ASSESSING BREAST CANCER-RELATED BREAST LYMPHOEDEMA USING INDOCYANINE GREEN LYMPHOGRAPHY

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Preface

The purpose of this thesis is to; i) explore the utility of indocyanine green (ICG) lymphography in the assessment of breast lymphoedema, and ii) investigate the lymphatic drainage pathways of the breast to determine if these pathways are altered following breast-conserving therapy for breast cancer.

This thesis is comprised of four chapters. To provide background relevant to the purpose of this thesis **Chapter 1** gives an overview of the lymphatic system (anatomy and physiology) and lymphoedema. It introduces terms and concepts that will be referenced in subsequent chapters. Additionally, it describes breast lymphoedema, investigating its aetiology, incidence, and risk factors. **Chapter 2** discusses the current assessment methods used in breast lymphoedema as described in the literature. It also introduces ICG lymphography as a potential assessment method. **Chapter 3** is a pilot study examining the viability of ICG lymphography in assessing breast lymphoedema. This chapter is presented in the format of the manuscript which has been submitted to the Journal of Breast Cancer Research and Treatment, with table and figure numbering aligned to the thesis document. The print version is accessible in Appendix 2. **Chapter 4** reviews and analyses the findings of this study with consideration to the clinical implications and future research directions. Specifically, it explores the role ICG lymphography has in the assessment, management, and monitoring of breast lymphoedema. References for Chapters 1, 2, and 4 are presented at the end of Chapter 4. The references for the pilot study (Chapter 3) are included in the manuscript in the format requested by the journal.

Abstract

Breast lymphoedema is a potential complication for women undergoing breast-conserving therapy for breast cancer. It is characterised by chronic swelling and tissue changes in the breast, which can cause discomfort, increased susceptibility to cellulitis, and negatively impact the sufferer's quality of life. Despite this breast lymphoedema remains under-recognised and under-reported. This can be attributed, at least in part, to the lack of a standardised assessment method. This thesis explores breast lymphoedema, specifically the current methods available to assess this condition and proposes a new method of assessment, indocyanine green (ICG) lymphography.

ICG lymphography is a validated assessment method for breast cancer-related arm lymphoedema. However, its use in breast lymphoedema has not been examined. Therefore, a pilot study recruiting two groups of participants (10 healthy controls, and 10 breast lymphoedema participants) was undertaken to determine if ICG lymphography could be utilised as an assessment method for this condition. Additionally, ICG lymphography was used to map the lymphatic drainage pathways of the breast in both participant groups to determine if these drainage pathways are altered following breast conserving therapy.

In this pilot study, ICG lymphography detected morphological changes to the lymphatic vasculature diagnostic of lymphoedema (dermal backflow and collateral lymphatic drainage) in all breast lymphoedema participants and none of the healthy control participants. Furthermore, lymphatic drainage pathways of the breast differed between the two groups. In the healthy control group lymph drained exclusively to the ipsilateral axilla region. In the breast lymphoedema group lymph drained to the: i) parasternal (6/10); ii) ipsilateral axilla (4/10); iii) contralateral axilla (4/10); iv) intercostal (3/10) and v) clavicular (2/10) regions.

These findings support the use of ICG lymphography in the assessment of breast lymphoedema. Additionally, understanding the lymphatic drainage pathways of the breast will have clinical implications for breast lymphoedema management. This pilot study serves to direct future research that examines the role of ICG lymphography not only in the assessment of breast lymphoedema but its diagnosis, management, and monitoring.

Candidate statement

I hereby declare that this thesis titled 'Assessing breast cancer-related breast lymphoedema using indocyanine green lymphography' is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. This work has not been previously submitted for a degree to any other university or institution and all sources have been referenced.

The research presented in this thesis was approved by Macquarie University Human Research Ethics Committee on the 16th May 2018 (reference number 5201800263).

Asha Heydon-White (45045399)

Signed:

Date: 13.05.2020

Supervisor statement

As supervisor of Asha Heydon-White's Masters of Research work, I certify that her thesis titled 'Assessing breast cancer-related breast lymphoedema using indocyanine green lymphography' is suitable for examination.

Signed: _____

Date: 14.05.2020

Dr Karen Peebles

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I would like to express gratitude to the participants of this study for their willingness to be involved and the provision of their time.

Lastly, I would like to thank my husband and son for their ongoing patience and support.

List of abbreviations

ALND	Axillary lymph node dissection
BCRL	Breast cancer-related arm lymphoedema
BCT	Breast-conserving therapy
BMI	Body mass index
ICG	Indocyanine green
MLD	Manual lymphatic drainage
MRI	Magnetic resonance imaging
SNB	Sentinel node biopsy
TDC	Tissue dielectric constant

Chapter One: The lymphatic system and breast lymphoedema

1.1 Overview

The lymphatic system comprises a complex network of lymphatic vessels and nodes which are found throughout the body. Lymphatic vessels start as a dense interconnected network of small blind ended capillary vessels which coalesce into progressively larger vessels (precollector and collecting vessels), eventually forming lymphatic trunks.[1] The lymphatic trunks connect to the venous system and therefore the wider systemic circulation through lympho-venous connections at the right and left subclavian veins.[2] Lymph nodes are small kidney shaped structures situated along the lymphatic vasculature, usually in clusters where lymphatic vessels merge to form lymphatic trunks.[3]

The lymphatic system transports fluid, macromolecules, immune cells, and products of tissue metabolism and catabolism[4] from the interstitial tissue to the systemic circulation. Once fluid in the interstitial tissue enters the lymphatic system through its capillary network it is termed lymph. The transportation of lymph is central to the maintenance of tissue fluid homeostasis within the body.[5] Other functions of the lymphatic system include the transportation of lipids from the digestive system to the systemic circulation, and the production and transportation of immune cells giving the lymphatic system a role in adaptive immunity.[6] Considering these functions, any lymphatic deficiency may therefore affect many vital processes in the body leading to a range of pathologies. One significant condition that develops as a result of lymphatic dysfunction is lymphoedema.[7]

Lymphoedema is the chronic swelling of a region of the body such as the arm or breast. Swelling develops when the lymphatic system's capacity to transport lymph is reduced. This leads to an imbalance between the volume of lymph to be transported and the transport capacity.[8] Consequently, fluid accumulates in the interstitial tissue resulting in tissue oedema. Lymphoedema is characterised as either primary or secondary. Primary lymphoedema occurs from congenital or hereditary disorders of the lymphatic system. Secondary lymphoedema is acquired as a result of trauma to the lymphatic system.[8] In

developed countries treatment for cancer is the most common cause of secondary lymphoedema.

Breast cancer and its treatment may predispose patients to developing secondary lymphoedema of their arm and/or breast.[8] While breast cancer-related arm lymphoedema (BCRL) is commonly reported in the literature, breast lymphoedema (sometimes referred to as breast oedema) is often overlooked. However, like BCRL, breast lymphoedema causes significant issues for these patients. For example, patients with breast lymphoedema are at an increased risk of developing cellulitis infections, suffer from negative body image, and have a reduced quality of life.[9] Therefore, further research investigating this condition is warranted.

This chapter on the lymphatic system and breast lymphoedema comprises four sections. The first section (**Section 1.2**) provides an overview of the superficial lymphatic system. Secondary lymphoedema such as breast lymphoedema largely involves this system. Therefore, knowledge of its anatomy and physiology will facilitate an understanding of the lymphatics of the breast and the pathophysiological changes that may develop in breast lymphoedema. The second section (**Section 1.3**) discusses the lymphatic anatomy of the breast in more detail, describing its unique characteristics, and exploring its relationship to the lymphatics of the anterior upper torso on which it sits. The third section (**Section 1.4**) focuses on breast lymphoedema, its incidence, aetiology, and risk factors. The final section (**Section 1.5**) takes a more pathophysiological approach. It outlines the long-term pathophysiological consequences of secondary lymphoedema on the lymphatic vasculature and subcutaneous tissue architecture. Additionally, it describes the morphological changes to lymphatic vasculature that can be used to identify lymphoedema.

Within each of these sections attention has been drawn to the relevant literature. Since the breadth of material related to this thesis is vast, it is impossible to cover it all in detail. Thus,

the reader will be directed to relevant review articles, for further detail on a given topic, if required.

1.2 Superficial lymphatic system

The lymphatic system is typically divided into three systems. Specifically, the deep, perforating, and superficial systems, which are illustrated in **Figure 1.1**. The deep system, which is relatively separate from the superficial system, lies close to muscle and bone draining lymph from these sub-fascial structures. The perforating system (not shown) runs alongside perforating arteries that travel from superficial to deeper tissue, perforating the deep fascia.[10] These vessels transport lymph from the superficial tissues to the deep system while remaining completely separate from the superficial lymphatic system. The superficial system lies just below the skin (epidermis). It functions to transports lymph from the interstitial tissues back to the systemic circulation.[8,10] In the context of this thesis the superficial system is most relevant because it is the system in which secondary lymphoedema typically manifests.[11] For this reason the superficial system will be described in detail below.

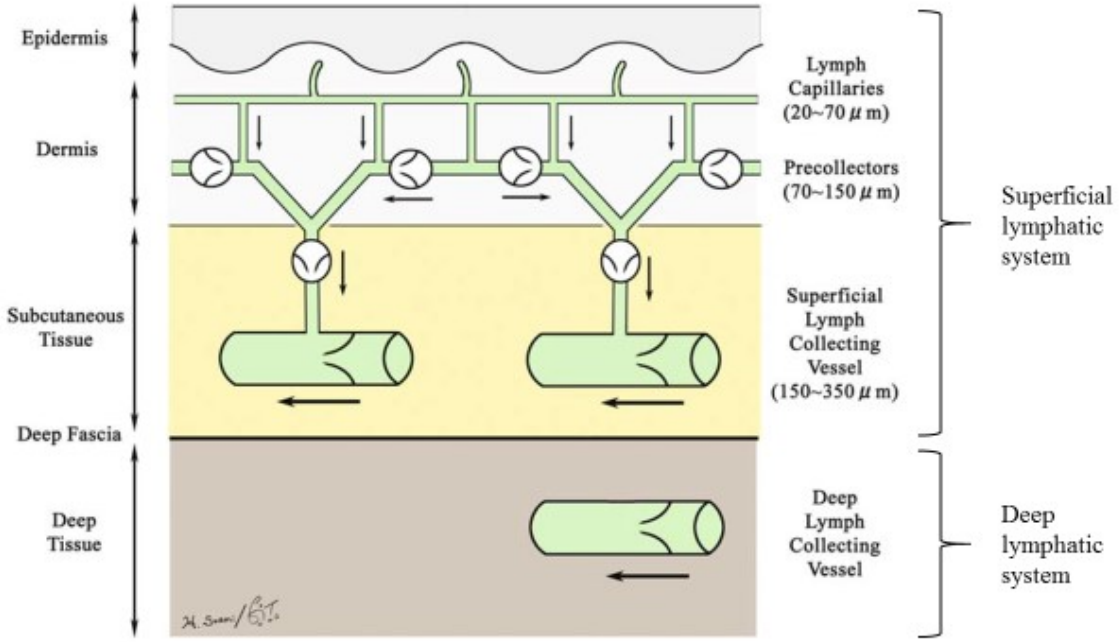

Figure 1.1 Schematic diagram of superficial lymphatic vessels Adapted from Suami H, Pan WR, Mann GB and Taylor GI. The lymphatic anatomy of the breast and its implications for sentinel lymph node biopsy: a human cadaver study. Annals of Surgical Oncology. 2008;15:863 under a CC-BY-NC.[12]

1.2.1 Anatomy of the superficial lymphatic system

The superficial system can be delineated by dividing it into lymphatic territories (**Section 1.2.1.1**), or structurally in terms of its vessels and nodes (**Section 1.2.1.2**).

1.2.1.1 Superficial lymphatic territories of the torso

A lymphatic territory is an area of skin that drains to a specific lymph node or a lymph node region via the superficial lymphatic system.[13] The concept of lymphatic territories were first described in the 1800s by an anatomist, Sappey. Sappey depicted four territories of the torso (excluding the breast) and their drainage regions. Two upper territories which drained

symmetrically to ipsilateral axillary lymph nodes and two lower territories which drained symmetrically to ipsilateral inguinal lymph nodes.[12]

As shown in **Figure 1.2** each territory was demarcated by a theoretical vertical midline that divided the torso in the sagittal plane and a horizontal line at the level of the umbilicus dividing the torso in the transverse plane. These lines were termed Sappey's lines.[14] More commonly known as watersheds,[15] Sappey's lines have been reported to be relatively void of lymphatic vasculature. Therefore, the transport of lymph between lymphatic territories is considered to be limited.[13] These findings have had implications on the management of lymphoedema such as the direction in which lymphatic drainage techniques are applied. However, the accuracy of these reports is questionable as later studies[13-16] have identified lymphatic drainage across these torso watersheds. This suggests that non-conventional lymphatic drainage pathways of the torsos may exist which would have consequences on the management of lymphoedema in this area.

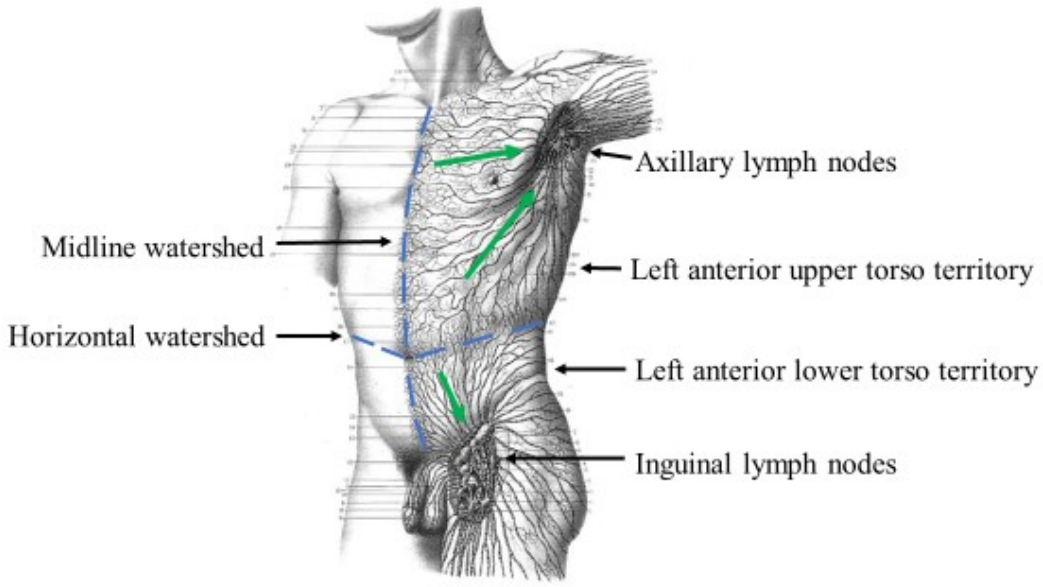

Figure 1.2 Sappey's drawing (1874) of the superficial lymphatic system of the torso. Edited to highlight the anterior territories of the torso, the lymphatic drainage of each territory towards corresponding lymph nodes (green arrows), and watersheds (blue dotted lines). Adapted from Suami H, Pan WR, Mann GB and Taylor GI. The lymphatic anatomy of the breast and its implications for sentinel lymph node biopsy: a human cadaver study. Annals of Surgical Oncology. 2008;15:863 under a CC-BY-NC.[12]

1.2.1.2 Structure of the superficial lymphatic system

The superficial lymphatic system, as shown in **Figure 1.1**, comprises three types of vessels (capillaries, precollectors, collecting vessels) and lymph nodes. The main characteristics and functions of each are as follows.

