsy, *ALND* axillary lymph node dissection, *IMC* internal mammary chain, *SCF* supraclavicular fossa, *ICF* infraclavicular fossa, + present, − absent

clinical management, such as informing clinicians of patent pathways during manual lymphatic drainage (MLD).

Methodological considerations

As ICG lymphography has not been previously used to assess breast lymphoedema, there are no studies to which we can directly compare our findings. However, ICG lymphography is known to be a valid and reliable technique for assessing BCRL [24]. Our protocol for breast lymphoedema was modified from our published protocol for analysing arm lymphoedema (BCRL) [31] with careful consideration of breast lymphatic anatomy to determine injection sites [28, 34–36]. In the late 1800s, Sappey described the lymphatics of the breast draining exclusively via a subareolar plexus to axillary lymph nodes [34]. This pathway has been confirmed by others and imaging studies using techniques such as lymphoscintigraphy have applied injections into this plexus to identify the sentinel lymph nodes in breast cancer patients [36]. The significance and exclusivity of the subareolar plexus in draining the breast is however an area of debate. Other researchers have identified lymphatic vessels of the breast that both bypass the subareolar plexus and drain to other lymph nodes including the internal mammary and intercostals [28, 35]. In cadaveric studies, Suami et al. [28] showed that superficial breast lymphatic vessels originate in the periphery of the anterior upper torso before travelling through the breast to the axillary lymph nodes. Based upon these findings, injection sites 1 and 2 were positioned in the anterior upper torso.

A third injection site in the breast (site 3) was selected with the knowledge that in cases of breast lymphatic obstruction, lymph drainage of the anterior upper torso may bypass the breast via collateral pathways (Fig. 1). As ICG dye has a high fluorescence intensity at injection sites, rather than injecting the subareolar plexus we placed site 3 at the periphery of the lower outer quadrant to ensure that the injection did not inhibit visualisation of breast lymphatic vasculature. Moreover, anecdotal evidence from our clinical experience suggests lymphatic drainage of the breast in breast lymphoedema patients occurs in a lateral to medial direction. Accordingly, we were confident that these injection sites were sufficient to give a comprehensive analysis of breast lymphatic vasculature, drainage patterns, and pathways.

Drainage patterns and pathways in breast lymphoedema

Our observation of linear drainage patterns in the control group and dermal backflow in the breast lymphoedema group was not unexpected. In accordance with our findings, Yamamoto et al. [24] employed ICG lymphography to assess BCRL and reported linear drainage patterns in the unaffected arm, and dermal backflow in the affected arm of patients with significant lymphoedema. Similarly, Akita et al. [23] visualised dermal backflow in patients

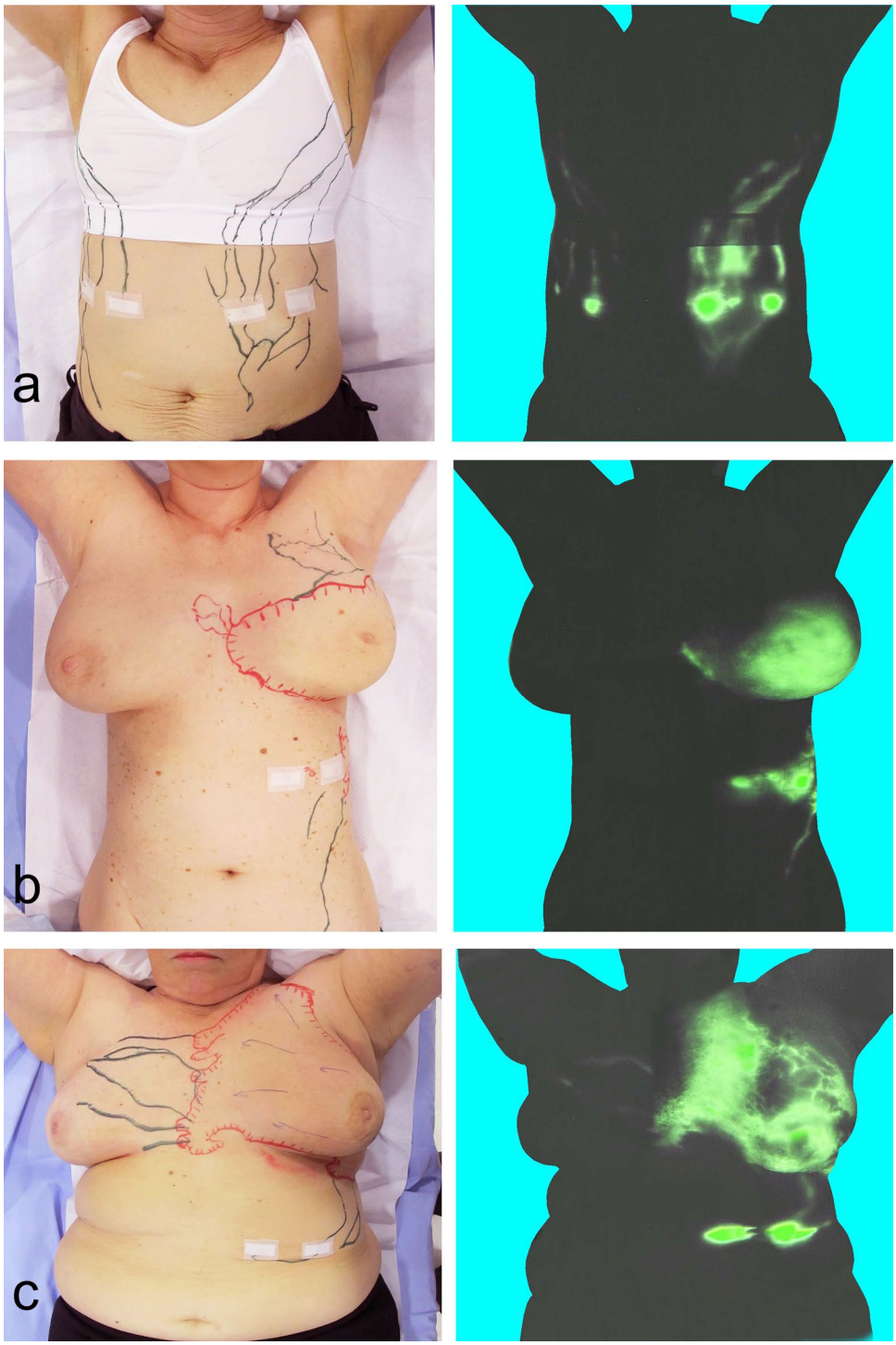

Fig. 2 Representative still photographs (left) and ICG lymphography montage images (right) in control and breast lymphoedema participants. ICG lymphography images were taken at the end of the scanning procedure thus incurring some washout of ICG dye from collecting vessels. Still photograph images: injection sites 1 and 2 (Micropore tape), lymphatic collecting vessels (green) and dermal backflow (red). **a** Control participant demonstrating bilateral linear drainage to ipsilateral axilla regions. Torso lymphatics observed draining bilaterally to the abdomen and inguinal region. **b** Breast lymphoedema participant demonstrating collateral lymphatic drainage of breast to clavicular and parasternal regions. **c** Breast lymphoedema participant demonstrating collateral lymphatic drainage of breast to clavicular and contralateral axilla regions

with BCRL, while linear patterns were observed in patients without BCRL. Linear drainage patterns represent normal unobstructed lymph drainage from the distal tissue, through patent collecting vessels, to upstream lymph nodes. These patterns are visualised as longitudinal lines with ICG lymphography, while dermal backflow, visualised as a reticular honeycomb pattern, indicates retrograde movement of lymph into microscopic dermal capillaries [26]. This occurs due to obstruction in upstream lymphatic vasculature and is diagnostic of lymphoedema [37, 38]. Our observation of dermal backflow in the breast indicates the same diagnostic features seen in BCRL are present in patients with breast lymphoedema. Additionally, our findings suggest that through analysis of these patterns, ICG lymphography may be useful in monitoring patients at risk of developing breast lymphoedema and identifying patients without the condition.

Our results showed that women with breast lymphoedema also displayed collateral drainage pathways, which differed according to the severity of lymphoedema. Collateral pathways, like dermal backflow, become evident when lymph flow is obstructed [39]. It remains unknown as to whether collateral pathways are pre-existing or represent lymphangiogenesis in the face of obstruction [40]. Irrespective of the mechanism, our results showed that as severity increased (TDC ratio ≥ 1.40), drainage to the ipsilateral axilla was impeded while collateral pathways external to the breast (e.g. contralateral axilla) were more likely. This raises the possibility of a hierarchy existing within drainage pathways relevant to severity. However, due to our small cohort we cannot make any conclusions regarding this and further studies are required to test this hypothesis.

The TDC results identified a higher water content in the affected breast with suggestions that the distribution of water differs between affected and unaffected breasts. The device however only detected breast lymphoedema with a whole breast TDC ratio ≥ 1.40 in half of the breast lymphoedema participants despite lymphoedema being identified with ICG lymphography in all participants. This raises questions about the sensitivity of using a whole breast TDC ratio to assess breast lymphoedema, especially with patients that have localised swelling. The role of breast quadrant rather than whole breast TDC ratios and the diagnostic threshold of this ratio should be explored further. Additionally, given the potential association observed between TDC ratios and lymphatic drainage pathways of the breast, there may be value in using this device alongside ICG lymphography in future research.