Lymphatic capillaries, commonly referred to as the initial lymphatics, are small blind ended vessels that form a dense interconnected lymphatic network just below the epidermis. They are the primary site for fluid exchange between the interstitium and the lymphatic system. Their vessel wall comprises a single endothelial cell layer with a discontinuous basement membrane.[17] Lymphatic capillary endothelial cells have a unique oak-leaf shape allowing

the formation of overlapping flaps between adjacent cells. Discontinuous button-like junctions present on the sides of each flap maintain structural integrity of the capillary while allowing the entry of fluid and solutes from the interstitium through openings at the tip of each flap.[18] These openings serve as the primary valves of the lymphatic system. In normal conditions these valves ensure the unidirectional movement of lymph, preventing it from escaping back into the interstitium.[19] Anchoring filaments which tether the endothelial cells to the surrounding interstitium additionally facilitate the opening of these primary valves in conditions of increased interstitial oedema by stretching the endothelial cell junctions.[20] A further suggested method of fluid entry into the superficial lymphatic system is the transport of water across the endothelial cells (transcellular transport) via aquaporin-1 channels.[2]

Precollectors are only found in the superficial lymphatic system. As seen in **Figure 1.1** they run vertically down from the capillaries (in the dermis) to collecting lymphatic vessels (in the subcutaneous tissue). This aligns to their primary role which is to transport lymph between the lymphatic capillaries and collecting vessels. In comparison to lymphatic capillaries, precollectors are larger in diameter and have a more developed vessel wall. However, their vessel wall structure can vary along its length. For example, some sections of a given precollecting vessel may have a single layer of endothelial cells (i.e. resemble a lymphatic capillary) whilst other sections may have a more developed structure with lymphatic valves every 2-3 mm,[20] similar to a lymphatic collecting vessel.

Lymphatic collecting vessels run horizontally through the adipose layer of subcutaneous tissue towards regional lymph nodes. Similar to veins (and arteries), the lymphatic collectors are more developed and consist of three coaxial layers. The inner layer (intima) is lined by endothelial cells, the middle layer (media) consists of lymphatic muscle cells and the outer layer (adventitia) comprises connective tissue, vasa vasorum and nerves.[1]

Another important component of the lymphatic collecting vessel structure is the presence of bicuspid valves at semi-regular intervals (every 1 -3 mm).[19] These valves are commonly referred to as secondary lymphatic valves. Similar to the primary lymphatic valves, secondary lymphatic valves prevent the backflow of lymph.[1,17] Additionally, they divide the vessel into functional units called lymphangions. Lymphangions have the ability to contract and therefore provide an intrinsic force that actively transports lymph proximally.[1] This is an important function of the lymphatic system that will be discussed in more detail in **Section 1.2.2**.

Lymph nodes are situated throughout the human body.[20] They are classified as either regional if located in clusters where lymphatic collecting vessels merge to form larger lymphatic trunks (e.g. axilla), or interval if they lie along a collecting vessel.[20] Lymph is transported to and from lymph nodes via afferent and efferent collecting vessels and all lymph passes through at least one lymph node before it is returned to the systemic circulation. Lymph nodes are composed of a number of compartments through which lymph is filtered. Additionally, they house immune cells which contribute to the lymphatic system's role in adaptive immunity. Lymph nodes have their own blood supply enabling the direct reabsorption of water back into the systemic circulation.[2] In diseases such as breast cancer, cancer cells may break away from the primary tumour and spread to other areas of the body. As cancer cells travel through the lymphatic system they typically spread to the lymph nodes first. Therefore, lymph nodes are often targeted in cancer treatments such as surgery or radiotherapy.

1.2.2 Physiology of lymph flow in the superficial lymphatic system

The formation of lymph and its transport within the lymphatic system is influenced by forces that bear resemblance to those in the wider circulation. For example, lymph moves centrally through progressively larger lymphatic vessels according to pressure gradients (e.g. from high to low pressure). However, unlike the wider circulation, the lymphatic system does not

have a centralised pump (heart). Instead pressure gradients are augmented by extrinsic forces (e.g. respiratory movements or skeletal muscle contractions) and intrinsic forces (smooth muscle contraction within lymphatic collecting vessels). The role of each force in the formation and transport of lymph will be considered below.

Pressure gradients are responsible for the formation of lymph at the lymphatic capillaries. When interstitial pressure becomes higher than capillary pressure the primary lymphatic valves open allowing the transport of fluid and solutes into the lymphatics. Once pressure in the capillaries rises these valves close preventing the backflow of lymph.[2] Lymph fluid that has accumulated in the capillaries is transported proximally down a partial pressure gradient. Additionally, suction forces generated through the active contraction of the upstream lymphatic collecting vessels, termed the retrograde pump, aid in capillary lymph transport.[21]

Extrinsic forces are external to the lymphatic system. For example, respiratory movements can create variations in central venous pressure, which may augment the formation and transport of lymph.[2] However, the influence of these forces depends on the location of lymphatic vessels within the body. In the context of the breast, it is expected that due to its anatomical location, changes in central venous pressure as a result of breathing will augment lymph transport. The influence of these forces may also be impacted by the precise central venous pressure. For example, in patients who have an increased central venous pressure (e.g. patients with heart failure), the effect of these cyclical changes may be attenuated. Accordingly, this may impede lymphatic drainage.

The above factors had several implications for the study design in **Chapter 3.** For example, participants with heart failure were excluded from the study. Additionally, although breathing was not measured, care was taken to ensure participants were in a resting state (in supine) throughout the study. In other words, there was an attempt to minimise inter-participant variations in lymphatic drainage due to breathing.

Another extrinsic force responsible for influencing lymph formation and transport is the contraction of nearby skeletal muscles. Skeletal muscle contractions create cyclical tissue deformation that superimpose pressure gradients.[5] While these forces are particularly relevant to limb lymphatic drainage, they would have less effect on the breast as it is not composed of any muscular tissue. Exogenous extrinsic forces may however contribute to lymph formation and transport within breast tissue. For example, intermittent direct external manual pressure, such as that applied by a lymphoedema therapist during manual lymphatic drainage (MLD) treatments, can aid lymphatic drainage of the superficial lymphatic system.

Intrinsic forces play a significant role in the transport of lymph once it enters the relatively deeper aspects of the superficial lymphatic system. In contrast to extrinsic muscle contractions evoked by skeletal muscle, intrinsic forces involve the active contraction of lymphangions in lymphatic collecting vessels. Lymphangions are arranged in series along the vessel and act as individual but coordinated pumps that sequentially contract, propelling lymph proximally.[17] Secondary lymphatic valves, which divide the lymphangions, compartmentalise lymph and thereby reduce the hydrostatic pressure within the lymphatic collecting vessel. This aids the proximal transport of lymph against a net hydrostatic pressure gradient.[17]

Lymphangions are capable of contracting due to a lymphatic muscle layer within the wall of the collecting vessel. Lymphatic muscle has unique properties with similarities of both blood vessels (smooth) and cardiac (striated) muscle allowing it to function in both tonic and phasic contractions.[2] Similar to blood vessels, lymphatic collecting vessels exhibit myogenic responses to changes in intravascular pressure.[1,17] Additionally, they undergo rapid phasic contractions, similar to those of cardiac pacemaker cells and their contraction cycle is analogous to that of the cardiac cycle with periods of systole and diastole.[1] Lymphatic muscle contraction strength, frequency, and amplitude are influenced by numerous inherent factors pertaining to the lymphatic vessel itself or endogenous substances that cannot be controlled for in the study undertaken in **Chapter 3**. While it is beyond the scope of this thesis to discuss these in detail (see Scallan et al.[1], Breslin et al.[2], Chakraborty et al.[17])

it is important to note that the relationship between these factors is complex and inextricably linked to allow coordinated, efficient contraction of lymphangions that are capable of adapting to changing lymphatic loads.

1.3 The lymphatic anatomy of the breast

The breast is situated on the anterior thoracic wall overlying the pectoralis major muscle. It extends from the second to the sixth rib with medial and lateral borders at the edge of the sternum and mid-axillary line respectively.[22] The breast consists of parenchymal, stromal (e.g. connective tissue), and adipose tissue. The parenchyma includes 15-20 lobes and lactiferous ducts that function to transport milk to the nipple during lactation.[23] Stromal tissue throughout the breast provides support to the parenchyma. For example, Cooper's ligaments run from the dermis of the skin through the breast parenchyma to the clavipectoral fascia giving support and shape to the breast.[22] Adipose tissue surrounds the lobes of the breast and lies within the subcutaneous tissue largely determining the size of the breast.

Overlying the subcutaneous tissue is the skin of the breast (dermis and epidermis). Its thickness varies across the breast with an average dermal thickness of 1-1.5mm.[24,25] The skin accommodates the nipple and areola. The nipple contains the openings of the lactiferous ducts[26] and surrounding it the areola has openings to glands (Montgomery glands) which provide lubrication during breastfeeding.[23]

Lymphatic vessels are present throughout the breast tissue. A highly dense network of lymphatic vessels can be identified superficially in the subcutaneous and areola tissue,[13,26,27] while fewer lymphatic vessels are present in the deeper parenchymal tissue.[28] Despite being an area of anatomical study since the 1700s, there continues to be aspect of the breast lymphatic drainage that remains unclear today. Specifically, the

anatomical course that the lymphatic vessels take to regional lymph nodes and the way in which lymph is drained from the breast's subcutaneous and parenchymal tissue.

In the 1800s Sappey proposed that all lymphatics of the breast (subcutaneous and parenchymal) drained in a centripetal direction to a subareolar plexus located beneath the breast areola. From the subareolar plexus lymph then drained exclusively to axillary lymph nodes via a few collecting vessels.[29,30] In 1959 Turner-Warwick[31] disputed the exclusivity of the subareolar plexus in draining the lymph of the breast. He reported observing lymphatic collectors of the breast that bypassed the subareolar plexus and concluded that Sappey had overemphasised the importance of this plexus. These findings have been supported by others[12,28-31] including Pavlista et al.[30] (2005) and Suami et al.[12] (2008) who both topographically mapped the lymphatic drainage of the breast in female cadaver specimens. They both observed a subareolar plexus and lymphatic collecting vessels that were also independent of this plexus. In addition to these, Suami et al.[12] described perforating lymphatic vessels in the medial aspect of the breast that followed the course of the internal mammary artery.

A key significant difference between these two studies (and other anatomical breast studies) was the locations of the injections used to investigate these lymphatic vessels. The majority of studies (including Pavlista et al.[30]) investigating the lymphatic drainage of the breast have done so by performing injections directly into the breast. While Suami et al.[12] performed similar injections they also injected the periphery of the anterior upper quadrant of the torso. By tracing the lymphatic collecting vessels from these injections to their corresponding lymph nodes (ipsilateral axilla) they were able to demonstrate that breast collecting vessels that were independent of the subareolar plexus originated in the periphery of the anterior upper torsos (**Figure 1.3**). In the majority of their specimens these collecting vessels ran superficially between the dermis and the breast tissue, however in some specimens collecting vessels also ran directly through the breast parenchyma.

The aforementioned findings have implications when considering assessment methods of the breast that utilise lymphatic tracers or dye to image breast lymphatics. Rather than performing injections directly into the breast tissue, injections should be placed closer to the vessel origin in the anterior upper torso. Furthermore, as the majority of these lymphatic vessels lie superficially in subcutaneous tissue, imaging techniques capable of penetrating the dermal and subcutaneous tissues should be adequate to assess the superficial lymphatics of the breast.

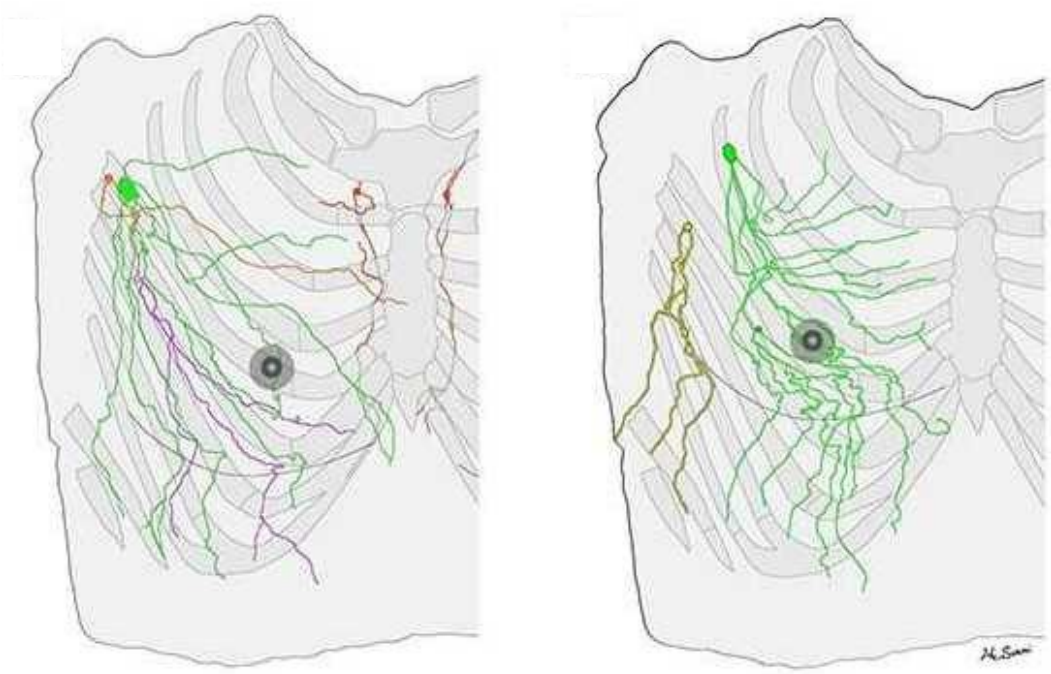

Figure 1.3 Direct radio-opaque injection of the anterior upper torso of two female cadavers. Lymphatic collecting vessels were traced from their first tier lymph nodes distally. These images demonstrate that lymphatics of the anterior upper torso travel from the periphery of this territory through the breast tissue to ipsilateral axillary lymph nodes. Reproduced from Suami H, Pan WR, Mann GB and Taylor GI. The lymphatic anatomy of the breast and its implications for sentinel lymph node biopsy: a human cadaver study. Annals of Surgical Oncology. 2008;15:863 under a CC-BY-NC.[12]

There are fewer reports in the literature pertaining to the lymphatics of the breast parenchyma. This could be related to the difficulty faced in identifying and assessing these deeper lymphatics and the fact that there are less lymphatic vessels in this tissue. Grant et al.[27] described two lymphatic drainage pathways of the parenchymal tissue. The first main group are the lymphatics of the lobules and ducts that drain to the subareolar plexus. In this way the parenchymal lymph can be said to mix with the lymph of the superficial lymphatics. The second group drain the deep breast tissue close to the base of the breast at the pectoralis major muscle. These lymphatics perforate through the pectoralis major muscle and drain only a small amount of lymph to both axillary lymph nodes and other regions such as the intercostal and intermammary lymph nodes. The role of these lymphatic vessels in the development of breast lymphoedema is yet to be clarified.

Ultimately, the lymphatic vessels of the breast (skin and parenchyma) drain to a number of regional lymph nodes. These lymph nodes include the: ipsilateral axilla, internal mammary chain, intercostal, clavicular, liver, sub-diaphragmatic, and contralateral axilla.[26,29,30,32] Of these, the ipsilateral axilla has been shown to be the dominant drainage pathway and the internal mammary chain lymph nodes the second.[33-35] The ipsilateral axillary lymph nodes are of particular interest in breast cancer treatment as they are the most commonly involved lymph nodes when breast cancer advances and spreads.[33] Therefore, treatments such as surgery or radiotherapy frequently target these lymph nodes, which can disrupt the main lymphatic drainage pathway of the breast.

1.4 Breast lymphoedema

Breast lymphoedema occurs following breast cancer when treatment disrupts or damages the lymphatics of the breast. It is characterised by an increase in breast volume or size, tissue fibrosis, and skin changes including; thickening, erythema, hyper-pigmented skin pores, and peau d'orange (dimpled texture of the skin due to cutaneous oedema).[36] Women suffering

from breast lymphoedema report symptoms such as heaviness and erythema in the affected breast.[9] Additionally, breast lymphoedema negatively impacts body image and quality of life.[36,37] Following breast cancer treatment lymphoedema may delay wound healing, increase susceptibility to tissue infections and cellulitis, or adversely affect cosmetic outcomes.[9,38,39] Despite this breast lymphoedema has received scant attention in the literature.

The paucity of research in breast lymphoedema can be attributed to a number of factors. First, breast lymphoedema can be considered as a relatively new condition. Traditional breast cancer treatments excised the entire breast tissue (mastectomy) thereby eliminating the risk of breast lymphoedema. Advances in breast cancer diagnosis and treatment have led to earlier diagnosis of breast cancer and more targeted treatments. Accordingly, treatments that conserve the breast tissue are now commonly employed in the management of breast cancer. While there are many advantages to these treatments (e.g. less extensive surgery) one potential treatment-related effect of conserving the breast tissue is the development of breast lymphoedema. Second, the impact of breast lymphoedema has been under-recognised in the literature. This is potentially due to a combination of factors. Traditional breast cancer treatments placed patients at a greater risk of developing BCRL compared with breast lymphoedema. Additionally, BCRL has been shown to have significant negative impacts on patients' physical function and quality of life.[40] Consequently, the majority of lymphoedema research pertaining to breast cancer has been done on BCRL rather than breast lymphoedema. Moreover, a few studies assessing breast lymphoedema have suggested that it resolves with time (one to three years).[24,41] These findings further reduce the clinical importance of this condition. Other studies however contradict this, demonstrating the chronicity of breast lymphoedema with incidence rates of 65.4% and 26% at two to five years and six years respectively.[37,42] Clearly there is a need for ongoing research to investigate breast lymphoedema and clarify its time course.

A further reason for the paucity of research in breast lymphoedema is the inaccurate reporting of this condition. The true incidence of breast lymphoedema remains unknown with a wide

incidence interval described in the literature (10% - 76%[37,41,43,44]). This can be attributed, at least in part, to the lack of a standardised definition and agreed upon assessment methods. Breast lymphoedema is defined by most as a secondary lymphoedema of the breast, implying it is an oedema of the subcutaneous tissues due to superficial lymphatic dysfunction.[11] Wratten et al.[24] however refers to breast lymphoedema as having an additional component, parenchymal oedema. They define parenchymal oedema as generalised oedema or enlargement of the whole breast. Kwak et al.[45] also references the parenchymal tissue of the breast in their definition of breast lymphoedema, reporting an increased parenchymal density on mammogram as a defining characteristic. The exact aetiology of parenchymal oedema however has not been determined and it is unclear if it is of lymphatic origin.[39] Additionally, it has been suggested that parenchymal oedema may not be present in all cases of breast lymphoedema.[24] In order to define breast lymphoedema further research is required to investigate parenchymal oedema and its relationship with subcutaneous tissue oedema. Irrespective of this, subcutaneous tissue oedema is clearly a significant component of breast lymphoedema and the superficial lymphatics of the breast have a distinct role in the development of this condition. Therefore, despite the lack of a standardised definition, identifying an objective assessment method that is capable of diagnosing and assessing breast lymphoedema on the basis of superficial lymphatic dysfunction would improve the accuracy of reported breast lymphoedema incidence rates.

As a direct result of more targeted breast cancer treatments and the growing prevalence of breast cancer among women, it is likely that an increase in the incidence of breast lymphoedema will be observed.[25] Breast-conserving therapy (BCT) is now routinely used for managing early-stage breast cancer. It offers similar outcomes of overall survival and disease free survival when compared to mastectomy.[46-49] While BCT has the advantages of being less complicated, achieving better breast cosmesis, and resulting in a more positive body image than mastectomy,[49] it presumably will lead to an increased incidence of breast lymphoedema.[25] The growing prevalence of breast cancer in Australia is another factor that is also potentially contributing to expanding breast lymphoedema incidence rates. In 2019 breast cancer was estimated to become the most commonly diagnosed cancer with over

19,000 new cases.[50] Moreover, Australia's excellent survival rates (5 year survival of 91% in 2015 [50]) means that treatment related diseases such as breast lymphoedema will become an increasing health burden. These factors highlight the need for more research to progress our understanding of this condition and determine the most effective ways to assess and manage it.

1.4.1 Aetiology and risk factors

Breast lymphoedema, like limb lymphoedema, occurs when the lymphatic load (volume of lymph) exceeds the lymphatic system's capacity to transport lymph.[51] As a result fluid accumulates in breast interstitial tissue and oedema develops.[8] The lymphatic transport capacity of the breast lymphatics can be damaged or obstructed following BCT to manage breast cancer. BCT involves breast-conserving surgery and radiotherapy treatment. Each of these treatments can impact the development of breast lymphoedema, and influenced the study design, as follows.

First, breast-conserving surgery involves the excision of the cancerous tissue in the breast. Axillary lymph nodes are also usually excised[33-35] to limit the spread of breast cancer to other areas of the body (metastases) and its recurrence. Of these surgical interventions excision of the axillary lymph nodes is the major factor in determining the development of breast lymphoedema.[52] In fact there appears to be no or minimal risk of developing breast lymphoedema when patients undergo excision of the breast tissue only.[9,24,41,52] Therefore, to be eligible to participate in the study undertaken in **Chapter 3**, breast lymphoedema participants were required to have undergone axillary surgery as part of their BCT.

An additional consideration is the number of axillary lymph nodes excised. A sentinel node biopsy (SNB) involves the removal of one or a few lymph nodes to which the breast cancer

drains first, while an axillary lymph node dissection (ALND) involves the removal of the majority or all of the axillary lymph nodes. ALND requires more extensive surgery and contributes to a significantly higher risk of developing BCRL (ALND 23% compared to SNB 6%).[51] However, in breast lymphoedema the risk of each procedure is yet to be determined with conflicting reports evident in the literature. Some studies suggest a greater risk for patients undergoing ALND compared with SNB.[24,41,44] For example, Wratten et al.[24] reported an increased incidence of breast lymphoedema in patients undergoing ALND and minimal to no incidence in patients undergoing SNB unless they developed a post-operative wound infection. In comparison, other studies report similar incidence rates between the two procedures.[9,43,52] Boughey et al.[52] suggests similar incidence rates exist in the breast compared with BCRL because a SNB will always disrupt the dominant lymphatic drainage pathway of the breast. Whereas the arm may have a different sentinel node and therefore this procedure may preserve its dominant lymphatic drainage pathway, reducing the risk of developing BCRL. Considering these reports the type of axillary surgery and the number of lymph nodes excised during surgery were recorded from the medical records of each breast lymphoedema participant in the study undertaken in **Chapter 3**.

Second, radiotherapy treatment is routinely administered following breast-conserving surgery for early-stage breast cancer to treat any residual microscopic disease in the remaining breast tissue.[49] When breast cancer involves regional lymph nodes radiotherapy may also be delivered to these lymph nodes and has been shown to increase the overall survival rate in these patients.[53] Lymph node irradiation nonetheless causes fibrosis of the lymph nodes and a reduction in their size.[54,55] This impacts the function of both the lymph nodes and the downstream lymphatic vessels (afferent vessels) that drain into them. For example, Jonsson and colleagues[55] suggested that lymph node irradiation increases lymphatic transport resistance in afferent vessels resulting in vessel dilatation. The direct impact of radiotherapy on the lymphatic vessels however remains understudied. While Avraham et al.[56] demonstrated that radiotherapy to lymphatic vessels depleted their number and caused lymphatic endothelial cell apoptosis in the animal model, further studies are needed to understand how radiotherapy may impact human lymphatic vessels. Additional research also

needs to consider the impact radiotherapy has on the development of breast tissue fibrosis, which may externally constrict lymphatic vessels impeding lymphatic function, and its inhibitory nature on lymphatic vessel regrowth (lymphangiogenesis).[38]

In addition to the pathophysiological changes that occur to the lymphatic system as a result of radiotherapy treatment, the type of radiotherapy, method of delivery, and treatment regimen can also influence the risk of breast lymphoedema. It is however difficult to determine the specific treatment factors that contribute to an increased risk as a variety of radiotherapy types and treatment regimens have been employed in the literature. When comparing types and delivery methods of radiotherapy, intensity modulated radiotherapy compared to wedge based radiotherapy[57,58] and hypofractionated rather than fractionated treatments[59] have been shown to reduce the incidence of breast lymphoedema. Kelemen et al.[60] suggested an increased risk of breast lymphoedema when radiotherapy is delivered with increased irradiated breast volume, increased boost volume, use of a photon boost, or increased breast separation. They did not find an association between breast lymphoedema and lymph node irradiation. In contrast, Wratten et al.[24] did report an increase in breast lymphoedema in patients who had undergone an ALND and radiotherapy to their regional lymph nodes. In the study undertaken in **Chapter 3**, the radiotherapy treatment details, including lymph node irradiation, were obtained from the medical records of the breast lymphoedema participants.

There are some additional factors which may increase the risk of breast lymphoedema, although they are less well studied compared to axillary surgery and radiotherapy. These factors include; post-operative complications of seroma formation[61] and infection,[44] a high body mass index (BMI),[37,43,44,52] and tumours of the upper outer breast quadrant.[43,52] In addition to upper outer quadrant tumours Boughey et al.[52] noted an increased risk of breast lymphoedema for patients requiring central and lower inner quadrant incisions. Conflicting results appear in the literature for factors such as bra cup size,[37,39,41,52,62] tumour size,[52,60] chemotherapy,[37,39,52] and hormonal therapy.[37,52] Additionally, comparing the results of each study is challenging due to the heterogeneity of study design and the variety of assessment methods employed. Therefore

the identification of a complete and accurate risk factor profile is difficult. In order to determine a risk factor profile breast lymphoedema needs to be defined and a standardised objective assessment method is required to ensure accurate identification and assessment. In the study undertaken in **Chapter 3** these additional factors were also reported from the comprehensive medical history and demographic information collected for each breast lymphoedema participant.

1.5 Pathophysiological consequences of secondary lymphoedema and its identification

As discussed in earlier sections, secondary lymphoedema develops as a result of damage sustained to the lymphatic system. Lymphatic dysfunction and a reduced transport capacity create lymph stasis. Fluid accumulates in the interstitial tissue and oedema develops. Lymphatic dysfunction and chronic tissue oedema promote chronic tissue inflammation along with a number of complex pathophysiological processes, many of which are yet to be classified. As lymphoedema severity progresses, these processes lead to irreversible remodelling of the lymphatic vasculature and surrounding subcutaneous tissue architecture which further impedes lymphatic transport and progresses severity.[51,63]

Remodelling of the lymphatic vasculature involves morphological and histological changes to lymphatic vessels. The structure of the lymphatic vessels change becoming enlarged and hyperplastic.[17,64] Enlarged, hyperplastic vessels are poorly functioning with elevated diastolic pressures and weak contraction ability. Elevated endolymphatic pressures within collecting lymphatic vessels facilitate histological changes to the vessel wall. Mihara et al.[65] investigated these histological changes in different stages of leg lymphoedema severity. They classified these changes into three types (ectasia, contraction, and sclerosis) which they found correlated with lymphoedema severity stages (**Figure 1.4**).[65] As

lymphoedema severity increased, progressive alterations to the lymphatic endothelial cells, lymphatic muscle, and collagen of lymphatic collecting vessels were observed. Additionally, the microvascular networks of the collecting vessels were gradually lost. This histological remodelling eventually resulted in a narrowing of the collecting vessel lumen, and fibrosis and sclerosis of the vessel wall. These changes impede the vessel's ability to actively contract and therefore obstruct the transport of lymph.

Chronic tissue oedema also causes the remodelling of the subcutaneous tissue architecture. The accumulation of protein rich interstitial fluid in the subcutaneous tissue promotes inflammatory responses and stimulates fibroblasts, keratinocytes and adipocytes.[66,67] These responses play a critical role in the development of tissue changes such as fibrosis, skin thickening, and adipose tissue deposition. Changes to the subcutaneous tissue architecture contribute to an increased lymphoedema severity. For example, tissue fibrosis reduces the lymphatic transport capacity and lymphatic regeneration, and adipose tissue deposition causes permanent increases in tissue volume that can only be reduced with surgical interventions (e.g. liposuction[68]).

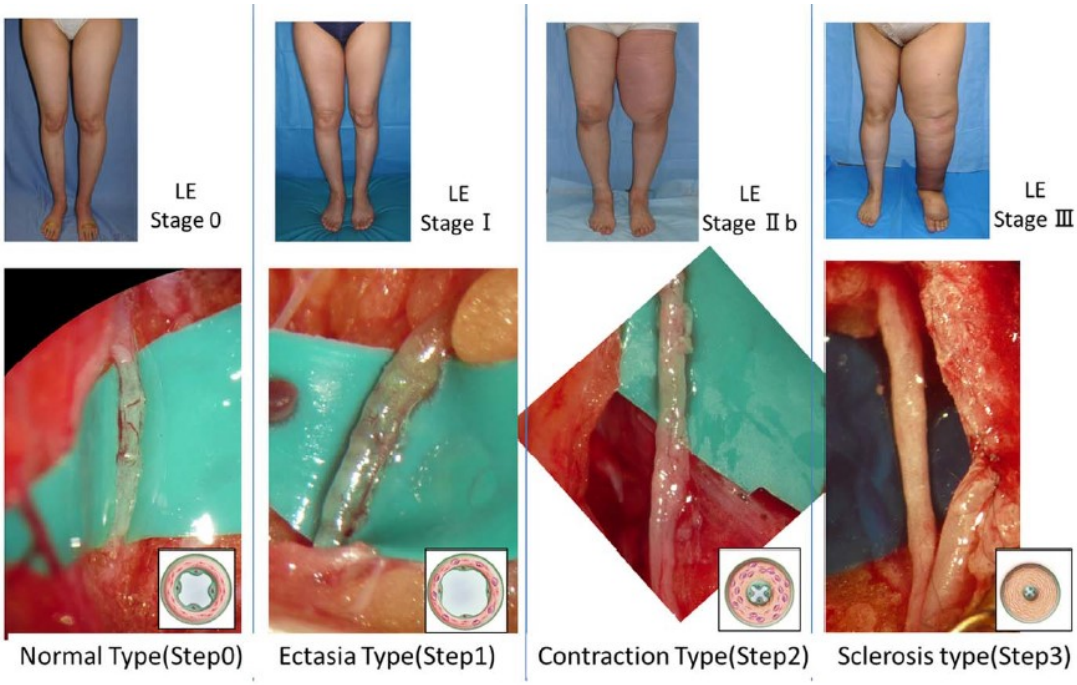

Figure 1.4 Changes to lymphatic collecting vessels according to lymphoedema severity. Collecting vessels' lumen was dilated in ectasia stage (mild lymphoedema), progressively becoming narrower and constricted in contraction and sclerosis stages (severe lymphoedema). Lymphatic endothelial cells which protruded into the lumen in normal collecting vessels were flattened and smooth in ectasia stage. The adhesion between these cells progressively deteriorated as severity increased exposing the collagen fibres of the vessel wall. The smooth muscle layer increased in size in contraction and sclerosis type occluding the vessel lumen. The proportion of different types of smooth muscle cells in the vessel wall changed as severity increased (increase in synthetic type compared to contraction type), resulting in increased collagen proliferation. Reproduced from 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 under a CC-BY.[65]

In order to prevent or limit the development of these pathophysiological consequences to lymphatic vasculature and subcutaneous tissue architecture, secondary lymphoedema needs to be diagnosed early at a subclinical stage. To diagnose lymphoedema early it must be identified when lymphatic dysfunction first occurs, prior to the manifestation of clinical signs such as tissue oedema or skin thickening. Early diagnosis enables timely management which can reduce lymph stasis within the tissue and associated inflammatory responses.[69] The impact of early diagnosis and management in reducing lymphoedema progression and

severity has been well documented in BCRL studies.[40,70,71] A retrospective study by Koelmeyer et al.[71] reported a significant reduction in both incidence and severity of lymphoedema for patients who were assessed within 90 days of undergoing breast cancer surgery when compared to patients assessed more than 90 days post-surgery. Furthermore, a systematic review by Shah et al.[40] highlighted the importance of early detection of BCRL in subclinical stages prior to progression to clinical lymphoedema. Consequently, it is now recommended practice that patients at risk of developing BCRL undergo a prospective surveillance model of care following their breast cancer treatment.[70]

1.5.1 Identifying morphological changes to lymphatic vasculature in secondary lymphoedema

Lymphatic imaging techniques have been shown to be able to accurately diagnose and assess clinical and subclinical secondary lymphoedema by identifying changes to the superficial lymphatic system as a consequence of lymphatic damage and dysfunction. For example, they can identify morphological changes to the patterns and pathways of lymphatic drainage.[72,73] To compensate for lymphatic damage associated with the development of secondary lymphoedema, the lymphatic system may attempt to re-route lymph from obstructed areas to working lymphatic regions. Lymph can be re-routed via the dermal capillary network (dermal backflow patterns) or collecting vessels to unobstructed lymph nodes (collateral lymphatic drainage pathways).