Limitations

This study has some limitations. First, the main limitation is the study's small participant numbers. However, this was a pilot study, which served to direct future research. A larger prospective longitudinal study recruiting at-risk breast cancer patients irrespective of breast lymphoedema symptoms would aid in determining the diagnostic validity of ICG lymphography. Additionally, this study could progress our understanding of breast lymphoedema, identifying a risk factor profile and the time course of this condition. Second, a recognised limitation of ICG lymphography is its limited penetration depth (20 mm) [33]. While this impedes assessment of deep lymphatics and breast parenchyma, it nevertheless allows minimally invasive real-time imaging of the superficial lymphatics where secondary lymphoedema manifests [41, 42]. Third, MLD was applied after 20 min if drainage pathways remained undetermined. This may influence the transit time of ICG dye as MLD facilitates lymph transport velocity [43]. However, transit time was not a focus of this study and the application of MLD served to reduce scanning time. This makes ICG lymphography a more viable assessment method in a busy clinical environment and less onerous on participants.

Finally, there are some practical considerations for using ICG lymphography in the clinical setting. Though ICG lymphography has been approved for breast sentinel node biopsy in some countries, the current off-label use of ICG dye in lymphatic imaging may limit the accessibility of this technique [44, 45]. Additionally, whist some training is required to undertake ICG lymphography this is expected of any medical imaging tool. Moreover, compared with other medical imaging techniques the cost is relatively low.

Clinical implications

Our findings have implications for the detection and monitoring of breast lymphoedema. We have shown that ICG lymphography is capable of identifying lymphatic morphological changes in breast lymphoedema. This is important because these changes often precede the clinical signs of lymphoedema and therefore ICG lymphography may facilitate earlier detection of breast lymphoedema. Early detection allows earlier intervention, reducing chronic tissue oedema and the development of irreversible changes to lymphatic vessels and tissue architecture. Akita and colleagues [23] demonstrated the effectiveness of ICG lymphography in diagnosing BCRL in patients earlier than conventional limb volume measurements. Consequently, through early detection and management they were able to demonstrate reversibility of dermal backflow patterns and lymphatic dysfunction in some patients. Moreover, an assessment method that is capable of identifying early breast lymphoedema would enable prospective monitoring of at-risk patients, especially high risk patients (e.g. patients who have undergone extensive axillary treatment). These findings support the need for future research to investigate the role of ICG lymphography in early detection, monitoring, and intervention of breast lymphoedema.

The high-resolution images and real-time feedback on lymphatic function provided by ICG lymphography have clinical implications on the management of breast lymphoedema. ICG lymphography could be utilised to evaluate the acute and long-term outcomes of various treatments such as MLD, intermittent pneumatic compression, or compression bras. Furthermore, understanding lymphatic drainage pathways of the breast following BCT is also important for the management of breast lymphoedema, specifically the manner in which MLD, a gentle massage that promotes lymph drainage from areas of congestion towards functioning lymphatics, is applied. Traditionally MLD for breast lymphoedema is directed towards the contralateral axilla and the inguinal regions. Our findings demonstrate other drainage

regions which may also be utilised. This could potentially optimise treatments and influence patient outcomes. Additionally, the variability between drainage pathways identified in our breast lymphoedema group (Table 4) highlights a unique benefit of ICG lymphography. It offers an assessment method capable of informing the patient's therapist of patent lymphatic drainage pathways, thereby enabling the development of personalised management plans.

Conclusion

ICG lymphography is a potential qualitative imaging method for the identification and assessment of breast lymphoedema following BCT. This pilot study supports the need for further research to investigate the role of ICG lymphography in diagnosing, monitoring, and managing breast lymphoedema. Furthermore, it supports the utility of ICG lymphography in the armamentarium of breast lymphoedema assessment tools.

Acknowledgements This research is supported by an Australian Government Research Training Program (RTP) scholarship and the Macquarie University ALERT research program.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were conducted in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and approved by Macquarie University Human Research Ethics Committee (Reference Number 5201800263).

Informed consent Informed consent was obtained from all individual participants included in this study.

References

1. Tailby E, Boyages Am J (2017) Conservation surgery and radiation therapy in early breast cancer: an update. Aust Fam Phys 46:214–219
2. Adriaenssens N, Belsack D, Buyl R et al (2012) Ultrasound elastography as an objective diagnostic measurement tool for lymphoedema of the treated breast in breast cancer patients following breast conserving surgery and radiotherapy. Radiol Oncol 46(4):284–295
3. Mihara M, Hara H, Hayashi Y et al (2012) Pathological steps of cancer-related lymphedema: histological changes in the collecting lymphatic vessels after lymphadenectomy. PLoS ONE 7(7):e41126
4. Rutkowski JM, Moya M, Johannes J, Goldman J, Swartz MA (2006) Secondary lymphedema in the mouse tail: lymphatic hyperplasia, VEGF-C upregulation, and the protective role of MMP-9. Microvasc Res 72(3):161–171
5. Aldrich MB, Sevick-Muraca EM (2013) Cytokines are systemic effectors of lymphatic function in acute inflammation. Cytokine 64(1):362–369
6. Zampell JC, Yan A, Elhadad S, Avraham T, Weitman E, Mehrara BJ (2012) CD4+ cells regulate fibrosis and lymphangiogenesis in response to lymphatic fluid stasis. PLoS ONE 7(11):e49940
7. Meek AG (1998) Breast radiotherapy and lymphedema. Cancer 83(S12B):2788–2797
8. Degnim AC, Miller J, Hoskin TL et al (2012) A prospective study of breast lymphedema: frequency, symptoms, and quality of life. Breast Cancer Res Treat 134(3):915–922
9. Verbelen H, Gebruers N, Beyers T, De Monie AC, Tjalma W (2014) Breast edema in breast cancer patients following breast-conserving surgery and radiotherapy: a systematic review. Breast Cancer Res Treat 147(3):463–471
10. Dylke ES, Benincasa Nakagawa H, Lin L, Clarke JL, Kilbreath SL (2018) Reliability and diagnostic thresholds for ultrasound measurements of dermal thickness in breast lymphedema. Lymphat Res Biol 16(3):258–262
11. Back MF, Guerrieri M, Wratten C, Steigler A (2004) Impact of radiation therapy on acute toxicity in breast conservation therapy for early breast cancer. Clin Oncol 16(1):12–16
12. Wenz F, Welzel G, Keller A et al (2008) Early initiation of external beam radiotherapy (EBRT) may increase the risk of long-term toxicity in patients undergoing intraoperative radiotherapy (IORT) as a boost for breast cancer. Breast 17(6):617–622
13. Vicini FA, Chen P, Wallace M et al (2007) Interim cosmetic results and toxicity using 3D conformal external beam radiotherapy to deliver accelerated partial breast irradiation in patients with early-stage breast cancer treated with breast-conserving therapy. Int J Radiat Oncol Biol Physics 69(4):1124–1130
14. Johansson K, Darkeh MH, Lahtinen T, Bjork-Eriksson T, Axelssqn R (2015) Two-year follow-up of temporal changes of breast edema after breast cancer treatment with surgery and radiation evaluated by tissue dielectric constant (TDC). Eur J Lymphol Relat Probl 27(73):15–21
15. Clarke D, Martinez A, Cox RS, Goffinet DR (1982) Breast edema following staging axillary node dissection in patients with breast carcinoma treated by radical radiotherapy. Cancer 49(11):2295–2299
16. Rönkä RH, Pamilo MS, Von Smitten KAJ, Leidenius MHK (2004) Breast lymphedema after breast conserving treatment. Acta Oncol 43(6):551–557
17. Boughey JC, Hoskin TL, Cheville AL et al (2014) Risk factors associated with breast lymphedema. An Surg Oncol 21(4):1202–1208
18. Greenhowe J, Stephen C, McClymont L, Munnoch DA (2017) Breast oedema following free flap breast reconstruction. The Breast 34:73–76
19. Kuzmiak CM, Zeng D, Cole E, Pisano ED (2009) Mammographic findings of partial breast irradiation. Acad Radiol 16(7):819–825
20. Li J, Dershaw DD, Lee CF, Joo S, Morris EA (2010) Breast MRI after conservation therapy: usual findings in routine follow-up examinations. Am J Roentgenol 195(3):799–807
21. Wratten CR, O'Brien PC, Hamilton CS, Bill D, Kilmurray J, Denham JW (2007) Breast edema in patients undergoing breast-conserving treatment for breast cancer: assessment via high frequency ultrasound. The Breast J 13(3):266–273
22. Brandon Dixon J, Weiler MJ (2015) Bridging the divide between pathogenesis and detection in lymphedema. Semin Cell Dev Biol 38:75–82