Dermal backflow is the retrograde movement of lymph from collecting vessels to the lymphatic capillaries and the transport of this lymph through the capillary network. It occurs as a result of lymphatic collecting vessel obstruction and incompetency of the secondary lymphatic valves in both precollector and collector vessels.[74] Suami and colleagues[75] have demonstrated that lymph in dermal backflow moves directionally through the capillary network from areas of obstruction towards functioning lymphatic vessels or lymph nodes. Therefore, dermal backflow acts as a bridge to bypass the obstruction. Using lymphatic

imaging techniques such as indocyanine green (ICG) lymphography, dermal backflow can be identified as a reticular, honeycomb-like pattern[76] (**Figure 1.5**) and is considered a diagnostic sign of lymphoedema.

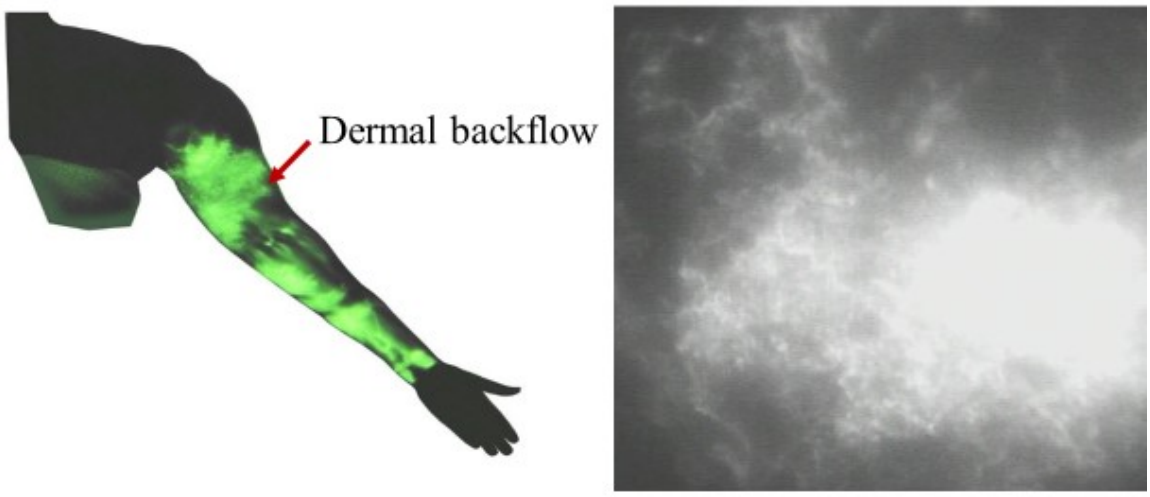

Figure 1.5 Indocyanine green lymphography image of dermal backflow in the arm of a patient with breast cancer-related arm lymphoedema (left). Close up image of dermal backflow showing lymphatic capillaries in the skin (right).

Collateral lymphatic drainage pathways are additional lymphatic vessels that serve to transport lymph from areas of obstruction towards working lymph nodes.[77] It remains unknown as to whether collateral pathways are pre-existing, or represent lymphangiogenesis in the face of obstruction.[78] A number of different types of collateral drainage pathways may exist including; connections between the superficial and deep lymphatic systems, lymphovenous shunts, and of particular significance to this thesis collateral drainage pathways of the superficial system. It has been suggested that the utilisation of collateral

lymphatic pathways may have a role in preventing lymphoedema progression.[79] Similar to dermal backflow patterns, collateral drainage pathways can be identified using lymphatic imaging techniques. In the superficial lymphatic system, collecting lymphatic vessels are observed transporting lymph from areas of dermal backflow or bypassing obstructed areas, draining to lymph node regions other than the dominant drainage pathway. For example, in BCRL superficial collateral lymphatic drainage pathways of the arm have been observed to drain to the ipsilateral clavicular, cervical, parasternal, and even across the midline watershed to the contralateral axilla region.[75,77,79]

While the morphological changes to the lymphatic vasculature of the breast have not been directly studied, it can be assumed that due to similar pathophysiological mechanisms between BCRL and breast lymphoedema a number of similarities exist. Therefore, similar morphological changes to the drainage patterns and pathways of the breast may develop in breast lymphoedema. Identifying these patterns and pathways may aid in the diagnosis and assessment of breast lymphoedema, including early subclinical stages.

1.6 Chapter summary

In summary, this chapter has provided an overview of breast lymphoedema. Specifically, it has highlighted the importance of progressing our understanding of this condition and of finding an assessment method that can identify breast lymphoedema early, before irreversible changes occur in the lymphatic vasculature and subcutaneous tissue of the breast.

This chapter has also highlighted how understanding the anatomy and physiology of the breast lymphatics and the pathology of breast lymphoedema has impacted the study design. The next chapter will examine the assessment methods currently used in breast lymphoedema and explore a potential new assessment method, ICG lymphography.

Chapter Two: Assessing breast lymphoedema

2.1 Overview

Accurate and reliable assessment methods are crucial to progress clinical knowledge and management of breast lymphoedema. **Chapter 1** highlighted the paucity of research and limited clinical knowledge (e.g. risk factor profile, time course and prognosis) that exists for breast lymphoedema. Therefore, valid, assessment methods with good diagnostic accuracy are needed to facilitate future research. Furthermore, an assessment method that can be utilised in clinical practice would; aid diagnosis, enable the monitoring of disease progression, guide appropriate treatment regimens, and permit the monitoring of treatment outcomes.

A variety of assessment methods have been considered in the literature. These range from fairly subjective methods such as breast lymphoedema questionnaires, to more objective methods of tissue dielectric constant (TDC) measures of breast tissue water, and medical imaging techniques. This chapter will review these assessment methods, examining the advantages and disadvantages of each. Additionally, it will highlight, where applicable, how these attributes influenced the study design in **Chapter 3** (**Section 2.2**). Finally, it will propose a new method of assessment for breast lymphoedema, ICG lymphography. It will discuss this technique in detail and explore the potential benefits this lymphatic imaging technique may provide in the assessment of this condition (**Section 2.3**).

2.2 Breast lymphoedema assessment methods

Due to the shape of the breast tissue, lymphoedema assessment methods commonly employed in limb lymphoedema cannot be directly applied to the assessment of breast lymphoedema. For example, volumetric measurements such as circumferential tape measurements determine the volume of the limb based upon a truncated cone formula[80]

and therefore cannot be utilised in breast lymphoedema. While three dimensional volumetric scanners have been used to measure breast volume their accuracy can be compromised in patients with larger breasts.[81] Additionally, volumetric measurements may not be the most appropriate assessment method in breast lymphoedema. BCT and subsequent reconstructive procedures to either the affected and/or unaffected breast have the potential to significantly alter breast size and shape. Therefore, it would be difficult to determine if a change in volume of the affected breast is due to tissue oedema or the structural changes as a result of these procedures. Consequently, limb lymphoedema assessment methods need to be adapted, or alternative assessment techniques need to be considered when assessing breast lymphoedema.

In the literature, the most frequently utilised breast lymphoedema assessment methods include; i) breast lymphoedema questionnaires, ii) physical examination, and iii) medical imaging techniques such as ultrasound, magnetic resonance imaging (MRI), and mammography. Each of these methods will be discussed in detail below.

2.2.1 Breast lymphoedema questionnaires

Questionnaires offer a quick, inexpensive, readily accessible assessment method that does not require any specialised equipment or training. They usually take the form of a self-administered questionnaire, which can be performed alone or to supplement other physical assessment measures.

A few studies have implemented questionnaires to investigate the incidence and severity of breast lymphoedema in women following BCT. In a study by Adriaenssens et al.[37] a self-devised questionnaire using Likert scales to rate the symptoms of breast lymphoedema was administered to determined its incidence and severity. The questionnaire was composed of eight questions and included the following symptoms; heaviness, swelling, redness, peau

d'orange, numbness, tingling, stabbing pain, and skin twitching of the breast. In another study by Degnim et al.[9] a similar self-devised questionnaire was administered but rather than determine the incidence of breast lymphoedema this study reported on the severity and number of symptoms in women with and without breast lymphoedema following BCT. In comparison to the previous study similar scales were used to report symptom severity, however fewer questions and different symptoms of breast lymphoedema were included (heaviness, discomfort, redness, visible swelling, and associated distress).

Although convenient to use, the present limitation in using symptom-related questionnaires is that an accurate symptom profile for breast lymphoedema is lacking. This is highlighted in the studies above. For example, aside from swelling, heaviness, and redness, each questionnaire utilised different symptoms. Furthermore, to be able to use symptom-related questionnaires to determine incidence rates of breast lymphoedema the diagnostic capabilities of these symptoms needs to be investigated. Another disadvantage of this method of assessment is the current lack of a validated and reliable questionnaire that is specific to breast lymphoedema. Both questionnaires in the above studies were self-devised and neither was validated. Therefore, while this assessment method may be appealing to clinicians for use in a busy clinical setting, further research is required to investigate the symptom profile of breast lymphoedema and to develop a valid and reliable questionnaire specific to this condition. Recognising these limitations, we used symptom-related questions in the study undertaken in **Chapter 3** but did so in conjunction with physical measures.

2.2.2 Physical examination

Physical examination of breast lymphoedema is the most frequently employed assessment method in the literature. Similar to questionnaires, this assessment method is appealing to clinicians as it can be undertaken quickly in the clinical setting without the need for equipment or specialised training. Physical examination involves the observation and palpation of the affected breast by a clinician to assess clinical signs of breast lymphoedema

(e.g. visible change in breast size, positive pitting, or peau d'orange). In the literature physical examination has been used to diagnose breast lymphoedema,[9,52] report its incidence,[9,41,44] grade severity,[9,41] and evaluate risk factors.[52]

Despite the frequent use of physical examination as an assessment method for breast lymphoedema several considerations exist when reviewing the literature. First, detailed study methodology is often lacking.[9,24,44,52] This limits reproducibility and comparison of study results. Second, diagnosis and severity staging is relatively subjective and arbitrary. Breast lymphoedema is commonly diagnosed by individual clinician assessment as either present or absent and/or assigned a severity grade of mild, moderate, or severe without description of the diagnostic or severity criteria used. One study undertaken by Clarke et al.[41] did quantify each severity stage. They assigned breast lymphoedema that was asymptomatic with a minor increase in size as mild. Moderate lymphoedema was a readily apparent disparity between breasts with some degree of peau d'orange, and severe lymphoedema had either poor cosmesis or significant symptoms such as pain. Third, the majority of studies using physical examination do so as part of a broader assessment investigating the tissue toxicity side effects of radiotherapy. These studies use validated toxicity assessment criteria such as the Radiation Therapy Oncology Group and European Organisation for Research and Treatment of Cancer (RTOG/EORTC) or the Late Effects Normal Tissue Task Force-Subjective, Objective, Management, Analytic (LENT-SOMA).[60,82-90] However, assessing breast lymphoedema is not the intended purpose of these assessment criteria and questions relating to it are limited. Furthermore, the role of physical examination as an assessment method for breast lymphoedema is not the focus of these studies.

In view of the above, physical examination was undertaken in the study in **Chapter 3**. However, it was not the focus of this study. Physical examination was used as a comparator with subjective reports and also aligned to clinical practice.

2.2.3 TDC measure of breast tissue water

Given that one of the main clinical signs of breast lymphoedema is interstitial fluid accumulation or oedema (**Chapter 1**) it is logical that measuring the tissue water of the breast provides an important assessment of breast lymphoedema. Simple devices to use are the MoistureMeterD or MoistureMeterD Compact (Delfin Technologies Ltd., Finland). These are hand held devices that provide a measurement of the water content of the skin and subcutis tissue to an effective penetration depth of 2.5 mm.[91] When placed on the skin the device emits a high frequency signal that is absorbed or reflected by free and bound tissue water. The reflected signal is used to calculate a TDC measurement providing information on the water content of the tissue.[91] The MoistureMeterD Compact device automatically converts this TDC measurement to a water percentage (0-100%) which is displayed on the device screen. These devices offer a relatively inexpensive, non-invasive, portable assessment method of oedema in the superficial breast tissue. Additionally, measurements are quick and repeatable.

To assess breast lymphoedema, analysis can be conducted on variations in the tissue water content of the affected breast over time and/or comparison with the tissue water content of the unaffected breast. As individual differences in breast tissue water content exist between patients the use of a ratio of the TDC values between the affected and unaffected breasts can be used to account for these differences. This provides a quantitative value that enables comparison of results between participants and studies. Furthermore, a TDC ratio can provide a diagnostic threshold for use in breast lymphoedema. Johansson and colleagues[92] proposed a TDC ratio diagnostic threshold for breast lymphoedema of ≥ 1.40 after examining the normal variation in TDC values in 15 healthy women.

In a later study, Johansson et al.[93] performed repeated TDC measurements on 65 women undergoing BCT over a two-year period. They demonstrated that the TDC device could be used to diagnose breast lymphoedema, determine its incidence, and assess its time course. TDC measurements may also have applications in the assessment of lymphoedema following

reconstructive breast surgery. Greenhowe et al.[94] used the MoistureMeterD Compact to investigate changes in the mean water content of the breast following breast reconstructive surgery for breast cancer. They reported significant increases in the mean water content of the native breast skin in the reconstructed breast with minimal changes in the unaffected breast. These studies support the use of TDC devices in the assessment of breast lymphoedema. However, there are a number of factors including age, BMI, gender, and race[95] that have been suggested to influence TDC measurements and their impact on the accurate assessment of breast lymphoedema needs consideration. Additionally, owing to the limited penetration depth of the TDC device, the accuracy of results need to be investigated in breast lymphoedema patients with increased skin thickness and scar tissue. Furthermore, Liang et al.[95] suggests that the use of diagnostic thresholds is debatable, therefore more research examining the diagnostic threshold proposed by Johansson and colleagues[92] is needed. The study undertaken in **Chapter 3** utilised the MoistureMeterD Compact as a comparative objective assessment method for ICG lymphography. Additionally, the TDC ratio was used in the breast lymphoedema participant group to rank lymphoedema severity.

2.2.4 Medical imaging

Medical imaging techniques including ultrasound,[24,25,39,44,96] MRI,[42] and mammography[97,98] have been used in the literature to identify and assess breast lymphoedema. These studies have reported the incidence and time course of breast lymphoedema based upon visualisation of its clinical signs (e.g. subcutaneous tissue oedema, parenchymal oedema, and skin thickening). The simplest and most commonly investigated imaging method is ultrasound.

Ultrasound is capable of identifying subcutaneous tissue oedema, and skin thickening of the breast. When using skin thickness as a diagnostic sign of breast lymphoedema, ultrasound has been shown to have excellent specificity, sensitivity, and reliability (inter-image, inter-rater, and intra-rater).[25,39] Additionally, it provides a quantitative measure of breast

lymphoedema. Adriaenssens et al.[39] and Ronka et al.[44] reported skin thickness of 2 mm to be diagnostic of breast lymphoedema, while Dylke and colleagues[25] described diagnostic thresholds of greater than 1.6 mm in the superior and lateral breast quadrants and more than 2 mm in the medial and outer quadrants. In comparison with other imaging techniques ultrasound is inexpensive, readily available, non-invasive, and does not require the use of a tracer or expose patients to radiation. Therefore, it is an appealing imaging method to use in breast lymphoedema.

Sophisticated imaging such as MRI creates three-dimensional images of the breast tissue with high contrast. Mammography which is an x-ray of the breast tissue constructs a two-dimensional image. Both techniques can visualise clinical signs of breast lymphoedema, however neither has been thoroughly studied in the literature to determine its validity and reliability in assessing breast lymphoedema. One advantage of both imaging methods is that they can visualise the parenchymal tissue of the breast and parenchymal oedema. Parenchymal oedema can be observed as trabecular thickening (thickening of the Cooper's ligaments and fibrous stroma), causing a diffuse increase in parenchymal density and reduced breast compressibility.[97] Therefore, if parenchymal oedema is considered a component of breast lymphoedema these imaging methods could be used in combination with other assessment methods that are adept at assessing subcutaneous breast oedema to provide a comprehensive assessment of breast lymphoedema. However, further research is required to understand both the role parenchymal oedema plays in breast lymphoedema and evaluate the effectiveness of these two techniques in assessing breast lymphoedema.

A considerable disadvantage of MRI is its accessibility and cost. This may limit its clinical use in breast lymphoedema, making prospective monitoring unrealistic. In comparison mammography would be more accessible and affordable. Additionally, it is already used as a routine investigation in breast cancer. Mammograms are regularly administered each year after breast cancer treatments and therefore could provide a method to monitor patients at risk of developing breast lymphoedema without additional imaging. However, more regular imaging may be required to prospectively monitor patients at risk of developing breast

lymphoedema to ensure it is detected in early stages. As mammography exposes patients to radiation, despite the dose being very low, more frequent imaging may not be realistic. Furthermore, due to the limitations of both imaging techniques the scope of using these assessment methods in the clinical setting to evaluate acute outcomes of breast lymphoedema treatment would be confined.

In consideration of the aforementioned assessment methods it is evident that assessment methods for use in breast lymphoedema need to be easy to use, repeatable, minimally or non-invasive, objective, valid and reliable, and clinically relevant. In view of this, ultrasound and TDC measures of breast tissue water appear to have the greatest potential to accurately assess breast lymphoedema. However, these methods assess breast lymphoedema based upon its clinical signs. As discussed in **Chapter 1, Section 1.5** the early detection of secondary lymphoedema is vital to limit disease progression and severity. Therefore, a method of diagnosing and assessing breast lymphoedema early in subclinical stages, before clinical signs manifest, would provide significant benefits. As secondary lymphoedema occurs as a result of damage and obstruction to the lymphatic system leading to a reduced transport capacity, an assessment method that can visualise lymphatic vasculature and lymphatic transport may enable subclinical diagnosis and early intervention.

2.3 ICG lymphography

ICG lymphography is a novel imaging technique that can evaluate the superficial lymphatic system in real time. It allows direct visualisation of superficial lymphatic vessels and the transport of lymph within this system. Additionally, it can identify changes to lymphatic vessel morphology indicative of lymphoedema (dermal backflow and collateral lymphatic drainage pathways).[73,75,99] Therefore, ICG lymphography may allow the visualisation of the superficial breast lymphatics and this imaging technique should be considered as a potential method to assess this condition.

Since the 1950s ICG lymphography has been employed safely in other areas of medicine such as ophthalmology to examine blood flow within the eye, and cardiology to determine cardiac output.[99-102] In 2007 Unno et al.[73] and Sharma et al.[103] explored its application in lymphatic imaging and lymphoedema. Unno et al.[73] investigated secondary leg lymphoedema, reporting that ICG lymphography was safe to use and effective in detecting lymphatic disorders through the presence of dermal backflow. Sharma et al.[103] visualised synchronised contractions of lymphangions and the propulsion of lymph in collecting vessels when using ICG lymphography in a swine model. Their findings demonstrated the capabilities of ICG lymphography in assessing lymphatic transport in real time. Since these initial investigations the use of ICG lymphography in lymphatic imaging has gained momentum and further research has proven it to be a valid and reliable assessment method for BCRL.[99,100]

ICG dye is relatively safe with minimal side effects making it a good tracer to use in lymphatic imaging. Moreover, it means that ICG lymphography is repeatable and could be used safely in longitudinal breast lymphoedema studies. ICG dye is a tricarbocyanine water soluble dye[104] with iodide properties. These properties mean that its use should be avoided in anyone with a known allergy to iodine, sodium iodide, or anaphylaxis to shell fish, an over-active thyroid or known benign thyroid tumour.[102] In the study undertaken in **Chapter 3** participants with any risk of a reaction to ICG were excluded. Aside from this, ICG dye has few side effects and minimal risk involved in its use. Severe allergic reaction is reported as very rare (less than 1 in every 10,000 patients).[105] Additionally, unlike some other lymphatic imaging techniques, ICG dye does not expose patients to radiation.[104]

Further advantages of using ICG dye in lymphatic imaging include; i) its affinity to bind with protein (albumin and alpha-1 lipoprotein)[106] which is readily transported in the lymphatic system, and ii) its fluorescence properties once bound to protein enabling visualisation of lymphatic transport and vasculature. Bound ICG generates a strong fluorescence in the near-

infrared wavelengths. The peak excitation wavelength for ICG in blood is approximately 800 nm and its peak fluorescence wavelength is approximately 840 nm.[106] These fluorescence properties have the advantage of allowing deeper penetration of ICG lymphography imaging (up to 20 mm)[107] as the near-infrared wavelength avoids influence of autofluorescence by haemoglobin (absorbs light at wavelengths < 650 nm) and water (absorbs light at wavelengths > 900 nm) in the tissue.[106] Additionally, the different wavelengths for ICG excitation and fluorescence emission enable a high contrast and clear image.[104]

2.3.1 ICG lymphography technique

ICG dye is unstable in solution therefore it is stored in a lyophilized form, protected from light and below 30°C. Once reconstituted it must be used within 6-10 hours.[102] Typically it is reconstituted with either distilled water or 0.9% sodium chloride and then this solution is injected intradermally or subcutaneously into the affected tissue. A small volume of ICG solution (0.1 – 0.3 ml) is administered at each injection site.

ICG lymphography injection sites are determined based upon anatomical knowledge of the superficial lymphatic system. In particular, the origin of lymphatic collecting vessels and the normal drainage pathways of these vessels to lymph nodes. In BCRL, injection sites are often located in the distal aspect of the arm close to the origin of collecting vessels. Additionally, as collecting vessels are fairly independent with few interconnections[10] injections are administered circumferentially. For example, four injection can be administered in the arm at the webspaces of the first and fourth fingers and the medial and lateral aspects of the anterior wrist.[75] This protocol enables a comprehensive assessment of the arm's superficial lymphatic system and identification of lymphatic drainage patterns and pathways.

To visualise ICG fluorescence a near-infrared camera system (e.g. Hammamatsu PDE-Neo II, Hammamatsu Photonics, Japan) is scanned over the tissue. The camera system is equipped with a light emitting diode to emit a near-infrared wavelength, different filters to both remove the fluorescent wavelength and the excitation light, and a charge coupled device to detect the emitted light and image.[104] The Hammamatsu PDE-Neo II camera system was employed in the study undertaken in **Chapter 3**. This camera system is relatively inexpensive, and its portability enables it to be use in a variety of clinical settings (e.g. outpatient clinics, or surgical theatres). The camera system's light emitting diode emits wavelengths centred at 760nm and uses a bandpass filter to block light from the charge coupled device at less than 820nm.[107] This camera system consists of a; i) hand held camera unit, ii) controller unit, iii) digital video processor, and iv) remote control.

The hand-held camera unit has a fixed focus at 15-25 cm allowing investigation of a 10 x 10 cm^2 field of view.[76] Additionally, it has a focus ring for near focus and close up imaging of lymphatic vessels (**Figure 1.5**). Control functions on the camera permit the user to switch between ICG fluorescent images (black and white or green colour images) and white light emitting diode images so that the lymphatic vasculature can be referenced to the patient's surface anatomy. The excitation light intensity can also be altered on the hand-held camera unit to improve visualisation of the lymphatic vasculature. The controller unit enables image enhancement through the adjustment of contrast and brightness. A digital video processor is connected to an external monitor so that the scanned image can be viewed in real time. A remote control adds additional features such as still image photography of the lymphatic vasculature.

During and after the scanning process images can be analysed to diagnose and assess lymphoedema. Morphological changes to the lymphatic vasculature (dermal backflow patterns and collateral lymphatic drainage pathways) are common diagnostic and assessment characteristics used in ICG lymphography (**Chapter 1, Section 1.5)**. Dermal backflow patterns are further delineated into subtypes based upon their visual appearance and can be used to stage lymphoedema severity. For example, Yamamoto et al.[99] classifies dermal

backflow patterns as splash, stardust, and diffuse. A splash pattern, which is equivalent to subclinical lymphoedema, is described as scattered ICG dye in tortuous lymphatic vessels. A stardust dust pattern is described as dimly luminous and spotted fluorescent ICG signals, and a diffuse pattern as widely distributed ICG dye without any twinkling or identifiable spots.[99] As lymphoedema severity increases these dermal backflow patterns progress from a splash to diffuse pattern. Alternatively, patent lymphatic vasculature with normal lymphatic function has been used to exclude a diagnosis of lymphoedema.[99,100] Referred to as a liner pattern, it can be observed as longitudinal lines travelling from injection sites, through the tissue, to lymph node regions (**Chapter 3**, **Figure 3.2**).

In comparison to other assessment methods used in breast lymphoedema, ICG lymphography may offer a number of advantages. The main advantage is its ability to directly visualise lymphatic vasculature and the transport of lymph in real time. Therefore, through the detection of early lymphatic dysfunction it may provide an objective assessment method capable of diagnosing breast lymphoedema earlier than other assessment methods. In BCRL ICG lymphography has enabled early diagnosis at subclinical stages with high sensitivity and specificity.[101,108] Moreover, Akita et al.[100] demonstrated reversibility of subclinical dermal backflow patterns (splash) to normal linear patterns and mild lymphoedema (stardust patterns) to subclinical (splash) or normal (linear) patterns on ICG lymphography in some patients with BCRL. This finding has significant implications for patients with secondary lymphoedema as it suggests a potential reversibility when early intervention is performed.

A second advantage of ICG lymphography imaging is that it can be used to map lymphatic drainage pathways. As discussed in **Chapter 1**, the lymphatic drainage pathways in the 'normal' breast drain predominately to the ipsilateral axillary lymph nodes.[12] In breast lymphoedema however the lymphatic drainage pathways may be altered as a result of BCT. Lymphoedema treatments direct lymph from affected areas towards unaffected areas utilising these lymphatic drainage pathways. Therefore, understanding these alterations may change current breast lymphoedema management and improve treatment outcomes for patients.

2.4 Chapter summary

This chapter reviewed the commonly reported breast lymphoedema assessment methods. Considering the limitations of each, specifically their constraints in identifying early breast lymphoedema, a new assessment method ICG lymphography was proposed. The unique characteristics and functions of this lymphatic imaging technique warrant the investigation of its use in the assessment of breast lymphoedema.

To date no studies have examined the role of ICG lymphography in breast lymphoedema. Therefore, a pilot study was undertaken (**Chapter 3**) to explore the potential utility of this technique in the assessment of breast lymphoedema and to investigate if the lymphatic drainage pathways of the breast are altered following BCT. Because the normal lymphatic drainage of the breast has not been previously reported using ICG lymphography, a healthy control group was required to allow comparison of these drainage pathways. Moreover, in addition to ICG lymphography, assessment methods commonly performed in the clinical setting (symptom-related questions, physical examination, and TDC measures of breast tissue water content) were undertaken on each study participant to confirm and compare ICG lymphography findings of breast lymphoedema.

Chapter Three: Assessing breast lymphoedema following breast cancer treatment using indocyanine green lymphography

This chapter is the exact format of the manuscript which has been submitted to Breast Cancer Research and Treatment with the exception of the tables and figures. These have been incorporated into the manuscript and numbering aligns with the thesis format.

Statement from co-authors

As co-authors of the paper 'Assessing breast lymphoedema following breast cancer treatment using indocyanine green lymphography', we confirm that Asha Heydon-White was primarily responsible for undertaking this study and writing the manuscript. She has made the following contributions;

- Conception and design of the pilot study in collaboration with the other authors.
- Screening and enrolment of participants onto the study.
- Participant assessments (questionnaires, physical examination, TDC measures of breast tissue water content).
- Data collection and analysis.
- Writing of the manuscript and critical appraisal of the content.

Dr Karen Peebles _____ Date: 22.04.2020

A/Prof Hiroo Suami _____ Date: 22.04.2020

Prof John Boyages _____ Date: 22.04.2020

Ms Louise Koelmeyer _____ Date: 22.04.2020

ASSESSING BREAST LYMPHOEDEMA FOLLOWING BREAST CANCER TREATMENT USING INDOCYANINE GREEN LYMPHOGRAPHY

Short title: Assessing breast lymphoedema using ICG lymphography

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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.

Key words: 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 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] Whilst 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 don't allow direct visualisation of the lymphatic system. This is important as

abnormal lymphatic morphology suggestive of lymphatic dysfunction precede 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.

i) *Physical assessment:* 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.

ii) *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]

iii) *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 **Figure 3.1**. 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 minutes. If ICG did not extend to the breast following injections at sites 1 and 2, a further injection was administered at site 3. Imaging was considered complete once lymphatic drainage pathways of the breast were identified and images became stable (no further extension of dermal backflow).

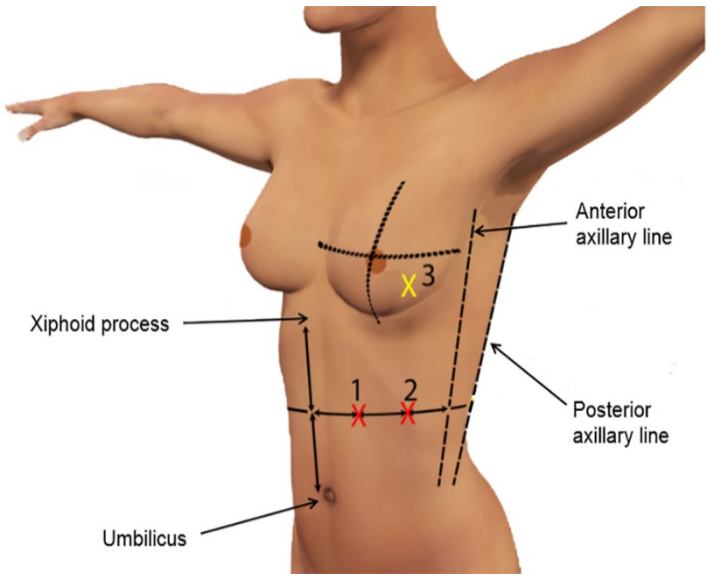

Figure 3.1 ICG lymphography breast lymphoedema injection sites. Injection site 1 and 2: the mid-point between the xiphoid process and umbilicus was identified. The horizontal distance from this mid-point to the anterior axillary line was then divided into thirds. The first injection site was marked at 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.