23. Akita S, Nakamura R, Yamamoto N et al (2016) Early detection of lymphatic disorder and treatment for lymphedema following breast cancer. Plast Reconstr Surg 138(2):192e–202e
24. Yamamoto T, Yamamoto N, Doi K et al (2011) Indocyanine green-enhanced lymphography for upper extremity lymphedema: a novel severity staging system using dermal backflow patterns. Plast Reconstr Surg 128 (4):941–947
25. Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA (2016) Indocyanine green: historical context, current applications, and future considerations. Surg innov 23(2):166–175
26. Suami H, Chang D, Skoracki R, Yamada K, Kimata Y (2012) Using indocyanine green fluorescent lymphography to demonstrate lymphatic architecture. J Lymph 7(2):25–29
27. Bruna J (2000) Collateral lymphatic circulation. Eur J Plast Surg 23(6):321–325
28. Suami H, Pan WR, Mann GB, Taylor GI (2008) The lymphatic anatomy of the breast and its implications for sentinel lymph node biopsy: a human cadaver study. Ann Surg Oncol 15(3):863–871
29. Diagnostic Green (2016) United Kingdom package leaflet: information for the user. https://www.diagnosticgreen.com.row/product-information/. Accessed 10 Feb 2020
30. Nuutinen J, Ikäheimo R, Lahtinen T (2004) Validation of a new dielectric device to assess changes of tissue water in skin and subcutaneous fat. Physiol Meas 25(2):447–454
31. Suami H, Heydon-White A, Mackie H, Czerniec S, Koelmeyer L, Boyages J (2019) A new indocyanine green fluorescence lymphography protocol for identification of the lymphatic drainage pathway for patients with breast cancer-related lymphoedema. BMC Cancer 19(1):1–7
32. Suami H, Heydon-White A, Mackie H, Koelmeyer L, Boyages J (2018) Cryogenic numbing to reduce injection discomfort during indocyanine green lymphography. J Reconstr Microsurg 35:31–e2
33. Marshall MV, Rasmussen JC, Tan I-C et al (2010) Near-infrared fluorescence imaging in humans with indocyanine green: a review and update. Open Surg Oncol J (Online) 2(2):12
34. Suami H, Pan WR, Taylor GI (2009) Historical review of breast lymphatic studies. Clin Anat 22(5):531–536
35. Turner-Warwick R (1959) The lymphatics of the breast. Br J Surg 46(200):574–582
36. Pavlista D, Eliska O (2005) Cutaneous and subcutaneous lymphatic drainage of the breast. Lymphol 38(2):92–102
37. Narushima M, Yamamoto T, Ogata F, Yoshimatsu H, Mihara M, Koshima I (2016) Indocyanine green lymphography findings in limb lymphedema. J Reconstr Microsurg 32(01):072–079
38. Shinaoka A, Koshimune S, Yamada K et al (2017) Accelerated lymph flow in early-stage secondary lymphedema detected by indocyanine green fluorescence lymphography. J Reconstr Microsurg 33(8):596–602
39. Suami H, Koelmeyer L, Mackie H, Boyages J (2018) Patterns of lymphatic drainage after axillary node dissection impact arm lymphoedema severity: a review of animal and clinical imaging studies. Surg Oncol 27(4):743–750
40. Blum KS, Proulx ST, Luciani P, Leroux J-C, Detmar M (2013) Dynamics of lymphatic regeneration and flow patterns after lymph node dissection. Breast Cancer Res Treat 139(1):81–86
41. Olszewski WL, Jain P, Ambujam G, Zaleska M, Cakala M (2009) Topography of accumulation of stagnant lymph and tissue fluid in soft tissues of human lymphedematous lower limbs. Lymphat Res Biol 7(4):239–245
42. Lahtinen T, Seppälä J, Viren T, Johansson K (2015) Experimental and analytical comparisons of tissue dielectric constant (TDC) and bioimpedance spectroscopy (BIS) in assessment of early arm lymphedema in breast cancer patients after axillary surgery and radiotherapy. Lymphat Res Biol 13(3):176–185
43. Tan IC, Maus EA, Rasmussen JC et al (2011) Assessment of lymphatic contractile function after manual lymphatic drainage using near-infrared fluorescence imaging. Arch Phys Med Rehabilit 92(5):756–764.e751
44. Verbeek FP, Troyan SL, Mieog JSD et al (2014) Near-infrared fluorescence sentinel lymph node mapping in breast cancer: a multicenter experience. Breast Cancer Res Treat 143(2):333–342
45. Toh U, Iwakuma N, Mishima M et al (2015) Navigation surgery for intraoperative sentinel lymph node detection using indocyanine green (ICG) fluorescence real-time imaging in breast cancer. Breast Cancer Res Treat 153(2):337–344