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 3.1** Participant characteristics. 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 is summarised in **Table 3.2**. 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.

Table 3.1 Participant characteristics

	Controls (n = 10)	Breast lymphoedema (n = 10)
Age (y)	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.

The TDC measures of tissue water percentage are shown in **Table 3.3**. 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 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 3.4**).

Representative ICG lymphography images of lymphatic drainage patterns and pathways for both groups are shown in **Figure 3.2**. 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 3.4**). 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 3.4**). 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 3.4**). 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.

Table 3.2 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 3.3 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.

Table 3.4 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.

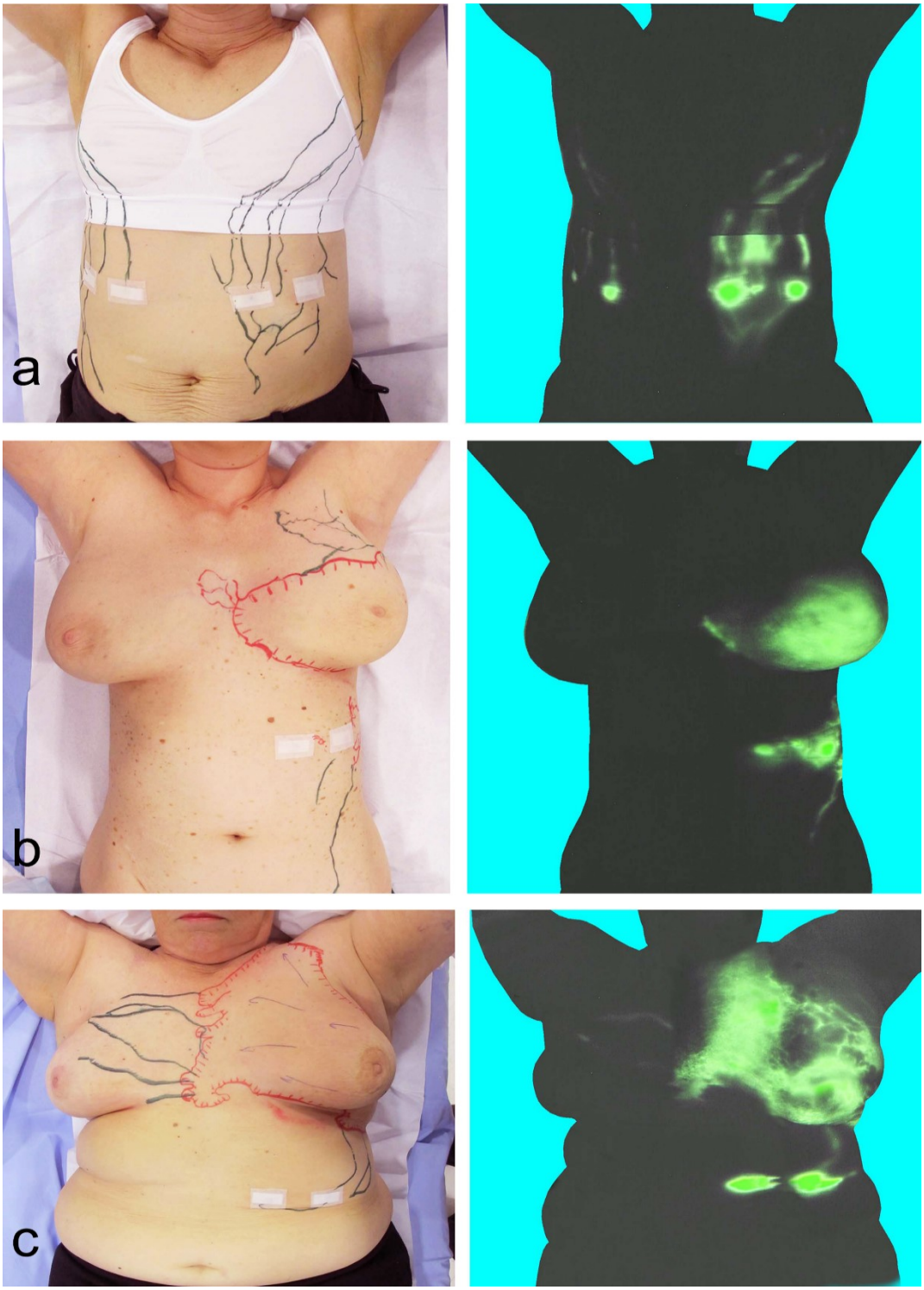

Figure 3.2 Representative still photographs (left) and ICG lymphography montage images (right) in control and breast lymphoedema participants. ICG lymphography images were taken at the end of the scanning procedure thus incurring some washout of ICG dye from collecting vessels. Still photograph images: 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

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 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 knowledge that in cases of breast lymphatic obstruction, lymph drainage of the anterior upper torso may bypass the breast via collateral pathways (**Figure 3.1**). 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 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, indicate 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 3.4**) 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.

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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.

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Chapter Four: Discussion

4.1 Overview

This thesis explored the utility of ICG lymphography in the assessment of breast lymphoedema. The original pilot study (**Chapter 3**) clearly demonstrated that ICG lymphography has a role in the assessment of this condition. Additionally, ICG lymphography was shown to be capable of mapping the lymphatic drainage pathways of the breast and identified alterations to these pathways in participants with breast lymphoedema following BCT. It is believed this is the first study to use ICG lymphography to assess breast lymphoedema. The question is now posed as to whether ICG lymphography could be incorporated into the clinical setting as an assessment method for breast lymphoedema. Moreover, whether it could be used in the management and monitoring of breast lymphoedema.

This final chapter will use a translational approach to explore how research utilising ICG lymphography in lymphoedema can be applied to the clinical area of breast lymphoedema. Specifically, it will use the study findings, in the context of the wider breast cancer-related arm lymphoedema (BCRL) arena, to explore the broader clinical implications of ICG lymphography in breast lymphoedema. The discussion will be posed around three clinically relevant questions as follows.

1. Can ICG lymphography be used to assess breast lymphoedema in the clinical setting?
2. Can ICG lymphography be used in the clinical management of breast lymphoedema?
3. Can ICG lymphography be used to monitor breast lymphoedema?

At the end of this chapter several practical considerations will also be addressed.

4.2 Question 1: Can ICG lymphography be used to assess breast lymphoedema in the clinical setting?

The assessment of breast lymphoedema requires methods which can objectively assess breast lymphoedema, measure its severity, and accurately identify the early subclinical stages of this disease. Based upon the pathophysiology of secondary lymphoedema (**Chapter 1, Section 1.5**), earlier detection requires assessment methods capable of identifying early lymphatic dysfunction and changes to the lymphatic system (e.g. alterations in lymphatic patterns and pathways) that precede the clinical signs of breast lymphoedema (e.g. oedema, skin thickening). In clinical practice, assessment methods also need to be minimally or non-invasive, safe to use repeatedly, cost-effective, and relatively easy to perform, albeit with some prior training. In the assessment of secondary lymphoedema, ICG lymphography meets many of these criteria and therefore its application in the clinical assessment of breast lymphoedema should be considered. To determine its capabilities in the clinical setting it needs to be examined if ICG lymphography can be used; i) to detect the presence or absence of breast lymphoedema (**Section 4.2.1**), ii) to assess the severity of breast lymphoedema (**Section 4.2.2**), iii) to detect subclinical breast lymphoedema (**Section 4.2.3**), and iv) as a composite tool in the assessment of breast lymphoedema (**Section 4.2.4**).

4.2.1 ICG lymphography can be used to detect the presence or absence of breast lymphoedema

The study undertaken as part of this thesis demonstrated that ICG lymphography is capable of visualising the lymphatic patterns and pathways of the breast. This included those of normal lymphatic anatomy (linear patterns and dominant lymphatic drainage pathways), and those that are diagnostic of lymphoedema (dermal backflow and collateral lymphatic drainage pathways).

Support for the use of ICG lymphography to detect the presence and absence of breast lymphoedema based on alterations in lymphatic drainage patterns is as follows. First, the

study in **Chapter 3** showed that dermal backflow was observed in all breast lymphoedema participants, whereas linear drainage patterns (and the absence of dermal backflow) were observed in all healthy control participants. From these results, it would appear that ICG lymphography has the required resolution to identify the presence and absence of dermal backflow with 100% accuracy in the breast. Whether this would have been the case in a larger population of participants requires further investigation. Nevertheless, these findings offer promise for the ability of ICG lymphography to clearly detect the presence or absence of breast lymphoedema, based upon this feature.

Second, ICG lymphography studies performed in body regions outside the breast have highlighted the importance of dermal backflow as a cardinal feature of lymphoedema. For example, studies using ICG lymphography to assess secondary lymphoedema including BCRL, lower limb lymphoedema, and abdominal lymphoedema[73,99,108-110] have proven that dermal backflow patterns are diagnostic of lymphoedema. Because the morphological changes to the superficial lymphatic system in secondary lymphoedema are similar across different limbs and body areas, it is reasonable to conclude that these changes can also be applied in the detection of breast lymphoedema.

Further support for the use of ICG lymphography to detect breast lymphoedema comes from its ability to identify collateral lymphatic drainage pathways in patients with breast lymphoedema. In the study undertaken in **Chapter 3**, collateral drainage pathways were observed in the majority (90%) of breast lymphoedema participants. In contrast, the dominant lymphatic drainage pathway to the ipsilateral axilla (and the absence of collateral lymphatic drainage pathways) were observed in all healthy control participants. These results imply that ICG lymphography can assess the lymphatic drainage pathways of the breast, detecting both normal lymphatic drainage and alterations to lymphatic drainage pathways that are diagnostic of lymphoedema. Therefore, similar to lymphatic drainage patterns, these findings are encouraging and warrant the further study of lymphatic drainage pathways in the detection of breast lymphoedema.

It is recognised that previous ICG lymphography studies in BCRL have not routinely analysed lymphatic drainage pathways. However, that is not to say these pathways are not worthy of investigation. A potential reason for this is that ICG lymphography assessment criteria for lymphoedema has been modelled upon earlier lymphatic imaging techniques such as lymphoscintigraphy. Lymphoscintigraphy has been used as an assessment method for lymphoedema since the 1950s and is considered the gold standard assessment method for limb lymphoedema.[63] While dermal backflow is classified as a cardinal diagnostic feature of lymphoedema using lymphoscintigraphy, collateral lymphatic drainage pathways are considered an additional feature with less diagnostic accuracy.[63,111] However, lymphoscintigraphy has poor resolution, creating two-dimensional images that are grainy in appearance. Furthermore, real-time imaging is not possible with this technique. Consequently, it be could be hypothesised that ICG lymphography is better equipped to identify superficial collateral lymphatic drainage pathways and that the role of these pathways in the detection and assessment of lymphoedema has not been fully established.

In the context of lymphatic drainage pathways, it is important to highlight ICG lymphography analysis also revealed unexpected lymphatic drainage pathways of the anterior upper torsos. In the pilot study in **Chapter 3**, lymphatic collecting vessels were visualised draining lymph from injection sites 1 and 2 in an inferior direction in a number of participants of both groups. These vessels were observed travelling towards abdominal regions and across the horizontal watershed to inguinal regions (**Figure 4.1**). The drainage pathways observed were variable between participants and even asymmetrical between sides in some control participants (**Figure 4.1**). These finding are discordant with Sappey's description of the lymphatic drainage of the torso territories.[12] In the breast lymphoedema participants, it is possible that these lymphatic collecting vessels were collateral pathways that had developed through lymphangiogenesis to reduce lymphatic overload[77,78] of the anterior upper torso. However, their presence in the healthy control group suggests that these vessels form part of the normal lymphatic anatomy. These findings are supported by others who also observed lymphatic collecting vessels of the torso draining in directions discordant with Sappey's descriptions, across watersheds, and asymmetrically between sides.[13,14,16] This suggests that the lymphatic anatomy of the torso is complex. The variable nature of lymphatic drainage between individuals and between right and left sides of the same individual highlights the challenges in determining the normal lymphatic anatomy of this area and the

accurate identification of changes to lymphatic drainage as a result of lymphoedema. However, these findings do demonstrate that ICG lymphography is capable of visualising lymphatic vasculature in the upper torso and could be considered as a potential method to assess lymphoedema of this area.

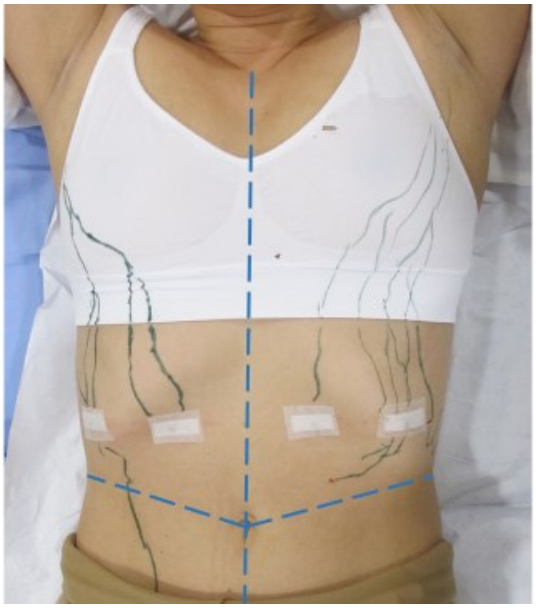

Figure 4.1 Control participant with asymmetrical inferior lymphatic drainage from left and right injection site 2. Lymphatic drainage was to abdominal region (left side) and crossing horizontal watershed to inguinal region (right side). Midline and horizontal watersheds marked on image (blue dotted lines).

4.2.2 ICG lymphography could be used to assess the severity of breast lymphoedema

The development of an ICG lymphography staging system would provide an objective measure to classify the severity of breast lymphoedema. Currently breast lymphoedema severity is determined using physical examination and/or questionnaires that assess clinical signs and symptoms.[9,37,82] As discussed in **Chapter 2**, these assessment methods are relatively subjective and the staging systems used vary between studies, or are not defined. This potentially limits the accuracy of results and reduces test-retest reliability.

The use of ICG lymphography to develop a staging system is not an entirely new concept as it has been employed successfully in the area of BCRL. Utilising lymphatic patterns, Yamamoto et al. (Arm dermal backflow stage),[99] Chang and colleagues (M. D. Anderson lymphoedema classification),[112] and Nguyen et al. (modified M. D. Anderson lymphoedema classification)[113] have each outlined a staging system to assess the severity of BCRL. Despite some differences between these staging systems, they all assign a severity stage of BCRL (e.g. stage 0 indicating no lymphoedema, stage 5 indicating severe lymphoedema) based upon the patterns observed in the arm (e.g. linear, splash, stardust, or diffuse), and the extent of dermal backflow. These studies indicate ICG lymphography has the ability to detect subtle changes in the pattern of lymphatic dysfunction which relates to disease severity. Accordingly, they demonstrate how dermal backflow patterns could be used to develop a breast lymphoedema severity staging system.

Finally, and most recently, Suami et al.[75] not only used the patterns of dermal backflow (modified M. D. Anderson lymphoedema classification) but also analysed the lymphatic drainage pathways in BCRL patients to provide information relating to severity. This retrospective cohort audit compared the lymphatic drainage pathways of over 100 patients with BCRL with their modified M. D. Anderson lymphoedema classification. They found that 95% of patients with a severity stage of 1 (mild lymphoedema) continued to have a patent dominant lymphatic drainage pathway. In regards to the collateral lymphatic drainage pathways of the arm, earlier stages of BCRL (modified M. D. Anderson stage 2 and 3) predominantly drained to the clavicular region. In more severe stages of BCRL (modified M. D. Anderson stage 4), collateral drainage pathways to the parasternal and contralateral axilla regions were more likely.

The study in **Chapter 3** demonstrated that both dermal backflow patterns and lymphatic drainage pathways could be identified in the breast. Therefore, based upon the aforementioned staging systems used to assess BCRL severity, there is potential to use both the patterns and pathways identified on ICG lymphography to develop a severity staging system for breast lymphoedema. Whilst the pilot study in **Chapter 3** was not designed to identify or analyse different patterns of dermal backflow, it is relevant to mention that patterns

similar to those observed in BCRL[99,112,113] were observed in the breast lymphoedema participant group. For example, some participants demonstrated dermal backflow patterns similar to the diffuse pattern described in the BCRL literature. Moreover, in the breast lymphoedema group, the ICG lymphography results demonstrated differences in the collateral drainage pathways observed in those with a high or low TDC ratio (see **Chapter 3** and **Section 4.2.4**). These findings support the undertaking of a larger study to confirm if ICG lymphography can detect subcategories of dermal backflow in the breast, and if lymphatic patterns and pathways of the breast can accurately assess breast lymphoedema severity.

Similar to the staging systems described in BCRL, an ICG lymphography breast lymphoedema staging system could use a five point scale (0 – 4) to assign severity. For example; stage 0 indicates no lymphoedema, Stage 1 describes subclinical lymphoedema, Stage 2 mild clinical lymphoedema, Stage 3 moderate lymphoedema, and Stage 4 severe lymphoedema. By implication, having a scale like this would enable the early detection of breast lymphoedema. The next section (Section **4.2.3**) will specifically discuss how ICG lymphography could be used to detect subclinical breast lymphoedema.

4.2.3 ICG lymphography could be used to detect subclinical breast lymphoedema

A fundamentally important stage in lymphoedema severity and one that has immense clinical implications is subclinical lymphoedema. As discussed in **Chapter 1, Section 1.5**, the early diagnosis and management of secondary lymphoedema is vital to limit disease severity and progression. Detection of lymphoedema in its subclinical stage implies lymphoedema is diagnosed at the earliest possible stages of lymphatic dysfunction before clinical signs of lymphoedema manifest. Consequently, management at this stage offers the greatest treatment outcomes, as irreversible changes to lymphatic vasculature and subcutaneous tissue architecture would be minimal or absent. Although it is yet to be investigated in breast lymphoedema, it seems reasonable to suggest that early detection and management would have similar implications. Therefore, assessment methods that enable the detection of subclinical breast lymphoedema would be beneficial in the management of this condition.

The study in **Chapter 3** was not specifically designed to determine if ICG lymphography could detect subclinical breast lymphoedema. Nevertheless, it was designed with this broader consideration in mind. This exploratory pilot study served to determine if ICG lymphography could be undertaken in the breast to identify breast lymphoedema. The next step would be to investigate if ICG lymphography can be used to not only to assess breast lymphoedema severity, but also early subclinical stages.

Support for the role of ICG lymphography to detect subclinical changes in breast lymphoedema, earlier than more conventional assessment methods, again comes from limb lymphoedema literature. A study by Akita et al.[100] demonstrated that ICG lymphography was able to detect BCRL in 50% of participants prior to any changes in limb volume measured using conventional limb volumetric assessments. Furthermore, they demonstrated that early detection enabled earlier management of BCRL, which reversed dermal backflow patterns to splash or linear patterns in some patients. Due to the chronicity of clinical lymphoedema, this finding is extremely important and highlights how crucial prospective monitoring, early diagnosis, and management are in secondary lymphoedema. Accordingly, it is likely that ICG lymphography could have similar applications in breast lymphoedema.

Support for the use of ICG lymphography in the assessment of early breast lymphoedema also comes from its known diagnostic accuracy in detecting subclinical secondary limb lymphoedema. Akita et al.[108] reported ICG lymphography to have a sensitivity of 0.98, specificity of 0.55, and an accuracy of 0.82 in detecting secondary limb lymphoedema (leg and arm) when compared with the gold standard, lymphoscintigraphy. Interestingly, they found that when subclinical lymphoedema (splash pattern on ICG lymphography) was reported as a negative result, sensitivity, specificity, and accuracy all improved (0.98, 0.93, 0.95 respectively). These results suggest that ICG lymphography may be better at detecting subclinical secondary limb lymphoedema compared to lymphoscintigraphy and support its use in the diagnosis of both clinical and subclinical limb lymphoedema. Mihara et al.[101] also reported that ICG lymphography had a high sensitivity (1.0) and specificity (1.0) in diagnosing early stage arm lymphoedema. While these results demonstrated even better

sensitivity and specificity, they may be slightly inflated because this study used physical assessment as a comparator test rather than lymphoscintigraphy.

Considering these findings in limb lymphoedema, it seems reasonable to conclude that ICG lymphography is able to identify early subclinical lymphoedema accurately and before more conventional assessment methods. However, further investigation is required to verify if ICG lymphography could have similar applications in breast lymphoedema. In order to address this clinical question, an adequately powered longitudinal prospective study involving two steps that monitors patients at risk of developing breast lymphoedema following BCT is now proposed. The purpose of step one is to develop a severity staging system for breast lymphoedema. As discussed in **Section 4.2.2**, the lymphatic patterns and pathways of the breast could be analysed in patients with breast lymphoedema to create a severity staging system for the breast. Step two would involve undertaking the study itself. The aim of step two is to determine if ICG lymphography can identify early subclinical lymphoedema of the breast. This study would recruit patients diagnosed with breast cancer planning to undergo BCT. Each patient would undergo a baseline ICG lymphography assessment prior to the commencement of BCT and then repeat ICG lymphography assessments at regular time intervals for a period of at least 12 months. Follow up assessment times could be as follows; after surgery and prior to radiotherapy treatment, six weeks after completion of radiotherapy treatment (approximately three months after surgery), then six, nine, and 12 months after the date of surgery. Based upon prior BCRL studies,[99,100] it is hypothesised that ICG lymphography would detect altered patterns of lymphatic drainage in patients with subclinical breast lymphoedema.

4.2.4 ICG lymphography can be used as a composite measure of breast lymphoedema

In the clinical setting, a range of methods are often employed to assess clinical lymphoedema. Undertaking a variety of assessments can provide a more comprehensive evaluation of this condition. For example, a patient with advanced BCRL may have the following assessments; i) volumetric measurements with either a tape measure or Perometer to determine the volume

difference of the affected arm compared to the unaffected, ii) a measure of the extracellular fluid component of the arm (e.g. bioimpedance spectroscopy), iii) ICG lymphography to assess the lymphatic vasculature and lymphatic transport, and iv) an MRI to determine the amount of adipose tissue and skin thickening within the arm.[114] Therefore, in clinical breast lymphoedema, ICG lymphography may be one of a few assessment methods utilised to complete a comprehensive assessment of this condition. This raises the question as to which assessment methods would be most appropriate to use alongside ICG lymphography. These assessment methods should be objective, repeatable, accessible, and able to quantitatively assess the clinical signs of breast lymphoedema.

One method that could be used alongside ICG lymphography is the TDC device. The benefits of the TDC device in assessing clinical breast lymphoedema have already been outlined in **Chapter 2**, **Section 2.2.3**. Additionally, the TDC results from the study in **Chapter 3**, identifying a higher percentage water content in all quadrants of the affected breast compared with the unaffected breast and control participants (**Chapter 3**, **Table 3.3**), further support the use of this device in assessing breast lymphoedema. As a composite measure with ICG lymphography, the findings in **Chapter 3** highlight the potential of use of these two techniques in assessing breast lymphoedema severity. Results from this study demonstrated that participants in the breast lymphoedema group who continued to have lymphatic drainage of the breast to the dominant pathway (ipsilateral axilla) had a lower whole breast TDC ratio (<1.40). Furthermore, aside from collateral drainage to the parasternal region which was equally present in participants with a higher or lower whole breast TDC ratio, these participants had a tendency to have collateral drainage towards the intercostal region. Alternatively, breast lymphoedema participants with a higher whole breast TDC ratio (≥1.40) did not demonstrate patency of their dominant lymphatic drainage pathway, and collateral lymphatic pathways were more commonly observed to drain towards clavicular and contralateral axilla regions (**Chapter 3**, **Table 3.4**).

Another potential assessment method that could be combined with ICG lymphography is ultrasound. Ultrasound has excellent reliability in detecting dermal thickness of the breast[25] (**Chapter 2, Section 2.2.4**) and therefore could provide a quantitative, objective, repeatable

measure of skin thickening in breast lymphoedema. As skin thickening is a common secondary tissue change of clinical breast lymphoedema,[24] its assessment in conjunction with the above-mentioned methods would further aid in evaluating breast lymphoedema. However, one disadvantage of this assessment method is that it is not as readily accessible as the TDC device in the clinical setting and requires more specialised training.

Further assessment methods which should be considered as composite measurements of breast lymphoedema include MRI and mammography. The advantage of these techniques, over and above the TDC device and ultrasound, is that these medical imaging techniques are capable of assessing parenchymal oedema (**Chapter 2, Section 2.2.4**). If it is confirmed that breast lymphoedema does involve a component of parenchymal oedema, either of these assessment methods could be incorporated alongside ICG lymphography to provide a more comprehensive assessment of this condition.

The use of ICG lymphography as a composite measure for breast lymphoedema is not limited to the clinical setting. Combining assessment methods in the research setting may improve clinical knowledge of breast lymphoedema. For example, ICG lymphography and the TDC device could be used in conjunction with a comprehensive medical and treatment history to identify a risk factor profile for breast lymphoedema. While the study in **Chapter 3** was not designed to identify a risk factor profile for breast lymphoedema, the medical history and anthropometric measurements for each breast lymphoedema participants was collected. Therefore, a comparison of the study findings with risk factors described in the literature (**Chapter 1, Section 1.4.1**) can be undertaken and will be discussed below.

In the breast lymphoedema group in **Chapter 3**, dermal backflow was identified in all participants irrespective of the extent of axillary surgery. This implies that both SNB and ALND pose a risk for the development of breast lymphoedema. However, due to the small study numbers, it cannot be determined if there is a greater risk associated with ALND compared to SNB. Additionally, breast lymphoedema was identified in participants who had undergone an uncomplicated SNB procedure (**Table 4.1**). This contradicts the findings of

Wratten et al.[24] who reported patients undergoing SNB were only at risk of developing breast lymphoedema if their procedure was complicated by a post-operative wound infection.

Comparing other breast lymphoedema risk factors commonly discussed in the literature (**Chapter 1, Section 1.4.1**) with the study findings in **Chapter 3**, the following observations can be made. All breast lymphoedema participants had whole breast radiotherapy. The majority also had chemotherapy treatment, including Taxane (8/10), and had tumours in the upper outer quadrant, central, or lower inner quadrants (9/10). Interestingly, when breast lymphoedema participants were ranked according to whole breast TDC ratio results, those with a higher TDC ratio (≥1.40) had more risk factors than those with a lower TDC ratio. **Table 4.1** highlights these findings, demonstrating that the majority of participants with a higher whole breast TDC ratio (≥1.40) had complications following BCT such as infection, breast cellulitis, or seroma formation (4/5), a BMI ≥25 (4/5), and/or regional lymph node irradiation (3/5) compared to participants with a whole breast TDC ratio <1.40 (0/5, 1/5, 2/5 respectively). While it is acknowledged that larger studies are required to make conclusions about these findings, it suggests that these factors may contribute to the severity of breast lymphoedema. Furthermore, it supports the composite use of ICG lymphography and the TDC device (in combination with a comprehensive patient medical and treatment history) to progress clinical knowledge of breast lymphoedema.

Table 4.1 Breast lymphoedema participant's potential risk factors ranked according to whole breast TDC ratio

Case	Whole breast TDC ratio	LN surgery	WBI	Regional LN irradiation	BMI ≥25	Tumour UOQ/LIQ/Central	Chemotherapy	Hormonal therapy	Post-op infection	Cellulitis	Seroma
1	(1.81)	ALND	+	+	+	+	+	+	+	-	-
2	(1.77)	SNB	+	-	+	+	-	+	-	+	-
3	(1.64)	SNB	+	-	+	+	-	-	-	-	+
4	(1.46)	ALND	+	+	-	+	+	-	-	-	-
5	(1.45)	ALND	+	+	+	+	+	+	-	-	+
6	(1.39)	SNB	+	-	-	+	+	+	-	-	-
7	(1.16)	ALND	+	+	-	+	+	-	-	-	-
8	(1.15)	SNB	+	+	+	+	+	+	-	-	-
9	(1.07)	SNB	+	-	-	-	+	-	-	-	-
10	(1.03)	SNB	+	-	-	+	+	+	-	-	-

Breast lymphoedema cases listed in descending whole breast TDC ratio value. Dotted line delineates participants with whole breast TDC ratio ≥ 1.40. TDC, tissue dielectric constant; LN, lymph node; WBI, whole breast irradiation; BMI, body mass index; UOQ, upper outer quadrant; LIQ, lower inner quadrant; post-op, post-operative; ALND, axillary lymph node dissection; SNB, sentinel node biopsy.

4.3 Question 2: Can ICG lymphography be used in the clinical management of breast lymphoedema?

Lymphoedema therapists commonly employ traditional lymphoedema treatments such as compression, MLD, skin care, and exercise in the management of breast lymphoedema. However, there is a paucity of research investigating their effectiveness in the management of breast lymphoedema. ICG lymphography could provide a method to evaluate the efficacy of these treatments. Additionally, it could be used to inform and improve the clinical management of breast lymphoedema.

4.3.1 Evaluating the efficacy of breast lymphoedema treatments

The efficacy of traditional lymphoedema treatments in the management of breast lymphoedema has not been established. The only studies evaluating breast lymphoedema treatment have investigated the role of additional treatments such as kinesiology tape[115,116] and deep oscillation therapy in combination with MLD.[117] While these studies reported improvements in breast lymphatic drainage, further high powered studies are required to support their use.

One of the challenges faced in evaluating breast lymphoedema treatments is identifying an appropriate assessment method that can be used to analyse treatment outcomes. An assessment method that can objectively assess breast lymphoedema reliably over repeated measurements is required. Considering the functionality of ICG lymphography in the assessment of lymphoedema (**Chapter 2**), this technique offers a feasible method to investigate and evaluate breast lymphoedema treatments. Additionally, owing to its real-time imaging capabilities, high resolution, and direct visualisation of lymphatic vasculature and transport, it offers unique benefits when compared to other assessment methods. The potential uses of ICG lymphography in the evaluation of breast lymphoedema treatments include the; i) study of the long-term outcomes of treatment, and ii) study of the acute outcomes of treatment.

ICG lymphography is safe to perform repeatedly at regular time intervals and is therefore capable of investigating the long-term consequences of treatment. This was demonstrated in a study by Akita et al.[100] who used ICG lymphography in a longitudinal prospective study to assess BCRL. In this study, patients that developed clinical lymphoedema (stardust pattern) were provided conservative management (skin care, exercise, elevation, and compression garments). By analysing changes to participants' dermal backflow patterns at regular time intervals they were able to evaluate the outcomes of these treatments. A similar study design could be employed in breast lymphoedema.

As treatments can be performed during the ICG lymphography imaging procedure, the acute treatment outcomes of breast lymphoedema could also be studied. The real-time imaging capabilities provide immediate feedback on the effects of these treatments. Moreover, the direct visualisation of lymphatic vasculature and transport would enable the investigation of the physiological impacts that treatments have on the superficial lymphatic system of the breast. Assessing physiological features such as lymphatic contractility rate and velocity could provide quantitative measures to evaluate the effectiveness of breast lymphoedema treatments and should be considered in future research. Assessment of these features have been explored in limb lymphoedema and show promising results. In a study by Tan et al.[118] the contractile function of the lymphatic system was assessed using ICG lymphography to determine the efficacy of MLD in the lymphedematous limb. They concluded that ICG lymphography could accurately quantify treatment-related effects on lymphatic function and that MLD increased lymphatic velocity and reduced lymphatic propulsion periods. In another study by Adams et al.[119] the acute effects of intermittent pneumatic compression, a device which uses an inflatable sleeve to provide intermittent external pressure to the lymphedematous tissue, were investigated using ICG lymphography in control and BCRL participants. This study reported an improvement in lymphatic function in both participant groups and demonstrated that ICG lymphography could evaluate acute treatment outcomes.

4.3.2 Informing and improving clinical management

ICG lymphography has the ability to inform and improve breast lymphoedema treatments thereby influencing clinical management and treatment outcomes. There are a couple of ways in which ICG lymphography could be applied in the clinical management of breast lymphoedema. First, ICG lymphography could be used to improve treatment techniques. Second, ICG lymphography could provide a personalised management plan for each patient.

First, ICG lymphography may offer a significant advantage in improving the technique of MLD. MLD is one of the main treatment methods utilised in the management of breast lymphoedema. It involves gentle massage of the tissue to stimulate the contractile function of the superficial lymphatic system[118] and promote the movement of lymph from areas of congestion towards functioning lymphatics. Various hand movements are performed to facilitate the uptake of lymph into the lymphatic system and to assist lymphatic transport through areas of dermal backflow to patent lymphatic drainage pathways and regions. MLD is regularly employed in managing breast lymphoedema as other conventional techniques such as compression are difficult to apply to the breast. However, prior to ICG lymphography there was no way in which to visualise and assess the effectiveness of these hand movements on lymphatic transport. Anecdotal evidence from clinical experience using ICG lymphography-directed MLD suggests that the pressure and speed in which MLD needs to be applied differs depending on the presence of dermal backflow and the patency of lymphatic collecting vessels in the breast. Therefore, research investigating the hand manoeuvres, pressure, and speed of MLD in breast lymphoedema may improve this treatment technique.

Another way in which ICG lymphography could improve and inform treatments such as MLD is by identifying the lymphatic patterns and pathways of the breast in patients with breast lymphoedema. Dermal backflow patterns indicate areas of fluid accumulation and obstruction within the breast tissue. Therefore, being able to demarcate the exact area of dermal backflow using ICG lymphography would enable the lymphoedema therapist to focus treatment on these areas. Additionally, identifying the lymphatic drainage pathways of the breast would aid lymphoedema therapists as to which pathways to utilise in MLD

treatments. For example, lymphoedema therapists are often taught to perform MLD of the breast away from the ipsilateral axilla and towards the ipsilateral inguinal and contralateral axilla regions.[13] However, in the study presented in **Chapter 3**, lymphatic drainage of the breast to the ipsilateral axilla continued to be evident in four breast lymphoedema participants and no participants drained to the ipsilateral inguinal region. While some participants did demonstrate lymphatic drainage to the contralateral axilla (4/10), the most frequently observed collateral pathway was to the parasternal region (6/10). Additionally, collateral drainage pathways were observed to the clavicular (2/10) and intercostal (3/10) regions. While the possibility of collateral drainage to the inguinal region cannot be excluded, these findings do suggest that the direction in which MLD is applied in breast lymphoedema should be reconsidered. A larger study examining the drainage pathways of the breast in patients with breast lymphoedema would confirm the frequency of each pathway and could change and optimise current practice.

ICG lymphography can also be applied to optimise other treatments, such as intermittent pneumatic compression, which are used instead of or in conjunction with MLD. For example, different pressures applied during these treatments may increase or reduce lymphatic function. The real-time feedback of ICG lymphography would enable the evaluation of different treatment pressures to determine which pressure has the greatest impact on improving lymphatic transport in the breast. Zaleska and colleagues[120] investigated the role of ICG lymphography in assessing the effectiveness of various compression modalities including; intermittent pneumatic compression, and compression bandaging. By combining ICG lymphography with pressure monitoring they were able to identify adequate pressures required to transport lymph in the lymphedematous tissue for each treatment method. For example, a pressure of 80 – 120 mm Hg was considered to be the most effective pressure to evacuate oedema from the affected tissue when applying an intermittent pneumatic compression device. Similar applications could be utilised in breast lymphoedema to advance and improve treatments.

A second benefit of applying ICG lymphography in the clinical management of breast lymphoedema is its ability to provide personalised management plans for patients with breast lymphoedema. Each breast lymphoedema participant in the study in **Chapter 3**

demonstrated individual variability in relation to the extent of dermal backflow and the lymphatic drainage pathways of their breast following BCT (**Chapter 3**, **Table 3.4**). Therefore, an ICG lymphography assessment prior to undertaking treatment could help to guide lymphoedema therapists in deciding which treatments to apply and the direction in which lymph from the breast should be moved. The breast lymphoedema participants from the study in **Chapter 3** were asked if they had previously undergone treatment for their breast lymphoedema. While this was not reported in **Chapter 3**, it is interesting to note that four participants had undergone treatment that included MLD. A comparison of the direction in which MLD was applied by their lymphoedema therapist to the direction in which lymph was observed to drain from their affected breast using ICG lymphography can be seen in **Table 4.2**. The differences between the direction in which MLD was applied and the direction in which lymphatic drainage of the affected breast was observed highlights the benefit that a personalised ICG lymphography treatment plan would provide to optimise clinical management of breast lymphoedema.

Table 4.2 Lymphatic drainage of the breast. Comparison of the direction of MLD applied by lymphoedema therapists and the direction of lymphatic drainage observed using ICG lymphography

Participant	Direction of MLD	Direction of ICG lymphography
1	Parasternal	Contralateral axilla
	Clavicular	Clavicular
		Intercostal
2	Contralateral axilla	Ipsilateral axilla
		Parasternal
		Intercostal
3	Inguinal	Contralateral axilla
4	Contralateral axilla	Ipsilateral axilla
	Clavicular	Parasternal
		Intercostal

MLD, manual lymphatic drainage; ICG, indocyanine green.

In view of the points discussed above there are many research directions in which ICG lymphography could be employed to evaluate breast lymphoedema treatment modalities. Additionally, they demonstrate the substantial and unique benefits that ICG lymphography would offer in the clinical management of patients with breast lymphoedema.

4.4 Question 3: Can ICG lymphography be used to monitor breast lymphoedema?

Monitoring breast lymphoedema is an essential component of its assessment and management. ICG lymphography can be used in the monitoring of breast lymphoedema to detect breast lymphoedema in at-risk patients and serve as an outcome measure to evaluate treatments. Therefore this final question is not an isolated question but rather a natural extension of the previous two questions (**Section 4.2**, **Section 4.3)**. As the role of ICG lymphography in the monitoring of subclinical breast lymphoedema has already been discussed (**Section 4.2.3**), this section will consider its role in the monitoring of breast lymphoedema following diagnosis.

In addition to evaluating treatment outcomes, ICG lymphography could also be applied to monitor the time course of breast lymphoedema. The time course of the development and progression of breast lymphoedema remains unclear. Conflicting reports exist in the literature, some state that breast lymphoedema improves with time (one to three years),[24,41,93] while others have observed persistent breast lymphoedema at least six years after BCT.[37,42] For ethical reasons, a study monitoring the time course of breast lymphoedema left untreated could not be undertaken, unless a participant refuses treatment or treatments are contraindicated. Therefore, a more likely scenario is that monitoring breast lymphoedema using ICG lymphography could be used to determine whether a given treatment changes the natural course of disease progression.

The study proposed to investigate this would be a longitudinal prospective study using ICG lymphography to monitor patients at risk of developing breast lymphoedema. This study could serve to investigate not only the time course of breast lymphoedema but evaluate the role of MLD in the management of breast lymphoedema and identify risk factors associated with breast lymphoedema. Therefore, the purpose of this study would be; i) to determine if breast lymphoedema resolves within two years, ii) identify a risk factor profile for breast lymphoedema, and iii) evaluate the efficacy of MLD (traditional MLD versus ICG lymphography-directed MLD) in managing breast lymphoedema.

To evaluate the time course and potential resolution of breast lymphoedema this study would be required to be a three-year study. The methodology for the study would be as follows. Patients diagnosed with breast cancer planning to undergo BCT would be recruited at inception (i.e. prior to commencing any breast cancer treatment) and monitored at regular time intervals for a period of three years. Similar follow-up times to those proposed for the study to assess subclinical breast lymphoedema could be used in the initial 12 months. For the remaining two years, follow-up assessments would be conducted every six months. Initial and follow-up assessments would include; a medical history (breast cancer pathology and treatment details), anthropometric measurements (e.g. height and weight to determine BMI, breast cup size) and ICG lymphography assessment of the affected breast using the protocol described in **Chapter 3**. ICG lymphography results would be assessed to determine improvement, resolution, or progression of breast lymphoedema based upon observed patterns and pathways of lymphatic drainage. Additionally, when a patient is diagnosed with breast lymphoedema using ICG lymphography, a comparison with anthropometric measurements and the patient's medical history could be undertaken to identify a risk factor profile for this condition.

To evaluate the efficacy of MLD treatments, this study would be a three-armed study. Participants with no signs of breast lymphoedema (linear pattern on ICG lymphography) would continue to be monitored for the three-year period. Patients that develop breast lymphoedema (dermal backflow patterns) at any time point would be randomly allocated to either traditional MLD treatment or ICG lymphography-directed MLD. MLD treatments would be undertaken by a trained lymphoedema therapist weekly for a set

period of time (e.g. 12 weeks). Following treatment intervention, patients would have a follow-up assessment either in line with their standard assessment timeline, or an additional assessment would be undertaken to evaluate the outcomes of treatment. Further consideration would need to be applied to participants with persistent breast lymphoedema that did not improve following MLD treatment intervention. These participants would require ongoing treatment that may involve MLD and/or another form of lymphoedema treatment such as a compression bra, and ongoing monitoring to evaluate the long-term outcomes of that treatment.

4.5 Practical considerations

Finally, before a firm conclusion can be made regarding the role of ICG lymphography in the assessment of breast lymphoedema in the clinical setting, a couple of practical implications need to be considered. These include; the availability of ICG lymphography, training requirements, and the cost of undertaking this imaging.

The current availability of ICG lymphography is a consideration that will have implications on its clinical use in breast lymphoedema. Despite ICG dye being relatively safe to use in lymphoedema and lymphatic imaging, its use in this field is currently off-label. Therefore, until this changes its application is limited to specialist centres using ICG lymphography in the research setting. However, future research could investigate ways in which ICG lymphography findings can be transferrable to the clinical setting. One approach would be to investigate potential relationships between ICG lymphography and other more readily accessible objective assessment methods such as the TDC device. A second approach could be to analyse associations between ICG lymphography results (patterns and pathways) and other breast lymphoedema factors that would influence management of this condition. For example, which collateral lymphatic drainage pathways are the most frequently observed in patients with breast lymphoedema that have undergone ALND. Identification of associations could enable extrapolation of research findings to the clinical setting.

Undertaking ICG lymphography and analysing the findings does require some training. Accurate interpretation of results relies on clinician experience and an adequate knowledge of lymphatic anatomy. The ICG protocol developed in the study in **Chapter 3** aimed to ensure consistency and improve reliability of ICG injections and imaging technique across participants in both groups. This protocol could be utilised to aid clinician training. Additionally, the development of a standardised protocol for the analysis of ICG lymphography findings in breast lymphoedema would further assist in training, and increase intra-rater and inter-rater reliability of this assessment technique. A final point for consideration is that a medical clinician with appropriate qualifications to undertake ICG injections and manage any potential adverse reactions is required to be present during the imaging procedure. This may pose some restrictions on the use of ICG lymphography, limiting it to use in lymphoedema clinics where a multidisciplinary team operates. As an alternative, similar to other medical imaging, tertiary centres could be set up to perform ICG lymphography. Detailed reports of ICG lymphography findings and a personalised management plan could be sent to referring lymphoedema therapists to assist them in managing patients with breast lymphoedema.

Aside from the initial start-up equipment costs, the cost of undertaking ICG lymphography is relatively inexpensive when compared to other medical imaging techniques. Ongoing costs include the ICG dye and clinician appointment fees. While these costs may be incurred by the patient, many patients are likely to be interested in having this assessment given the benefits a personalised ICG lymphography management plan could provide.

4.6 Conclusion

ICG lymphography should be considered in the armamentarium of breast lymphoedema assessment tools. This thesis has demonstrated its utility in assessing the lymphatic drainage patterns and pathways of the breast to identify breast lymphoedema and map alterations to lymphatic drainage pathways following BCT. Additionally, it has examined

the current breast lymphoedema assessment methods, highlighting benefits that ICG lymphography may contribute. The most significant of these being the detection of early subclinical breast lymphoedema. Furthermore, this thesis has explored the clinical implications of ICG lymphography in the assessment, management, and monitoring of breast lymphoedema. While it recognises there are some practical considerations to the implementation of ICG lymphography in the clinical setting, the potential benefits to the assessment and management of breast lymphoedema warrant further exploration and investigation.

The findings and recommendations raised in this thesis offer many future research directions. Those considered of most immediate importance are as follows. Determining the diagnostic accuracy and reliability of ICG lymphography in the assessment of breast lymphoedema, in particular the detection of subclinical lymphoedema. The development of a severity staging system to provide an objective tool to clinically assess breast lymphoedema and compare research findings. Finally, future research should employ ICG lymphography to evaluate and improve the efficacy of breast lymphoedema treatments, optimising patient outcomes.

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Appendix 1: Macquarie University ethics approval

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CRICOS Provider No 00002J

16 May 2018

Dear Dr Peebles

Reference No: 5201800263

Title: *Use of Indocyanine Green Fluorescence Lymphography (ICG-LG) to assess breast oedema and identify lymphatic drainage pathways of the breast after cancer treatment*

Thank you for submitting the above application for ethical and scientific review. Your application was considered by the Macquarie University Human Research Ethics Committee (HREC (Medical Sciences)).

I am pleased to advise that ethical and scientific approval has been granted for this project to be conducted at:

- Macquarie University

Approval Date: 26 April 2018

This research meets the requirements set out in the *National Statement on Ethical Conduct in Human Research* (2007 – Updated May 2015) (the *National Statement*).

Standard Conditions of Approval:

1. Approval is contingent on continuing compliance with the requirements of the *National Statement,* which is available at the following website:

http://www.nhmrc.gov.au/book/national-statement-ethical-conduct-human-research

2. This approval is valid for five (5) years, subject to the submission of annual reports. Please submit your reports on the anniversary of the approval for this protocol.

3. Proposed changes to the protocol and associated documents must be submitted to the Committee for approval before implementation.

It is the responsibility of the Chief investigator to retain a copy of all documentation related to this project and to forward a copy of this approval letter to all personnel listed on the project.

Should you have any queries regarding your project, please contact the Ethics Secretariat on 9850 4194 or by email ethics.secretariat@mq.edu.au

The HREC (Medical Sciences) Terms of Reference and Standard Operating Procedures are available from the Research Office website at:

https://www.mq.edu.au/research/ethics-integrity-and-policies/ethics/human-ethics

The HREC (Medical Sciences) wishes you every success in your research.

Yours sincerely

Professor Tony Eyers
Chair, Macquarie University Human Research Ethics Committee (Medical Sciences)

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research* (2007) and the *CPMP/ICH Note for Guidance on Good Clinical Practice*.

Appendix 2 of this thesis has been removed as it contains published material. Please refer to the following citation for details of the article contained in this appendix.

Heydon-White, A., Suami, H., Boyages, J. *et al.* Assessing breast lymphoedema following breast cancer treatment using indocyanine green lymphography. *Breast Cancer Res Treat* **181,** 635–644 (2020).
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